SYMPOSIUM ON CRUSTACEA

PART III

MARINE BIOLOGICAL ASSOCIATION OF INDIA

MARINE FISHERIES P.O., MANDAPAM CAMP

INDIA

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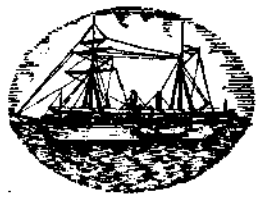

MARINE BIOLOGICAL ASSOCIATION OF INDIA

MARINE FISHERIES P.O., MANDAPAM CAMP

INDIA

PROCEEDINGS

OF THE

SYMPOSIUM ON CRUSTACEA

HELD AT

ERNAKULAM

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PART III

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ECOLOGICAL ASPECTS OF DIAPAUSE IN COPEPODS

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ABSTRACT

Among the arthropods the phenomenon of diapause has so far mainly been studied among the insects. Recent studies of freshwater copepods have shown diapause to be present also in this group. Resting stages are found in the egg, copepodid, and adult stages, and periods of dormancy are confined to both winter and summer, varying with the species. The dormant phase in the bottom mud may last for nearly a year under anaerobic conditions. The dormant copepodids of cyclopoids may be concentrated in the deepest part of the lake or on the slope, dependent upon the ecological conditions of which oxygen depletion is important.

The factors regulating the initiation of the diapause are still obscure, but photoperiodicity may be involved as among the insects. The diapause may be broken by physiological changes alone in a constant environment, but ecological factors also act as reviving factors of which change of temperature seems to be important.

An interesting aspect in freshwater cyclopoids is that a dormant bottom phase in the copepodid stage is found in some of the most limnetic species living in the plankton of the largest lakes of America and Europe. The resting stage is, therefore, not restricted to pond or littoral forms as previously supposed.

In marine copepods, no demonstration of a bottom resting stage seems to be on record. But there are indications that the phenomenon of diapause may be present also in marine copepods. Whether the scarcity of diapause in marine copepods is due to physiological differences or to lack of investigations remains to be shown.

A PHASE of dormancy in the life-cycle is known in most groups of animals. Among invertebrates it is best known among the insects where the so-called 'diapause' has been relatively well studied (Andrewartha, 1952; Lees, 1955). The evidence accumulated, especially in the last 10 years, indicates that the phenomenon of diapause is present also among the copepods.

It has been known from the turn of this century that freshwater calanoids could be hatched from dry mud, and that two types of eggs exist in many species of *Diaptomus*, one of which is a diapause egg capable of long resting periods and increased tolerance against unfavourable periods. In a recent paper Brewer (1964) has given ample evidence of diapause in *Diaptomus stagnalis* involving two periods of blocked development in the egg stage, the first being obligatory and confined to summer, the second facultative and confined to winter.

In the two other groups of copepods, the harpacticoids and the cyclopoids, there is so far no satisfactory demonstration of resting eggs, but resting stages are present among the freshwater harpacticoids as encysted adults (Fig. 1), and among the freshwater cyclopoids in some of the copepodid stages II–V and possibly also adults. These resting stages are characterized by a dormant phase in the bottom mud in which no development or growth takes place. For a review of literature on this subject up to 1958, *see* Elgmork (1959). The cyclopoid species with known resting stages so far are given in Table I. They are all freshwater forms.

The first copepods with resting stages discovered were encysted forms and these were the only known species with resting stages for about 4 decades, to the middle of the 1950's. This led to the use of the term encystment as synonymous with resting stage, and these terms can still be seen used indiscriminately. After the mid-1950's, however, several other species of cyclopoids with a resting

stage showed no encystment, and the presence of a cyst is not a necessary condition for the diapause stage in the freshwater cyclopoids.

TABLE I

Survey of diapause stages in freshwater cyclopoids 1964

No.	Species	Diapause stage. Copepodid stages	Encystment	Habitat	Start of resting period s = spring–summer a = autumn	Main references
1	*Cyclops bicuspidatus thomasi* Forbes	IV	+	Limnetic in lakes	s	Birge and Juday (1908), Moore (1939), Cole (1953)
2	*Mesocyclops leuckarti* (Claus)	IV + V	–	Limnetic in lakes, ponds	a	Fryer and Smyly (1954), Smyly (1961, 1962), Szlauer (1963), Elgmork (1964)
3	*Cyclops bicolor* Sars	III + IV	+	Ponds, littoral	a ?	Fryer and Smyly (1954)
4	*Cyclops strenuus strenuus* Fischer	IV	–	Ponds	s	Elgmork (1955, 1959)
5	*Mesocyclops oithonoides* (Sars)	V	–	Limnetic in lakes	a	Elgmork (1958)
6	*Cyclops insignis* Claus	IV	– ?	Ponds, littoral	s	Elgmork (1959: 35)
7	*Cyclops kolensis* Lilljeborg	IV	–	Limnetic in lakes, littoral	s	Patalas K. in Elgmork (1959: 170) Szlauer (1963)
8	*Metacyclops minutus* (Claus)	Adv. cop.	+	Temporary pools	After dry period	Rzóska (1961)
9	*Cyclops vicinus* Ulj.	IV	–	Limnetic in lakes, ponds	s	Patalas, *ibid.*, Wierzbicka (1962), Szlauer (1963)
10	*Cyclops bohater* Koz.	V	–	Limnetic	s	Wierzbicka (1962), Szlauer (1963), Einsle (1964 *a*)
11	*Cyclops scutifer* Sars	II, III, IV, V	–	Limnetic in lakes	s	Elgmork (1962, 1965)
12	*Mesocyclops hyalinus* (Rehb.)	IV	–	Limnetic in lakes, ponds	?	Smyly (1964)
13	*Cyclops strenuus* f. *landei* Koz.	IV	–	Limnetic	s	Einsle (1964 *a*)
14	*Cyclops vicinus lobosus* Kiefer	IV	–	Limnetic	s	Einsle (1964 *a*)
15	*Cyclops gigas* Claus	V	?	Ponds, littoral and profundal of lakes	s	Einsle (1964 *a*: 162)
16	*Mesocyclops edax* (Forbes)	IV ♀	–	Limnetic	a	Elgmork (1964)
17	*Eucyclops lilljeborgi* (Sars)	♀	–	Ponds, littoral	a	Elgmork (1964)
18	? *Macrocyclops albidus* (Jurine)	♀	–	Ponds littoral	a	Elgmork (1964)

In some species of cyclopoids only one stage is capable of dormancy, in other species two stages, and in *Cyclops scutifer* even 4 stages are found in diapause. In insect diapause usually only one stage is involved, and the copepods thus seem to deviate from the insects in that more stages are capable of diapause in the same species. The 4 stages in *C. scutifer* are especially interesting as they are combined with an unusually long life-cycle of 3 years in Southern Norway. The younger copepoid stages go into diapause one year, and the older ones in the next (Elgmork, 1965).

Some species of cyclopoids have been found to be able to overwinter as adult females in the bottom mud of both ponds and lakes (Elgmork, 1964). At the present stage of knowledge, however, it is impossible to decide whether this is a quiescent condition directly induced by adverse environmental conditions or is a diapause determined by hormonal changes not necessarily regulated by obviously unfavourable factors (*cf.* Elgmork, 1959–63).

It is generally accepted that resting stages are primarily confined to species living in the littoral of lakes or in ponds where the environmental factors show the greatest fluctuations. It is therefore of great significance that the cyclopoids with known resting stages include some of the most limnetic cyclopoids such as *C. bicuspidatus thomasi, M. leuckarti, M. oithonoides, C. scutifer* found in the plankton of the largest lakes of America and Europe. These observations therefore contradict the theory that resting stages are restricted to shallow water forms where their function seems most obvious.

These facts should lead to a re-evaluation of the traditional plankton technique. So far only the open water has been sampled in plankton studies and the bottom mud has generally been neglected. It is now clear that a proper plankton study in freshwater cannot be made without also taking into consideration the possibility that a large part, or even the total population, of some of the cyclopoids present may be hidden in the bottom mud during a large part of the year (Fig. 2). Nor can it be ascertained whether an increase in a planktonic cyclopoid population is due to the present production of new individuals or only to the emergence from the mud of a population produced the year before, but which had escaped consideration by descending to the mud. Neglect of the bottom resting stage may thus lead to false results in studies of geographical distribution, community composition, secondary production and related problems.

The cyclopoids with resting stages so far known can be divided into two phenological groups (Elgmork, 1959), one with a 'summer resting stage' initiated during the beginning of summer, and

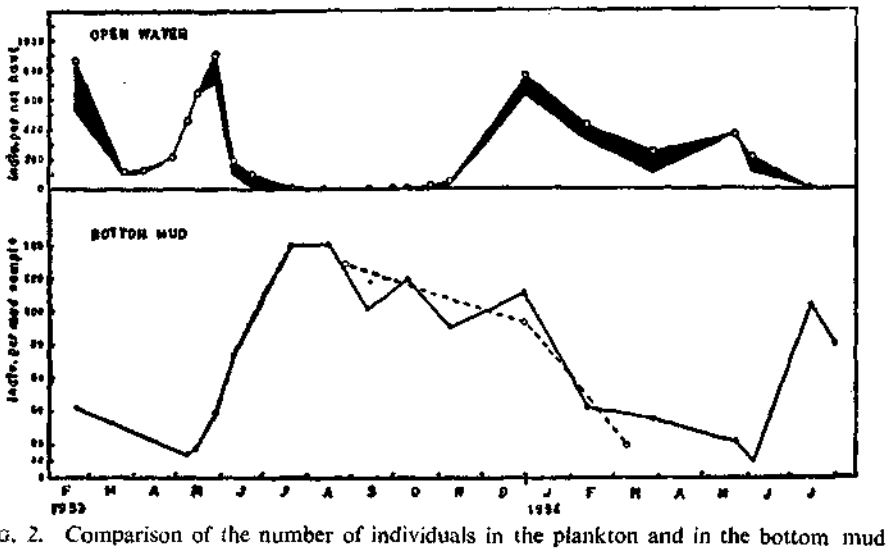

FIG. 2. Comparison of the number of individuals in the plankton and in the bottom mud in a population of *Cyclops strenuus strenuus*. *Black space* in upper curve represents copepodid IV which is the resting stage. *Stippled line* in lower curve is from 1955–56 (from Elgmork, 1959).

the other with a 'winter resting stage' initiated in the autumn. Both resting periods can be prolonged until the next spring, and the resting period can thus last for at least 10 months, often under severe anaerobic conditions.

The individuals in dormancy do not sink passively to the bottom from wherever they are in the water, but select a special area of the lake and actively penetrate deep into the bottom deposits. In some species there seems to be a tendency to concentrate in the deepest part of the lake, leading to tremendous concentrations in a restricted area. *C. scutifer, e.g.*, has been found at a density of about 4 million individuals per $m.^2$ in Norway (Elgmork, 1962). The horizontal distribution over the bottom, however, is influenced by environmental factors of which oxygen most probably is important. *C. strenuus strenuus* showed two different patterns of horizontal distribution in two subsequent years in a lakelet in Norway (Elgmork, 1959), one year with concentrations in the deepest anaerobic part, the other year on the slope above the oxygen limit (Fig. 3). In Esthwaite Water in England the resting stage of *M. leuckarti* was found in the deepest basin (Smyly, 1961), while in a lake in Poland the same species was confined to the slope of the basin (Szlauer, 1963). This difference may be attributed to the fact that the resting period starts after the autumn overturn in the English lake, but before the overturn in the Polish one, the low oxygen tension in the deeper part being apparently responsible for the avoidance of this part of the lake (Szlauer, 1963).

Resting copepodids of cyclopoids have been found as deep as 30 cm. down into the mud (Elgmork, 1959). The majority of resting individuals, however, are generally found in the uppermost layers of mud.

An important aspect in the diapause of copepods is the possible factors terminating and initiating the resting period. From the study of *C. strenuus strenuus* (Elgmork, 1959) it is clear that the resting condition may be terminated by internal physiological changes alone in a constant environment. There thus seems to be an internal clock that can wake the animals in the absence of environmental fluctuations. That changes in the intensity of dormancy take place was demonstrated in pond populations of *C. strenuus strenuus* as shown in Fig. 4. Dormancy was at its deepest in the warmest part of the summer, but in the autumn a small fraction of individuals start to emerge, and in the winter the majority of individuals in the mud emerge at the first filtering of the water above the mud. Similar results with a demonstration of revival in a constant environment and changes in the intensity of dormancy with time have also been demonstrated in *M. leuckarti* (Smyly, 1962).

But changes in the environment are usually important as reviving factors even if they are not a necessary condition for revival. It seems as if temperature changes, especially rise in temperature, act as arousing factor in the field (Elgmork, 1959; Smyly, 1961). Wierzbicka (1962) states that temperature is of no significance in the emergence from a resting to an active state in the laboratory, and surmises, as does Szlauer (1963), that a rise in the oxygen pressure may have a reviving effect. Even if this may be so in the laboratory it is difficult to understand how this factor can have any influence down in the sediments except in the uppermost layers of mud, which, according to Mortimer (1941–42), are the only layers of deposits influenced during a change from reducing to oxydizing conditions in the water above the mud. Smyly (1962), on the other hand, found similar rates of revival of resting copepodids of *M. leuckarti* in aerated and stagnant mud samples.

In the diapause eggs of *Diaptomus stagnalis* a reduction of oxygen is a necessary condition for termination of the diapause (Brewer, 1964).

As early as 1908 Birge and Juday noted the difficulty of correlating the induction of the resting condition with environmental factors. Different possibilities, such as temperature, lack of food (Smyly, 1961), and a reducing agency (Wierzbicka, 1962) have been proposed, while Szlauer (1963) states that a favourable respiratory condition is a necessary factor. Elgmork (1959) showed that

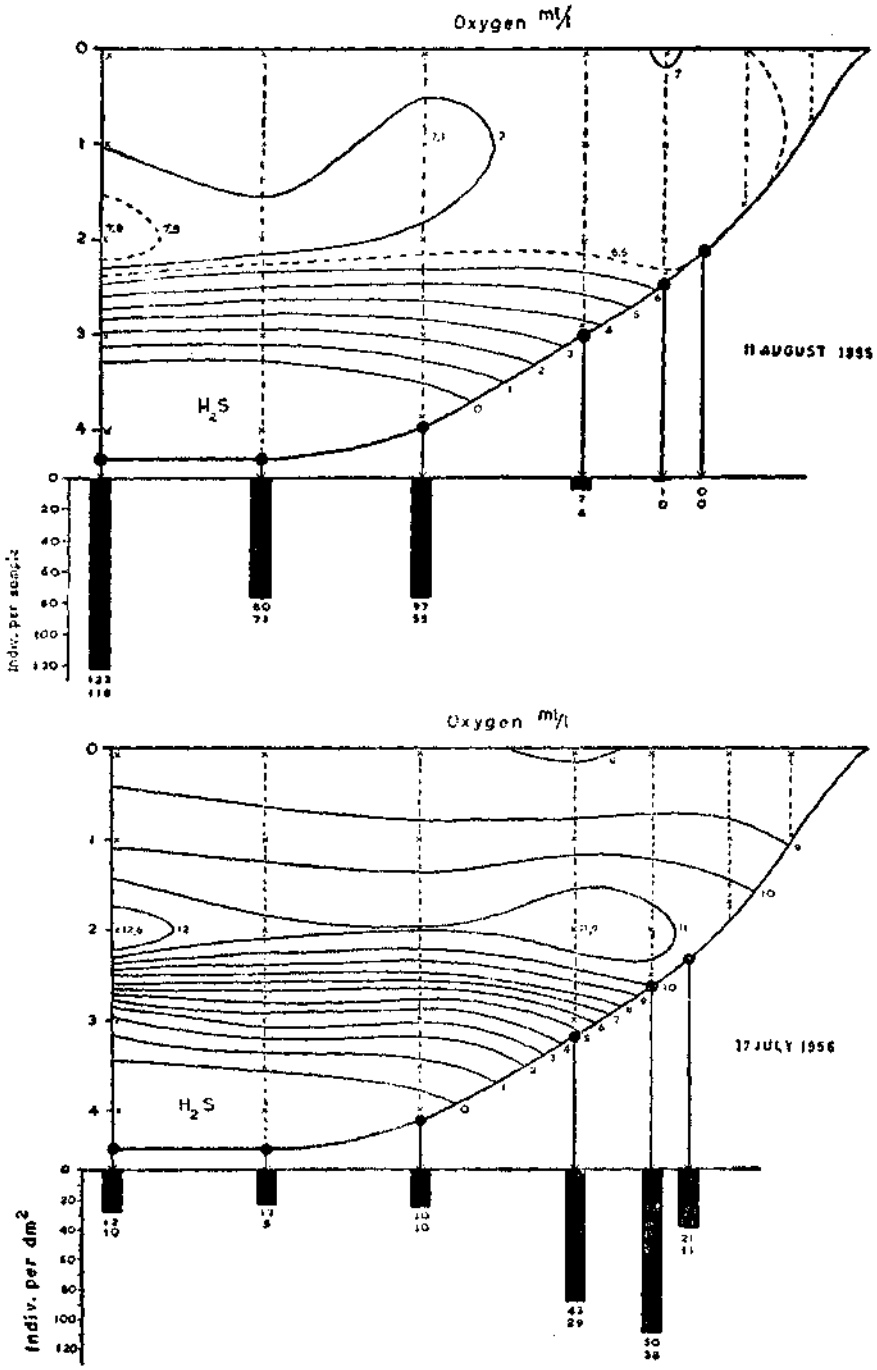

FIG. 3. Horizontal distribution in relation to oxygen conditions of resting stages of *Cyclops strenuus strenuus* in two subsequent years. Horizontal scale about 1 : 250. depth in m. (from Elgmork, 1959

in *C. strenuus strenuus* diapause started at the same time in small littoral, warm, and well aerated ponds as in a stratified lakelet with much colder water and an anaerobic hypolimnion. This apparent independence of the local environment led to the hypothesis that diapause is initiated primarily by changes in length of day, a factor so important in insect diapause (*cf.* Lees, 1955). Later Smyly (1961) and Einsle (1964 *a*) considered the same possibility, and Einsle (1964 *b*) has recently demonstrated in laboratory experiments that photoperiodicity can induce dormancy in a cyclopoid of the *strenuus* group. Smyly (1962) found that low temperature must prevail if the dormant phase in *M. leuckarti* was to last for a long time, but he could not re-induce dormancy by low temperatures.

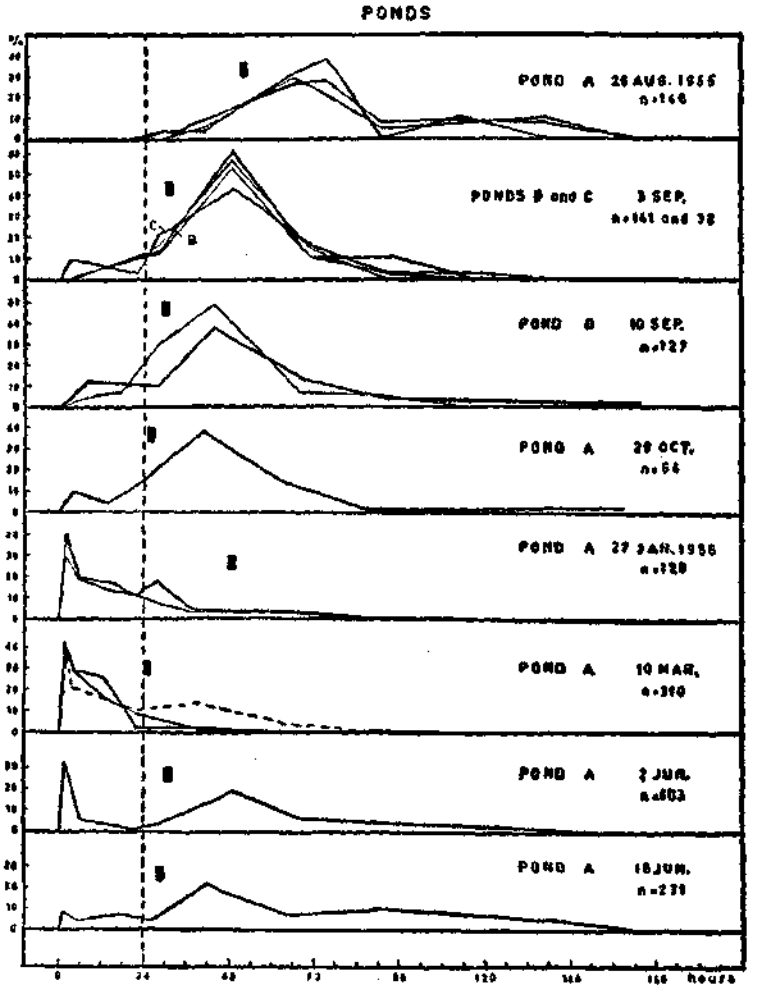

FIG. 4. Rate of emergence of resting copepodids IV of *Cyclops strenuus strenuus* from shallow ponds in Norway as recorded by repeated filterings of the water above the mud in the laboratory. *Black rectangle* indicates period of transport from a field laboratory (from Elgmork, 1959)

So far only freshwater copepods have been considered. The phenomenon of diapause seems, at this stage of knowledge, to have evolved primarily in the freshwater forms. This may seem natural in view of the more variable freshwater environments, but diapause would also be expected to exist at least in marine near-shore and estuarine species. Its apparent absence in marine biotopes

therefore may possibly be due to insufficient study, as resting stages of these small creatures may easily have been overlooked.

There are, however, some indications that a resting phase may be present also in marine copepods, even though no direct demonstration in the bottom mud seems to be on record. Some species of calanoids, *e.g.*, *Calanus finmarchicus*, pass the winter as copepodids, usually in stages III to V and in the warmer European waters as cop. V (Marshall and Orr, 1955). There seems to be a period of blocked development which is broken in mid-winter, when the copepodids start to develop, independently of any change in temperature, but no dormancy has been reported. The hypothesis that this is a diapause phenomenon is supported by the results of Carlisle and Pitman (1961) showing differences in neurosecretion between summer and winter copepodids analogous with diapause changes in insects. Conover (1965) reports that *Calanus hyperboreus* enters a state of blocked development without dormancy in cop. V which is not broken for some time, irrespective of changes in the environment. Similar arrests in development but without dormancy were found in *Cyclops vernalis robustus* studied by Coker (1933); *see also* Einsle (1964 *a*) and Smyly (1964) for further evidence of a possible diapause without dormancy. It is evident, however, that a retardation of development may also follow from low temperature or scarcity of food, and it is difficult at present to assess the importance of a possible physiological mechanism in these cases.

The possibility of a morphologically different resting egg in the neritic calanoid *Temora longicornis* is reported by Agot Berner from Scottish waters (personal communication). There is also some indication that marine planktonic copepods may have a resting period in the mud, although proof of this is still lacking. The estuarine copepod *Acartia tonsa* is said to appear in the plankton as cop. V on the American Atlantic coast (Jeffries, 1962) and abruptly in the adult stage on the Pacific coast (Russel, personal communication). These sudden occurrences may indicate a resting stage in the mud during the winter. Furthermore, Conover (1956 and personal communication) reports adult *Acartia clausi* from the mud in Long Island Sound, although it is not certain that the individuals were alive at capture or whether they were contaminations from the water above. However, there were at that time few *A. clausi* in the plankton tows and the individuals were all well preserved.

Further studies are necessary to evaluate the significance of a possible resting stage in marine copepods, so important in the freshwater forms.

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FIG. 1. Encysted harpacticoid copepod. (from Lauterborn and Wolf, 1909)

ON THE FEEDING BEHAVIOR OF PLANKTONIC MARINE COPEPODS AND THE SEPARATION OF THEIR ECOLOGICAL NICHES

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ABSTRACT

Because many investigators necessarily work with preserved zooplankton rather than the living animals, our knowledge of the feeding of the planktonic copepods, especially the oceanic species, is based largely on studies of the morphology of the mouthparts and the gut contents of preserved specimens. However, feeding rates of some readily obtainable, hardy species have been measured in the laboratory using various types of food. Methods used in making these measurements are reviewed. Factors which may affect the feeding rate of a particular species of copepod on a particular type of food include temperature, light, size of experimental container, duration of the experiment, the concentration and age of the food, and the developmental stage and sex of the copepod. Methods used by copepods to capture food are discussed.

The results of various studies permit many species to be classified as herbivores, carnivores or omnivores. In general, however, few cases of high food specificity are known: most planktonic copepods show some selectivity in feeding but each species apparently feeds on a rather wide variety of food. This is especially true of the species feeding by filtration. Further work is needed, especially to determine the role of detritus and heterotrophic micro-organisms in the nutrition of copepods.

The extent to which the niches of the planktonic species of an area are separated by differences in feeding behaviour is of special interest. The high species diversity of tropical zooplankton, the low standing crop of available food, and the relative stability of the environment suggest that diversification in feeding may be particularly important for tropical copepods. The availability of food in the Indian Ocean and the feeding behaviour of Indian Ocean copepods are discussed in relation to these theoretical considerations.

INTRODUCTION

THE feeding behavior of various planktonic copepods has been studied frequently because of the importance of these organisms in the food webs of the ocean, so that the basic mechanism of feeding and the general type of food eaten are known for some of the more common species. Because several good reviews of this subject have appeared recently (*e.g.*, 2, 57, 68, 70, 86, 94), this paper will not discuss the results of previous work in detail. Emphasis will be placed instead on some unsolved problems concerning the role of feeding behaviour in the organization of planktonic communities. Related problems concerning the assimilation of food and the spatial distribution of copepods relative to their food supply will not be discussed here.

METHODS OF STUDYING FEEDING BEHAVIOR

Because many investigators necessarily work with preserved zooplankters rather than living animals, analysis of gut contents has been a primary method of determining what oceanic copepods eat (*e.g.*, 7, 19, 27, 45, 59). The usefulness of this approach is limited by the fact that many potential food organisms leave no recognizable remains in the gut. Thus, the ingestion of organisms with hard parts (diatoms, foraminiferans, radiolarians, coccolithophorids, armored dinoflagellates, etc.) is easily seen; feeding on bacteria, naked flagellates, some types of detritus, soft-bodied larvae, or any prey not swallowed whole may be difficult to detect. Examination of the gut is often supplemented by a study of mouthparts (*e.g.*, 47, 94). Anraku and Omori (4) have recently correlated the mouth-part morphology of several species with experimentally demonstrated preference for plant or animal food.

Visual observation of copepods during feeding has shown how several species capture particulate food by filtration or other means (12, 14, 27, 36, 60, 83, 94). Digestive enzymes have been analysed to show that this food can be assimilated (9, 43).

Copepods have often been kept in the laboratory in the presence of various kinds of food, and the success of molting and survival (13, 33, 85, 87, 91), amount of fecal pellet production (15, 65, 83, 87), and number of eggs laid (64, 87) have been used as measures of the suitability of the offered food. More useful information is gained by measuring the rate of feeding on different foods. This is usually done by placing the copepods in a suspension of food particles for a relatively short time and measuring the change in concentration of food during the experiment. Concentrations of food may be assessed by direct visual counting of particles (32, 34), by photometric measurements of turbidity (*cf.* 56), by measuring the level of activity of radioisotope-labeled food (65, 66, 67, 96), or by determining the weight of the food after filtration (16). Because of uncertainty concerning the metabolic pathways of radioisotopes (8, 69), the relative insensitivity of turbidimetry and gravimetry, and the possible interference by fecal material in all three methods, a direct visual count is usually most satisfactory. This is especially true when feeding on various types of food in a mixture is to be measured (4, 41, 76). Electronic counting of food particles (44, 75) removes the tedium of visual counting for some types of food when no extraneous particles are present.

The grazing or filtering rate (F) is usually computed from the following formula:

$$F = V/T.\ \ln(Ci/Ct)$$

where V is the volume of water available per copepod in the experimental containers, T is the duration of the experiment, Ci is the concentration of food particles in control containers with no copepods, Ct is the final cencentration of food particles in containers where copepods were present, and F is the volume of water swept clear of food particles per unit time by a copepod (32, 34). The rate of ingestion of food may then be calculated as the grazing rate F times the biomass (volume, weight, carbon content, caloric content, etc.) of food available per unit volume of water (18). Factors known to affect the laboratory measurement of grazing rate include temperature (3, 14, 32, 65); the size of the experimental container (8, 18, 65); the concentration and age of phytoplankton cells used as food (14, 76); the duration of the experiment (76); the species and size of the food particles (41, 76); and the size and developmental stage of the copepod (8, 34, 67, 76). The effects of light and time of day are uncertain (14, 32, 35, 65, 82). Generally speaking, grazing experiments should be run in the dark at constant temperature in containers of at least 500 ml. volume. The containers must be agitated in some way to keep food particles in suspension, and the concentration of food particles should be as low as is consistent with precise counting. Sedimentation or filtration may be used to concentrate food particles prior to counting after an experiment.

Present Status and Unsolved Problems

It should be pointed out that, with the possible exception of North Atlantic *Calanus*, generalizations concerning the feeding of planktonic copepods are based on inadequate evidence. There is no study of marine forms comparable to Fryer's (30, 31) work on freshwater cyclopoids. Nevertheless, some marine copepods may be placed in general trophic categories on the basis of studies cited in the previous section of this paper and some unpublished observations by the author in the Indian Ocean. These categories follow the basic outline used by Wickstead (94), but the incomplete and sometimes contradictory nature of the evidence and the fact that many species may change trophic categories, either seasonally or during ontogeny, should be emphasized. Little is known about the feeding of juvenile stages even when the adult has been studied (*cf.* 36, 67).

Adult copepods apparently relying mainly on phytoplankton for food include *Acartia*, *Calanus s.l.*, the Eucalanidae, the Paracalanidae, *Pseudocalanus*, and *Oithona*. These forms usually have

many blunt, grinding mandible teeth and an abundance of fine setae on the mouthparts, and capture food largely by filtering or raking particles out of the water. However, the first three groups feed on brine shrimp nauplii when these are offered as food, and *Calanus* and *Acartia* spp. in particular often contain crustacean remains in the gut. All may feed on small animal protozoans. Omnivorous copepods—those thought to feed commonly on both plant and animal food—include *Temora, Centropages,* and the Metridiidae. Copepods feeding mainly by predation include *Tortanus, Candacia, Euchirella,* the Euchaetidae, the Pontellidae, and probably *Corycaeus* and *Oncaea* Most of these forms have sharp mandible teeth for piercing or tearing, and are able to grasp active prey. Instances of plant food in the guts of some of these groups are reported.

Very little is known concerning the role of detritus and bacteria in the nutrition of planktonic copepods. Since particulate organic detritus in the sea is approximately five times more abundant than phytoplankton and bacteria and fifty times more abundant than zooplankton (79), it constitutes a large potential food source for filter-feeding organisms. Copepods may be unable to assimilate much of the carbon in detritus, however, of particular interest are recent studies concerning rather large aggregates of organic detritus (58, 89) which may be produced from dissolved organic matter and may be eaten and assimilated by zooplankton (6, 89). Detritus, together with organisms migrating downward from surface waters (93), has been considered an important food source for deep-water species. While single bacteria cells are probably too small to be filtered out of suspension by most copepods, bacteria may be readily eaten when absorbed on inorganic or organic particles as aggregates (*cf.* 61).

Cases of selective feeding within the general trophic categories are known from field and laboratory investigations. Although most predatory species appear to be rather unspecialized as to diet and opportunistic in what they catch, some may be specialized to feed on a particular food, such as marine eggs (94). Unpublished laboratory experiments show that some omnivores, such as the Metridiidae, may eat only the animal food from a mixture of diatoms and brine shrimp nauplii. Anraku and Omori (4) demonstrated similar selectivity in *Centropages typicus.* Filter-feeding *Calanus* prefers large phytoplankton cells to small, especially as its own body size increases, (41, 76), and does not eat some cells in the natural phytoplankton, such as spiny *Chaetoceros* or dinoflagellates like *Ceratium. Acartia* shows some selectivity of feeding when food is scarce (84). In spite of these cases of selectivity, however, the gut contents of filter-feeding species usually reflect fairly accurately the composition of the food available in the water around them (7, 62). Each species apparently feeds on a wide variety of food, rather than showing high food specificity, and the size and availability of a potential food particle is usually more important than its taxonomic status.

The Niches of Zooplankton—Preliminary Considerations

A niche will here be defined in a qualitative way as a place in the spatial arrangement and a role in the functional organization of a community, thus including both physical habitat and trophic relationships with other organisms. Strictly speaking, the presence of organisms is not a necessary requirement of a niche, but it is common to define a niche by the occupancy of a single species, so that "niche" and "species" are functionally equated. The term "population" refers to the individuals of one species within a physically definable area. Two populations inhabiting the same area are termed sympatric. The exclusion principle (or Volterra-Gause hypothesis) may therefore be stated as follows: The niches of two sympatric populations cannot be identical indefinitely, nor can the overlap of two niches ever be identical to the entire niche of either population for very long (51, *see also* 39). Stated positively, when two populations are persistently sympatric, they must be ecologically distinct in some way.

Two fundamental conditions must be met before the exclusion principle is applicable to any pair of sympatric populations. First, there must be a single, density-dependent, limiting factor which controls both populations (40, 95). Thus both populations must belong to the same trophic

level. Further, if one of the two populations is limited by a predator rather than by food or space, and the other population is either not so limited or is limited by a different predator, coexistence between the two competing populations is possible. Second, sufficient time must be allowed so that competition can reach its conclusion and one of the species can be eliminated. The environment must therefore be stable enough so that natural selection favors the same species for several generations. The two populations can coexist if changes in the environment reverse the direction of competition faster than extinction can occur (17, 50, 51, 52).

In view of the exclusion principle, it is of interest to determine what factors are responsible for (or at least related to) the coexistence of sympatric populations of planktonic copepods of the same trophic level. The possibilities for separation of niches may be rather limited in the relatively unstructured planktonic environment (*cf.* 26, 81). Pejler (80) summarized the possible mechanisms through which zooplankton populations may avoid or reduce direct interspecific competitions as follows: (1) Different horizontal and/or vertical distributions; (2) differences in selective feeding, each species feeding preferentially on a different food; (3) different seasonal distributions, the maximum abundances of the different species occurring at different times of year; and (4) occasional periods during which food is not a limiting factor.

Mechanisms 1 and 2 provide ecological distinctness through spatial and functional separation of niches respectively. Mechanism 3—a temporal separation of niches—is usually related to seasonal changes in the environment which change the direction of competition. Seasonal differences in breeding cycles resulting in temporal separation may be genetically regulated, just as selective feeding or vertical distribution may be. Periodic excess of food (mechanism 4), such as a phytoplankton bloom, is also a temporal escape from the consequences of continued competition. For a period of time, potentially competing populations are no longer controlled by the same limiting factor.

Even within a moderately small and homogeneous area, copepod populations are seldom distributed randomly or evenly, but are usually aggregated (patchy) both horizontally and vertically. If the patches have some stability over time and if the species aggregate so that patches of each species differ in location from those of other species, then spatial separation between species may occur even within one water mass. A marked instance of horizontal and vertical spatial separation on a larger scale is reported for the genus *Rhincalanus* in the Indian Ocean (90).

Because plankton communities usually consist of diverse species subjected to considerable intermixing, it is difficult for a given species of planktonic copepod to become closely associated with one food species for any length of time. It is possible, however, for different species of filter-feeders to feed most effectively on different sizes of food organisms and therefore fill somewhat different niches (50, 92, p. 150). A wide spectrum of food particle sizes can thus support several species of filter-feeders if each is specialized to feed on a different size class with maximum efficiency. The diversity of food must be great enough for each species to find enough of that type of food on which it feeds more effectively than competing species. Factors other than particle size, such as shape, presence of spines or a thick frustule or armor, or some chemical factor, might also permit planktonic copepods to discriminate between and preferentially utilize certain foods.

The Separation of Niches of Planktonic Copepods

Few field investigations of marine zooplankton have been directly concerned with niches and competition or contain sufficient information to be examined from this point of view (*cf.* 71, p. 72). The zooplankton communities of various temperate and arctic areas have been studied by several workers in recent years (*e.g.*, 20 through 25, 37, 63, 78). The cited works suggest that occurrence of congeneric species in the same water mass at the same season is somewhat unusual in temperate areas (*cf.* 29), and Fager and McGowan (28) found evidence for selection against congeneric species pairs in recurrent groups of non-copepod zooplankton in the North Pacific.

However, there is insufficient information in most cases to discuss more than seasonal or gross distributional differences between the populations studied. In Marshall's (63) study particularly, the similarities between the annual life-histories, seasonal vertical distributions, and seasonal variations in body size of most of the populations are more striking than the differences. Of the eight genera and species examined by Marshall, three were omnivorous and the rest were herbivorous. There was no evidence of selective feeding by these species, so that the niches cannot be defined more closely. Particularly in arctic waters, the broods of most herbivorous zooplankton coincide with the summer bloom of phytoplankton, so that niches are not separated temporally. However, different carnivorous species produce broods at different times throughout the year, which may reduce interspecific competition (25, 37).

Geographic (hydrographic) separation is evident in the sibling group *Calanus finmarchicus, C. helgolandicus, C. glacialis, C. pacificus, C. australis,* and *C. chilensis* in the world ocean (5, 11, 53, 54), and in the sibling pair *C. lightii* and *C. tenuicornis* off the North American western coast (10). Mullin (77) was unable to attribute the coexistence of *C. finmarchicus, C. glacialis* and *C. hyperboreus* in the Gulf of Maine to differences in selective feeding or vertical distribution of the three species, and emphasized the importance of the periodic excess of food represented by the spring bloom for the continued survival of *C. glacialis* and *C. hyperboreus.*

Somewhat more extensive information is available on the comparative ecology of species of the genus *Acartia.* Conover's (14) study of *A. clausi* and *A. tonsa* in Long Island Sound demonstrated some selective feeding, which led to the suggestion that these two species might select slightly different food. Both fed mainly on phytoplankton captured by a raking movement of the second maxillae, although some animal food was also taken. The niches of the two species were separated largely by differences in seasonal abundance, *A. tonsa* being dominant in winter and spring and *A. clausi* most abundant in summer and fall. In early spring and late summer, there was probably considerable overlap of the niches. Seasonal alternation of abundance was confirmed for other areas (1, 55). In the Black Sea, a seasonal separation of *A. clausi* and *A. latisetosa* was found, the former being present throughout the year but the latter being strictly a summer form (84). Some selective feeding was demonstrated, but no major differences between the species were indicated. Both species fed omnivorously.

Raymont and Miller (88) studied the interactions between natural populations of plankton in large tanks enriched with nitrate and phosphate. Although this artificial community was initially quite diverse in numbers of species present, the final "climax" community was of low species diversity, being dominated by one species of phytoplankton and one species of zooplankton, *Acartia tonsa.* Interspecific interactions other than competition affected the outcome of these experiments, but the results do suggest that under these confined, artificially controlled conditions, exclusion may occur so that all but one species are eliminated from a rather generalized niche.

In temperate waters, therefore, spatial or temporal separation of congeneric species is frequently related to the seasonal cycle of temperature or the movement of different water masses past geographically fixed sampling stations. However, the niches and competition of sympatric species, even those of different genera, are poorly defined, particularly with regard to food. It is generally thought (*e.g.* 42) that herbivorous copepods are food-limited in temperate waters much of the year, but evidence for this is largely indirect. The lack of evidence for highly selective feeding among most filter-feeding copepods has already been mentioned.

Because of the generally greater climatic stability of tropical areas, populations are usually more stable in size than in temperate waters. Grice and Hart (38) confirmed earlier studies in finding a much higher species diversity of surface-dwelling zooplankton in the tropical Sargasso Sea than in temperate neritic waters, although the total zooplankton biomass was much greater in the latter region. The biomass and species diversity of phytoplankton follow the same geographic pattern (49). Similar findings have been reported for North Pacific Plankton (46). Grice and Hart interpreted their results to mean that intraspecific competition is more important than interspecific competition in the Sargasso Sea. An alternate or more complete interpretation is that

intense interspecific competition in a stable environment has caused the Sargasso Sea populations to become ecologically specialized, permitting higher species diversity. Within the resulting small niches, intraspecific competition for food keeps the individual populations small. Since the environment and populations are rather stable, and the food supply apparentlylimits the zooplankton most of the year (74), some sort of niche separation between zooplankton populations is to be expected. The nature of this separation remains unclear, lacking an extensive functional study of the populations involved.

Although there may be a regular seasonal variation in phytoplankton productivity in tropical waters (72, 73), many tropical copepods apparently breed throughout the year (48). If this is generally the case, temporal separation of niches is of little importance. Analysis of the gut contents of tropical copepods (45) and selective feeding experiments (Mullin, unpublished) do not reveal significant differences in the feeding of the species within one trophic category. Although the diversity of species of phytoplankton is greater in tropical than in temperate waters (49), Mullin (in press) found that the size distribution of particulate organic carbon in the tropical Indian Ocean is similar to that in the temperate Gulf of Maine. If this finding is generally applicable to tropical waters, and if filter-feeding copepods "select" their food mainly on the basis of size, the effective diversity of food is no greater in tropical than in temperate oceans.

Examination of raw data of Grice and Hart shows that herbivorous and omnivorous calanoid copepod species restricted to temperate shelf and slope waters outnumber "strictly Sargasso Sea" species of these trophic categories, while the "strictly Sargasso Sea" predatory calanoids outnumber the "strictly temperate shelf and slope" predatory calanoids by almost two to one. This suggests that much of the increase in copepod diversity in tropical as compared to temperate communities is attributable to the predatory species rather than to the filter-feeders. Grice and Hart (38) and Heinrich (46) noted that in tropical waters the biomass of carnivorous zooplankton was greater relative to the biomass of herbivores than in temperate waters.

As is apparent from this discussion, conclusions concerning the factors permitting planktonic copepods to coexist sympatrically are speculative at best. Feeding behavior serves to divide the species into different, but frequently overlapping, trophic categories. Feeding is rather nonselective within each trophic category, however, so that several sympatric populations apparently utilize the same food resources. There is at present no reason to believe that these populations are limited by different predators. Spatial separation of closely related species occurs commonly, and seasonal fluctuations in the environment permit temporal separation of niches in temperate waters. Of special interest is the situation in which a seasonal phytoplankton bloom provides temporary release from food limitation for polar and temperate populations. Further study of limiting factors is obviously necessary, and attention must also be paid to small-scale environmental diversity, at present either undetected or uncorrelated with what is known about copepod biology, which may be related to the observed diversity of planktonic communities.

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OBSERVATIONS ON THE ECOLOGY OF BARNACLES IN THE ELBE-ESTUARY

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ABSTRACT

There are found four species of barnacles in the Elbe-Estuary: *Balanus balanoides* (L.), *Balanus crenatus* Brug., *Balanus improvisus* Darw. and *Elminius modestus* Darw.

The biotop is characterized with its changes of abiotic factors especially salinity and temperature and the spreading of the balanids is recorded in the estuary. *Elminius modestus* was found first in the Elbe-Estuary in 1953 near Cuxhaven. After the strong winter of 1962-63 the *Elminius* population was decreased in a high degree, because this species is more sensitive to low winter temperatures in the tidal zone of the estuary than *B. balanoides* and *B. improvisus*.

The nauplii and cypris stages occur from April/May to October/November, the season of settling is at the same time or a little later. The maximum of cypris larvae in the plankton is not quite in accordance with the attachment of the cypris, as a considerable part is being destroyed by predatory plankton and benthic organisms.

The calcification of the shell after metamorphosis takes place far more quicker in *Balanus balanoides* in the tidal zone than in *Balanus improvisus* living mainly under LWM. The calcareous reserves for the primary calcification of the chitinous shell after metamorphosis are already laid out at the metanauplius.

IT is a well-known fact that barnacles found damaging ships' bottoms can survive great changes in abiotic factors. Some of the species occur in regions where such environmental variations are the rule. According to their ecological potential their distributional limits are determined horizontally and vertically. Under favourable conditions reproduction is possible; otherwise only a sterile dispersal takes place. These conditions may be very clearly observed in estuaries with a high rate of change in abiotic factors. The main factor determining their distribution is salinity.

In the Elbe-Estuary four species of barnacles occur: *Balanus balanoides* (L), *B. crenatus* Brug., *B. improvisus* Darwin and *Elminius modestus* Darwin. Their distribution and ecology has been studied for many years. Before discussing the ecology of these species an understanding of the biotop and the abiotic factors, especially water temperature and salinity are necessary.

The brackish-water region of the Elbe-Estuary has been already divided into haline districts on the basis of biological observations by Kirchenpauer (1859) and Dahl (1891). The euhaline zone ("Westwasser") occurs in the western part near the lightship "Elbe 1" (Fig. 1), the polyhaline zone between it and Cuxhaven, the mesohaline zone from Cuxhaven up to Brunsbüttel and the oligohaline zone between Brunsbüttel and the Glückstadt Region. Here brackish water ends and freshwater begins. These zones are naturally not fixed, but change somewhat with the tides and weather conditions. The bottom salinities are usually higher, the differences being often significant Fig. 1 (Kühl-Mann, 1953; Lucht, 1964).

During the year the water temperatures show a regular gradient, which underlies certain veriations, the maximum temperature being found in July and August with values up to 22° C. at Cuxhaven, and the minimum temperatures of + 1 to −1° C., in January and February.

In cold winters the Elbe can carry ice for shorter or longer periods and the Wadden sea is covered with ice. These conditions determine the beginning and end of the period of the barnacle attachment season. The long-term salinity changes caused by the waterflow of the Elbe are not so regular but they are highly important for biology. When the waterflow is reduced the salinity is high, *e.g.*, in the years 1947–53, 1959–60, 1962–64. The salinity was lower from 1954 to 1958 and in 1961, sometimes considerably below 15‰. The haline zones and also the favorable conditions for attachment of marine larvae are shifted upstream or reversed. Short-term changes of the salinity and other hydro-chemical factors such as pH, alkalinity, seston and detritus are caused by tides, weather conditions and winds (Kühl, 1964).

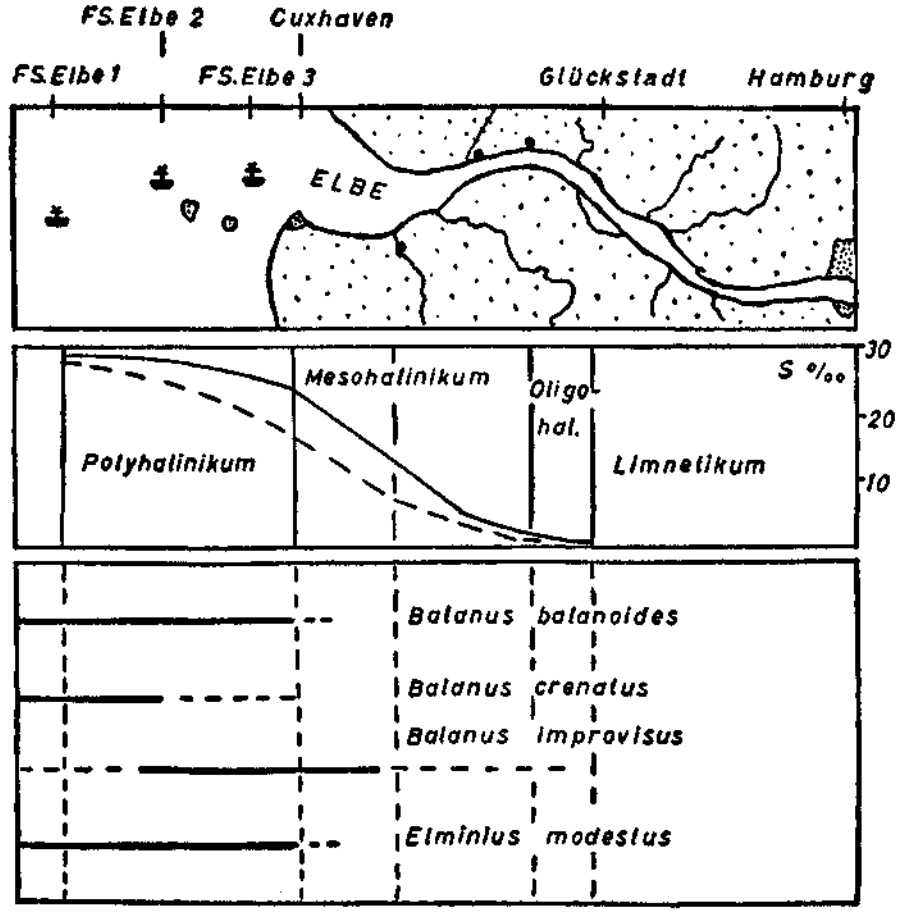

FIG. 1. Map showing the Elbe-Estuary, average salinity at flow and ebb (broken line) based on records between 1949 and 1962, haline zones and distribution of the observed barnacle species.

In the Elbe-Estuary vertical plankton samples were taken with the "Helgoländer Larvennetz" during 14 trips with the research vessel "Uthörn" from 1949 to 1962 (Kühl-Mann, 1953). At the "Alte Liebe"-Pier-Cuxhaven regular plankton samples were taken for many years. 50 L water samples were sieved or vertical catches were made with a small net of 40 cm. aperture and a mesh width of 280 μ. For investigating the attached cyprid larvae and young barnacles test plates of different inert materials as glas, plexiglas, PVC or slate were used. The cyprids or barnacles were counted from an area of 100 or 1,000 cm.^{2} (Kühl, 1957). In the figures mean values from several samples or the prozental distribution are given.

Balanus balanoides (L.) is found from the Elbe-Estuary to the limits of the polyhalinikum a few kilometers towards Cuxhaven. On the light ships and buoys *Balanus balanoides* settles in the upper parts below the green-algae zone. The density is in general not very high compared with that of *Balanus crenatus*. Higher quantities of *Balanus balanoides* are found on the jetties and piers in the Cuxhaven region. On the stone they mainly settle on the vertical sides of the stones. No *Balanus balanoides* were observed on the horizontal surfaces of the big stones formerly. Although Cuxhaven populations are dense no cylindric forms have been observed as they were found also at *Balanus crenatus* in the Elbe-Estuary. The patella type was observed in wave protected areas as well. The carino-rostral diameter in general measured up to 12 mm. After the cold winter of 1962 the diameter of *Balanus balanoides* increased very significantly up to 22 mm. and 10 mm. high. At the wooden pier of the "Alte Liebe" the distribution of *B. balanoides* increased 0·5 m. reaching the green algae zone. *Balanus balanoides* has been described by Krüger (1927) as mainly stenohaline but also occurring in the Kattagat at a salinity of 30–25‰. Krüger (1940) also noted that *Balanus balanoides* settled in the litoral region in salinities of 13‰. The observations at Cuxhaven show that *Balanus balanoides* can endure at times very low salinities and that reproduction is the rule. Since 1962 the salinities, caused by a low waterflow, have been relatively high at Cuxhaven (for long-term more than 20‰). It is possible that the mean increase of the diameter growth of *B. balanoides* by 3 to 4 mm. is caused by the higher salinity, in the same way as the increase of the settled area.

Balanus crenatus Brug. normally inhabits the outer parts of the Elbe-Estuary (lightships Elbe 1 and Elbe 2 and buoys). In general the upper limit in the region of the lightship Elbe 2 (Fig. 1) occurs at an average salinity of 20 to 30‰. If the waterflow of the Elbe is lower than 500 m.^{3}/sec. at Artlenburg—the salinity at Cuxhaven is in summer for a longer time higher than 15 (—20)‰ and *Balanus crenatus* occurs also in this region. The minimum values at which a new attachment can start are at 20‰. The settlement starts in June. The lightships Elbe 1 and 2 have primarily a first settlement of *Balanus crenatus*. Here the barnacles reach a considerable size, with a dense settlement causing cylindric forms up to a height of 40 mm. and 15 mm. basis diameter. In the Cuxhaven region *B. crenatus* does not attain such growth. We found a height of 25 mm. and 8 mm. basis diameter. At a single settlement the patella type was observed to have a basis diameter of 10 mm. and a height of 10 mm. Krüger has remarked that *Balanus crenatus* can endure salinities of 9‰. It is possible that *B. crenatus* is able to live in the Elbe region at such low salinities, but a new settlement respective reproduction is impossible. *Balanus crenatus* has also been reported to occur in the Elbe by Kirchenpauer (1859) and Dahl (1891).

Balanus improvisus Darwin is an euryhaline species and is therefore found in the poly- and meshohaline zones. On the lightship "Elbe 3" and the buoys towards Cuxhaven this species settles in abundance and occurs up the river to the limnetic zone. Kirchenpauer (1859) also recorded a *Balanus crenatus*, which was observed by him on the buoys at St. Margarethen a little village only 15 km. downstream from the brackish limit. Dahl confirmed this record (1891). As Schaper (1922) has stated it was at least partly *Balanus improvisus*. The cyprids of *Balanus improvisus* drift up the river with the tides and if the water-flow diminishes the haline zones shift upstream and the settlement can start. The adult animals have a higher capacity for resistance and it is therefore later possible to find *Balanus improvisus* or its remains in the freshwater regions. Thus Caspers (1949) stated *B. improvisus* occurs at Pagensand about 15 km. upstream from the brackish-water limit. The reproduction takes place only in the polyhaline zone. *B. improvisus* settles from the bottom up to about 0·5 m. above the mean low watermark on all available substrates.

Elminius modestus Darwin introduced from Australian waters to Europe was first seen in 1963 on the Cuxhaven piers (Kühl, 1954, 1963) from whose population the entire estuary was inhabited. Today *Elminius* occurs at Heligoland (Den Hartog, 1959) and in the Wadden sea. The distribution is the same as for *Balanus balanoides*. *Elminius* settles from the bottom up to the green algae zone, the vertical distribution being somewhat greater than for *Balanus balanoides*. *Elminius* attached itself also to the horizontal surfaces of the stones on the jetties while *B. balanoides* settled mainly on the vertical walls as before mentioned. In the tidal zone *Elminius* is more sensitive

to low winter air temperatures than the endemic species. Former observations in the Wadden sea showed that after longer cold periods in ice-covered waters *B. balanoides* and *B. improvisus* survived on the stones and mussels, whereas all the *Elminius* died. After the long cold winter 1962–63 in which the Wadden sea was covered by an ice layer for three months the *Elminius* population was diminished so completely that only one single *Elminius* appeared on the testplates very late in September 1963 whereas in the previous years in June and July all substrates were settled completely in the tidal zone. In 1964 the population was regenerated, that a strong *Elminius* settlement could be observed, but not in the same density as in the years before. *Elminius* settles somewhat later than *Balanus balanoides* and *B. improvisus*, in general in June. At the end of the attachment season in October *Elminius* have reached a mean basic diameter of 10 mm. and a height of 6–8 mm. Cylindric forms were not observed in a very dense maximum settlement.

It was mentioned that *Elminius* begins to settle somewhat later than *Balanus improvisus*. This is clear where the new attachment of *Balanus improvisus* and *Elminius modestus* are compared in a favorable year such as 1959. During June 2,090 barnacles settled monthly on testplates on an area of 100 cm.² of which 80% were *B. improvisus*; in July there were 748 barnacles, 55% being *B. improvisus*; in August, 511 barnacles w th 60% *B. improvisus*, in September 597, with 53% *B. improvisus*, in October 932 barnacles, but only 3% *Balanus improvisus*, the rest was *Elminius modestus* (Fig. 2). The long and severe winter 1962–63 proved that *Elminius* is more sensitive against cold winter temperatures, than the endemic species. Therefore in September and October only 1% *Elminius* could be observed on the testplates, on which *Balanus improvisus* mixed with some *Balanus crenatus* were dominant. The stock of *Elminius* was to a high degree wiped out. In 1964 the conditions were more favorable to *Elminius* densities of 20–30% were again reached but not with the same development as in the previous years.

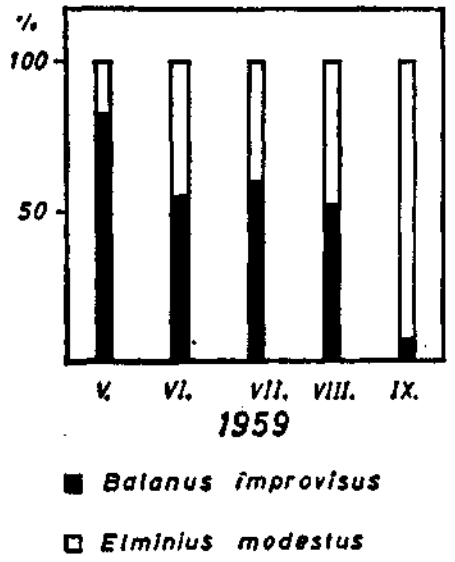

FIG. 2. Relation between the settlement of *Balanus improvisus* (black) and *Elminius modestus* between May and November 1959.

The distribution of *Balanus* nauplii and cyprids in the Elbe-Estuary is given from investigations on Elbe zooplankton made over a period of many years (Kühl-Mann, 1962). From the mean values it is clearly seen that the maximum of the nauplii and cyprid larvae is situated in the region of the lightship "Elbe 3" to "Elbe 2". The nauplii and cyprids drift back and forth with the tides. This district is also a maximum field for the other plankton—phyto- and zooplankton—also the fouling of the lightship "Elbe 3" is more severe than of the other lightships. The intensive development is caused by hydrochemical conditions (Kühl-Mann, 1953) (Fig. 3).

The season of attachment by the cyprids is from May to October/November in the Cuxhaven region. The larvae of *Balanus balanoides* arrive often already in April, followed by *B. improvisus* and *B. crenatus*. The cyprids of *Elminius modestus* appear at latest in June. The limitary effect

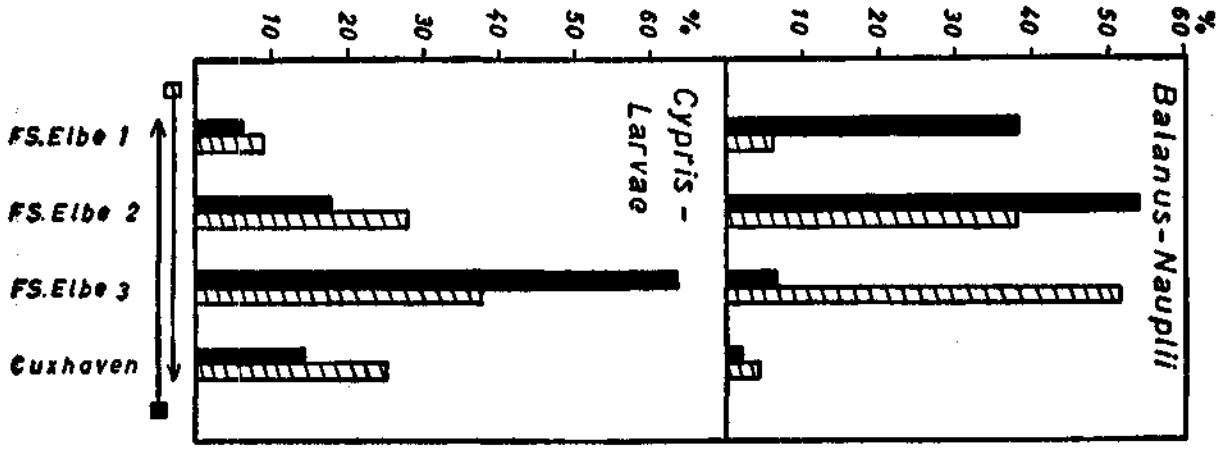

FIG. 3. Occurrence of barnacle nauplii and cyprids in the Elbe-Estuary expressed as percentage, at flow and ebb (indicated by arrows).

of the 10° C. water temperature on many barnacles is very significant, *e.g.*, *Balanus improvisus* (V. Breemen, 1934). The barnacle larvae are often abundant in the Cuxhaven plankton samples and sometimes they characterize the plankton community. In the beginning of May the barnacle nauplii first appear in enormous masses, then their number decreases very quickly, and in June and July the cyprids are dominant. The sudden decrease of the barnacle nauplii is caused in part by the metamorphosis, but also by the increase of the number of plankton feeders in June, especially medusae (*Rathkea, Eucheilota, Nemopsis, Ctenophora, Rhizostoma*) and crustaceans and their larvae (Plagmann, 1937; Kühl, 1964) (Fig. 4).

In some years no direct relationship has been noticed between the number of cyprids in the plankton and the number of attached cyprids and young barnacles; in other years the relationship is more pronounced. It could be observed that in the beginning of the season of attachment in June, enormous numbers of cyprids had attached up to 7,000 larvae and juvenile barnacles had settled on 100 cm.^{2} per week (Fig. 5). After a short time the number of the newly attached cyprids decreased quickly to 1,000 specimen/100 cm.^{2}; later the number increased again (3,000/100 cm.^{2}).

The number of the cyprids in the plankton increased and decreased as well, but the differences were not so remarkable and a second increase is also not seen. Observations in the field and in aquaria indicated that this was also caused by predators which reduced the stock. In most cases the feeders were the megalopa larvae of *Carcinides maenas* which were crawling on the surfaces and feeding on the newly attached cyprids and young barnacles. If the differences were very high, the numbers of megalopas in July and August were also great in the plankton (Fig. 5). *Crangon crangon* in all stages is also an important cyprid feeder, especially from May to September (Plagmann, 1937). *Crangon* takes the cyprids from the plankton and also from the surfaces.

In some years the numbers of *Balanus* nauplii and cyprids occurring in the plankton during a tide were estimated, and the number of attached cyprids was counted on testplates of 1000 cm.^{2} after a fixed time (in Fig. 6 one hour exposure). Characteristic observations could be made. The number of nauplii was high two hours before tidal low water, whereas the cyprids were very common in the plankton two hours before tidal high water (Fig. 6). Compared to these data the numbers of the cyprids attached to an area 1000 cm.^{2} after one hour exposure is greatest at tidal

low water: up to 500 larvae/1000 cm.²/hr. These results are significant, they were obtained by many investigations in several years.

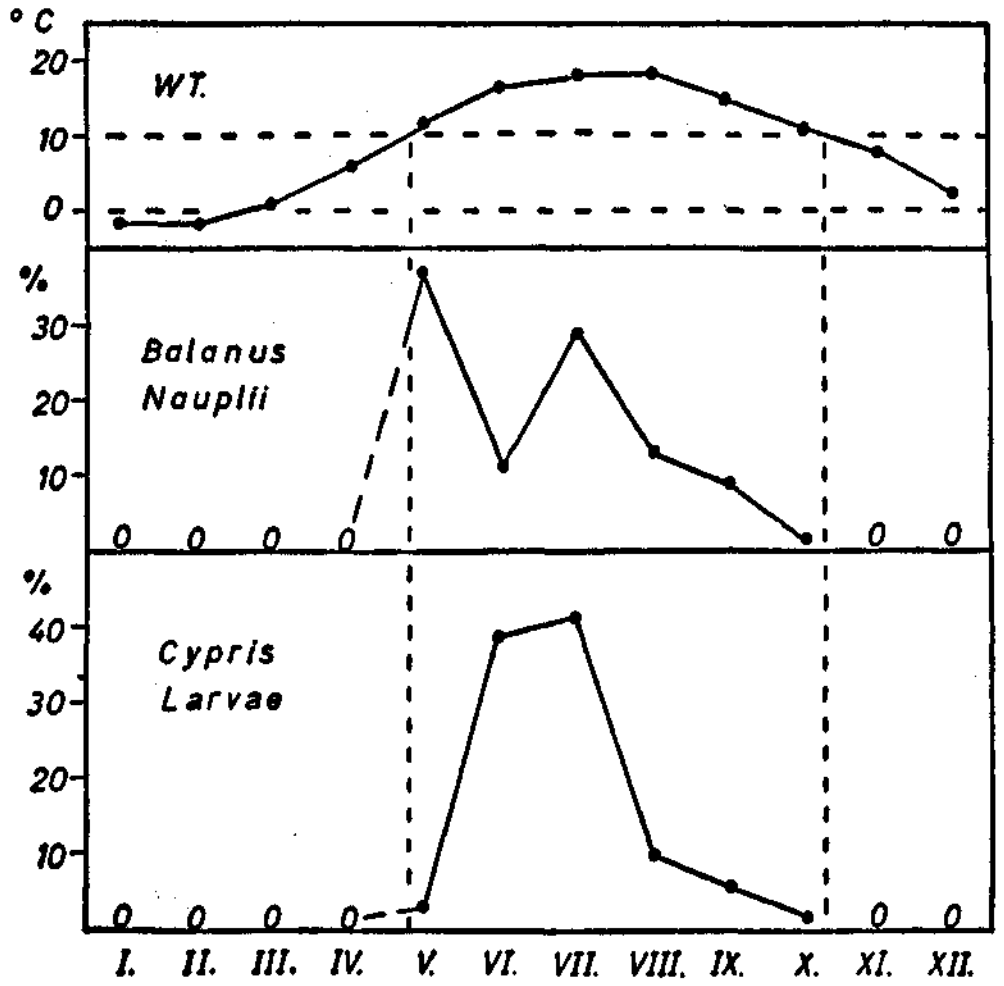

FIG. 4. Water temperature (average monthly) in 1962, occurrence of *Balanus* nauplii and cyprids between April and October 1962, expressed as percentage of total numbers (based on 48 plankton samples).

The duration of the cyprid metamorphosis is for *Balanus improvisus* about 20 to 24 hours, for the intertidal *Balanus balanoides* only half that time. Already in the metanauplius the calcarous reserves for the primary calcification of the chitinous integument are laid out before the nauplius eye and on both sides of the caudal spines (after Fuchs, not published). In the cyprid these reserves are in the anterior and posterior end of the body between the oily globules. In *Balanus improvisus* there are compact masses and in *Balanus balanoides* we find numerous small spots (Fig. 7A) of calcarous reserves laying closely together at the same places. This is also a good way to distinguish living cyprids of *Balanus improvisus* and *Balanus balanoides* (Fuchs). During the metamorphosis the calcarous deposits are distributed over the body in small spots in median directions (Fig. 7 B). After hatching of the barnacle the chalky spots are seen in the terga, scuta and also in the paries (Fig. 7 C). In *Balanus balanoides*, the intertidal barnacle, the formation of the calcarous shell plates is far quicker than in *B. improvisus*, which lives mainly under the low water level (Kuhn-Fuchs, 1954). In this manner the juvenile *Balanus balanoides* is well protected against

desiccation. The shell of *Balanus improvisus* is completely calcified after 3–5 days, the cirri are developed and the barnacle begins to feed.

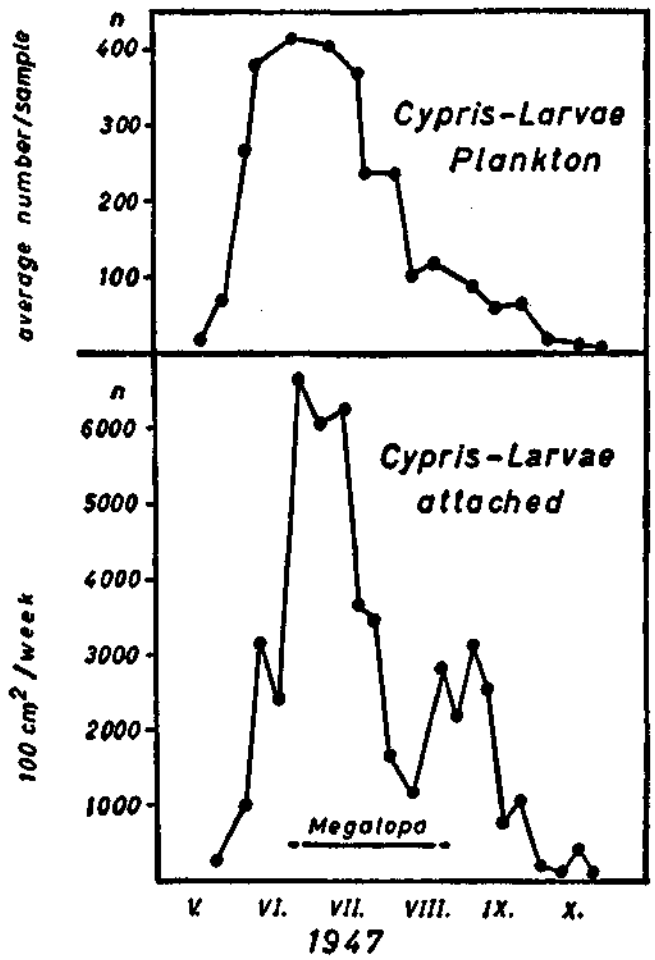

FIG. 5. Average numbers of cyprid larvae in the plankton (*n*/haul) and attached on 100 cm./week between May and October 1947.

The results mentioned above show that the observations and findings concerning the barnacles of the Elbe-Estuary, especially *Balanus balanoides* and *Balanus crenatus*, differ in some points from the data given in literature. *Balanus balanoides*, for example, is in the Elbe-Estuary euryhaline, similar to *Elminius modestus*, but *Balanus crenatus* is less euryhaline than assumed in literature. In the Elbe-Estuary we find great differences in the intensities of salinity and other abiotic factors (*e.g.* pH, O_2 saturation, alkalinity, transparency, seston) vertically as well as horizontally. Therefore it is not easy to determine in general the limits of these factors for the resistance and reproduction of these living organisms and all data given are restricted to the Elbe-Estuary. There is some evidence for the existence of physiological or ecological "local forms". The organisms must develop and grow under hard conditions. Therefore, they are more resistant against changes in abiotic factors. Alterations in the distribution of sessile animals can also be produced by artificial harbour constructions. Thus a new guide jetty has been constructed from Cuxhaven

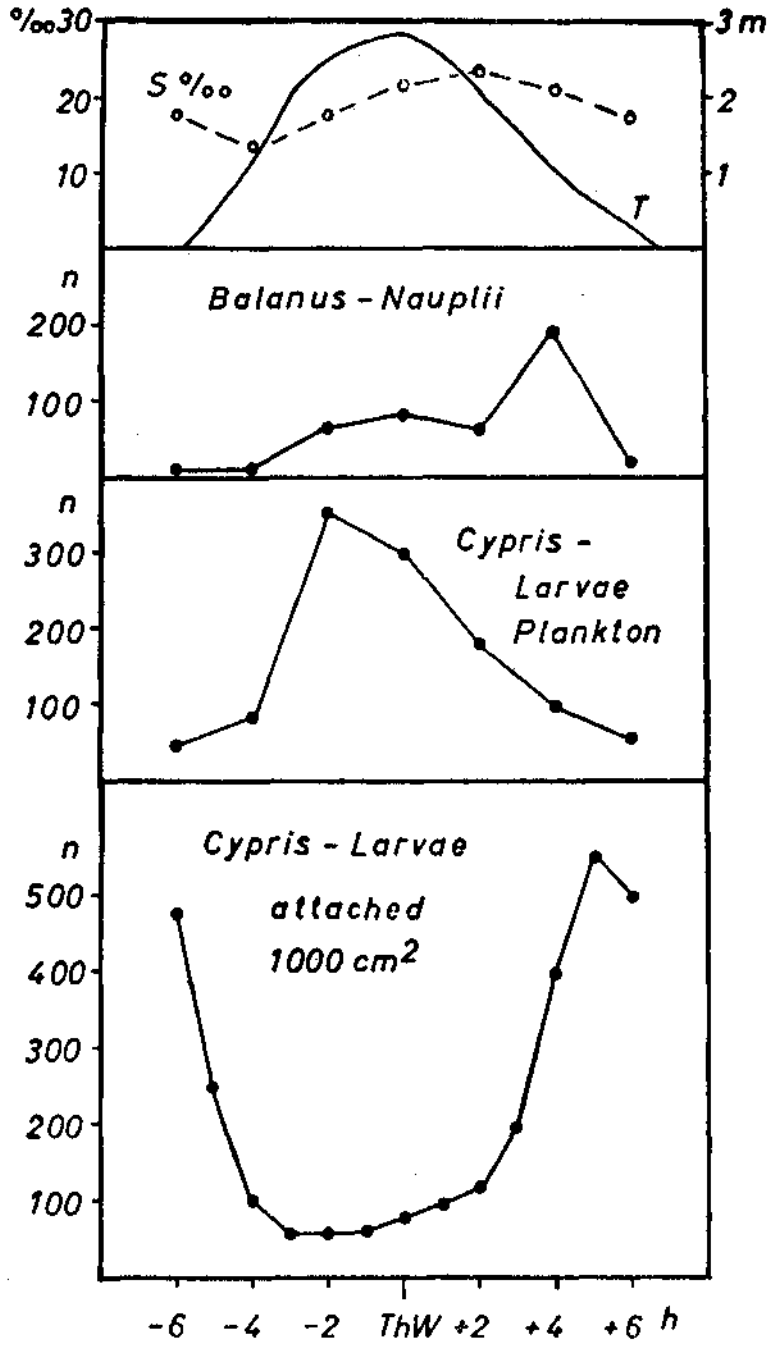

FIG. 6. Salinity (S‰) and tide (T), average numbers of *Balanus* nauplii and cyprid larvae in serial plankton hauls and of attached cyprids on one hour exposure on 1000 cm.² whilst a tide. (THW = highwater).

toward the Isle of Neuwerk, thereby creating a permanent place for settling in the lower polyhaline zone.

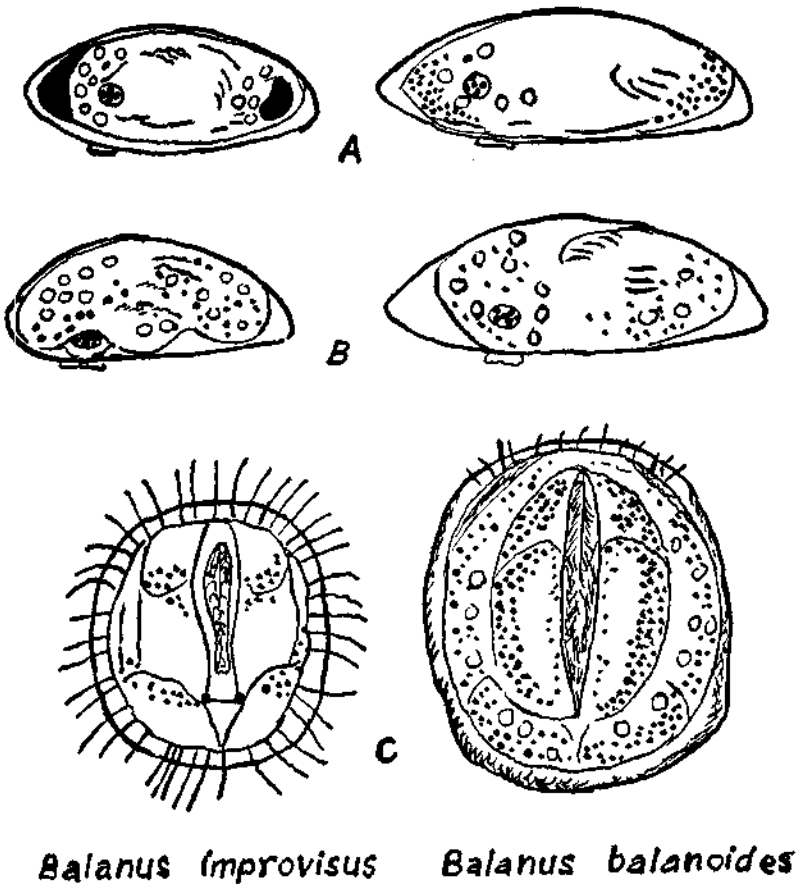

FIG. 7. The Ca-reserves (black spots at the anterior and posterior end) in *Balanus improvisus* and *Balanus balanoides* immediately after the attachment of the cyprid (A) and some hours later after the distribution of the spots (B) and after metamorphosis in the shellplates (C). Orig.

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TEMPERATURE AND CHLORINITY DISTRIBUTIONS, AND SPECIES' ASSOCIATIONS OF SOME SUBTROPICAL EUPHAUSIID AND HYPERIID CRUSTACEA

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ABSTRACT

The zooplankton of 636 plankton net hauls from the slope of the Eastern Australian Continental Shelf, and of 662 hauls from the shelf itself South-West Pacific, 44° S. lat. to 23° S. lat. was examined. These net hauls contained 28 species of Euphausiids and 20 of Hyperiids. Of these, 12 species of Euphausiids and 7 of Hyperiids were present a sufficient number of times to warrant the setting up of temperature and chlorinity distributions. Males were present of all species.

The range of temperature was 9°–25° C., and of chlorinity, parts per thousand, was 19·30–19·80. When the species' means were plotted on a temperature/chlorinity diagram and latitude ranges were allowed for the species fell into the following groups:—

I. Slope only:

(*a*) Originating in southerly water masses: *Euphausia spinifera, E. similis* var. *armata*; (*b*) in central water masses: *Euphausia gibba.*

II. Slope and Shelf:

(*a*) Southerly water masses: *Euphausia similis, Streetsia challengeri*; (*b*) Central: *Euphausia recurva, Nematoscelis difficilis, Thysanoessa gregaria, Brachyscelus crustulum*; (*c*) Northerly: *Stylocheiron carinatum, S. abbreviatum, Nematoscelis microps, Phrosina semilunata, Anchylomera blossevillei, Primno macropus.*

III. Mainly Shelf:

(*a*) Southerly: *Nyctiphanes australis, Euthemisto gaudichaudi*; (*b*) Northerly: *Pseudeuphausia latifrons.*

The number of times each species occurred with each of the others was recorded for each set of samples; slope, and shelf. By the use of chi-square, the degree of significance of the excess or deficit of each such number of joint occurrences, compared with that "expected" (number of single occurrences of Species A times of Species B, divided by the appropriate number of samples, slope or shelf) was determined.

Of the Euphausiids and Hyperiids in *slope* waters, *E. spinifera, E. recurva*, and *N. difficilis* formed a group of which the species were commonly found together with the Hyperiid, *P. semilunata* conjoined, except with *E. spinifera.* Otherwise, *P. macropus* occurred with *N. difficilis* and *B. crustulum* with *T. gregaria.*

Of the 19 species recorded in detail, the majority occurred only a few times in the *shelf* plankton hauls. Of the three species which occurred most often in shelf hauls, *N. australis* and *E. gaudichaudi* occurred jointly, significantly in excess; each species was significantly in deficit of joint occurrence with the northern, *P. latifrons. T. gregaria* was coupled significantly in excess with *N. australis.*

The joint occurrences of each of the crustacean species with each of the 14 common Tunicate and 14 Chaetognath species were recorded. Of the 420 possible combinations 42 were significantly in excess and 46 significantly in deficit. The majority of those in excess had closely related temperature and chlorinity ‰ means, those in deficit were widely separated in those characteristics.

INTRODUCTION

EUPHAUSIID species are of considerable importance in the fisheries economy of the oceans. The importance of Hyperiid species in that regard is less. In cold and temperate seas various

* We regret to inform his passing away.

characteristics of the species have been studied in some detail, particularly the Euthausiacea of the Southern Ocean by members of the Discovery Committee, later incorporated in the National Institute of Oceanography, and published in the Discovery Reports, and those in the North Atlantic (Einarsson, 1945). Until the classic study by Brinton (1962) appeared, as part of the plankton investigations of the Pacific by the Scripps Institution of Oceanography, followed by the preliminary study of Zooplankton species groups in the North Pacific by Fager and McGowan (1963) also of that Institution, and by the comprehensive study of the distribution of oceanic Euphausiids by Lomakina (1964) of the Zoological Institute of the U.S.S.R. Academy of Sciences, there had been little ecological work published on the subtropical Euphausiacea. That is still the position in the case of the subtropical Hyperiid Amphipoda.

In spite of the recent increase of interest in these crustacea, there have been few detailed records published of the temperature and chlorinity preferences of the species of either Order.

In this contribution, such preferences are discussed for subtropical species in the zooplankton of the South West Pacific on the basis of the presence of species in plankton hauls. Data exists for a more detailed study in terms of relative abundance in the net-hauls. The simpler analysis is considered here. It is extended to a consideration of the extent to which Euphausiid and Hyperiid species form a significant part of each other's biological environment; and also to the extent to which those species and species of the Tunicata and Chaetogantha are similarly coupled.

Materials and Methods

Species of the above classes were identified from plankton hauls made at 17 pairs of stations, approximately one degree of latitude apart, between latitudes 23° S. and 44° S. along the Eastern Australian Continental Shelf and Slope, during the research operations of F. R. V. *Warreen*, of C. S. and I. R., Division of Fisheries, 1938–42 (Fig. 1). The slope stations, sited approximately along the 650 metre contour, provided 636 plankton samples at depths varying from the surface to 500 metres; the shelf stations, along the 60 metre contour, at depths ranging from the surface to 50 metres, 662 plankton samples.

Temperature and chlorinity observations were made at standard depths (see *Oceanographical Station Lists*, C.S.I.R.O., 1951, Vols. 1 and 2).

The depths of the plankton hauls and those of the hydrological observations did not always coincide. Accordingly, the hydrological data within the vertical range of a plankton haul were regarded as being equally applicable to the species present in that haul. The number of hauls made at each temperature or chlorinity range was not the same. To enable comparison, or quantity, "times present per 100 hauls," was calculated for each species (Sheard, 1965). These data, and the resulting means, are given in Tables I and II.

The method employed in assessing species groupings was as follows:

(*a*) A hand punch-card was set up for each net-haul, each species being given a code number. The haul count for each species present was entered opposite the appropriate, numbered hole. Temperature and chlorinity, and other relevant data were entered on the body of the card. Summary cards, slope and shelf separately, were then prepared for each species. These indicated the number of times each species was present; and the number of joint occurrences of each with each of the others. The summary cards also indicated the necessary hydrological data from which the various means were calculated.

(*b*) An 'expected' number of joint occurrences was estimated for each pair of species (slope and shelf separately) by multiplying together the occurrences of each and dividing by the number

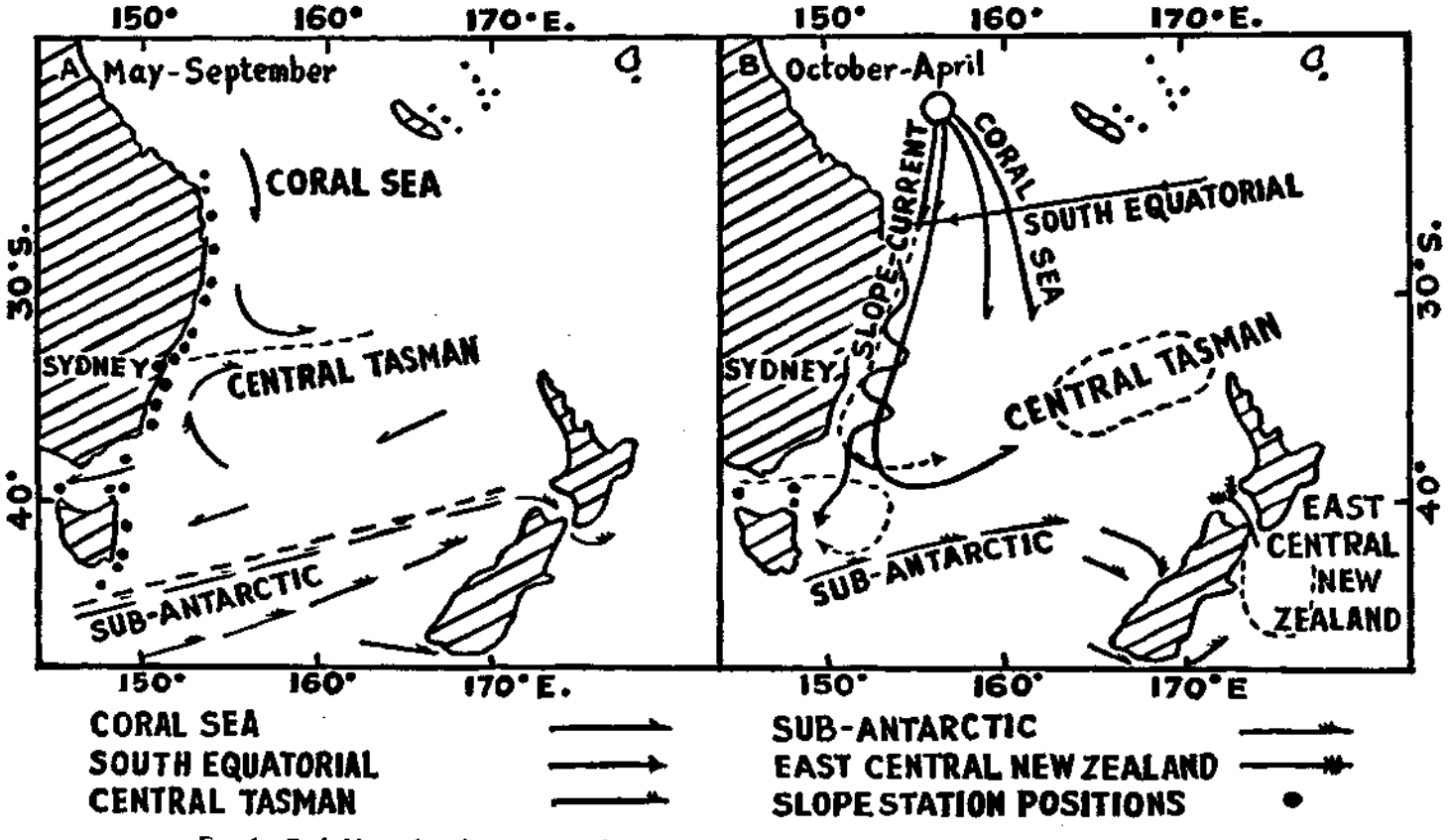

FIG. 1. Probable paths of movement of water masses of the Tasman Sea (after Rochford, 1957, Fig. 25)

TABLE I

Temperature distribution, Euphausicacea and Amphipoda—Hyperida Eastern Australian zooplankton, latitudes 43° S to 23° S
(Values expressed as times present per 100 hauls at each degree of temperature) (Ranges of larval or juvenile stages underlined)

	Temperature range (*a*) Continental slope																	Mean Temp. °C.	Temperature range (*b*) Continental shelf													Mean Temp. °C.
	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25		11	12	13	14	15	16	17	18	19	20	21	22	23	
EUPHAUSIACEA:																																
1. *Euphausia spinifera* ..	9	11	11	13	12	8	9	8	9	4	4	1						13·4														
2. *E. similis* var. *armata*	6	5	4	5	14	7	3	6	7	1	1	2						13·5														
3. *E. similis* ..	2	5	3	6	8	6	2	6	8	2	3	4	3					14·8			1		1	1								
4. *Nictyphanes australis* ..	3	2	4	3	6	4	6	5	6	4	5	3	5					15·5	5	27	26	16	13	38	34	25	17	14	6			15·7
5. *Thysanoessa gregaria* ..	15	11	20	27	12	29	30	32	40	27	30	19	24	10	10	9		16·4		5	6	8	8	5	6	6	3	6	6	3	4	17·0
6. *Euphausia gibba* ..	2	1	2	5	3	2	2	6	5	8	6	1	2					16·9														
7. *Nematoscelis difficilis* ..	18	14	12	19	21	19	20	23	24	17	20	16	20	17	20	18	11	17·			6		2	2	2	1	2	1	4		4	17·8
8. *Euphausia recurva* ..	15	18	16	16	23	23	30	30	40	27	29	26	38	22	13	14		17·0					4	8	7	11	7	7	11	16	15	19·8
9. *Pseudeuphausia latifrons*			1	1	2	1	2	2	3	1	1	1	3	5	3	5		19·2			1	2	3	1	2	3	2	16	7	27		20·0
10. *Stylochiron carinatum* ..			2	2	6	10	9	9	16	12	15	16	14	27	27	14		19·4													1	
11. *S. abbreviatum* ..				2	3	5	5	6	9	9	10	5	7	7	10	14	22	19·4						1	2	1	1	2	4	3		20·3
12. *Nematoscelis microps* ..			2	2	3	2	3	4	3	6	7	8	7	9	12	1		19·3								1	1					
HYPERIIDAE:																																
13. *Euthemisto gaudichaudi* ..			1	2	3	3	2	2	2									14·1	5	17	12	7	8	11	6	4	2					14·5
14. *Streetsia challengeri* ..			1	2	3	2	2	2	2	1	1	1	1					15·3						1		1		1		1		
15. *Brachyscelus crustulum* ..	3	5	3	1	2	4	5	9	7	6	7	2	8		3			16·3					1	1	1	1	1					
16. *Vitilia australis* ..					1	1	2	1	2	1	1		1					16·5					1	3	3	1	1	1			4	17·6
17. *Phrosina semilunata* ..		2	1	2	4	5	4	6	12	9	13	11	15	12	17	18		19·4					1	1		1		1		4		
18. *Anchylomera blossevillei* ..					1	2	3	3	6	6	5	5	8	7	13	14		20·5					2			1		1				
19. *Primno macropus* ..	3	2	1	2	2	6	8	8	12	15	14	15	18	24	37	45	33	21·0		5				2	2	1	1					

of hauls in each case. Chi-square was then calculated as the number of observed joint occurrences *minus* the 'expected' number; this result was squared and divided by the 'expected' number. Those joint occurrences in significant excess ($p < 0{\cdot}01$, one degree of freedom) were entered on Tables III and IV with a plus sign, those in significant deficit by a minus sign. The test was used only when the 'expected' number was 5 or more.

Temperature and Chlorinity Characteristics of the Species

(i) *Temperature.*—The temperature distributions and mean temperatures of the species are given in Table I. Temperature ranges within which larval or juvenile stages were found are underlined. Slope and shelf distributions are given separately. (The number of times each species was recorded in each region is given in Table III.) Three species, *Nyctiphanes australis*, *Pseudeuphausia latifrons* and *Euthemisto gaudichaudi* are present a greater number of times in the shelf hauls than in those of the slope. *Thysanoessa gregaria* and *Euphausia recurva* occur rather frequently on the shelf but the remainder are rare visitors. Several oceanic species, *Euphausia spinifera*, *E. similis* var. *armata* and *E. gibba* did not occur in the shelf net-hauls.

There were sufficient data to estimate a mean temperature for each of the slope species but this was possible with less than half of the shelf occurrences. Where the data were sufficient the means were similar. The temperature ranges were typical of subtropical species.

(ii) *Chlorinity.*—These data are given in Table II in the same manner as for temperature. The range of chlorinity ‰ is small and the means less reliable.

Even so, some preference is indicated by the majority of the species, although this might be less a direct preference for some one chlorinity range and more a reaction to the overall characteristics of the various water bodies which, when mixed, form the slope and shelf water masses.

(iii) *Water mass affiliations of the species.*—From Fig. 1 it can be realised that the slope and shelf waters are derived from a number of sources, ranging from the warm Coral Sea and South Equatorial water masses to the cooler Central Tasman and derived Tasman subantarctic waters. These all form a highly mobile system and it can be expected that the waters of the continental slope and shelf wil contain contributions in varying degrees from each source from time to time.

The position is set out in a more static form in a Temperature/Chlorinity ‰ diagram (Fig. 2), where the general temperature/chlorinity boundaries of the various contributing water masses are indicated.

From time to time, and from place to place, the temperature/chlorinity characteristics of the slope and shelf waters lie generally within a rectangle of which the temperature boundaries are generally 11°–23° C. and chlorinity boundaries 19·4–19·7, although the occasional range might be from 9°–25° C. and 19·3–19·8 Cl.‰.

It can be expected, with the slope and shelf waters derived from so many unlike sources, that they will contain, from time to time, species whose primary origin was in one or the other component water masses.

To test this the mean temperature/chlorinity of each species was entered on the diagram (Fig. 2). It can be seen that these means group into two sections. The one more characteristic of the warmer water masses, and the other of the cooler. (In the diagram that water mass labelled South-West Tasman is regarded as being formed by mixing between the waters of the central Tasman Sea and those Tasman Sea waters derived more directly from Subantarctic sources.)

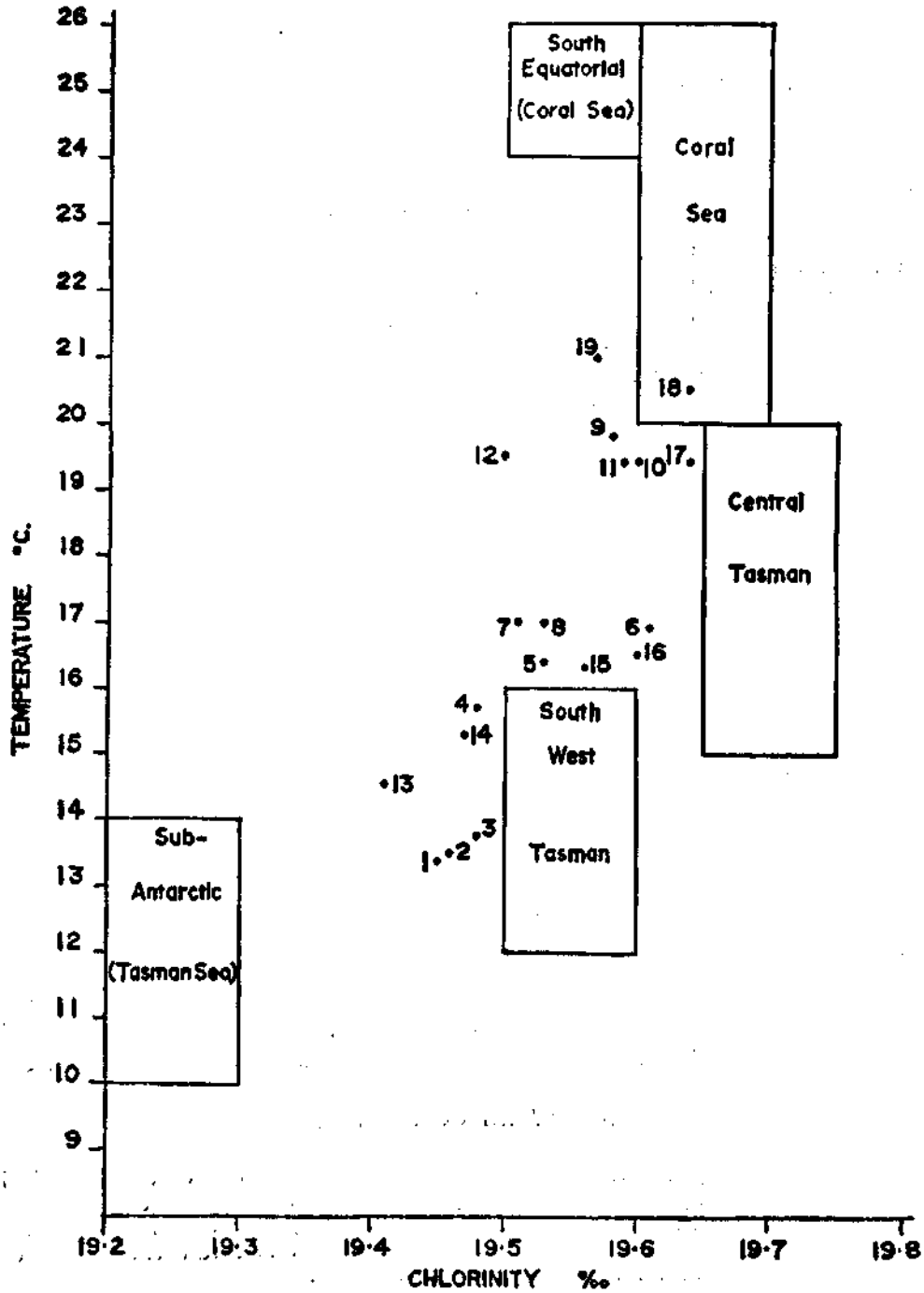

FIG. 2. The relation of mean temperature/chlorinity ‰ of Euphausiid and Hyperiid species to the generalised T/Cl ‰ distribution of nominated water masses of the South Pacific (adapted from Rochford, 1958, Fig. 3). Numbers 1–12 (Euphausiids), and 13–19 (Hyperiids) are in order of mean temperature, separately (*see* Tables I and II)

TABLE II

Chlorinity distribution, Euphausiacea and Amphipoda—Hyperida
Eastern Australia zooplankton latitude 43° S to 23° S
(Values expressed as times present per 100 hauls at each point of chlorinity ‰)
(Ranges of larval or juvenile stages underlined)

	Chlorinity ‰, range											
	(*a*) Continental slope					Mean Cl‰	(*b*) Continental shelf					Mean Cl‰
	19·3	19·4	19·5	19·6	19·7		19·3	19·4	19·5	19·6	19·7	
EUPHAUSIACEA:												
1. *Euphausia spinifera* ..	13	10	12	7	4	19·45						
2. *E. similis* var. *armata*	5	6	9	5		·46	1		1			
3. *E. similis* ..	11	5	8	4	4	·45						
4. *Nyctiphanes australis* ..	8	4	6	4	6	·48	26	21	29	25	14	19·48
5. *Thysanoessa gregaria* ..	21	23	33	23	43	·53	12	1	6	5	11	·51
6. *Euphausia gibba* ..		1	2	3	5	·61						
7. *Nematoscelis difficilis* ..	16	17	22	20	22	·51	9	1	2	2	3	·41
8. *Euphausia recurva* ..	21	16	25	23	41	·53			4	8	9	·62
9. *Pseudeuphausia latifrons* ..	3	3	1	2	1	·45			4	4	2	·58
10. *Stylocheiron carinatum* ..		2	5	11	9	·60			1	1		
11. *S. abbreviatum* ..		2	3	5	6	·59			3	1		(·52)
12. *Nematoscelis microps* ..	3	3	4	3	3	·50				1		
HYPERIIDAE:												
13. *Enthemisto gaudichaudi* ..			3	1	1	(·56)	26	17	6	7	4	·41
14. *Streetsia challengeri* ..		2	2	2	1	(·47)				1	1	
15. *Brachyscelus crustulum* ..	3	1	4	6	7	·56		1	1	1	1	(·55)
16. *Vibilia australis* ..			1	2	1	(·60)		1	2	2	1	(·55)
17. *Phrosina semilunata* ..		1	2	7	12	·64			2	1		
18. *Anchylomera blossevillei* ..			1	5	6	·64				2	2	
19. *Primno macropus* ..		2	5	8	11	·57	8	1	1	1		

SPECIES JOINT OCCURRENCES

(i) *Joint occurrences of the crustacean species.*—Those species joint occurrences which are in excess or deficit of their expected number (using the chi-square test outlined earlier) are set out in Table III, which is in two parts; (*a*) slope occurrences, (*b*) shelf occurrences. Some species do not occur sufficiently often for the non-exact chi-square test to be used because this requires the expected number of joint occurrences to be greater than five.

In slope waters species associated with the cooler water masses tend to occur jointly in excess of expectation. Of these, *E. spinifera, T. gregaria, E. recurva* and *M. difficilis* form a group.

TABLE III

Significant joint occurrences, Euphausiacea and Amphipoda—Hyperiidae. Eastern Australian Continental slope and shelf between latitudes 44° S and 23° S (+ = Joint occurrence significantly > than expected; − = Joint occurrence significantly < than expected)

(*a*) = Slope stations; (*b*) = Shelf stations.

Species	Continental slope 636 plankton hauls			*E. spinifera*	*E. similis* v. *armata*	*E. similis*	*N. australis*	*T. gregaria*	*E. gibba*	*N. difficilis*	*E. recurva*	*E. latifrons*	*S. carinatum*	*S. abbreviatum*	*N. microps*	*E. gaudichaudi*	*S. challengeri*	*B. crustulum*	*V. australis*	*P. semilunata*	*A. blosseville*	*P. macropus*	Continental shelf 662 plankton hauls		
	Times present	Mean temperature	Mean chlorinity ‰																				Times present	Mean temperature	Mean chlorinity ‰
EUPHAUSIACEA:																									
Euphausia spinifera ..	38	13·4	·45																		(*b*)		0		
E. similis var. *armata* ..	27	13·5	·46																				0		
E. similis ..	21	14·8	·45																				3	14·7	·49
Nyctiphanes australis ..	28	15·5	·48					+			−					+							157	15·7	·48
Thysanoessa gregaria ..	118	16·4	·53	+	+	+	+																39	17·0	·51
Euphausia gibba ..	14	16·9	·61																				0		
Nematoscelis difficilis ..	91	17·0	·51	+				+															15	17·8	·44
Euphausia recurva ..	114	17·0	·53	+	+			+		+													39	19·8	·62
Pseudeuphausia latifrons	7	19·2	·45													−							24	20·0	·58
Stylocheiron carinatum ..	43	19·4	·60					+															3	23·0	·55
S. abbreviatum ..	18	19·4	·59																				8	20·5	·52
Nematoscelis microps ..	14	19·5	·50																				2	19·0	·60
HYPERIDA:																									
Euthemisto gaudichaudi	9	14·1	·56																				49	14·5	·41
Strectsia challengeri ..	6	15·3	·47																				3	19·0	·65
Brachystelus crustulum	32	16·3	·56					+															4	17·0	·55
Vibilia australis ..	5	16·5	·60																				10	17·6	·55
Phrosina semilunata ..	32	19·4	·64					+		+	+												5	20·1	·53
Anchylomera blossevillei	18	20·5	·64																				4	17·0	·60
Primno macropus ..	34	21·0	·57		(*a*)					+													11	18·4	·50

TABLE IV

Significant joint occurrences, Euphausiacea and Hyperida with Tunicate and Chaetognath species.
Eastern Australian Continental shelf and slope between latitudes 44° S and 23° S
(+ = Joint occurrences significantly > than expected; − = Joint occurrence significantly < than expected)

	Euphrosina hamata	*S. serratodentata tasmanica*	*Sagitta decipiens*	*Oikopleura dioica*	*Sagitta planktonis*	*S. lyra*	*Iasis zonaria*	*Krohnitta subtilis*	*Salpa fusiformis*	*Oikopleura albicans*	*Oikopleura longicauda*	*Thalia democratica*	*Fritillaria pellucida*	*Doliolet gegenbauri*	*Oikopleura fusiformis*	*O. rufescens*	*Stegosoma magnum*	*Sagitta hexaptera*	*Pterosagitta draco*	*Sagitta regularis*	*Megalosercus huxleyi*	*Krohnitta multitentaculata*	*Doliolum denticulatum*	*S. serratodentata pacifica*	*Sagitta bipunctata*	*S. enflata*	*Oikopleura cophocerca*	*Sagitta robusta*	Mean temperature	Mean chlorinity ‰	Latitude range
E. spinifera ..		+								+		−		−	−							−							13·4	19·45	44–35
E. similis var. *armata* ..		+																											13·5	·46	42–34
E. gaudichaudi ..		+												−	−								−				−		14·5	·41	43–34
E. similis ..																													14·8	·45	43–35
N. australis ..		+										−		−	−						−		−						15·7	·48	43–30
E. crustulum ..																		+											16·3	·56	43–30
T. gregaria ..	+				+	+	+		−												−						−		16·4	·53	44–23
N. difficilis ..			+														−		+								−		17·0	·51	41–28
E. recurva ..				−		−			−		+		+			+							−			+	−		17·0	·53	39–24
S. carinatum ..					+	+								+			+		+	+		+	+	+	+	+		+	19·4	·60	39–24
S. abbreviatum ..																			+										19·4	·59	39–24
P. semilunata ..		−																+								+			19·4	·64	39–30
N. microps ..								+																					19·5	·50	36–28
P. latifrons ..												+		+									+			+			20·0	·58	35–23
P. macropus ..														+				+	+				+		+	+			21·0	·57	43–28
Mean temperature °C. ..	12·4	13·8	14·2	14·5	14·7	15·5	16·3	16·4	16·8	16·8	17·6	17·8	18·4	18·8	18·8	19·4	19·5	19·8	19·9	20·1	20·2	20·2	20·6	20·6	20·7	20·7	20·8	21·6			
Mean chlorinity ‰ ..	19·41	·50	·50	·57	·42	·49	·52	·55	·48	·52	·53	·55	·54	·63	·60	·57	·56	·60	·53	·60	·55	·60	·54	·60	·56	·61	·58	·57			
Latitude range ..	43–28	44–34	43–27	42–28	43–28	44–27	43–35	44–28	43–24	44–24	44–24	42–24	41–28	41–24	42–24	42–24	42–24	43–27	43–24	37–27	39–24	41–24	37–24	37–24	43–27	37–27	38–24	36–28			

The Hyperiid, *Phrosina semilunata*, is grouped with the three latter species but not with *Euphausia spinifera*.

T. gregaria is joined in excess with nine of the crustacean species. It occurs throughout the slope region and at all temperatures and chlorinities present there. *E. recurva*, the next species most commonly present in slope plankton hauls, although more restricted in range, is similar in temperature and chlorinity preferences. It is joined significantly with six of the other species. *N. difficilis*, which is also similar in the above preferences but whose range lies within that of *T. gregaria*, is joined with five.

In shelf waters few of the crustacean species are present a sufficient number of times to provide an adequate expected number of joint occurrences. It is noteworthy that *T. gregaria* is coupled with *N. australis* and with *Euthemisto gaudichaudi*, these three species are important, either directly or indirectly, in the food chains of important economic species of the lower New South Wales, Victorian, and Bass Strait shelf and slope regions. These include the short-tailed Shearwater or Tasmanian Mutton Bird (*Puffinus tenuirostris*), the Barracouta (*Thyrsites atun*), the Australian Salmon (*Arripis trutta*), and several species of Tuna.

The Euphausiid, *P. latifrons*, a warm subtropical species which replaces *N. australis* in the northern part of the Eastern Australian region, is significantly in deficit in its occurrence with that species, and with the cooler water Hyperiid, *E. gaudichaudi*.

(ii) *Joint occurrences with Tunicate and Chaetognath species.*—These are recorded in Table IV. In general, there is a tendency for species with like mean temperatures to occur together significantly above expectation, and for those of which the mean temperatures are unlike to occur jointly below expectation.

Table IV has been ruled into four sections at the 17° mean temperature points. That ruling also divides the mean chlorinity distributions approximately into higher and lower sections. In general there is a tendency for species of which the means occupy the same section of the table to occur together, significantly above expectation, and for those with means lying in different sections to occur together, significantly below expectation.

If the data of Table IV are considered in conjunction with those relating to the paths and temperature/chlorinity characteristics of the water masses whose mixing, from time to time, forms the waters of the slope and shelf (Figs. 1 and 2), it can be suggested that the extent to which the various source water bodies maintain their identity could be examined using the degree of joint association of various species as a measure.

Some aspects of this type of problem have been discussed by Cassie (1963), using a more sophisticated statistical treatment based on species abundance and by Bary (1964).

The techniques sketched in this communication have been described in more detail, and the history of this type of investigation reviewed in Sheard (1965). The chi-square method of selecting those species which occur commonly together is an arbitrary one as it deals only with the presence or absence of a species in a sample. It can be used, however, as an objective method of selecting species for abundance studies.

In cases where sets of species selected as above occur commonly together such groups can be selected objectively by a grouping method due to Fager (1957). The groups so selected can be more useful than pairs of species in the preliminary analysis of ecological data, particularly if environmental relationships can be measured and used to set up diagrams of the type of Fig. 2.

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SOME ASPECTS OF ADAPTATION IN DECAPOD CRUSTACEA IN THE NORTH-WEST ATLANTIC

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ABSTRACT

The adaptation of diverse species of decapod Crustacea to available habitats in the area of study in the north-west Atlantic has resulted in recognisable features of (*a*) competitive exclusion as illustrated by *Pandalus borealis* and *P. montagui* communities; (*b*) thermal stress as shown by altered reproductive potential in some species particularly at low extremes of temperature; (*c*) social orientation in *Homarus americanus*, and (*d*) limitation in numbers of separate decapod species by probable micro-communities in an unstable or varied habitat subject to temperature, etc., extremes.

INTRODUCTION

DURING the period 1946–60, research vessels of the Fisheries Research Board of Canada collected decapod Crustacea when making surveys primarily for groundfish in the Newfoundland-Labrador area but also when surveying for shrimp off Newfoundland and when making special benthos studies in the Canadian eastern Arctic. The areas surveyed included deep waters of the continental shelf; deep channels with Atlantic water incursions occurring in bays of Newfoundland such as Hermitage Bay and Trinity Bay and in the Gulf of St. Lawrence; deep troughs with permanently low temperatures such as in Placentia Bay, Bay of Islands and Conception Bay; and the shallow offshore Grand Banks as well as shallow coastal areas (the latter mostly in the Arctic) (Fig. 1). A detailed study of about 25,000 specimens including approximately 50 species of decapods has been concluded (Squires, 1957, 1961, 1962 and 1963 and in preparation). The purpose of the present paper is to examine four concepts arising from the larger study. These concepts include competitive exclusion, reproductive adaptation to low temperatures, social orientation in *Homarus* and the occurrence of low numbers in the communities of many of the shrimp species examined.

COMPETITIVE EXCLUSION

The limitations of this principle have been discussed by Hardin (1960). The principle that two completely competing species cannot occupy the same niche also implies that if an advantage such as a higher rate of reproduction is held by one, then the other becomes excluded (even to the point of extinction). I suggest that this principle affords a possible explanation for the interaction of two species with fairly similar life-histories, *Pandalus borealis* and *P. montagui* in the area of investigation. The distribution of the present-day populations of these species is different in the north-west Atlantic, although some overlap occurs (Squires, 1963).

P. borealis is characteristically stenothermal and stenobathic in reaction to its environment compared with the form of *P. montagui* that has survived in this area. *P. montagui* on the other hand may have always possessed a greater eurythermal and eurybathic facility than *P. borealis*, but it is evident from our collections that it is at present excluded from the large communities of *P. borealis*. *P. borealis* occurs in great numbers where the temperature is moderately high (4–6° C.) and where a smooth detritus bottom is available at depths of 200–300 m. These conditions are found over considerable distances in the Gulf of St. Lawrence and off the south-west coast of Newfoundland (Squires, 1961). *P. montagui* has not been collected at the depths indicated in these

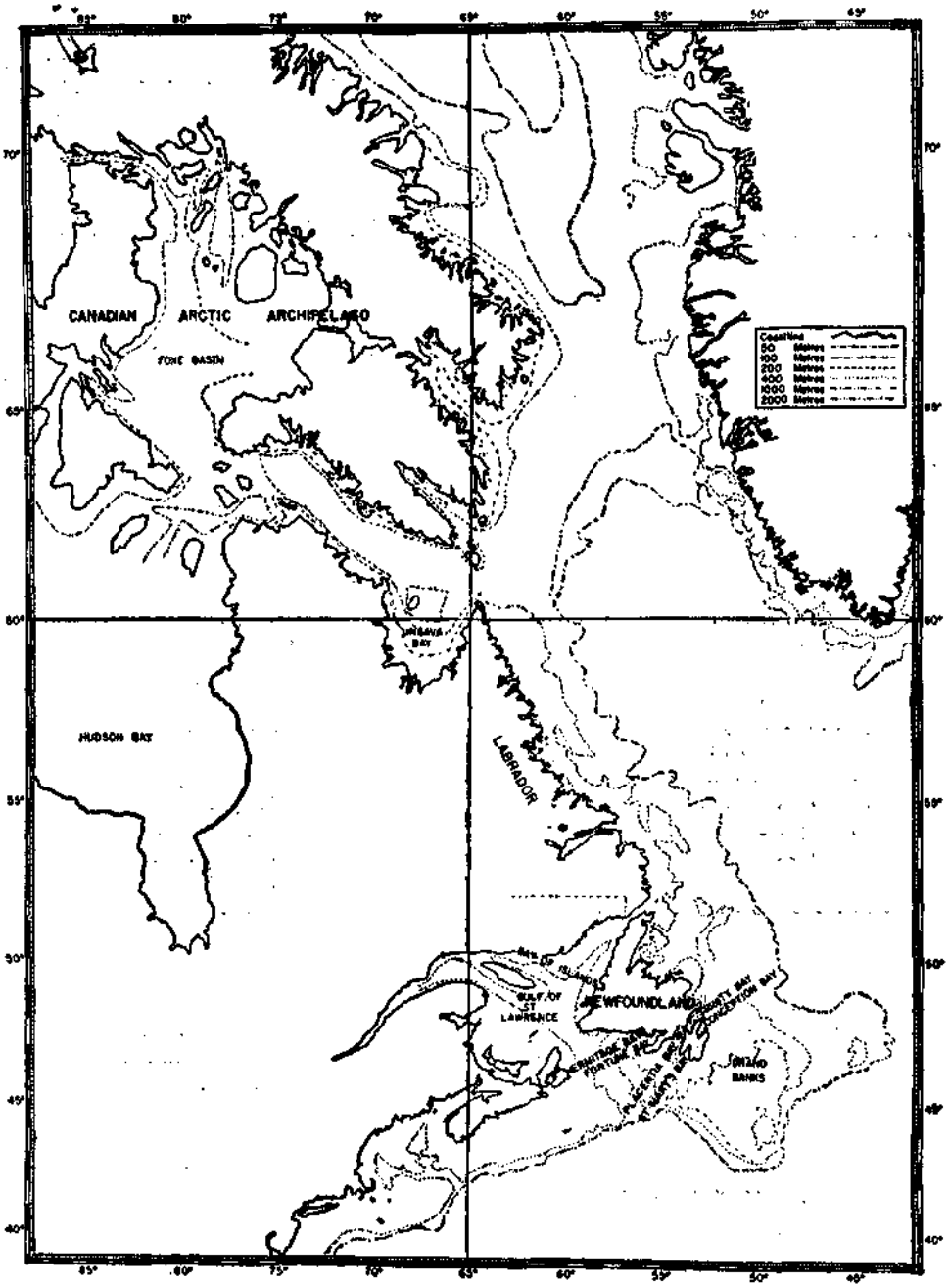

FIG. 1. Map of most of the area in which decapod Crustacea were collected during the period 1946–1960 by vessels of the Fisheries Research Board of Canada. Place names mentioned in the text are included.

areas. At lower temperatures (about −1 to 3° C.), and in depths over 200 m., however, *P. montagui* appears to have a reproductive advantage over the *P. borealis* present, and the former is then more abundant than the latter. (In these low temperatures the ratio of females to males in *P. borealis* communities became lower than in those of *P. montagui*: *see* also under "reproductive adaptation to temperature" below.) As a corollary, *P. montagui* was also the dominant species in depths less than 200 m. (except in Ungava Bay) and at temperatures lower than 3° C. in this areas.

Discussion

Apparently stenothermal and stenobathic forms of *P. montagui* could not survive in the North-west Atlantic [in the north-east Atlantic, however, this species thrives in comparatively high temperatures (Mistakidis, 1957; Allen, 1963)]. These forms could not compete successfully with *P. borealis* but in the same way the eurythermal and eurybathic forms of *P. borealis* could not survive in competition with *P. montagui*. At the present time the overlap in distribution of these species in deep and cold water occurs where a marginal population of *P. borealis* is maintained by expatriate larval drift.

A stenothermal (but to low temperature) and stenobathic species of shrimp, *Eualus macilentus*, can compete successfully with *P. montagui* in some parts of the area, but *P. montagui* appears to be the dominant shrimp species among many others in shallow water (excluding the very shallow sublittoral areas where *Crangon septemspinosus* is the dominant species).

Reproductive Adaptation to Temperature

Thermal stress is apparent in the low reproductive potential of several species of decapods in collections from low temperature localities, but it is particularly evident in *P. borealis* from such collections. *P. borealis* is a protandrous hermaphrodite (Berkeley, 1930; Hjort and Ruud, 1938; Allen, 1959) and its populations are found to comprise approximately equal numbers of males and females under optimal conditions (Squires, 1963). (Very small specimens including those of the 1st year are excluded from the numbers under consideration since they are not usually caught by the fishing gear used.) Samples of *P. borealis* from populations subjected to permanently low temperatures (near 0° C.) have a considerably lower ratio of females to males than those from areas with temperatures of 4–6° C. (Fig. 2). *P. montagui*, on the other hand, shows a successful adaptation to varied temperatures by the almost 1 : 1 ratio of males to females found in samples from permanently low temperature areas as well as from more moderate temperatures (Fig. 3).

In species of decapods other than *P. borealis* and *P. montagui* where protandry does not occur, low temperature stress may be shown by a lowered reproductive potential represented by the small proportion of females carrying eggs in any year. High reproductivity is defined as a high percentage of females spawning annually in a population. However, in low temperature areas, decapods may take several years to reach maturity, and since they are long-lived, as a rule, in such areas, they may spawn more than once in a lifetime. In a population sample, therefore, the percentage of ovigerous females which may represent high reproductivity in some species, may not do so in others. The hypothetical percentages of ovigerous females of any species in a population sample may be calculated from the equation:

$$R = y/(a - 1) + c \times 100 \qquad (1)$$

where

R = the hypothetical percentage of ovigerous females in a sample,
a = the number of years to reach maturity,
c = the number of times individual females may spawn in a lifetime,
and y = the number of year-classes spawning in the present year.

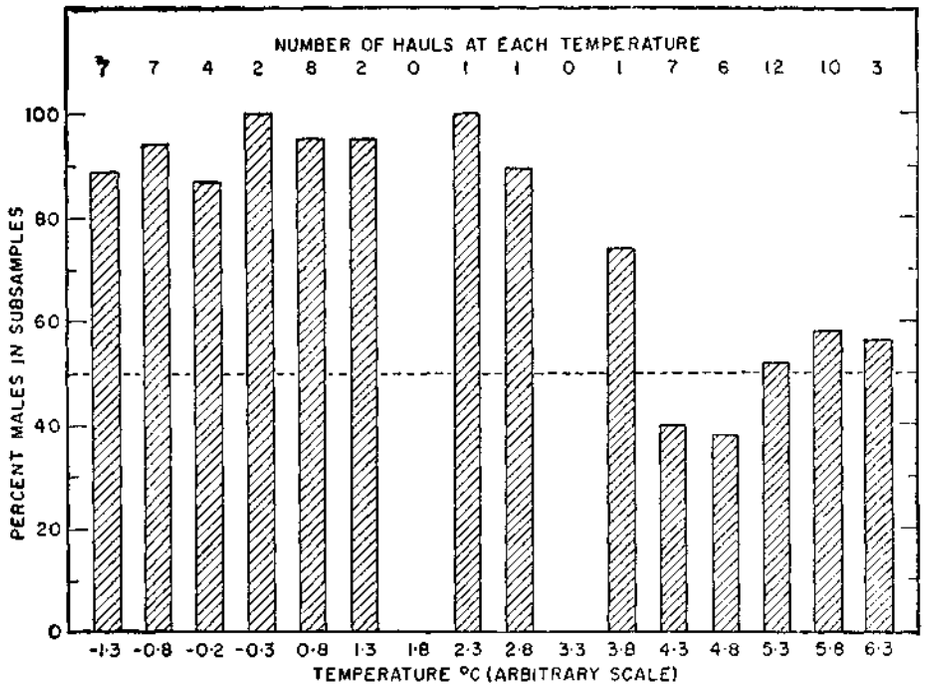

FIG. 2. Bar graph showing the per cent. of male *Pandalus borealis* in subsamples from different temperatures where hauls were made in the northwest Atlantic during 1946–1960 by vessels of the Fisheries Research Board of Canada.

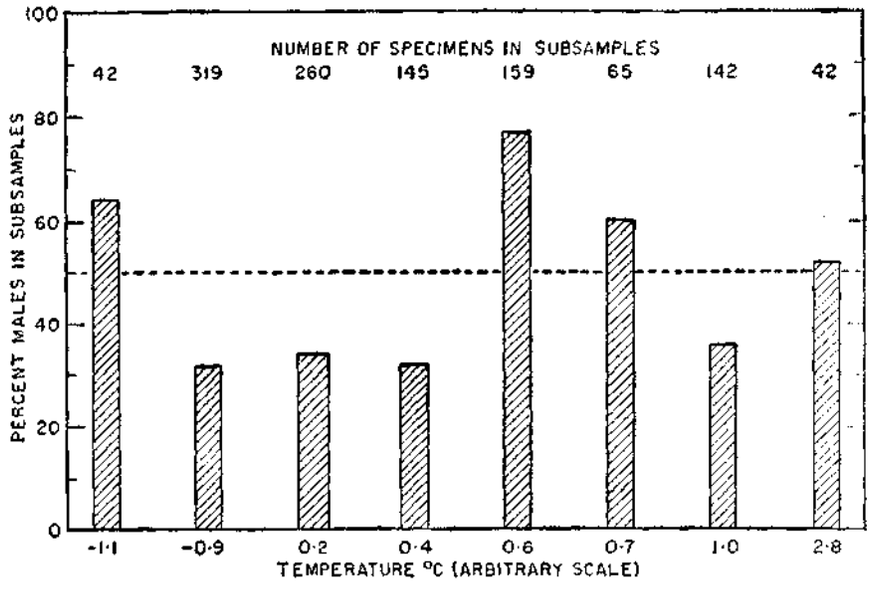

FIG. 3. Bar graph showing the per cent. of male *Pandalus montagui* in subsamples from different temperatures where hauls were made in the northwest Atlantic during 1946–1960 by vessels of the Fisheries Research Board of Canada.

This is an equation representing high reproductivity or annual spawning , but for comparison the following modification is introduced to calculate percentages for low reproductivity in which individual females spawn only in every second year:

$$R = y/(a - 1) \times b + c \times 100 \quad (2)$$

where

b = the number of year-classes mature but not ovigerous in the present year.

The calculated percentages are shown in Table 1.

TABLE 1

Expected percentages of female decapods potentially ovigerous in autumn if spawning takes place (A) annually and (B) biennially, as calculated from the equations $y/(a-1)+c\times100=R$ for annual spawning and $y/(a-1)+b+c\times100=R$ for biennial spawning

Years to reach maturity	Frequency of spawning during lifetime				
	Once	Twice		Three times	
	A	A	B	A	B
	R	R	R	R	R
	%	%	%	%	%
1	100	100	50	100	50
2	50	66	50	75	50
3	33	50	40	60	43
4	25	40	33	50	38

Thermal stress in *P. borealis* appears to occur also in areas with temperatures above those which are optimal for the species (*viz.*, above 6° C.). This is evinced by the occurrence of "primary females" (females which are not males when first hatched), by a small maximal size of individuals and possibly by small egg size in populations from areas with comparatively high temperatures. Primary females occur in samples of *P. borealis* from British Columbia (T. H. Butler, personal communication) and from the North Sea, where "....more than 30% may never show male characteristics" (Allen, 1959). Mistakidis (1957) also found about 30% primary females in *P. montagui* from the Wash and the Thames estuary. No primary females were found in about 12,000 specimens of *P. borealis* and 3,000 specimens of *P. montagui* examined from collections made in Newfoundland and Labrador.

The maximal size of *P. borealis* in subsamples from an unfished population off Northumberland, England, was 26 mm. in carapace length (Allen, 1959). Off the south-west coast of Newfoundland the maximal carapace length was 32 mm. in *P. borealis*. Also in an unfished population of *P. montagui* off Northumberland the maximal carapace length was 22 mm. (Allen, 1963), while off Lincolnshire from a fished population it was 19 mm. Off Newfoundland and Labrador the maximal carapace length in *P. montagui* was 28 mm. In both species, therefore, the maximal size of individuals taken from comparatively warm water (6–14° C.) was considerably less than from comparatively cool water (4–6° C.) as in *P. borealis*, or than from cold water (−1 to 3° C.) as in *P. montagui*. In the high temperature area the diameter of eggs when first laid was 0·8 mm. in diameter in *P. montagui* and 1·1 mm. in *P. borealis* (Mistakidis, 1957; Allen, 1959 and 1963). These diameters were significantly less than those from the low temperature area of the north-west Atlantic where the egg size in both species was 1·3 mm. in diameter.

Discussion

In *Pandalus borealis*, a protandrous hermaphrodite, permanently low temperatures appear to slow the rate of growth and other life processes so that male characters may be retained until an age limit is reached. In many instances, therefore, in such populations of *P. borealis* in the area of investigation, natural mortality ensues before the change from male to female sex occurs. The possibility that females might migrate to other areas than those sampled is negated by the apparent stenobathic characteristic of large females—none are taken in shallow water, and the troughs with permanently cold water are surrounded by considerable areas of shallow water. In some other species of shrimps found in these low temperature troughs, however, cold adaptation has progressed to the point where full reproductivity is maintained. *Eualus macilentus* is one of the species in which about 100% of the females from cold water troughs were found to be ovigerous when sampled in autumn (Table II). However, only 61% of the females of this species were ovigerous from shallow water in the Arctic. Other shrimps show this difference in adaptation to low temperature environments although species such as *Lebbeus polaris* do not (Table II). On the other hand, differences in a species taken in the Arctic and in the other areas considered may not be real since, as pointed out above, the percentage potentially ovigerous in any year may depend on the age at maturity, the number of times spawning in a lifetime and whether spawning takes place annually or biennially in the species (Table I).

Table II

The percentage of females of some decapods probably ovigerous annually. These were examined from collections made in the north-west Atlantic during 1946–60

Species	Numbers examined		Percentage probably ovigerous annually	
	Foxe Basin	Nfld. and Labrador	Foxe Basin	Nfld. and Labrador
Eualus fabricii ..	41	12	64	100
E. gaimardi belcheri ..	205	19	50	70
E. macilentus ..	249	332	61	100
Spirontocaris spinus ..	59	101	68	25
Lebbeus groenlandicus ..	39	20	57	57
L. polaris ..	333	137	75	75

Social Orientation in *Homarus*

Social hierarchies have been conclusively demonstrated in some birds and mammals (Wynne-Edwards, 1960). These hierarchies are exhibited in stable groups of individuals and may be said to favour survival where population density tends to threaten survival (Allee *et al.*, 1949). Hierarchies have been demonstrated for several types of invertebrates also among which the decapod crustaceans have been given special attention. Individual dominance has been shown to occur in hermit crabs (Allee and Douglis, 1945; Reese, 1962), crayfishes (Boovbjerg, 1953; Lowe, 1956), the spiny lobster (Olsen, 1958; Fielder, in press) and in the American lobster (Douglis, 1946).

In preliminary work with the American lobster we also have found an almost straight line ranked dominance. Only seven female lobsters of approximately the same size were used but the results were the same in several trials. On the first introduction of two lobsters to each other a struggle for the best area of shelter took place. One was able to overcome the other without apparent injury to either and took over the area of shelter. The subservient lobster assumed a posture with lowered antennules which were beaten feebly while the dominant lobster kept its

antennules erect beating the water vigorously and frequently. By a series of introductions over a period of several days the most dominant animal was found and an order of dominance ascertained. Following the first introduction and struggle, subsequent introductions of the same animals resulted in shorter struggles for dominance. These animals had been caught in hand traps in approximately the same area over a short period and had been kept singly in a wooden compartmented crate following capture. While our work was in the nature of a preliminary check to determine what happened when lobsters were released on already occupied grounds, it appears to substantiate the work of others and suggests the need of further elaboration.

Discussion

Social orientation in *Homarus* appears to be a reaction to stress concomitant to a degree of overcrowding in a strictly limited type of habitat. In our area of investigation the preferred habitat of lobsters is generally small in area for the size of the population present. It is probable that in this habitat aggregations of lobsters are formed, perhaps depending upon the grouping of shelters, and that the orders of dominance are built up in the group which may be partly conditioned by size (as in the spiny lobster; Olsen, 1958) although aggregations by size and sex are suspected from some underwater observations and from trap catches.

Although many of the commercial-sized lobsters are removed periodically from the fished populations in Newfoundland, approximately eight levels of size of lobster remain on the grounds. (These size groups may be of approximately similar age, presuming that it takes a lobster eight years to reach commercial size and excepting the O-group in any year.) Since the numbers of eggs and larvae produced by a given population appear to be considerable in any year (Scarrett, 1964), the megalopa which settle at any one time will exceed in number any one age group on the grounds. This settling may be in a nursery area due to planktonic drift but the survival of these small lobsters invading the grounds annually will depend on the number of appropriate shelters available. Individual dominance, if present at this stage, will be an important factor in the competition for shelter and food, and it will continue to be important as each size level is reached by moulting, and as competition for a diminishing number of larger shelters takes place. The end effect in this process is that the lobsters interact to maintain exact numbers on the grounds at all size levels. Whenever population increase threatens the environment, the weaker or less dominant individuals are exposed to predation from fish or other lobsters. The probable result is that recruitment to the fishery is kept at approximately the same number each year. The first year recruits which may represent 40–70% by number of the total annual catch is the primary concern of the fishery.

Low Numbers of Single Species in Multi-Species Communities

Trawl or dredge hauls from localities which are shallow or subjected to variable conditions of temperature or permanently low temperatures appear to always contain several species of shrimps in relatively small numbers. The numbers of these species taken singly or together contrast unfavourably with the large number of *Pandalus borealis* found in single species communities in deep water and under comparatively uniform conditions including moderate temperatures. In the multi-species communities combinations of species occur in sufficient regularity that they may be said to characterize certain areas. Quite possibly in such areas they may not be representative of single mixed species communities but rather of a mosaic of discrete communities (Morgans, 1959) such as are found in other animal species particularly in shallow marine environments. The decapods furnish a good example of a greatly diversified group of species which have adapted to or become specialized in a considerable variety of habitats.

Some of the commoner decapod species combinations taken in catches in the area of investigation were as follows:

(*a*) *Lebbeus polaris, L. groenlandicus, Eualus fabricii, E. gaimardi, Spirontocaris spinus, Pagurus pubescens* and *Hyas coarctatus*. This combination occurred particularly in the cold shallow waters of Ungava Bay (with the addition of *Argis dentata* and *Sclerocrangon boreas*; Squires, 1957) but was also common with some minor variations in many shallow bays or on off-shore shoals from Foxe Basin to the Grand Banks.

(*b*) *Eualus gaimardi, Sabinea septemcarinata, Spirontocaris phippsi, Sclerocrangon boreas, Argis dentata* and *Lebbeus polaris* occurred in shallow water embayments (0–35 m.).

(*c*) *Argis dentata, Sabinea septemcarinata, E. gaimardi belcheri* and *Lebbeus polaris* occurred at medium depths (70–145 m.) and moderately low but variable temperatures in localities such as St. Mary's Bay and Bay of Islands, Newfoundland.

(*d*) *Eualus macilentus, Pandalus montagui* and *P. borealis* occurred in deep and permanently cold troughs in bays such as Fortune, Conception and Placentia Bays.

Discussion

The species of shrimps found in northerly and shallow marine areas may be subjected to relatively moderate increases in temperature in summer. This increase may last several months in the southerly part of the geographical area under consideration but may be as short as a month in the more northerly localities. As we have seen, some species when subjected to long periods of low temperature may have a low reproductive potential (*e.g., Eualus fabricii*) while others maintain relatively high reproductivity under all conditions (*e.g., Lebbeus polaris*) (Table II). High reproductivity is probably a best indication of the successful adaptation of these decapod species to the varied conditions of shallow and cold or variable marine environments. However, unlike *P. borealis* which appears to have eliminated all competing shrimp species in its relatively warm and deep soft-mud areas, such species have many apparent competitors and an area to live in which is limited also by their own specialised preferences for type of habitat and food. The limitations of food-supply and space coupled with environmental extremes militate against large numbers of each of these species, and in addition the stresses of interspecies competition and predation contribute to their occurrence in small numbers. Therefore, although successful adaptation to temperature, etc., extends the geographical range of these species their communities tend to consist of a small number of individuals.

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PRELIMINARY OBSERVATIONS ON THE BEHAVIOR OF YOUNG HERMIT CRABS*

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ABSTRACT

Due to the new techniques available for the culture of larval crustaceans, it is possible to obtain some numbers of larvae and young crabs, the social history of which are known. This allows us to observe the initial reactions of young stages of hermit crabs when presented with gastropod shells of the appropriate size and when placed with conspecific individuals of the same stage of development. The preliminary results of the study now in progress at the Institute of Marine Science have shown that such observations can be very productive for the study of behavior.

It is usually stated that the behavior of invertebrates is largely "instinctive" (innate, inborn, genetically controlled). Indeed many of the aggressive behavior patterns observed in adult forms are executed by naive young crabs, when first encountering a conspecific crab. Also the crab's initial reactions to a gastropod shell indicate that many behavior patterns can be executed without prior experience. However, in all behaviour patterns, particularly those of shell-fighting, many "mistakes" are made and the effects of experience can be seen.

NEW techniques in the laboatory culture of larval crustaceans have presented numerous new possibilities to those interested in crustacean behavior. The rearing of postlarval stages from eggs is now a routine procedure, and even the laboratory rearing of socially naive, mature adults from eggs is a possibility (unpublished data Costlow; Provenzano). Present techniques of rearing in compartmented plastic trays (Costlow and Bookhout, 1949; Provenzano, 1962) do not totally isolate crabs from possible visual contact with conspecific individuals. Slight modification of the trays now widely used (*i.e.*, making the sides of the compartments opaque), would provide numbers of socially naive animals of any stage of development desired.

My present studies at the Institute of Marine Science of the University of Miami are concerned with the behavior of glaucothoe and early crab stages of several species of hermit crabs (Paguridae, Diogenidae and Coenobitidae). The species and stages studied have been obtained as a result of the rearing work of A. J. Provenzano, Jr., and his staff at that Institute. The number of species and stages that I have had the opportunity to observe has been considerable and the results of these observations very interesting. A detailed report of the observations on all species studied is being prepared (Hazlett and Provenzano, in prep.). Young stages of *Pagurus pygmaeus, Pylopagurus operculatus, Calcinus verrilli, Clibanarius tricolor, Dardanus venosus* and *Coenobita clypeatus* have been observed when (1) first presented with a gastropod shell of the appropriate size and (2) placed with conspecific individuals of the same stage of development.

Rather than attempting at this time to present data on all the patterns observed, I wish to mention just two results of these observations—the shell-investigating behvaior of very young *Calcinus verrilli* and the shell-fighting behavior of very young *Pylopagurus operculatus*. The behavior of adult *Calcinus verrilli* is very similar to that of *Calcinus tibicen*, which has been described elsewhere, as has been the behavior of adult *Pylopagurus operculatus* (Hazlett, in press). The results obtained from the observations on early stages of these species illustrate certain principles of crustacean behavior which may prove to be of general applicability.

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When a 'naked' adult hermit crab is presented with an empty shell it quickly goes through a rather stereotyped pattern of orientation, investigation, and entrance. Each of these activities can be seen in late glaucothoe and early crab stages when individuals first come in contact with a shell. Even upon initial contact, first stage crabs quickly get into an 'opposed' position on the shell and begin moving their chelipeds about in the aperture. They actively clean out any debris in the aperture for some minutes and may move around on the shell before attempting their initial entrance into it. Up to this point, the only difference between the behavior of young crabs and behavior of adults is the longer length of time spent in this opposed position by the young animals. The crab's entrance into the shell is a complex series of movements of the adbomen and cephalothoracic appendages. The abdomen is bent forward under the cephalothorax and then, as the crab moves its whole body forward, it lowers the abdomen, inserting the telson and uropods into the aperture; the crab must then twist first its abdomen and then its whole body around to back into the shell aperture (*see* Reese, 1963).

It is in this series of movements that some early stage crabs make 'mistakes'. Among six first and second stage crabs of *Calcinus verrilli*, three made some mistake in their initial attempt to get into a shell. The most common mistakes observed were moving forward too little or two much (thus the downward motion of the abdomen missed the aperture) or failing to twist the abdomen properly (making more than partial entrance into the shell impossible). Both mistakes were in the co-ordination of behavioral elements, not the absence of such elements. In the case of these early stages, these 'mistakes' were quickly corrected. However, there is some question as to whether later stage crabs can orient as correctly (and correct their mistakes as rapidly) as early stages. Preliminary observations indicate the possibility of certain 'critical periods' in the behavioral development of these crabs. Experiments are now in progress to see if naive 10th to 12th postlarval instars can correctly orient and enter gastropod shells.

Shell-fighting interactions are complex and the co-ordinated responses of the combatants is rather remarkable. Both the winning and the losing crab have definite "roles"—patterns invariably observed in adult interactions. What seems even more remarkable is that these "roles" may be partially learned. The most consistently correct behavior pattern shown by young *Pylopagurus operculatus* was rapping. However, a higher percentage of diogenid type raps was observed than is normal for the adults of this species. Although there is some overlap, pagurid and diogenid crabs have distinctive patterns of rapping. Diogenid aggressors rapidly bring their own shell into contact with that of the defending crab, while pagurids pull the defending crab's shell toward their own (Hazlett, in press). Adult *Pylopagurus operculatus* very rarely execute any diogenid type raps, whereas about half of the rapping movements executed by young crabs were diogenid-like.

Young crabs (7th to 9th postlarval instars) tended to orient to their opponents approximately correctly, although some rather amusing fights were observed in which the attacking individual showed little regard for correct orientation. Raps were executed not only from the usual opposed position (with the defensive crab withdrawn into its shell), but from 180° out of opposed, 90° out, too far back from the defender's aperture or too far forward and with the defending crab up in the aperture and the combatants' appendages intermingled. In fact, defending individuals quite often partially emerged and executed aggressive movements and displays (Hazlett and Bossert, in press) toward attacking crabs—a situation rather uncommon among adult crabs. In general young crabs acted much more frequently on a physical contact level than do adult crabs.

Equally glaring mistakes were made as losing crabs left their shells. In several fights, the defending crab started to come out, but when the attacker stopped rapping the defending crab pulled back into its shell. Often the losing crab moved out very slowly and even stopped when almost completely out (adult losers quickly vacate their shell and move onto the back of their own or the attacker's shell). Several losers were pinched rather hard on the abdomen by the winning crab while dallying in such a manner (one was dead the next day). After vacating shells, losers

did not 'properly' let the winner get into the shell it had won, but either displayed at or physically attacked the winner. Several winners were pinched hard by losers while trying to get into vacated shells. The great majority of shell exchanges by adults occur without such mishaps.

One ninth stage *Pylopagurus operculatus* lost two fights on consecutive days. In its first fight, it made almost all the mistakes mentioned above for losing crabs. However, the next day, when it lost again, it moved out of its shell rapidly and moved onto the back of the winner's shell and did not touch the winner with its chelipeds at all. It quickly moved into the winner's old shell when it was vacated—it was a good loser in contrast to its behavior in its first fight.

It appears that the behavior of these crabs is not solely dependent upon inherited patterns, as has been generally assumed for invertebrates, but is a product of experience as well. Just as in the case of vertebrates, the establishment of effective behavioral interaction is developed both by maturation and by experience. It is hoped that further research of this type will show more of the interplay of experience and the crab's genetic framework in the development of behavior patterns. These observations will provide data for systematic purposes as well as the study of behavior *per se*.

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NON-GENETIC ADAPTATION IN CRUSTACEA

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ABSTRACT

The status of our present knowledge concerning non-genetic adaptation of Crustacea to temperature, salinity, pressure, illumination, and dissolved gases is briefly reviewed. The information available is rather scarce. Most of the non-genetic apaptations reported in literature are similar to those known to exist in other groups of animals; examples are shiftings of lethal limits, changes in rates and efficiencies of metabolism, activity, regulative potential, reproduction, pigmentation, haemoglobin synthesis, respiration, etc. Other adaptations appear to be related to specific crustacean features, such as their primary aquatic way of life, respiration through gills, urine formation in antennal or maxillary glands, exoskeleton and body shape. Examples are the different mechanisms and structures involved in ion and osmoregulation, respiratory adaptation and adaptations to life on land.

INTRODUCTION

ONE of the most exciting fields in modern biology is the study of the various abilities and mechanisms available to individuals and populations for coping with the ill-effects of their environment. Critical discrepancies between a living system's immediate potential and the demands imposed by a stressful environment may be reduced through personal adjustments or through changes in the genotype composition of populations *via* selective processes. Of the various phenomena involved in such compensations, some lead to actual changes in the living system concerned; they modify its capacities, take a relatively long time to develop and persist beyond the impact of the environmental circumstance which induced them. It is my intention to talk to you about such phenomena; they are known as adaptations.

More specifically, adaptations have been defined as adjustments of organisms to their environment, which ultimately result in a relative increase in their capacity to survive, reproduce and compete; they favor survival in a changing or changed surrounding and make for increased fitness under stressful conditions (*e.g.*, Kinne, 1958, 1962, 1963, 1964 *a*, *b*). Adaptations thus are "advantageous" or "useful" in an ecological sense. The amount of a given adaptation may be assessed quantitatively by measuring differences in functional or structural performance of individuals with different environmental histories. End points used as criteria may be rates of survival at extreme factor intensities (*e.g.*, temperature, pressure, salinity, light), rates and efficiencies of metabolism, activity and reproduction, changes in internal state or in behavioral patterns, recovery from extreme stresses, etc.

Adaptation to a given environmental situation may consist of a non-genetic *plus* a genetic component. Non-genetic adaptation (also known as acclimation or acclimatization) involves adjustments which are directly induced by the environment and are not passed on as such to the next generation; they may, however, be retained by the individual concerned for various lengths of time beyond the immediate stress situation or even be—at least in part—irreversible throughout the individual's life. Reversible acclimations need reinforcement if they are to be maintained indefinitely. Genetic adaptations involve changes in the genotype. Their main mechanism appears to be selection acting upon genetic variation. Genetic changes which are retained in the process of selection are usually *per se* adaptive or are linked to other adaptive characteristics; strictly "neutral" or "disadvantageous" genes could hardly be retained for any length of time. Non-genetic and genetic

components of a given adjustment may be differentiated experimentally by cross-acclimation and breeding tests.

The present contribution deals with non-genetic adaptations in Crustacea and is based on more comprehensive reviews (Kinne, 1963, 1964 *a*, *b*). It briefly reports on acclimations to some of the more important abiotic factors: temperature, salinity, pressure, illumination and dissolved gases. Biotic factors, while presumably also important with respect to the phenomenon of non-genetic adaptation, have hardly been studied and consequently will not be treated here.

Knowledge on adaptation in Crustacea is still rather limited, and, on the basis of the small amount of information available, it is often quite difficult to decide whether a functional or structural response is truly adaptive or not. Proper assessment of the adaptive value of a given response requires more information, especially on ecology and phylogeny of the organism concerned, than is frequently available. An effort was made, however, to select examples from literature that appear to represent adaptations according to the definition presented above.

General Considerations

The phenomenon of non-genetic adaptation has been discussed recently by Kinne (1964 *a*, *b*). Following the enviornmental change which ultimately causes the relative increment in the efficiency of performance (acclimation), three phases may be distinguished: (*a*) immediate responses commencing seconds or minutes after the change; they involve shock reactions, over- or undershoots or increased fluctuation of performance and may have a positive survival value but do not seem to be necessarily an integral part of the subsequent acclimation process; (*b*) stabilization commencing minutes, hours or days after the change and leading towards increased constancy of performance, thereby gradually approaching a steady level; (*c*) the new steady state commencing hours, days or weeks after the changes, that is, after completion of the most important adjustments. Only the new steady state will be considered here in some detail.

The period of time which elapses between a given environmental change and the beginning of the new steady state (*i.e.*, the length of the stabilization period) varies in different species and for different life processes. Within a given species it depends on age (it usually increases with increasing age), physiological condition and the type and the degree of the environmental stress employed. In general, the velocity of acclimation seems to be proportional to metabolic rate. In *Homarus americanus* transferred from 14·5 to 23·0° C., thermal acclimation is completed after 22 days, and substantial acclimation to low salinity and to low oxygen occurs within one week (McLeese, 1956). In *Astacus astacus* transferred from freshwater into blood-isosmotic water of 15‰ salinity, blood osmoconcentration reached a new steady level after 12 days, and O_2-consumption after 20–30 days (Schwabe, 1933). The freshwater crab *Potamon* acclimated to new salinity levels in about 12 days (Drilhon-Courtois, 1934). Considerably shorter intervals were found in *Asellus aquaticus* and *Heloecius cordiformis*: *Asellus*, after being transferred at 15–18° C. from freshwater into a salinity of about 10‰, established a new equilibrium of blood osmoconcentration after 24 hours (Lockwood, 1959); in *Heloecius* transferred from freshwater into a salinity of about 9‰, blood osmoconcentration reached a new equilibrium after some 12 hours—this period was, however, longer after transfer into more extreme salinities (Edmonds, 1935). The planktonic copepod *Acartia tonsa* shows a rapid decline in body fluid osmoconcentration following transfer from about 35‰ (full strength sea water) to about 17‰ (50% sea-water) within minutes; after one hour no further osmotic change was detected (Lance, 1965). From these data it appears that the new steady state of performance following a significant change in temperature or salinity may be reached within hours, days or weeks. In some cases the stabilization process begins within minutes or hours; in others there is a latent period of considerable length. Thus in *Homarus americanus* a detectable gain in thermal acclimation commences only after a latency period of some 10 days (McLeese, 1956).

Temperature

Considering the quantitative performance of living systems on earth, temperature appears to be the most effective single environmental factor. Non-genetic adaptation of crustaceans to low or high temperatures is well documented. Acclimation to reduced temperatures tends to shift the lower lethal limit downward, and acclimation to increased temperatures tends to shift the upper limit upward. Such variations in lethal limits are often maintained for days, weeks or months after removal of the stressor. They have been observed in quite a number of crustaceans, *e.g.*, in *Streptocephalus seali* (Moore, 1955), *Homarus americanus* (McLeese, 1956), *Artemia salina* (Grainger, 1958) as well as *Armadillidium*, *Porcellio*, *Oniscus*, *Ligia* and other macro-crustaceans (Edney, 1951 *a*, *b*; 1960).

Lethal temperature limits may also be affected by seasonal changes in complex, natural environments, leading to increased cold resistance in winter animals and to increased heat resistance in summer animals. Examples are *Emerita talpoida* (Edwards and Irving, 1943) and *Gammarus limnaeus* (Krog, 1954). In *Hemigrapsus nudus* and *H. oregonensis* urine and blood osmoconcentrations are influenced by seasonal acclimation of these crabs as well as by the experimental temperature. In both species, summer and winter acclimation tended to favor stronger hyper-osmotic regulation at the respective seasonal temperatures than at temperatures foreign to the seasons; furthermore, seasonal adaptation of osmoregulatory mechanisms has been shown to alter the balance of active processes so that for a given range of experimental conditions, urine is lower in winter than in summer animals, both in absolute concentration and relative to the blood (Dehnel and Stone, 1964).

Acclimation of metabolic rate to new intensities or patterns of temperature has been studied in many cases. Based on experiments and theoretical considerations, five types of such metabolic adjustments have been distinguished (Precht, 1949; Precht *et al.*, 1955): compensations (types 1-3), no compensation (type 4), and inverse compensation (type 5). Type 3 represents the normal and most frequent case, namely, partial compensation. Type 1 involves overcompensation, and type 2, perfect compensation resulting in quasi-constant rate functions after complete acclimation to different levels of experimental temperature.

Precht's acclimation types have later been modified to some extent by Prosser (1958), who distinguishes four basic patterns (Fig. 1). Pattern I shows no compensation and is characterized by little or no change in the position of rate-temperature curves, the curve for warm-acclimated individuals practically being a continuation of the curve for cold-acclimated ones. No convincing reports on crustaceans showing a complete lack of compensation have come to my attention. Pattern II A shows shift in position (translation) of rate curves without change in slope; examples are: rates of heart-beat in *Daphnia* (Precht, 1949; Precht *et al.*, 1955), molting frequency of *Gammarus* (Kinne, 1953), and O_2-consumption in *Pachygrapsus* (Roberts, 1957 *a*, *b*). Pattern II B shows reverse translation: the curve of cold-acclimated individuals lies to the right and below that of warm-acclimated ones; it may occur in situations where other environmental factors, such as extreme salinity or modified concentrations of dissolved gases interfere; this may be exemplified by seasonal changes in O_2-consumption of *Gammarus limnaeus* (Krog, 1954). Pattern III is rare; it is characterized by a rotation of rate-temperature curves without a shift in position. Most common is a combination of clockwise rotation and translation leading to an extrapolated intersection of the two rate-temperature curves above the normal temperature range and to a reduction of Q_{10} by cold acclimation (Pattern IV A); this may be exemplified by Q_{10} curves of cold acclimated *Uca rapax* (Vernberg, 1959). Counter-clockwise rotation and translation with the two rate curves intersecting at a low temperature, often by extrapolation below the normal biological range, constitute pattern IV C. Patterns IV B and IV D have rarely been indicated.

Acclimation to different temperature levels may also involve changes in the temperature preferendum in behaviour, orientation, migration, territorialism, etc., and in the quantity or activity of physiologically important substances (hormones, enzymes, pigments, etc.) produced, as well as in

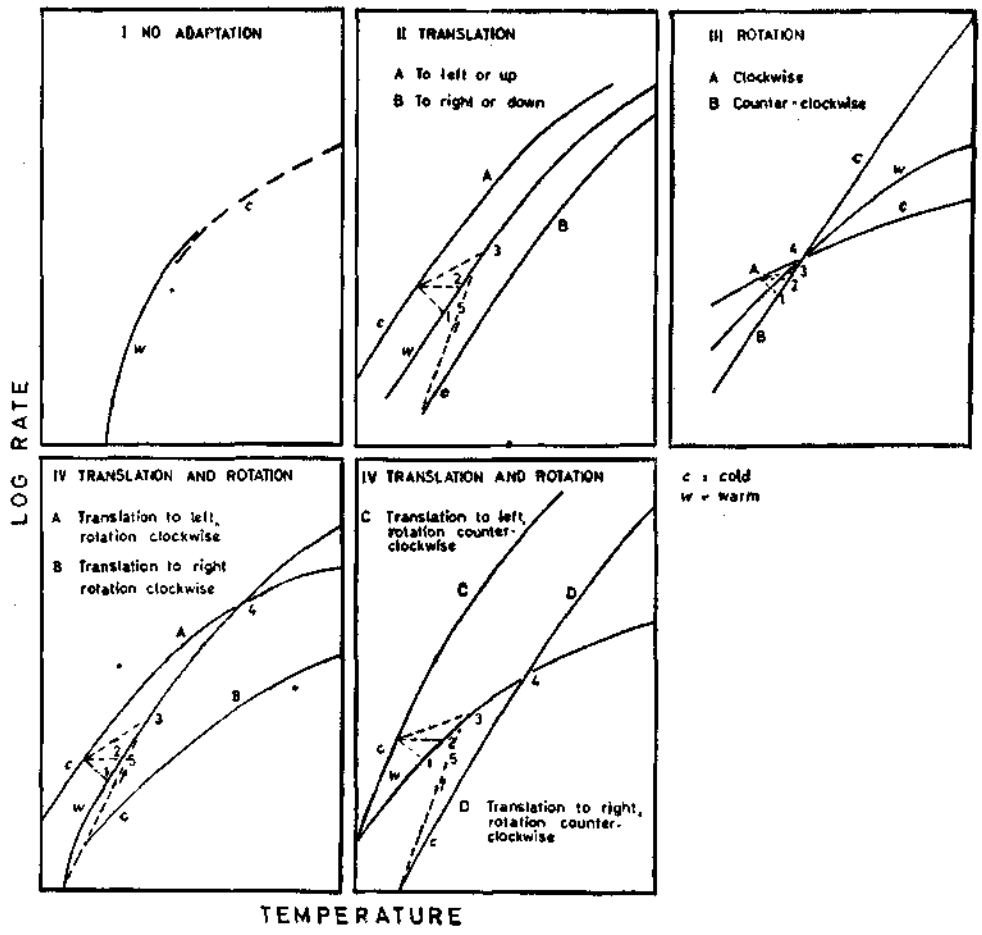

FIG. 1. Patterns of non-genetic adaptation of rate functions to different constant temperatures. All patterns are indicated for cold acclimation. *c.*, cold; *w.*, warm. Broken lines and numbers indicate Precht's types of acclimation. Curves are plotted with decreasing Q_{10} at higher temperatures. Clockwise rotation means reduced Q_{10}, counter-clockwise rotation increased Q_{10}. (After Prosser, 1958, modified.)

body size and body structure; structural non-genetic adaptations, however, are documented by relatively few reports (*see* Kinne, 1964 *a*, *b*). The effects of temperature on the process of non-genetic adaptation may be significantly modified by simultaneously effective additional factors, such as salinity, pressure, illumination, oxygen and carbon dioxide.

SALINITY

The simplest and probably most ancient type of non-genetic adaptation to salinity appears to be overall tissue acclimation in osmoconformers lacking specific organs for effective regulation. Osmoregulators employ specialized regulatory organs such as gills, gut and excretory glands, which then presumably tend to represent the primary site of functional or structural acclimation.

Following a significant change in salinity, the new steady level of metabolic rate may be (*a*) higher in subnormal salinities and/or lower in supranormal salinities, (*b*) higher both in subnormal and supranormal salinities, (*c*) lower both in sub- and supranormal salinities, (*d*) essentially unaffected (for examples, *see* Kinne, 1963, 1964, *b*). Changes in salinity not only modify the rate of metabolism but also the efficiency of metabolic processes, reproductive potential, activity and behaviour as well as structural aspects (for examples, *see* Kinne, 1964 *a, b*). They may thus cause functional and structural variations in individuals subjected to different salinity histories. Such environmentally induced variations may result in considerable differences in the response patterns, even in individuals with identical genotypes.

In terrestrial or semi-terrestrial crustaceans, changes in atmospheric humidity seem to induce adjustments that are to some extent comparable to those induced by salinity variations in their aquatic relatives. Next to nothing has been done, however, to investigate acclimation to various levels of humidity.

Pressure

Non-genetic adaptation to different hydrostatic pressures (water depths) is poorly investigated (Kinne, 1964 *a*). In nature, differences in light conditions, temperature and water movement as well as in quality and quantity of the food available are usually intimately correlated with significant differences in water depths. Such concomitant changes of other environmental factors may modify the resulting acclimation; they make a detailed analysis more difficult.

Lack of light, a rather constant physical environment and scarcity of food are also characteristic for cavernicolous habitats. Acclimation to cave life may therefore show somewhat similar trends as does acclimation to deep-sea conditions. Cavernicolous crustaceans have lower metabolic rates than their epigean relatives and exhibit greater sensitivity to changes in temperature, salinity, light and other environmental factors.

The known effects of high hydrostatic pressures on protoplasmic viscosity, enzyme activity, cell division and muscle contraction may to some extent also apply to living conditions in the deep sea. Critical experiments under ecological conditions are wanting. In a variety of larvae and adults of planktonic crustaceans, increased locomotory activity and upward swimming have been observed in response to small increments in pressure (Knight-Jones and Qasim, 1955; Baylor and Smith, 1957). In the shallow water shrimps *Palaemon, Crangon* and *Pandalus*, a pressure increase of 50 atm. (equivalent to a water depth of 500 m.) leads to temporary increase in locomotor activity, brief exposure to 150 atm., however, causes reversible paralytic effects. Other invertebrate groups such as Cnidaria, Ctenophora, Echinodermata or Mollusca appear to be less pressure sensitive than the Crustacea (Ebbecke, 1935; *see also* Fontaine, 1928). The intertidal amphipod *Synchelidium* n. sp. is able to perceive pressure changes of less than 0·01 atm.; its reactions imply rapid adjustments (Enright, 1961).

There is some evidence that high hydrostatic pressures can be tolerated more successfully at relatively high temperatures (Schlieper, 1963). The high pressures and relatively low temperatures of the deep sea would then represent a severe stress combination into any newcomer in the oceanic abyss.

The ability to acclimate to different depths may be of considerable ecological importance. New technical devices will hopefully bring more light into the fascinating aspects of functional and structural acclimations caused by prolonged exposure to new levels of hydrostatic pressure.

Illumination

Immediate responses to sudden changes in light intensity may involve increase or decrease in activity or metabolic rate. Thus abrupt increase in illumination reduces locomotion rates in *Onis-*

cus asellus, Asellus communis, Hyalella knickerbockeri, Cambarus sp. (Stehr, 1931) and in *Uca pugnax* (Holmes, 1908). Increasing illumination causes acceleration of limb movement in *Branchipus* (McGinnis, 1911), *Triops* (Seifert, 1930), and *Hemimysis lamornae* (Franz, 1914).

After acclimation to certain light conditions, photopositive or photonegative responses may be inversed in *Artemia salina* (v. Frisch and Kupelwieser, 1913), Cladocera (Kikuchi, 1938; Koehler, 1924; v. Frisch and Kupelwieser, 1913), Copepoda (Rose, 1925), and larvae of *Homarus vulgaris* (Blum, 1934). *Armadillidum* becomes photopositive after long exposure to light (Müller, 1926), and *O. asellus* becomes photopositive after exposure to darkness (Cloudsley-Thompson, 1952). Individuals of *Simocephalus vetulus* raised under continuous fluorescent lighting (complete acclimation) reproduce at a faster rate than individuals raised in the dark (Parker, 1959). Dark-acclimated *Daphnia* and *Simocephalus* respond to sudden illumination with a transient decrease in heart rate, while light-acclimated individuals respond to shadow with a transient increase (Schulz, 1928).

Functional and structural pigment changes in response to illumination and background have been observed in *Orconectes* (Bowman, 1942); *Palaemonetes* (Brown, 1934; Fingerman and Tinkle, 1956) and other crustaceans. Such acclimations tend to provide the individual involved with indistinct, obliterative coloration; they are best developed in benthic forms. Crayfishes acclimated for extended periods to different backgrounds seem to accumulate the color-change hormone (lightening or darkening) which is not used under the condition of acclimation. In addition to the few examples presented, many other known responses seem to cause actual changes in the responding individual and may be of adaptive value. Most of the information available represents, however, more or less a by-product of research that was not primarily concerned with problems of adaptation. Critical experiments are wanting. Responses of crustaceans to various conditions of illumination have been reviewed recently by Waterman (1961) and by Pardi and Papi (1961).

Dissolved Gases

In aquatic crustaceans the physiological importance of changed concentrations of dissolved gases has been documented in a number of papers. Only a few of these papers, however, pertain to our present topic, and these few are concerned exclusively with the effects of dissolved oxygen and carbon dioxide.

Most frequently aquatic crustaceans are confronted with problems of low oxygen pressure. *Astacus fluviatilis* triples its breathing rate and doubles its ventilation volume when oxygen concentrations drop from 6·6 to 2·1 ml./l. (Lindroth, 1938). Modifications in CO_2 concentration had little effect until after the calcareous carapace was coated with collodion to prevent exoskeletal carbonates from affecting the results; breathing frequency then increased proportionally with increase in CO_2 from 5–20% (Peters, 1938). Changes in O_2 or CO_2 concentration do not affect the ventilation rate in *Homarus vulgaris*. This decapod increases its percent O_2 withdrawal presumably by improvement of circulation; it cannot prevent, however, a gradual decline in O_2-consumption (Thomas, 1954).

Homarus americanus acclimated at 15–16° C. for 4 days to 3·7 mg. O_2/l. survived longer when transferred into 0·8 mg. O_2/l. than individuals that had been kept previously in 6·4 mg. O_2/l. Increased survival times after acclimation to reduced O_2 levels were also obtained at 24–25° C. (McLeese, 1956). Some decapods increase their blood sugar level under conditions of asphyxiation (Stott, 1932). Such adjustments may be accompanied by increase in reduced glutathione in the hepatopancreas (Monier, 1936).

When kept under low oxygen stress for several days, a number of *Daphnia* species synthesize additional hemoglobin in considerable amounts. Consequently, they may change from a pale color to bright red (Fox, 1948, 1955). After re-acclimation to well-aerated water, hemoglobin values slowly drop to normal again (Fox *et al.*, 1949; Fox and Phear, 1953). Similar reversible non-genetic adaptations occur in other Cladocera, *e.g.*, in *Simocephalus vetulus* (Hoshi, 1957), *Ilyocryptus sordidus*

(Fox *et al.*, 1951), *Chydorus sphaericus* (Fox, 1955), in the anostracan *Artemia salina* (Gilchrist, 1954), in the notostracan *Triops cancriformis* (Fox, 1949), and in the conchostracans *Caenestheria inopinata* and *Leptestheria mayeti* (Fox, 1955). Increase of blood hemoglobin in *Daphnia* at low oxygen pressures has been shown to result in a relative increase in its potential to compete: acclimated individuals enjoy a longer life-span and an increased reproductive capacity (Fox *et al.*, 1951).

In general, metabolic adjustments to alterations in oxygen and carbon dioxide levels are presumably manifold and rather complex. They may involve adaptive changes in the permeability of respiratory surfaces, efficiency of gaseous exchange, O_2 and CO_2 affinity of blood, quantity and quality of respiratory pigment, pigment transport, metabolic rate and metabolic pathways as well as in rate and volume of ventilation, body shape and size. They may further lead to modifications in the ability to build up a temporary oxygen dept and initiate enzymatic acclimations liberating energy anaerobically.

Summary

1. The information available on non-genetic adaptation in Crustacea is scarce. There is great need for critical experiments under adequate conditions.

2. The types of non-genetic adaptations occurring in crustaceans are universal among animals rather than specific for that group. They involve shiftings in lethal limits, changes in rates and efficiencies of metabolism, activity, regulative potential, reproduction, pigmentation, hemoglobin synthesis, respiration, etc.

3. Non-genetic adaptations result in modifications in the actual response amplitude relative to a given environmental situation. They may lead to functional and structural variation in individuals subjected to different environments. Such variations may be retained beyond the immediate effects of the stressor. It seems possible that in special cases environmentally induced variations due to acclimation may lead to repeated breeding discontinuities (disengagement, isolation) of groups of individuals with different environmental experiences and thus provide a basis for selective processes.

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FIELD INVESTIGATIONS ON THE SHORE CRABS OF THE GULF OF MANNAR AND PALK BAY, WITH SPECIAL REFERENCE TO THE ECOLOGY AND BEHAVIOUR OF THE PELLET CRAB *SCOPIMERA PROXIMA* KEMP[1]

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ABSTRACT

In the course of carrying out observations on the ecology and behaviour of shore crabs of Palk Bay and Gulf of Mannar in 1959–60, special attention was given by us for studying the burrowing habits, sexual dimorphism, mating behaviour, etc., of the pellet crab *Scopimera proxima* Kemp details of which are embodied in this paper.

INTRODUCTION

THERE is a paucity of information on the habits and behaviour of shore crabs from the Indian Region. Some observations in earlier literature may be found in the works of Henderson (1893), Anderson (1894), Alcock (1900, 1902), Kemp (1915, 1919) and Sewell (1922). Recently in a series of papers, Altevogt (1955 *a* & *b*, 1956, 1957 *a*, 1957*b*, 1957 *c* and 1959) has given accounts on the ecology, biology and ethology of shore crabs, especially of fiddler crabs of the genus *Uca* Leach [*U. annulipes* (H. Mlne-Edwards), *U. marionis marionis* (Desmarest), *U. m. nitidus* (Dana), and *U. triangularis* (A. M. Edwards) and the closely related genus *Dotilla* Stimpson [*D. blandfordi* Alcock and *D. myctiroides* (H. Milne-Edwards)] from India. On account of this want of information, we were prompted to make some observations during our spare time. In view of the short period during which the observations could be made, the study is incomplete in several aspects.

Our observations were restricted to the period from early September to the end of December 1959 in the areas close to Mandapam Camp and Rameswaram Island when field investigations were conducted both during daytime as well as at night. Special observations were made on the ecology and behaviour of the pellet crab *Scopimera proxima* Kemp in relation to other shore crabs in the eco-system.

Frequent field trips were made to the following three selected areas (as indicated in Fig. 1):

Area I. Beach west of C.M.F.R.I. Jetty, Mandapam Camp (Gulf of Mannar).

Area II. Between Pamban and Kundugal Point, Rameswaram Island (Gulf of Mannar).

Area III. Beach adjacent to C.M.F.R.I. Field Laboratory (Palk Bay).

It was observed that *S. proxima* is restricted to certain types of sandy beaches and in such areas they show a definite zonation in the intertidal region. Collection of an allied species, *S. pilula* Kemp indicated that both these species do not co-exist in the same area as they show preference to certain types of soil and also differ in habits.

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Distribution of Shore Crabs in Relation to the Tide

A list of the shore crabs encountered in the areas of study is given below:

Species	Area-I	Area-II	Area-III
Family Ocypodidae			
Ocypode ceratophthalma (Pallas)	X	X	X
Ocypode cordimana Desmarest	X	..	X
Ocypode macrocera H. Milne-Edwards	X	..	X
Ocypode platytarsis H. Milne-Edwards	X	..	..
Scopimera proxima Kemp	X	..	X
Scopimera pilula Kemp	..	X	..
Uca annulipes (H. Milne-Edwards)[1]	..	X	..
Dotilla myctiroides (H. Milne-Edwards)	X	X	X
Macrophthalmus depressus Ruppell[2]	..	X	..
Family Grapsidae[3]			
Metapograpsus thukuar (Owen)[4]	..	X	..

[1] An allied species *U. marionis* as well as the variety *U. m. nitidus* have been recorded by Gravely (1927) from Area-II.

[2] *M. telescopicus* and *M. convexus kempi* have also been reported from Area-II by Gravely (1927).

[3] *Grapsus albolineatus* and *Plagusia depressa* var. *tuberculata* found habitually on the granite blocks of the pier close to Area-I are not included in the present study.

[4] *Metapograpsus frontalis* found among dead coral blocks in the intertidal zone in the vicinity of Area-III is also not included in this account.

The species mentioned as occurring under the three areas in the above list are primarily specific to the particular areas. However, a species such as *O. platytarsis* could occur in Area-III though we have not collected it from there. On the other hand a species such as *Macrophthalmus depressus* will not normally occur in areas I and III.

Areas I and III are sandy beaches, while area II is typically marshy, a considerable part of which is exposed during low tide and the soil shows gradations from clayey to sandy condition.

The general distribution of the shore crabs in the three areas in relation to low and high water marks is shown in Figs. 2, 3 and 4. In area-I, the beach has a gentle slope between low and high water-marks, but rises more sharply above the latter, and about 4–6 meters of beach is exposed during low tide. Ocypodids were found inhabiting the area above the high water-mark but the burrows of these crabs found closer to the HWM are smaller in size inhabited by smaller crabs. *S. proxima* shows a distinct zonation immediately below the HWM. *D. myctiroides* was rare, but invariably was found to occur very close to LWM.

Area-II is located between Kundugal Point and Pamban covering a shallow bay which is mainly a mudflat with hardly any sandy beach. This area is submerged under water during high tide and during low tide a considerable part is left exposed. Our observations were restricted to

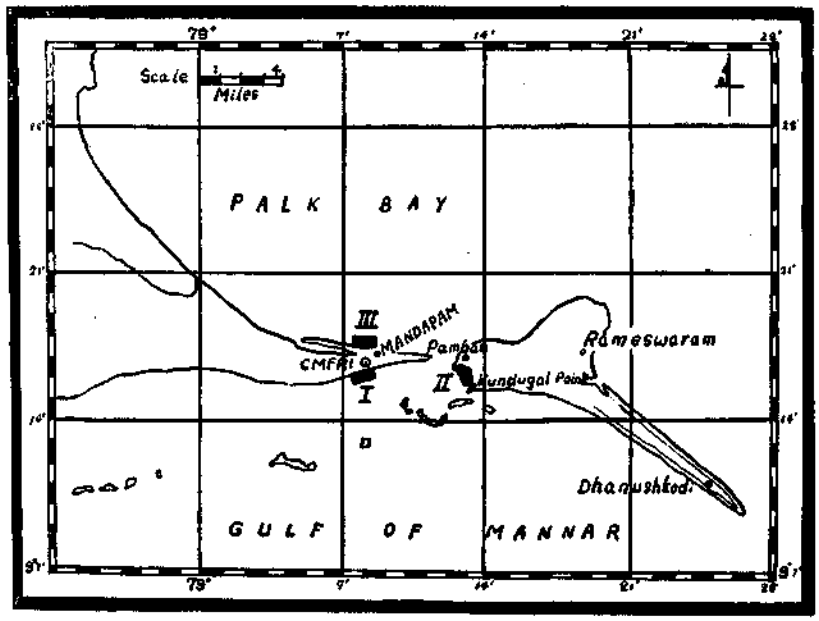

FIG. 1. Map showing Areas-I, II and III from where observations were conducted and collections made.

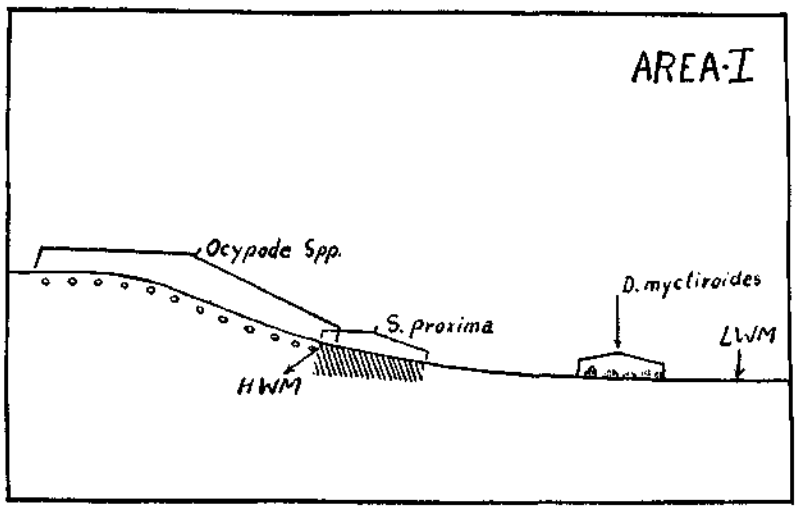

FIG. 2. Schematic section of Area-I showing zonation of shore crabs.

the area between HWM and LWM, in which was also present a dense congregation of gastropods (*Potamidis, Cerethium,* etc.) in a distinct zone just above LWM. The mudflat below LWM during low tide may have large puddles of water and only *Macrophthalmus depressus* was seen occurring. Inclusive of the gastropod zone above the LWM, about 9–10 meters of beach is generally exposed in this area. The areas above the gastropod zone to the HWM is ploughed up in large patches

here, as a result of the burrowing and feeding of the crabs. *U. annulipes*, *S. pilula* and *D. myctiroides* occur in this zone of which the last-mentioned species was always present close to the gastropod zone which is nearest the LWM. At the time of our observation quantities of sea-weeds were washed up in patches along the intertidal zone which areas were not generally found to be occupied by the crabs. Fig. 3 shows the details of occurrence.

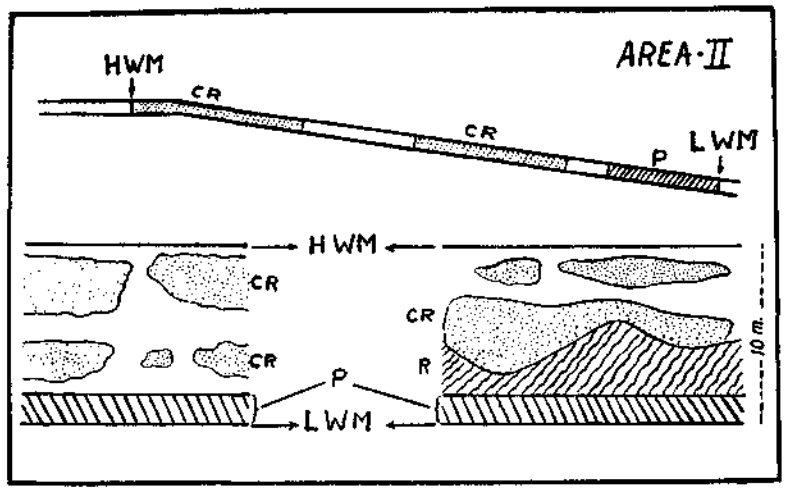

FIG. 3. Schematic section of Area-II showing zonation of shore crabs. (CR—areas 'ploughed' by crabs; P—'gastropod zone' of chiefly *Cerethium* and *Potamidis*; R—washed up sea weeds; HWM—high water mark; LWM—low water mark).

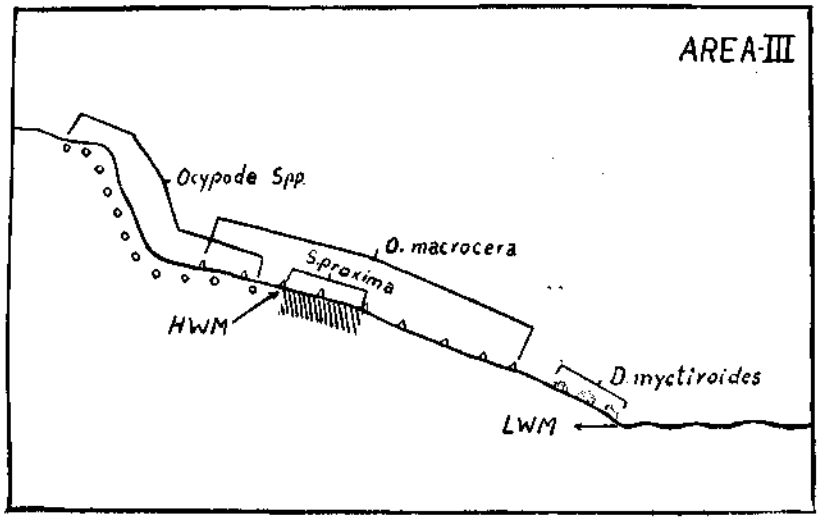

FIG. 4. Schematic section of Area-III showing zonation of shore crabs.

Area-III (Fig. 4) resembles Area-I except that the beach slopes steeply as a result of which the intertidal zone is narrower. Another notable difference is the occurrence of *O. macrocera* in larger numbers even extending to well below the HWM where they are generally found making mudballs, etc.

Occurrence of Species of *Scopimera* in Relation to Tide

It was observed that generally *Scopimera proxima* has its burrows closer to the HWM than to the LWM. Sample counts were made as the one given below to find out the frequency of occurrence of burrows at different levels in the inter-tidal zone. For instance in Area-I two test plots 4 meters square were chosen at random as soon as the tide had receded. The exposed intertidal zone at the time was 4 meters wide in this area. Each plot was further subdivided into two as shown in Fig. 5 and observations were made at intervals between 10·30 and 14·00 hours on

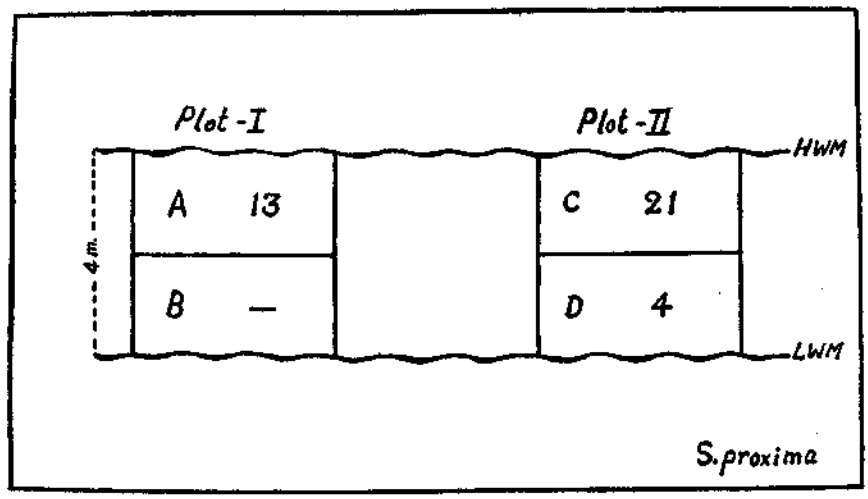

Fig. 5. Test plots in Area-I indicating occurrence of burrows of *Scopimera proxima* more towards HWM than LWM.

11–9–1959. The total number of burrows in the different squares are given in the figure and the frequency of the appearance of the burrows were as follows:

Plot numbers				Time (hr.)
I			II	
A	...	C	..	10·30
B	—	D	...	10·30
A	1	C	14	11·45
B	—	D	1	11·45
A	4	C	17	12·25
B	...	D	4	12·25
A	13	C	21	14·00
B	...	D	Burrows plugged	14·00

By about 14·00 hours the rising tide had caused the crabs in square C of plot II to plug their burrow entrances and a similar action by the crabs of squares A and C of plots I and II was noticed as the water approached the HWM by about 14·00 hours. These observations lead us to deduce that:

1. *S. proxima* has its burrows in a definite zone having a width of about 1·5 meters from HWM. This may also depend on the slope of the beach as the zone occupied by the crabs will be relatively narrow when the slope is greater (as in Area-III) or extended when the slope is very gentle.

2. The surface activity of the crab is restricted to a period of about 3 to 4 hours from the time of setting in of low tide to high tide.

3. The feeding habits appear to influence the zonation in this species.

In the case of *S. pilula* occurring in Area-II a difference was noticed in the zonation as well as the frequency of occurrence in relation to HWM and LWM. Briefly stated this is as follows: The species occupies a fairly wide zone between HWM and LWM separated from the latter only by the gastropod zone. Within this area of occurrence, it was found to be more abundant closer to the LWM as shown in Fig. 6.

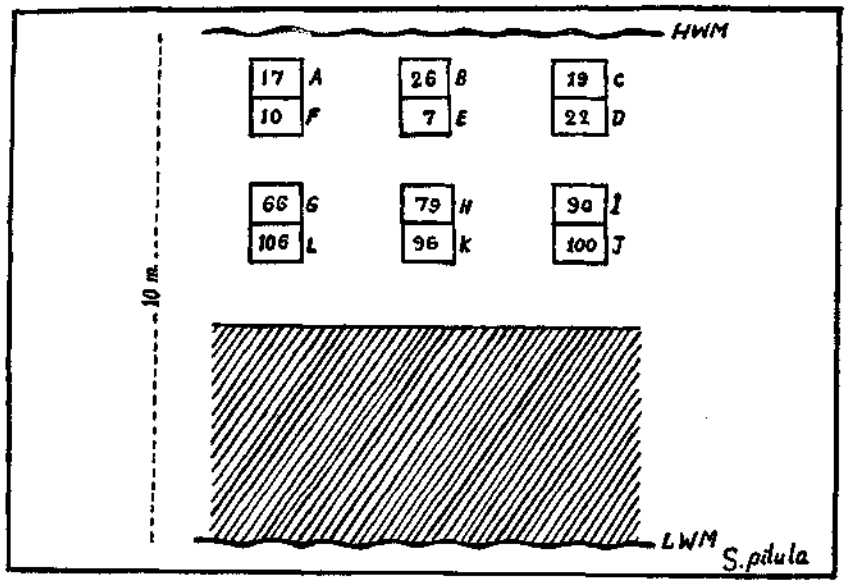

FIG. 6. Test plots in Area-II indicating occurrence of burrows of *Scopimera pilula* in relation to HWM and LWM. Area shown in hatching indicates 'Gastropod zone'.

BURROWING HABITS OF SHORE CRABS

Scopimera proxima.—Adult crabs were not generally found to wander about at the surface seeking new locations for burrowing, the exception being males seeking females. Within a short time after the water recedes, openings of burrows were seen with one or two pellets outside the entrance. Vertical sectioning of such burrows indicated their extension to even 100 mm. suggesting that the burrows were not freshly made. Normally the burrows have a 'neck' about one-fourth their length followed by a slightly wider 'chamber' where the crab rests. This is followed by a narrow tube going vertically downwards. The burrows do not have secondary escape routes (Fig. 7).

When settling on the beach, very small crabs occupied areas in the *S. proxima* 'zone' nearer to LWM. This would mean that there may be a certain amount of shifting of burrow sites with growth, the larger crabs occurring closer to HWM.

Scopimera pilula.—The burrow in this species is slightly different from that of *S. proxima*, being deeper, having a longer chamber and not generally vertically descending downwards below the level of the chamber. A typical burrow of 180 mm. shows the following dimensions: Length of 'neck' 55 mm.; diameter of surface opening 7·0 mm.; diameter at base of 'neck' 10 mm.; length of 'chamber' 50 mm.; diameter at widest part of chamber 18 mm.; diameter at base of 'chamber' 12 mm.; length of tube below 'chamber' 75 mm. No lining was noticed in any part of the burrow (Fig. 7).

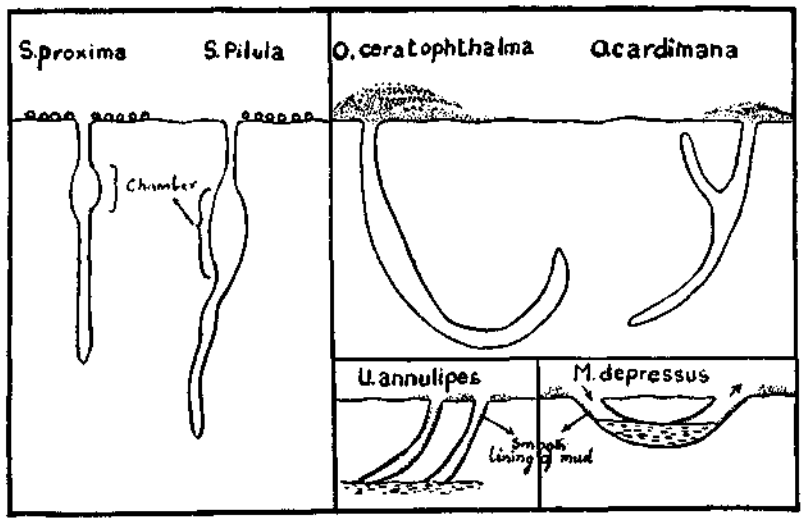

FIG. 7. Typical burrows of shore crabs in the areas investigated.

Uca annulipes.—The burrow typically descends almost vertically for about one-third its length and then slants and goes obliquely downwards to the level of the water. The burrow wall is lined by a thin layer of clay (Fig. 7).

Macrophthalmus depressus.—In the mudflats in Area-II large burrows, each with also an exit opening of the same size as the inward opening were seen, and this species was found to inhabit such burrows. The shallow loop of the burrow is always found to have water in it and the walls of the burrow above this (inward and outward routes) are lined with mud (Fig. 7).

Ocypode ceratophthalma.—The burrow is deep often exceeding a depth of even a meter from the surface, as the burrows of the larger crabs are situated further away from HWM. The burrow usually descends vertically for some distance and then runs obliquely downwards. In one case a slight U-shaped bend was noticed at the lower end but there are no indications of secondary escape routes. The burrow opens out at an angle, and will have heaps of sand at the entrance dug up and brought from below in addition to the wet sand brought up as mud balls and discarded away from the entrance in one direction.

Ocypode cordimana(?).—The burrow of this species (?) has a striking resemblance to that of *O. arenaria* described by Cowles (1908), as shown in Fig. 7. The notable feature is that the burrow has a secondary escape route reaching to below the surface giving the burrow a Y-shaped appearance.

Closing of burrows.—As the tide comes up towards the HWM many of the burrows were found to have large wet pellets or mudballs covering the entrances. This was specially noticeable in the burrows of larger crabs and occurring progressively from LWM to HWM in relation to the

incoming water. A typical instance of plugging of the entrance of the burrow by *Scopimera proxima* was observed as follows: In a burrow about 0·75 m. away from water, we noticed a crab come out and move about the entrance and cover a distance of about 5 cm. from the entrance and on the way making about four or five pellets. Just as it was making a fresh pellet, a wave brought the water to about 10 cm. from the entrance of the burrow, and immediately the crab scampered to the burrow drawing behind it a few pellets which were close to the entrance with its right walking legs, the pellets rolling into the entrance of the burrow at the same time, and thus effectively plugging the opening. The action of drawng in the pellets by the crab was intentional, and not accidental, and the movement was such that the pellets did not roll in one after the other which would have only resulted in their falling straight into the burrow.

Temperature inside the burrows.—In Area-II temperature inside the burrows of *S. pilula* at 5 cm. below surface at 10·40 hours (on 9–9–1959) was found to be 28·5° C. while the sea-water temperature (Surface) opposite the burrows had risen to 33·0° C. [The shallowness of the water beyond the LWM (about 0·5 m.) is responsible for the higher water temperature].

The temperature recorded at 09·00 hours in Area-I from a burrow 5 m. away from LWM (of *Ocypode* sp.) was 28° C. at a depth of 220 mm. from the surface and 30·5° C. at a depth of 50 mm. below surface. At this time the surface sea-water temperature was 28·5° C.

In the same area on 11–9–1959 temperature readings were taken between 08·00 and 10·45 hours in burrows of ocypodids at 10 cm. below surface, and these are given below:

Diameter of burrow (mm.)	Temperature at 10 cm. depth °C.	Time hr.
15	30·5	09·45
20	29·5	09·50
20	29·5	10·00
10	29·25	10·00
15	30·0	10·05
9	30·0	10·05

The surface water temperature beyond LWM at 10·45 hours was 29° C.

On the same day, in one burrow 15 mm. in diameter, the temperature at 09·50 and 10·55 hours were 30·5° C. and 31·0° C. respectively when the surface water temperatures were 29·0° C. and 29·5° C. In view of the fact that burrows of ocypodids were very deep and not vertical it was not possible to take the temperatures at greater depths.

Observations made at night.—Observations were made at various times during the night to note the habits of the shore crabs, especially *Scopimera proxima*. Species of *Ocypode* were found invariably to be active foraging on the beach, especially during the earlier part of the night. The burrows of *S. proxima* were found with the entrances plugged with pellets and the crabs not seen at the surface. This inactivity of the crab at the surface was noticed at night when the level of water was far away from the HWM. However, the species was seen to be active as early as 05·00 hours in the morning. It was not possible to see the conditions in Area-II during night time.

Reactions of *Scopimera proxima* Towards External Disturbances

1. In Area-I large quantities of sea grass (*Cymodocea* sp.) are generally washed ashore and in the afternoon and evening when there is a steady sea to land breeze, bits of grass are blown over the sand. We found that when this happened for the first few times *S. proxima* scuttled into the burrow when the weeds were blown close to it or over the burrow. However, watching individuals for some time, we noticed that the crabs once they were used to the weeds being carried by the wind did not run into their burrows when disturbed thus. However, if they were working in one of the paths, they showed momentary alertness when a bit of the grass or weed was blown closeby. When there were very strong breeze, especially in the evenings, very few crabs were seen to come out of the burrows to make pellets. So also when there were heavy downpours of rain, the crabs were not seen outside and the pellets disintegrate and after the rains the crabs are active.

2. The crab was not seen to evince any reaction when sunlight was reflected with a mirror when it was normally working in a path.

3. We tried to see whether the crab would show any reaction to its image reflected from a mirror placed close to its burrow. The animal working on a path parallel to the mirror was not seen to show any reactions to the image as well as to its own movements reflected by the mirror.

4. In order to see whether the crab reacted to sound or vibrations, an alarm clock was set repeatedly at various distances from 15 cm. from the burrow to 200 cm. from the burrow, and also placed in a pit below the surface about 20 cm. away from the burrow when the crab was normally working in a path. Prior to this the crab's activities of visiting the burrow was watched for some time and the alarm was adjusted when the crab visited the burrow and set to go off when it was on a path. No reactions were noticed.

These experiments carried out in the field are of a very preliminary nature and could not be repeated several times to arrive at conclusive results.

Natural Enemies of *Scopimera proxima* and Other Shore Crabs

Altevogt (1959) records three birds as the natural enemies of the fiddler crabs at Bombay, namely the Indian house crow *Corvus splendens*, the paddybird *Ardeola grayii* and the whimbrel *Numenius phaeopus*. The following birds were found to be common on the mudflats and shores in Area-II: the curlew *Numenius arquata orientalis*, the spotted sandpiper *Tringa glareola*, the redshank *Tringa totanus*, the common sandpiper *Actitis hypoleucos*, the little ringed plover *Charadrius dubius*, the paddybird *Ardiola grayii* and the little egret *Egretta garzetta*. All these birds except the first mentioned have been seen to chase and peck at objects in the ploughed up areas in the intertidal zone where more than one species of crab occurs. It is likely that some were at least feeding on crabs. The spotted sandpiper and the common sandpiper were most commonly seen in Areas-I and II. Solitary individuals of both these species are found moving about in the intertidal zone and the beach above HWM and they were found to be active even at night. Only once did we find chelipeds of male fiddler crabs in a spot where a little egret was seen chasing and pecking at objects in the 'ploughed' intertidal zone in Area-II which indicated that the bird had been preying on these crabs.

On Pellet making by *Scopimera proxima*

Observation was made from the time the crabs started coming to the surface for feeding to find out the quantity of sand worked into pellets. The crab scrapes the sand at the surface with its chelae and brings it to the mouth where the specialized setae of the second maxilliped may help it to sort out the interstitial fauna and reject the sand which appears as a wet ball (covered copi-

TABLE I

Table showing details of size of crabs, pellets, etc., of Scopimera proxima*

No. of crabs in burrow	..	1	1	1	1	1	1	1	1
Sex	..	..	M	M	..	..	M	F	F
Length of carapace	..	3·8	2·9	3·2	..	..	2·8	4·0	3·7
Width of carapace	..	5·9	4·5	5·0	..	..	4·6	5·5	5·8
Diameter of burrow at surface	..	5·3	5·0	5·0	5·8	4·7	..	5·0	5·0
Depth of burrow	..	60	..	..	..	..	100	160	124
Number of paths	..	3	5	6	8	4	4	2	1
Combined length of paths	..	180	320	410	730	510	228	90	100
Wet weight of 10 pellets (gm.)	..	..	0·170	0·220	0·200	0·191	0·095	0·320	0·270
Wet weight of 20 pellets (gm.)	..	..	0·353	0·408	0·390	0·365	0·195	0·658	0·490
Total wet weight of all pellets	..	..	8·688	17·030	14·425	22·645	9·107	25·150	5·190
Dry weight of all pellets	..	..	8·390	16·450	14·215	22·495	8·975	..	4·980
Diameter of largest pellet	..	3·0	3·0	3·2	2·9	2·7	2·4	4·0	3·0
Diameter of smallest pellet	..	2·4	2·7	3·00	2·7	2·5	1·9	3·5	2·7
Duration taken for making total No. of pellets (minutes)	..	..	250	255	265	255	270	200	65

* All measurements in millimeters and weights in grams.

ously with water). On completion of the pellet the water is 'sucked' in and the pellet is knocked off by a sharp movement of the right chela. The information given in Table I would give some idea of the activity of the crab. To this may also be added the following data on the number of pellets made by adults during specified time intervals (Table II). This was found to vary from

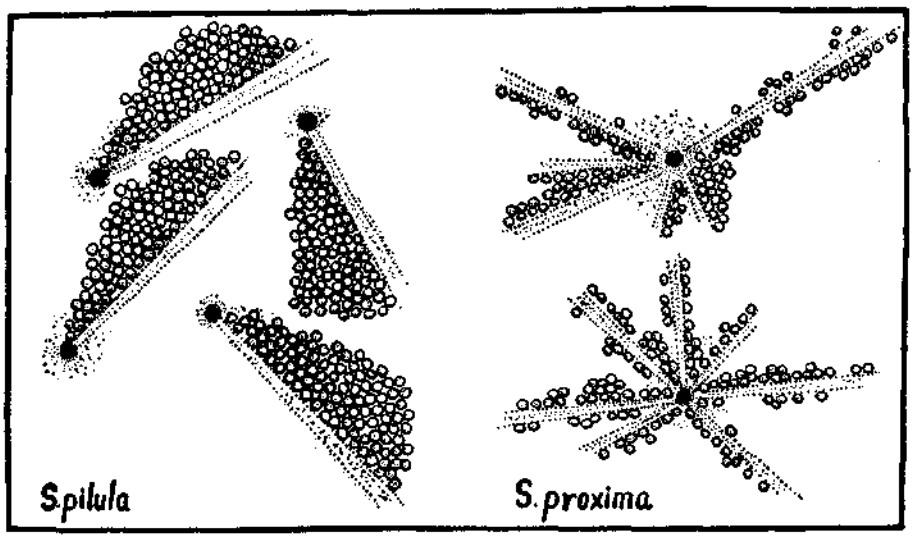

FIG. 8, Pattern of paths and arrangement of pellets in two species of *Scopimera*, *S. proxima*, and *S. pilula*.

specimen to specimen depending also on the size of the crab, the visits it made to the burrow, the periods it remained quite at the burrow entrance, etc. It was not possible to record and time all these activities for correlation with the number of pellets made.

TABLE II

No.	Time (hr.)	No. of pellets	L/W of carapace of crab (mm.)	Weight of crab (gm.)	No. of paths	Length of each path *plus* total length of paths
1	10·50	38	2·9/4·5	0·021	1	70 mm.
	12·45	230			4	45, 40, 90, 40 = 215
	14·30	494			5	50, 50, 90, 50, 80 = 320
2	10·52	81	3·2/5·0	0·023	2	25, 40 = 65
	12·45	(Disturbed			2	90, 40 = 130
	14·35	783			6	90, 70, 60, 110, 40, 40 = 410
3	10·55	37	Crab not collected	..	1	65
	12·50	350			3	111, 120, 30 = 261
	14·45	759			8	120, 120, 100, 80, 40, 100, 110, 60 = 730
4	11·22	47	..	..	1	22
	14·50	*ab*1,500			4	80, 110, 100, 120 = 410
5	11·30	208	2·8/4·6	0·020	1	60
	14·45	997			4	70, 50, 50, 58 = 228
6	11·34	13	..	..	..	..
	12·40	322	..	..	4	40, 40, 70, 80=230
7	11·35	14	4·0/5·5	0·025	..	..
	12·30	206			2	40, 50 = 90
	14·20	785				(Paths not distinct)

The above observations were taken on 11–9–1959 from Area-I. The average time taken for making a pellet was found to be 4 seconds. The longest single path measured at any time was 410 mm. and at this burrow there was only one additional short path of 40 mm. However, the average lengths of path was found to be about 75 mm. The width of the path depended on the size of the crab, being as wide as the carapace *cum* the space covered by the walking legs.

The pellets made by the same individual were found to vary slightly in diameter even in the fresh condition. Desiccation of pellets exposed for longer periods may also result in very slight changes in the diameter. During the period when mating was noticed, it was found that the males (generally smaller) working in the same paths as the female (generally larger) made smaller pellets.

In the case of *S. pilula* which is a larger species being about double the size of a normal adult *S. proxima*, the pellet size was also consequently larger. The average diameter of the pellets for a crab with carapace length and width of 5·7 and 7·0 mm. respectively was 6·5 mm. This species also differs from *S. proxima* in the habit of working the path as well as the disposition of the pellets which are cast off. Typically it has only a short broad path and the pellets are cast off on one side, so much so the pellets appear to be arranged in a triangular area with the path bordering one side. In addition to the path, which on account of the size of the species is broader and slightly deeper, often the space covered by the discarded pellets was also seen to be scraped. This species has a

tendency to wander even beyond the path, but when disturbed scuttles back along the path to the burrow. Figure 8 shows schematic drawings of the types of paths made by these two species and the arrangement of pellets. The second maxillipeds and the spatulate setae help in pellet making. The spatulate setae (Fig. 9) are relatively longer and well developed in *S. pilula*, apparently as the surface sand grains in areas inhabited by this species are much finer than that seen in areas where *S. proxima* abounds. Typical sizes of the surface sand grains seen from pellets made by the two species are shown in Fig. 10.

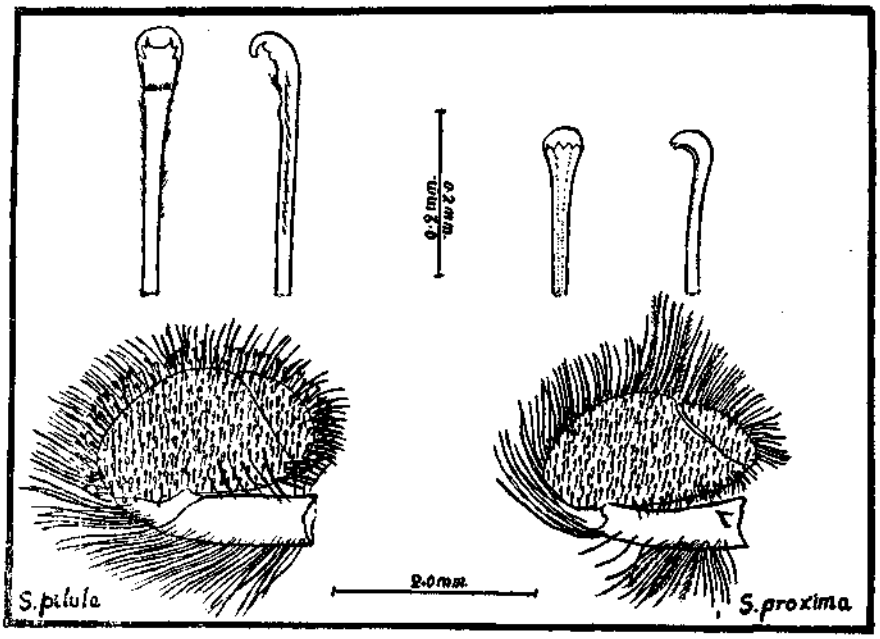

FIG. 9. Second maxilliped and spatulate setae (spoon-shaped) in *S. pilula* and *S. proxima*, of carapace size L/W 4·7/6·0 mm. and 4·3/5·8 mm. respectively.

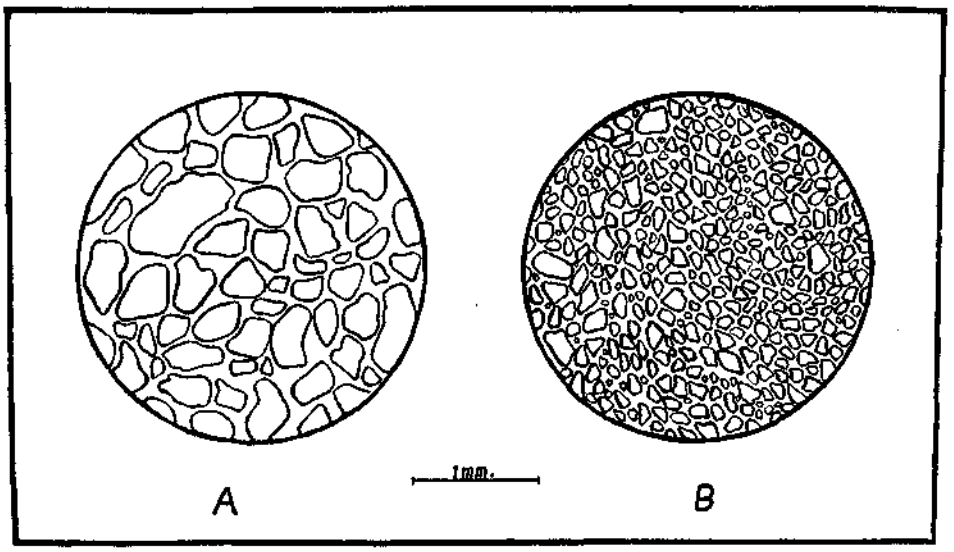

FIG. 10. Surface sand grains in Areas-I and II inhabited by (*A*) *S. proxima* and (*B*) *S. pilula* from pellets made by the two species.

MATING BEHAVIOUR OF *Scopimera proxima*

Information pertaining to direct observation on mating of shore crabs from the Indian Region is very meagre, practically nothing has been known but for the observations of Altevogt (1955, 1959) on the fiddler crabs of the genus *Uca* (*U. annulipes, U. marionis* and *U. triangularis*). Altevogt (1959) remarks that "···nobody has so far seen any copulation in the Indian species, the total number of fiddler copulation seen in the field amounting to a meagre five witnessed in the Americas by Miss J. Crane (1941–44)." We have been able to make some observations on the mating behaviour and copulation in *Scopimera proxima* on a few occasions between 19–9–1959 and 4–10–1959. These have helped in elucidating some problems relating to: the mating duration including approaching and leaving; number of times copulation is effected by the male during each active phase in relation to the tide; actual duration of copulation; successful and unsuccessful copulations; intervals between copulations; wandering habits of male in seeking female; burrowing habits of male during this period, etc. The instances and the conditions observed are given below datewise. The various activities noted were recorded with a stopwatch, and notes taken in the field. These are given below.

1. *On* 11–9–1959 *at* 10·00 *hours in Area-I.*—For the first time we found two crabs entering into the same burrow. At the time it was not known that each burrow was occupied only by a single animal. Collections made between 11–9–1959 and 19–9–1959 confirmed this and indicated the possibility that the two crabs at one burrow may indicate that they were male and female.

2. *On* 19–9–1959 *at* 10·05 hours in *Area-I.*—Two crabs were found disappearing into one burrow. There were no burrows atleast for a distance of about 30 cm. from this. The smaller of the two crabs collected and identified as male and indicated here as A came out and remained outside the burrow in a path which had just been begun, while the larger crab identified as female and indicated here as B often came upto the burrow entrance, but at the slightest disturbance of our movement darted back into the burrow. On the path close to the entrance A remained unconcerned. After sometime B came just out of the burrow entrance with its right side foremost and its legs almost extending up to the legs of the right side of A. They were found to keep still in this position for 14 seconds after which A approached B which slightly withdrew to the entrance of the burrow until A moved directly opposite it and pressed against the flexed abdomen of the female and started copulation. B was found to take a stance slightly inclined over A. They remained in this position for exactly 1 m. 40 sec. after which with a quick backward movement they separated and both were active in a path, B making a few pellets. After 3 minutes B which was about 5 cm. away from A approached the latter. A became alert and immediately moved to the side of B and tried effecting copulation which lasted for only 4 seconds, the partners then separating. After an interval of about 3 minutes A was found trying to excavate a burrow itself 3 cm. away from burrow of B at the end of a short path made by the latter. For some reason or other it gave up its efforts after a few digging movements for a few seconds and moved to a spot 5 cm. from the burrow of B and started again to excavate its own burrow. In the meantime B was found to be normally active, entering its burrow and coming up at intervals to make pellets. At 10·12 hours B was seen to start a new path leading directly towards the new burrow of A where the latter was busy bringing up excavated sand, to the mouth of the burrow and had not started making its own path or pellets. At 10·15 hours B approached the entrance of A and when the latter went in followed it into the burrow, but immediately reappeared at the entrance. A also came out of the burrow and after a few moments made a quick sidewise movement with chelipeds extended towards B and tried effecting copulation which lasted for 4 seconds when a piece of wind blown sea grass disturbed them, separating the two, each darting back to its own burrow. After this B resumed pellet making and frequently entering its burrow, while A was busy excavating its own burrow. The pair was watched during the next 30 minutes, but did not come together.

A few points of interest are that before the first mating (unsuccessful) took place, A was not disturbed by slight external movements, but only two or three times when there was some marked movement in the surroundings by our shadow falling across the burrow entrance did it follow

B into the latters burrow. The surroundings of the entrance of the burrow of B did not have any loose sand in heaps as was seen in the case of the new burrows started by A. From this and other such observations it is deduced that the presence of such loose sand at the entrance of burrows may indicate that such burrows have been freshly dug from the surface and does not represent plugged old burrows from which the crabs have come out. It may also be stated that a few of the latter type of burrows had the pellets around the burrow entrance loosened by amphipods, giving the false appearance of fresh burrows made from the surface. But by experience these could be easily identified.

During mating, A was slightly supine and exhibited slight movement of both chelipeds, at the same time trying to right itself by its walking legs pressed against the sides of B. The latter remained passive except for some slight movement of the dactyli of the first walking leg of one side.

Some of the questions that come up are, whether the pair had copulated prior to 10·05 hours when they were first spotted by us; whether closely adjacent burrows of one larger than the other usually placed about 5 cm. apart are those of male and female; whether two sizes of pellets seen in the path of a burrow could have been made by the female as well as a visiting male, one of which may be a smaller animal and consequently make smaller pellets; and whether the occurrence of such mixed pellets could indicate easily the mating period of the crabs.

3. *On* 19-9-1959 *at* 11·22 hours *at Area-I.*—At 11·22 hours in a burrow with eight paths, the longest being 12 cm. a crab later found to be a female[5] and hence indicated here as B was found to be normally active. Approaching the burrow from about half a metre away was found a second crab caught and identified later as a male and indicated here as A. When A approached B along one of the paths, the latter darted into its burrow. Still on one of the paths about 10 cm. from the burrow entrance of B, the former showed markedly excited behaviour and rapidly approached the burrow resulting in the pushing in of a few pellets, which were in turn brought up to the surface by it. In the present case A was larger than B and this helped to keep track of the movements of both the specimens; the length and width of carapace of A and B being 4·7 and 6·0 and 4·3 and 5·8 mm. respectively.

Between 11·20 and 11·27 hours A came upto the surface of the burrow three times bringing up excavated sand, while B was never seen to come up. At 11·30 hours A once again came upto the burrow entrance bringing up some excavated sand and remained at the entrance for 12 seconds. Between 11·30 and 11·40 hours A made three more visits to the burrow entrance each time remaining for a few seconds shoveing a little bit of sand away from the entrance of the burrow. Between 11·35 and 11·40 hours A made frequent visits at almost regular intervals to the surface of the burrow each time again bringing up a little bit of sand and also smoothening the sides of the burrow entrance. The time it remained at the burrow entrance each time it came up during the last five minutes was noted to be roughly 5 seconds each, and equal time was spent by it inside the burrow. During all this time, B never came up to the surface. It is unlikely that copulation could have taken place inside the burrow in the 'chamber', as A was coming up and going down at such regular intervals. It is also not known whether this action on the part of the male was a type of sexual display in order to excite the female. Between 11·40 and 11·45 hours A made four quick visits to the burrow opening returning immediately (not staying each time for even 2 seconds at the entrance) as though beckoning B to come up to the surface. At 11·45 hours A came out of the burrow followed by B and for 14 seconds both animals were found exhibiting an offensive defensive display which made us wonder whether B was trying to drive away A or *vice versa* or whether they were of the same sex. After this display, which appeared to be more in the nature of a struggle, each holding the chela of the other the animals without loosing their holds moved a few millimeters to one side. In this position they remained without showing any movements for 40 seconds after which quickly closing up copulation was effected. This lasted for exactly 1 minute

[5] Secondary female.

and 42 seconds after which with a jerky movement both the animals separated, A working on the path and B remaining near the entrance of the burrow. After an interval of 45 seconds the animals once again effected copulation without any preliminaries and were together for exactly 3 minutes and 10 seconds. During the copulatory phase, twice the animals were observed to show slight side to side swaying movement and more often an attempt by A to press down on B. But for this movement, appendages (chelipeds and legs) showed no movements. Soon after this second copulation A moved to the end of the path about 10 cm. from the entrance of the burrow and after making three pellets was seen moving off and was captured ¾ meter away from the burrow of B. The latter was found to resume normal activity of making pellets and excavating the sand from the burrow. This specimen was also later caught and preserved, and it turned out to be a secondary female showing the narrow abdomen.

4. *On* 20–9–1959 *at* 11·09 *hours in Area*-I.—At 11·09 hours at one burrow with none others in the immediate vicinity a medium sized crab was seen outside the burrow entrance (later identified as male and indicated here as A) while a larger crab (female indicated here as B) was seen entering the burrow and coming out. Both crabs were moving the chelipeds and maxillipeds as during pellet making for seven seconds when A approached B which in turn darted into the burrow and emerged after 25 seconds. Soon A left the burrow of B and wandered to about 50 cm. away to another burrow and rolled a few pellets into this burrow from near the opening and waited a few moments. At 11·17 hours it moved on to another burrow nearby after taking a circuitous route. No crab was seen at the entrance of this third burrow to be visited by A. Entering a path, A went into the burrow and after a few seconds emerged followed by another crab which had been inside the burrow (female and designated here as C). A and C entered the burrow once again and immediately came out. A was completely out, while the left side of C was out, the legs almost touching A. For exactly 1 minute and 20 seconds they remained still in this position and then A made a slight movement with its first walking leg touching C which immediately darted into the burrow. Soon A and C changed positions, A going to the opposite side of the burrow and C coming up from the burrow to the position occupied previously by A. In this position they were for 20 seconds when both the animals come together and copulation was effected. When this lasted for 50 seconds, the animals got disturbed by outside movements. C was seen entering the burrow and coming out at regular intervals and each time it came up it was moving its chelipeds as though trying to clean them, probably rubbing off adhering sand. All this while A was seen to show no marked movements, but everytime part of the body of C showed outside the burrow entrance it became alert. At 11·20 hours soon after C disappeared into the burrow, A followed suit, but emerged 16 seconds later followed by C which seemed extremely agitated as it was darting in and out of the burrow at very short intervals. A again tried to approach C following it into the burrow but again came out after 15 seconds followed immediately by C. A was then seen touching the legs of C with the hind two dactyli and both animals remained still for 14 seconds, soon after which C again darted into the burrow. Every time C came up to the burrow entrance A made a slight movement towards it and at 11·27 hours copulation was effected without any further display or preliminaries. For the first 40 seconds both crabs were jogging for position holding on to each others chelae, C eventually occupying a slightly inclined position over A. After coming to this attitude both animals were completely still. After 4 minutes 45 seconds slight movements were noticed and then the animals were still and 6 minutes from the commencement of the copulation again slight movements were noticed the animals separating after 6 minutes and 35 seconds of being together. Soon after separating, C was poised threateningly over A and all of a sudden it resumed normal poise and activity of cleaning the chelipeds and making pellets. After a few moments A darted towards C which became alert and moved towards A which in turn ran 6 cm. away from C. Latter entered the burrow and A once again approached the burrow entrance, but when C appeared at the burrow entrance A left along one path and started digging about 10 cm. away from burrow of C. The characteristic sand mount appeared as the burrow was dug from the surface. The distance travelled by A from burrow of B–C was 630 cm. and enroute it passed close to three burrows which it did not visit, except at the first of these burrows it stopped at a path and moved two or three pellets with the chelipeds as though 'tasting' them. Enroute it also stopped at the side of small stones, shells and weeds for short periods. By 11·42 hours A had started making pellets having completed its burrow.

5. *On* 21-9-1959 *at* 10·00 *hours in Area-I.*—Photographs of male and female at burrrow prior to copulation and male digging burrow from surface were taken.

6. *On* 4-10-1959 *at* 10·00 *hours onwards in Area-*I.—A small crab (male indicated here as A) was seen clearing a path near burrow entrance littered with pellets. A left the burrow and travelled one metre reaching the burrow of another bigger crab (female indicated here as B) which was staying outside the burrow entrance. Without any preliminaries both the crabs were seen to copulate and the duration of the copulation was 5 minutes and 50 seconds. During this period, for the first 20 seconds both crabs were making adjusting movements, the walking legs of A pressing against the sides of B and during the next 20 seconds the crabs showed slight rocking movements from side to side. After 3 minutes and 15 seconds further side to side rocking movements were noticed for 15 seconds after which the crabs were still, and at 5 minutes 20 seconds both crabs showed slight movement of the legs, for 30 seconds when they separated. After this B entered the burrow twice and came up. A tried to dig its burrow about 3 cm. from burrow of B in the midst of pellets made by the latter. A was seen thrusting sand towards the burrow of B. Both assumed an aggressive stance with extended chelipeds for 3 seconds, the female as though guarding its burrow entrance. A made a detour to opposite side of burrow of B and approached the latter. No attempt was made to copulate and B was in a defensive attitude. A returned to the new burrow it had started and was found digging for 20 seconds and started making a path towards burrow of B. At least once A was seen to push some sand into the brurow of B. When B came out, A was alert at the burrow entrance while the former was trying to clear the entrance. B made a quick movement towards A, touching the latter's legs with its own on which A moved away from the burrow entrance and B returned to its own burrow. After a few seconds B was seen approaching the burrow of A while A was 2 cm. away from its burrow entrance. Both were making pellets and B was seen to raise and lower itself on its legs a few times. After a few moments B started making pellets close to the burrow of A and the latter moved away when B was seen deliberately to push some sand and roll one pellet into the burrow of A. Just then A made a quick movement towards B and the latter raised itself on its limbs with extended chelipeds in an offensive attitude; A also assuming a similar pose, both animals were seen trying to cling on to each other's chelipeds parting with a quick jerky movement after a few seconds. Thence B was seen to deliberately shove sand and pellets into the burrow of A completely covering up the burrow and was seen to walk and turn around the covered burrow. All this time A which was 5 cm. away, started moving away from the area while B was trying to readjust pellets in a new path.

At 10·52 hours A started digging a second burrow about 5 cm. from the burrow of B in another direction. B was seen coming as close as 3 cm. away from this and making pellets while A was actively digging the burrow. B approached A, but when disturbed darted into its own burrow, all the while A undisturbed was digging its burrow. B again approached A's burrow within 2 cm. This was at 10·54 hours. A had started to make its own path, with pellets. It is not known whether the deliberate closing of the partly made burrow of A by B represents territorial behaviour. At 10·59 hours A was busy with the digging of its burrow, while B was resting at the entrance of its burrow. No unusual behaviour was seen on the part of the animals.

At 11·00 hours B walked to about 2 cm. from burrow of A at the same time rolling a few pellets. A was seen going in and out of its new burrow removing sand. A also started making a path with pellets, leading towards B, meeting the path on which B was at one extreme. The movement of A resulted in the rolling of a few pellets on to the path made by B which made the latter to move with lightning speed to this point of intrusion, seeing which A returned (fled) to its burrow. This was noticed happening 5 times in five minutes. After this B started making another path from this point towards the burrow of A, which when reached, it again deliberately shoved some sand into the burrow. When disturbed by our shadow, B ran back to its burrow. At 11·08 hours A tried to roll pellets from the new path made. At 11·10 hours B again approached the burrow of A showing some sand in front at which A left its burrow and moved in an opposite direction for 5 cm. and made 4 pellets before moving on to path of B and approaching the latter tried effecting copulation lasting hardly 5 seconds when the animals separated with a jerkey movement. This was

caused by external disturbance. Both animals returned to their respective burrows, and again at 11·12 hours B was seen approaching the burrow of A and showing sand into it. A coming outside the burrow entrance assumed an offensive pose as B, but after a few seconds both animals returned to their respective burrows.

In summing up it may be stated that in *S. proxima*:

1. The courting males wander seeking the females, but were not seen to enter the burrows of other males.

2. The female, if outside the burrow, evinces little excitement on the approach of the male.

3. When the female is within the burrow, the male enters the burrow without any pause at the opening of the burrow. However, immediately it enters an active phase of ascending to the burrow opening and disappearing within, in quick succession, suggesting courting. The female follows the male to the burrow opening usually after several such 'passes'. While outside the burrow, during the phase of courtship, the male was not seen to nip, nibble or jab at the female with its chelae.

4. Mating took place outside the burrow, at the burrow entrance or in one of the 'paths'.

5. More than one successful mating by the same pair has been observed. At the same time, unsuccessful mating attempts have also been noted when the pairs came in close contact of each other, but one of the pair (male) left.

6. The duration of successful mating (copulation) varies, the maximum duration noted being 6 minutes and 35 seconds.

7. During one tidal phase, it was found that a single male in addition to successfully mating one or more times with a single female, also wandered entering the burrows of other females.

8. Aggressive behaviour of the female towards the male after successful mating was also observed. This was seen when the female deliberately plugged the freshly excavated burrow of the male with which it had mated.

9. Males of *S. proxima* are slightly smaller and lighter in colour than females (the width/length of carapace in the largest male and female seen being 6·1/4·6 mm. and 6·5/4·7 mm. respectively. However, such large specimens are rare). Apparently males predominate as out of 137 specimens collected from the burrows in Area-I, as many as 78 were males, 37 normal females and 12 'secondary' females. It is likely that the lighter body colour of males which merges with the colour of the sand and the higher male sex ratio may be adaptive features as during the breeding season the males wander about and may be subject to predation.

10. At least once a male was found to have a successful mating with a 'secondary' female. Egg bearing 'secondary' females with the characteristically narrow abdomen have also been collected. The presence of such females, first reported by Kemp (1919) in *S. proxima* has not been fully explained and calls for further investigation, as to: (*a*) the effect of parasites (not evident in our specimens); (*b*) whether they are abnormal and if so whether the abnormality is genetic; or (*c*) whether these females begin their development as males.

In conclusion we may say that the pellet crab *Scopimera proxima* is an extremely interesting animal for studies on behaviour.

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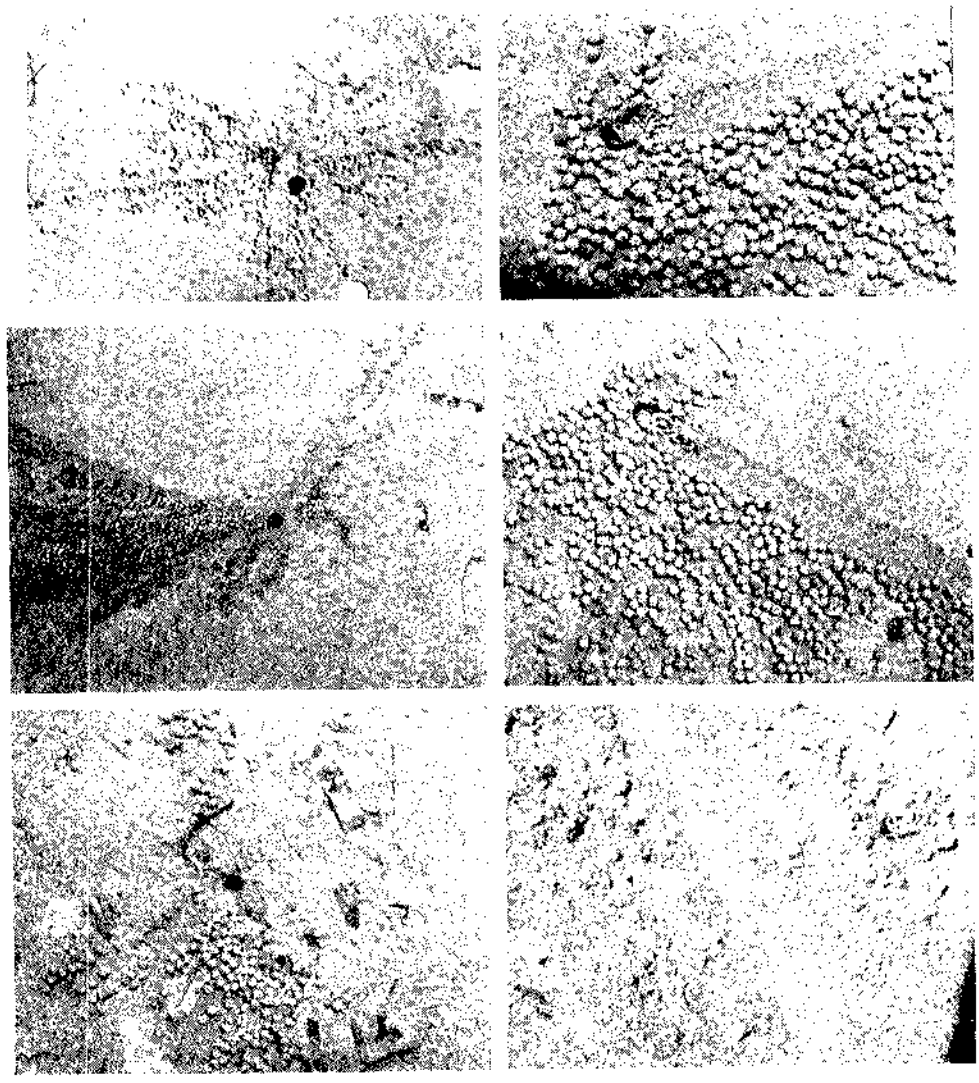

TOP LEFT AND MIDDLE LEFT: Patterns of paths and pellet arrangements in *Scopinera proxima*.
TOP RIGHT: Female *S. proxima* clearing burrow entrance.
MIDDLE RIGHT: Male and female of *S. proxima* at the same burrow.
BOTTOM LEFT: Male *S. proxima* just finishing making a pellet.
BOTTOM RIGHT: Male of *S. proxima* digging a burrow from surface, after mating with female in adjacent burrow.

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FREE AMINO ACIDS IN THE BLOOD OF *OCYPODA PLATYTARSIS* AND *SCYLLA SERRATA*

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ABSTRACT

The paper deals with the quantitative studies of free amino-acids in the blood of *Ocypoda platytarsis* and *Scylla serrata.*

There are a few striking differences between the two species in the free amino-acid content of their blood. In *Ocypoda* the main constituent is tyrosine (0·18 mg./ml. of blood) and in *Scylla,* the group glycine, serine, aspartic acid (0·35 mg./ml. of blood) are the main constituents. Leucine, isoleucine, phenylalanine, valine, methionine and histidine are lacking in *Scylla* but present in *Ocypoda.* In *Scylla,* glycine, serine, aspartic acid are more than twice than in *Ocypoda.* Glutamic acid, threonine is nearly twice in *Scylla. Scylla* has also a lower concentration of tyrosine and alanine.

The findings generally agree with similar observations made by Camien *et al.* (1951) in the lobster *Homarus vulgaris* and the crayfish *Astacus fluviatilis.* It has been suggested that the high concentration of some amino-acids may have a bearing on the animals' ecological habits and may be osmoregulatory.

INTRODUCTION

THE free amino-acid composition of the blood of crustacea is little known. The non-protein amino-acids in the blood have been investigated quantitatively in *Astacus fluviatilis, Homarus vulgaris* and *Eriocheir sinensis* (Camien *et al.*,1951; Florkin, 1960; Mendel, 1904) and qualitative studies on free and protein amino-acids have been carried out in the blood of *Paratelphusa guerini, Panulirus polyphagus* and *Scylla serrata* (Ragnekar, 1955 *a, b*). Apart from these studies we do not have any information about the composition of free amino-acids in the blood of other crustaceans. However, the free amino-acid composition of the whole animal has been investigated in *Penaeus aztecs, Clibanarius vittatus, Pagurus pollicaris* and *Neopanope taxana* (Awapara, 1962). The non-protein amino-acid content of the muscle has been studied in a number of crustacea. (Awapara, 1962; Camien *et al.*, 1951; Duchateau *et al.*, 1959 (cited by Awapara, 1962); Mendel, 1904). Notwithstanding these studies there is need to know the free amino-acid composition of crustacea, particularly in their blood, in relation to their ecological habits.

In the present study, the free amino-acid composition of the blood in the two crabs *Ocypoda platytarsis* and *Scylla serrata* has been investigated, and the constituent amino-acids, quantitatively determined.

MATERIAL AND METHODS

Fresh and live animals were used for the estimation of the amino-acids. *Ocypoda platytarsis* was collected from the seashore and *Scylla serrata* from the estuarine banks at Porto Novo. Blood pooled from 15 animals of *Ocypoda platytarsis* and 12 animals of *Scylla serrata* was used in the study.

The blood was withdrawn from the live animal puncturing the heart through a hypodermic syringe. Care was taken not to mix it with the body fluid of the animal. As coagulation sets in

immediately, the syringe was rinsed very often in a very dilute solution of potassium oxalate. The blood was immediately transferred to a container kept on ice.

For the study of amino-acid composition, the free amino-acid extract was prepared by adding absolute alcohol thrice the volume of blood and centrifuging it. The supernatant was taken and to it was added thrice its volume of chloroform; it was centrifuged and the supernatant was taken for further studies.

Unidimensional and two-dimensional chromatographic analysis was carried out on Whatman No. 1 filter-paper. For unidimensional chromatograms N-Butanol, acetic acid and water mixed in the ratio 24 : 6 : 10 was used and for two-dimensional chromatograms, buffered phenol was used as the second solvent and the N-Butanol-acetic acid-water mixture as the first solvent. Ninhydrin of 0·25% concentration was used as the colour developing reagent.

For quantitative studies of amino-acids, a densitometer was used with a green filter (excepting for imino-acids) and the maximum density method was followed as given by Block and Weiss (1955).

A standard amino-acid solution was prepared as described by Block and Weiss (*op. cit.*). A known volume of it was spotted with a micropipette and a chromatogram was run. The concentration of constituent amino-acids was determined using the densitometer.

The known volumes of free amino-acid extracts were run and chromatograms prepared, and the content of constituent amino-acids determined, taking the known amino-acid concentration as standard. The volumes were calculated in mg./ml. of blood.

Spot tests were also carried out as given by Smith (1960) to determine the constituent amino-acids in cases of uncertainty.

The results are presented below:

Amino-acids	Concentration in mg./ml. of blood	
	Ocypoda platytarsis	*Scylla serrata*
Leucine + isoleucine ..	0·14	..
Phenylalanine ..	0·14	...
Valine + methionine ..	0·09	...
Tyrosine ..	0·18	0·10
Alanine ...	0·08	0·02
Glutamic acid + threonine ..	0·11	0·21
Glycine + serine + aspartic acid ..	0·14	0·35
Histidine ..	0·03	..

Observations and Discussion

It will be observed that there are a few striking differences between the two species in the free amino-acid content of their blood. Leucine, isoleucine, phenylalaline, valine, methionine and

histidine are lacking in *Scylla serrata* but are present in *Ocypoda platytarsis*. In *Scylla*, glycine, serine and aspartic acid are more than twice than in *Ocypoda platytarsis*. Glutamic acid + threonine is nearly twice in *Scylla serrata*. *Scylla serrata* has also a lower concentration of tyrosine and alanine.

Camien *et al.* (1951) found that in the blood serum of lobster, *Homarus vulgaris*, the main constituent of the non-protein amino-acids is glycine, and in the crayfish, *Astacus fluviatilis*, it is glutamic acid. Camien *et al.* (1951), and Mendel (1904) have recorded relatively low concentrations of amino-acids in the blood when compared to muscle amino-acids in the animals they investigated. Camien *et al.* (1951) used amino-acid (non-protein) extract prepared by treating the serum samples with tungstic acid and hydrolysing the samples by refluxing for 24 hours with 6 N HCl.

Most of the studies on the non-protein amino-acids in crustacea have been on their muscle, as already stated. These studies reveal that certain amino-acids like glycine, proline, arginine, glutamic acid and alanine are the most abundant of the amino-acids which are probably important for the regulation of osmotic pressure in their muscle (Awapara, 1962, Duchateau *et al.*, 1959, Camien *et al.*, 1951).

As stated before, excepting for the work of Mendel (1904) and Camien *et al.* (1951) and Rangnekar (1955 *a*, *b*,) we do not seem to have any literature on the amino-acids of the crustacean blood, for purposes of comparison. In the present study it has been found that tyrosine is the main constituent amino-acid in *Ocypoda platytarsis* and glycine, serine, aspartic acid in *Scylla serrata*. Generally speaking, the amino-acid values in the present study compare favourably with the findings of Camien *et al.* (1951).

It is interesting to note the absence of sulphur-containing amino-acids in *Scylla serrata*. Rangnekar (1955 *b*) also did not record any.

It may be that the high concentrations of some amino-acids have a bearing on the ecological habits of the animals, and may be osmoregulatory.

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PROBLEMS IN CRUSTACEAN ENDOCRINOLOGY

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ABSTRACT

Current problems in crustacean endocrinology are considered under the general categories, morphological, physiological and chemical.

Study of neurosecretory cells might profitably follow along lines of descriptive and experimental ultrastructure to furnish information on the synthesis, transport and release of active principles by the neurosecretory neuron. Resolution of the number of active principles in a complex like the X-organ sinus gland system of the eyestalk might be aided by differential centrifugation studies in which separated subcellular granules could be tested for specific biological activities. Immunochemical staining of cell bodies and axons of neurosecretory cells in such a complex might also indicate sites of synthesis. Continued routine histological descriptions of neurosecretory cells in the nervous systems of an ever-increasing variety of crustacean species hold little promise for progress in understanding neurosecretory processes.

Several physiological questions in well-known areas of crustacean endocrinology are still unanswered; little is known of the mechanisms whereby retinal pigments and chromatophores respond to the hormones which activate them; the relation, if any, between molt hormone and insect ecdysone is unclear; to what extent are such metabolic changes as respiration, water metabolism and calcium metabolism dependent upon a specific molt hormone or upon other hormones coming from presently known or as yet unknown glands. Only recently has a beginning been made in the study of larval metamorphosis as influenced by the eyestalk endocrine system.

The multiplicity of physiological effects attributed to hormones from the eyestalk raises the question of number of hormones involved and their physiological specificity. Does one hormone have more than one function? This question can be most readily answered after separation and purification studies have resulted in the availability of chemically homogeneous preparations whose physiological specificity can then be investigated.

CRUSTACEAN endocrinology, in the modern sense, may be considered to have begun in 1928 with the discoveries by Perkins and by Koller that the integumentary chromatophores of prawns were regulated by a blood-borne substance apparently originating in the eyestalks. Not long afterwards, Kleinholz (1936) demonstrated that retinal pigments, which undergo photomechanical movements in response to light and to darkness, may similarly be under hormonal regulation by a principle found in the eyestalk. The discovery (Carlson, 1935, 1936; Abramowitz, 1937) that melanophore response in brachyurans to eyestalk removal and to injection of eyestalk extracts was opposite to that of the erythrophores of macrurans, coupled with observations on the effects of reciprocal injections of eyestalk extracts among several crustacean genera, raised the question of the number of different hormones involved in regulating the various pigmentary effectors. Hanström's (1937) discovery of the sinus gland in the crustacean eyestalk established a morphological basis for what had, up to this point, been almost exclusively a physiological approach to crustacean endocrinology. This, along with the development of surgical techniques permitting removal of the sinus gland with relatively little damage to the rest of the eyestalk, made possible critical deficiency and replacement experiments in physiological tests of a variety of new responses believed to have an endocrine basis (*i.e.*, respiratory metabolism, carbohydrate metabolism, water regulation, molt, and gonadal growth). In turn, results from some of such tests led to the important discovery (Passano, 1953; Bliss and Welsh, 1952; Bliss, Durand and Welsh, 1954) that the sinus gland is the focus of axons of neurosecretory cells located both in the medulla terminalis of the eyestalk and in other ganglia of the central nervous system.

More recently additional regulatory mechanisms, believed to be endocrine in nature, have been described. The discovery of the Y-gland (Gabe, 1956) and its demonstrated role in regulating molt (Echalier, 1959) offer experimental control in studies of the various physiological and biochemical changes found associated closely with the molt process. The pericardial organ, described by Alexandrowicz (1953) and its morphology explored in detail by Maynard (1961, 1962) was shown to contain a cardioaccelerator principle (Alexandrowicz and Carlisle, 1953; Maynard and Welsh, 1959) whose physiological and chemical properties have been also studied (Cooke, 1962; Belamarich, 1963). Charniaux-Cotton's discovery of the androgenic gland in male *Orchesia* (1954) and her description of its role in regulating the differentiation of male primary and secondary sex structures (1957) have done much to clarify what had long been a puzzling problem in crustacean endocrinology. The diabetogenic principle of the eyestalk (Abramowitz *et al.*, 1944) was found to mediate a number of hyperglycemic responses (Kleinholz and Little, 1949; Kleinholz *et al.*, 1950; Scheer and Scheer, 1951). At this time we find crustacean endocrinology to include diverse functions which can be compared with those found among vertebrates and insects. Figure 1 is a diagrammatic summary of many of the presently-known endocrine functions in Crustacea.

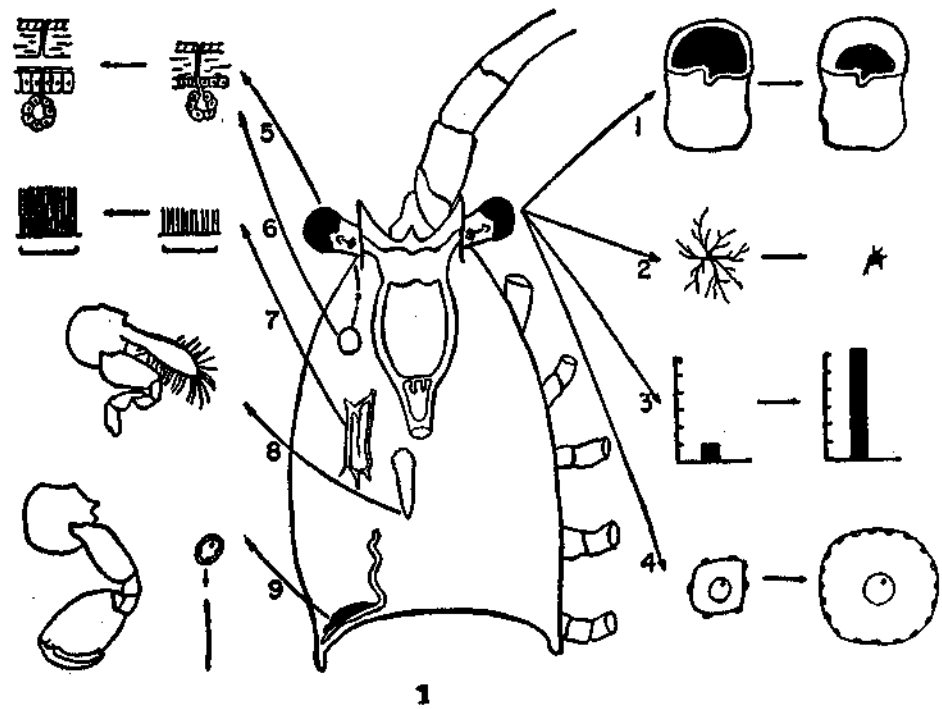


FIG. 1. Summary of hormonal functions in crustaceans. (1) light-adapting distal retinal pigment hormone; (2) chromotophorotropins; (3) hyperglycemic hormone; (4) eyestalk removal results in ovarian growth; (5) molt-inhibiting hormone; (6) molt hormone from the Y-gland; (7) cardio-accelerator hormone from the pericardial organ; (8) ovarian hormone regulating secondary sex characters; (9) androgenic hormone regulating primary and secondary sex characters in the male. (Modified from Kleinholz, 1959)

For purposes of review here, some of the current problems in crustacean endocrinology might conveniently be considered under three general categories: morphological, physiological and chemical.

Morphological Studies

1. *Neurosecretory Structures*

Neurosecretory cells are demonstrable by several histological methods in a substantial number of crustacean species (Scheer, 1960). Many predominantly descriptive studies have indicated the presence of such secretory cells in specific regions of the nervous system. The functional role of particular neurosecretory cells is less well known and might be further indicated by studies seeking correlations between histological changes and experimentally-induced or cyclical physiological states of the animal; the striking demonstration of the part of the Y-gland in the molt cycle (Gabe, 1953, 1956; Echalier, 1959) and the recent studies of Matsumoto (1962) are cases in point.

Progress in understanding neurosecretory processes is desirable and may be better achieved by new approaches to morphology rather than by continuation of routine histological descriptions of the occurrence of neurosecretory cells in an ever-increasing variety of crustacean species. Potter's (1956) cytological and Rehm's (1959) cytochemical studies on the X-organ sinus gland complex of the crustacean eyestalk might be exploited in similar directions, as indeed Durand (1956; 1960) has begun to do. The demonstration (Hodge and Chapman, 1958) by electron microscopy of two types of fine granules in axon terminals constituting the sinus gland and evidence that probably similar granules are bounded by a semi-permeable membrane that can be lysed to release chromatophorotropic hormone (Perez-Gonzalez, 1957) suggest a combination of morphological and physiological studies of such granules in the neurosecretory cells of the X-organ and the axons proceeding from these cells to the sinus gland. Another approach identifying particular hormones with specific cell types, as has been done with the anterior lobe of the pituitary gland, might be possible after eyestalk hormones have been isolated and purified; such preparations which possess a sufficient degree of antigenicity might then permit localization of hormone origin by use of fluorescent antibody methods.

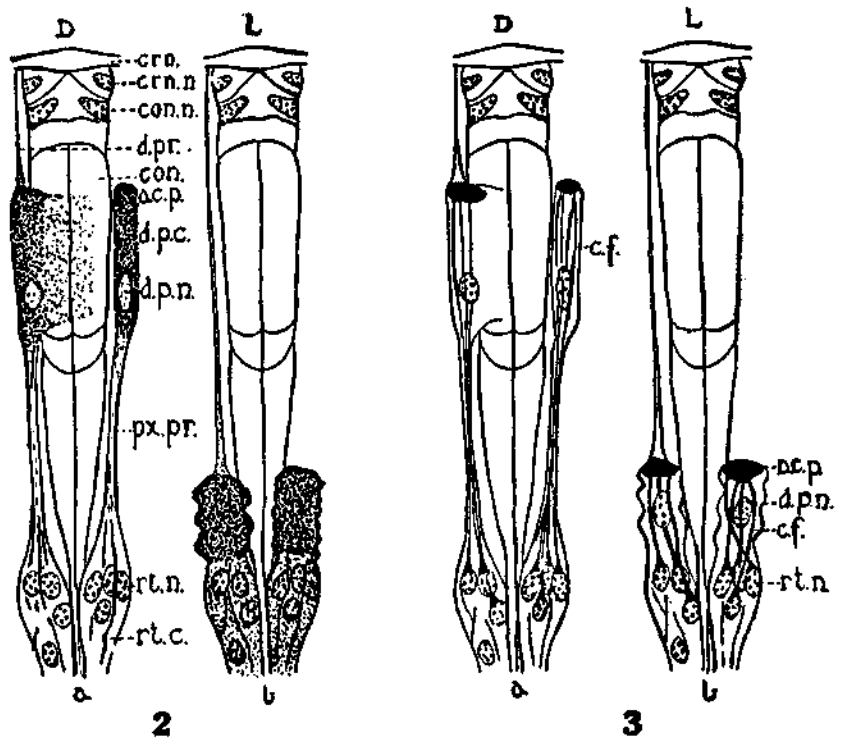
Studies of fine structure with the electron microscope may also be helpful in clarifying a number of obscure areas in neurosecretory activity. The sites of synthesis of such substances within the neuron, and the mechanisms of release of active principles into the circulatory system are not well known at present. Knowles' examination of the pericardial organ (1962) indicates that aggregate bodies containing fine vesicles may represent the droplets reported in axons by other investigators using normal optical and phase microscopy, that such aggregate bodies may arise from mitochondria-like bodies, and that they may represent assemblage or even synthesis of hormone along the course of the axon.

2. *Non-neurosecretory Structures*

Electron microscopy could also give new insights into non-nervous structures. Because the term "androgenic" has been used to name the gland regulating primary and secondary sex characters in Crustacea (Charniaux-Cotton, 1957) some investigators have supposed that the active hormone is steroid in nature. Few chemical studies of the chemical nature of the sex hormone have been published, but Charniaux-Cotton (personal communication) has found no hormonal activity in lipid-solvent extracts of androgenic glands. King (1964), in fact, has recently shown that the ultrastructure of the androgenic gland resembles that of protein-producing vertebrate cells rather than that of steroid-secreting cells. The cells of the androgenic gland possess well-developed granular endoplasmic reticulum and long mitochondria with flat transverse cristae; they lack electron-dense lamellae in the Golgi centers, and, surprisingly lack formed secretory granules.

So far as I know, there is no published account of the cytology of crustacean pigmentary effectors. Hypotheses as to the mechanisms by which movements of pigments within crustacean chromatophores and retinal cells are effected in the responses to backgrounds and to light and to darkness are largely speculative, save for the study of Welsh (1930). This author reported the presence of what appear to be contractile fibrils (Figs. 2 and 3) which originate near the nuclei of the retinular

cells and pass through the distal retinal pigment cells to terminate distally near the caps of accessory reflecting pigment. The appearance of such fibrils in the dark-adapted and light-adapted distal retinal pigment cells indicates an active role in the adaptive responses of these effectors. Examination of the ultrastructural characteristics of such intracellular fibrils for cytological features associated with contractile elements should be interesting.



FIGS. 2–3. Fig. 2. Ommatidia of *Palaemonetes*, (*a*) in the dark-adapted position; (*b*) in the light-adapted position. *ac.* P., accessory pigment; *con.*, cone; *con. n.*, nuclei of cone cells; *crn.*, cornea; *crn. n.*, nuclei of corneal hypodermal cells; *d.p.c.*, distal pigment cell; *d.p.n.*, nucleus of distal pigment cell; *d. pr.*, distal process of distal pigment cells; P*x. pr.*, proximal process of distal pigment cells; *rt.c.*, retinular cell; *rt. n.*, nucleus of retinular cell. Fig. 3. Depigmented ommatidia: (*a*) from a dark-adapted eye showing contractile fibers in the distal pigment cells in a relaxed condition; (*b*) from a light-adapted retina showing fibrils in a contracted condition. *ac. p.*, accessory pigment; *c.f.*, contractile fibril; *d.p.n.*, nucleus of distal pigment cells; *rt. n.*, nucleus of retinular cell. (From Welsh, 1930)

PHYSIOLOGICAL STUDIES

1. *Pigmentary Effectors*

Relatively early in the contemporary studies of pigmentary effectors in crustaceans, the problem of the number of different eyestalk hormones involved in the regulation of these effectors was raised. Brown (1935) examined chromatophoral behaviour of *Palaemonetes* kept on backgrounds of different colors and found that each type of chromatophore responded independently of the others, leading him to propose that separate hormones were involved in regulating each specific type. This view came to be known as the multiple hormone hypothesis, as distinguished from the unitary hypothesis

resulting from studies (Abramowitz, 1937) of the comparative effects of eyestalk removal and of reciprocal injection of eyestalk extracts between several crustacean species. Such studies indicated that responses of the various types of chromatophores to the extraspecific eyestalk extracts were identical with those produced when each test species was injected with extract prepared from its own eyestalks. Hence, according to the unitary hormone hypothesis, one eyestalk chromatophorotropic hormone, common to all crustaceans, produces a diversity of response in various species, depending on the specific organization of the chromatophores.

This problem can probably best be resolved by a direct approach involving chemical separation of the various fractions believed to act on the pigmentary effectors. It is, however, not a simple procedure; prerequisites are a knowledge of the chemical nature of the particular hormones, tests that will quantitatively assay the chromatophorotropin, and large amounts of eyestalks of a given species for use in extraction steps.

Substantial progress can be reported in the direction of separating pigmentary effector hormones. Separation of the distal retinal pigment light-adapting and the erythrophore-concentrating hormones has been achieved by chromatography on Dowex and D.E.A.E.-Sephadex columns (Josefsson and Kleinholz, 1964); the chromatographic results indicate that these hormones are two different molecules. Further chromatographic study (Kleinholz and Kimball, 1964) has also led to the separation of another chromatophorotropin, *Uca*- melanophore-dispersing hormone, which is distinct from erythrophore-concentrating hormone.

While hormonal control of one or more of the retinal pigments has been demonstrated in several macruran species (*see* Charniaux-Cotton and Kleinholz, 1964, for bibliography), few direct effects of eyestalk extract on the retinal pigments of brachyurans have been described. Smith (1948) reported the results of a variety of deficiency and injection experiments with the brachyuran retina, chiefly on the basis of presence or absence of retinal glow of the dark-adapted retina when momentarily illuminated. This method avoids the technical difficulties of preparing sections of eyestalks with heavy exoskeletons, but, unfortunately, does not reveal which of the retinal pigments are affected by the experimental procedure. With suitable species of crabs it should be possible to observe the retinal pigments and their photomechanical changes to light, darkness, and retinal pigment hormone directly while avoiding or skirting the difficulties encountered in preparing sections of such retinas.

2. *Molt and Associated Phenomena*

Endocrine regulation of crustacean molt implicates a molt-inhibiting hormone from the eyestalk and a molt-accelerating principle from the Y-organ. Some physiological relation probably exists between these two hormone sources, the Y-gland presumably being prevented from releasing its molt hormone by the inhibitor from the X-organ sinus gland complex of the eyestalk. When environmental factors halt secretion of the molt-inhibiting hormone from the eyestalk, release of Y-gland hormone can follow and molt ensue. To date, however, the only demonstration of such a possible relation has been that made by Carlisle (1957). Confirmation of his observations by a direct demonstration of the effect of eyestalk hormone on the secretory cycle of the Y-gland, similar to that reported by Durand (1960), would be desirable.

Gabe (1956), the discoverer of the crustacean Y-gland, was impressed by its morphological similarity to the prothoracic gland of insects in which it controls molt. Karlson (1956) has isolated ecdysone, the insect molt hormone. An ecdysone-like substance capable of inducing puparium formation in test *Calliphora* has also been isolated from crustaceans (Gallego and Menéndez, 1959), but no direct evidence is yet available that ecdysone will cause molt in crustaceans, or even that the crustacean molt hormone and the ecdysone-like material extractable from crustaceans are physiologically or chemically related. Nothing is yet known of the chemical nature of the crustacean molt hormone. A direct test of the efficacy of ecdysone in inducing molt in crustaceans from which the Y-glands have been removed is desirable, as is also information on the properties of the Y-gland

hormone. The possibility of using limb-bud growth rate (*see* Bliss and Boyer, 1964,for literature) as an assay for molt hormone (Passano and Jyssum, 1963) should be explored.

Molt is a complex process accompanied by marked physiological and biochemical changes. A number of environmental factors are known to affect the molt cycle, either inhibiting or accelerating it (Charniaux-Cotton and Kleinholz, 1964; Passano, 1960). In our present hypothesis of the endocrinology of molt the pathway for such inhibition or acceleration may be the central nervous system, but the mechanism by which molt is influenced might be the medulla terminalis X-organ (stimulated or arrested *production* of hormone inhibiting the Y-gland) or the sinus gland (stimulated or arrested *secretion* of this hormone) resulting either in the maintained inhibition of Y-gland or in release of its molt hormone.

Among a variety of metabolic effects influenced by the endocrine system of the eyestalk or accompanying the molt cycle might be listed respiratory metabolism, water balance, calcium metabolism, and the mobilization of metabolic reserves such as carbohydrate, protein and lipids. These are described and discussed in the two reviews just mentioned. The basic endocrine problem concerned with these associated phenomena is whether the particular change is regulated by a separate hormone from the eyestalk complex, or regulated by the molt-inhibiting hormone of the X-organ sinus gland system, or whether control comes by way of the Y-gland. Experimental design for further exploration of endocrine regulation of any of these features must consider the variety of possible hormone sources. The most recent illustration of a problem of this nature comes from the observations of Costlow (1963) on metamorphosis, molt and growth of megalops of *Callinectes* which were destalked at various periods following the final zoeal molt; the results indicate that increased growth rate can follow in such destalked post-larval crabs without increase in the molt frequency.

3. *Pericardial Organ*

Study of the cardioaccelerator substance found in the pericardial organ (Alexandrowicz and Carlisle, 1953; Maynard and Welsh, 1959) has led to conflicting reports. Carlisle (1956) found two active spots on paper chromatograms of extracts of this organ, one of which he believed to be 5, 6-dihydroxytryptamine. Maynard and Welsh (1959), however, found the concentration of 5-hydroxytryptamine in pericardial organ extracts to be insufficient to account for the physiological activity of such extracts and presented evidence that the cardioaccelerator substance was a peptide. Smyth (1959) observed the R_f values of 5-hydroxytryptamine and the cardioaccelerator substance to be markedly different after paper chromatography. Belamarich (1963) found two active compounds resolved by paper chromatography of pericardial organ extracts; both of the compounds were stainable with ninhydrin and as a result of acid hydrolysis were identified as peptides. Cooke (1962) reports further that when test hearts are perfused with 5-HT to maximum response, perfusion with pericardial organ extract produces additional stimulation equivalent to its effect alone; furthermore, hearts made unresponsive to 5-HT with lysergic acid diethylamide respond normally to pericardial organ extract.

The finding of two active spots by chromatography is interesting in connection with the report that at least three kinds of neurosecretory cells send fibers to the pericardial organ complex (Maynard, 1961 *b*), and three size classes of vesicles can be distinguished with the electron microscope in some pericardial organs (Maynard and Maynard, 1962). Speculation from the anatomical position of the pericardial organ complex added to the observations from electron microscopy lead Maynard to suspect the possibility of other general regulatory effects. Two such mentioned are increased oxygen transport rates by rise in ventilation rate in the gill chamber, and reduction of peripheral resistance to blood flow in the gill veins; physiological studies to test these possibilities have not yet been made.

Chemical Studies

Chemical studies in crustacean endocrinology may take several desirable directions. Tests that combine study of a particular physiological activity with determination of simple solubility and

other chemical properties (*i.e.*, molecular size, stability to heat, stability to enzymes) of the crude extract may result in information on the chemical nature of the active substance. The peptide nature of several chromatophorotropins, of retinal pigment hormone, and of the pericardial organ cardioaccelerator substances, as well as the protein nature of the diabetogenic hormone have been shown by such studies. Practically nothing is known of the properties of the molt-inhibitor from the eyestalk and the molt hormone from the Y-gland. Of the androgenic hormone it is known only that electron microscopy indicates that the gland cells show features of fine structure associated with protein-secreting cells rather than steroid-secreting cells.

Once the nature of the active substance is indicated, it is possible to do simple separations by paper chromatography or paper electrophoresis, or to undertake the more elaborate separation and purification methods that permit treatment of the substance on a large scale, such as column chromatography or countercurrent separations. With a suitable assay method, it is possible to monitor the steps of the extraction procedure with regard to quantitative distribution and recovery of the active substance.

It is not known at this time how many individual chemical substances are involved in the variety of physiological responses attributed to endocrine control in crustaceans. Is each particular type of physiological response produced by a separate hormone, or may one hormone molecule be involved in several different kinds of response? The answer to this question can come from tests of specificity of action of separated and purified principles.

After individual hormones are separated in pure form, new chemical approaches of considerable interest in comparative physiology and comparative biochemistry become possible. Molecular structures of invertebrate hormones are practically unknown. What interrelations will be shown by comparative biochemical studies of such purified protein and polypeptide hormones; what portions of such molecules will be found necessary for evoking the characteristic physiological response in the test animal; what similarities will be demonstrable with the molecular structure of comparable hormones from vertebrates? These are questions which cannot yet be answered; study of crustacean hormones in such directions will undoubtedly show them to be physiologically and biochemically as complex as the hormones of vertebrates. Some of us may view this prospect ruefully, but the challenge is irresistible.

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ON PHYSIOLOGY AND BEHAVIOUR OF ESTUARINE BARNACLES

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ABSTRACT

Knowledge of cirriped physiology is rudimentary (Waterman *et al.*, 1960 and 1961) and available data do not afford a physiological explanation for the distribution of estuarine forms. Some data have been published (*cf.* Krüger, 1940) but only one or two relatively reliable studies on osmoregulation and resistance to desiccation have appeared recently (Belyaev, 1949; Suzuki and Mori, 1963). The present study was undertaken in an attempt to better understand the basis for the vertical and horizontal distributions of three species of *Balanus* in the San Francisco Bay Estuarine System, California.

There are no freshwater cirripeds and those found in estuaries are either confined to them or are marine invaders. *Balanus glandula*, a marine species, is tolerant of mesohaline conditions. It can maintain blood and mantle cavity fluid hypertonic to dilute sea-water, but below 25% sea-water feeding activities cease. This species is relatively resistant to desiccation, losing 70% total water in two days (V.P.D. between 14 and 17 mm. Hg.). *Balanus amphitrite*, an introduced warm-water species, appears physiologically similar in these regards although the temperature regime of the estuary is clearly suboptimal and restricts its distribution. *Balanus improvisus*, also an introduced species, but coming from temperate and subtropical waters, inhabits infrahaline conditions for at least 10 months of the year. However, contrary to expectations, it conforms, the blood remaining essentially isotonic through dilutions to less than 3% sea-water. Correlated with this is a high permeability to water, 70% total water being lost in 6-8 hours through desiccation. A lack of osmoregulatory ability in an estuarine species has previously been unknown in Crustacea. But this appears to parallel the situation in certain bivalve Mollusca (Schlieper, 1935), and a hard shell and sessile way of life are certainly other apparently convergent similarities.

1. INTRODUCTION

THE thoracic Cirripedia are generally considered strictly marine Crustacea (Pennack, 1953), and it is true that no species is known to complete its life-cycle in freshwater or terrestrial conditions. Yet certain species spend sufficient time in air to be included among the semi-terrestrial Crustacea (Edney, 1960), while others are truly estuarine (Bousfield, 1955).

Probably because of their relative abundance, sessile mode of existence and conspicuousness, intertidal and estuarine barnacles have been studied extensively and the ecology of some species is rather well known. The literature has been reviewed by Hedgpeth (1957) and Moore (1958). However, surprisingly, if one judges by the current literature, their physiological mechanisms of adaptation to intertidal and estuarine conditions are virtually unknown (Lockwood, 1961; Nicol, 1960; Prosser and Brown, 1961; Waterman, 1960).

The objective of the present study has been to investigate adaptations made by three sessile barnacles to intertidal and estuarine conditions in order better to understand the factors limiting the distribution of these animals in nature, specifically in the San Francisco Bay estuarine system.

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II. The Estuary

A. Physical Conditions

San Francisco Bay (Fig. 1), in providing access to the sea for the large rivers of the Sacramento-San Joaquin Valleys, constitutes a substantial estuarine system into which marine organisms penetrate to varying degrees (Filice, 1958; Newman, 1954; Packard, 1918; Schmitt, 1921; Sumner *et al.*, 1914). The system extends some 50 miles from the marine environment of the Pacific Ocean to purely freshwater in the Sacramento-San Joaquin Delta Region.

The coastal tidal regime extends throughout the system to varying degrees. The mean tidal range of four feet is amplified somewhat as one progresses up the system to Crockett in Carquinez Strait, where it has increased by half a foot. It then declines riverward until, near Port Chicago, it is essentially the same as at the Bay Entrance, or Golden Gate. From there the range continues to attenuate gradually until there is no tidal fluctuation; but this is well into freshwater where no marine organisms are found.

Unlike a number of estuaries (Bousfield, 1955), this estuary is unstratified. Strong tidal currents, the great extent and shallowness, and the usually strong prevailing winds—especially throughout the summer months, keep the waters well mixed.

The temperature regime is comparable in pattern to other such bodies of water in that the ocean is relatively stable, while the inland reaches tend to be more variable and of greater range. Surface water temperatures (Fig. 1) have a mean annual range in the ocean of 12–14° C., and 8–21° C. in the delta region. While the ocean has a stabilizing effect on water temperatures, the extremes become greater as one progresses up the system. Naturally, this is a very general picture, and local situations within the system differ considerably from it.

The salinity regime of the system is complex, being affected daily by the tides, and annually by variations in river flow. Currents have predictable patterns within the navigable portions of the Bay, and ebb velocities range between two and six knots at the Bay Entrance. The run-off varies considerably, ranging from 10,000–130,000 cubic feet per second in January and 2,000–10,000 cubic feet per second in August. In general, these two months reflect the extremes. During most of the year, the current pattern reverses itself during the flood, well up into the freshwater reaches of the system.

The estuarine environment then is not only variable, but very complex, being the result of tides, currents, run-off, the configuration of the system and local conditions. Generalisations are difficult to make. The chlorinity profiles given in Fig. 1 are a compromise and reflect the situation seen at a particular station in a broad way. The profiles have been constructed from six years of high tide surface water chlorinity data (State Calif. Dept. Water Resources), and depict the following information: The height of each profile in Fig. 1, read against the chlorinity scale at the left, represents the extreme range at a particular station at the highest reaches of the tide, while the thickness of each profile represents the percentage of time spent at any given chlorinity during an annual cycle. It can be seen that the annual range increases away from the Bay Entrance, becoming greatest in San Pablo Bay, and then decreasing gradually from the marine end as one progresses up the system towards freshwater conditions. This is the reverse of the temperature regime, where the range becomes greater as one penetrates further up the system.

A "freshwater" barrier exists at Pittsburg, through the control provided by Shasta Dam, and chlorinities generally remain at or less than 0·5‰ for nine or ten months of the year. The barrier ordinarily breaks down briefly during August and September, when river flow is minimal, and brackish water penetrates further into the system.

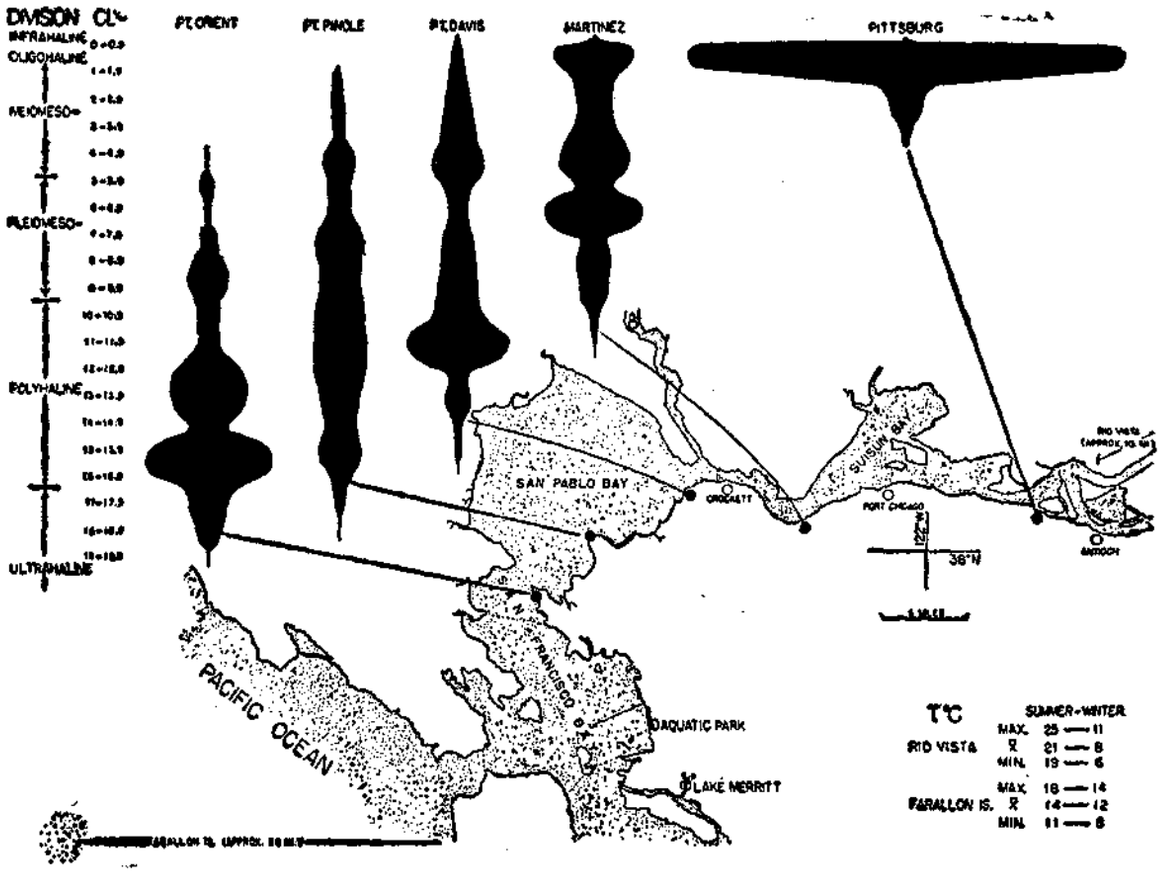


FIG. 1. San Francisco Bay Estuarine System: Surface water temperatures for the Farallon Islands in the open ocean and Rio Vista on the Sacramento River are summarized at the lower right. The schematic chlorinity profiles represent extreme range and mean per cent of time at a particular chlorinity level, during an annual cycle. The white horizontal line, across the profile for Pt. Davis, indicates a profile width of 10%.

The invertebrate fauna of the estuary has three components: tolerant or hardy marine species penetrating to varying degrees; estuarine species largely of marine origin, occurring predominantly in the estuary, and a few freshwater species in the upper brackish portions of the system. This faunal complex is comparable to that of other large estuaries (Remane, 1934), although here a large fraction of the purely estuarine species have been introduced through the activities of man (Ricketts and Calvin, 1952; Newman, 1963). All the sessile cirripeds found in the estuary are indigenous, except two. One of these is rather inconspicuous and has a restricted polyhaline distribution, while the other is a dominant estuarine species extending from polyhaline conditions into freshwater.

B. Distribution of Barnacles

The distribution of barnacles in the San Francisco Bay Estuarine System has been studied by Newman (1954), Paulson (1955) and Filice (1958); and for the present report is depicted in Fig. 2.

Balanus crenatus Brugiére, *B. nubilus* Darwin, and *B. improvisus* Darwin are found both subtidally and intertidally in the system, but the remainder are primarily intertidal forms. Most of the marine species penetrate only into the protected portion of San Francisco Bay proper. Two of these species, *Chthamalus dalli* Pilsbry and *Balanus crenatus*, extend up through North San Francisco Bay. While the latter progresses subtidally into San Pablo Bay (Filice, 1958), it is not known that it survives there through the entire annual cycle. *C. dalli*, on the other hand, frequents only the high intertidal and has not been known to penetrate into San Pablo Bay. San Pablo Strait, just north of Point Orient, is where the first really sharp faunal break occurs in the relatively protected portions of the system.

Balanus glandula Darwin, although a marine or protected outer coastal form, extends up the system beyond San Pablo Strait into Carquinez Strait, in the vicinity of Crockett, and this area constitutes the second faunal break in the system. It should be mentioned here that the intertidal distribution and range of *B. glandula*, and its tolerance of estuarine conditions, suggest it is the ecological counterpart of *B. balanoides* (L.) of the North Atlantic.

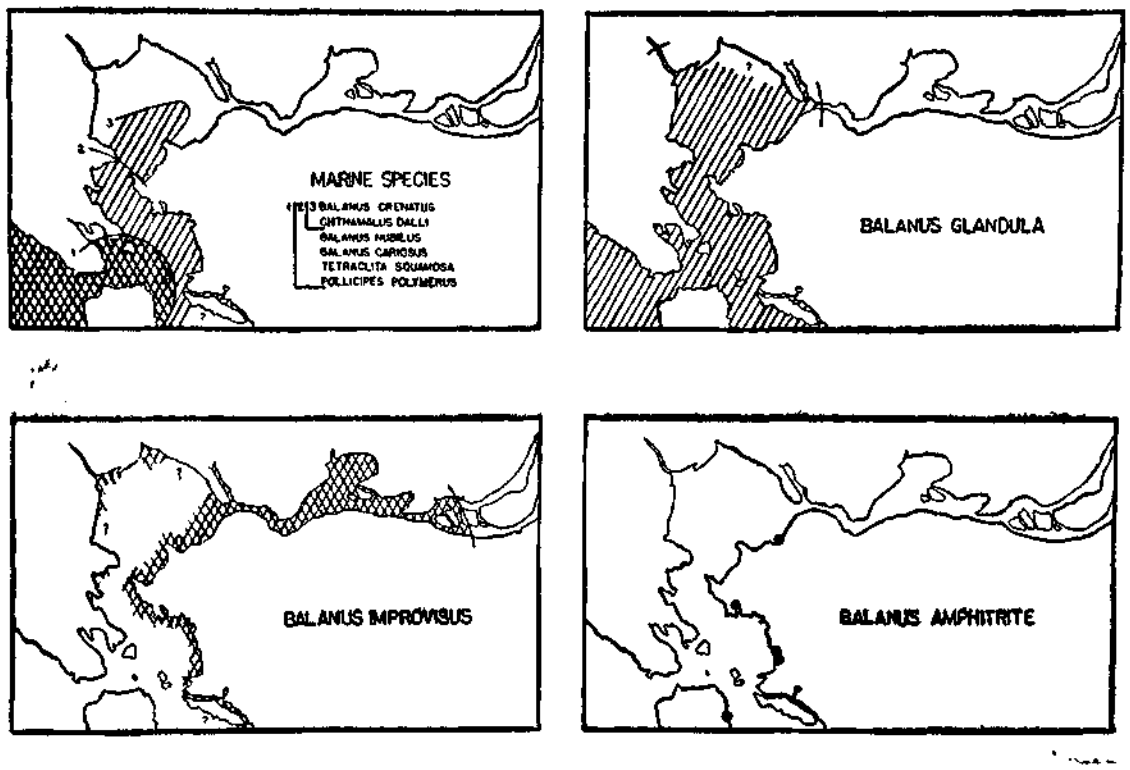


FIG. 2. Distribution of Barnacles in the San Francisco Bay Estuarine System.

Balanus improvisus is a true estuarine species and does not occur on the outer coast. It is commonly found subtidally as well as intertidally, and this suggests that it was not present at the time of the "Albatross" survey, in that it was not recorded by Pilsbry (1916). It was undoubtedly introduced by ships. It is widely distributed in the system, and is the only form found above Carquinez Strait, where it becomes extremely abundant intertidally. At Antioch, it survives in freshwater for some ten months of the year (Newman, 1954).

The last form to be considered, *Balanus amphitrite amphitrite* Darwin, was also introduced by ships. Although it is an estuarine form elsewhere (Millard, 1950), in the Bay it is restricted to

relatively few localities, and in some of these it is the only barnacle present. In the Oakland Estuary and Richmond Harbor, it is found with *B. glandula*, *B. improvisus* and *C. dalli*, but in Aquatic Park in Berkeley and Lake Merritt in Oakland, it is found alone. In San Francisco, the situation occupied by *B. amphitrite* is completely artificial; an area along the shore where warm sea-water is being discharged from the condensers of a power plant (Rodgers, 1949). In the Oakland Estuary it is known to breed only on rising temperatures (Graham and Gay, 1945). *B. amphitrite* is in fact a warm temperate to tropical species and although it will tolerate temperatures as low as 12° C., it requires 18° C. or warmer in order to breed (Hutchins, 1947). This, and its distribution in the Bay, suggest that suboptimal temperatures, rather than competition, account for its restricted distribution. Conceivably a rapid lowering of the overall temperature regime by a few degrees would lead to its local extinction.

Although the observed distribution of a species is the result of complex interactions of numerous factors, one of the most important is no doubt the transition from marine to freshwater conditions. By comparison with other marine invertebrates that enter estuaries, it may be assumed that some adaptations to reduced salinity have been made by these barnacles. In other invertebrates, particularly the Crustacea, the most obvious adaptations are concerned with osmoregulation: the reduction of permeability and maintenance of body fluids hypertonic to dilute media. The following experiments are concerned with this aspect of the problem and related phenomena.

III. Osmoregulation

A. Material and Methods

1. *Animals.*—Living barnacles were collected locally. All specimens were taken intertidally from approximately the middle of each species' intertidal range.

Balanus amphitrite is abundant along the retaining walls of the south model boat pond at Aquatic Park, Berkeley. It may be easily removed on a pallet of substratum by hammer and chisel. *Balanus glandula* is readily obtained at Berkeley Yacht Harbour and Pinole, and was removed from wooden substrata by delaminating thin layers with a knife. *Baolanus improvisus*, also occurring on wood, was collected at Martinez, where the intertidal population in San Francisco Bay is perhaps best developed.

In the laboratory, specimens were inspected for injuries that may have occurred by their removal. Even a small amount of damage to the basis or basal margin of the wall usually proved fatal within a few days and damaged specimens were disposed of. Specimens appearing in good condition were cleaned by a light scrubbing in sea-water, much of the substratum remaining attached to the basis being removed in the process. These specimens were then placed in 12-inch finger bowls in sea-water, and kept at room temperature without aeration. No animals were used for experimental purposes that had not survived at least two days in the laboratory, and, having been fed on *Artemia* larvae on the second day, had not discharged fecal pellets within 12 hours.

Specimens of *Balanus amphitrite* and *B. improvisus* that survive this preliminary treatment will survive in the laboratory indefinitely. Specimens of the former species have been kept for more than a year. However, *Balanus glandula* usually did not survive well for more than two or three weeks under these conditions, although a few individuals survived for several months. This species is basically a coastal form, and the bulk of the population is exposed daily by the tides. Poor survival in the laboratory may have been due to the materials having been constantly submerged.

The method of handling living material described here undoubtedly imposed some selection as well as conditioning within the experimental population. Whatever the effect, the treatment of the various samples was kept reasonably uniform.

2. *Sampling techniques.*—Capillary-tubing (approximately 0·78 mm. O.D. and 0·39 mm. I.D.) was flame-drawn to form micropipettes five to six centimeters long. A sample of about 0·12 microliters of mantle cavity fluid or blood was withdrawn by capillarity, the fine tip of the pipette was then broken off, and the volume of the sample reduced to about 0·02 microliters by shaking. By shaking in the reverse direction, the sample was then brought to the middle of the capillary tube, the ends of the tube being sealed by forcing them into vaseline, and the sample frozen by placing it on a block of dry ice. In this condition, samples were stored until their freezing point depression could be determined.

Samples were taken from experimental animals in a number of ways. If it is necessary to be certain that blood is being sampled, rather than mantle cavity fluid, the animal must be sacrificed. In this case, the shell is broken open, mantle cavity fluid is removed by a brisk shake, and a site selected from which to draw blood. In large individuals (greater than 15 mm. basal diameter), blood can readily be drawn from either the right or left branchial sinus, where the branchiae attach along the under margin of the sheath behind the tergal spurs, from the rostral sinus near the point of dorsal attachment of the prosoma with the basi-occludent angles of the scuta, or from the epineural sinus along the base of the cirri (*cf.* Darwin, 1854; Nussbaum, 1890 and Cannon, 1947 for details of the circulatory system).

Two methods were used in drawing blood without sacrificing the animals, which then could be used for further experimentation. By inserting the pipette from the outside, through the arthrodial membrane at the basi-occludent angles of the scuta, the rostral sinus may be reached; or by inserting near the base of the tergal spur the branchial sinus may be reached. The latter proved most satisfactory. In either case, the pipette is rotated between the fingers and moved up and down very slightly, a procedure that apparently frees the tip and prevents it from clogging. However, for purposes of this investigation, as will be described later, it was not crucial in two of the three species studied, to be able to distinguish between blood and mantle cavity fluid, for they appear to be essentially isosmotic.

Mantle cavity fluid was obtained with the same type of pipette and the sample was handled in the same manner. The pipette could be inserted between the valves, assisted by first forcing an opening with a dissecting needle, or in the same manner as that applied in obtaining blood from a branchial sinus, only driving the pipette deeper and along the margin of the scutum near the lateral eye-spot. Specimens large enough to be sampled in this way, either for blood or mantle cavity fluid, showed infection at the sites of injury, usually after two punctures on each side. Such survivors nearly always returned to good condition within a week in normal sea-water.

3. *Freezing point depression determinations.*—The method of determining freezing point depressions used in this study was essentially that of Jones (1941) as modified by Gross (1954). Samples of unknowns, and standards of known freezing point depressions, are frozen and then allowed to warm at a uniform rate. The times at which samples and standards melt are noted. The known freezing point depressions of the standards are then plotted against the observed time of melting and, by referring the time at which each sample melted to this curve, an estimate of the freezing point depression is made.

The apparatus (Fig. 3) consisted of a clear plastic tank of approximately 1·7 litres capacity, contained in a chamber formed of plastic foam insulation two inches thick. The tank is filled with 20% alcohol which is then chilled to about — 10° C. by the addition of small bits of dry ice. Frozen samples and standards in capillaries are inserted into a stainless steel rack submerged in a separate tray of 20% alcohol also cooled to about — 10° C. The rack containing the specimens is quickly transferred to the tank and the stirrer started. A thermometer may be observed in order to anticipate the melting of the first sample or standard.

Light from behind the chamber, polarized by the first polaroid screen, passes through the specimens *via* windows in the insulation and out the front of the chamber through the second polaroid-screen. The planes of polarization of the screens are at 90°. Thus, the frozen specimens appear

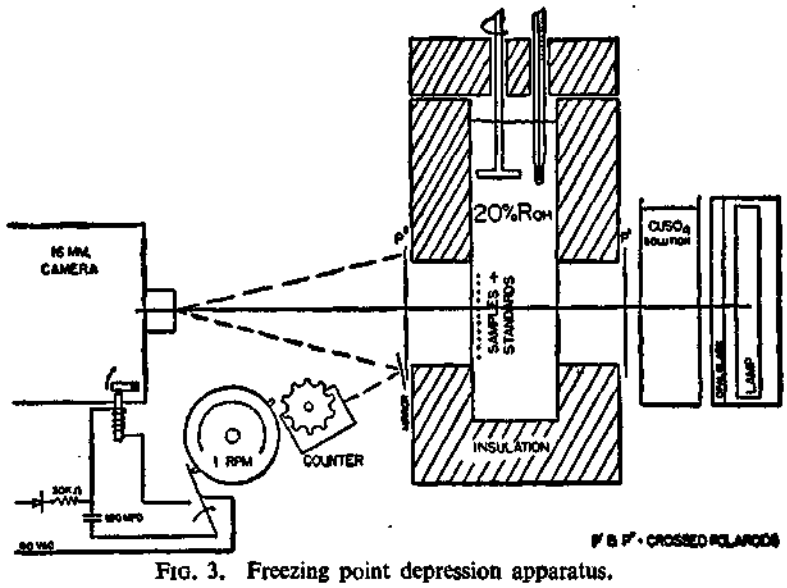


FIG. 3. Freezing point depression apparatus.

as bright spots against a dark field, and disappear upon melting. To prevent beads of condensation from forming on the optical surfaces of the tank, they should be swabbed with a liquid detergent.

Usually the observer sits in front of the chamber and notes the time at which each specimen melts. During the present study, a 16 mm. camera actuated by a timer took pictures of the specimens at fifteen or thirty second intervals. A counter geared to the timer was photographically recorded as a number on each frame. The purpose of this arrangement was to eliminate the necessity of making observations during the course of the run, and to obtain a permanent record of the results. In practice however, for most of the runs, direct observations were made for fear of some failure either in the picture taking mechanism or developing process.

Standards were made up from a solution containing 29·23 grams of sodium chloride per liter. Three standards were used: straight stock solution 0·50 M NaCl, 0·25 M NaCl, and distilled water. The salt solutions had freezing point depressions of 1·7° and 0·85° C. respectively. Initially, capillaries containing approximately 0·02 microliters of each standard were made up for each run, and only used once. However, "permanent" standards prepared in flame sealed capillaries and color coded were found satisfactory when used repeatedly over a period of two months. These were used either singly or in pairs and occasionally checked against fresh standards.

B. Experiments on Osmoregulation

The following experiments were performed in order to gather data on osmoregulatory behaviour. Experimental series E-1 and E-8 were with *Balanus glandula*; E-2 through E-7 were with *B. amphitrite*; E-9 was with *B. improvisus*. The data for all the experiments are summarized in Table I.

E-1.—The purpose of this experiment was to gain experience in the technique of sampling blood and in using the freezing point apparatus. Samples of mantle cavity fluid were also taken.

Specimens of *Balanus glandula* were collected on sticks from Berkeley Yacht Harbour, 12–29–59. They were separated from the wood, cleaned, and placed in normal sea-water in the laboratory. On 12–31–59, twelve specimens appeared in good condition, and were divided between four 4-inch finger bowls: 100, 75, 50 and 25% sea-water. After 18 hours, mantle cavity fluids and blood from

epineural sinuses were sampled, sacrificing the animals. Freezing point determinations for the animals in 100 and 75% sea-water, the media, and standards, were run as one group, those from 50 and 25% sea-water as another. The results indicate two things: *Balanus glandula* maintains itself hypertonic to dilute media, and the mantle cavity fluid is essentially isosmotic to the blood. These findings were borne out in the E-9 series of experiments also concerned with *Balanus glandula*, and the numerous experiments to follow, concerned with *B. amphitrite*.

E-2.—There are certain difficulties in handling specimens of *Balanus glandula* in comparison with *B. amphitrite*. They do not keep well in the laboratory, the opercular valves are placed rather deeply within the sheath, and individuals from the population at Berkeley are rather small. Therefore, attention was shifted to *Balanus amphitrite*. From the preceding experiment, the assumption was made that balanids in general hyper-regulate to some degree in dilute media. The question arises then, as to how they would behave in concentrations greater than normal sea-water?

TABLE I

Dilution series %	Time hrs.	Delta° °C.	Delta^4 Mantle cavity	Delta^4 Blood
		Balanus glandula		
(1)	18	1·31	1·54	1·63
			2·05	1·79
			1·89	1·92
(1)	18	0·88	0·88	
			1·00	
			1·24	
(1)	18	0·86	1·57	1·38
			1·28	
			1·47	1·38
(9)	24	0·86	0·96 (3)	
(1)	18	0·51	0·69	0·69
			0·93	
			0·56	
(9) 50, 25	24, 24	0·37	0·70	
			0·72	
			0·77	
			0·50	
(9) 50, 25, 12	24, 24, 24	0·21	0·68	
			0·64 (2)	
(9) 50, 25, 12, 6	24, 24, 24, 24	0·09	0·69	
			0·69	
			0·54	
				0·60
				0·58
				0·67
(9) 50, 25, 12, 6	24, 24, 24, 24	0·07	0·66	
				0·84
		Balanus improvisus		
(8)	24	0·77		0·92
				0·80
(8) 50, 25	24, 24	0·42		0·53 (3)
				0·50
(8) 50, 25, 12	24, 24, 24	0·17		0·23 (3)
				0·38
(8) 50, 25, 12, 6	24, 24, 24, 24	0·09	0·12	0·12
			0·09	0·12
				0·12
(8) 50, 25, 12, 6, 3	24, 24, 24, 24, 24	0·01	0·04	0·04
			0·04	0·01
			0·02	0·01

TABLE I (*Contd.*)

Dilution series %	Time hrs.	Delta° °C.	Delta' Mantle cavity	Delta' Blood
		Balanus amphitrite		
(2) By evaporation	48,	3·90	3·70 (4)	3·70 (3)
(3) 100, 150	20, 27	3·60	3·60	
			2·40	
(6) By evaporation	35 days	2·97	2·91 (3)	2·91 (3)
(5) 100, 50	24, 46	1·10	1·06 (2)	
			1·40	
(5)	70	0·94	1·17 (3)	
(7)	24	0·74	0·77	
			0·84 (7)	
(3)	47	0·70	0·71 (2)	
(5) 100, 50, 25	24, 22, 24	0·52	1·17 (3)	
(5) 50, 25	46, 24	0·49	1·06 (3)	
(5) 50, 25	24, 46	0·47	1·17 (3)	
(4) 50, 25	24, 47	0·39	0·83	
			1·21	
(3) 50, 25	16, 31	0·36	0·87	
			0·77	
(4) 50, 25	24, 24	0·36	0·48	
			0·57	
(4) 25, 12	24, 47	0·28	1·23	
			1·27	
(4) 50, 25, 12	24, 24, 23	0·25	1·00	
			1·30	
			0·90	
(5) 50, 25, 12	24, 22, 24	0·23	1·01 (2)	
(5) 25, 12	24, 46	0·23	1·03 (2)	
(5)	70	0·23	0·97	
			1·01	
			0·83	
(5)	70	0·23	1·14 (2)	
(5) 50, 25, 12	24, 24, 24	0·23	0·43 (3)	
			0·48	
			0·37 (2)	
			0·45	
(4) 25, 12, 6	24, 24, 23	0·16	1·20	
			0·64	
			1·21	
(3) 50, 12, 6	16, 21, 10	0·12	1·30	
			1·22	
(7) 50, 25, 12, 6	24, 24, 24, 24	0·08	0·30	
			0·39	
			0·38	
			0·42	
			0·48	
			0·45	
			0·68	
			0·33	
(5) 25, 12, 6	24, 22, 24	0·07	1·01 (2)	
			0·68	
(7) 50, 25, 12, 6, 3	24, 24, 24, 24, 24	0·02	0·47 (3)	
			0·45 (2)	
			0·29	
			0·56	
			0·36	
(7) 50, 25, 12, 6, 3	24, 24, 24, 24, 24	0·02		0·26
			0·31	
			0·21 (2)	
			0·38	
			0·36	
			0·26	
			0·34	

Specimens of *Balanus amphitrite* were collected at Aquatic Park, Berkeley, 4–6–61. Waters of Aquatic Park, at the time of the collection, had a chlorinity 65% that of sea-water, as determined by titration.

The seven animals were placed in normal sea-water. The dish was shallow and the surface area relatively large. In two days, the volume of the medium was reduced to half the original by evaporation. A double set of standards and samples of medium and samples of mantle cavity fluid and blood were prepared.

The freezing point depression data indicate that this species hyporegulates slightly in a medium twice the concentration of normal sea-water, and that the mantle cavity fluid and the blood are isosmotic. These observations are supported by the findings in experiment E-3, and E-6 to follow.

E-3.—In order to gain an overall view of the osmotic behaviour of *Balanus amphitrite*, the following experiments were carried out, beginning on 4–4–61. Mantle cavity fluid was sampled:

Time schedule and dilution series (Balanus amphitrite, *E*-3)*

Groups	100%	150%	50%	25%	12%
	hrs.	hrs.	hrs.	hrs.	hrs.
1	20	27			
2	47				
3			20 and 27		
4			16	31	
5			16	21	10

* Two changes in last dilution.

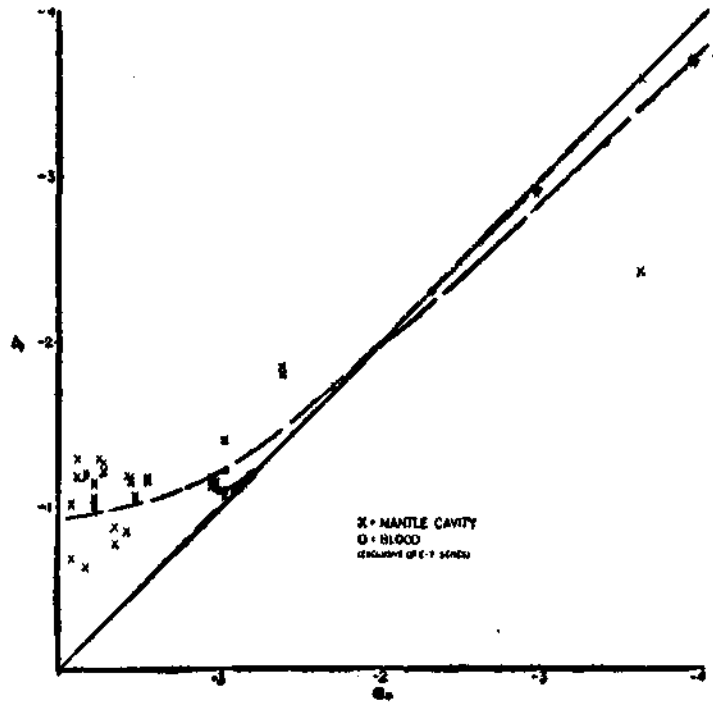


FIG. 4. Osmoregulation in *Balanus amphitrite*.

The animals in hypertonic media hyporegulate slightly in high salinities. In 50% sea-water, they essentially are isotonic, while below this point, they hyper-regulate strongly. There is an apparent rise in osmotic pressure in the individuals in 12% sea-water.

E-4.—From the results obtained so far, it was observed that *Balanus amphitrite* hyper-regulates in dilute media. It also appears there may be an increase in its internal osmotic concentration in media below 12–25% sea-water. This correlates with cessation of feeding activites, which reduced the permeable areas exposed, and may account for the apparent osmoregulatory improvement. That is, the animals may not be regulating any more strongly; they simply may be able to catch up when areas permeable to water are reduced by the withdrawing of feeding appendages into the protection of the mantle cavity.

Time schedule for dilution series (Balanus amphitrite, *E*-4)

Group	50%	25%	12%	6%
	hrs.	hrs.	hrs.	hrs.
1	24	47		
2	24	24	23	
3		24	47	
4		24	24	23

The results indicate that both groups in 12% sea-water regulate at a higher level than those in 25% sea-water. Those exposed the longest do best in terms of maintaining a higher osmotic pressure. However, the number of individuals is too small and the following experiments were designed to further explore the problem.

E-5.—This experiment, involving *Balanus amphitrite*, is comparable to the last and was designed to explore osmotic behaviour in the range of concentrations between normal and 6% sea-water. As in all the experiments so far, animals were divided into groups, and the groups exposed to different media. The combinations were such that when the experiment terminated, animals were found in media ranging from 50–6% sea-water. The animals were usually sacrificed when samples were taken, but this time a sample of mantle cavity fluid was drawn off by forcing the tip of the micropipette through the arthrodial membrane supporting the valves within the sheath, as described earlier.

Time schedule for dilution series (Balanus amphitrite, *E*-5)

Group	100%	50%	25%	12%	6%
	hrs.	hrs.	hrs.	hrs.	hrs.
1	24	46			
2	24	22	24		
3		70			
4		46	24		
5		24	46		
6		24	22	24	
7			24	46	
8			24	22	24
9				70	
10				46	24

Again, the animals regulate through 12% sea-water. In this series, no increase in osmotic pressure is observed at lower dilutions, except the last, and it is not entirely clear whether an increase actually occurs, or is simply a result of experimental procedure, as suggested earlier. If animals are induced to close by being brought abruptly into a very dilute medium in a few steps or one single step, conceivably they could maintain themselves at a relatively higher osmotic concentration, awaiting more favourable conditions. If such were the case, it would explain the apparent rise seen so far. The E-7 series of experiments were designed to test this possibility.

E-6.—Experiments E-2 and E-3 indicated that in concentrations greater than normal sea-water, *Balanus amphitrite* hyporegulates weakly, essentially conforming to the medium. This could be due to short-term conditioning in the laboratory, and may not reflect behaviour in nature or under long-term acclimatization. Therefore, specimens in a 2-liter aquarium were gradually acclimated to a high concentration by allowing the water to evaporate slowly for 35 days. In this way, they were brought up to a salt concentration approximately 150% that of sea-water. Blood and mantle cavity fluids were then sampled. The freezing point depression of the medium was 2·97, and 2·91 for the samples, comparable to the results obtained earlier.

E-7.—Within the limitations of the apparatus and experimental procedure, it has been demonstrated that the mantle cavity fluid is virtually isotonic with the blood when *Balanus amphitrite* is hyporegulating in concentrated media, and hyper-regulating in dilute media. In the foregoing experiments, mantle cavity fluid could be withdrawn by perforation of the arthrodial membrane with a micropipette, without appreciable damage to the animal. Because of the uncertainty regarding increased degree of hyper-regulation in dilute media, and because of the more desirable aspects of utilizing the same animals throughout a complete dilution series, a different approach was undertaken in the following series.

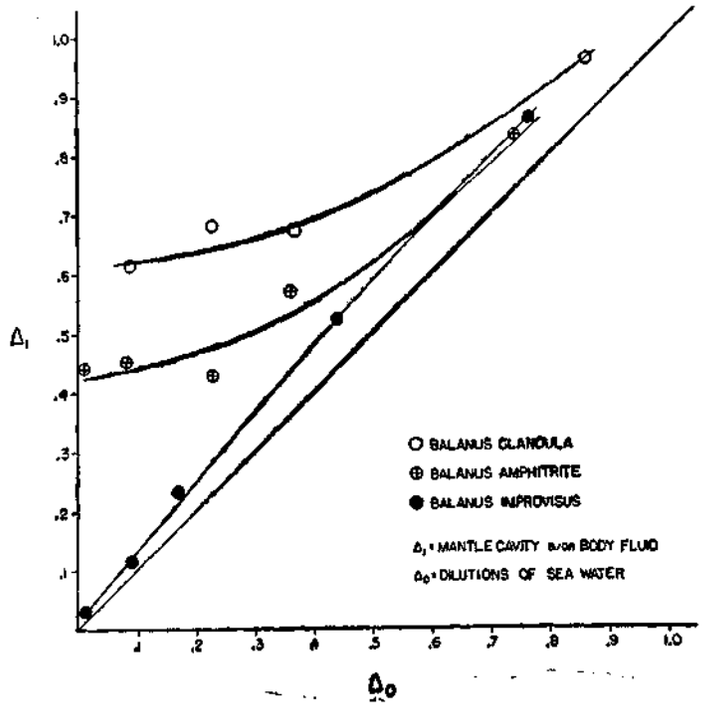


FIG. 5. Osmoregulation in *Balanus glandula*, *B. amphitrite*, and *B. improvisus*.

A group of animals were acclimated for 24 hours at a particular salinity, at the end of which time, mantle cavity fluids were sampled. After each sampling, the animals were transferred to the next dilution, where they remained for 24 hours before being sampled and transferred again. In the final dilution (the second twenty-four hour period in 3% sea-water) blood was sampled, sacrificing the animals. The same animals were sampled at each step of the series, and one is clearly following their progress as dilution increases. If there should be an increase in their osmotic pressure at low concentrations, it should be readily detected. Mantle cavity fluid was sampled in all cases except last period of 3% sea-water, when blood was taken.

The data demonstrate again that *Balanus amphitrite* regulates strongly down to 6% sea-water. Even in 3% sea-water, it holds its own for the first 24 hours, but it shows signs of failing during the second. Again, there is a tendency (Fig. 5) to increase in osmotic concentration below 25% sea-water.

E-8 and 9.—Balanus improvisus and *B. glandula* were run through dilution series in the same manner as *B. amphitrite* (E-7). As has already been observed, *B. glandula* (E-1) follows much the same pattern as *B. amphitrite*, although regulating at a somewhat higher level. But it is surprising to note from the results of this experiment that *B. improvisus* (Fig. 5), although hyper-regulating slightly, follows the line of isotonicity all the way down to 3% sea-water. *Balanus improvisus* is the most estuarine species in San Francisco Bay and, as will be described shortly, it differs from the others in its behaviour in dilute media and its permeability to water in air.

IV. Behaviour

During the course of the experiments on osmoregulation, it was observed that these species of *Balanus* behaved somewhat differently in response to dilute media. The differences in behaviour seemed to be correlated with their osmoregulatory capacities. It also had been observed, in maintaining animals in the laboratory, that resting individuals in favourable media could be stimulated to feed by the addition of appropriate food to their medium. Therefore, a group of four or five individuals was placed in each step of a dilution series ranging from 50–6% sea-water (to 12% sea-water for *Balanus glandula*). They were observed intermittently while being acclimated for about 24 hours, after which *Artemia* larvae were added, and observations continued during the subsequent day.

Balanus amphitrite appeared in good condition and behaved normally in 50% sea-water. In 25% sea-water, the valves remained closed and only rarely were mantle margins seen exposed. During this period, some would feed with a slow beat upon the introduction of *Artemia*. In 12% sea-water, the animals showed signs of weakness, that is the valves could be moved with the light pressure of a dissecting needle, and they would not respond to food. Below this dilution, the weaker individuals died, while the remainder survived even in 3% sea-water. When feeding in dilute media, the cirral beat was slow, and the trophic structures worked at a level always visible from the exterior. This differs from the slow beat seen in normal or slightly dilute sea-water, and its significance will be taken up shortly in regard to a mechanism which maintains the mantle cavity separate from the external medium while feeding.

Balanus glandula behaved in much the same manner as *B. amphitrite*, showing, however, more signs of weakness and distress below 50% sea-water. This was manifested by weakly held valves, exposed mantle edge, and noticeable gap in many individuals. It should be mentioned that *B. glandula* is essentially an intertidal species, and the specimens used would not normally be exposed to such prolonged periods of submergence. Even in normal sea-water, although most individuals survived for several weeks, they did not behave in the same manner as they did when first brought from the field, and placed in aquaria.

Balanus improvisus was conspicuously different in its behaviour from both the aforementioned species. Spontaneous feeding beats were observed down to 12% sea-water. From 12–3%

sea-water, most of the animals continued a slow cirral rhythm without extending the cirri fully. They did not hold the trophic region particularly high in relation to the aperture. By viewing the animals from above, a passage to the mantle cavity appeared regularly behind the cirri with each forward stroke. In 6% sea-water, some individuals were stimulated to greater activity and appeared to feed when *Artemia* were added, but in 3% sea-water, only the slow rhythmical movements in which the coiled cirri appeared above the aperture were observed.

While *Balanus glandula* and *B. amphitrite* reduced and eventually ceased their activity in response to dilution, *B. improvisus* showed only reduced activity and was active between intermittent periods of complete closure.

V. Anatomical Considerations

Balanus amphitrite and *B. glandula* regulate their internal fluids against dilute media. It would not be surprising to find the mantle cavity fluid hypotonic to the blood, for it is generally assumed that when the animals are feeding, the mantle cavity is in communication with the external medium and that its fluid is being circulated by the motion of the trophic structures (Crisp and Southward, 1961). It was therefore surprising to find the mantle cavity fluid isotonic to the blood, even when samples were taken as quickly as possible from animals feeding in dilute media. How could this be possible if the mantle cavity fluid were being circulated during the feeding process? Clearly, it is not being circulated, or at least, not to an appreciable extent. If it is a fact that under certain conditions, the fluid is being circulated, how then is circulation prevented during feeding in those forms that regulate strongly in dilute media?

In a barnacle, the structures supporting the trophic apparatus can assume three basic positions (Fig. 6). When withdrawn, in the resting or closed position (Fig. 6, A), the thorax is telescoped somewhat, folding forward on the prosoma, and together these portions lie nearly parallel to the basis. The opening or aperture of the mantle sac is controlled by the complex musculature operating the opercular valves, and, when the aperture is completely closed, the body, suspended in mantle cavity fluid, is entirely surrounded by the mantle.

In the second position (Fig. 6, B), the body is rotated toward the vertical, the thorax being straightened somewhat, and the cirri uncoiled slightly. In this position, the animal can be observed to oscillate slowly up and down, usually simply pumping water in and out, although this behaviour is also associated with the moulting process or in ejecting newly hatched larvae.

The feeding position (Fig. 6, C), is apparently assumed by contraction and an upward movement of the prosoma, forcing the thorax to telescope outward, and further unfold. In this way, the basal positions of the trophic structures are forced up into the apertural cone formed by the opercular valves. The degree to which they are forced upward can vary, and in a "low" feeding position, the dimensions of the thorax and prosoma are such that they do not occlude channels to the outside. The feeding strokes, in this position, create a current in and out of the mantle cavity by small rhythmical alterations of its volume. However, if the body is thrust up further, to the "high" feeding position (Fig. 7), it fits snugly into the apertural cone and effectively blocks the aperture. In this position the animal is quite capable of feeding, for all the trophic structures are elevated just above the apertural constriction. The seal is made below them, posteriorly and laterally, by the expanded portions of the thorax and prosoma against the velum and opercular membranes, and anteriorly by the basal positions of the labrum and first cirri.

This anatomical arrangement, by which the mantle cavity can be mechanically separated from the exterior during the feeding process, is confined to sessile barnacles. It can be observed that the body cannot be pulled out through the aperture, even when the muscles and other attachments have been severed. This is not the case in the pedunculate barnacles and the plugging arrangement is lacking in such animals. The difference between sessile and pedunculate barnacles, in this regard,

is of considerable phylogenetic interest, but need not concern us here. However, a brief comparison between the two groups does aid in an understanding of the anatomical relationships involved and can be taken up for illustrative purposes.

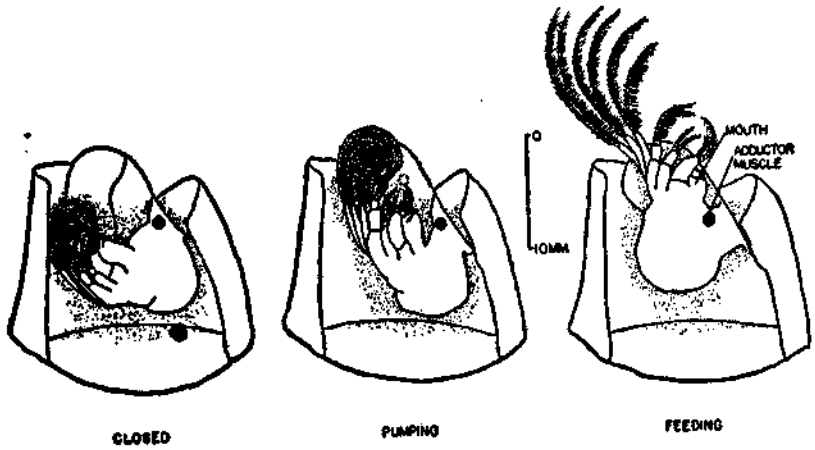


FIG. 6. Behaviour of *Balanus glandula*, illustrated from the left side after the tergum, scutum, and lateral and carinolateral wall plates have been removed (The mantle, which normally lines the interior of the shell, and the right cirri, have been deleted).

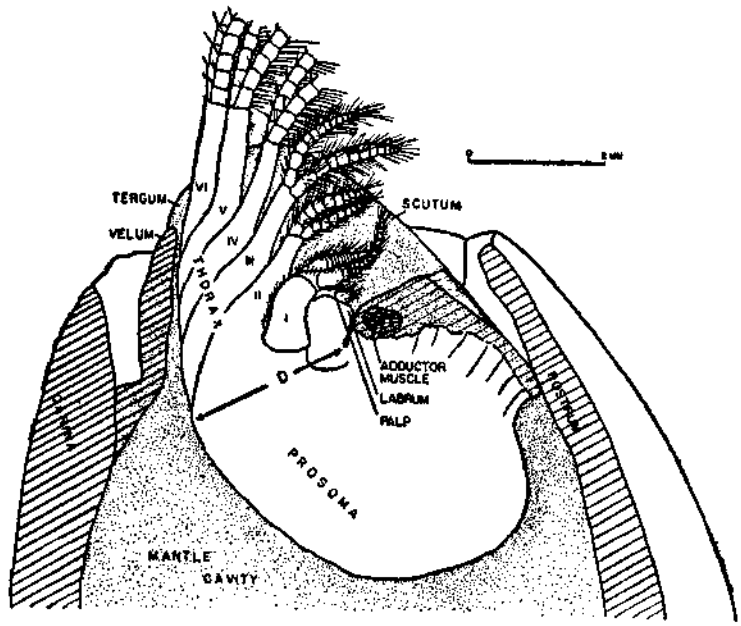


FIG. 7. *Balanus glandula*, in a moderately high feeding position, illustrating anatomical relationships that allow the animal to mechanically separate the mantle cavity from the external medium while feeding [Hatching = cut portions of wall plates; hatching *plus* stippling = cut portions of body and membranes; stippling = mantle membranes lining inner portions of wall and valves; Roman numerals indicate left cirri; (D) = depth of body, from base of labrum to base of cirrus II].

The aperture is closed by drawing the mantle or opercular flaps together, and, when closed, length is its only dimension. In opening the length becomes somewhat less, as a modest width is attained. When open, the shape of the aperture approximates the shape of those parts of the body that come into the aperture when the animal is feeding. For simplicity and comparative purposes, let us assume that the size of the aperture is a function of its length. The length of the aperture is measured from the lower angle near the attachment of the prosoma to the scuta or at the adductor muscle, to the upper angle where the velar membrane joins the lower carinal margins of the terga (Fig. 7). The size of the body then, where it comes to rest in the aperture, can be expressed as a measurement of the depth of the body. The body will always be somewhat smaller than the aperture, and the relationship can be expressed as a ratio (Fig. 8).

Lepas anatifera L. (Fig. 8, A), of the family Lepadidae, lives attached to floating objects and is quite comparable to one of the oldest known barnacles, *Praelepas*, from the Carboniferous (Withers, 1935). It will be noted that the orientation of the aperture, represented by a line passing along its length, is some 35° from the long axis of the entire animal. The feeding position in *Lepas* is assumed by rotation of the body, bearing the trophic structures, around the adductor muscle and out through the aperture. The valves, being flap-like, do not form an opercular cone. The depth of the body, at the margins of the aperture when the animal is feeding, is but 0·75 the length of the aperture (wide line overlying the narrow line in Fig. 8). Upon inspection, there is room to spare, and, anatomically, there is no provision for occluding the aperture while feeding.

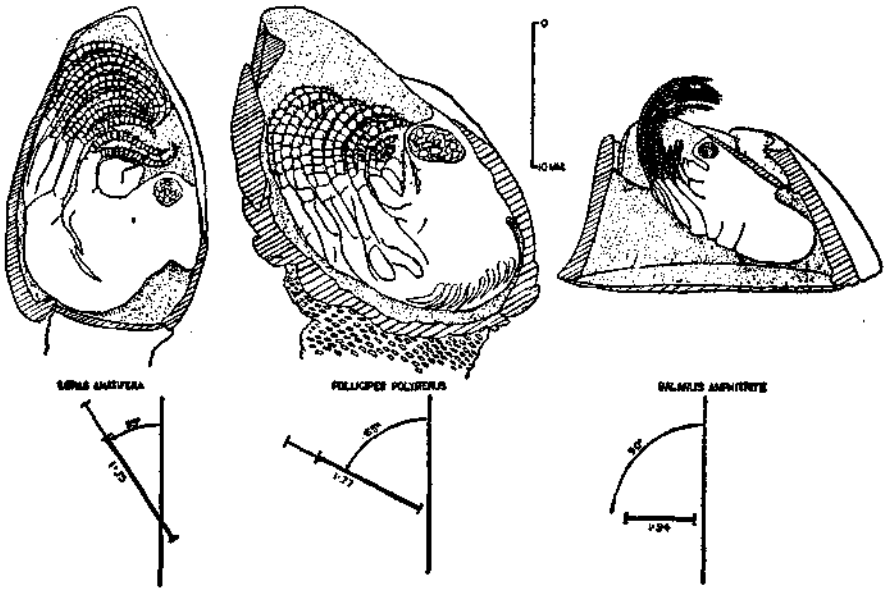


FIG. 8. Comparative anatomical relationships between three distinct forms, suggesting evolution of mechanism effecting separation between the mantle cavity and the external medium during feeding.

Pollicipes polymerus Sowerby, is an intertidal representative of the family Scalpellidae. While most scalpellids have essentially the same anatomical relationships described here for lepadids, intertidal forms tend to be rotated so that the angle of the aperture to the long axis of the entire animal is greater, as indicated (Fig. 8, B). This arrangement brings the aperture up over the animal and would seem to be an adaptation for feeding under crowded intertidal conditions. The valves, although limited more to the apertural region, are still simple flaps, and do not form an operculum. The ratio of the depth of the body to the length of the aperture is 0·77; the aperture cannot be occluded while feeding.

In *Balanus amphitrite* Darwin (Fig. 8, C), the angle of the aperture is some 90° to the vertical and the valves form an opercular cone or tentorium, into which the basal regions of the trophic structures fit when feeding. The depth of the body is 0·94 the length of the aperture, and in the "high" feeding position, communication between the mantle cavity and the external medium is mechanically blocked. This is a highly evolved arrangement, as compared with the two primitive examples given above, and it should have some adaptive value. As has been described, it allows the animal to close the aperture while feeding.

VI. Desiccation

The three species of *Balanus* used in this study have different intertidal distributions in San Francisco Bay, and it might be expected that they would differ in their ability to resist desiccation. In shelled organisms that close during unfavourable periods, resistance to desiccation quite likely entails reduction of water loss as well as toleration of simply drying out. Permeability is also an important physiological factor in estuarine organisms, especially in those that osmoregulate. It therefore seems most appropriate to investigate certain aspects of this problem.

The experiment was simple. By allowing specimens to dry in air, the rate of water loss can be readily determined gravimetrically. The procedure was as follows: specimens that had been submerged in sea-water were removed and dried lightly with paper towelling. Since some water loss may occur through spontaneous activities of the animal and incomplete sealing of the valves, the apertures of control animals were sealed with hot paraffin. Although the seal may have appeared perfectly tight, it was not, for during drying the mantle space became filled with air, replacing the water lost. The only way that air could have entered was around the plug and a very small leak would allow this to happen.

After the initial preparation, the animals were weighed on a Roller Smith Precision Balance of five grams capacity. They were then allowed to dry in air and were weighed at intervals throughout a 48-hour period. At each weighing, temperature and relative humidity were taken with a sling psychrometer, the data being converted into vapor pressure deficit by use of standard tables. At the end of each run, the sealed animals were unplugged and, with the unplugged individuals, returned to sea-water in order to determine survival. After this, they were broken open and dried to constant weight at 60° C.

The differences between the initial weight and the final weight were taken as "total water," and from these data "% water loss" was calculated and plotted against time (Fig. 9). Estimates of the rates of water loss are given below. Vapour pressure deficits ranged between 14 and 17 mm. Hg during the course of the experiments.

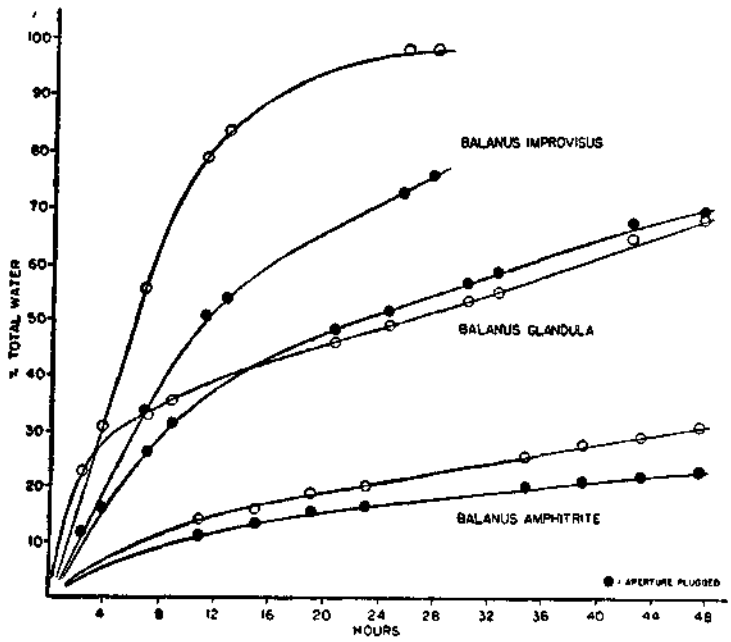
Summary of desiccation data

Species	Total water (c.c.)	Loss/hour (c.c.)	Surface area ($mm.^2$)	Loss/$mm.^2$/hr.
B. amphitrite ..	0·503	·00169 (0·34%)	27·4	$6·2\times10^{-6}$
B. glandula ..	0·1215	·00091 (0·75%)	10·0	$9·1\times10^{-6}$
B. improvisus ..	0·1001	·00547 (5·5%)	8·3	$68·0\times10^{-6}$

Balanus amphitrite and *B. glandula* differ conspicuously from *B. improvisus* in their respective rates of water loss under the conditions tested, and this correlates well with their survival. At the

end of the experiments, all specimens of *B. amphitrite* and half the specimens of *B. glandula*, regardless of being plugged or unplugged, were alive, while all *B. improvisus* had perished. The difference between the behaviour of *B. amphitrite* and *B. improvisus* is surprising since they are very closely related. They differ conspicuously from *B. glandula* in the relationships of the valves to the wall plates, the gross structure of the plates themselves, and their usually uneroded condition (Pilsbry, 1916). These similarities and differences have a quantitative basis; for example, in the ratio of total weight to total water, given below.

	B. amphitrite	*B. improvisus*	*B. glandula*
Total weight (gm.) ..	1·186	0·201	0·553
Total water (c.c.) ..	0·544	0·088	0·139
Ratio ..	2·18	2·28	3·98



WATER LOSS IN AIR

FIG. 9. Resistance to desiccation in *Balanus amphitrite*, *B. glandula* and *B. improvisus* (Vapour pressure deficit = 14–17 mm. Hg).

It can be seen from the data for "total weight" and "total water" that *B. improvisus* is considerably smaller than *B. amphitrite*. The question arises as to whether or not the apparent difference in rate of water loss, although real enough for the animals, may not be primarily a function of surface to volume relationships, rather than physiological differences in the organisms themselves. The ratio of total weight to total water is virtually the same in *B. improvisus* and *B. amphitrite*, and the animals are very similar in form.

The mean values for total water in *B. improvisus* and *B. amphitrite* are 0·1 and 0·5 c.c. respectively. This is a ratio of 1 : 5. Surface area increases disproportionately with size, as a function

of the volume to the $\frac{2}{3}$ power. For the volumes involved here, the ratio would be $1^{\frac{2}{3}}$: $5^{\frac{2}{3}}$, or approximately 1 : 3. Thus, while *B. amphitrite* has five times the volume of *B. improvisus*, it has but three times the surface area. If an arbitrary rate of water loss is assumed, say ·005 c.c./hr. per unit area, and this is taken to be the same in both species, *B. improvisus* would lose ·005 c.c. or 5% of its intital volume per hour, while *B. amphitrite* would lose. · 105 c.c. or 3% of its in itial volume per hour. Therefore, *B. improvisus* would be expected to lose water, initially, more than twice as fast as *B. amphitrite*, because of its smaller size.

When actual rates are compared, this value is found to be inadequate to account for the remarkable difference between these two species. Specifically, selecting the relatively uniform slope of the curves (Fig. 9), between 3·75 and 11·25 hours for *B. improvisus*, and 19·25 and 43·5 hours for *B. amphitrite*, mean values of ·00547 and ·00169 c.c./hr. are obtained. These rates represent losses of 5 and ·34% per hour respectively, meaning that *B. improvisus* is losing water 10-15 times faster than *B. amphitrite*, and is therefore intrinsically more permeable to outward passage of water while in air.

In previous observations and experiments, it was found that *B. amphitrite* closes up and regulates strongly in dilute media, while *B. improvisus* does not. It would be disadvantageous for *B. amphitrite* to be as permeable to water as is *B. improvisus*. However, it is not clear why *B. improvisus* should be highly permeable to water and therefore vulnerable to desiccation, for it occurs intertidally.

VII. Mantle Cavity Water and Desiccation

The question now arises as to just how much water the mantle cavity contains, for this would give some idea what it contributes to the water lost during periods of desiccation. Ten non-ovigerous specimens of *Balanus amphitrite* were dried with towelling and then observed in air for two hours. At the end of this time no more moisture could be seen about the opercular parts. The specimens were then weighed in the usual manner, the valves forced open and water in the mantle cavity forcibly removed by vigorous shaking. The animals were weighed again and then oven-dried to constant weight. The results are given below:

Total water	$\bar{x}$ 0·4842 ± 0·0474 c.c.
Mantle cavity water	$\bar{x}$ 0·1336 ± 0·0101 c.c.
Mantle cavity water as % total water	$\bar{x}$ 27·6 ± 2·4%

In an animal that is closed tightly or sealed with wax, the only way water can leave the mantle cavity, while drying is taking place, is by passing first through tissues lining the entire interior, and then through the shell itself. Since *Balanus amphitrite* has a mantle cavity that holds about one-quarter as much water as is contained in the tissues, it would seem that the mantle cavity water must make a substantial contribution to the water lost in situations where the animal survives until the next high tide.

If the body and mantle cavity fluids are essentially in osmotic equilibrium, as demonstrated by the freezing point depression data, and approximately one-quarter of the total water contained by the animal is in the mantle cavity, these two systems should lose water in proportion to the volume of water each contains; approximately 3/1, provided the mantle cavity fluid is not in communication with the exterior. Therefore, at the end of 24 hours, the mantle cavity should still contain some 27% of the total water remaining at that time. In order to test this, a group of ten animals was allowed to dry for two hours, and then weighed. We noted in a previous experiment that normal *Balanus amphitrite* lost approximately 20% of their total water in 24 hours when allowed to dry in air under laboratory conditions. So in this experiment, they were allowed to dry for 24 hours, at the end of which time they were weighed again. Immediately following this

weighing, the valves were forced open and any remaining mantle cavity fluid expelled by vigorous shaking, the animals then being weighed and oven-dried to a constant weight. The results are given below:

Total water	.. ..	$\bar{x}$ 0·4600 ± 0·0352 c.c.
Water remaining after 24 hours	.. ..	$\bar{x}$ 0·3620 ± 0·0300 c.c.
Water remaining in mantle cavity after 24 hours	..	$\bar{x}$ 0·0640 ± 0·0080 c.c. (17·5 ± 2·2%)
Water lost after 24 hours	.. ..	$\bar{x}$ 0·0975 ± 0·0115 c.c. (21·2 ± 2·6%)

From these data, it is obvious that the relationship assumed above (that the body and mantle fluids are in a simple osmotic equilibrium, and that all water lost escapes *via* the tissues and then through the shell), is questionable. At the end of the 24-hour period, the animals had lost 21% of their total water, as expected from the earlier experiment, but the remaining water in the mantle cavity turned out to be 17% rather than the predicted 27% of the total water present at that time. Even if the standard errors are considered in a direction favourable to reducing the difference between these two means, the data are still some 5% short of agreement. Utilizing the criterion that if the difference between two means (10·1) is greater than twice the standard error of the difference (6·4), the means are significantly different, then these two means are significantly different and water is being lost more rapidly from the mantle cavity than from the body tissues.

VIII. Discussion

A. Distribution

As one progresses from the marine environment into polyhaline waters of an estuary, the number of species of marine barnacles diminishes rapidly, and when conditions become predominately mesohaline, none are found. However, certain species that do not occur on the outer coast may be found in the estuary. The barnacle fauna of estuaries may then be of two components: tolerant, marine and strictly estuarine forms.

In the San Francisco Bay estuarine system, tolerant marine species are *Balanus glandula*, *B. crenatus* and *Chthamalus dalli*, and the strictly estuarine forms, *B. improvisus* and *B. amphitrite amphitrite*. All of these vary in degree of penetration and vertical range within the system.

There are two general considerations that should be evaluated when attempting to analyze the factors responsible for the observed distributions. Balanoid barnacles are attached forms, and adults must be able to endure the conditions prevailing where the planktonic larval stages settle. As pointed out by Smith (1955), although the observed distribution is undoubtedly a reflection of the conditions under which the adults are able to survive, the actual inhabitable range available to the population may or may not be reached by the planktonic stage ready to settle. If current patterns of the system and larval behaviour are such that larvae are not carried to certain reaches of the available habitat, adults of course will not occur there, even though conditions might otherwise be most favourable.

Bousfield (1955) has studied the occurrence of barnacles in the Miramichi Estuary in eastern Canada. He discusses current and stratification patterns, comparing them to the distribution of larvae of three species of *Balanus* at different times in the daily cycle of the tides, and has been able to relate differences in distribution of the adult barnacles to these relationships.

The San Francisco Bay estuarine system is, for the most part, unstratified. Earlier investigations (Sumner *et al.*, 1914; Miller *et al.*, 1928) report surface and bottom salinities to differ by 1–5 parts per thousand. Sumner *et al.* show that the differences become greater as one progresses up the system, and Miller *et al.* give a mean difference of 1·78 ppt. for all observations at Crockett

where bottom samples were taken in the channel, 31 feet below zero water. Since their data are for surface and bottom salinities, and because the differences are generally rather small, we do not actually have evidence for stratification. According to the US. Army Engineers (1962, personal communication), who have been engaged in an extensive study of San Francisco Bay for a number of years, true stratification occurs only during periods of peak runoff. Runoff varies much from year to year, but in general, in the vicinity of Pittsburg, conditions are stratified for but a few weeks of the year. During the remainder of the year, waters range from partially mixed to completely mixed, usually the latter condition prevailing.

One of the three species of *Balanus* studied here is different from one of the three species occurring in the Miramichi. Two of the species, *Balanus improvisus* and *B. crenatus*, are the same in both estuaries, while *B. glandula* in the Pacific is in a different subgenus than *B. balanoides* in the Atlantic. However, *B. glandula* is the ecological counterpart of *B. balanoides*. They have very similar latitudinal distributions and bear the same intertidal relationships to *Chthamalus* on their respective coasts (Pilsby, 1916; Ricketts and Calvin, 1952; Moore, 1958).

If this ecological similarity is granted, then San Francisco Bay and the Miramichi Estuaries have comparable barnacle faunas, but the distribution of comparable species differs somewhat. Subtidally, *B. crenatus* penetrates relatively deeper into the Miramichi than it does in the San Francisco Bay estuary. This correlates with the nature of the system, since the Miramichi is stratified and San Francisco is not. By the same token, the relative distance between the fully estuarine *B. improvisus* and its marine intertidal competitor, *B. balanoides* would be expected to be greater in a stratified estuary because of the surface wedge of reduced salinity encountered by the marine form. In this way, the apparent difference in distribution of the barnacles in these two estuaries can be explained. It probably corresponds to the salinity pattern rather than marked differences between the population involved.

Since the distribution of adults is effectively the same with respect to salinity in these two very different estuaries, one may ask whether or not the adults actually occupy all the space that adult adaptations allow? Bousfield (1955) stresses larval distribution but admits that low salinities probably set the upper limit of riverward distribution of each species in the Miramichi. Since currents carry the larvae about, it seems probable that they are carried far beyond the range inhabitable by the adults. This does not imply that the larvae must be adapted to a broader range of conditions than are the adults. On the contrary, as is well known, the larvae appear during the more favourable part of the year. It is likely, although currents are undoubtedly important in the retention and distribution of larval forms in an estuary, the distribution of the adults is a reflection of some mean range tolerated by them.

The means of dispersal apparently must differ in these two estuaries. The distribution of the adults then must be more a measure of their tolerance of estuarine conditions, rather than simply of a relationship between complex larval behaviour and the current and drift system within the estuary. This proposal is less mechanistic, and perhaps less of a credit to nature, but seems the more plausible one.

If the adults essentially fill the range available to them, ecological analysis of their distribution, correlated with factors of the environment, should suggest what sorts of physiological adaptations have been made. The range of environmental factors differs rather dramatically within an estuary, and species of similar appearance segregate readily in such a system. One might expect rather marked differences in physiological adaptations. This should make differences relatively easy to detect. If salinity is admitted to be a primary factor in governing the distribution of the adults, osmoregulation is the adaptation to investigate first.

B. Physiology

1. *Osmoregulation.*—Osmoregulation in animals has been reviewed by Nicol (1960) and by Prosser and Brown (1961). Special attention has been given the Crustacea by Robertson (1960)

and by Lockwood (1961). In general, marine Crustacea are conformers, remaining essentially isosmotic with their environment. Those that occupy the high littoral zone, and those that penetrate into estuarine conditions, tend to remain hyperosmotic in dilute media, but most of these conform in concentrations above normal sea-water. The so-called semi-terrestrial, and freshwater forms, have the ability to maintain a relative homiostatic state in either dilute or concentrated media.

In that barnacles are successful in both intertidal and estuarine conditions, they might be expected to behave osmotically in much the same manner as other Crustacea. Yet, despite their being relatively well known from an ecological point of view, little is known of their adaptations to estuarine and intertidal situations. Prosser and Brown (1961) list *Balanus balanoides* among the marine poikilosmotic Crustacea. Their reference is to Barnes and Barnes (1958), who say that *Balanus balanoides* normally remains closed in 50% sea-water. But these authors have no data on osmotic behaviour.

The earliest study made on osmotic ability known to me, is that of Borsuk and Kreps (1929). They studied the respiratory rates of *B. balanoides* and *B. crenatus*, in media of different dilutions. In their introduction, they state that these species have the ability to endure salinities from 0-70‰, that from their not yet published experiments they find the body fluids change in response to changes in the external medium, and that these balanids appear to be poikilosmotic animals (conformers). On the other hand, Krüger (1940), while neither citing the work to which he refers, nor stating what species he is considering, says that animals transplanted from sea-water to freshwater endure the latter more than three months, and after this time there is irreversible damage, the animals become edematose and die. In that he is taking a view contrary to that of Borsuk and Kreps, it would seem that whatever barnacle he is referring to regulates in freshwater for as long as three months, after which time regulation breaks down and the animals die. Whether or not this interpretation of Krüger's statement is correct, Belyaev (1949) reports hyper-regulation in *B. balanoides*, *B. balanus*, *B. crenatus* and *B. improvisus*.

Thus, from the literature we have conflicting reports on the osmotic behaviour of barnacles in dilute media: they may regulate or they may conform. Both of these views appear to be right, for in the present study, both types of adaptation have been found to occur, depending upon the species.

Osmotic behaviour in three species of *Balanus* has been investigated. *B. glandula* and *B. amphitrite* are intertidal forms. The former is an indigenous marine species that penetrates into conditions that are predominantly mesoshaline, while the latter has been introduced and is restricted to certain polyhaline reaches of the San Francisco Bay estuarine system. Both species remain hypertonic in dilute media, as would be expected in truly intertidal or estuarine Crustacea. Also it was observed that these two species are relatively resistant to desiccation. Although, *B. glandula* loses water faster than *B. amphitrite*, the individuals were about one-fifth as large, and the greater part of this difference is a function of proportionately greater surface area, rather than a difference in permeability.

Osmoregulation and relatively low permeability are expected in high intertidal or estuarine Crustacea. It was, then, a little surprising to find that *B. improvisus*, a species rather closely related to *B. amphitrite*, should be a conformer rather than a regulator, for it is the most estuarine species in the Bay. Both in the field and in the laboratory, it tolerates dilutions down to 3% sea-water. The ability to survive by conformity in such dilute media is known in no other Crustacea.

Correlated with this remarkable adaptation is an equally remarkable high permeability to water. This species loses water some ten times faster than *B. glandula* and *B. amphitrite*, under identical conditions in the laboratory.

From the foregoing, it appears that estuarine barnacles have taken two approaches in solving the problems of an estuarine existence: that of osmotic regulation and that of near-conformity.

The latter is out of keeping with our general knowledge on how Crustacea behave. Yet, there may be a plausible explanation underlying both of these approaches, and these involve characteristics that set the sessile barnacles apart from other Crustacea.

First, it is clear that, as attached organisms, barnacles must tolerate all the conditions of their immediate environment. For intertidal forms, these conditions may be quite extreme. During unfavourable periods, the intertidal barnacle stops most activities, closes the operculum, and awaits an improvement. During the waiting period, it must resist desiccation, and water-proofing the shell in order to reduce water loss is the usual method. With a relatively water-proof shell, a modest osmoregulatory ability is more effective, and may be advantageous during rainy periods when the animal is exposed. The shutting down of activities, the water-proofing, and osmotic regulation would appear to be "pre-adaptations" to moderate estuarine conditions, since the animal could withstand periods of dilution. However, the survival technique of discontinuing activities under periods of stress would preclude full adaptation to rigorous estuarine or freshwater conditions, for the animal is avoiding carrying out normal activities in dilute media.

We cannot say, of course, why such forms have not overcome this difficulty and evolved the ability to remain active during periods of severe dilution, and to regulate as well. But one would suspect that this may be because of their predominantly intertidal way of life, where selection pressure is towards successfully waiting out unfavourable periods that occur during nearly every tidal cycle.

Subtidal forms, although attached, are not compelled to wait out unfavourable times. Yet they are equipped, in a morphological sense, in the same way as intertidal forms. Whether they are pre-adapted to an intertidal life, or have descended from intertidal forms, is not known, although it does seem likely that the transformation from the pedunculate to sessile form went hand in hand with attainment of a high littoral, although probably not intertidal, existence. In any event, subtidal forms apparently meet the environment in a different way. As Barnes and Barnes (1957) have pointed out, many behave peculiarly when brought out into air; they do not close down, but rather "struggle in an erratic fashion and soon become desiccated." This suggests that they are not behaviourally "familiar" with the waiting out of unfavourable periods. Conceivably, upon entering estuaries, they would continue to carry on normal activities. If they are quite permeable to water as are other marine subtidal Crustacea, selection might favour tolerance to dilution of body fluids. To what degree such a form could become estuarine would then depend upon how much dilution its physiology could adjust.

Balanus improvisus is found at moderate heights in the intertidal, where other species are not present or are marginal inhabitants, but it is also a successful subtidal form. *B. improvisus* would appear, then, to be somewhat of a compromise between a subtidal and intertidal barnacle, with the behavioural adaptation of closing when out of water, but conforming osmotically when submerged in hypotonic media and remaining active during the process. This last behavioural adaptation was quantitatively analyzed by Pora and Rosco (1955) as well as being observed in the present study in the same species. Perhaps then, it is in consequence of its subtidal background that this form has utilized the method of conformity in the estuarine environment. Although this method is unusual among Crustacea, it should not be regarded as a passive adaptation. Considerable physiological adjustment must take place if an animal that virtually conforms is to survive at very low salinities. Apparently, this approach has allowed *B. improvisus* to become the most successful estuarine barnacle known.

Again, we may ask why the most direct method, that of regulating and carrying on normal activities, has not been developed in this group, while it has been strongly developed in so many other Crustacea. Although no really appropriate answer can be suggested here, there is one that seems to have some merit. The sessile barnacle is completely enclosed in its shell, and the shell can essentially seal the animal off from the outside world. Although this arrangement, and the attached mode of life, is unique among the Crustacea, it is quite comparable in a sense, to the situation seen

in many shelled Mollusca. It is certainly this life-form that contributes to the considerable success of these two groups in the high intertidal zone, and both behave in much the same way when conditions are unfavourable. On entering the estuary then, conceivably the mollusc could adapt in much the same way. It is perhaps instructive that the bivalved molluscs, such as *Mytilus edulis* and *Cardium lamarcki*, conform under estuarine conditions (Peterson, 1958). Is it simply that it is more economical for sedentary animals to adapt in this way?

2. *The Mantle Cavity.*—The sessile barnacles are completely surrounded by a mantle, and when withdrawn, the animals reside within a mantle cavity. It has been demonstrated, in *Balanus glandula* and *B. amphitrite*, the mantle cavity fluid is essentially isotonic with the blood, even when the animals are sampled when feeding in hypotonic media. Since it is widely held that the mantle cavity is in communication with the external milieu during feeding (Cannon, 1947; Crisp and Southward, 1961; Monterrosso, 1933), and that the feeding process provides a current within it, the hypertonic state of the mantle cavity fluid is unexpected. A simple mechanism appears to provide a reasonable explanation. It consists of a mechanical separation of the mantle cavity from the exterior, established by the position of the animal when feeding, accomplished by the unique relationships of the trophic apparatus and the thorax and prosoma, to the conical tentorium formed by the opercular valves. When an animal assumes the "high" feeding position, the trophic structures are presented at the margin of the aperture. The underlying fleshy positions of the prosoma and thorax act as a plug against the mantle lining of the opercular valves. In this way, feeding can be carried out with the trophic apparatus completely outside, while the soft body beneath seals the mantle cavity from the external milieu.

The evidence for this mechanism is manifold: the osmotic conditions, observations on animals when feeding in dilute media, and the anatomical relationships observed in diverse genera and species. The concept of this mechanism also gains support from the findings of Crisp and Southward (1961). By photographically recording the movement of currents, they demonstrated during the "fast" feeding beat carried out when *Balanus balanoides* is in the high position, almost no exchange occurs between the mantle cavity and the exterior. They were studying currents with regard to respiration, and were confused by this finding, since they attribute respiratory function to the branchiae lying within the mantle cavity. Clearly, during this most active process, the branchiae would not be involved in respiration because there was no exchange. To account for the inconsistancy, they proposed that the cirri serve as respiratory structures under these conditions.

There are perhaps several desirable attributes in the adaptation of separating the mantle cavity from the external medium while feeding. In marine forms, it would prevent the entrance of debris and other organisms. Conceivably, this would be advantageous for both the animal and the developing larvae within. In estuarine forms that regulate, even weakly, this arrangement prevents the mantle cavity fluids from being diluted during feeding. This reduces work in maintaining a hypertonic internal milieu, and provides a favourable environment for developing young, and for the trophic apparatus when withdrawn from hypotonic conditions. Other attributes are discussed below, with regard to desiccation.

3. *Desiccation.*—The ecology of desiccation in marine invertebrates has received attention by Moore (1958). Special attention has been given the terrestrial Crustacea by Edney (1960). It is generally agreed that intertidal barnacles are subjected to severe desiccation, yet it is surprising that relatively little study has been done on them concerning this matter.

Vaillant (1871) reports that high intertidal specimens of *Balanus balanoides* spend as much as nine-tenths of their time out of water. He found experimentally that this species would survive at least 44 days if protected from desiccation.

Monterosso (1933) studied what he termed "anabiose," or suspension of vital phenomena during unfavourable conditions. He made very detailed observations on morphology, behaviour,

and alterations of internal water spaces during desiccation, of the high intertidal barnacle *Chthamalus stellatus depressus*. In one experiment, animals survived out of water for 119 days, and Monterosso mentions that survival was enhanced by high humidity. However, no temperature or humidity data are given. He concluded that internal water in different parts of the body was used up at different rates in a sequence of steps. In particular, water contained in the extensive excretory sacs (maxillary glands) appeared important and it was given up to the tissues as the animals dried.

Barnes and Barnes (1957) claim that resistance to desiccation is achieved in a variety of ways in different species. They mention that it is frequently stated that resistance to desiccation is achieved by enclosing a small quantity of water in the mantle cavity (no references given). But this, they say, is not so. They go on to discuss differences in behaviour between intertidal and subtidal species, and describe how detailed observations on the intertidal form *Balanus balanoides* revealed active expulsion of quantities of water, over a short period of time after specimens were removed from the sea. They imply that the bulk of the water in the mantle cavity is forcibly expelled, but give no quantitative data to support this observation.

Suzuki and Mori (1963) studied the distribution of mantle cavity, body and shell water, and the differences in rates of desiccation between high and low intertidal populations of *Tetraclita squamosa japonica*. They report that high intertidal individuals contain twice as much extra-visceral (mantle cavity) water, relatively, than do low intertidal individuals. They show that exposed barnacles on hot substrates remain cool, presumably due to evaporation. However, during desiccation, water was lost primarily from the shell, and some from the body tissues, the amount in the mantle cavity remaining relatively constant. From their data, the following percentages and V.P.D.'s can be calculated:

	% water: whole animals	Mantle cavity water as % total water	Water lost
High Intertidal ..	76	43	9% (in 5 hrs.); (V.P.D. 10·9 mm. Hg)
Low Intertidal ..	71	27	4·5% (in 2 hrs.); (V.P.D. 7·5 mm. Hg)

The experiments on desiccation carried out in this study show that in the first six hours, *Balanus glandula* behaves differently when the aperture is plugged than when it is unplugged. In the latter condition, the animals lose water at a substantially faster rate. After 12 hours however, this difference has had little effect on the total water lost, and by the end of 48 hours the amount of water lost is the same. The initially higher rate, in unplugged individuals, seems most likely due to the expulsion of water from the mantle cavity as described by the Barneses.

It has already been described how the intertidal species *Balanus amphitrite* is considerably more resistant to desiccation than is *B. glandula*. This is probably chiefly due to proportionately greater surface area in the latter. However, *B. amphitrite* does not show the same rapid loss in the first few hours. This, and the fact that the mantle cavity holds some 17 rather than 28% of the total water remaining after 24 hours indicates that mantle cavity water is important in survival during desiccation in this species. That is, the predicted and observed values for mantle cavity water remaining after 24 hours differ appreciably, even when the extreme standard errors are allowed, more water being lost from the mantle cavity than from the tissues. This suggests that mantle cavity and body fluids are not in a simple osmotic relationship.

Balanus improvisus, relative to the other species examined, is highly permeable to water. As in *B. glandula*, about 30% of its total water (V.P.D. 14–17 mm. Hg) is lost in an exposure period of some six hours. Longer periods of exposure, and/or higher vapour pressure deficits, would allow more water to be lost. This species apparently lacks the margin of safety seen in the first two species.

Suzuki and Mori (1963) found a substantial difference in the relative amounts of mantle cavity water held by low intertidal and high intertidal individuals of *Tetraclita squamosa japonica*; 27 and 43% of the total water respectively. In the present study on intertidal *Balanus amphitrite*, the mantle cavity contained 28% of the total water. This is quite comparable to the low intertidal *Tetraclita squamosa*, a species with a very different shell structure. On the other hand, low and high intertidal *Tetraclita* contained about the same relative amounts of total water; 71 and 76% respectively, or two to three times as much as the species studied here. The two closely related species, *B. amphitrite* and *B. improvisus*, contained 46 and 44% respectively, while the high *B. glandula* had but 25% of its total weight as water.

Vaillant (1871) proposed there are physiological differences between species of intertidal barnacles regarding their resistance to desiccation. And as suggested by the Barneses (1957), behaviour of the animals is also significant. From the foregoing it is clear, the problem is complicated and detailed studies of each species are necessary if a common basis for the differences is to be found, if indeed there is one.

In this study, it has been assumed that loss of water during desiccation in air is a function of permeability to water. When an animal is placed in hypotonic media, the same relative permeability should allow water to flow in, and presumably salts to flow out, in response to the osmotic and diffusion gradients. If permeability is high, it would be difficult for an organism to regulate osmotically in dilute media. It is interesting that the most permeable species, *B. improvisus*, is also the poorest regulator, and this is in keeping with these initial assumptions.

IX. Summary and Conclusions

1. The region extending from San Francisco Bay proper, through San Pablo Bay and Suisan Bays to the Sacramento and San Joaquin Rivers, constitutes a large estuarine system. The environment at the Bay entrance is marine, with sea-water becoming increasingly dilute until freshwater is reached in the vicinity of Pittsburg and Antioch in the Delta Region.

2. The fauna of the estuary is made up of three components: tolerant marine forms, a primarily brackish-water or estuarine element, and a few freshwater species. Of the barnacles, there are no truly freshwater forms, and those found in the estuary are either confined to it, or are marine invaders. *Balanus glandula*, a marine intertidal species, extends throughout the predominantly polyhaline reaches of the system, tolerating moderate mesohaline conditions for as much as half an annual cycle. Inhabiting the high intertidal, it receives more saline water during high tides than do subtidal forms in the same area when the tide is low, since the estuary is unstratified. *Balanus glandula* penetrates further into the estuary than does its subtidal counterpart *B. crenatus*.

The two species confined to the estuary are *B. amphitrite* and *B. improvisus*. Both are believed to have been introduced by ships. *Balanus amphitrite* is an intertidal form restricted to a few localities that are, for the most part, polyhaline. The somewhat marginal existence of this tropical and warm temperate species is probably best accounted for by a suboptimal temperature regime prevailing in regions of suitable salinity. On the other hand, *B. improvisus*, one of the most "estuarine" barnacles known, is found in both intertidal and subtidal habitats from polyhaline to essentially infrahaline conditions.

If *B. glandula* of the Pacific Coast is regarded as the ecological counterpart of *B. balanoides* of the Atlantic, the distribution of *B. glandula*, *B. improvisus* and *B. crenatus* in San Francisco Bay does not directly correspond to the distribution of *B. balanoides*, *B. improvisus* and *B. crenatus* of the Miramichi Estuary in north-eastern Canada. However, the Miramichi is a stratified estuary, and the apparently greater penetration of *B. crenatus* than *B. balanoides* is due to the greater riverward encroachment of saline water along the bottom than at the surface. When this is taken into account, the distributions of forms in the estuaries is essentially the same.

3. The distribution of adult barnacles in the Miramichi and San Francisco Bay estuaries suggests that the degree of penetration is a reflection of the ability of the respective species to tolerate dilution, rather than the degree to which larvae are retained and distributed by currents within the system. Larvae appear and settle during the more favourable time of the year, and it appears that the area of settlement is more extensive than the range inhabitable by the adults.

4. Osmoregulatory ability, behaviour in dilute sea-water and resistance to desiccation have been investigated in *Balanus amphitrite*, *B. glandula* and *B. improvisus*. The intertidal species *B. glandula* and *B. amphitrite* regulate below 50% sea-water, the former somewhat more strongly than the latter. Both species, but in particular *B. amphitrite*, continue to feed below 50% sea-water, during which time the mantle cavity is maintained hypertonic to the medium and isotonic to the blood. A particular posture assumed under these conditions allows the animals to feed while effecting a mechanical separation between the mantle cavity and the external milieu. This adaptation would appear to aid in preventing entrance of foreign material, dilution of the mantle cavity during osmotic stress, and in controlling the conditions under which the larvae develop. The anatomical arrangement providing for the separation is not seen in the pedunculate barnacles, but rather it correlates with the more highly evolved sessile mode of life.

Osmotic regulation in intertidal and/or estuarine barnacles is in keeping with the sorts of adaptations made by other Crustacea to these habitats. However, in *B. improvisus*, occurring both intertidally and subtidally, blood remains only slightly hypertonic to all dilutions of sea-water, that is, it essentially conforms. This would not be particularly surprising if it were not for the fact that *B. improvisus* is a most successful estuarine barnacle and is known to survive almost indefinitely in 3% sea-water.

5. Relative rates of desiccation correlate for the most part with habitat and osmoregulatory ability. *Balanus glandula* and *B. amphitrite* are considerably less permeable to water than *B. improvisus*. *Balanus amphitrite* and *B. improvisus* are very closely related systematically, yet the latter is some ten times more permeable to water, and its near conformity in dilute media is concomitant with this.

It is commonly held that intertidal barnacles are highly resistant to desiccation and species have been reported to survive out of water for as long as 119 days, at least under conditions of high humidity. Experiments and observations on these three species indicate, at rather modest vapour pressure deficits (14–17 mm. Hg), *B. improvisus* survives but little more than one day, *B. glandula* only two to three days, and *B. amphitrite* six to ten days.

6. It appears that sessile barnacles have taken two different approaches in adapting to estuarine conditions. Strictly intertidal forms are subject to the daily fluctuations of the tides and are thus constantly being conditioned to waiting out unfavourable conditions experienced between high tides. In keeping with their high intertidal habitat, they have modest osmoregulatory ability, and this is put to use in moderately dilute sea-water. When osmotic stress becomes severe, however, they revert to their usual method of riding over unfavourable periods by shutting down until conditions improve. Such behaviour might impede or even preclude penetration into regions of extreme dilution since the animals are curtailing vital activities as a solution to the immediate problem.

Predominantly subtidal barnacles are not adapted to the cyclic exposure by the tides and presumably shut down only for intrinsic reasons or when disturbed. Like other subtidal marine Crustacea, they are probably relatively permeable to water, isosmotic with the environment and without regulatory abilities. They would be expected, then, to be poorly equipped for an estuarine existence. However, *B. improvisus* can adjust to extreme dilution while vital processes continue. This mode of adaptation is the most successful, since it is a fact that where *B. improvisus* occurs in temperate waters, it is the most estuarine of the barnacles.

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OBSERVATIONS ON THE OSMOTIC BEHAVIOUR OF *SPHAEROMA WALKERI*, A STENOHALINE ISOPOD

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ABSTRACT

It is known that the estuarine wood-boring isopods, *Sphaeroma terebrans* and *S. annandalei* are able to tolerate hypotonic conditions confronted in their natural habitat. But in the other Indian species *S. walkeri*, which is exclusively marine in distribution, the limits of tolerance to dilution of the medium has not hitherto been investigated. Experimental studies on the salt and water transport in this marine isopod, when subjected to 8·5‰ and 17‰ sea-water, were made by measuring the changes in volume of the animal and increase in the salt concentration of the medium with the aid of a "conductivity bridge". The volume changes were assessed by the method outlined by Gnanamuthu (1961) without removing the animal out of water. The volume (V) of the isopod was calculated from the loss of weight it sustained through displacement when weighed (W_1 and W_2) in sea-water of two different densities (d_1 and d_2), applying the formula $V = \frac{W_2 - W_1}{d_2 - d_1}$.

It was found that in both the media used salt loss appeared to be irrecoverable and proved lethal. That there is no evidence of absorption of salt at any stage may indicate the stenohaline character of this isopod. It was of interest to note that at definite levels static conditions prevailed in the transport of water and salt for periods ranging from one to twelve hours. The factor responsible for this feature is obscure.

INTRODUCTION

Of the four Indian species of the wood-boring isopod genus *Sphaeroma*, *S. triste* and *S. walkeri* are exclusively marine in their distribution whereas *S. annandalei* and *S. terebrans* occur in the mouth of rivers Vellar and Puduar in the east coast of India. This distribution of the Indian species, as also evidenced from the reports of occurrence of these species by earlier authors (Erlanson, 1936; Pillai, 1955; Becker, 1958; George, 1963, 1964; and Purushotham, 1955–59) has prompted the present study of the limits of tolerance of dilution as well as endurance under unfavourable osmotic conditions. The present paper deals with the stenohaline behaviour of *S. walkeri*.

Though several authors have studied the osmotic behaviour of Crustacea, most have confined their attention to the larger decapod crustacea (Krogh, 1919; Beadle, 1957; Lockwood, 1962). Further, these authors studied the changes in weight by measuring them in air after towelling the adherent water with filter-paper (Margaria, 1931; Hukuda, 1932; Schwabe, 1933). The smaller crustaceans such as the isopods can be studied only by more precise methods, since they suffer mutilation of appendages during towelling and since a larger number of individuals may have to be used for any determination.

In the present study the volume has been determined without removing the animal out of water. This method also facilitated a closer analysis of the osmotic response, that is, transport of salt and water in these stenohaline isopods in anisotonic media.

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Methods

(a) *Determination of Exact Initial Volume*

The accurate volume of these small living isopods in water at regular intervals was determined by applying the principle outlined by Gnanamuthu (1961). A polythene basket is suspended by a nylon thread from the left arm of the balance and immersed in a known volume of 100% sea-water in a beaker. After determining the weight of the basket in sea-water, the animal is placed in the basket and the weight of the animal in 100% sea-water is obtained. One-fourth of the sea-water is replaced by filtered distilled water and stirred to make it 75% sea-water. The weight of the basket and the animal in 75% sea-water is found. The animal is immediately removed and the weight of the basket is found to derive the weight of the animal alone. The apparent weight of the animal in the 100% sea-water is W_1 and in 75% sea-water W_2.

The densities of the above two media (d_1 and d_2) are found by using specific gravity bottles. The volume of the animal was first calculated from the loss of weight the animal sustained through displacement when weighed in sea-water of two different densities, applying the formula

$$V = \frac{W_2 - W_1}{d_1 - d_2}.$$

Since a preliminary weighing in the two media of chosen densities showed that within the five minutes required for weighing in the dilute medium there is no osmotic intake of water, it may be taken for granted that the two weights of the isopods in sea-water as well as in dilute water is the same. This very necessary assumption was tested to be true by a retransfer to sea-water and weighing the isopods in sea-water. It is probable that these crustaceans take a much longer time, about fifteen minutes at least, to recover from the shock of transfer to anisotomic media.

(b) *Estimation of Volume Changes in Hypotonic Media*

After determining the initial volume, the isopods encased in the polythene basket, were kept immersed for a while in sea-water of salinity 17‰ and then transferred to the experimental medium of 200 c.c. of 17‰ sea-water. Sea-water with salinity of 17‰ and 8·5‰ were prepared by addition of distilled water. The volume of the isopods subjected to lower dilutions was determined every fifteen minutes from the displacement and from this the percentage increase in volume was calculated.

(c) *Measurement of Salt Transport*

The salinity changes in the experimental medium were traced by titration and also by employing the Mullard's conductivity bridge with its accessory dip type immersion cell. The resistance offered by the medium, expressed in μmhos $\times 10^4$, for sea-water of 10‰, 20‰, 30‰ and 40‰ salinities were found out five times in each case and a standard curve showing the linear relationship between salinity and the conductivity bridge readings was statistically drawn on a graph sheet. The immersion cell was dipped into the experimental medium and during the course of experiments the salinity changes were followed by noting the resistance reading (μmhos $\times 10^4$) in the conductivity bridge. From these readings the exact change in the salinity of the medium was traced out with the aid of the standard curve.

Experiments

Tolerance of S. walkeri *to Changes in Salinity*

Salt and water transport in two different hypotonic media (Sea-water of 8·5‰ *and* 17‰).—Ten isopods of 10 mm. length were left in the polythene basket (*vide* Material and Methods) suspended

from the arm of the balance. The basket dipped in 200 c.c. of the medium of sea-water and the true volume of the ten isopods was first determined. The salinity of sea-water was next altered to 17‰. The changes in the volume were recorded every hour upto 48th hour, every two hours upto 112th hour. Side by side with these volume determinations, the changes in the salt concentration were followed by dipping the immersion cell of the Mullard's conductivity bridge. Since the isopods died in the medium of 17‰ about the 112th hour, the determinations were stopped. The experiment was repeated, using a medium of 8·5‰ and in this case, the isopods collapsed after 18 hours. The data regarding the percentage increase in the volume due to osmotic entry of water in medium of 17‰ and 8·5‰ are set forth in Tables I and II. The corresponding loss of salt in these media is shown in Tables III and IV.

TABLE I

Percentage increase in volume in Sphaeroma walkeri *in sea-water of* 17‰

Hours	Volume of one isopod (average of 10) in c.c.	Percentage increase in initial volume
0 (initial)	0·0416	0
1, 2	0·0416	0
3, 4, 5	0·0444	6·73
6, 7, 8, 9	0·0485	16·58
10, 11, 12	0·0625	50·34
16	0·0694	66·80
17	0·0625	50·34
18, 19, 20, 21, 22	0·0570	37·02
23	0·0555	33·43
24, 25, 26, 27, 30, 31	0·0564	33·17
32, 33, 34	0·0530	27·40
35, 36, 37, 38	0·0512	23·07
39, 40, 43, 44, 45, 46, 47, 48, 49, 51	0·0542	30·28
52, 54, 56, 58, 60, 62, 64	0·0485	16·58
66, 68, 70, 72, 74, 76, 82, 88, 90, 92, 104, 106, 108, 110, 112	0·0416	0

RESULTS

From the tables it will be obvious that during the first two hours there is no change in the water and salt content. Thereafter, the isopods survive till the 112th hour in 17‰ and collapse within 18 hours in the more dilute medium. From the tables it will be clear that in 17‰ the maximum osmotic entry of water is upto the 18th hour and this is roughly the same period during which the animal survives in the more dilute medium. In both media water enters the body to the same extent, namely 66–68% of the initial volume. In the medium of 17‰, however, after this 17th hour, the volume is reduced, probably due to the output of water by the kidneys and the isopods regain the original volume about the 66th hour. But the collapse of the animal is

TABLE II

Percentage increase in volume in Sphaeroma walkeri *in sea-water of* 8·5‰

Hours	Volume of one isopod (average of 10) in c.c.	Percentage increase in initial volume
0 (initial)	0·0532	0
1, 2, 3	0·0532	0
4	0·0552	3·06
5	0·0584	7·96
6, 7	0·0592	9·20
8	0·0624	14·11
9	0·0690	25·00
10	0·0738	32·50
11	0·0784	39·90
12	0·0832	47·40
13, 14	0·0860	51·90
15	0·0920	61·40
16	0·0940	64·56
17, 18	0·0964	68·30

TABLE III

Salt loss from Sphaeroma walkeri *in sea-water of* 17‰

Hours	Conductivity reading (μ mhos$\times 10^4$)	Salinity in 200 c.c. of 17‰ sea-water in gm.	Amount of salt lost by isopod in mg.
0, 1, 2 (initial)	1·45	3·400	..
3, 4, 5, 6	1·46	3·430	3·0
7, 8, 9, 10	1·465	3·445	4·5
11, 12, 13, 14, 15, 16, 17, 18	1·470	3·450	5·0
19, 20, 21, 22	1·490	3·485	8·5
23, 24, 28, 36	1·500	3·510	11·0
40, 42, 44, 48, 50, 52	1·520	3·550	15·0
55, 57, 59, 61, 63, 65, 68, 70, 74, 78, 82, 90, 92, 94, 96, 98, 104, 106, 108, 112	1·523	3·560	16·0

clearly due to the loss of salt, to the extent of 16 mg. upto the 18th hour and becomes more marked later. Table III shows that the salt loss is slow But in the more dilute medium the rate of

TABLE IV

Salt loss from Sphaeroma walkeri *in sea-water of* 8·5‰

Hours	Conductivity reading (μ mhos $\times 10^4$)	Salinity in 200 c.c. of 8·5‰ sea-water in gm.	Amount of salt lost by isopod in mg.
0, 1 (initial)	0·850	1·70	..
2	0·860	1·72	2·0
3	0·880	1·76	6·0
4	0·890	1·78	8·0
5	0·895	1·79	9·0
6	0·900	1·80	10·0
7, 8	0·915	1·83	13·0
9	0·920	1·84	14·0
10, 11, 12	0·930	1·86	16·0
13, 14	0·950	1·90	20·0
15, 16, 17, 18	0·970	1·94	24·0

salt loss is markedly rapid. In four hours as much salt as 8 mg. is lost. In the more dilute medium by 17th hour 24 mg. is lost. From these it will be obvious that salt loss proves lethal in both cases.

It will be clear from the above two series of experiments on endurance of the isopods to dilutions of the medium that the isopods survived normally for about 17 hours in 17‰ medium. Salinity changes in the Madras harbour recorded throughout the year ranged from 28‰ to 32‰. Since the maximum dilution is only upto 28‰ and exceptionally upto 19·45‰ (Muthu, 1955), the isopods can easily survive for twelve hours when changes in tide will restore the salinity again.

REMARKS

Though the osmotic behaviour of the stenohaline isopods has not excited as much biological interest as the osmotic behaviour of euryhaline isopods, yet because of the methods of measuring, the continuous water transport and salt transport used in the present study, the pattern of behaviour sheds light on osmotic regulation in general. The data of transport of water and salt given in Tables I–IV, are illustrated in Figs. 1 and 2. From those graphs the pattern of osmotic behaviour of the stenohaline isopods will be evident. We can draw the following inferences:—

(i) The salt transport is much slower than that of water for about 20 hours. Thereafter salt loss is heavy. The fact that there is no evidence of gain of salt at any stage indicates the stenohaline character of these isopods.

(ii) In both salt and water transport, there are definite levels at which the transport is suspended, probably by effort. The escape of salt and water and the entry of water are not uniform and continuous as in a physical system. The changes in the volume clearly indicate that osmotic effort is spasmodic and not sustained.

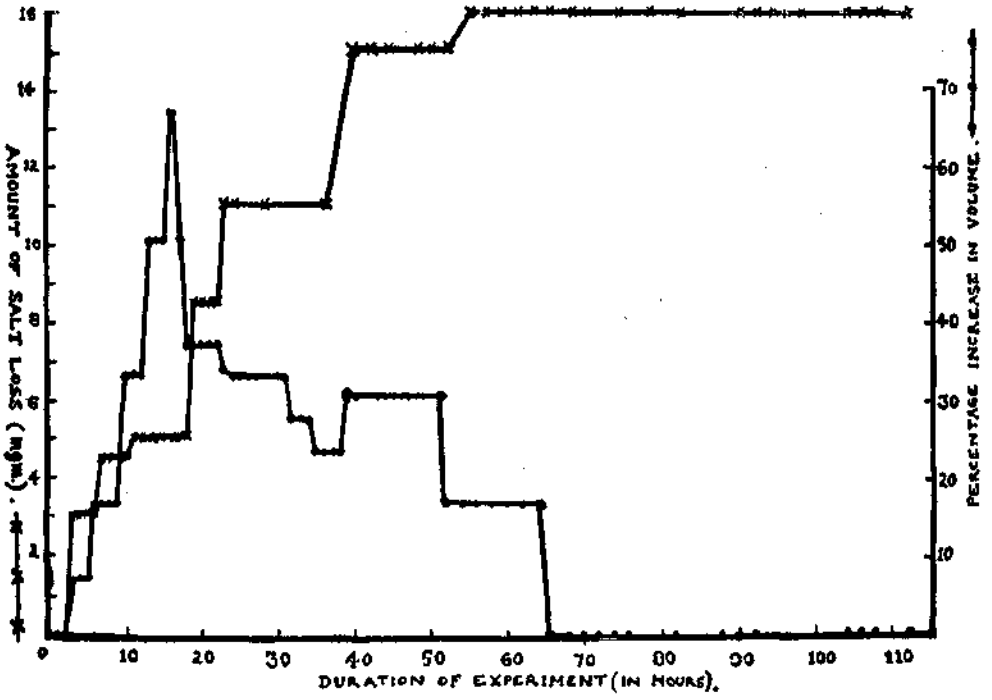

FIG. 1. Percentage increase in volume and loss of salt in sea-water of 17‰.

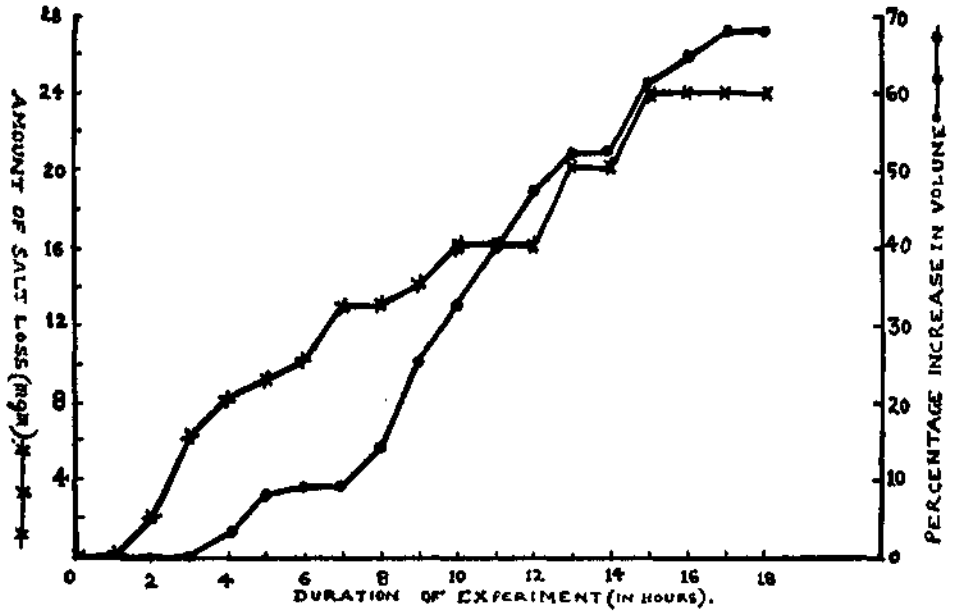

FIG. 2. Percentage increase in volume and loss of salt in sea-water of 8·5‰.

(iii) The loss of water is definitely controlled after the 16th hour, probably through the activity of the kidneys. From the 66th hour to the 112th hour when it dies, the amount of water in the body has been restored to the normal but the animal collapsed because of having lost salt to an irrecoverable degree.

(iv) The stepwise loss of salt and water transport suggests the possibility of some obscure factor, either nervous or endocrinal, responsible for the static conditions.

Acknowledgements

I have great pleasure in acknowledging my indebtedness to Prof. C. P. Gnanamuthu for suggesting this problem and guidance. I also wish to express my gratitude to Dr. A. Purushotham, Officer-in-charge, Wood Preservation Branch, F.R.I. and Colleges, Dehra Dun and Prof. G. Krishnan, Director, Zoology Department, University of Madras, for their help and encouragement.

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Discussion

Dr. A. N. P. Ummerkutty: Won't it be better to give the true salinities instead of the percentage of sea-water?

Dr. R. Y. George: In fact 100% sea-water in this paper refers to a salinity of 34‰.

Mr. K. N. Sankolli: What does the term "Collapse" mean?

Dr. R. Y. George: Stimulation with a needle was used and if no response was obtained, the animal was assumed to be dead.

Dr. N. B. Nair: Could this effect be a case of suspended animation in the adverse conditions, with recovery following a return to better conditions.

Dr. R. Y. George: At the end of the experiment the animals were returned to the normal medium and no recovery occurred.

PIGMENTS OF EUPHAUSIID EYES

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ABSTRACT

Pigments in the compound eyes of the euphausiid crustacean *Meganyctiphanes norvegica* include astaxanthin, ommochromes, melanin, rhodopsin and possible pterins.

Astaxanthin in the region of the basement membrane has been shown to migrate towards and away from the bases of the rhabdomes in response to light and dark adaptation respectively. Both proximal and distal screening pigments, which also migrate under these conditions, appear, from histochemical tests, to be melanin in the eyes of the euphausiids, whereas in those of pelagic decapods the distal pigment between the crystalline cones is melanin but the proximal pigment around rhabdomes is not. The presence of melanin in extracts from euphausiid eyes has been confirmed from its infra-red absorption spectrum and chemically by Ehrlich's reaction. Ommochromes, probably ommins, have been extracted from euphausiid eyes, but their precise location has not yet been determined.

A homogeneity test by Dartnall's method of partial bleaching on a 2% digitonin extract of the visual pigment from the eyes of *M. norvegica* indicates that the difference spectrum, produced by bleaching with white light, with its absorption maximum at 465–470 mμ, may consist of a major component with absorption maximum at 454 mμ, sensitive to light of wavelengths about 490 mμ, and a minor component, with absorption maximum at 481 mμ, sensitive to red-light at 630 mμ. This finding is the subject of further study.

Attempts have also been made to determine whether any photo-sensitive pterin pigments are present in these eyes, but so far without success.

THREE physiological categories of pigments occur in the compound eyes of crustaceans. Of these three types, one is light-reflecting and the other two light-absorbing. In one of the latter groups reversibly photolabile substances function as visual pigments, and in the other substances absorbing light partially or entirely form screening layers within the eye.

According to Kleinholz (1959) the white reflecting pigment in the eyes of decapods consists of a mixture of several components some of which have been identified as pteridines and purines. There appears to have been no reference to the occurrence of reflecting pigments in euphausiids.

A visual pigment was first observed in an euphausiid by Kampa (1955) who reported the existence of euphausiopsin, with absorption maximum at 462 ± 1 nm., in the eyes of *Euphusia pacifica*. Fisher and Goldie (1959) found a similar pigment in digitonin extracts from the eyes of *Meganyctiphanes norvegica*, with absorption maxima in the range 460–465 nm. Retinene was liberated during the bleaching of this pigment, indicating it to be a rhodopsin. Subsequently these authors (Fisher and Goldie, 1961) reported the occurrence of rhodopsins in other euphausiids with absorption maxima as follows: *Thysanoessa raschii* 460–465 nm.; *Thysanopoda acutifrons* 480 nm.; *Nematoscelis megalops* 465 nm.; and *Stylocheiron maximum* 470 nm.

Previous work on the screening pigments of euphausiid eyes appears to be confined to the somewhat inconclusive results of Fisher and Goldie (1961).

METHODS

(*a*) *Reflecting and screening pigments.*—Since normal histological techniques involve the use of fat solvents and consequent removal of the fat-soluble pigments, sections of eyes fixed in 1%

* We regret to inform his passing away.—Editor.

formalin in sea-water were cut by freezing microtomy, permanent records being obtained by colour photomicrography using either transmitted light or dark-ground illumination. In this way the distribution of astaxanthin in the eye was determined (Fisher and Goldie, 1959). Melanin could be located in such sections by the method of Stinson, Lea, Sunbury and Ford (1959), in which the sections were floated several times on dilute ammonia in tap-water (10–12 drops in 50 ml) and then on silver nitrate solution (1 g. in 50 ml tap-water). The reaction was not entirely specific for melanin but provided, with other reactions mentioned below, cumulative evidence of its anatomical distribution. By irrigation of sections treated by the Stinson method with ethanol to denature any proteins combined with carotenoids, for example as rhodopsin, these fat-soluble pigments could be removed by washing with light petroleum (b.p. 40–60° C), leaving the black silver granules to mark the position of the melanin.

Other tests for identifying melanins or for distinguishing melanins from ommochromes were applied to sections cut from eyes embedded in ester wax. Only two such tests produced clear-cut results. The first, devised by Lillie (1957), probably depends on chelation of Fe^{++} by some part of the melanin complex, resulting in the production of a dark green colour when potassium ferricyanide is subsequently applied. The second is the Bodian reaction, as used by Dublin (1943), whereby silver in the silver-protein complex, protargol, is reduced so that melanin appears black.

A method for the extraction of melanin (Bonner and Duncan, 1962) was also applied to a batch of eyes from *Meganyctiphanes norvegica*. They were subjected to trypsin digestion at 37° C. for one week, followed by hydrolysis with 6N HCl. Humin from trypsin was separated from the remainder of the black insoluble residue by refluxing with tetrahydrofuran, which dissolved humin and was miscible with water, so that a water-free sample of melanin could be obtained. The dried pigment was removed from the flask with carbon tetrachloride and prepared on potassium bromide discs for examination by infra-red absorption spectrophotometry, using commercially prepared melanin (L. Light & Co.) as a control.

The possible occurrence of ommochromes in euphausiid eyes has been investigated, using the techniques of Butenandt and his co-workers at Munich. To study the possible occurrence of ommins, macerated eyes from *Meganyctiphanes norvegica* were extracted with benzene, acetone and methanol to remove lipid-soluble materials, and then with 2% hydrochloric methanol. Sulphur dioxide was passed into the solution, which was subsequently evaporated to dryness under reduced pressure on a steam-bath. The residue was taken up in 50 ml. ethanol and re-precipitated with a few drops of concentrated hydrochloric acid. The precipitate was removed by centrifuging and re-dissolved in ethanol. This purification procedure was repeated several times and the final precipitate was dissolved in M/15 phosphate buffer pH 7·38. The absorption spectrum of the solution was determined over the wavelength range 300–600 nm.

An attempt was made to detect xanthommatin in eyes of *Meganyctiphanes norvegica*, by grinding 500 pairs with 2 N hydrochloric acid. After centrifugation, the supernatant was mixed with *n*-butanol in the ratio 3:5. Sulphur dioxide was bubbled for 45 minutes through the mixture which was then shaken for the same period, and finally centrifuged. The absorption spectrum of the supernatant was determined against *n*-butanol in the range 300–600 nm.

(*b*) *Visual pigments.*—Groups of light- or dark-adapted eyes were finely ground in McIlvaine's buffer pH 4·6 and centrifuged. The supernatant was discarded and the whole process repeated. The residue was ground in a 4% solution of alum and again centrifuged. After being washed with 0·9% saline, the residue was freeze-dried and then repeatedly extracted with light petroleum (b.p. 40–60° C.) until all colour due to carotenoid pigments was removed. After being dried under vacuum the residues were washed with phosphate buffer pH 6·4 and then with 0·9% saline. The centrifuged residues were treated with 2% aqueous digitonin. After suitable periods of extraction the pH of the extracts was adjusted to 9·3 with sodium carbonate solution. Absorption spectra of extracts in the range 300–600 nm. were determined before and after various light bleaching procedures.

The possible occurrence of photolabile pterins, of the type reported in the eyes of the blue-bottle fly *Calliphora erythrocephala* by Autrum and Langer (1958), was investigated in acid and alkaline extracts of eyes of euphausiids by the paper chromatographic methods of these workers and of Ziegler (1960).

Results

(*a*) *Reflecting and screening pigments.*—Examination of dissected eyes of *Meganyctiphanes norvegica* and *Thysanoessa raschii* and of histological sections of eyes from several species of euphausiids revealed no evidence of a white reflecting pigment layer of the type described by Knowles (1950) in the decapod *Leander serratus*.

In the dark-adapted euphausiid eye astaxanthin is concentrated in a dense layer just proximal to the rhabdomes with a dark-brown pigment surrounding the bases of the rhabdomes and another around the crystalline cones (Fisher and Goldie, 1959). In the light-adapted eye, the astaxanthin moves up to surround the bases of the rhabdomes and the proximal dark-brown layer forms a screen at the distal ends of the rhabdomes. The distal pigment surrounding the crystalline cones remains unchanged in position.

When the method of Stinson *et al.* (1959) was applied to frozen sections cut from eyes of *Meganyctiphanes norvegica*, reduced silver granules appeared in the positions of the proximal and distal pigments, indicating these to be composed of melanin. After removal of astaxanthin, the only other pigmented area in the eye was that of the rhabdomes which remained red in colour.

Application of the Lillie and Bodian reactions to sections from eyes of three species of euphausiids, eight species of decapods, and one mysid gave the results shown in Table I. Both reactions confirm the presence of melanin in proximal and distal pigments in the eyes of *Meganyctiphanes norvegica* and of *Stylocheiron maximum*, although the latter species appears to possess very little

Table I

Histochemical detection of melanin in crustacean eye-screening pigments

Species	Lillie reaction		Bodian reaction	
	Distal pigment	Proximal pigment	Distal pigment	Proximal pigment
Meganyctiphanes norvegica ..	+	+	+	+
Stylocheiron maximum ..	+	+	+	+
Nematoscelis megalops ..	+	−	+	+
Pandalus bonnieri ..	+	+	+	−
Crangon allmani ..	+	−	+	−
Systellaspis debilis ..	+	−	+	−
Acanthephyra purpurea ..	+	−	+	−
Sergestes arcticus ..	+	−	+	−
Amalopenaeus elegans ..	+	−	+	−
Pasiphaea sivado ..	+	−	+	−
Ephyrina hoskyni ..	+	−	+	−
Eucopia sculpticauda ..	+	+	+	+

proximal pigment. The absence of a positive Lillie reaction in the proximal pigment of *Nematoscelis megalops* is at variance with the positive Bodian reaction given by the same pigment. It is interesting to note that the proximal pigment in the eyes of all the decapods except *Pandalus bonnieri* did not appear to be melanin and also that both distal and proximal eye pigments in the mysid were melanin.

The extract prepared according to the technique of Bonner and Duncan (1962) was examined in the infra-red spectrophotometer by my colleague, Dr. J. D. S. Goulden, of the Physics Department, National Institute for Research in Dairying, who showed that its absorption spectrum was more closely similar to those obtained by Bonner and Duncan for melanin from *Arenicola* and *Holothuria* than to that from the control prepared from commercial melanin. Further evidence that the euphausiid eye pigment was melanin resulted from the application of Ehrlich's reaction (Fellers and Clough, 1925) in which a characteristic wine-red colour due to the presence of an indole nucleus was produced, with an absorption maximum in ethanol of 549 nm., compared with 550 nm. for the same product from commercial melanin. The absence of tryptophan was confirmed by the absence of a petroleum ether-soluble pink colour when the pigment was boiled with diluted bromine water.

The extract prepared from eyes of *Meganyctiphanes norvegica* for ommochrome study exhibited an absorption spectrum, in M/15 phosphate buffer pH 7·75, with a maximum at 525 nm. compared with those for ommin recorded by Butenandt, Biekert and Linzen (1958) at pH 7·5 of 520 nm. and by Fuzeau-Braesch (1960) at pH 8·0 of 530 nm. It is evident that the absorption peak depends upon pH. Absorption curves exhibited by alkaline degradation products of the ommin in alkaline ethanol indicated the presence of 2-amino-3-hydroxy-acetophenone and xanthurenic acid, but so far attempts to prepare the acid degradation product, 3-hydroxykynurenine, by the technique of Butenandt *et al.* (1958) have been unsuccessful.

The absorption maximum of the extract prepared by the hydrohloric acid and *n*-butanol treatment showed λ max. at 493 nm. compared with a value of 492 nm. for xanthommatin in this solvent, reported by Butenandt, Biekert, Kübler and Linzen (1960). Further purification of the pigment to confirm its identity has not so far been achieved.

(*b*) *Visual pigments.*—Table II shows the results of 84 experiments with eye extracts of *Meganyctiphanes norvegica* in which, despite various initial bleaching conditions, the final treatment has always been exposure to a 5, 6 V, 30 W tungsten filament bulb for at least 5 minutes, which would be adequate to bleach rhodopsin. Despite the relatively small numbers of experiments in each month, there is some indication that bleaching occurred more readily in the summer months.

Examination of absorption spectra of extracts of eyes from euphausiids caught in winter indicated that absorption in the wavelength range 400–550 nm. was lower in relation to that below 400 nm. than it was in extracts from eyes of those caught in summer, the difference apparently being due to the presence of much more rhodopsin in summer than in winter.

The homogeneity test described by Dartnall (1957) using successive bleachings at different monochromatic wavelengths was applied to an extract from the eyes of *M. norvegica* caught in July 1964. The absorption spectrum of the unbleached extract was determined over the range 280–600 nm. The extract was then exposed to light of wavelength 630 nm. for 65 min. The difference curve between the absorption spectra of the extract before and after this treatment exhibited λ max. at 485 nm., after correction according to Dartnall (1962). The extract was stored in darkness for 40 min. and then re-examined in the spectrophotometer. During this period the loss of absorption was maximal at 502 nm. with a corresponding increase at around 382 nm. corrected values. On further exposure to light of wavelength 490 nm. for 10 min. there was considerable bleaching with a maximal peak in the difference curve at 452 nm. but with a second peak at 492 nm. After a final 10 min. exposure to white light the integrated curve for bleaching by all the light sources combined showed a corrected absorption maximum at 462 nm.

So far no photolabile pterins have been found in extracts from eyes of *M. norvegica* by the techniques of Autrum and Langer (1958) or of Ziegler (1960). A number of fluorescent substances have been separated by their paper chromatographic methods, but none has exhibited a positive murexide reaction characteristic of pterins.

TABLE II

Seasonal variation in light bleaching of extracts from eyes of Meganyctiphanes norvegica

Month specimens collected	Number of extracts examined	Number of extracts bleached	Percentage of extracts bleached
January ..	6	2	33
February ..	10	1	10
March ..	2	1	50
April ..	4	1	25
May ..	9	6	67
June ..	4	4	100
July ..	9	7	78
August ..	4	2	50
September ..	12	3	25
October ..	5	2	40
November ..	16	2	12
December ..	3	1	33

DISCUSSION

As Zimmer and Grunner (1956) have pointed out, the euphausiid eye produces a superposition image thereby exploiting the light rays better than an eye that produces an apposition image. The perimeter of the euphausiid eye contains beneath the cornea a melanin screen through which project the crystalline cones, forming the dioptric system. Similarly in the light-adapted eye the tip of each rhabdome is surrounded by a screen of melanin. Kampa (1964) has postulated the existence of a filament of high refractive index connecting the proximal end of each crystalline cone to the distal end of the rhabdome opposite to it, with the suggestion that these filaments act as light guides. The function of the astaxanthin in the light-adapted eye would presumably be to screen the nerve fibres associated with the rhabdomes by absorbing stray light, particularly at about its absorption maximum of 490 nm., this wavelength being very close to the absorption peak of the visual pigment. It is perhaps significant that the photophore of the eye of *Meganyctiphanes norvegica* is surrounded by a dense layer of screening pigment, including astaxanthin, that may well be concerned in absorbing any light reflecting towards the photoreceptors from the luminescent organs. Boden and Kampa (1959) have demonstrated that this luminescence in *Thysanoessa raschii* has an emission spectrum maximal at 476 nm. and in *Euphausia pacifica* at 472 nm. The light emitted by the photophores of *Meganyctiphanes norvegica* is similar in colour to that emitted by *Thysanoessa raschii* and so presumably would be absorbed by the astaxanthin screen.

The location and function of the ommochromes has not so far been elucidated. Butenandt *et al.* (1960) drew attention to difficulties in the histochemical detection of ommatins. The only pigmentation unaccounted for in the frozen sections after removal of the carotenoids and location of the melanin was the reddish-pink colour of the rhabdomes themselves but whether or not this was due to ommochromes is not known.

Obviously, the possibility of the occurrence of more than one visual pigment in euphausiid eyes, suggested by the results merits further investigation. Unfortunately, a further experiment using extracts from eyes of animals collected in October 1964 was unsuccessfulg owing to the relatively smaller amount of rhodopsin present than in the July extracts, indicatin the need to continue these studies next summer.

In various aspects of this work I am much indebted for experimental assistance to Mrs. E. H. Evans (nee Goldie), B.Sc., Mrs. J. M. Martin (nee Fitt), B.Sc. and D. J. Pritchard, B.Sc.

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Discussion

Dr. L. B. Kirschner: Can the pigments so far found in these Euphausiacean eyes be related to a particular function in the organ?

Dr. L. R. Fisher: It is known that the melanins form screens in the eye. Thus in light-adapted eyes the crystalline cones become surrounded by melanin so that the light is funnelled. Kampa thinks that a filament of pigment connects the rhabdomes and crystalline cones acting as a passage way for the light. We have no idea of the function of ommochromes. It is interesting that at 100 fathoms, where the animals may be caught, the activity spectrum of the light is at above 470 μ. The photophores produce light with a maximum at above 470 μ and the visual pigments have a maximum absorption at 460 μ. This is a close correspondence but no real explanation is offered for this.

Dr. J. H. Wickstead: When do the pigments appear during development?

Dr. L. R. Fisher: This is not known since the larvae have not been examined from this point of view.

Dr. J. H. Wickstead: I could think that an examination of *Pseudoeuphausia latifrons* would prove useful since this species shows very different habits from those of other euphausiids.

Dr. L. R. Fisher: This would certainly be interesting.

Dr. S. Z. Qasim: Can the findings be related to optomotor responses at all?

Dr. L. R. Fisher: We have made no attempts to record movements of the eyes.

RESPIRATORY BEHAVIOR OF THE FRESHWATER CRAB *PARATELPHUSA MASONIANA* (HENDERSON) UNDER DIFFERENT CONDITIONS

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ABSTRACT

The freshwater crab *Paratelphusa masoniana* Henderson is commonly available along the banks of the rivers and ponds of this locality. It is amphibious in nature. The course of the water current through the body of the animal was determined by putting the living crab in a water filled trough containing carmine particles. For studying the activity of respiration, an apparatus was designed so that the entire water expelled through the exhalent pore within specific period could be measured. It was found that the temperature of the water affects the rate of respiration considerably. The animal ceases to respire at temperature below 16° C. and above 45° C. Normally, the rate of respiration increases with the rise of temperature. The light also has some effect on respiration. It was found that the rate decreases when the crab is kept in intense light. When the crab is kept in darkness, the rate of respiration decreases considerably. It was also found that the rate of respiration is more in larger specimens.

INTRODUCTION

THE freshwater crab *Paratelphusa masoniana* is of some medicinal and food value. The animal is very common on the banks of rivers and ponds in this locality. Although considerable work has been done on the morphology of the respiratory organs in crustacea, very little is known about their respiratory behavior. Edwards (1946) and Clark (1955) have discussed the respiratory behavior of some arthropods. Ellenby (1951) has correlated the body size with oxygen consumption in *Ligia oceanica*. Recently, Arudpragasam and Naylor (1964) have studied the oxygen consumption and respiratory rhythm in a marine crab *Carcinus maenas*. As the respiratory behavior of none of the Indian crab has been studied so far, the present investigations were conducted.

MATERIAL AND METHODS

About twenty specimens of the freshwater crab, *Paratelphusa masoniana*, were procured from the river Kalinadi, and were kept in an aquarium under artificial conditions.

Before proceeding with the experiments on the respiratory behavior under different conditions, the course of the respiratory current was determined. To study the course of the water current the carapace of the crab was removed and it was kept in a trough filled with water to which Carmine particles were added.

To calculate the rate of water expulsion during respiration a water-tight apparatus was prepared using a rubber tube and a french letter open at both the ends. One end of the french letter was tied with a rubber tubing which was placed in a large measuring cylinder. The cephalothorax of the experimental crab was inserted into the other end of the letter. Thus the water coming out through the exhalent aperture in a given time was collected into the cylinder. The water collected in a fixed time from the crabs under different conditions gave a fairly good idea of the respiratory activity of the animal.

RESPIRATION

In the crab *Paratelphusa masoniana*, respiration is mostly aquatic but some times aerial respiration also takes place. During the course of studies on the morphology and physiology of the above crab, it was observed that it is well adapted for an amphibious life. It requires only a small amount of water for respiration. That is why their occurrence has been reported even from places far from the banks of rivers and ponds (Ramkrishna, 1950).

The respiratory organs of *Paratelphusa* include 7 pairs of gills, scaphognathite of the maxilla and the epipodite of the 1st maxillipede. The gills are arranged on the two skeletal heaps (S.H.), situated one on either side of the heart (Fig. 1).

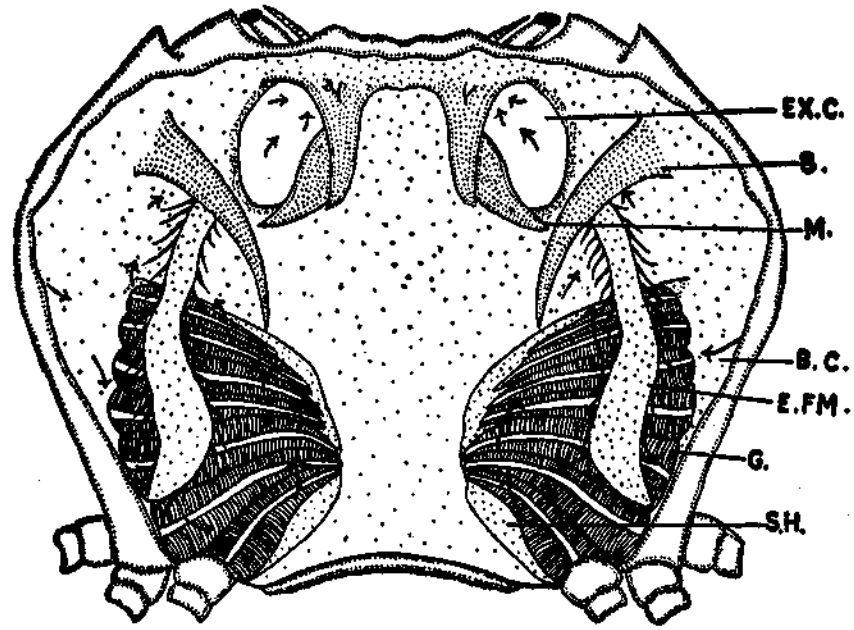

FIG. 1. Respiratory organs of *Paratelphusa masoniana*, showing the way of respiratory current. B.C., branchial chamber; EX.C., exhalent chamber; E.FM., epipodite of first maxillipede; G., gill; M., mandible; S., skeletal piece; S.H., skeletal heap.

Before starting the experiments the course of the respiratory current was determined. To study the course of respiratory current the crab, with its cephalothoracic carapace removed, was kept in a water filled trough containing carmine particles. After some time it was noted that water entered the branchial chamber through openings situated at the bases of the chelipede and of the last walking leg. The basal portion of the epipodite of the first maxillipede acts as a valve to close the inhalent aperture while the movements of blade-like portion of the rest of the epipodite, which covers the dorsal surface of all the gills, accelerates the incoming water current and also removes the foreign particles which may adhere to the gills. The same process is described by Lim (1918) in *Carcinus maenas*. However, the scaphognathite helps in the circulation of water from the branchial chamber towards the exhalent chamber.

The scaphognathite hangs through the roof of the exhalent chamber. It moves rapidly so as to attract the water present in the respiratory chamber. This water which is deficient in oxygen is passed out in the form of a water jet through the exhalent opening, situated just below each orbit, on either side of the mouth, above the 3rd maxillipedes.

RESPIRATORY BEHAVIOR

To sutdy the effect of physical factors on the respiratory behavior of *Paratelphusa masoniana* the apparatus described above was used and the water expelled in an hour by the animal subjected to experimentation was collected to see the comparative rate of respiration under different conditions.

Effect of temperature

It has been observed that the optimum temperature for the activity of the crab lies between 25° C. and 35° C. When the temperature of the water was decreased by adding ice, it was noted that the process of respiration slowed down and at 15° C. the animal died (*vide* Table I).

TABLE I

Rate of water expulsion at decreased temperatures

S. No.	Temperature of water °C.	Water exyelled in an hour in ml.
1	20	2,000
2	18	1,700
3	16	849
4	15	Nil

On the other hand it was observed that when the temperature of water was raised by adding hot water the rate of respiration increased considerably. However, a temperature higher than 45° C. proved fatal for the animal (*vide* Table II).

TABLE II

Rate of water expulsion at increased temperatures

S. No.	Temperature of water in °C.	Water expelled in an hour, in ml.
1	20	2,000
2	25	2,300
3	30	2,650
4	35	3,000
5	40	2,950
6	45	Nil

It is evident from the above experiments that an increase in the temperature of the water upto a certain limit, increases the rate of respiration. But when the temperature is increased beyond 45° C. it kills the animal. With the decrease in temperature of the water, the rate of respiration slowed down and at 15° C. the animal dies.

Effect of light

It was found that any change in the degree of illumination effects the respiratory behavior considerably as shown in Table III.

TABLE III

The effect of light on rate of respiration

S. No.	Condition of light		Water expelled in an hour, in ml.
1	Normal daylight	..	4,720
2	Complete darkness	..	3,012
3	Increased light intensity (100 wts.)	..	4,530

Table III shows that when light intensity is increased there is a slight decrease in the rate of water expulsion while in complete darkness the effect is more marked.

Effect of size

The experiments have shown that the rate of water expulsion is higher in larger specimens as compared to those of small size. The rate of respiration in crabs of different sizes is given in Table IV.

TABLE IV

Rate of water expulsion in the crabs of different size

S. No.	Breadth of cephalothorax in mm.	Water expelled in an hour, in ml.
1	32	210
2	40	305
3	50	703
4	60	810

DISCUSSION

The freshwater crab *Paratelphusa masoniana*, which is very common on the banks of the local rivers and ponds, is amphibious in nature. This nature of the animal can well be correlated with its respiratory organs. In *Paratelphusa* the number of gills is restricted to 7 pairs. Of these the first pair is greatly reduced and takes little part in respiration. It may be argued that with the reduced number of functional gills the amount of water required decreases considerably and hence accounts for its amphibious nature.

It is evident from the above description that physical factors like temperature, light, etc., effect the rate of respiration to a considerable extent. Edwards and Irving (1943 *b*) observed that the optimum temperature for the metabolism of *Talorchestia megalophthalma* is 33° C. and that at a higher temperature of 43° C. and a lower temperature of 10° C. it dies.

Almost similar conditions are found in *Paratelphusa masoniana* as the animal dies above 45° C. and below 15° C. If the temperature is decreased, the rate of water expulsion also decreases while increase in the temperature of the water quickens the rate of respiration. Further, the animal is found to expel water at the miximum rate in normal daylight, but darkness as well as bright light reduces the rate of respiration considerably. Although the animal, being nocturnal in habit, is more active at night, its rate of respiration decreases considerably in darkness. The size of the animal can also be correlated with the rate of water expulsion as it is more in a bigger animal.

Acknowledgements

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PHYSIOLOGY OF DIGESTION OF THE FRESHWATER CRAB, *POTAMON MARTENSI* WOODMASON

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ABSTRACT

The common freshwater crab *Potamon martensi* is predominantly a carnivorous animal. The food consists of small fishes, crustaceans, insects, etc. However, sometimes it feeds on algal filaments also.

The physiological studies include the determination of pH and the qualitative estimation of enzymes in the different parts of the gut of the animal.

It has been investigated that in oesophagus, midgut caecum, hindgut and hindgut caecum, the medium is weakly acid while in the stomach and digestive gland it is more acidic. However, in rectum the medium is weakly alkaline.

Studies on the qualitative estimation of enzymes show that most of the carbohydrases such as amylase, invertase, maltase, lactase, etc., are secreted by the digestive gland; weak inulinase and salicinase are also present. The proteases and lipase are very active in the extract of the digestive gland. In the oesophageal extract, the invertase, maltase, lactase, amylase, raffinase, proteases and lipase are present. Almost all the enzymes are present in the midgut, while none are found in the extracts of the midgut caecum, hindgut caecum and rectum.

INTRODUCTION

THE common edible freshwater crab, *Potamon martensi* is abundant on the banks of rivers and ponds of Muzaffarnagar. In the present paper the pH in the different parts of the gut of *Potamon* and the site of enzyme secretion has been described in detail.

A review of the available literature shows that very little work has been done so far on the morphology and physiology of the alimentary canal of any Indian crab. Hardy and Mac-Dougall (1895) described the anatomy of the alimentary canal of *Daphnia*, Balss (1926) studied the anatomy of the gut of certain Crustacea. Pearson (1908) gave a good account of the alimentary canal of the British marine crab, *Cancer*. Patwardhan (1935), Reddy (1937) and Agrawal (1962, 1963 and 1964) have worked on the morphology and the physiology of digestion in some of the decapods and amphipods. Nicholls (1931) studied the alimentary canal of *Ligia* while Yonge (1924) studied that of *Nephrops*.

MATERIAL AND METHODS

The specimens of *Potamon martensi* were collected from the river Kalinadi, which runs through Muzaffarnagar. They were kept in an aquarium under stones, with very little water.

To determine the hydrogen-ion concentration of the normally feeding crabs, freshly collected alive specimens were dissected. The different parts of the gut were separated and the pH was determined with the help of the pH meter, and indicator papers.

For qualitative estimation of the enzymes, the extracts from the different parts of the gut and digestive glands were prepared. Fifteen crabs, starved for 15 days, were dissected alive and the

different parts of the alimentary canal were ground up in a mortar with a little thymol and a few drops of glycerol to form a thick uniform emulsion. This was diluted with 50% glycerine to make a solution of about 10% strength. The solution after being centrifuged at 3,000 r.p.m. was kept in a tube which was filled with toluene, for about a week, before being treated for the different enzymes.

The Alimentary Canal

Potamon martensi is primarily a carnivorous animal. However, in the absence of the preferred food, it may also feed on small aquatic plants. Normally, it feeds on dead animals, and has never been observed to catch living animals.

The alimentary canal (Fig. 1) of *Potamon* is quite long. The mouth is a small gape guarded by the 3rd pair of maxillipeds. It lies in between paired, stout mandibles, which are provided at their base with a glandular mass. The small oesophagus leads into a spacious, sac-like, compactly-built cardiac stomach, which is provided with complicated masticatory processes. The cardiac stomach is followed by a small pyloric stomach where the food is filtered and then passed on to the midgut. The midgut is very small; it gives off a pair of narrow coiled caeca, one on either side of the gut. The midgut leads into the thin-walled hindgut. From the left side of the hindgut arises a long narrow hindgut caecum. The rectum is a wide tube and opens to the exterior through the ventral anus.

The digestive gland or hepatopancreas is a large highly branched structure occupying most of the visceral space. It is yellowish in colour. The digestive glands serve mainly for the secretion of the enzymes but it also acts as a storage centre for reserve food materials.

pH Determination

pH determination is an important aspect of the physiology of digestion, for different enzymes act best under different hydrogen-ion concentrations. The average pH of the different parts of the gut of the normal feeding crab is tabulated in Table I. It could be seen from this that the medium is almost similar throughout the alimentary canal. It is throughout weakly acidic except in the rectum where it is alkaline.

A few animals were then starved for about a week by keeping them in filtered water, and the pH of the different parts of the gut was tested as before. The results showed that the medium in the different parts became more acidic while it became more alkaline in the rectum. A few crabs, after being starved for a week, were fed on some selected food for about 4 hrs. and the pH in the different parts of the gut was again noted. It is found that the readings almost agreed with those given in Table I.

Qualitative Estimation of Enzymes

The extracts of the different parts of the gut were prepared as mentioned earlier. A few drops of the extracts were incubated with a few drops of the different substrates at room temperature; control experiments were also set up in each case. These incubated solutions were tested after 24 hrs., 48 hrs. and 72 hrs. to determine the digestion of the substrates if any. For the determination of amylase, invertase, glycogenase, raffinase, inulinase and salicinase; Benedict's tests (Agrawal, 1964) were performed while for maltase and lactase, osazone and Barfoed's (Agrawal, 1964) tests were performed.

The presence of lipase was tested by conducting experiments with condensed milk. Two drops of bromo-thymol blue were added to 25 ml. of a 10% milk solution; 1% sodium hydroxide

solution was added until the solution turned light blue in colour. One ml. of blue milk solution was added to a few drops of the different extracts and the rest of the tubes were filled with toluene. After a few hours of incubation, the colour of the solution, wherever lipase was present, changed to yellow. The control experiments in each case gave negative results. The presence of lipase was further confirmed by experimenting with olive oil. Ten drops of olive oil were dissolved in 4 ml.

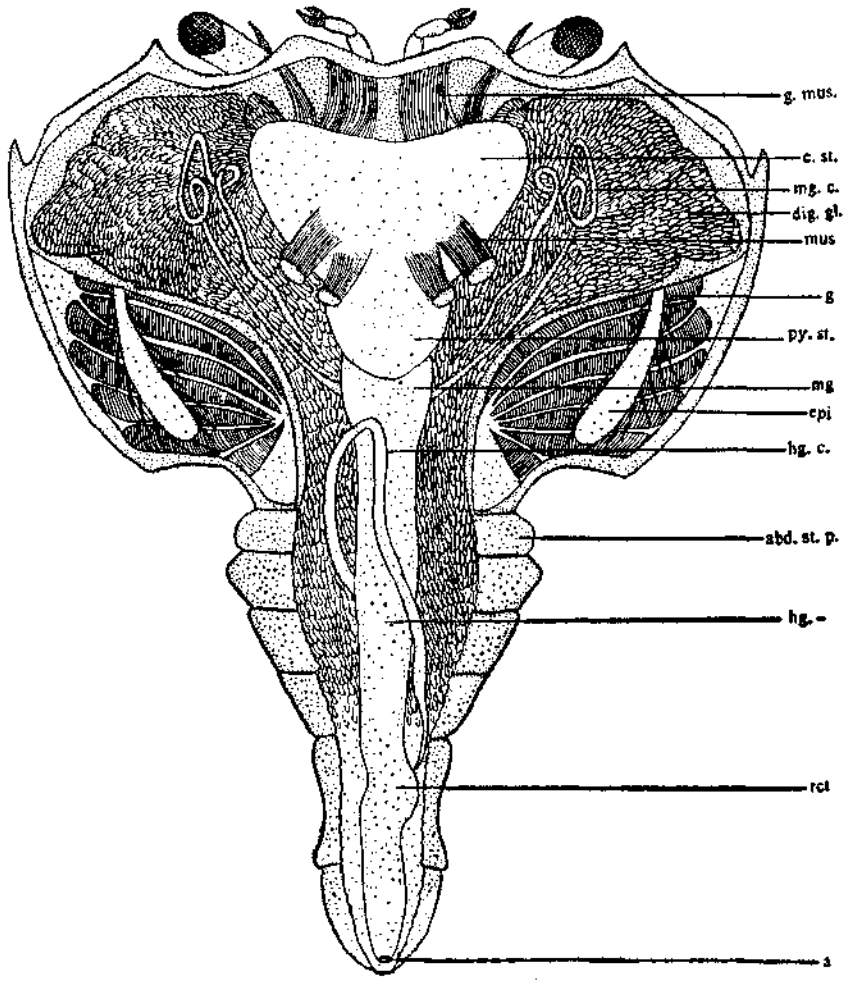

FIG. 1. Alimentary canal of *Potamon martensi*. abd. st. p., abdominal sternal plates; a, anus; c. st., cardiac stomach; dig. gl., digestive gland; epi., epipodite of first maxilliped; g, gill; g. mus., gastric muscles; hg., hind gut; hg. c., hindgut caecum; mg. c., midgut caecum; mg, midgut; mus., muscles; py. st., pyloric stomach; rct., rectum.

of absolute alcohol; 4 ml. of hot water was added to it. The mixture was then allowed to cool after which 10 drops of phenol red were added. A few drops of 0·01 N NaOH were added to make the emulsion faintly pink. Two ml. of this mixture was incubated with 1 ml. of the different extracts. Soon, the pink colour of the mixture changed to yellow, confirming the presence of lipase.

The presence of proteases was investigated by incubating a few drops of the different extracts with 10% gelatine solution. The gelatine gets completely liquified in those cases where proteases are present while in control experiments, the gelatine remained solid.

TABLE I

pH of the alimentary canal of normal feeding crabs

S. No.	Oesophagus	Cardiac stomach	Pyloric stomach	Midgut	Midgut caecum	Hindgut	Hindgut caecum	Rectum	Digestive gland
1	6·4	5·6	5·8	5·6	6·6	6·2	6·1	7·4	5·9
2	6·5	5·9	5·9	5·8	6·8	6·3	6·2	7·2	6·0
3	6·4	5·6	5·9	5·9	6·8	6·4	6·3	7·4	5·9
4	6·7	5·7	6·0	5·6	6·7	6·4	6·1	7·3	5·9
Average pH	6·4	5·6	5·9	5·6	6·8	6·4	6·1	7·4	5·9

TABLE II

Results with the digestive gland of Potamon

S. No.	Substrate	Duration of reaction and extent of digestion			Control experiments	
		24 hrs.	48 hrs.	72 hrs.	24 hrs.	72 hrs.
1	1% starch solution ..	+	+	+	—	—
2	Saturated glycogen solution ..	++	++	++	—	—
3	5% sucrose solution ..	++	++	++	—	—
4	2% maltose solution ..	++	++	++	—	—
5	2% lactose solution ..	++	++	++	—	—
6	1% raffinose solution ..	++	++	++	—	—
7	1% inulin solution ..	±	++	++	—	—
8	1% salicin solution ..	+	++	++	—	—
9	1% gelatine solution ..	Completely dissolved within 4 hrs.			Remained solid	
10	Condensed milk, etc. ..	Colour changed to yellow			No change	

The following tables contain the summary of the results. The sign ++ means a vigorous reaction; + a positive reaction; ± traces of reaction while — indicates no reaction.

Table II shows that most of the carbohydrates are digested by the enzymes secreted by the digestive gland. However, inulinase and salicinace take a longer time for complete hydrolysis. Protease and lipase, as could be expected in a carnivorous animal like *Potamon*, are very active.

Similar experiments were set up with the extracts of the oesophagus, stomach, midgut, midgut-caecum, hindgut, hindgut-caecum and rectum, the results are presented in Tables III, IV, V and VI.

TABLE III

Results with the oesophagus

S. No.	Substrate	Duration of reaction and extent of digestion			Control experiments	
		24 hrs.	48 hrs.	72 hrs.	24 hrs.	48 hrs.
1	1% starch solution ..	+	+	+	–	–
2	Saturated glycogen solution ..	++	++	++	–	–
3	5% sucrose solution ..	–	+	+	–	–
4	2% maltose solution ..	++	++	++	–	–
5	2% lactose solution ..	++	++	++	–	–
6	1% raffinose solution ..	+	++	++	–	–
7	1% inulin solution ..	±	+	+	–	–
8	1% salicin solution ..	+	+	+	–	–
9	10% gelatine solution ..	Completely dissolved within 4 hrs.			Remain solid	
10	Condensed milk, etc. ..	No change in colour			No change in colour	

TABLE IV

Results with the extract of stomach

S. No.	Substrate	Duration of reaction and extent of digestion			Control experiments	
		24 hrs.	48 hrs.	72 hrs.	24 hrs.	72 hrs.
1	1% starch solution ..	+	+	+	–	–
2	Saturated glycogen solution ..	++	++	++	–	–
3	5% sucrose solution ..	–	+	+	–	–
4	2% maltose solution ..	++	++	++	–	–
5	2% lactose solution ..	++	++	++	–	–
6	1% raffinose solution ..	+	++	++	–	–
7	1% inulin solution ..	±	+	+	–	–
8	1% salicin solution ..	+	+	+	–	–
9	10% gelatine solution ..	Completely dissolved within 4 hrs.			Remain solid	
10	Condensed milk, etc. ..	No change in colour			No change in colour	

Experiments with the oesophageal extracts indicate the presence of most of the carbohydrases, proteases are also present while lipase is absent. In the stomach almost all the enzymes, except lipase, are present. Most of the enzymes are present in the midgut while only a few are present in the midgut ceacum and hindgut, and none in the hindgut caecum and rectum.

TABLE V

Showing presence or absence of enzymes in the midgut and midgut-caecum

S. No.	Substrate	Midgut	Midgut caecum
1	1% starch solution ..	+	+
2	Saturated glycogen solution ..	+	+
3	3% sucrose solution ..	+	−
4	2% maltose solution ..	+	±
5	2% lactose solution ..	+	±
6	1% raffinose solution ..	+	−
7	1% inulin solution ..	+	−
8	1% salicin solution ..	+	−
9	10% gelatine solution ..	Completely dissolved	Become semi-liquid
10	Condensed milk, etc. ..	No change in colour	No change in colour

TABLE VI

Results with hindgut, hindgut caecum and rectum

S. No.	Substrate	Hindgut	Hindgut caecum	Rectum
1	1% starch solution ..	+	±	−
2	Saturated glycogen solution ..	+	±	±
3	5% sucrose solution ..	+	−	−
4	2% maltose solution ..	±	−	−
5	2% lactose solution ..	±	−	−
6	1% raffinose solution ..	±	−	−
7	1% inulin solution ..	−	−	−
8	1% salicin solution ..	−	−	−
9	10% gelatine solution ..	Dissolved within 4 hrs.	Remain solid	Remain solid
10	Condensed milk, etc. ..	No change in colour	No change in colour	No change in colour

DISCUSSION

The freshwater crab *Potamon martensi* is a nocturnal animal. It feeds mainly on dead animals. However, it could also be fed on aquatic plants. It may be pointed out that the form and size of the gut are adapted to the character of the food taken. The crab, *Potamon*, is mainly carnivorous, feeding on dead animals. This finds relation in the short alimentary canal, having a

sac-like cardiac stomach for the mastication of food and the pyloric stomach for filtering the food particles. Ide (1892) while discussing the functions of the stomach in different Crustacea mentioned about the masticatory function of the cardiac stomach and filtering mechanism of the pyloric stomach. Jordan (1909), Patwardhan (1935) and Agrawal (1963 *a*) also found the same condition in the different crustaceans studied by them.

According to Yonge (1937) there is a definite correlation between the food of an animal and the nature and relative strength of its digestive enzymes. As also observed by Hasler (1935), Vonk (1955) and Agrawal (1963) in other crustaceans, the medium in the different parts of the alimentary canal of *Potamon* is almost similar being weakly acidic except in rectum where it is weakly alkaline.

The enzymological investigations show that the crab can consume a large amount of protein, fat and carbohydrates. This is in accordance with the carnivorous diet of the animal which can also subsist on vegetarian diet. It has been found that most of the enzymes are secreted by the digestive gland.

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PHARMACOLOGY OF THE HEART OF FRESHWATER CRAB, *POTAMON MARTENSI* WOODMASON

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ABSTRACT

The crab *Potamon martensi* is commonly found in the freshwater ponds and rivers of this locality, mostly buried in the holes on the banks of the rivers and ponds. The animal has a well-developed heart. An attempt has been made to find the effect of a few sedative medicines on the heart of this crab and results are given below:

The drugs like adrenalin, camphor and strychnin are found to act as stimulants and increase the heart-beat of the crab to a great extent. The injections of atropine and digitalin are found to be harmful, as a light dose of these medicines reduces the heart-beats considerably. The drugs like aspirin, chloroform, ether, caffein and arsenite are found to be deadly poisonous as the animal dies immediately. Similar experiments when performed with another freshwater crab *Paratelphusa masoniana* showed that this latter crab is much more resistant to these medicines and the effect is not so fatal as in *Potamon*. The above observations show that the medicines like aspirin, caffeine and atropine which are taken as medicines to relieve pains by human beings, are toxic to crabs.

INTRODUCTION

STRUCTURALLY and functionally the heart of arthropods differs markedly from that of other animals. Generally, the blood vascular system of arthropods is of the open type. However, in the crab, *Potamon martensi*, the venous system is of the open type having a few sinuses while the arterial system consists of closed blood vessels. As it lacks a completely closed type of blood vascular system, it is able to offer less resistance to the flowing blood and the heart does not act as a pressure pump.

The heart of *Potamon martensi*, like that of other decapods, is a single-chambered sac of striated muscles. Its dorsal wall is provided with a pair of valved ostea. The heart is lodged within a pericardial cavity.

Dearborn (1903) and Carlson (1906) were the first to study the physiology of the crustacean heart. Since then quite a few workers made investigations on the physiology of the heart. However, very little has been done on the pharmacology of the heart of any of the Indian crustacea. Bain (1929) who first studied the problem seriously observed the effect of adrenalin and some other drugs on the isolated crustacean heart. Bain's work was followed by that of Davenport (1941 and 1942) who studied the cardiac pharmacology of *Cancer magister* Dana. Baylor (1942) reported the effect of certain drugs on the heart of *Daphnia*. Recently, Prosser (1942) and Krijgsman (1952) made some valuable contributions in this field.

MATERIAL AND METHODS

The crabs for this study were procured form the river Kalinadi and for experimentation the crabs were stored in the aquarium. The drugs atropine, digitalin, arsenite, camphor, adrenalin and strychnin were obtained in the liquid form while aspirin and caffeine were procured in powder form and were dissolved in distilled water, so as to make them suitable for injection.

All these drugs were diluted to about 1% solution to keep their potency low. The living animals after removing their carapace, were kept free for some time so that they could restore their normal heart-beat. The normal heart-beats per minute were noted. A dose of 0·2 ml. of each drug was injected into the heart of the animal. The piston of the syringe was pushed very slowly as its sudden push may result in the bursting of the heart. The heart-beats were noted after each injection. The experiments with all the drugs were performed at room temperature.

OBSERVATIONS

The effect of the different drugs on the rate of heart-beat has been studied. Adrenalin is found to stimulate the heart of the freshwater crab *Potamon martensi*. Brucke and Satake (1912) in *Homarus* and Elliott (1905) in *Cancer*, have reported that adrenalin inhibits the heart-beat. However, our observations have the support of Bain (1929) who conducted experiments in *Cancer* and *Carcinus*. Davenport (1941 and 1942) has also reported that adrenalin is a heart stimulant. Camphor also acts as a heart stimulant as the heart-beat increases after the injection.

TABLE I

Showing the effect of drugs on the heart of Potamon

S. No.	Weight of the crab in gm.	Normal heart-beats (per minute)	Name of the drug injected	Beats after injection (per minute)	Death in minutes
1	10·0	24	Caffeine	No beating	2
2	17·5	15	Aspirin	No beating	8
3	14·0	20	Adrenalin	32	17
4	10·0	29	Camphor	89	12
5	10·0	23	Chloroform	No beating	1
6	8·0	25	Strychnin	98	30
7	7·0	18	Digitalin	10	13
8	10·0	23	Ether	No beating	2
9	10·0	25	Atropine	No beating	5
10	9·0	17	Arsenite	No beating	8

Strychnin has a specific effect on the heart of the crab *Potamon martensi* as it also acts as a heart stimulant. Rijlant (1932) while working on *Limulus* and Krijgsman and Krijgsman (1951) working on *Periplaneta* reported a heart blocking action of strychnin, when doses of high concentration were used. But we observed that a dose of low concentration excites the heart of *Potamon martensi*. Aspirin has an adverse effect on the heart-beats of *Potamon* as even a very low dose damages the heart. Chloroform, as also reported by Dearborn (1903), has a toxic effect on the heart of *Potamon*. Even a very light dose may considerably reduce the rate of heart-beats. The injection of ether decreases the rate of heart-beats and finally causes the death of the crab. The observations of the authors agree with that of Needham (1950). Injection of caffeine also effects the heart of *Potamon* adversely and makes it inactive. The injection of even a low dose of arsenite decreases the rate of heart-beating and soon causes the death of the animal. Atropine injection is found to be deadly poisonous for the heart of *Potamon*, as the heart immediately ceases to function. Davenport (1941, 1942) has also reported similar results in *Cancer*, while Bain (1929) has denied such effect. Digitalin also slows the rate of heart-beats in *Potamon*. However, Krijgs-

man and Krijgsman (1951) have given contradictory results in *Periplaneta*. The results of the injections of these different drugs on the heart of *Potamon* are summarised in Table I.

It is evident from Table I that drugs like Caffen, Aspirin, Atropine, Ether, Chloroform and Arsenite kill the animal within no time; Digitalin reduces the heart-beat while drugs like Adrenalin, Camphor and Strychnin acts as a heart stimulant and increases the heart-beats considerably.

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THE PERMEABILITY AND LOCATION OF THE FILTRATION SITE IN THE CRAYFISH ANTENNAL GLAND

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ABSTRACT

Recent investigations indicate that the antennal gland of the cray-fish operates on a filtration-reabsorption basis. Since little is known about the mechanisms underlying either the filtration process or those involved in reabsorption, experimental tests have been made to investigate permeability of the filtration site and its location. The results of these experiments are discussed in the paper.

RECENT studies (Riegel and Kirschner, 1960; Riegel, 1961; Riegel, 1963) indicate that the antennal gland of the crayfish operates on a filtration-reabsorption basis. While little is known about the mechanisms underlying either the filtration process or those involved in reabsorption, two questions arose which were immediately accessible to experimental test. The first concerned the permeability of the filtration site, and the other its location.

To investigate the permeability problem a series of polymers of graded size were employed. These included inulin, a pair of dextrans (one with a molecular weight range of 15–20,000, the other 60–90,000), human serum albumin labelled with I^{125} and a mammalian serum globulin labelled with a fluorescent dye. Both inulin (molecular weight 5,000) and the low molecular weight dextran (LMWD) were excreted in the urine after being injected into the ventral hemocoel. The urine concentration of both exceeded the blood concentration and the urine to blood concentration ratio (U/B ratio) for both averaged 2–3. The high molecuar weight dextran (HMWD) was also excreted in the urine but its concentration appeared to be somewhat lower than that of the other polymers. Table 1 shows mean U/B ratios for the three carbohydrate compounds. The means for inulin and LMWD were not significantly different ($P > 0{\cdot}1$), but the urine concentration of the HMWD appeared to be low. In order to eliminate uncertainties due to the use of different groups

TABLE I

Excretion of polymers C^{14}-labelled compounds

Polymer used	Number		U/B ratio
	Animal	Periods	
Inulin ..	10	23	2·22 (± 1·22)
LMWD ..	3	10	3·03 (± 1·52)
HMWD ..	3	16	1·35 (± 0·70)

of animals for obtaining such U/B data, a series of experiments were undertaken in which inulin-H^3 and one of the dextrans labelled with C^{14} were injected simultaneously into the same animal and the blood and urine concentrations compared periodically. Table 2 shows that the ratio of LMWD to inulin in the urine is essentially the same as that in the blood as suggested by the data in Table 1. However, the ratio HMWD: inulin was lower in the urine showing that excretion of

HMWD is depressed. This would appear to suggest that restraint on filtration occurs as the molecular weight approaches 60–90,000.

TABLE II

Excretion of polymers H^3-inulin, C^{14}-dextrans (double labelled)

Polymer pair	Number		Dextran: Inulin ratios		
	Animals	Periods	Urine	Blood	U/B
Inulin LMWD ..	3	7	2·04	2·22	0·92 (±0·24)
Inulin HMWD ..	4	16	0·93	1·36	0·69 (±0·14)

Excretion of proteins is even more limited. Serum albumin (MW 68,000) was excreted after being injected into the ventral hemocoel, but Fig. 1 shows that the urine concentration never approached that in the blood. This was true even when measurements were made as long as 96 hours after the injection. The mean U/B for albumin was 0·51 ± 0·19 (S.D.) in sharp contrast to the carbohydrate polymers all of which had a U/B greater than 1. Fluorescein-labelled globulin (MW 180,000) was not excreted in the urine, and Evan's Blue, which becomes conjugated to blood protein, also fails to appear in the urine.

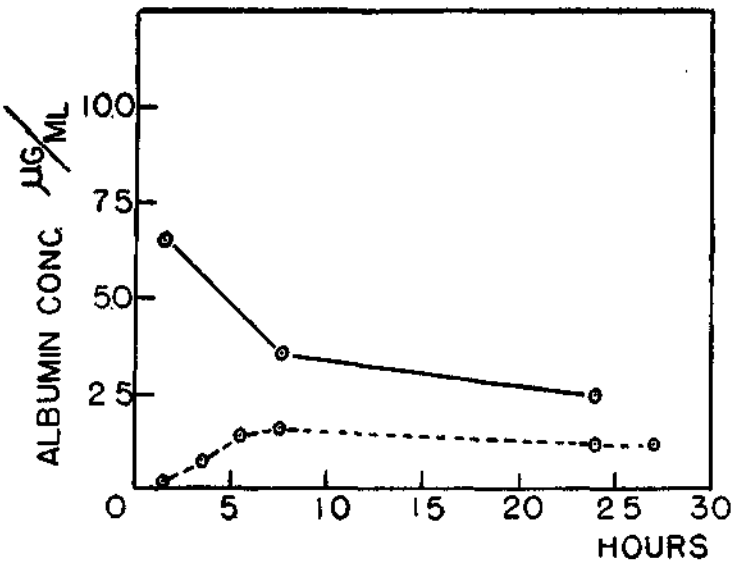

FIG. 1. Concentration of albumin in the blood (—⊙—) and urine (··⊙··) following injection of 0·024 mg. into the ventral hemocoel. The U/B was 0·52 at 24 hours. It was 0·61 after 98 hours when the blood concentration had fallen to 1·84 microgm. per ml.

These data indicate that compounds as large as the LMWD are freely filtered in the antennal gland but that restraint on filtration begins to become apparent in the molecular weight range 50–100,000. Proteins with molecular weight exceeding 200,000, including those normally found in crayfish blood, do not appear in the urine.

TABLE III

Albumin, Inulin ratios in the antennal gland

Number		Blood	Coelomosac	Labyrinth	Tubule
Animal	Organs				
2	4	1·00	16·3	0·55	0·81

We have also observed that protein becomes concentrated in the coelomosac. Thus when albumin-I^{125} and inulin-C^{14} are injected together into the ventral hemocoel, the albumin inulin ratio in the coelomosac exceeds that in the blood by an order of magnitude although in other parts of the antennal gland the ratio is less than one. These data are shown in Table 3. Fig. 2 shows that when Evan's Blue is injected into an animal the dye becomes very concentrated in the coelomosac only. Since this compound is bound tightly to blood protein these data provide evidence for protein concentration in the coelomosac. Fluorescein-labelled, mammalian globulin is also concentrated in this region. The marked rise in protein levels in the coelomosac could be explained if it results from the ultrafiltration of water and small solute molecules into the tubular lumina of the organ. The elevated protein concentration seems to occur exclusively in the peritubular region, a fact which would also be explained if an ultrafiltrate is being expressed into the tubular lumen leaving the protein behind. Location of the filtration site in the coelomosac is also supported by Riegel's (unpublished) observation that the tubular fluid has a composition close to that of an ultrafiltrate of blood.

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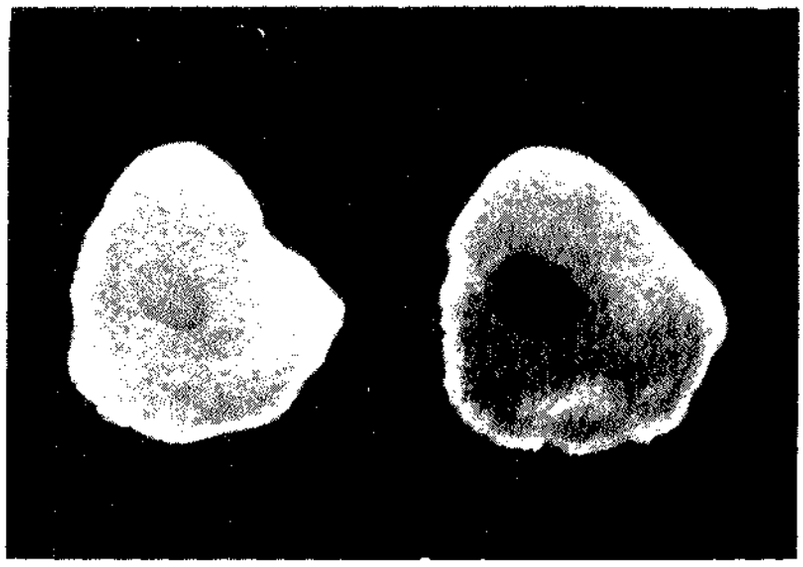

FIG. 2. Evan's blue accumulation in the coelomosac after injection of the dye into the hemocoel. The photographs show a view of the dorsal surface. The gland on the right was taken from an animal that had been injected two hours earlier. The other gland is from a control (non-injected) animal.

STUDIES ON THE MOULT CYCLE IN THE CRAB, *OCYPODA MACROCERA*

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ABSTRACT

From the data collected on the moult stages of crabs from the Visakhapatnam beach, it was concluded that *Ocypoda macrocera* moults continuously throughout the year. At different stages of the moult cycle, samples of integumentary tissue and hepatopancreas were subjected to histological and histochemical study. The rate of regeneration of autotomized limbs was correlated with the observed changes in the epidermis and hepatopancreas. It was found that autotomized limbs complete their regeneration during the period of proecdysis. Bilateral ablation of eyestalks resulted in the acceleration of moulting frequency. Starvation lengthened the intermoult cycle and had marked inhibitory influence on the initiation of basal limb regeneration in the eyestalkless crabs. The results were discussed in the light of previous reports on moulting in brachyuran Crustacea.

THE moulting process is one of the interesting aspects of crustacean physiology. Baumberger and Olmsted (1928) proposed a division of the intermoult cycle into six stages, based on the gross consistency and appearance of the integument: (1) hard, (2) pillans, (3) about to moult, (4) newly moulted, (5) soft, (6) papershell. This was the first scheme of classification of the moult stages. Drach (1939) was the first to emphasize that growth in crustaceans is a continuous process, occurring long before and after ecdysis. This idea was expressed in the term "intermoult cycle". Drach (1939) subdivided the crustacean moult cycle into stages A through D, based on the state of exoskeleton. With slight modifications, Hiatt (1948), Scheer and Scheer (1951), Travis (1954), Passano (1960 *a*) and Skinner (1962) used the scheme proposed by Drach (1939), to study the moult cycle of different crustaceans. Carlisle (1960) is of opinion that Drach's classification, though of practical value, would be especially useful if the broad classifications are then related to the stages of the scheme of Carlisle and Dohrn (1953). Carlisle and Dohrn (1953) divided the moult cycle into four stages: (1) Proecdysis: During this stage the animal prepares to moult, most noticeably by removal of calcium from exoskeleton. The epidermis becomes heightened and the new cuticle is laid down; (2) Ecdysis, the actual process of exuviation where the animal sheds the old skeleton; (3) Metecdysis, is the period when the new exoskeleton is hardening and the animal is returning to normal in its physiological state; (4) Intermoult: The stage of normality to which the altered conditions of the other stages are referred. This is of two types (1) anecdysis, is a long period of rest between the conclusion of one metecdysis and the beginning of next proecdysis and occurs in crabs that moult seasonally and (*b*) diecdysis, is a short period during which a metecdysis passes imperceptibly into the succeeding proecdysis and is found in animals that moult round the year.

The onset of proecdysis is the most significant event in the intermoult cycle. Extensive researches of Bliss (1956, 1960), Bliss and Boyer (1964) on *Gecarcinus lateralis* revealed that the regenerating limb bud can be used as a useful criterion to identify the onset of proecdysis. In this crab, a marked increase in size of a limb bud reflects rapid growth on the part of the entire animal, indicating that the animal is in the proecdysis stage of the intermoult cycle, preparing to shed its shell. Hodge (1956 *a*, *b*) and Skinner (1962) obtained similar results. The works of Durand (1960) on *Orconectes virilis*, Bauchau (1961) on *Carcinus maenas* and *Eriocheir sinensis* and Passano

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and *Jyssum* (1963) on *Sesarma reticulatum* also revealed the relationship of rapid limb growth to overall growth. The literature pertaining to the physiology of moult cycle was recently reviewed by Carlisle and Knowles (1959) and Passano (1960 *a*).

The present investigation was undertaken to learn (1) the normal moult cycle of the crab *Ocypoda macrocera*, (2) whether limb bud growth is related to the moult stages, (3) the effect of eyestalk ablation on ecdysis and limb regeneration, (4) to determine the influence of feeding and starvation on the proecdysial duration and regeneration and (5) to study histochemically the localization of fat and glycogen in the integument and hepatopancreas of the crab in the different stages of moult cycle.

Material and Methods

The specimens of *Ocypoda macrocera* used in the present study were collected from the Visakhapatnam beaches. To determine the normal moult cycle of the species, collections were made at least once in a week during the period from October 1962 to October 1964. A random sample of animals from each collection was dissected to determine the incidence of proecdysis and other moult stages in the different crabs of the sample.

On arrival to the laboratory, crabs were placed in community tanks. They were acclimated to laboratory conditions for one to two weeks and then kept individually in white enamel basins (22 cm. in diameter) with sufficient sea-water to cover the branchial area. Experimental animals were maintained at temperature of $28 \pm 1^{\circ}$ C. A strict photoperiod of 12 hours light and 12 hours darkness was maintained for animals with intact eyes. The bilateral ablation of eyestalks was followed by the cauterization of eyestalk stubs with a hot needle. At the beginning of each experiment dealing with limb regeneration, the experimental crabs were forced to autotomize their right third walking legs. The degree of limb regeneration was determined for each animal by measuring the regenerate with vernier calipers to the nearest 0·1 mm. Regeneration was expressed as a regeneration index following the method of Bliss (1956):

$$R = \frac{\text{length of limb bud in mm.}}{\text{Carapace width in mm.}} \times 100.$$

For the histological and histochemical studies, pieces of the integument and integumental tissues of the branchio-stegite area and hepatopancreas were removed from crabs of different moult stages. Portions of these tissues, fixed in Bouin's fluid and Helly's fluid, were stained by Mallory's triple. Material fixed in Serra's was stained in methyl green pyronin according to the technique of Brachet (1953). The periodic acid-schiff method was used to demonstrate the presence of glycogen. Control sections in this series were pretreated with salivary amylase. Material fixed in formal-calcium and postchromed was used for the detection of lipids using Sudan Black B (Pearse, 1960).

Results

In the present study, individual crabs were studied one moult through the other, following day-to-day changes in the exoskeleton. The data of individual crabs was pooled and the average values for the size group were determined. The nature of exoskeleton and the duration of each intermoult stage, for crabs measuring 27–29 mm. carapace width are given in Table I. The following is a brief summary of characters of each stage.

Stage A

The carapace is completely soft. Even slight pessure causes a depression on the carapace. This stage is equivalent to the early phase of metecdysis and is divisible into two sub-stages.

A_1.—This is the period upto 12 hours following exuviation. The crab has a soft membranous integument and is not able to move freely. It cannot lift its body on its legs. Water absorption is in progress.

TABLE I

Postexuvial sclerotization and loss of flexibility in varions integumental areas of specimens of O. macrocera *(27 to 29 mm. in carapace width)*

Days after moult	Carapace						Thoracic sternites	Carpus and propodus of chela	Merus and carpus of walking legs	Stage in the inter-moult cycle
	Proto-gastric area	Meso- and Urogastric areas	Anterior branchial area	Posterior branchial area	Branchio-stegites	Cardiac area				
½	++	++	++	++	++	++	++	++	++	A_1
1	+	++	++	++	++	++	++	++	++	A_2
2	+	++	++	++	++	++	++	+	++	A_3
3	–	+	+	++	++	++	++	+	++	B_1
4	–	+	+	++	++	++	++	+	++	B_1
5	–	+	+	++	++	++	++	+	++	B_1
6	–	+	+	+	++	++	+	–	++	B_2
7	–	+	+	+	++	++	+	–	++	B_2
8	–	+	+	+	++	++	+	–	++	B_2
9	–	–	–	+	+	+	–	–	+	C_1
10	–	–	–	+	+	+	–	–	+	C_1
11	–	–	–	+	+	+	–	–	+	C_1
12	–	–	–	–	+	+	–	–	+	C_2
13	–	–	–	–	+	+	–	–	+	C_2
14	–	–	–	–	+	+	–	–	+	C_2
15	–	–	–	–	+	+	–	–	+	C_2
16	–	–	–	–	+	+	–	–	+	C_2
17	–	–	–	–	–	–	–	–	–	C_3
18	–	–	–	–	–	–	–	–	–	C_3

++ Integument completely soft.
\+ Integument partially rigid.
– Integument completely rigid.

A_2.—The duration of this stage is from 1 to 2 days. The crab has a parchment-like skeletion and can elevate on its legs. The water absorption is completeld. The crab has a water content of 82%. The exoskeleton of the protogastric area of the carapace and that of carpus and propodus of chela become partially rigid.

Stage B

This is the period of hardening of the shell and corresponds to the middle phase of metecdysis. This stage is subdivided into two.

B_1.—This is of 2–3 days in duration. The cuticle of the protogastric area of the carapace becomes completely rigid. The meso and urogastric areas of carapace and the anterior branchial area become partially rigid. The posterior branchial area, branchiostegites and cardiac area of the carapace, thoracic sternites, merus and carpus of walking legs remain soft.

B_2.—The anterior branchial area and thoracic sternites become partially rigid. The carpus and propodus of the chela becomes completely rigid. The water content of the crab is 80%. The duration of this stage is 2–3 days.

Stage C

The stage 'C' is divided into four sub-stages.

C_1.—This is of 3–4 days duration. The meso and urogastric areas, the anterior branchial area and thoracic sternites become completely rigid. The posterior branchial area, the branchiostegites, cardiac area and the merus and carpus area of walking legs become partially rigid and depressible. The water content of the crab is 78%. The crab cannot yet take food.

C_2.—It is of 4–5 days duration. Except the branchiostegites, the cardiac area and the merus and carpus of walking legs, the exoskeleton is completey rigid. The water content is about 73%. The crab initiates feeding in this stage.

C_3.—The exoskeleton is completely rigid. The membranous layer has not yet formed. This stage is of 7–9 days duragion. The water content of the crab is 66%.

C_4.—This stage is the longest period of intermoult cycle. The membranous layer is formed. This is evident only by dissecting the integument and is the only difference between C_3 and C_4. The water content of the crab is 58%. The stages C_1–C_3 correspond to the late phase of metecdysis. 'C_4' is equivalent to the 'intermoult' stage of the scheme proposed by Carlisle and Dohrn (1953).

Stage D

This stage represents the preparation for the following moulting. This represents the proecdysis stage of Carlisle and Dohrn (1953).

D_1.—18–25 days in duration. The first sign of proecdysis initiation, the precocious formation of new setae in the cores of the old, denotes this stage.

D_2.—3–6 days duration. The secretion of pigmented layer of the exoskeleton occurs. Chitinization of new spines takes place.

D_3.—1–3 days in duration. Resorption is completed. Membranous layer is gelatinous. The new integument becomes completely detached from the overlying old exoskeleton. The animal refuses food and reduces its activity.

D_4.—1 day. Epimeral line splits along its entire length. The crab is completely inactive.

Stage E

Ecdysis.—Two distinct phases are recognised. (1) Passive phase which is initiated a day prior to exuviation and is marked by absorption of water, which serves to exert pressure to separate the pleural groove on branchiostegites. (2) Active phase involves the actual muscular activity involved in the moulting process. Prior to the splitting of the epimeral line, the new underlying integument is wrinkled. After the fracture along the epimeral line, the old carapace is elevated allowing the new integument to stretch to the size to which it will harden. In the passive phase this epimeral split extends anteriorly almost to the mouth. In this respect, *Ocypoda macrocera* resembles *Callinectes sapidus* (Churchill, 1918) and *Pachygrapsus crassipes* (Hiatt, 1948) and differs from *Cancer pagurus* (Pearson, 1908) where the epimeral fracture appears first at the anterior end and proceeds posteriad.

After the body expansion is considerably completed the periopods are moved slightly in a rhythm, for a period of 10–12 minutes, resulting in the withdrawal of the 4th and 5th pair of periopods from the old shell. In *Maia squinado* (Drach, 1939) this movement occurs for 10–15 minutes and in *Pachygrapsus* (Hiatt, 1948) it occurs for 7 minutes. It is interesting to note that the mature king crab, *Paralithodes camtschatica* (Sakuda, 1961), completes the process of exuviation in 4 minutes. After the withdrawal of the posterior 4 pairs of appendages from the old shell, the old sternal integument is separated from the new.

An elongation of the body together with upward and backward movement of the crab, exerts the necessary pull on the abdomen which is subsequently withdrawn. At this stage, the limbs are directed forwards. Later, rapid movement of limbs occur and this helps to remove the buccal appendages and chelae from the old shell. Considerable variability occurs in the time required for the maximum post-exuvial body expansion. The greatest part of post-exuvial expansion takes place prior to an hour after ecdysis. Most of this expansion occurs during ecdysis, for, swelling of the body is itself a factor to facilitate exuviation. After exuviation, the crab lies on its dorsal side for an hour or two and it is during this period, additional swelling of the body occurs.

To determine whether any seasonal variation in the moulting frequency of *O. macrocera* occurs in this area, a total of 2,416 crabs (20–30 mm. in carapace width) were staged to the respective intermoult stages, soon after collection. In each month, from November 1962 to November 1964 the male and female ratio was 1 : 1. 10–12% of the crabs were in proecdysis in every month except in October, November and December, when this percentage increased upto 18–25. From these observations it is evident that *O. macrocera* moults round the year. The high percentage incidence of proecdysis in samples of October, November and December may be due to the females that moult soon after they liberate their eggs. Unpublished observations of the authors showed that the reproductive activity is at its height during the period from October–December and the megalops and first crab stages are present from late December upto April–May, with maximum abundance in late January and February. In the remaining months of the year they occur very rarely.

Histochemical observations

The integumental tissues from the branchial area of an intermoult *Ocypoda* are bordered by an outer and an inner integument. Four layers are present in the outer integument: (1) thin epicuticle, (2) the pigmented layer, (3) principal layer and (4) the membranous layer. The epicuticle and pigmented layer are secreted before moult. The inner integument is about 1/25th of the outer integument in thickness and is composed of a thin epicuticle and an endocuticle. The epithelial cells are columnar and the large oval reserve cells (Hardy, 1892) are abundant.

Integumentary tissues removed from early D_1 stage animal is identical to that of C_4 animal. In the late D_1 stage resorption of membranous layer is initiated. By the end of D_2 stage, the

epidermis is completely separated from the old shell. The epicuticle synthesis is completed by the end of D_3. Sections of the integumentary tissue from crabs of different moult stages showed that the number of lipoprotein cells (Sewell, 1955) increased by the end of C_4. During the early period of proecdysis secretory granules are found in these cells. In the late proecdysis, their contents reach their maximum, the secretory granules coalesce to form one droplet and the cells move towards the epidermis. By the end of proecdysis, after the formation of epicuticle, they disappear.

Tests for glycogen in the integumentary tissues revealed that the glycogen content varies with the moult stage. During the period of proecdysis, glycogen is maximum. The glycogen content of the 'cells of Leydig' (Cuenot, 1893) also increases during this period, decreasing after the ecdysis. The glycogen content of intermoult stage epidermal tissue was very low. Similar results were reported previously by Verne (1924, 1926) in *Carcinus maenas*, Renaud (1949) in *Cancer pagurus*, Travis (1955, 1957) in *Panulirus argus*, Schwabe *et al.* (1952) in *Panulirus japonicus* and *Skinner* (1962) in *Gecarcinus lateralis*,

Droplets of lipid are found in the apices of epidermal cells, reserve cells and connective tissue of the integumentary tissue of a crab from early metecdysis. In the middle phase of metecdysis, in the newly formed endocuticle, lipid is found in pore canals and in the form of a distinct zone. The same condition continues to the intermoult stages.

Within the tubular epithelium of the hepatopancreas two major cell types are present. (1) secretory cells: swollen cells containing very large vacuoles at the distal ends. (2) absorptive cells: tall and cylindrical with a basal or central nucleus. In addition, two more cell types occur. Fibrillar cells are few and are distributed throughout the tubule while embryonic cells are restricted to the blind end of the hepatopancreatic tubules. All these cell types are essentially similar to the cell types reported in the hepatopancreas of *Panulirus* (Travis, 1955).

During the early metecdysis glycogen is present in considerable quantities and is distributed in the distal and basal ends of absorption cells. In the secretory cells, they are present at the periphery of vacuoles. By the end of B_2, or by the end of middle phase of metecdysis, glycogen decreases to a great extent. In the intermoult stage, very few glycogen granules are evident in the hepatopancreas. These are found at the bases of secretory and absorbing cells. In the proecdysis stage, glycogen is abundant in the epithelial tissue of hepatopancreatic tubules. Similar results were previously reported by Bernard (1879) in *Astacus*, Vitzou (1882) in *Homarus* and Von Schönborn (1910) in *Carcinus*.

Lipid droplets are found in considerable quantities in the hepatopancreatic tubules of crabs in metecdysis. The lipid content is very abundant in the intermoult stage. This resembles the observations of Renaud (1949) in *Cancer pagurus* and Travis (1955) in *Panulirus argus*. There is little change of the lipid content in the hepatopancreas tubules, in proecdysis, over that observed in intermoult stage. Paul and Sharpe (1916), Renaud (1949), Travis (1955) reported similar instances of accumulation of lipids in the hepatopancreas preceding moult, in other crustaceans.

Experiments on limb regeneration and moulting

Experiments were carried out on normal *Ocypoda*, kept under laboratory conditions following forced autotomy of one of their walking legs. At the start of the experiment the crabs (23–25 mm. carapace width) were in the intermoult stage. The crabs of the group A (10 individuals) were starved while those of group B (10 individuals) were fed once in three days. The observations were followed for the next 40 days and the degree of limb regeneration was determined. After an initial lag period, a small limb bud has grown in the place of an amputated leg. This increased in size and reached an R value of 8·3–11·0 in fed crabs and 7·7–9·7 in starved crabs. None of these crabs entered proecdysis.

To determine whether bilateral ablation of eyestalks results in the onset of proecdysis, 8 crabs were deprived of their eyestalks on the 25th day following autotomy. The operation initiated proecdysis and all the eight crabs moulted with an average proecdysial duration of 19·0 days. Four days before ecdysis, the limb buds had final R values ranging from 22·4 to 26·2.

In the next experiment the influence of starvation on the moulting and regeneration was considered. Two groups of 10 crabs each in the intermoult stage (22-24 mm. carapace width) were selected, one set was starved and another was fed. After a week, each crab of both the groups was forced to autotomize its third walking leg. On the same day, eyestalk ablation was performed. Both the groups entered proecdysis and moulted. The duration of proecdysis was not significantly different in the two groups of crabs (proecdysis duration was 18·8 days in starved crabs and 18·6 days in fed crabs, the values being the averages of individuals). All the crabs of the fed group regenerated their legs whereas only one crab of the starved group did so.

Discussion

The results of present study of the moult cycle of *Ocypoda macrocera* are in general agreement with the previous observations on brachyuran moult cycles. *Ocypoda macrocera* moults round the year and this lengthy span of moulting frequency is probably associated with the small range of temperature changes of the area. The range of temperature changes of the area is small (Ganapati and Rao, 1959; Ganapati and Rao, 1962). The results of observations on the distribution of glycogen and fat in the hepatopancreas and integumental tissues support the previous histophysiological studies of the moult cycle in other crustaceans. Lipoprotein cell granules coalesce to form one large droplet, in the late proecdysis, in *Ocypoda*. Similar situation occurs in *Carcinus* (Sewell, 1955). But, in *Gecarcinus* the granules do not coalesce (Skinner, 1962).

The observation that eyestalk ablation precipitates moulting, initiating the onset of proecdysis indicates that the moult inhibiting hormone is secreted in the eyestalk; which is the general rule in most of the brachyura studied (Passano, 1960 *a*). Observations on the regeneration in *O. macrocera* confirm Bliss (1956, 1960), Passano and Jyssum (1963) and Bliss and Boyer (1964) in their studies on the interrelationship between limb regeneration and intermoult cycle. In normal crabs, starvation has no significant effect on regeneration. In eyestalkless crabs, if autotomy occurs on the same day of eyestalk ablation, starvation inhibited the initiation of limb bud growth. Crisp and Patel (1960) found that after anecdysis, the moulting rate was low in *Balanus balanoides* but it recovered much more rapidly in those individuals that were fed. Passano (1960 *b*) observed that starvation had no effect on limb regeneration, but the total percentage of moults in cold treated *Uca* was reduced. Since amputation of legs and removal of eyestalks alter the metabolic balance of the individuals, the crabs need certain period to return to their normal state, until which limb bud growth may not be initiated. In such individuals, feeding helps the animal to recover rapidly than starved individuals. It might be that the inhibition of basal limb bud growth in the starved crabs, is due to the delayed recovery of the individuals to initiate growth of new limb bud tissue.

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PIGMENTATION AND COLOUR CHANGE IN DECAPOD LARVAE

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ABSTRACT

The author gives a description of the chromatophores and their basic reactions in the first zoeal stages of one natantian (*Crangon crangon* L.) and three brachyuran species (*Carcinus maenas* L., *Rhithropanopeus harrisi* Gould and *Geograpsus lividus* Milne-Edwards). The larval chromatophores can be classified as belonging to two systems, the primary and the secondary one. The latter represents the anlage of the adult chromatophores. In all the species investigated the chromatophores are arranged according to a constant pattern, characteristic of the species, being thus of some taxonomic value. However, in the case of the brachyuran zoeas it has been shown that the general scheme of the larval chromatophores in this group, proposed by Aikawa, is not true.

In all the larvae investigated the pigments in the chromatophores are capable of the quick movements characteristic of the physiological colour change. With the exception of *Rhithropanopeus*, the larvae do not adapt themselves to the colour of the background (secondary reaction). The incident light only is effective in producing dispersion or concentration of the pigments (primary reaction). This behaviour is in contrast to the adult forms in which, again with the exception of *Rhithropanopeus*, the secondary type of reaction is prevailing. The larval chromatophores show a clearly expressed diurnal rhythm. Chromatophorotropins from adult eyestalks have no effect on the chromatophores of the larvae.

UNTIL recent times very little attention was given to the study of the chromatophores of decapod larvae. Even now our knowledge of the pattern formed by the chromatophores and of their reactions is rather poor. It was even uncertain if the larval chromatophores of forms which at the adult stage show a typical physiological colour change (for instance Brachyura) are capable of pigmentary movements. Most of the observations were made on fixed larval material with the majority of chromatophores destroyed.

The situation is certainly not satisfactory, especially if one considers the great interest shown by many scientists in the chromatophores of adult decapods, known to be highly specialised effectors of a neuroendocrine reflex along a pathway running from the eyes, the receptors of light rays, over the nervous centres controlling the release of hormones carried by the blood stream to the chromatophores.

The description of the chromatophoral patterns of a number of decapod larvae, given by Keeble and Gamble as early as 1904, had not been followed, for many years. Some time later, Hyman (1925) mentioned the chromatophores of the larvae of some representatives of the Xanthidae (Brachyura). Lebour (1928) published good illustrations of decapod larvae showing the chromatophores in their natural colours, but without mentioning them in the text. More recently, the papers of a few authors brought out illustrations of this kind, accompanied by short descriptions, Costlow and Bookhout (1959, 1960, 1961 *a*, 1961 *b*), Pike and Williamson (1961). But only in Aikawa's (1929) paper are the features of the larval chromatophoral system discussed as a problem. This author, who compared the patterns formed by the chromatophores in the larvae of a number of Brachyura from Japanese waters, was struck by their regular invariable topographic location. Aikawa concluded that the chromatophoral system of the larva is an important taxonomic character, characteristic of not only species and genera but also of families.

In the earlier literature, observations on the response of the larval chromatophores to various stimuli are even rare. Keeble and Gamble (*loc. cit.*) noticed changes of the chromatophores in the zoeas of *Palaemon* and of *Hippolyte varians*. Koller (1927) made similar observations on the larvae of *Crangon crangon* and Carstam (1947) on the larvae of *Nephrops norvegicus*. Although none of these investigations was very detailed, the general conclusion could be drawn that in the larvae the capacity of background adaptation is nil or rudimentary. So there seemed to exist a difference in this respect between the behaviour of the larvae and that of the adults.

From these results it seems probable that in the development of the Decapoda a change of the type of response to light rays may occur, similar to that detected earlier in some vertebrates undergoing metamorphosis. Babák (1910) first observed a difference in the response of the melanophores between the larval and the metamorphosed forms of *Amblystoma mexicanum*. The melanophores of the latter show a certain degree of background adaptation. The animal's skin pales on a light-coloured bottom and darkens on a dark one. On the other hand, the colour change of the larvae is independent of the background. Their colour turns from a light to a dark hue with the growth of the intensity of the illumination. Metamorphosed animals react to reflected rays but the larvae react only to incident rays. The type of response observed in the larva is known as the primary and that found in the adult as the secondary reaction. Later on a similar change of reaction was found also during the development of other amphibians, *e.g.*, *Rana pipiens* (Hooker, 1914) and in some fishes, *Perca* and *Salmo* (Duspiva, 1931) and *Macropodus* (Tomita, 1936).

Beginning my investigations I tried first, to confirm and to complete the earlier results. After a thorough investigation it became clear to me that in the first zoea of the shrimp, *Crangon crangon* (L.) the number and the pattern of the chromatophores are principally the same in specimens from the Baltic Sea and in those from the Plymouth area, as described by Keeble and Gamble (Pautsch, 1951).

The pattern of the chromatophores is very regular (Fig. 1). The chromatophores of the cephalothorax are paired, whereas in the abdomen, they are median and unpaired. Majority of them are dichromatic and contain a black-brown and a yellow pigment. Some monochromatic chromatophores are also present, black-brown as well as yellow ones. In some animals the light-coloured pigment is white and not yellow. The number of different pigments is smaller in the larva than in the adult, where at least four different pigments are present, black-brown, yellow, white and red.

In almost all individuals the number and the pattern of the chromatophores in the cephalothorax were found to be identical. An exception was the monochromatic chromatophores in the antennulae. In most individuals these chromatophores were single, but in some cases three chromatophores could be found in each of the antennulae. Individual variation was observable also in a pair of small dichromatic chromatophores in the lateral portion of the carapace. In individuals with generally yellow pigment these chromatophores were in some cases black and white and not black and yellow.

In the abdomen, the most important are the big unpaired chromatophores, situated on the ventral side of each segment. They show no individual variation, contrary to the dorsally located chromatophores of the abdomen. The latter are present principally in the first four segments only, but in a number of individuals some of them may be lacking. These cells are smaller and less important than the ventral chromatophores of the abdomen. Besides this, the fourth and the fifth abdominal segments contain lateral dichromatic cells, while in the sixth segment yet another small cell is present. It is located caudal to the main chromatophore and also contains both pigments. In the telson lie three dichromatic cells forming the corners of a triangle, the top of which is directed cephalad. In some individuals the top chromatophore can be a paired one.

The larval chromatophores of *Crangon* are much less numerous than those of the adult. Regularly arranged, the chromatophores of the larva belong, almost all, to the primary system

of chromatophores (Keeble and Gamble, *loc. cit.* ; Aikawa, *loc. cit.*). At later stages of development the primary system is replaced by the secondary system, characteristic of the adult. The anlage of the secondary system can be found already in the larva. In the zoea of *Crangon* it consists of a pair of dichromatic chromatophores present in the carapace, the anlage of the chromatophores in the thorax of the adult shrimp, and of the chromatophores found in the antennulae and in the maxillipedes.

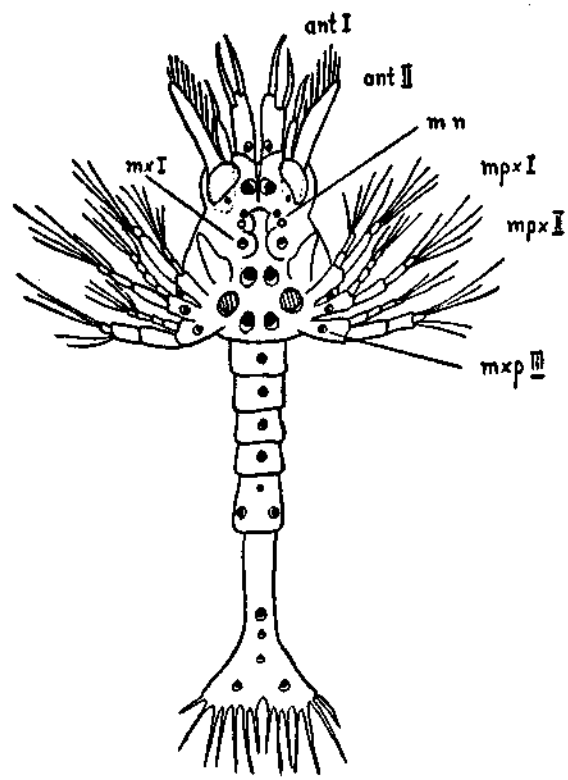

FIG. 1. Chromatophoral system of the first zoea of *Crangon crangon*, ventral view. Black—the black-brown pigment, white circles—the yellow pigment, checked—a deeper lying dichromatic chromatophore, *ant I*, antennulae, *ant II*, antennae, *mn*, mandibulae, *mx I*, maxillae I, *mxp I–III*, maxillipedes.

In Keeble and Gamble's paper there is no mention of the individual variation of the chromatophores in the *Crangon* zoea. Since the description given by these authors is based on observations of only a few individuals, it is impossible to decide, without re-examining the larvae from the Plymouth area, if there is any difference in this respect between the populations of Plymouth and those of the Bay of Gdansk.

The remainder of my observations were made on brachyuran larvae. My aim was, among other things, to estimate whether the value of the general scheme of the chromatophoral system in brachyuran larvae, worked out by Aikawa and claimed by him to be valid for the whole group, is really so.

In the first zoea of *Carcinus maenas* (L.) dichromatic chromatophores prevail, the two pigments are a black-brown and a light yellow, the latter assumes in some cases an almost white appearance (Fig. 2). Monochromatic chromatophores containing a black-brown pigment are less numerous. In some individuals a single, small monochromatic chromatophore, containing a red pigment, could also be detected (Pautsch, 1961).

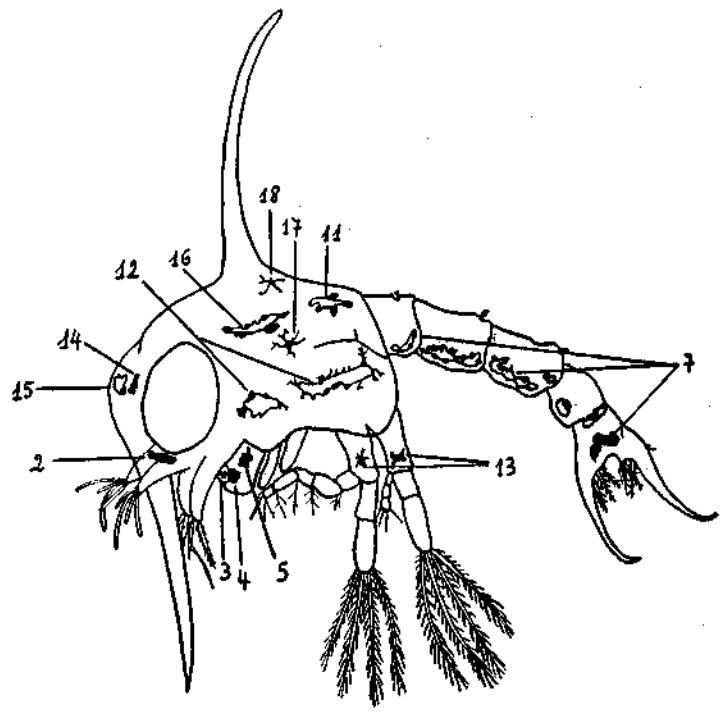

FIG. 2. Chromatophoral system of the first zoea of *Carcinus maenas*, (2) antennal chromatophores, medially coalesced, (3) labral chromatophore, (4) mandibular chromatophores, (5) maxillar chromatophores (?), (7) abdominal chromatophores, (11) postcardiac chromatophores (?), (12) carapacial centre, (13) maxillipedal chromatophores, (14) optic centre, (15) median ocular centre, (16) dichromatic chromatophore, unclassified, (17) dark chromatophore, unclassified, (18) red chromatophore, unclassified.

Here, too, the chromatophores are arranged in a regular and almost invariable pattern. According to Aikawa's scheme (Fig. 3) they could be classified as follows:

Aikawa's general scheme

Carcinus maenas

I. *Primary system of chromatophores* :

A. *Neural group*:

1. Supracerebral chromatophores	0	
2. Antennal chromatophores	X	medially coalesced
3. Labral chromatophores	X	a single chromatophore
4. Mandibular chromatophores	X	
5. Maxillar chromatophores	X	coalesced (?)
6. Maxillipedal chromatophores	0	
7. Abdominal chromatophores	X	

B. *Vtsceral group*:

8. Median gastric chromatophores	0	
9. Precardiac chromatophores	0	
10. Subcardiac chromatophores	0	
11. Postcardiac chromatophores	?	

II. *Secondary System of chromatophores*:

12. Carapacial centre	X	two pairs
13. Maxillipedal chromatophores	X	two pairs
14. Optic centre	X	
15. Median ocular centre	X	

0 = chromatophore absent.
X = chromatophore present.

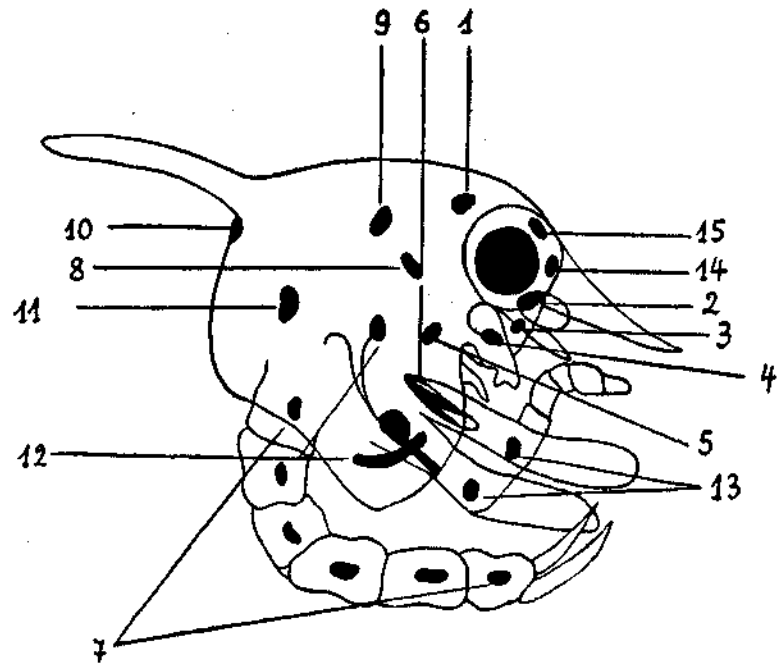

Fig. 3. Chromatophoral system of brachyuran larvae, general scheme after Aikawa. Explanations in text.

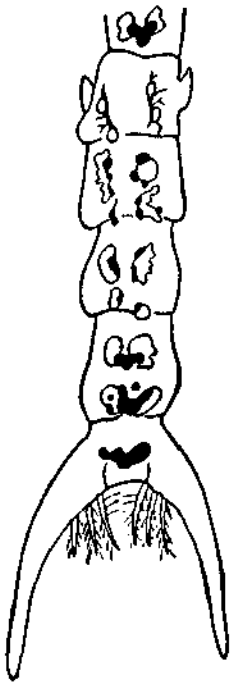

FIG. 4. Abdominal chromatophores of the first zoea of *Carcinus maenas*, ventral view.

It can be seen that only a part of the chromatophores of the *Carcinus* larva fits in Aikawa's scheme. The supracerebral chromatophores of Aikawa are lacking in *Carcinus*. An unpaired monochromatic chromatophore, lying between the basal segments of the antennulae, at the base of the rostrum, may be homologous with the antennal chromatophores of Aikawa, fused together. An unpaired monochromatic dark chromatophore, homologous with the labral chromatophores of Aikawa is also present as well as a pair of dark mandibular chromatophores. A big dark chromatophore lying just behind the antennae near the oral opening seems to represent the fused maxillar chromatophores of the general scheme. The maxillipedal chromatophores of the primary system are missing in the *Carcinus* larva.

The abdominal chromatophores are rather numerous (Fig. 4). The first segment contains a large single dichromatic chromatophore, probably the result of the fusion of a paired structure. The classification of this chromatophore is dubious. Since the first segment of the abdomen is situated inside the carapace and its anterior border is shifted cephalad, as far as the base of dorsal spine. So this structure could also be classified as homologous with the postcardiac chromatophores of Aikawa.

The systematic position of the remaining abdominal chromatophores is clear. The second segment has a pair of dichromatic chromatophores and the third and fourth segments each has two pairs of dichromatic chromatophores though varying in size. In the fifth segment two dichromatic chromatophores are present, each of them apparently resulting from the fusion of a paired structure. In the telson, a single monochromatic dark chromatophore can be seen, perhaps also the result of a fusion of a former pair.

In the visceral group of the primary system, the median gastric chromatophores of Aikawa are lacking in *Carcinus*. There is a fairly large single chromatophore, ventral to the heart, but this

is shifted too far caudally to allow of its comparison with the precardiac chromatophores of Aikawa (Fig. 2). The subcardiac chromatophore is also missing.

The secondary system of the *Carcinus* larva consists of a few conspicuous chromatophores The two pairs of dichromatic chromatophores forming the carapacial centre are particularly responsible for the coloration of the larva. In the state of dispersion the branches of these chromatophores reach ventrally the border of the carapace and dorsally the middle of the carapace. Secondary chromatophores are also present in the first and second maxillipedes. A big unpaired dichromatic chromatophore, situated medially between the eyes, represents Aikawa's median ocular centre. Two monochromatic dark chromatophores, lying between the ocular centre and the margin of the eye, are homologous to Aikawa's optic centre.

From the above it seems clear that the general scheme of Aikawa cannot be applied to all the constituents of the chromatophoral system of the zoea of *Carcinus*. Evidence to prove this is given mainly by the almost complete absence of the visceral group of chromatophores.

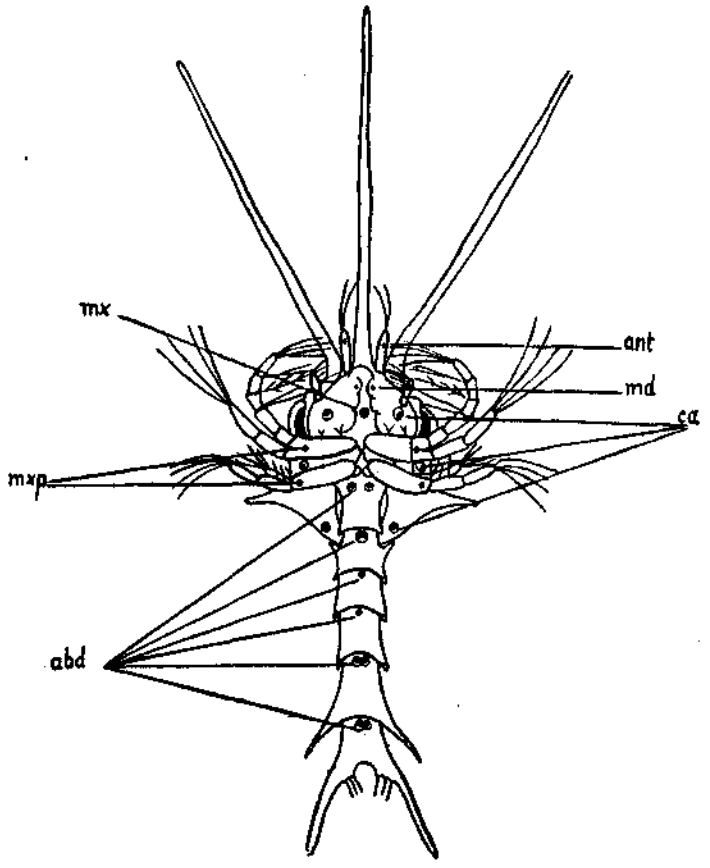

FIG. 5. Chromatophoral system of the first zoea of *Rhithropanopeus harrisi* subsp. *tridentatus*, ventral view. *abd*, abdominal chromatophores; *ant*, antennal chromatophores; *ca*, carapacial chromatophores; *md*, mandibular chromatophores; *mx*, maxillar chromatophore; *mxp*, maxillipedal chromatophores.

According to Aikawa the chromatophores of the maxillipedes and of the abdomen are of great taxonomic importance. But the scheme of distribution of the chromatophores in these parts,

proposed by Aikawa as characteristic of the Portunidae, does not apply to the larva of *Carcinus* or to that of *Callinectes sapidus*, described by Costlow and Bookhout (1959).

In the later investigated brachyuran species, *Rhithropanopeus harrisi* (Gould) subspecies *tridentatus* (Maitland) of family Xanthidae (Lawinski and Pautsch, 1965) the chromatophores of the first zoea form a regular pattern with only minor individual variation (Fig. 5). Only a part of them corresponds to Aikawa's classification. The chromatophores are dichromatic, dark and sulphur-yellow, or monochromatic dark. In some individuals an orange pigment may be present instead of the yellow one.

In the first zoea of *Rhithropanopeus* chromatophores homologous with the antennal, mandibular, precardial and post-cardial chromatophores of Aikawa can be identified. Aikawa's paired maxillar chromatophores are replaced by a single dark chromatophore. The remainder of the parts of the primary system of the thorax are lacking. In the abdomen, each segment contains chromatophores, paired or unpaired, some of them being monochromatic, others dichromatic, forming a pattern somewhat different from that of *Carcinus* larva.

The secondary system is represented, as in *Carcinus*, by the carapacial centre (two pairs of dichromatic chromatophores) and by monochromatic chromatophores in the first and second maxillipedes. In some individuals a pair of dichromatic chromatophores are present at the caudal margin of the carapace, but these show no homology to any element of Aikawa's system. The other parts of the secondary system are absent.

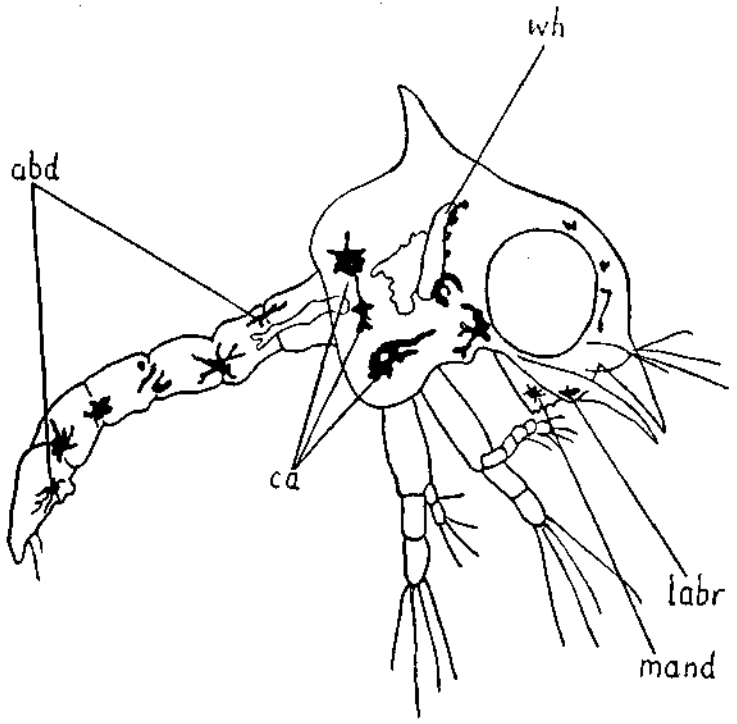

FIG. 6. Chromatophoral system of the first zoea of *Geograpsus livdus*. *abd*, abdominal chromatophores; the one in the third segment shown in strong concentration, optically desintegrated. *ca*, carapacial chromatophores, two of them joined by a fine branch; *labr*, labral chromatophore; *mand*, mandibular chromatophores; *wh*, the white chromatophores with adjacent black chromatophores, the one deep lying, situated caudally and shown in grey colour. In the first abdominal segment a single deep lying chromatophore is shown, also in grey colour.

The third larva studied belonged to the tropical species *Geograpsus lividus* (Milne Edwards) of family Grapsidae (Pautsch, 1965). In the first zoea almost all the chromatophores are monochromatic dark, of nearly black colour (Fig. 6). Their branches are very long and nearly always completely filled with pigment. Only one chromatophore situated in the thorax, adjacent to the dorsal side of the intestine is white. Its branches are very short. It seems hardly possible to identify this chromatophore with the median gastric chromatophores of Aikawa. The carapacial centre is very well developed and is made up of three pairs of dark chromatophores. In the state of dispersion the branches of these chromatophores join together, making it impossible to identify the individual cells. No secondary chromatophores are present in the maxillipedes. This is contrary to Aikawa's view, that the presence of these chromatophores is characteristic of the family Grapsidae.

In all the four species described an analysis of the main responses of the chromatophores to light rays was attempted. Generally, it can be stated that all the larval chromatophores are capable of dispersing and concentrating their pigments. It follows from this that the zoeae may change their colour.

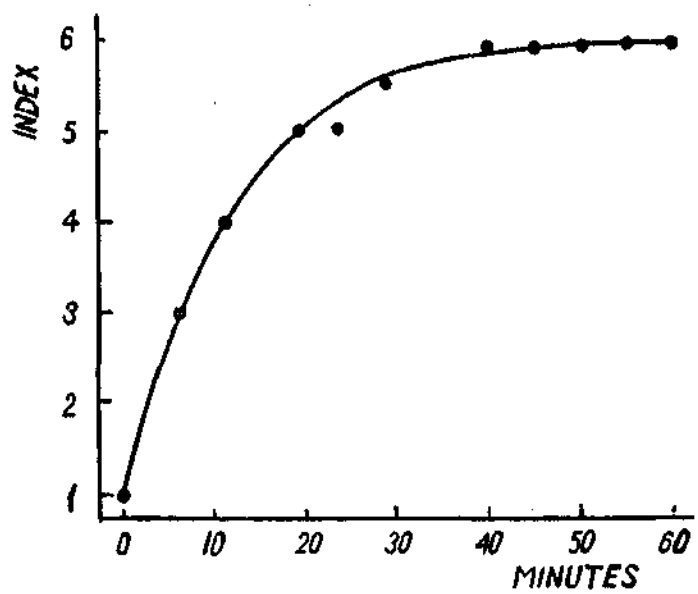

FIG. 7. The response of the chromatophores of the carapacial centre in the first zoea of *Carcinus maenas* to daylight, after a 24-hour stay in the dark-room.

In the first zoea of *Crangon crangon* (L.) there is indeed no background reaction, as claimed by previous investigators (Pautsch, 1951). In daylight the black-brown and the yellow pigments are dispersed, independent of the coloration of the background. During night or when kept in the dark, they begin to concentrate. Reflected light is therefore of no significance and only incident rays are effective. From the results of more detailed experiments it seems probable that besides intensity the wave-length also has an influence. Short waves, ultraviolet-violet (300–400 μ) and blue (315 μ) produce a stronger dispersion than long waves, like red (660 μ) even if their intensity is lower than that of the long ones.

The responses of the first zoeas of *Carcinus maenas* (L.) are generally similar (Pautsch, 1961). They also show a lack of background adaptation. If larvae with their chromatophores in submaximal dispersion are transferred to the dark-room, they adapt themselves slowly to the absence of light. In approximately 36 hours the new state of the pigments, a submaximal concentration

is reached and stabilized. The response to light after a longer stay in the dark-room needs much less time (Fig. 7). Already after five minutes the value of the index is much higher and after about 30 minutes it reaches its maximum.*

The degree of dispersion of both the pigments, black-brown and yellow, is in general the same, but not without exceptions. In maximal dispersion the branches containing the yellow-pigment are longer than those filled with the dark one. In this situation the big chromatophores of the carapacial centre are of a yellow hue. But after very long stay in the dark-room (seven days) the yellow pigment was more concentrated than the dark one.

The larval chromatophores of *Carcinus* are subject to diurnal rhythm. Their pigments are more dispersed during day than at the night (Fig. 8). The rhythm is partially independent of the diurnal changes in illumination. In larvae placed in the dark room the rhythm continues for some time.

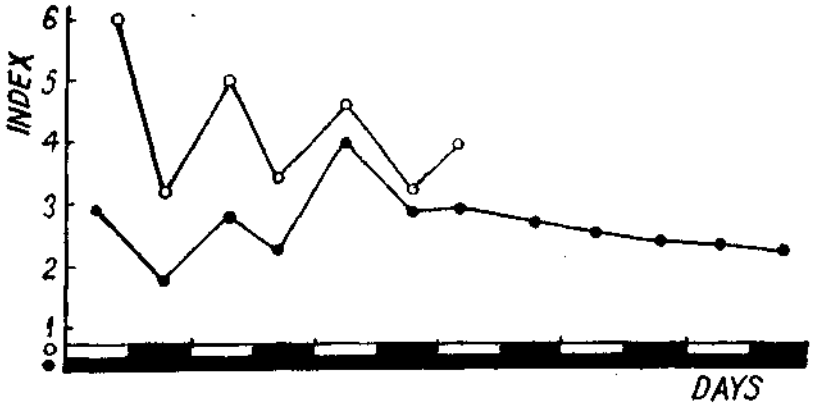

FIG. 8. Diurnal rhythm of the chromatophores of the first zoea of *Carcinus maenas*. White circles, larvae under natural conditions of illumination; black circles, larvae in the dark-room.

The responses to light of young metamorphosed *Carcinus* were investigated by Powell (1962 *a*). In these crabs three types of monochromatic chromatophores are present, black, red and white. So in this species, too, the pigmentation of a young individual is richer than that of the larvae. The crabs show the secondary type of chromatophoral reaction. The black chromatophores disperse, if the animal is placed on a black bottom. In the same surroundings the white chromatophores concentrate. On a white background the two types of chromatophores change in opposite directions. A diurnal rhythm of the chromatophores exists also in young crabs.

In the first zoea of *Rhithropanopeus harrisi* (Gould) also the primary reaction to light prevails (Lawinski and Pautsch, forthcoming). In complete darkness the pigments remain in a constant submaximal concentration. In larvae transferred from the dark room to the light of an electric bulb a rather prompt response of the chromatophores can be seen. In an illumination intensity of 1000 lux during the first 15 minutes there occurs a strong, but not yet maximal, dispersion of the dark and the yellow pigments (Fig. 9).

But unlike the *Carcinus* larvae, the zoeas of *Rhithropanopeus* possess the capacity of background adaptation, although only to a limited degree. After one hour's stay in the light of an electric bulb of 40 lux intensity the degree of dispersion of the black-brown pigment was somewhat higher in the larvae placed on a black background than in those on a white background. The

* The dispersion of the pigments was tested by the modified Hogben/Slome scale, Index 1·0 means maximal concentration, 2·0–5·0 growing dispersion and 6·0 maximal dispersion.

difference was about one degree of the Hogben/Slome scale. If white-adapted larvae were placed on a black background, the index of the dark pigment rose. The index of the same pigment in black-adapted larvae placed on white background decreased. Only the dark chromatophores show this secondary reaction while the yellow ones react primarily.

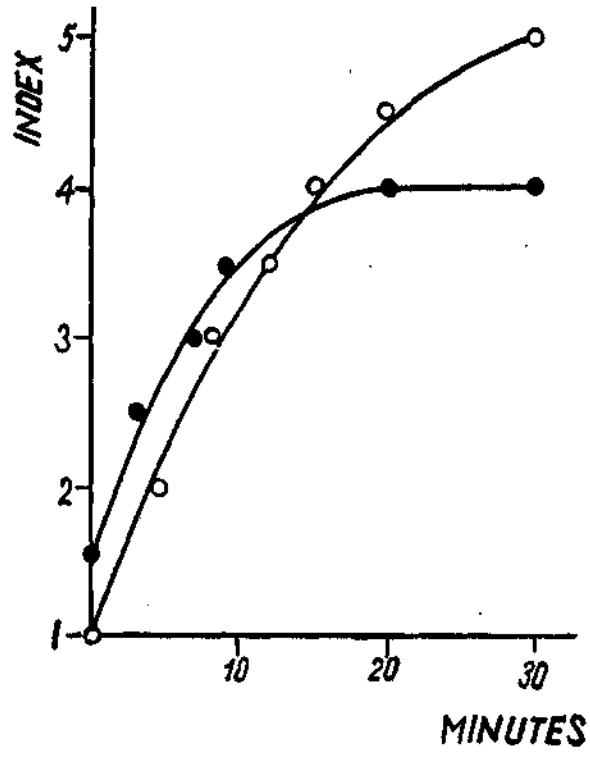

FIG. 9. Response of the chromatophores of the first zoea of *Rhithropanopeus harrisi* subsp. *tridentatus* in an illumination of 1,000 lux after a longer stay in the dark room. Black circles, black chromatophores; white circles, yellow chromatophores.

When the intensity of illumination is very high, for instance 1000 lux, the dark chromatophores do not adapt themselves to the background any longer. It may be assumed that in high-light intensities the stimulus from the incident rays is strong enough to annihilate the influence of the reflected rays. Further experiments (Pautsch and Lawinski, 1967) on later zoeal stages and on megalops of *Rhithropanopeus* revealed the existence of a more effective background adaptation of the melanophores.

The dark and the yellow chromatophores undergo a regular diurnal rhythm (Fig. 10). The dark pigment is constantly somewhat more dispersed than the yellow one. The maximal diurnal difference for the dark pigment was 2·5 degrees of the scale and for the yellow 2·7 degrees. In constant darkness the rhythm is abolished and the chromatophores remain in a state of submaximal concentration. The periodicity is regulated by external stimuli only. In this respect the larvae of *Rhithropanopeus* are different from the larvae of *Carcinus*.

The chromatophores of adult *Rhithropanopeus* are black, red, white and yellow (Pautsch *et al.*, 1960). The black and the white chromatophores possess slight ability of background adaptation,

The mean values of the indices of 100 adult chromatophores after a 24-hour stay on a black and on a white background in normal daylight were:

	Back ground	
	Black	White
Black chromatophores ..	4·5	4·1
White chromatophores ..	4·3	5·0

The pigment of the black chromatophores is a little more dispersed on a black background than on a white background. The pigment of the white chromatophores is more dispersed on a white background. This very slight secondary reaction causes no microscopically visible colour change, except the fact that in adult crabs the chromatophores are screened by the thick and opaque integument.

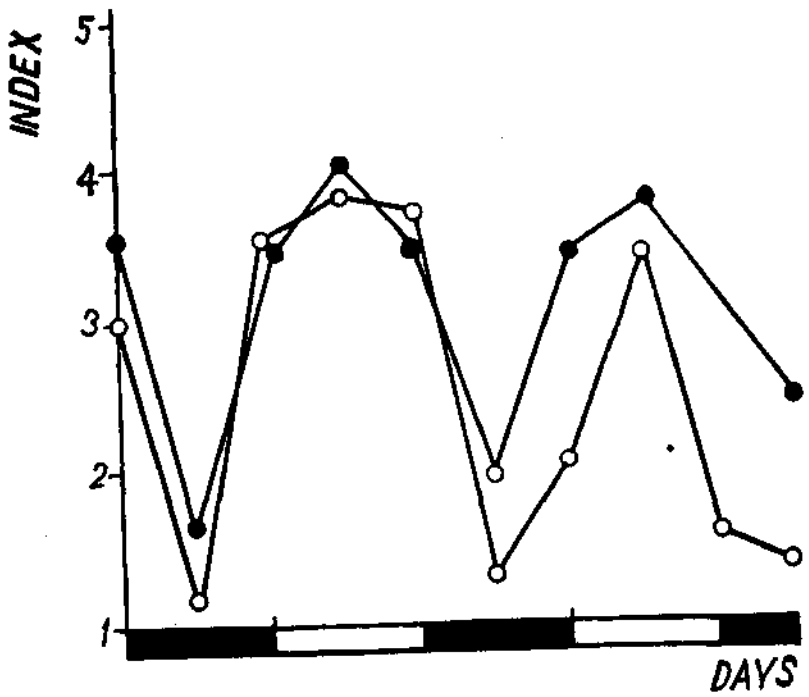

FIG. 10. Diurnal rhythm of the chromatophores of the first zoea of *Rhithropanopeus harrisi* subsp. *tridentatus*. Black circles, dark chromatophores; white circles, yellow chromatophores.

Incident light produces relatively strong effects on the chromatophores of adult *Rhithropanopeus*. In all the chromatophores the dispersion increases with the increase in the intensity of light. This is the case with normal as well as with blinded animals. But in the latter higher intensities are needed to cause dispersion. In blinded crabs chromatophores show no response in an illumination below 500 lux intensity. In all the four types of chromatophores a clearly expressed diurnal rhythmicity develops. In crabs placed in the dark-room the rhythm still goes on in the black and in the red chromatophores and is abolished in the white ones. When the animals are kept in a constant illumination of 500 lux the rhythm of all types of chromatophores is destroyed. The above proves that the rhythm is only partly dependent on the normal diurnal changes of light. Probably there also exists an internal regulating mechanism.

Of all the larvae investigated the first zoea of *Geograpsus lividus* (Milne Edwards) appeared to be the most susceptible to incident light. In daylight the degree of dispersion of the pigment in the chromatophores is higher than in the other larvae (Pautsch, 1963, 1965). This can be seen especially in the dark abdominal chromatophores, the long branches of which were in almost all larvae completely filled with pigment and interlaced, due to which it was impossible to distinguish the individual cell bodies.

After transferring the larvae to the dark room the decrease of the chromatophoral index is not so strongly expressed as for instance in the larva of *Carcinus*. The course in time of the response to light of the chromatophores of larvae that have been kept in the dark room is about the same as in *Carcinus*. There is no secondary reaction.

The diurnal rhythm of the black chromatophores and of the white one is clearly expressed, but its course in time is somewhat different than in the larvae of *Carcinus* or *Rhithropanopeus* (Fig. 11). In the latter maximum dispersion appears about noon while in *Geograpsus* the index begins to rise already in the morning, about 6 A.M., this process continuing during later hours, being, however, a little slower. After 6 P.M. the phase of concentration begins, reaching its maximum just before 6 A.M. Possibly, these differences may be explained by the more abrupt change of the day into the night, and *vice versa*, in the tropics where the observations on the *Geograpsus* larvae were performed. The rhythm of these larvae is exclusively environmentally controlled. Under constant light or darkness this rhythm is absent.

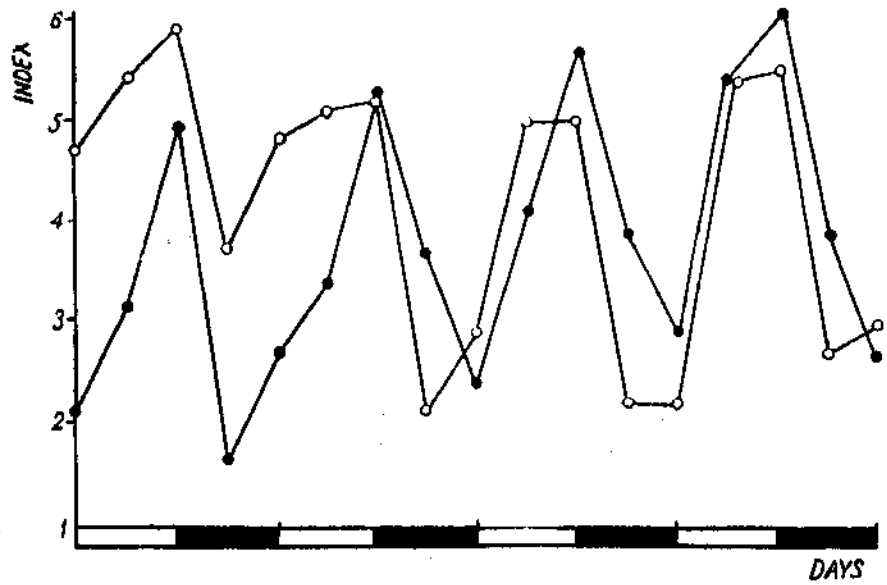

FIG. 11. Diurnal rhythm of the chromatophores of the first zoea of *Geograpsus lividus*. Black circles, black chromatophores; white circles, white chromatophores.

When I began my study on *Geograpsus*, nothing was known about the responses of the chromatophores of the adult of this species. My own observations were of a rather elementary character. Adult crabs possess black and white chromatophores. The black ones show a very distinct secondary reaction. On a black or a dark background the dispersion of the pigment is maximal and the colour of the crab changes visibly. The white chromatophores do not become adapted to the background. All the chromatophores of the adult undergo a diurnal rhythm, with its course in time very similar to that of the rhythm in the zoea (Fig. 12).

Summarising all these results one may state that, generally, in the first zoeas of decapods the primary type of reaction is represented. In the later stages the reaction changes over to the secondary type. In this respect there is a true analogy to the larvae of vertebrates. But this rule is not without exceptions. In *Rhithropanopeus* the larvae show a rudimentary secondary reaction, whereas in the same species the reaction is poorly developed in the adult.

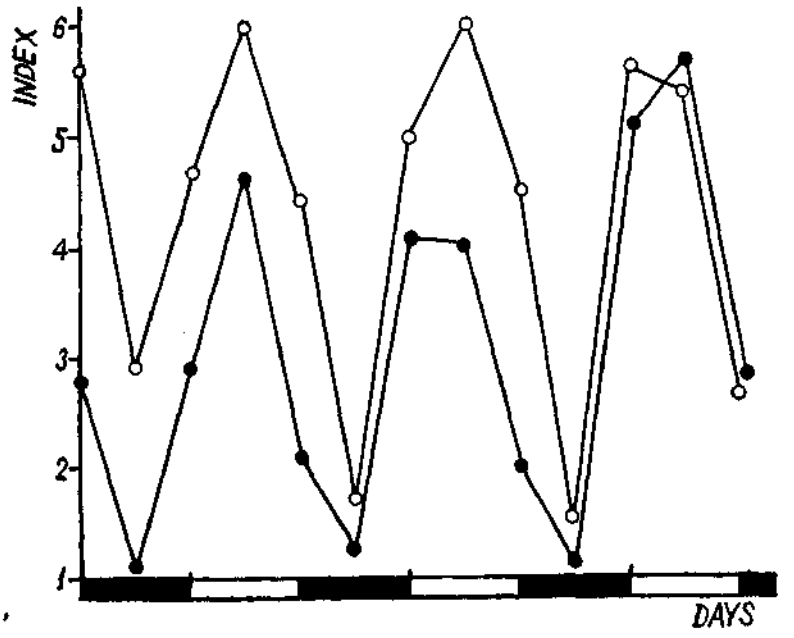

FIG. 12. Diurnal rhythm of adult chromatophores of *Geograpsus lividus*. Black circles, black chromatophores; white circles, white chromatophores.

No definite conclusions can be drawn as to the ecological importance of the different responses of the larval and adult chromatophores. It may be assumed that in forms like adult *Crangon crangon* and *Geograpsus lividus* the chromatic adaptation is of protective value. In some others, as for instance in young *Carcinus maenas* the change of colour may be a kind of mechanism controlling the temperature of the body (Powell, 1962 *a*). In adult *Rhithropanopeus* the chromatic adaptation seems to be without any importance and may be classified as a rudimentary physiological function.

Since the mode of life of the zoeas is planktonic in general, their limited background adaptation can hardly be assumed to play a protective role. On the other hand it might be possible that the primary reaction, accompanied by an increase of the body surface covered by pigment, gives some protection against the harmful effects of irradiation on the internal organs. It must be emphasized here that in the larvae investigated the central nervous system is dorsally covered by big chromatophores. In dispersion these form a kind of screen. Experiments with solar light of different wavelength were made on the larvae of *Crangon crangon* only. But it seems to be noteworthy that in this species the strongest response of the chromatophores was to short rays including violet, which are known to produce the most deleterious effects.

When summing up all these results one finds it difficult to avoid the temptation of touching on phylogenetic considerations—even at the risk of going wrong. Keeble and Gamble were the first to notice that in adult mysids the chromatophoral system is of a primary type. Its pattern is very similar to that of a zoea. From the observations of Chicewicz (1950) it is known that notwith-

standing this fact the chromatophores of *Praunus flexuosus* (Müll) react in a secondary way. There exists in this species a neuroendocrine regulatory mechanism of colour change, comparable to the one found in the Decapoda. On the other hand, it seems to be true that the chromatophores of leeches, capable of a physiological colour change, show the primary reaction only (Janzen, 1932). May be, all this could be interpreted by the primary reaction as being an earlier achievement in the phylogeny than the secondary reaction. Forms like *Praunus flexuosus* would represent an intermediate stage with not yet developed secondary chromatophores, but with primary chromatophores having already reached the level of secondary reaction.

The last point to be discussed in brief is the problem of neuroendocrine regulation of colour changes in the four species described in this paper. In the adults of all active chromatophorotropins are present. In the case of *Crangon crangon* this is a well-known fact that since the first investigations of Koller (1925), later continued and developed by many authors. Powell (1962 *b*) described the chromatophorotropins of adult *Carcinus maenas*. The chromatophorotropic activity of the neurosecretory products in *Rhithropanopeus* has also been proved experimentally (Pautsch *et al.*, 1960). Only the most basic experiments could be performed on adult individuals of *Geograpsus lividus* (Pautsch, 1965), but the results are sufficient to prove the existence of active hormones in the tissues of the eyestalk. Extirpation of the latter is followed by a concentration of the black and of the white chromatophores. When eyestalk-extracts are injected to eyestalkless *Geograpsus* the two pigments change from concentration to dispersion.

The placing of the first zoeas of all the four investigated species into diluted extracts from adult eye-stalks did not affect the chromatophores. But in contrast to this Broch (1960) noticed in the second zoea of *Palaemonetes vulgaris* pigmentary movements after a treatment with adult chromatophorotropins. Our own results (Pautsch and Lawinski, 1967) showed a similar response to adult hormones in the second and later zoeal stages of *Rhithropanopeus harrisi*. A reverse experiment, *i.e.*, treatment of adult brachyurans with extracts from larval bodies, was made by Costlow (1961) and by Costlow and Sandeen (1961). The chromatophores of adult *Uca* appeared to react to extracts from the first and later zoeas of *Sesarma reticulatum*. These varying results do not seem to be contradictory, but they cannot give any clear explanation regarding the first appearance of the neuroendocrine control of the chromatophores in the course of development. It can only be stated that it is highly improbable that this type of regulation develops already in the first zoea. The presence of substances acting on adult chromatophores does not prove that the larval chromatophores are regulated by them, and still less so since the adult chromatophorotropins appear to be inactive in this case. When interpreting the presence of the latter type of response in the later zoeal stages, it may be pointed out that these larvae show a clear background adaptation. But this does not necessarily mean that the second and the later zoeas have already developed the neuroendocrine reflex, characteristic of the adult stage. Hence, for the time being, there is no clear evidence as to the time of onset of a hormonal regulation of the colour change in a decapod's individual life.

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ADDENDUM

After submitting this paper to print, Ranga Rao* described positive responses to adult eyestalk extract in the chromatophores of excised pieces of larval carapaces *Ocypode macrocera*. Analogous experiments performed in my laboratory with first zoeae of *Rhithropanopeus* gave negative results.

* *Experientia*, 1967, **23**: 231–232.

THE ORIENTATION OF THE PRESSURE RESPONSES OF SOME MARINE CRUSTACEA

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ABSTRACT

A number of planktonic or temporarily planktonic marine crustacean species have been subjected to changes of hydrostatic pressure in unidirectional light and in darkness.

The results obtained show that there are considerable variations in the relative importance of light and gravity as orientating factors among the Crustacea as a whole and that it may even vary within a species, either between individuals or during the development of a single individual. There seems to be a strong correlation between the relative importance of the structures for their perception.

It is becoming increasingly clear that pressure sensitivity among planktonic animals is the rule rather than the exception. It is, therefore, suggested that the non-directional nature of pressure change as a stimulus to movement makes it a useful tool in the elucidation of the controversial role of geotaxis in the vertical migrations of these animals.

INTRODCUTION

IN recent years responses to changes in hydrostatic pressure have been reported in a number of crustacean species. Though there are some notable exceptions, which are dealt with by Knight-Jones and Qasim at this Symposium, pressure increase in darkness usually results in increased activity and upward swimming, while pressure decrease is followed by decreased activity or downward swimming. In the presence of directional light, however, there is considerable variation in the nature of the responses. During a study in which the pressure responses of planktonic animals of various taxonomic groups were investigated, experiments were conducted in which the particular aim was to compare the roles of light and gravity in the orientation of the responses.

MATERIAL AND METHODS

Most of the animals used were collected in relatively short hauls using conical tow-nets towed at depths of up to 70 m. Mysids were collected by D-net in Port Erin Bay. The larvae of various decapods and of the barnacle *Balanus balanoides* (L.) were hatched from the eggs in the laboratory.

The animals were observed while they were confined within a rectangular tank 60 cm. long and with a 5 cm. square cross-section, constructed of transparent 'Perspex'. The tank was completely filled with sea-water and the pressure within it was varied by raising or lowering a flexible tube communicating with the tank near one of its ends and filled for part of its length with mercury (Fig. 1). Pressures of up to about 2,000 mb. above atmospheric could be produced by this method, but generally the pressure changes used were within the range 500–1,000 mb.

The observation tank could be held vertically or horizontally, and illuminated from either end. All experiments were conducted in a darkroom where the lighting conditions could be controlled. The light source was a 500 W projector lamp mounted in a housing, producing a slightly divergent beam arranged to pass along the length of the tank. The intensity was controlled with a variac transformer and in the orientation experiments it was adjusted to produce about 1,000 lux at the end of the tank nearest to the light source.

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No temperature control was available and while most experiments were carried out within the range 8–12° C., the temperature occasionally rose to 15° or 16° C.

The animals were usually used either singly or in small numbers and their distribution in the observation tank was recorded at frequent intervals during the experiments. Where the individuals were so small as to make individual observations difficult large numbers were used and the distribution of the population was assessed objectively.

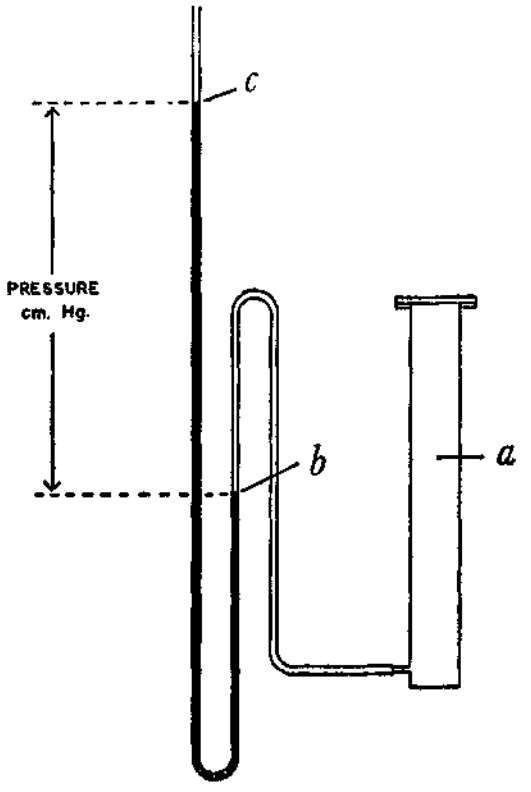

FIG. 1. Apparatus used to investigate the responses of marine animals to pressure changes. *a*, observation tank; *b*, proximal and *c*, distal end of the mercury column.

RESULTS

The responses observed in the species examined can be grouped into several categories depending on the effect of light direction on the orientation of the movements following pressure changes.

The first of these groups or response types contains those animals in which orientation to gravity seems to be of major importance. Here vertical light, whether from above or below, has little effect on the nature of the pressure responses, with increased pressure causing upward movement and decreased pressure causing downward movement in both cases. In horizontal light, on the other hand, light orientation is apparent in the responses with pressure increase causing lightward movement and pressure decrease producing either decreased activity or active movement away from the light. This category would include the mysids *Schistomysis spiritus* (G. O. Sars), *Praunus flexuosus* (Muller), *P. neglectus* (G.O. Sars) and *Leptomysis lingvura* (G. O. Sars). A fifth

species, *Siriella armata* (Milne-Edwards), does not fit quite so clearly into this category, since when illuminated from below the upward movement in response to increased pressure is not as well marked as in overhead light, so that there is some indication of an influence of light on the orientation in this case (*see* Rice, 1961).

A second response type seen in the Crustacea examined is in a way the antithesis of the first type for in this case light seems to be the dominant orientating factor. Here increased pressure causes or enhances movement towards the light source whether this involves upward, downward or horizontal swimming. Within this group various types of response to pressure decrease were observed.

In some species decreased pressure usually results in active swimming away from the light. Knight-Jones and Qasim (1955) reported this active response to decreased pressure in the copepod *Temora longicornis* (O. F. Müller) and in the nauplii of the barnacle *Balanus balanoides* (L.) and these observations were confirmed in the present work. In both of these species, a population of the animals placed in the observation tank would become segregated into two groups, one photonegative and one photopositive. At constant pressure there would be very little interchange between these two groups in *Temora*, but appreciable interchange in *Balanus*. In both cases pressure changes caused many of the animals to reverse their phototaxis in the appropriate manner. These changes in phototaxis were temporary in both species but were of much longer duration in *Temora*, where a pressure change of 1,000 mg. would produce a significant change in the distribution of the population for up to thirty minutes, whereas in the barnacle nauplii the original distribution would be reformed within a few seconds of a similar pressure change. An interesting feature of the responses of *Temora* is that the movements following pressure increases are much more rapid than those following a decrease. Thus, after an increase of 1,000 mb. some animals would cover the 60 cm. from one end of the tank to the other in less than ten seconds, whereas after a decrease of 1,000 mb. the fastest animals would take at least one minute to reach the far end of the tank.

Another case in which pressure decrease was followed by negatively phototactic swimming was the furcilia larvae of the euphausiacean *Meganyctiphanes norvegica* (M. Sars). In this case, however, since almost all the animals examined were negatively phototactic at atmospheric pressure, even with intensities as low as 50 lux at the end of the tank nearest the light, the movement following pressure decrease might be considered to be a return to the 'normal' behaviour rather than as a true change in phototaxis in response to the pressure change. Increased light intensity enhanced the negative phototaxis of the furcilias and could mask the response to pressure increase (Fig. 2).

In the copepods *Acartia clausi* Giesbrecht and *Calanus helgolandicus* (Claus) the responses to decreased pressure were rather variable, with both active and passive elements. In the case of *A. clausi*, the animals were consistently positively phototactic and moved towards the light source in a jerky manner in which they progress by a series of jumps between which the body appears to have no constant orientation. A decrease of pressure of 1,000 mb. in overhead light would cause about 10% of the population to swim actively downward, away from the light source, while most of the other animals sank passively, with only occasional periods of down-swimming. Similarly, in light from below, about 10% of the population responded actively to pressure decrease by swimming away from the light, this time vertically upwards, while the rest of the animals became orientated so that they faced directly away from the light but did not move more than about 5 cm. from the bottom of the tank. With horizontal illumination a similar result was obtained with 10–20% moving away from the light and the rest simply facing away from the light and ceasing to swim. An interesting feature of the active response to decreased pressure in this species was that during the photonegative movement the orientation of the body during the passive periods between the jumps appeared to be fairly accurately directed away from the light, in contrast with the situation during the photopositive movements. In all the situations considered above the animals would gradually return to their original positions if no further pressure change occurred, so that within fifteen to thirty minutes the original distribution would have been re-established. Increases of

pressure during the period after a pressure decrease and before the original distribution had been reformed caused very rapid movements towards the light by many individuals. In this way movements of 30 cm. in 6–7 seconds were frequently recorded.

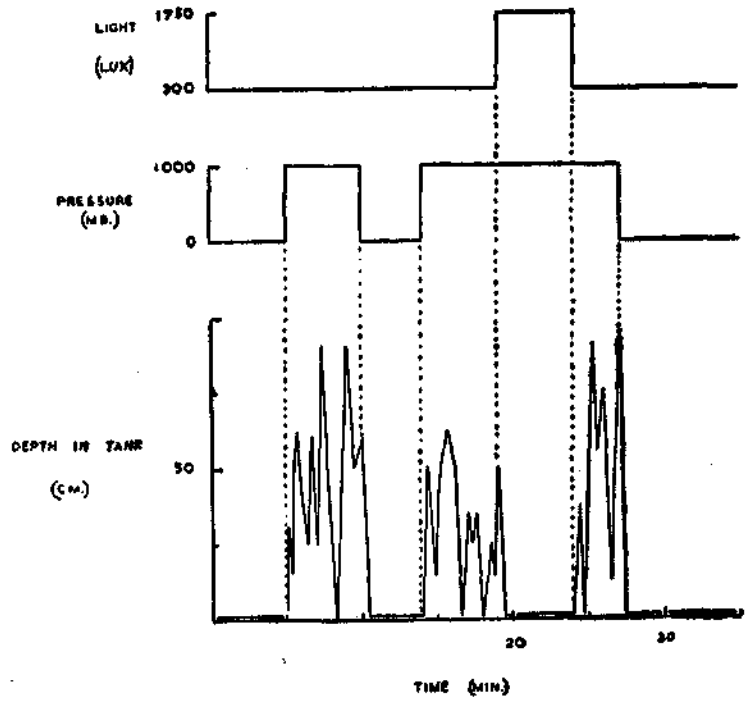

FIG. 2. Movements during pressure changes of a single furcilia larva of *Meganyctiphanes norvegica* in overhead light.

Though it was the field experiments of Hardy and Paton (1947) on *Calanus finmarchicus* which had first indicated that pressure might be an important factor in the control of the vertical distribution of planktonic animals, early laboratory experiments on *Calanus* by Hardy and Bainbridge (1951) and by Knight-Jones and Qasim (1955) failed to reveal any sensitivity to pressure changes. In the presently reported work specimens of the *helgolandicus* form were collected mainly in routine collections by townet in the Irish Sea at depths from 10 to 70 m. More than half of about 200 of these animals which were examined showed no change of behaviour whatsoever when exposed to pressure changes of up to 1,000 mb. The rest of the animals exhibited definite, but generally rather shortlived, pressure responses. Thus, in overhead light they remained mostly close to the bottom of the tank and pressure increase caused steady upward swimming lasting usually for less than one minute after which they would begin to sink. In a very small proportion of the population the pressure responses were of longer duration, lasting for fifteen minutes or more (Rice, 1962).

Rather different results were obtained with animals which were collected from a large surface swarm which appeared in Port Erin Bay in July 1961. The sea was extremely calm at this time and the animals were concentrated very close to the surface so that they could be collected by dipping them from the water's edge. Whereas there was considerable variation in the light responses of the townet caught animals, these 'surface' caught specimens were all very definitely positively phototactic to all intensities used (upto about 20,000 lux) in horizontal light and in vertical light from above and below. The pressure responses of these animals were basically similar to those seen in *Acartia clausi* with the change in distribution following pressure decreases

being apparent for up to fifteen or thirty minutes, though a rather smaller proportion responded by active swimming away from the light source than in *Acartia*. These surface caught animals were also much more sensitive than the townet specimens, with pressure changes as small as 50 mb. producing definite responses.

In light from below, a rather peculiar pressure response was exhibited by some of the deep caught photonegative animals and many of the shallow caught animals after they had moved away from the light to the top of the tank following a pressure decrease. In this situation a pressure increase was followed, not by active lightward swimming, but by passive sinking which in this case carried them towards the light.

In neither of the previous reports of experiments on *Calanus* in which no responses to pressure changes had been demonstrated was the form used specified, but from the known distributions of the two forms it seems probable that Hardy and Bainbridge's animals were mainly *finmarchicus*, while those used by Knight-Jones and Qasim were mainly *helgolandicus*. The animals used in the experiments reported above were all *helgolandicus*, but a sample of *finmarchicus* was obtained from Millport for comparison; they were collected by townet at a depth of about 115 m., flown to the Isle of Man in insulated flasks and used in experiments on the same day. Changes of pressure of up to 1333 mg. produced no apparent responses in any of these animals. However, no definite conclusions can be drawn from this result since examination of the animals after the experiments had been completed revealed that in all cases the caudal setae were broken, indicating that they had experienced considerable mechanical disturbance which might have destroyed any pressure sensitive mechanism which they possess.

In the larvae of brachyuran Decapoda the orientation of the pressure responses is not as clear cut as in the cases discussed above, since there is a tendency for the relative importance of light and gravity to change during development. In the first zoeal stage of *Macropipus depurator* (L.), *M. pusillus* (Leach), *M. puber* (L.), *Carcinus maenas* (L.), *Cancer pagurus* L., *Atelecyclus rotundatus* (Olivi), *Xantho couchii* Bell, *Pinnotheres pisum* (L.), *Ebalia tuberosa* (Pennant), *Eurynome aspera* (Pennant), *Macropodia rostrata* (L.), *Inachus dorsettensis* (Pennant) and *Hyas coarctatus* (Leach) pressure increase always causes, or enhances, movement towards the light source, while pressure decrease produces decreased activity and sinking but no orientated movement away from the light. Although there was this unanimity in the orientation of the pressure responses of these stage I zoeae, they did show some differences in behaviour which seem to be correlated with their size. The smaller zoeas, those of *Macropipus*, *Carcinus*, *Cancer*, *Atelecyclus*, *Xantho*, *Pinnotheres*, *Ebalia* and *Eurynome*, were very strongly positively phototactic under the lighting conditions used, and equally negatively geotactic in darkness. The larger zoeas of *Macropodia*, *Inachus* and *Hyas*, on the other hand, though being quite definitely photopositive in horizontal light, remained mainly close to the bottom of the tank in darkness or when illuminated from above.

Since the first zoeas were usually obtained from laboratory hatchings, large numbers of specimens were available for study. The later stages, on the other hand, were obtained entirely from plankton collections and were therefore available in much smaller numbers, so that fewer specimens and fewer species were examined. In the ultimate zoeas of *Macropipus* sp., *Ebalia tuberosa* and *Carcinus maenas*, while the light direction is still very important in the orientation of the animals' movements in response to pressure changes, gravity is becoming more important as an orientating factor. This change in orientation becomes obvious only in horizontal light where the two orientating factors are operating at right-angles to one another. In this situation the late zoeas respond to pressure increase by movement towards the light, but with a marked upward component (Fig. 3). Under the comparatively normal situation with light from above, pressure increase causes movement towards the light and decreased pressure causes sinking, as in the early zoeas. When illuminated from below, pressure changes cause changes in the general activity level, but no tendency to move upwards, away from the light, again similar to the early zoeas. Thus, when the two orientating factors are in direct opposition, orientation to gravity is not sufficiently strong to overcome the effect of light direction.

Megalopas of only two species, *Macropipus* sp. and *Carcinus maenas*, were examined, but in both cases there was a significant development in the orientation to gravity beyond the state seen in the late zoeas. In these animals increased pressure in all lighting conditions, including light from below, caused upward movement and decreased pressure caused downward movement. Even in the megalopas, however, the light direction was not entirely disregarded in the orientation of the movements, since after a pressure increase in horizontal light some individuals did move towards the light after having moved upwards, across the light beam, as was seen in the late zoeas. In addition, in light from below, some of the megalopas which were positively phototactic at constant pressure moved upwards only a few centimeters after a pressure increase, before once more returning to the bottom.

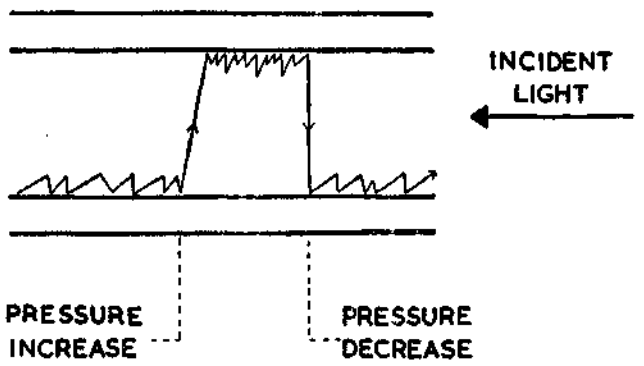

FIG. 3. Typical path followed by late brachyuran zoeas during pressure changes in horizontal light.

The first one or two zoeal stages of a number of other decapod species were examined, including the carideans *Pandalus montagui* Leach, and *Crangon crangon* (L.), the anomurans *Galathea squamifera* Leach, *Munida rugosa* (Fabricius), *Pagurus bernahardus* (L.), *P. pubescens* Krøyer and *Anapagurus laevis* Bell and in the astacuran *Nephrops norvegicus* (L.). All of these larvae exhibited similar pressure responses to those of the early brachyuran zoeas.

The only non-brachyuran species in which both the early and late larval stages were examined was the lobster, *Homarus gammarus* (L.). In this case the pressure responses of both the first and the fourth larva, which is equivalent to the megalopa of the Brachyura, are orientated entirely to light.

DISCUSSION

It is apparent that in all the Crustacea examined light is a very important orientating factor at one stage or another, at least under the experimental conditions used. During the investigation as a whole, a class of animals was encountered in which the light direction had no demonstrable effect at all on the orientation of the pressure responses. This was the case in the ephyra larvae of the jelly fish *Aurelia aurita* L. and the ctenophores *Pleurobrachia pileus* (O. F. Müller) and *Bolinopsis infundibulum* (O. F. Müller), where increased pressure caused only upward movement and decreased pressure caused downward movement under all lighting conditions (Rice, 1964). The nearest approach to this type of behaviour seen in the crustaceans was that of the brachyuran megalopas, but even in those species where light direction was completely dominant in orientation, gravity orientation was apparent in total darkness. In many species these gravity orientated movements obviously involved an appreciation of 'up' and 'down' and a definite behavioural geotaxis. In some cases, such as most calanoid copepods, however, this apparent gravity orientation could be purely mechanical since the body assumes a position during rest such that any forward swimming results in upward movement.

In the results obtained in unidirectional light the relative importance of light and gravity in the orientation of the observed pressure responses is generally as might be expected from anatomical considerations alone. Thus, those animals in which gravity orientation is well marked, such as the mysids, have well-developed organs of balance, whereas the presence of functional statocysts or their equivalent in the animals in which light orientation is dominant is less certain. The change in orientation noted during the course of development of the brachyuran larvae is probably correlated with the development of the statocysts since these organs are said to become functional only at the moult to the megalopa. However, the fact that the late zoeas examined appeared to orientate partly to gravity in horizontal light suggests the possibility that the statocysts may become functional before they are fully formed anatomically. A significant exception to this general correlation between gravity orientation and the possession of statocysts is in the larvae of *Homarus gammarus* where light was found to be the dominating factor even in the fourth stage which possess well-developed statocysts. The first larva of *Homarus gammarus* swims with the abdomen flexed and the chelae hanging beneath the body and rolls appreciably in forward movement. It would seem that the main function of the statocysts in this case is to enable the fourth larva to swim efficiently with the chelae, thorax and abdomen held in a more or less straight line, as suggested for *H. americanus* H. Milne-Edwards by Prentiss (1901).

To date, pressure responses have been demonstrated in almost one hundred marine species, including more than fifty crustaceans, and it seems that pressure sensitivity may be the rule rather than the exception among such animals. Pressure is a non-directional stimulus and as such has been used in the presently reported work to elucidate the relative importance of light and gravity in orientation. Similarly, it may prove to be a useful laboratory tool in the study of other aspects of behaviour, such as the effects of salinity and temperature gradients or discontinuities on the animals' movements, or the maximum swimming speeds of which they are capable. The pressure stimulus in darkness might also be used to investigate the occurrence of true geotaxis among migrating planktonic animals, a problem which has been the subject of considerable controversy in the past.

Pressure is still a relatively 'new' factor to be considered in attempts to account for the distribution and migrations of marine animals, and the whole question of the significance of the pressure responses in nature requires much more attention.

The responses considered above are all orientated in such a way that the movements following pressure changes tend to return the animal to the pressure existing before the change. It appears that in shallow water species these responses, as well as the reversed type of response in which increased activity follows decreased pressure, may be geared to the tidal cycle of pressure changes (Enright, 1961, 1962 and Morgan, Nelson-Smith and Knight-Jones, 1964). However, pressure responses of a depth regulatory nature have been observed in many typically deep-water planktonic species and it is likely that pressure changes other than those associated with the tidal rise and fall are important in the behaviour of these animals. In the laboratory experiments on these depth regulatory pressure responses the extent of the movements of the animals is generally much less than would be necessary to compensate for the pressure change experienced. The pressure changes involved in these experiments have usually been abnormally rapid and the lighting conditions have remained unaltered during the changes. Probably of much more importance in nature are the slow pressure changes which the animals would experience as a result of their own movements through the water and which would be accompanied by changes in the light intensity during the daytime. Some of the laboratory experiments indicate that light does have an effect on the extent of pressure responses so that experiments involving simultaneous changes in light intensity and pressure could furnish useful information on the role of pressure in diurnal vertical migration.

SUMMARY

1. A simple apparatus used to investigate the responses of marine crustaceans to pressure changes is briefly described.

2. In about thirty crustacean species, pressure responses of a depth regulatory nature have been observed in which the movements following pressure changes would tend to return the animals to the pressure existing before the change.

3. The responses observed are of two main types depending on the relative importance of light and gravity on the orientation of the movements. These are (*a*) those in which orientation to gravity is dominant and the light direction is of minor importance, and (*b*) those in which the light direction is dominant as the orientating factor.

4. There is a strong correlation between gravity orientated responses [type (*a*) above] and the possession of statocysts.

5. It is suggested that in deep water planktonic species pressure may be an important factor in diurnal vertical migration and that experiments involving simultaneous control of light intensity and pressure will be necessary to investigate this possibility since there are indications that the pressure responses may be affected by the intensity of the light as well as by its direction.

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RESPONSES OF CRUSTACEA TO CHANGES IN HYDROSTATIC PRESSURE

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ABSTRACT

Though many larval and post-larval marine Crustacea, including copepods, cirripedes and malacostracans, readily respond to small pressure changes in a way that is depth-regulatory in sense, they do not, as a rule, regulate their depths within narrow limits. The pressure responses merely make a contribution to complex behaviour patterns. Indeed, the significance and importance of the pressure sense vary greatly in different species, and may be involved in functions other than depth-regulation and in behaviour which is opposite in effect. For instance, *Corophium volutator*, which is normally benthic in the tidal mud-flats, is stimulated to swim by decreasing pressure. It can perceive pressure cycles similar in range and frequency to those caused by the tides and hence swims mostly during the ebb.

Cyprid larvae of the littoral barnacle, *Balanus balanoides*, settle more readily in the laboratory if they are given changes of pressure at frequencies similar to those of onshore waves. The responses of barnacle nauplii however, are of a more stereotyped kind and, therefore, seem suitable for studies on the mechanisms which confer pressure sensitivity.

INTRODUCTION

MANY of the accounts which have appeared in recent years, of the sensitivity of marine animals to small changes in hydrostatic pressure, have referred to Crustacea. The first demonstration of such sensitivity (if we leave aside studies on animals with gas-filled organs, such as teleosts and insects—*see* Harden-Jones and Marshall, 1953; Thorpe and Crisp, 1947) was with decapod zooea larvae by Hardy and Bainbridge (1951). Various interim reports (Knight-Jones and Qasim, 1955; Baylor and Smith, 1957; Qasim and Knight-Jones, 1957) described typical responses of planktonic crustacea as being depth-regulatory in sense, swimming activity being increased at higher pressures. Methods of orientation were analysed particularly by Rice (1964), who found that copepods and zooea larvae responded to increased pressure by becoming more positive to light, megalopa larvae by becoming more negative to gravity, whilst mysids orientated to both light and gravity. Finally, possible mechanisms for perceiving pressure changes were studied by Digby (1961) and Enright (1963).

At the same time it has become clear that some of these pressure responses are not concerned with simple depth-regulation in the plankton (Knight-Jones and Morgan, 1965). The careful studies of Enright (1961 and 1962), on the littoral amphipod *Synchelidium*, indicate a mechanism for controlling migration up and down surf-swept beaches. Probably mysids (Rice, 1961) and more certainly *Corophium* (Morgan, 1965) perceive and respond to pressure cycles of tidal range and frequency.

This symposium offers a good opportunity for reviewing what is evidently a complex of study, and including unpublished data which should be taken into consideration,

METHODS

The animals were kept in comparatively large volumes of sea-water and were studied within two days of collection unless otherwise stated. Experiments were usually carried out in conical flasks, containing sea-water 10 cm. deep, but smaller forms, such as barnacle larvae, were put into test-tubes connected together by a branching manifold of tubes. The vessels were immersed in water-filled, rectangular troughs, to keep the temperature constant and make viewing easier and safer. The lighting was diffuse and mostly from above. Records were kept of numbers swimming above half-depth. Total numbers in a given vessel were kept small, to make counting easier and to prevent the animals from disturbing each other, and the experiments were repeated as often as necessary, with fresh individuals.

In most experiments the pressure was changed with the help of the laboratory compressed air system, which was at nearly two atmospheres. The experimental vessels, with bungs secured by clamps, were connected to a partially open compressed air tap by a length of pressure tubing. This had an open sidearm and was also connected to a mercury manometer. To raise the pressure, the aperture of the sidearm was constricted by means of a screw-clip, gradually restricting the escape of air until the manometer level had risen to the required height. Widening the aperture again allowed the pressure to fall. At high pressures the escaping air made a hissing sound, but the more active swimming of the animals then could not be attributed to auditory stimulation, for they swam most strongly at the highest pressures, when the outlet was closed and the hissing had stopped.

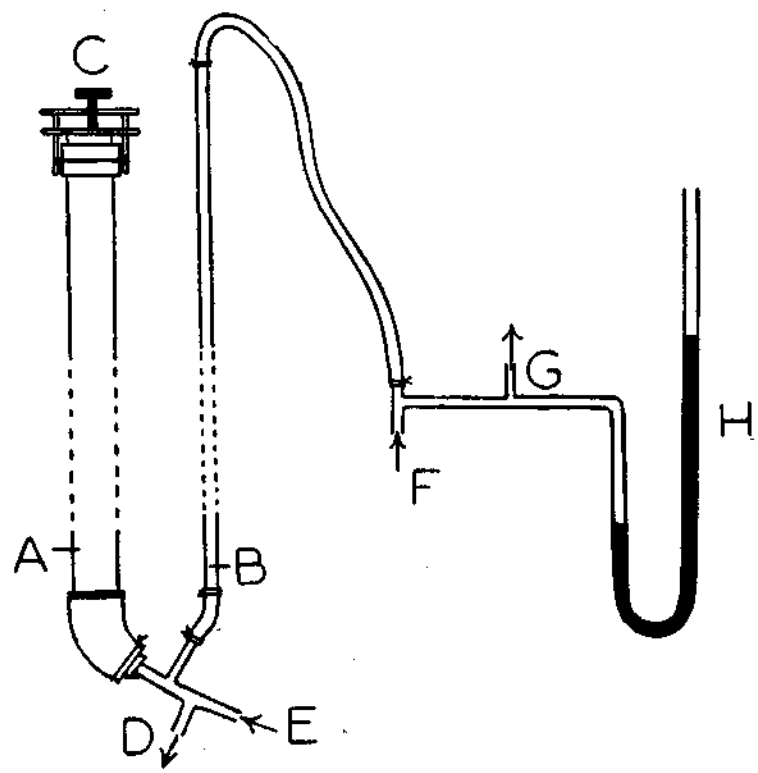

FIG. 1. A. Experimental tube 6·3 M long and 12 mm across, used to study extensive vertical movements of the type shown in Figs. 2 and 3. B. Water manometer. The system was filled to the level of C by removing the bung there, closing D and running in sea-water through E. Then, having added a plankton animal and with C, D and E closed, compressed air was turned on through F and the pressure raised, by gradually closing G, and registered by the mercury manometer H. The pressure could be lowered by opening G and further, by draining B through D.

Moreover they showed the same responses in experiments without compressed air, in which the whole experimental system was filled with water and closed, except for a connection to a flexible mercury column. This terminated in an open reservoir, which could be raised or lowered to change the pressure.

To study extensive vertical movements we suspended, in the axis of a zig-zag staircase, a tube 6·3 M long and 12 mm in internal diameter, of glass with a few plastic joints. The lighting was diffuse, mostly from above and from the sides. Alongside the tube, and connecting with it at the bottom, there was a finer tube to act as a water manometer. By opening a tap at the bottom both tubes could be filled from the laboratory sea-water supply, which had a head of 7 M. We would then introduce an animal into the wider tube and close this at the top. The pressure could be raised by applying compressed air, or lowered by releasing the compressed air and further by draining the water manometer tube. The procedures followed are explained in Fig. 1. The movements of the animals were recorded, against a vertical scale, by viewing them from the staircase.

Responses to Frequent Small Pressure Changes

Hyas araneus (L.) [Brachyura]

Zooea larvae were obtained from eggs hatching in the aquarium. They were much denser than sea-water, but most of them kept at a fairly uniform depth by swimming upwards (on the lighted side of the vessel), at a speed which exactly compensated for their tendency to sink. With pressure increases equivalent to 25 and 50 cm. of water, many of them increased their swimming speed for a few seconds and some for as much as 30 seconds, so that there was a general tendency to move upwards slightly, but the responses produce no striking increase in the numbers above half-depth (Table I). When the pressure was lowered they sank passively, but only for a few seconds. Pressure changes of 1 M or more produced responses which were somewhat greater, but the larvae seemed reluctant to depart from their mean level of swimming activity and tended to resume this long before they had changed depth enough to compensate for a pressure change.

Table I

Numbers of Hyas araneous *zooea larvae (out of 25), swimming above half-depth in sea-water 10 cm. deep, when given gradually increasing changes in pressure imposed at intervals of a minute. Numbers were recorded every 15 sec. Time in minutes is shown in brackets*

Atmospheric p.					⇌	At. + 25 cm.				
(1)	9	9	9	10		(2)	12	11	12	11
(3)	10	9	9	9		(4)	10	11	14	14
(5)	11	8	8	9		(6)	10	9	9	8
(7)	8	8	8	8		(8)	9	10	9	9
(9)	10	10	10	10		(10)	9	9	9	9
Means ..	10	9	9	9			10	10	11	10

Atmospheric p.					⇌	At. + 50 cm.				
(11)	8	8	7	7		(12)	11	12	11	10
(13)	10	8	9	9		(14)	11	12	10	10
(15)	10	7	6	7		(16)	8	9	8	9
(17)	9	9	9	9		(18)	13	10	10	9
(19)	7	9	9	9		(20)	9	8	7	9
	9	8	8	8			10	10	9	9

Atmospheric p.					⇌	At. + 1 M				
(21)	8	8	9	9		(22)	12	13	11	11
(23)	9	5	6	6		(24)	9	10	10	10
(25)	8	8	6	6		(26)	12	9	10	10
(27)	7	6	6	7		(28)	10	12	10	7
(29)	7	7	7	8		(30)	8	9	9	9
Means ..	8	7	7	7			10	11	10	9

Atmospheric p.					⇌	At. + 6 M				
(31)	9	5	6	9		(32)	19	23	21	23
(33)	19	13	9	9		(34)	19	22	17	17
(35)	15	10	8	5		(36)	12	17	18	16
(37)	13	10	10	9		(38)	13	16	16	17
(39)	10	8	6	6		(40)	11	14	12	13
	13	9	8	8			15	18	15	17

Carcinus maenas (L.) [Brachyura]

Megalopa larvae gave records (Table II) indicating greater sensitivity (for 10 cm. changes $p = 0{\cdot}01$), but otherwise behaved like the zooea larvae. The increased activity which small changes evoked was rarely strong enough to change, by more than 2 or 3 cm., the remarkably constant depth at which they swam. Increases of 1 M of water produced a response which was initially enthusiastic but soon tended to wane. Repeated pressure changes of this range were followed by a marked fall in activity, so that the numbers in contact with the bottom were greater than before this disturbance. Greater increases were needed to induce a majority to swim above half-depth and some individuals remained always in contact with the bottom.

TABLE II

Response of Carcinus maenas *megalopa larvae to gradually increasing changes in pressure, as in Table I*

Atmospheric p	⇌	At. + 10 cm.	→	Atmospheric p.	⇌	At. + 20 cm.
(1) 3 3 3 3		(2) 4 5 6 4		(11) 2 2 3 3		(12) 4 2 3 3
(3) 4 4 3 3		(4) 6 4 3 3		(13) 3 3 3 4		(14) 5 4 4 4
(5) 3 3 3 3		(6) 3 3 5 5		(15) 3 4 4 3		(16) 5 4 4 4
(7) 4 3 4 3		(8) 4 3 4 4		(17) 4 5 4 3		(18) 4 4 3 3
(9) 3 3 4 5		(10) 6 4 4 5		(19) 3 3 3 3		(20) 5 4 4 4
Means .. 3 3 3 3		5 4 4 4		3 3 3 3		5 4 4 4

Atmospheric p	⇌	At. + 50 cm.	→	Atmospheric p.	⇌	At. + 1 M
(21) 3 3 3 3		(22) 6 7 5 4		(31) 4 4 4 3		(32) 10 9 8 8
(23) 2 2 2 4		(24) 4 3 3 4		(33) 3 1 1 1		(34) 5 4 4 5
(25) 2 2 2 2		(26) 3 4 2 2		(35) 3 2 2 2		(36) 7 7 5 5
(27) 2 2 2 2		(28) 5 5 5 3		(37) 2 2 0 0		(38) 3 3 3 2
(29) 2 3 2 1		(30) 4 4 3 3		(39) 1 1 1 0		(40) 2 3 2 2
Means .. 2 2 2 2		4 5 4 3		3 2 2 1		5 5 4 4

Atmospheric p.	⇌	At. + 6 M
(41) 0 0 0 0		(42) 10 14 18 16
(43) 3 3 3 3		(44) 8 10 12 12
(45) 4 1 0 2		(46) 7 10 9 13
(47) 4 4 3 3		(48) 9 11 10 11
(49) 2 2 1 2		(50) 4 6 9 10
Means .. 3 2 2 2		8 10 12 12

Pinnotheres pisum (Pennant) [Brachyura]

A few of the first crab stage (Christensen, 1959) emerged from *Spisula solida* brought into the laboratory. They swam readily, suspended a few centimetres above the bottom. Placed in tall vessels they continued to swim near the bottom. In response to pressure increases they swam up momentarily and in response to decreases they sank down passively, but they quickly resumed their normal level of activity, which kept them suspended near the bottom.

Galathea sp. [Anomura]

Young post-zooea larvae, tow-netted from shallow water over sand, swam readily and behaved very like megalopa larvae in response to pressure changes (Table III). Evidently this is a searching phase, like the megalopa stage of crabs. The figures indicate great sensitivity (for 10 cm. changes $p = 0{\cdot}01$).

TABLE III

Response of Galathea *larvae to gradually increasing changes in pressure, as in Table I*

	Atmospheric p.				⇌	At. + 10 cm.			
(1)	0	0	0	0	(2)	2	2	1	2
(3)	0	1	1	1	(4)	2	1	1	1
(5)	1	0	0	0	(6)	0	0	0	0
(7)	0	0	0	0	(8)	1	2	0	1
(9)	0	0	0	0	(10)	0	0	0	0
Means ..	0	0	0	0		1	1	0	1

	Atmospheric p.				⇌	At. + 20 cm.			
(11)	0	0	0	0	(12)	0	1	1	0
(13)	0	0	0	0	(14)	0	0	0	0
(15)	0	0	0	0	(16)	0	0	0	0
(17)	0	0	0	0	(18)	0	1	0	1
(19)	0	0	0	0	(20)	0	1	1	0
Means ..	0	0	0	0		0	1	0	0

	Atmospheric p.				⇌	At. + 50 cm.			
(21)	0	0	0	0	(22)	4	3	2	1
(23)	1	1	1	1	(24)	2	3	1	1
(25)	1	1	1	1	(26)	1	1	1	1
(27)	1	1	1	1	(28)	1	1	1	1
(29)	1	1	1	1	(30)	1	1	1	1
Means ..	1	1	1	1		2	2	1	1

	Atmospheric p.				⇌	At. + 1 M			
(31)	1	1	1	1	(32)	3	4	6	6
(33)	1	1	1	1	(34)	3	4	6	5
(35)	0	0	0	0	(36)	2	2	4	3
(37)	1	1	0	0	(38)	2	3	4	3
(39)	2	1	1	1	(40)	3	3	4	4
	1	1	1	1		3	3	5	4

	Atmospheric p.				⇌	At. + 6 M			
(41)	1	1	1	1	(42)	5	10	16	20
(43)	1	1	1	1	(44)	2	7	12	9
(45)	2	1	0	0	(46)	1	3	9	7
(47)	0	0	0	0	(48)	1	7	8	7
(49)	0	0	0	0	(50)	1	2	6	4
Means ..	1	1	0	0		2	6	10	9

Eurydice pulchra Leach [Isopoda]

When placed in experimental flasks, *E. pulchra* swam rapidly about in all directions, but mostly more or less horizontally. Pressure changes equivalent to 10 and 20 cm. of water had little effect (Table IV), but 50 cm. increases induced much faster swimming, with increased numbers above half-depth ($p = 0{\cdot}001$). On subsequent decreases many *Eurydice* became immobile and sank quickly to the bottom. The responses followed the changes with little delay. After an increase the swimming was often directed upwards initially, but soon became more random, so that after 30 sec. the numbers above half-depth tended to decrease.

After a few hours in the flasks, most *Eurydice* became sluggish and swam only occasionally. Some sank to the bottom, ventral side upwards, and lay there, occasionally fanning with their pleopods. Small increases in pressure induced more rapid movements of the pleopods, large increases usually started them swimming again. Provided with sand, the *Eurydice* usually burrowed into it without delay and remained buried. Large pressure increases failed to induce them to leave the sand.

Some other Malacostraca were studied more briefly.

Idotea linearis (L.) [Isopoda]

This isopod swam upwards after increases in pressure and sank passively after decreases. Some littoral amphipods and some Cumacea from a shallow sandy bottom were excited to apparently random activity by frequent pressure changes.

TABLE IV

Response of Eurydice pulchra *to gradually increasing changes in pressure, as in Table I*

Atmospheric p.				⇌	At. + 10 cm.				Atmospheric p.				⇌	At. + 20 cm.			
(1)	3	1	4	3	(2)	2	3	2	2	(11)	2	3	6	4	(12)	7	4	4	1
(3)	2	2	1	3	(4)	4	1	2	2	(13)	3	2	2	4	(14)	2	5	2	4
(5)	2	2	1	3	(6)	2	2	4	6	(15)	3	1	3	5	(16)	5	4	3	3
(7)	3	3	3	2	(8)	5	4	2	6	(17)	1	2	3	2	(18)	4	4	4	2
(9)	5	2	6	4	(10)	3	1	3	5	(19)	3	4	2	3	(20)	4	4	7	3
Means ..	3	2	3	3		3	2	3	4		2	2	3	4		4	4	4	3
Atmospheric p.				⇌	At. + 50 cm.				Atmospheric p.				⇌	At. + 1 M			
(21)	3	2	3	1	(22)	9	3	4	5	(31)	2	0	1	2	(32)	5	4	4	4
(23)	2	3	2	4	(24)	6	5	3	4	(33)	0	1	2	1	(34)	4	6	5	2
(25)	1	2	5	1	(26)	5	4	4	5	(35)	1	3	1	3	(36)	6	7	7	3
(27)	1	2	2	0	(28)	4	3	4	4	(37)	1	3	1	2	(38)	5	5	6	4
(29)	3	2	1	2	(30)	5	5	4	7	(39)	1	1	0	2	(40)	6	4	7	6
Means ..	2	2	3	2		6	4	4	5		1	2	1	2		5	5	6	4
Atmospheric p.				⇌	At. + 2 M				Atmospheric p.				⇌	At. + 6 M			
(41)	3	2	1	2	(42)	8	8	6	7	(51)	3	4	5	3	(52)	13	12	6	7
(43)	1	3	3	3	(44)	6	6	4	4	(53)	3	2	3	2	(54)	10	9	11	6
(45)	2	3	4	3	(46)	8	9	8	7	(55)	2	2	4	5	(56)	9	10	9	10
(47)	2	1	5	4	(48)	9	9	10	8	(57)	2	3	2	3	(58)	9	11	8	7
(49)	4	2	3	3	(50)	10	6	7	5	(59)	3	2	3	2	(60)	12	12	9	10
Means ..	2	2	3	3		8	8	7	6		3	3	3	3		11	11	9	8

Elminius modestus Darwin [Cirripedia]

Stage 1 nauplii were liberated into dishes of sea-water from adults scraped off stones. An hour after liberation some which had become negative to light were transferred to test-tubes, in which a high proportion remained negative. These showed a very sensitive response to pressure, swimming up towards the light when the pressure was increased. An increase of 75 cm., produced by blowing with the lips, induced an obvious response; an increase of 4 M made all positive to light. The response followed the stimulus with a delay of only 1 or 2 seconds.

Balanus balanoides (L.) [Cirripedia]

Freshly liberated nauplii of this species, which are larger and easy to count, behaved rather similarly, but not all became positive even at 2 atmospheres (Table V). Most individuals changed from being negative to positive about 5 sec. after an increase in pressure, but the delay in responding to a decrease was longer, usually about 30 sec.

Older larvae tow-netted from the plankton clearly responded to pressure changes of less than 1 M (Table VI). But about 30 sec. after each increase in pressure, some individuals, which had started to swim up towards the light, turned round and swam downwards again, even though the pressure remained high.

The pressure response of barnacle nauplii was clearly orientated to light, so by illuminating them from below they could be induced to swim downwards in response to a pressure increase (Table VII). But there was also the tendency, seen in many planktonic animals, for swimming activity to increase after a pressure increase and decrease after a decrease. Thus after a decrease

nauplii illuminated from below swam upwards only sluggishly, scarcely counteracting their negative buoyancy. For this reason the figures in Table VII provide a less impressive demonstration of sensitivity to small pressure changes than those of Table VI.

TABLE V

Numbers of freshly hatched nauplii of Balanus balanoides *(out of 50) swimming above half-depth in water 2 cm. deep, when given alternate minutes of high and low pressure and illuminated from above. Records were made every 15 sec. Time in minutes is given in brackets*

	Atmospheric p.				⇌	At. + 10 M			
(1)	6	7	6	6	(2)	17	22	26	27
(3)	23	14	7	6	(4)	19	28	28	29
(5)	28	24	13	9	(6)	20	27	30	27
(7)	23	11	5	3	(8)	17	20	24	28
(9)	22	17	10	5	(10)	20	24	23	21

TABLE VI

Response of older Balanus nauplii, *illuminated from above, to gradually increasing changes of pressure; as in Table I but in water 4 cm. deep*

	Atmospheric p.				⇌	At. + 25 cm.			
(1)	5	5	6	5	(2)	6	7	7	8
(3)	8	9	10	8	(4)	8	9	7	8
(5)	8	7	7	7	(6)	7	7	7	9
(7)	7	6	6	6	(8)	7	7	6	6
(9)	4	4	4	5	(10)	5	6	6	7
Means ..	6	6	7	6		7	7	7	8

	Atmospheric p.				⇌	At. + 50 cm.			
(11)	5	5	5	4	(12)	7	5	8	7
(13)	7	8	6	6	(14)	10	10	9	9
(15)	7	7	7	6	(16)	9	9	9	9
(17)	8	8	7	7	(18)	7	8	8	9
(19)	7	6	6	6	(20)	7	7	7	7
	7	7	6	6		8	8	8	8

	Atmospheric p.				⇌	At. + 1 M			
(21)	7	8	8	8	(22)	9	11	8	8
(23)	8	8	8	8	(24)	11	10	8	8
(25)	7	6	5	4	(26)	8	11	9	8
(27)	7	7	6	5	(28)	10	7	7	8
(29)	7	7	7	7	(30)	8	10	9	8
Means ..	7	7	7	6		9	10	8	8

	Atmospheric p.				⇌	At. + 3 M			
(31)	6	6	7	6	(32)	12	13	10	9
(33)	8	7	6	5	(34)	9	12	11	8
(35)	7	7	6	5	(36)	11	10	10	9
(37)	7	7	6	6	(38)	9	8	9	9
(39)	7	7	6	6	(40)	11	14	12	10
	7	7	6	6		10	11	10	9

	Atmospheric p.				⇌	At. + 8 M			
(41)	8	8	8	5	(42)	14	15	15	14
(43)	9	7	6	6	(44)	15	15	13	12
(45)	9	7	5	5	(46)	16	15	13	14
(47)	10	8	7	6	(48)	15	16	14	13
(49)	10	8	7	6	(50)	17	17	15	14
Means ..	9	8	7	6		15	16	14	13

In cyprids this effect was still more marked and those illuminated from below, if only by a dim red light, would not swim upwards in response to a pressure decrease, but lay passively on the bottom. When illuminated from above, cyprids maintained their depth in a jerky fashion, within fairly narrow limits, by frequently alternating periods of swimming and sinking. They sank dorsal side downwards but, when starting to swim again, orientated anterior end upwards with the first few kicks of their cirri. This position reflex probably helps them to orientate to gravity, for they swam up to the surface in complete darkness (to sink again when brightly illuminated).

TABLE VII

Response of Balanus nauplii *as in Table VI and recording the numbers above half-depth, but illuminating from below*

Atmospheric p.					⇌	At. + 8 M					Atmospheric p.				⇌	At. + 8 M			
(1)	19	18	19	18		(2)	16	18	20	19	(11)	22	20	20	21	(12)	12	15	16	19
(3)	21	22	21	21		(4)	17	19	20	20	(13)	23	21	18	20	(14)	16	18	17	18
(5)	20	19	20	19		(6)	18	18	18	18	(15)	23	22	20	20	(16)	9	14	15	16
(7)	20	20	20	18		(8)	16	19	18	18	(17)	20	17	18	17	(18)	13	17	17	17
(9)	17	16	19	19		(10)	17	18	18	19	(19)	23	22	21	19	(20)	13	15	17	18
Means..	19	19	20	19			17	18	19	19		22	20	19	19		13	16	16	18

Swimming cyprids were very sensitive to pressure changes (Table VIII) but scarcely so to 5 cm. of water. Their most striking response was passive sinking following a decrease of 10 cm. or more. They swam up only slowly to subsequent increases, and the net effect of pressure changes was to reduce swimming activity.

TABLE VIII

Response of Balanus balanoides *cyprids, illuminated from above, as in Table VI*

Atmospheric p.					⇌	At. + 5 cm.					Atmospheric p.				⇌	At. + 10 cm.				
(1)	10	10	8	12		(2)	12	10	12	9	(11)	10	11	13	10	(12)	14	13	12	11
(3)	8	11	9	7		(4)	8	12	12	12	(13)	8	6	5	5	(14)	7	11	10	12
(5)	12	7	7	11		(6)	13	11	12	11	(15)	8	7	7	8	(16)	11	10	9	11
(7)	11	10	9	11		(8)	12	10	11	13	(17)	7	5	5	4	(18)	6	10	10	9
(9)	13	11	10	11		(10)	10	10	9	11	(19)	8	9	8	7	(20)	11	10	12	10
Means..	11	10	9	10			11	11	11	11		8	8	8	7		10	11	11	11

Atmospheric p.					⇌	At. + 25 cm.					Atmospheric p.				⇌	At. + 50 cm.				
(21)	9	11	9	8		(22)	11	9	11	8	(31)	3	4	5	5	(32)	8	6	7	6
(23)	6	8	7	9		(24)	8	10	12	13	(33)	1	0	0	0	(34)	2	4	4	6
(25)	2	2	2	3		(26)	9	9	10	12	(35)	1	1	1	1	(36)	4	3	4	6
(27)	3	2	2	2		(28)	5	6	6	6	(37)	1	1	1	1	(38)	4	4	6	5
(29)	3	2	3	3		(30)	5	5	4	4	(39)	2	1	1	2	(40)	3	2	3	3
Means..	5	5	5	5			8	8	9	9		2	1	2	2		4	4	5	5

Atmospheric p.					⇌	At. + 1 M					Atmospheric p.				⇌	At. + 3 M				
(41)	1	1	1	1		(42)	4	6	6	7	(51)	2	1	1	1	(52)	5	6	11	14
(43)	1	1	1	1		(44)	5	5	11	11	(53)	2	0	0	0	(54)	1	4	3	7
(45)	1	1	1	1		(46)	7	9	15	13	(55)	0	0	0	0	(56)	1	4	6	8
(47)	3	2	2	3		(48)	6	7	8	7	(57)	0	0	0	0	(58)	3	5	5	6
(49)	2	3	3	3		(50)	3	4	3	6	(59)	0	0	0	0	(60)	3	5	7	7
Means..	2	2	2	2			5	6	9	9		1	0	0	0		3	5	6	8

Stones which bear the bases of recently detached barnacles are very suitable for the settlement of cyprids, since they elicit the gregarious response (Knight-Jones, 1953; Crisp and Meadows, 1963). When such stones were introduced into flasks containing cyprids and the water was stirred, many cyprids found the stones and settled upon them, apparently stimulated by turbulence. But if the stones were placed in sea-water on the darker side of flasks and cyprids were added without stirring, to the side nearest the light, the cyprids did not readily find the stones.

Three flasks were set up in that way, one was kept at atmospheric pressure, another was kept at two atmospheres and the third was subjected to pressure changes fluctuating between one and two atmospheres at 15 sec. intervals. At constant pressure the cyprids lay on the bottom or swam up constantly on the lighted sides of the flasks, but at fluctuating pressure their behaviour was strikingly varied. They swam up and sank down as the pressure rose and fell, and occasionally one would become negative to light after a pressure decrease and would dash across to the stone. Thus they readily found the stone and settled upon it. In a series of experiments, many more settled in the flask at fluctuating pressure than in the others (Table IX).

TABLE IX

Numbers of Balanus balanoides *cyprids (out of 25 in each flask, making 75 in each experiment) which were attached and settling after 1 hr in six experiments, each involving three flasks, which were respectively subjected to:—*

Constant At. p.	At. p. + 10 M water	At. p. ⇌ At. + 10 M
1	1	20
1	1	10
0	1	7
0	0	17
0	0	9
0	0	8
Total .. 2	3	71

Temora longicornis (Müller) [Copepoda]

Adults were obviously sensitive to changes of about 10 cm. water. Their reaction was orientated to light, so when illuminated from below they swam downwards in response to an increase in pressure. Some other marine copepods and some freshwater copepods and Cladocera showed no obvious or predictable response to pressure changes.

MOVEMENTS IN VERTICAL TUBES

Carcinus moenas

Megalopa larvae, placed individually in the tube 6·3 M long, allowed themselves to sink until they were at or near the bottom. They then swam very persistently, less than a metre from the bottom. Increases in pressure, equivalent to another eight M, induced no more than transient increases in activity. Falling pressure was often followed by decreased activity. As a net effect of pressure changes the larvae tended to sink still nearer to the bottom, but they usually continued to swim, paddling at a very constant rate, and they often seemed to ignore the pressure changes.

Eurylice pulchra

This species also stayed mostly in the bottom half of the long tube, but these rapid swimmers were capable of changing their depth quite quickly. Two individuals given, on separate occasions, the same predetermined routine of pressure changes (Fig. 2), tended to migrate up and down the tube in a compensatory fashion. However, their movements were often reversed before the reverse pressure changes were given and long before the animals had equilibrated.

Caligus rapax (Milne Edwards) [Copepoda]

Two adults showed similar responses (Fig. 3), but their vertical movements were not extensive.

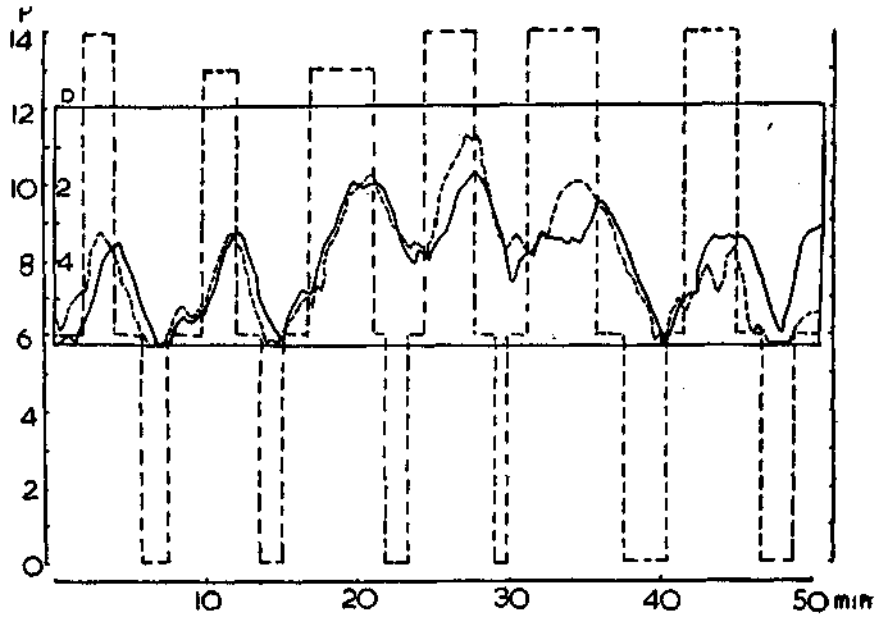

FIG. 2. Vertical movements of two *Eurydice pulchra* in the tube 6·3 M long, in response to pressure changes given as in Fig. 1. The time scale is along the bottom, in minutes. An animal's depth at a given time can be read, in metres, against the scale D. The interrupted line, which follows a rectangular course, indicates the pressure, at a depth of 6 M in the experimental tube, and should be read against the scale P, which shows metres of water above atmospheric pressure. This scale is inverted, so that it can be seen to what extent the movements of the animals compensated for the pressure changes imposed upon them.

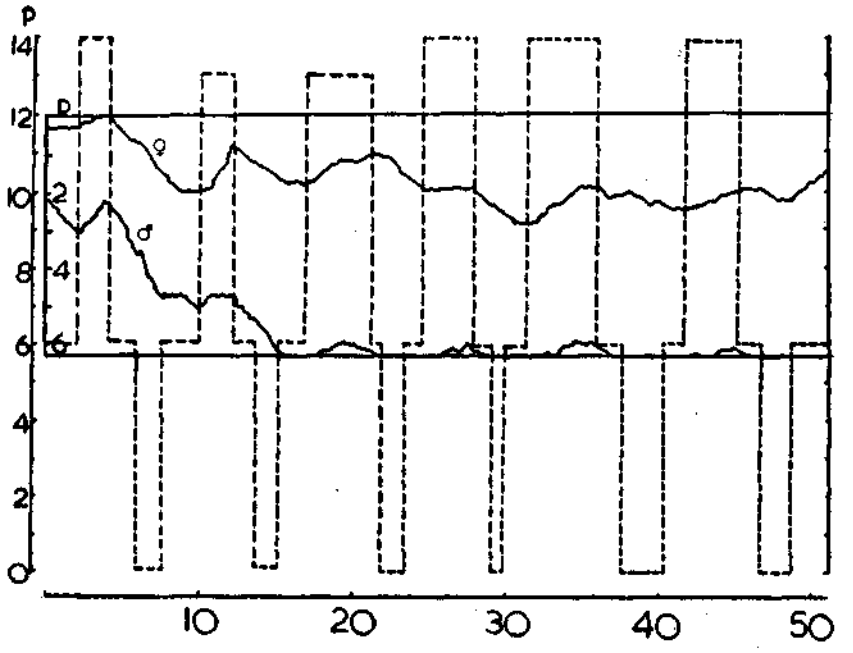

FIG. 3. Vertical movements of a male and a female *Caligus rapax*, as in Fig. 2.

An experiment was carried out in the sea with *Eurydice pulchra*, as a test of behaviour in conditions of natural submarine illumination. Two glass tubes, 150 cm. long and 4 cm. in diameter, were fixed together and weighted, so that they could be lowered and raised together. Both were fitted with rubber bungs, but each of the upper bungs was bored to take a narrow tube. One narrow tube was closed at its distal end, the other was open. Working from a dinghy, each tube was filled with sea-water, including twenty *Eurydice*, and the bungs replaced. Pads of gauze removed any risk of the *Eurydice* entering the narrow tubes. The tubes were suspended vertically, just below the surface and, after 5 min. without disturbance, the vertical distribution of the *Eurydice* was recorded, with the help of an aqualung, pencil, under-water pad, and transverse marks which divided each tube into five equal parts.

The tubes were then alternately lowered and raised, at intervals of a few minutes, the vertical distribution of the *Eurydice* being determined at successive depths, and on each return to the surface. The narrow tube which was closed at its distal end included a column of air, which served as a manometer. This showed that the pressure in the closed tube increased very little with depth. At 10 M depth the increased pressure recorded was equivalent to only 0·3 M of water. The other tube was open to pressure changes.

The day was calm but wintry, without bright sunshine. Just before the experiment the vertical extinction coefficient of the water was determined, with the help of matching photocells (Atkins, Poole and Warren, 1949), as being 0·45. Throughout the experiment most of the *Eurydice* stayed in the bottom 30 cm. of the tubes, but some swam up in the open tube, on being lowered. Those in the closed tube did not swim up, not even when the light was less than 2% of that at the surface (Table X).

TABLE X

Vertical distribution of 20 Eurydice pulchra *in each of a pair of glass tubes which were alternately lowered and raised in the sea. One tube was open to pressure changes. The other was closed and the pressure changes inside it were negligible. Vertical extinction coefficient was about* 0·45. *Light intensity at surface is taken to be* 100

Surface			Surface			Surface			Surface	
Open	Closed		Open	Closed		Open	Closed		Open	Closed
0	1		1	0		1	0		1	0
0	0		1	0		1	0		2	1
1	0		0	1		0	1		0	0
0	0		0	0		1	0		1	0
19	19		18	19		17	19		16	19
		2 metres			4 metres			10 metres		
	Open	Closed		Open	Closed		Open	Closed		
	2	0		3	1		5	0		
	1	0		0	0		1	1		
Light intensity 65	3	1	Light intensity 25	0	0	Light intensity 1·5	2	0		
	2	0		1	0		1	0		
	12	19		16	19		11	19		

FURTHER EXPERIMENTS WITH BARNACLE LARVAE

Adaptation ?

To seek evidence of adaptation, we distributed cyprids of *Balanus balanoides* randomly between two batches of three test-tubes, each tube containing 10 cyprids in sea-water 10 cm. deep. One batch was kept overnight at atmospheric pressure, the other at atmospheric + 20 M water. On the following day the distribution of cyprids between the upper and lower halves of the test-tubes was recorded every 5 min. Those at high pressure were still swimming more actively than the other batch, the proportions above half-depth being about 49% and 13% respectively (Fig. 4). After recording for 20 minutes, the pressure of both batches was changed to atmopsheric + 5 M of water,

Nearly all those previously at higher pressure sank down, many of the others swam up. The contrast between the two batches, both at the same pressure, was initially very striking. But the upward swimming in response to the increase of 5 M water soon became weaker and, after another 45 min., the difference between the batches had disappeared. Then a return to the earlier pressures was followed by a resumption of the earlier distribution (Fig. 4).

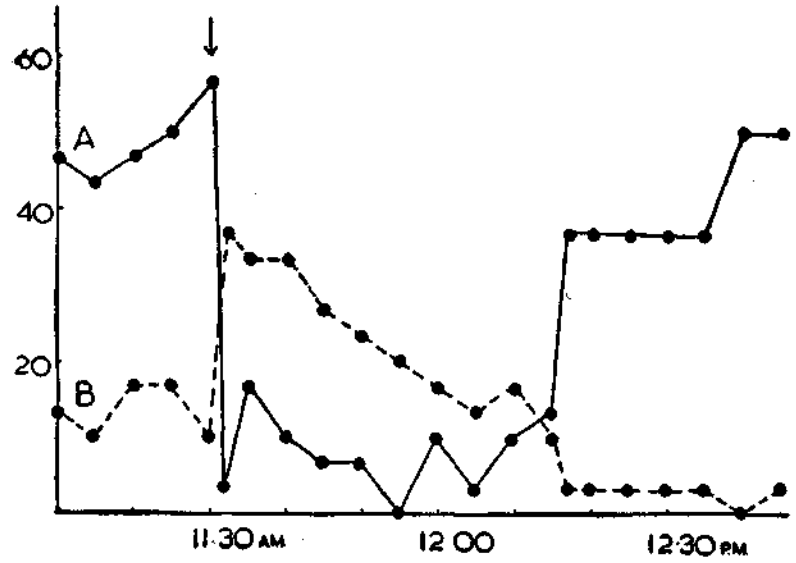

FIG. 4. Percentage above half-depth of two batches of cyprids of *Balanus balanoides*, one of which, A, had been kept overnight at 3 atmospheres, the other, B, at 1 atmosphere. At the time indicated by the first arrow, the pressure of both was changed to 1·5 atmospheres, at the time of the second arrow it was changed back again.

These results suggest that something like adaptation may take place, but to a limited extent. One batch was still excited after being at three atmospheres overnight, but the other showed only a transient response to the additional 5 M of water.

Falling Follows a Fall in Pressure

An extraordinary effect, which indicates rapid adaptation, is shown if cyprids are given a gradual increase in pressure to 5 M above atmospheric and if the pressure is then allowed to fall slowly. As long as the pressure is increasing the cyprids swim strongly, though they do not succeed in climbing very far. But as soon as it begins to fall and whilst it is still only just submaximal, they sink, completely inactive. If the pressure is allowed to return to atmospheric, their inactivity is long-lasting. Figure 5 illustrates recovery after such a slow positive pulse of pressure, upto plus 5 M of water and back to atmospheric over a period of 50 sec. Most of the cyprids lay inactive for over 5 min. Then, one by one, they began to swim again, until after 30 min. the previous situation was restored. They were then given a similar negative pulse of pressure. Whilst the pressure was decreasing the majority sank to the bottom, but after it had increased again they swam up readily. After 5 min. the proportion above half-depth was back to its former level (Fig. 5). Those cyprids had been collected nearly two weeks previously and seemed rather feeble after their long planktonic life. A fresh batch (Fig. 6 D) recovered to full activity more quickly, but again recovery was much quicker after a negative pulse than after a positive pulse (3 min. and 8 min. respectively).

Though we have seen above that the effects of a fairly large increase in pressure are cancelled by a small decrease, the converse is not true. Table XI records an experiment with cyprids which

TABLE XI

Responses of Balanus balanoides *cyprids, which had nearly all been immobilised by return to atmospheric pressure after being kept for an hour at two atmospheres, to increases in pressure of 50 cm. water, imposed and recorded as in Table I. The tests were made 2, 30, 60 and 90 min. after immobilisation*

After 2 min At.				⇌	At. + 50 cm.			
0	1	0	0		0	0	1	0
1	1	1	1		1	1	1	1
1	0	0	0		0	0	0	0
0	0	0	0		0	0	0	0
0	0	0	0		0	0	0	0
After 60 min At.				⇌	At. + 50 cm.			
3	2	1	2		3	5	4	4
0	2	2	1		3	2	3	3
0	0	1	2		1	2	2	2
1	1	0	0		2	2	3	1
0	0	1	1		1	1	1	1

After 30 min At.				⇌	At. + 50 cm.			
0	0	0	0		0	0	0	0
0	0	0	0		0	0	0	0
0	0	0	0		0	0	0	0
0	0	0	0		0	0	0	0
0	0	0	0		0	0	0	0
After 90 min At.				⇌	At. + 50 cm.			
4	4	4	5		6	5	3	4
0	0	0	1		2	2	5	6
1	0	0	0		1	2	4	4
2	2	2	2		3	2	1	2
0	0	0	0		2	2	2	2

had been immobilised by the decrease to atmospheric pressure, after they had been kept for an hour at two atmospheres. The cyprids then failed to respond to pressure increases of 50 cm. of water. Thirty min. later, when the cyprids were still inactive, such increases induced only feeble movements. After an hour, however, some cyprids had begun to swim and responded well to small pressure increases. After 90 min. recovery seemed complete.

Experiments with Brief Pulses of Pressure

These, using rapid movement of a mercury column, indicated that the paralysing effects of a pressure decrease are cancelled immediately by an increase of similar magnitude. Thus negative pulses lasting 4 sec. had very little effect, whereas similar positive pulses induced prolonged inactivity (Fig. 6 A & B). Prolonging the negative pulses to 30 sec. gave enough time for the cyprids to sink to the bottom during the first half of the pulse, whilst the pressure was falling, but they quickly resumed their former level of activity when the pressure rose again (Fig. 6 C).

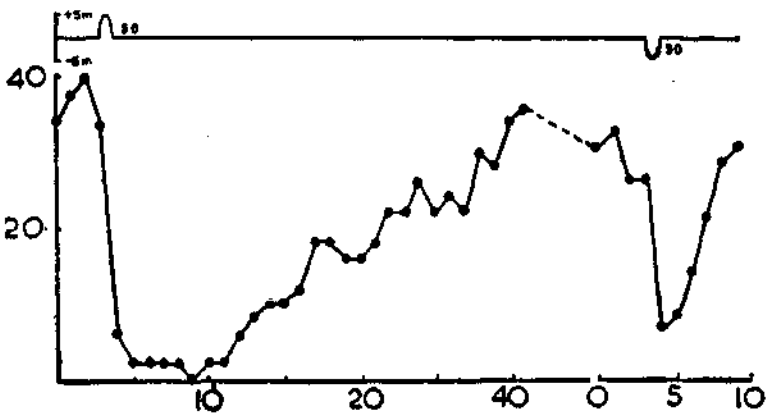

FIG. 5. Percentage of cyprids of *Balanus balanoides* above half-depth recorded every minute. The top line shows pressure, which was constant except for a slow positive pulse and a similar negative pulse, each lasting 50 sec. Interrupted line indicates a time lapse of about 1 hour.

To summarise our findings with cyprids so far, it seems clear that they do not adapt readily to pressures of several atmospheres, but within a small range (5 M of atmospheric), something like

adaptation seems so complete and rapid that they scarcely respond to absolute pressure levels, but only to change. They respond to whatever change happened to them last. They therefore treat a negative pulse as an increase in pressure and their behaviour is not much modified by it. They react to a positive pulse as to a decrease in pressure. They thereupon lie immobile for a long time, on the bottom of experimental vessels, for they are waiting for an increase in pressure which does not come, but which would come to them quite quickly under natural conditions, as they sink through the water.

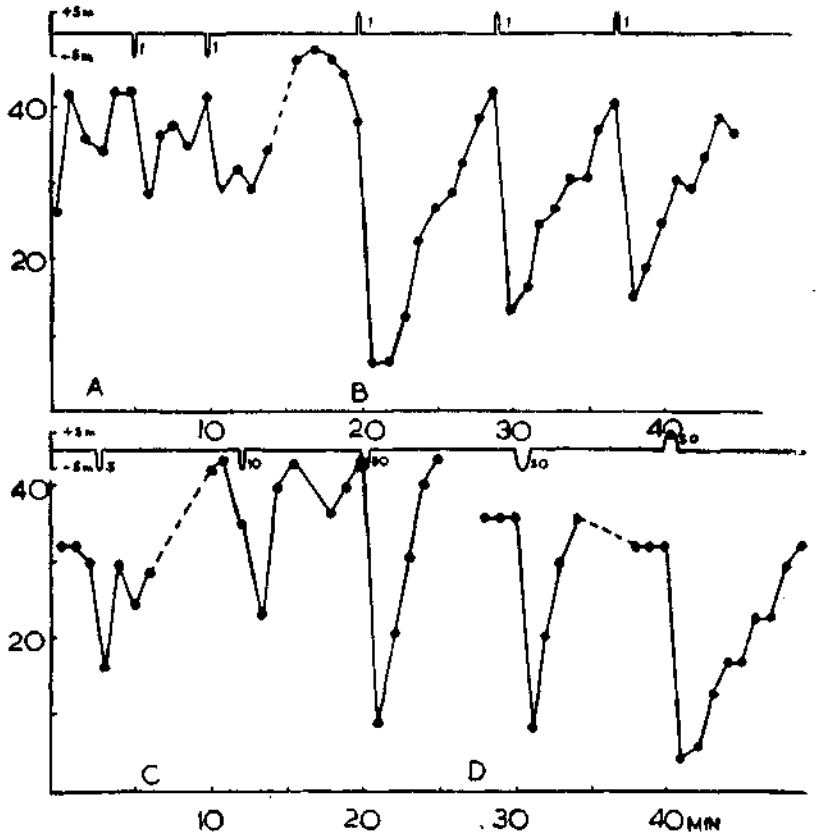

FIG. 6. Like Fig. 5, but illustrating the effects of brief pulses lasting 1 sec., which were A, negative and B, positive; C, the effects of longer negative pulses and D, of still longer and equal negative and positive pulses. The number by each pulse indicates the time in sec. which it occupied.

Absence of a "Boyle's Law Effect"

Enright (1961) found that *Synchelidium* at 5 atmospheres retained a sensitivity to small pressure changes similar to that which they showed at one atmosphere. He remarked that this was evidence against a method of perception involving gas bubbles.

We kept three similar batches of cyprids of *Balanus balanoides* for 24 hrs. at pressures of 0·5, 1 and 2 atmospheres respectively. Then we gave each batch a similar routine of small, gradually increasing pressure changes, equivalent to 25 cm., 50 cm. and 1 M of water, and recorded the numbers swimming up in response to these increases. There seemed to be no significant difference in sensitivity between the three batches (Table XII).

Like Enright's results, these speak against gas vesicles being involved in the mechanism of perception. Gas bubbles at pressures of 0·5, 1 and 2 atmospheres, given an increase in pressure

equivalent to, say, 1 M of water, would contract by about 20%, 10% and 5% respectively. If perception involved such a mechanism, cyprids at 0·5 atmospheres might therefore be expected to be more sensitive to small pressure changes than cyprids at 2 atmospheres would be, but this is not so.

TABLE XII

Numbers of Balanus balanoides *cyprids, out of* 3 *batches of* 90, *swimming above half depth* (5 cm), *recorded at* 15 *sec. intervals, the pressures being changed every minute. The three batches had been kept for* 24 *hrs. at* 0·5, 1 *and* 2 *atmospheres respectively*

0·5 At.				⇌ 0·5 At.+25 cm.				0·5 At.				⇌ 0·5 At.+50 cm.				0·5 At.				⇌ 0·5 At.+1 M			
8	11	7	6	18	14	15	10	3	3	3	6	11	15	17	19	6	8	9	10	12	16	19	23
7	8	8	10	13	17	17	17	1	1	3	5	8	15	17	22	6	7	6	8	9	12	21	17

Mean increase in numbers swimming up, in response to this p increase: 7 — Mean increase 12·4 — Mean increase 8·6

1 At.				⇌ 1 At.+25 cm.				1 At.				⇌ 1 At.+50 cm.				1 At.				⇌ 1 At.+1 M			
14	10	11	14	23	27	28	24	5	7	6	8	15	14	18	18	4	6	8	10	19	25	34	33
4	11	11	14	10	20	22	20	3	0	1	5	9	16	15	14	5	4	6	7	16	20	30	32

Mean increase 11·7 — Mean increase 10·5 — Mean increase 19·7

2 Ats.				⇌ 2 Ats.+25 cm.				2 Ats.				⇌ 2 Ats.+50 cm.				2 Ats.				⇌ 2 Ats.+1 M			
10	13	16	24	20	23	21	24	4	7	13	13	16	20	26	25	7	9	14	16	20	27	35	28
5	7	11	15	17	18	18	24	8	8	11	12	22	24	25	20	9	11	9	14	25	30	29	36

Mean increase 8 — Mean increase 12·7 — Mean increase 17·5

Nauplii, Ions and Galvanotaxis

Some State I nauplii of *Elminius modestus*, an hour after liberation from their parents, were about half negative and half positive to a light from above. Decreases in pressure had little effect upon them (the positive nauplii seemed determined to remain so), but increases had a marked effect. A rise of 4 M of water made them all positive. Brief negative pulses, dropping to atmospheric pressure minus 5 M of water and back to atmospheric again within a second, had no apparent effect, but similar positive pulses induced an obvious response. Nothing was visible for one or two sec. after the pressure had returned to atmospheric but then all the negative nauplii turned, like a flock of birds flying off, and swam towards the light for about 5 sec. They then mostly became negative again. Their response, in strange contrast to that of cyprids, seemed to be to the first half of the pulse, when the pressure was increasing and it was very transient. The ctenophore *Pleurobrachia* also responds to the first half of a pulse (Knight-Jones and Qasim, 1959).

As Ewald (1912) noted, an increase in concentration of Na^{+} makes barnacle nauplii more positive to light, whilst an increase of Mg^{++} makes them more negative. We found that a 25% increase in concentration of Mg^{++} abolished the usual response to an increase in pressure. The effect was to make the nauplii actively negative to light, not to narcotise them. Although the general effect of lowered pressure on various animals is to reduce activity, this effect is not readily reproduced by increasing the concentration of magnesium ions, in spite of their well-known narcotising properties. The response of barnacle nauplii to Na^{+} and Mg^{++} may perhaps be explained by these ions playing some part in the mechanism of pressure perception.

Digby's (1961) hypothesis, relating to this mechanism in shrimps, invokes charges on external surfaces. If pressure sensitive mechanisms, in general, involve the presence and transport of ions, his may account for some of the effects of lowered pH on phototaxis in various animals (Loeb,

1918). It seems remotely possible, too, that some examples of " galvanotaxis " may involve stimulation of some pressure sensitive depth-regulatory mechanism. But barnacle nauplii (and *Temora* adults) did not react markedly to electric currents unless those were quite strong (and apparently nearly lethal). Then they tended to go towards the light and also towards the anode. When the pressure was reduced their behaviour was similar, though perhaps less obvious.

Functional Significance of the Response

Our observations do not suggest that the animals studied regulate their depth within narrow limits, by the pressure sense alone. Given slight pressure changes only a small proportion respond and with large changes few respond to a fully compensatory extent. But many of the heavier animals are evidently accustomed to swim at rates which just balance their tendency to sink and the pressure sense helps to control those rates.

Small nauplii, which are not much affected by gravity but apparently have to swim more or less constantly in order to feed, avoid large depth-changes by repeatedly changing the sign of the orientation to light. Here again the depth is regulated primarily by the pattern of activity, and the pressure sense is linked to this.

In general, striking responses are produced only to large pressure changes, which would indicate some danger of being removed from the right habitat, or of not being in the best position to find it. *Eurydice pulchra* is a littoral animal, which buries itself in the beach during the ebb tide. The late larval stages of *Carcinus maenas* and *Balanus balanoides* need to find the littoral environment of the adults, so should keep fairly near to the surface. Planktotrophic animals, of whatever trophic level, should not stray far from the eutrophic zone. As Rice (1964) has remarked, the pressure sense helps them to maintain depth at night or in turbid water. Searching *Pinnotheres* or megalopa stages, incidentally, seem concerned not so much with maintaining constant depth as a constant small distance from the bottom.

Pressure responses seem to be particularly well marked in plankton from shallow inshore waters *Calanus*, which lives typically in deeper water, seems to have less obvious overt responses (Rice, 1962). Inshore waters are often turbid. It seems possible, too, that the inactive sinking, shown by many animals after a decrease or frequent changes in pressure, may have been evolved to protect animals from being cast ashore by heavy surf. Sinking then would tend to take them down into the undertow, which would transport them safely seaward. The data of Colman and Segrove (1955) indicate that the percentage of *Carcinus* megalopa larvae, swimming away from the bottom on a rough night, was not quite so great as on calm nights. Sinking and occasional photonegative swimming of *Balanus balanoides* cyprids, in response to changing pressure and turbulence, would help them to find littoral rocks.

Rice (1961) suggested that pressure responses might be important in the shoreward migrations of mysids with the flood tide and Enright (1962) showed that tidal migrations of the amphipod *Synchelidium* were probably brought about by a pressure-sensitive mechanism, involving swimming in the surf. Rice (1964) also remarked that the pycnogonid *Nymphon gracile* and probably the amphipod *Caprella acanthifera* were stimulated to swim by falling pressure, perhaps to promote seaward transport during the ebb-tide. It was later confirmed that *Nymphon* could perceive and respond in this way to artificial pressure cycles of tidal frequency and amplitude (Morgan, Nelson-Smith and Knight-Jones, 1965).

Corophium volutator (Pallas) can do this too. This amphipod burrows in mud quite high on the shore, but is often induced to leave its burrows by the incoming tide (Vader, 1964). Morgan (1965) found that it is not disposed to swim for long at that time, however, for it has a well-marked activity rhythm of tidal frequency, with maxima during the early ebb. This rhythm can be rephased experimentally, with the aid of pressure cycles of tidal range and frequency, peak activity being

induced during the early ebb of the artificial cycle, when the pressure begins to fall exceedingly slowly. But *Corophium* (and *Nymphon* too) can link the time sense with the pressure sense, often responding in a depth-regulatory fashion to pressure changes changing at rates similar to those which they experience during swimming and sinking. Given slower pressure cycles, changing at tidal rates, their response is invariably of the ebb-transport type, which is opposite to the depth-regulatory one.

Possibly the real significance of some responses of the depth-regulatory type may lie in the phasing of tidal rhythms of activity. Many animals show activity maxima at about high water, including *Carcinus* adults (Naylor, 1958 and 1964). After planktonic life the megalopa larva of *Carcinus* have to make their way inshore and up tide-swept estuaries. For this, it would help them to swim more on the flood than on the ebb, and their responses to changing pressure should ensure that they do so. Verwey (1958) remarked that adult *Portunus holsatus* seem to be capable of navigating up and down estuaries in this way and Morgan (1964) found that these adults waved their swimming legs in response both to rising and falling pressure. They were obviously pressure sensitive and appeared fully capable of using this modality for navigating in the way that Verwey (1958 and 1959) envisaged.

Finally, it seems remarkable that pressure responses have not been recorded in freshwater animals, so far as we are aware, apart from fish and insects with gas-filled organs (Thorpe and Crisp, 1947), and possibly the branchiopod *Chirocephalus*, the eggs of which seem to develop slowly if the water is deeper than a few centimetres (Hall, 1959). Various freshwater Cladocera and Copepoda, which we studied briefly, seemed insensitive to pressure changes. This may perhaps support Digby's (1961) suggestion, that the basis of perception is a phenomenon which involves ions and the body surface, but which is not well understood.

Summary

A variety of larval and/or adult copepods, cirripedes and malacostracans respond to pressure changes in a way that is depth-regulatory in sense. The most usual response is to fall with falling pressure. But small changes (10 or 20 cm. of water) evoke responses from only a small proportion and larger changes induce movements that are less than compensatory, so it is doubtful whether depths are regulated within narrow limits. If they are, this is due to the pressure sense controlling mechanisms which primarily involve the pattern, speed and direction of locomotion. Thus zooea and megalopa larvae swim up at rates which compensate closely for their negative buoyancy, barnacle nauplii frequently reverse the sign of phototaxis and *Eurydice* tend to swim horizontally. Megalopa larvae and the searching "first crab" stage of *Pinnotheres* swim not so much at a constant depth as a more or less constant small distance above the bottom.

With barnacle nauplii, rising or falling pressure evokes not only a change to positive or negative phototaxis respectively, but also some increase or decrease in activity, as in many larger, negatively buoyant animals. With cyprids of *Balanus balanoides*, the effect is primarily on activity. Adaptation is slow or non-existent to increases of several atmospheres, but very rapid and complete to a few metres of water. Swimming stops after a fall in pressure, but begins again after a corresponding rise. Otherwise rising pressure has only a slight stimulating effect, which is immediately cancelled by a small fall. The net effect of imposed pressure changes is to take the cyprids to the bottom and promote their attachment.

Some forms respond in a way that is opposite to depth-regulatory, being stimulated to swim by falling pressure. Recent studies by Morgan (1965) on *Corophium* indicate that this is to promote ebb-transport by tidal currents. It seems likely that the more usual response may sometimes be concerned with flood-transport, or with seeking the undertow to escape from surf.

Acknowledgements

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DISCUSSION

Dr. J. H. Wickstead: Do these animals suffer from the bends; that is, can the upward movement after a pressure-decrease be explained as a result of gases coming out of solution in the blood?

Dr. S. Z. Qasim: As far as I am aware no such phenomenon has been reported so far. Perhaps it might be useful to keep that in mind while making further observations. But the mere fact that such small changes in pressure create a response raises no possibility of such a mechanism.

Dr. L. B. Kirschner: I am interested in the effect of these ions. Did the magnesium solution affect other activity levels?

Dr. S. Z. Qasim: High concentrations did narcotize the animals, but at lower concentrations the general activity level was not affected; only the pressure responses were abolished.

CONTRIBUTIONS TO THE PROBLEM OF THE ROLE OF PROTEOLYTIC ENZYMES IN AMPHIPODS

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ABSTRACT

The proteolytic enzymes of the alimentary canal of the amphipods, *Dicerogammarus haematobaphes balatonicus* Ponyi and *Gammarus* (*Rivulogammarus*) *roeselli* var. *triacanthus* Schäferna and the decapod, *Astacus leptodactylus* Eschscholtz were investigated. The results indicate that the proteolytic enzymes of alimentary canal of the different genera may be different even within the group of malacostracans.

ACCORDING even to data of recent comprehensive works (Buddenbrock, 1956; Mansour-Bek, 1954; Vonk, 1960, 1964) very little information is available on the protein digestive ferments in Crustaceans. Similarly up to now the optimal pH for the endopeptidase activity in Crustaceans is an uncleared problem.

MATERIAL AND METHODS

We investigated amphipods *Dicerogammarus haematobaphes balatonicus* Ponyi and *Gammarus* (*Rivulogammarus*) *roeseli* var. *triacanthus* Schäferna, and a decapod (*Astacus leptodactylus* Esch scholtz) for purposes of comparison, in the period June–September 1964. For a fortnight before the experiments the animals were kept at 20–24° C. in big aquaria supplied with running lake water.

In order to secure the alimentary canal of both amphipods we cut the head of the animals and with careful separation of the 3 last segments of the abdomen the alimentary canal was pulled out. In the case of the decapod (*Astacus*) first the stomach juice was drained out with a glass canula (Vonk, 1960), and washed with distilled water and then the extract was prepared from its hepatopancreas as follows:

The extracts from the alimentary canal of the amphipods and the hepatophancreas of *Astacus* were made in 2 ways:

(*a*) The material was homogenised in destilled water and after centrifugation (4,000 r.p.m. 15 min.) applied (homogenised hepatopancreas).

(*b*) The material was cut with scissors in distilled water into pieces and then centrifuged (hepatopancreas lumen juice).

The stomach, the stomach juice and the midgut of the investigated amphipods contain quite significant amounts of juice compared to the relatively high juice content of the hepatophancreas tubules of these animals. Practically the extract was obtained from the content of the hepatopancreas tubules. According to these we speak in this paper of homogenised hepatopancreas and hepatopancreas lumen juice.

The extracts, stored in ice, were worked up within 1 hour.

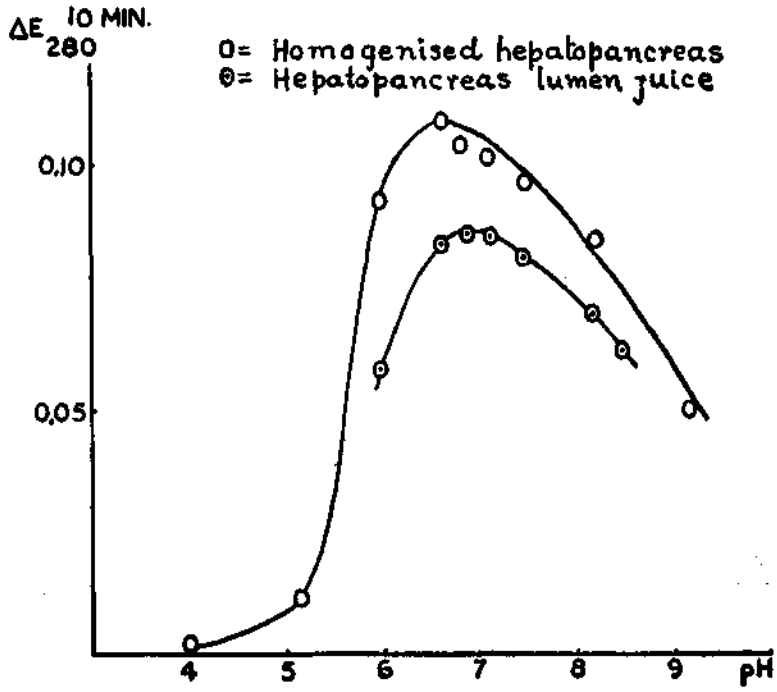

FIG. 1. pH-dependence of endopeptidase activity in *Dicerogammarus haematobaphes balatonicus*. The values are calculated for 0, 2 ml. of the water-soluble extracts.

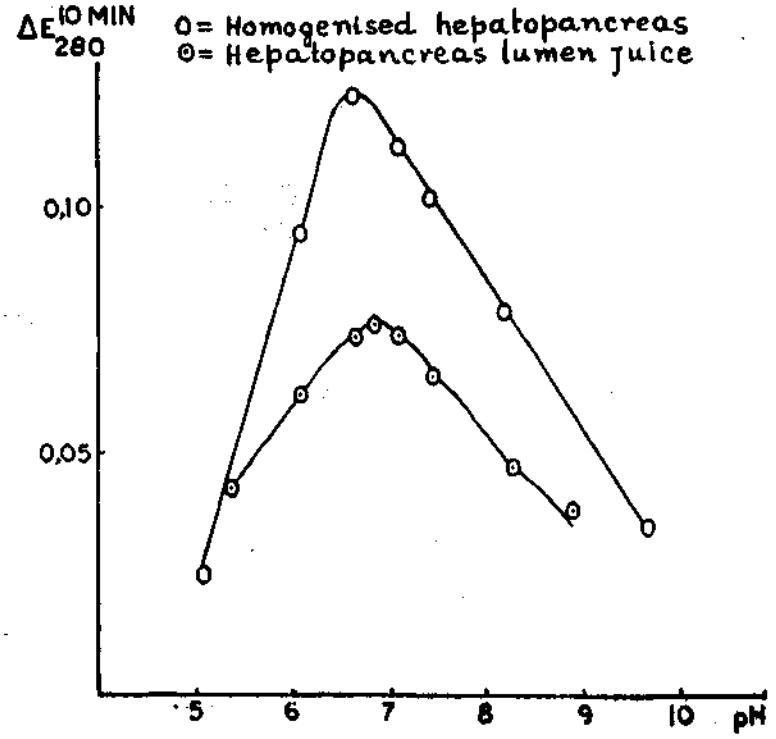

FIG. 2. pH-dependence of endopeptidase activity in *Gammarus (Rivulogammarus) oeseli*. The values are calculated for 0, 2 ml. of the water-soluble extracts.

The activity of the water-soluble extracts was determined according to Anson (1939) with denatured hemoglobin. The necessary pH (between 3·0–9·5) was obtained by adding of N HCl and N NaOH. Within the experimental series at each pH 3 samples (water-soluble extracts used in mounting quantities: 0·05, 0·1, 0·15 ml.) and 3 corresponding controls were used. Incubation took place at 37° C. for 10 min. Precipitation was carried out with 5·0 ml. 10% trichloracetic acid. After 1 hour the material was centrifuged (4,000 r.p.m. for 15 min.). The optical density of the decantated liquid was measured with Beckman's Spectrophotometer at 280 mμ. The extinction of the control was subtracted.

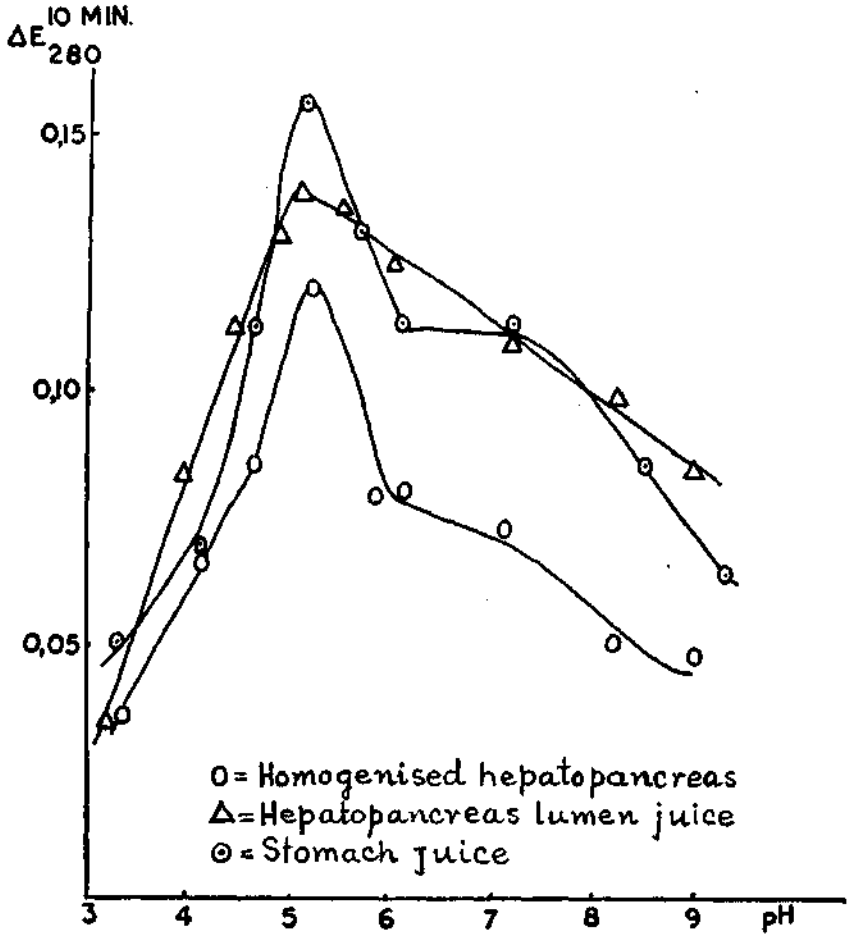

FIG. 3. pH-dependence of endopeptidase activity in *Astacus leptodactylus*. The values are calculated for 0, 2 ml. of water-soluble extracts respectively of the diluted stomach juice.

RESULTS AND CONCLUSIONS

Two organisms of quite different sizes were investigated. Preliminary estimations were made in order to choose the time of reaction and the number of animals for 1–1 series in a way, that we might obtain an adequate basis for the control and an identic ΔE 280. We took in consideration by choosing the beginning concentration of the enzyme solution that by applying it in increasing

quantities, the increase of the extinction values should result in a linear connection. With regards to the mentioned view-point the ΔE 280 measured at the optimal pH were found between the 0,100–0,150 values.

The optimum pH can be given for the amphipods equally in 6·6–7·1 (Figs. 1 and 2). No differences were found in the activity curves of the homogenised hepatopancreas and the hepatopancreas lumen juice.

It has to be noted that the 2 species investigated differ from the view-points of their feeding-habit as well as their food (Ponyi, 1961). Still these differences are not manifested in the endopeptidase activity curves.

Informatory experiments were carried out by the method of Birk and co-workers (Birk *et al.*, 1962) to know more about the native pH relations of the hepatopancreas of amphipods. The pH of the extracts were found to be 6·6–6·8. These values cover the optimal pH values of the above-mentioned endopeptidases.

The pH optimum for the homogenised hepatopancreas, hepatopancreas lumen juice and stomach juice of *Astacus* were measured at pH 5·2 (Fig. 3). Conspicuous differences are clearly observable in the form of the curves of the stomach juice and of the homogenised hepatopancreas at pH 6. We assume that here another endopeptidase may interfere, however its optimal pH value could not have been detected with our applied methods.

With regards to the different results obtained from amphipods and the decapod *Astacus* we suppose that the proteolytic enzymes of the alimentary canals of the different genera may be different even within the group of malacostracans.

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THE SIGNIFICANCE OF THE COPEPODS AS PARASITES ON SEA ANIMALS USED ECONOMICALLY

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ABSTRACT

Up to now one has nearly only been interested in the copepods, which are parasitising the fishes, from the zoological side. But they are also of great economic importance. In the freshwater fishery it has been known for a long time that copepods can create great damages to the fishes, as for example, *Ergasilus sieboldi* on the gills of *Tinca tinca*.

Examinations of different sea fishes have shown that the parasites can influence the growth of fishes, here too. Gadid, which have been infected by *Lernaeocera*, in most cases have a smaller weight than healthy fish of the same size. In connection with the contamination a secondary anaemia would appear. With shells as well (*Mytilus edulis*) a contamination with the copepod *Mytilicola* creates damages, which are to be seen in the metabolism as well as in the smaller weight.

Parasitic copepods which attach on the skin of the fish are generally not so dangerous.

THE parasitic copepods of marine fish have received until recently the attention of only biologists, though they were to a small degree the subject of fishery biological research in general. They were found harmful to a very small extent, if at all.

At the inland fishery, however, the damages caused by parasitic copepods on fish gill are well known. A strong attack of *Ergasilus sieboldi* Nordmann on the gill of tensh (*Tinca tinca* L.) for intstance could cause the complete extinction of all tensh in the lakes. In order to prevent this attack the management of the sea has to be readjusted. Even main parasites such as *Argulus foliaceus* L. or *Lernaea cyprinacea* L. can cause considerable damage as we know from fish farming with carp (Schäperclaus, 1954).

Parasites of the Skin

Marine fish is subject to attack by various kinds of copepods. Forms which settle on the skin of the fish, mainly on the head or on the eyes are very conspicuous. Thus *Lernaeenicus sprattae*, parasitic on sprats, settles around the eye and cannot be overlooked because of its long stretched form. These and similar forms which are rather rare will on the whole not cause any serious damage to the fish. But the case is different with parasitic copepods, such as *Lepeophtheirus pectoralis* O. F. Müller, a caligid which is found in great numbers on the pectoral fins, and on the gills of flounders.

When examining flat fish on several occasions I noticed that the skin around the fins was damaged by the attack. Also a reddish colour and inflammation appeared on the skin under the fins. In some cases even the skin between the fin rays was damaged. It is possible that such sites of attack may facilitate bacterial infection.

Parallels to this are known from the inland fishery. Freshwater fish are often attacked by *Lernaea* or *Tracheliastes* (Mann, 1963; Schäperclaus, 1954) which pierce the skin. Once the parasite is dead or if removed through other ways, holes remain into which fungi (*Saprolegnia*) spread out. Once that has taken place spots and other damages of the skin occur which can reach a diameter of a few centimeters. If for instance carp are attacked by *Argulus foliaceus* these

parasites can transfer the instigator of an infectious carp dropsy (Schäperclaus, 1954). It is also to be expected that diseases caused by parasitic copepods can be transferred to sea fish. The attack of red fish [*Sebastes marinus* (L.)] by *Sphyrion lumpi* can cause serious economic problems. The parasite causes such severe deformities to the muscles that the fish becomes unsuitable for sale (Priebe, 1963). *Sphyrion* inclines to infest the region of the lateral muscular tissues, and hence the cloacal region is preferred most. The head is burried deep into the muscular tissue and this causes reactions in the tissue resulting in the formation of grey and brown cysts. They often have the size of a pea or a bean, and usually acquire a bad smell. Once the young larvae leave the egg sacks shrink and finally fall to the side. Slowly the outer parts of the parasite degenerate and only the head remains. The muscular tissue surrounding it develops as a cyst which gets filled with a brown liquid. Normally one cannot see anything strange in the appearance of the host, but the degeneration of the muscles can be easily seen when filleting the fish. When filletting red fish, however, only small deviations are noticed in the muscular tissue. These derive most probably because of an increased attack by *Sphyrion*. One must expect that the red fish go through several *Sphyrion* invasions during their lives.

This is the reason why the head of *Sphyrion* is found more frequently in old fish than in young ones. To date there is no evidence to show that the attack by *Sphyrion* disturbs the natural growth of the fish.

The attack on red fish is quite diversified at the various fishing grounds. Templeman and Squires (1960) found near Labrador-East (Hamiltonbank) an attack as strong as 0·49%. It was noticed at the fish market at Bremerhaven that 1–3% of red fish which came from the Norwegian coast (Tampen) were infested with *Sphyrion* (Priebe, 1963).

Beside the red fish *Cyclopterus lumpus, Anarrhichas lupus, A. denticulatus*, and others are also infested by this parasite.

Gill Parasites

The attack of copepode on the gills of fishes seriously affects the health of the fish. At the Inland Fishery the most common parasite is *Ergasilus sieboldi* Nordmann. It is widely spread and it infests many cyprinds, especially the tensh (*Tinca tinca*).

When the attack is very strong it destroys the epithelium of the gills and causes deterioration in the condition of the host and sometimes causes outbreaks of epicootics. Schäperclaus (1964) observed that Ergosilosis seriously affects the feeding capacity of the fish. The condition factor of the fish with 60–80 parasites was 1·40, while that of a fish with 3063 was 0·88.

I have had opportunity to make a similar statement on gadoids which were infested by *Lernaeocera branchialis*. This parasite, however, does not live on the gills, but on the inner wall of the gill chamber. The head of the adult animal passes through the wall of the gill chamber and reaches the heart of the host. The subsequent activities of the parasite as observed by Kabata (1958) are: (*a*) attachment, resulting in destruction and dislocation of tissue; and (*b*) feeding resulting in the loss of tissue (blood).

As the head and the neck of the parasite penetrate into the tissue, the host counters with various reactions, as has been fully explained by Stekhoven (1936). But the effect of these developments on tissues and the damage, as well as the Haemoorrhogis seem to be of local importance only. The effect on the feeding activity of the host is more serious. When examining gadoids (whiting, cod and haddock) it was recorded (1952) that the weight of infected whiting is usually diminished by 5–10%. The young of cod and haddock are infected upto 80% and weight is diminished by 20–30%. These examinations have shown that the loss ranged quite considerably (0–47%).

This might be due to the different degrees of development of the parasite and different length of parasitisation (Kabata, 1958). It is shown in Fig. 1 how widely the weight of infested and

uninfested fish can vary. In this figure are also marked the length and the weight of a few goby (*Pomatoschistus minutus*) which were caught from the German North Coast. Out of 100 fish measured, 9 were infested with one parasite each. It was clearly seen that in almost every case the fish which were not infested were heavier than those of the same size, which were infested. The decrease in weight of infested fish in condition factor is very noticeable.

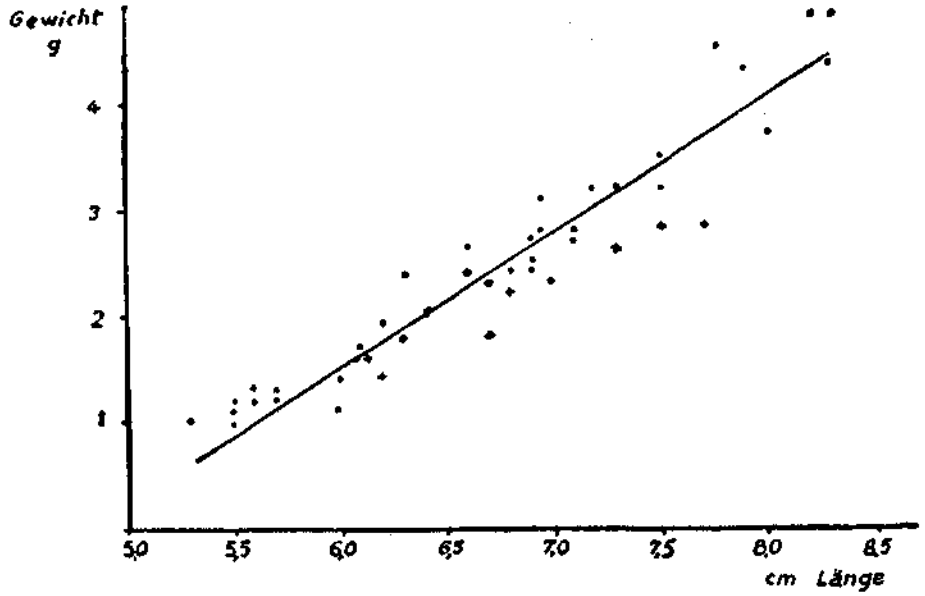

FIG. 1. Length and weight of not infested ● and infested + goby (*Pomatoschistus minutus*) (Mann, 1964).

Furthermore it was proved by Mann (1952) and Kabata (1958) that the infestation by *Lernaeocera* causes an anaemia. The following figures show us this:

	Haemoglobin content	Erythrocyte count
	%	
Normal whiting ..	38–40	902,500
Infested whiting ..	20–22	847,500

Kabata (1958) found similar figures in the haddocks infested by *L. obtusa*.

As shown in a recent study (Mann, 1964) infestation of *Pomatoschistus minutus* by *Lernaeocera minutus* also causes familiar changes in the combination of the blood.

Parasitisation also causes a disturbance in the metabolism of fat of the infested fish. The quantity of fat of uninfested *Pomatoschistus* was between 2·3% and 3·8%, the average was about 2·86%, indicating the live weight. In comparison to this the quantity of fat of infested fish did not go beyond 2·0%. The lowest value was by 1·76%. Kabata (1958) obtained similar figures after examining liver fat of gadoids infested by *L. obtusa*. Once whitings have been infested by *L. branchialis* the quantity of fat of these fish, considering the amount and the size of the parasites, decrease to half of the normal weight (Mann, 1960).

On the whole we can state that the attack by *Lernaeocera* on all species of fish influence the metabolism. This is noticeable in the lesser weight, loss of haemoglobin, and loss of liver fat. All the changes occurring in the infested fish show an amazing degree of parallelism, as is pointed out on Fig. 2 (Kabata, 1958).

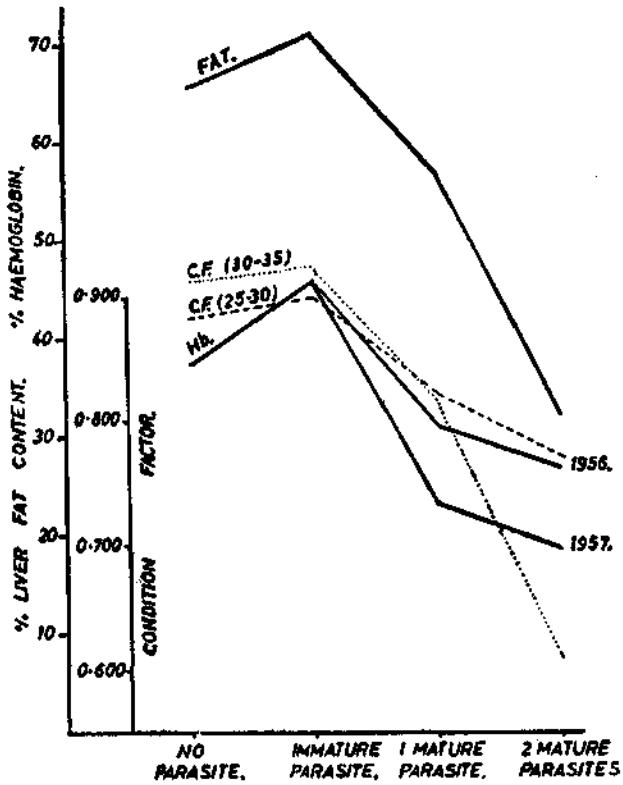

FIG. 2. Summarising the effects of infestation of haddock with *L. obtusa*. The values for haemoglobin content (Hb), fat content of the liver (FAT) and condition factor (C.F.) show initial increases followed by considerable drop (Kabata, 1958).

Internal Parasites

So far we have discussed the effect of parasitisation by copepods which live on the skin or on the gill of fish. It is quite obvious that parasites, which live in the body cavity or in the muscular tissue of the host must cause far greater damage. A few years ago the rapid reduction of the culture of mussels (*Mytilus edulis*) in the North Sea, at the Belgian, Dutch, and German coasts caused quite a sensation. Thorough examinations proved that the mussels were infested by parasitic copepods called *Myticola intestinalis* which live in the gut of the host.

The infestation occurred in nearly all the large natural and cultivated mussel-beds along the Atlantic and North sea coasts (Bolster, 1954; Hockley, 1951; Korringa, 1951; Meyer, Waarden and Mann, 1954 *a*). This parasite also attacks *Mytilus galloprooincialis* on the Mediterranean seacoast (Korringa P. *et* L. Lambert, 1951; Meyer-Waarden and Mann, 1954 *b*). Physiological investigations (Meyer-Waarden and Mann, 1950) proved that the damage done by *Mytilicola intestinalis* was very considerable. It effects predominantly the metabolism of the mussels. This resulted in deficient development and a decreased meat content in the infested mussels. This loss of con-

dition was not apparent where the intensity of the parasite was light (1–3 per mussel), but it was appreciable where infection was in the order of 5–10 *Mytilicola* per host.

The following specification was made when examining mussels which were collected from the German North Sea (Meyer-Waarden and Mann, 1950):

	Normal mussels %	Infested mussels %
Content of meat ..	27·1	22·6
	(17·5–37·5)	(15·3–33·8)
Content of protein (dry substance) ..	58·2	55·6
Fat (dry substance) ..	6·7	6·4

The reason for the inferior quality of the infested mussels is due to the fact that the digestion of protein is accelerated, and the need of oxygen is increased, but at the same time the filtering ability and consequently the capacity to take food, is decreased. It was expected that the parasites would effect the development of the gonads. The weight of gonads in mussels with and without *Mytilicola intestinalis* was therefore ascertained in relation to the weight of the meat in the mussels. In order to obtain an exact idea of the development of the gonads affected by the parasites, we examined a large quantity of mussels from an area which was, as we knew by experience, hardly infested. The gonads in the infested mussels were on the average more than 20% lighter in weight than in the healthy species. The percentage of weight of the gonads was under normal conditions between 10 and 19%, whereas that of infested mussels was between 7–15% (Mann, 1956). The fluctuating weight of the gonads in the various months depends on the varying degree of maturity of the gonads.

How great the economic importance of the attack on mussels by *Mytilicola intestinalis* can be shown when we compare the yield of cultivated mussels in Holland, which decreased from 50,000 t in the year 1949 to 5,000 t in the year 1950 (Schäperclaus, 1954).

It is of special interest that a related species called *Mytilicola orientalis* was found in the Olympia oyster, *Oystrea lurida*. Thorough examination revealed that the presence of the copepod in the intestinal tract brought about a lower condition factor in the infected individuals when compared with those oysters which are free of the parasite (Odlaugh, 1946).

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ON AN INSTANCE OF PARASITISATION BY THE PEA-CRAB (*PINNOTHERES* SP.) ON THE BACKWATER CLAM [*MERETRIX CASTA* (CHEMNITZ)] FROM INDIA, WITH A REVIEW OF THE WORK ON THE SYSTEMATICS, ECOLOGY BIOLOGY AND ETHOLOGY OF PEA CRABS OF THE GENUS *PINNOTHERES* LATREILLE*

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ABSTRACT

A few species of the genus *Pinnotheres* Latreille are known to be definite parasites of some bivalves, while the great majority of the pea-crabs may be classed as obligate commensals and live in bivalve molluscs, echinoderms and ascidians.

The backwater clam *Meretrix casta* (Chemintz) from Malpe, on the west coast of India was found to be infested with a species of the pea-crab and a careful study of the infected clams indicated definite damages caused by the crab to the gills, mantle, gonad and digestive gland of the host indicating a clear case of parasitism. Descriptive account of the pea-crab, the meat weight-total weight ratio in the infected and uninfected clams, and other details are dealt with here.

This study has led us to draw up a brief review of the work so far carried out on the species of the genus *Pinnotheres* on aspects such as, growth stages; sex ratio and abundance; sexual dimorphism; ethology; ecology; physiology; effects of parasitism on host; parasites of pea-crabs of the genus *Pinnotheres*, etc. This coverage also includes a list of the known species of *Pinnotheres* from the Indo-Pacific, Atlantic and the contiguous seas with their known hosts and other details.

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INTRODUCTION

ON November, 25, 1960, one of us (E.G.S.) made a collection of backwater clams, *Meretrix casta* (Chemnitz), from a creek at Malpe on the west coast of India. A few of the clams opened at the time revealed the presence of one or more pea crabs within the mantle cavity, and the larger females were all found to be ovigerous. In order to see the incidence of infestation, as well as to identify the crab, about 450 clams were collected at the time. Unfortunately, it has not been possible to revisit Malpe subsequently to make any further observations on this association. In the course of examining the material in detail we have found damages in the soft tissues of the host which could have been caused only by the crabs they were harbouring, thereby justifying the categorising of the crabs as facultative parasites.

A perusal of the literature in this connection, as well as for identifying the crab, indicated the great need for a review of the available work on the systematics, biology, ecology, and behaviour of pea-crabs, especially of the genus *Pinnotheres* Latreille. Thus, in addition to our observations on the aforesaid instance of parasitic association, we have tried to cover here the general problem of commensalism and parasitism as pertaining to pea-crabs; a list of the known species of pea-crabs of the genus *Pinnotheres* with data on distributional range and host records wherever available; and an annotated bibliography of the works hitherto carried out on the species of *Pinnotheres*. An up-to-date list of species of *Pinnotheres* from the Indo-Pacific and Atlantic Oceans and contiguous Seas, it was felt, would be useful, as during the last 47 years since the publication of a review of the Indo-Pacific Pinnotheridae (excluding west coast of America) (Tesch, 1918) at least 21 new species have been described from various parts of the Indo-Pacific.

In a critical treatment of some of the species of the genus *Pinnotheres*, Gordon (1936) has laid stress on one important character, namely asymmetry in the appendages of pea-crabs, which has not been given due consideration by most of the earlier workers while describing and drawing up synopsis for the identification of the species. As to the biology of pea-crabs, recent studies on species such as *Pinnotheres ostreum* Say, *P. pisum* Pennant, *P. sinensis* Shen, etc., have added considerably to our knowledge about the habits and activities of these crabs as commensals and facultative parasites. Excellent studies on the physiology of commensalism of some marine invertebrates conducted during the last few years by Davenport and others have added to our understanding of the commensal behaviour of pea-crabs. The complete life-history stages is now known for at least one species, *P. ostreum*, while one or more stages have been described for a few of the other species from different parts of the world. In a way a review of this nature is also meant to draw attention to the large gaps that have yet to be filled in the study of pea-crabs. The scope of the work has necessitated the division of the subject-matter into four parts as dealt with below.

We wish to express our sincere thanks to Dr. S. Jones for his kind encouragement throughout the course of this work. Our thanks are also due to Mr. S. K. Banerji and Mr. S. K. Dharmaraja of this Institute for their help in the statistical part of this paper, and to Mr. N. K. Prasad for assisting in the preparations of the drawings. In the course of this work we have also received much needed literature from Dr. Aage M. Christensen, Marinbiologisk Laboratorium, Helsingor, Denmark; Dr. C. H. Edmundson, B. P. Bishop Museum, Honolulu, Hawaii; Dr. John S. Garth, Allan Hancock Foundation, University of Southern California, U.S.A.; Dr. J. J. McDermott, Department of Biology, Franklin and Marshall College, Lancaster, Pa., U.S.A.; and Dr. A.Daniel, Zoological Survey of India, Calcutta, to whom our thanks are due.

I. AN INSTANCE OF PARASITISATION OF THE BACKWATER CLAM *MERETRIX CASTA* (CHEMNITZ) BY *PINNOTHERES* SP.

MATERIAL AND METHODS

Material.—450 specimens of the clam *Meretrix casta* ranging from 18·4–30·5 mm. in length, from a creek mouth at Malpe, west coast of India, collected on 25-11-1960. Most of the clams

were infested with *Pinnotheres* sp. and the material was preserved in formalin diluted to 5% strength with water from the locality.

Methods.—The total wet weights of the clams were noted to the nearest milligram. When the preserved clams were opened, the location and number of pea-crabs on either side of the viscera were noted along with damages, if any, caused to the soft parts of the host. In instances where data were taken for study of the clam meat weight—total weight ratio, the actual weight of the crab or crabs in the individual clams were noted. The wet meat weight of the clam was taken after pressing it gently between blotting-paper to remove excess moisture. For this study clams whose shells were tightly closed were used. The crabs once removed from the host have been preserved in 70% alcohol.

A small subsample of unopened clams numbering 40 specimens has been deposited in the research collections of the Central Marine Fisheries Research Institute, Mandapam Camp.

DESCRIPTION OF *PINNOTHERES* SP.

An examination of 214 crabs showed that they are referrable to a single species. The existing literature does not permit keying down the specimens to any of the known species, except indicate affinities to *P. parvulus* Stimpson in a few diagnostic characters such as the insertion of the dactylus of the external maxilliped to the inner margin of the propodus, the dactylus being styliform and not surpassing end of propodus; the dactyli of the fourth pair of walking legs being the longest; and the carapace being hardly 1·2 times as broad as long. Since it is generally felt that the genus *Pinnotheres* Latreille is badly in need of a revision on a world-wide basis, we do not want to add to the burden of a reviewer by designating a new species name for our material. The description given should help in its eventual placement in the system.

Descriptions and illustrations are given here of the ovigerous female which predominate in numbers; the hard-shelled (Stage I) and the soft-shelled (Stage II) stages of female; non-ovigerous female; and hard-shelled (Stage I) and soft shelled (stage II) stages of male.

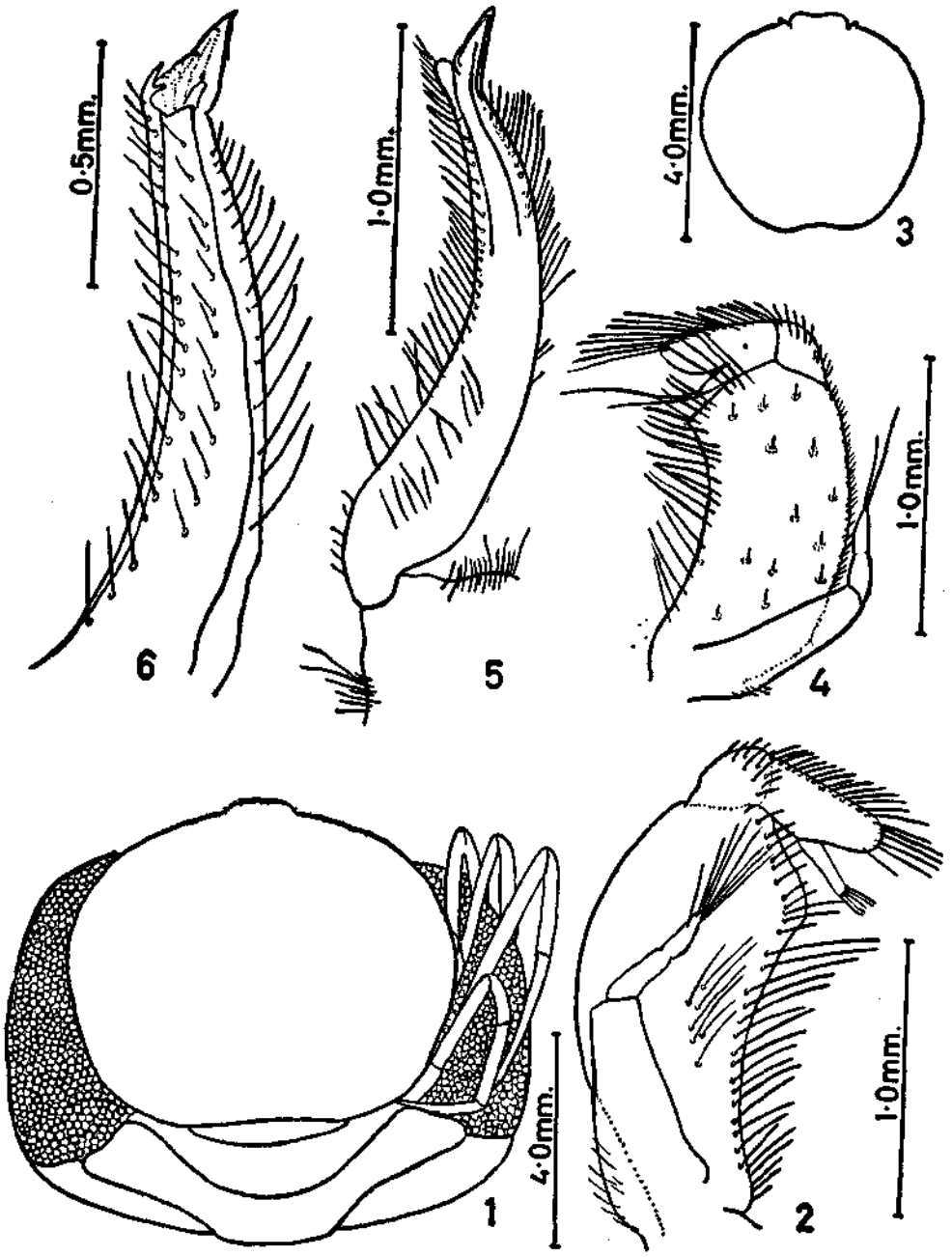
1. Ovigerous Female

(*Text-Fig.* 1: 1, 2; *Pl. I, Figs. C-D*). The carapace is slightly broader than long (about 1·2 times) and consequently appears subquadrate to almost orbicular in outline, curving downwards towards the anterior and lateral margins. The surface of the carapace is smooth and naked. The front which is inconspicuous, hardly projects beyond the anterior margin of the carapace and has a straight anterior border. The minute ovate orbits are barely visible dorsally.

The merus-ischium of the external maxilliped is large, the outer (anterior) margin evenly curved and so also the inner border which abruptly tapers towards the distal end. Long hairs are implanted along the inner border from base to distal end and a few such hairs are also seen laterally about mid-length of the segment. The greatest width of the merus-ischium is about 2·3 times contained in its length. The carpus is conspicuously short, being hardly 1·5 times longer than broad. The propodus is relatively longer and more or less uniformly broad and bluntly rounded at the tip. Its breadth at the point of insertion of the dactylus is about 2·3 times contained in its length. The dactylus is long, slender and uniformly broad (not tapering) and reaches to a level with the tip of the propodus. The distal margin of the carpus, the outer margin and tip of the propodus and the tip of the dactylus bear short to moderately long setaceous hairs (Text-Fig. 1: 2).

The palm of the cheliped is slender, smooth laterally and elongate, being 3·25 times its greatest height and about 2·5 times as long as the finger. The latter is also narrow and slightly curved towards the tip. The fixed finger is also of almost the same size and shape as the movable finger and both have their inner margins fringed with hair. No conspicuous tooth is seen on the inner margin of the fingers except a fringe of minute serrations or undulations. Along the distal half

of the base of the palm and continued along the lower margin of the fixed finger to its tip is a row of short hairs.



TEXT-FIG. 1. *Pinnotheres* sp. (1) Dorsal view of ovigerous female. (2) external maxilliped of same. (3) Carapace of hard stage male. (4) External maxilliped of same. (5) Copulatory pleopod of same. (6) Distal half of copulatory pleopod of soft stage male (Stage II) of same.

The walking legs are slender, moderately elongate and subequal, the third pair being the longest. The second pair is longer than the first pair, but slightly shorter than the fourth pair. The propodus is longest in the third walking legs which on its inner side distally bears two or three long silky hairs. The dactyli of the walking legs show a progressive increase in size from the first leg to the fourth leg. Those of the first three legs end as slightly recurved claws. Barb-like short hairs are present along the inner margin of the dactyli of the first two walking legs and the dactyli of the third pair of walking legs have in addition a few long silky hairs. The dactyli of the fourth pair of legs are conspicuously elongate, being almost as long as the propodus, and having moderately elongate hairs along the inner margin.

No marked colour pattern is noticeable except the light brown broad transverse band across the palm of each cheliped.

2. Female—Stage I (Hard-shelled Stage)

This is represented by a single specimen in the collection which happens to be also one of the smallest specimens examined having a carapace length of 3·0 mm. and width of 3.4 mm. Externally it shows a strong resemblance to the hard-shelled male (Stage I), but the differences are many. The chelipeds and walking legs appear relatively longer and are not hairy. The pleopods are digitiform and bifid, bearing long setae apically. This contrasts with the copulatory pleopods of the male at this stage which, when the abdomen is flapped open, stands erect prominently.

3. Female—Stage II (Soft-shelled Stage) (*Plate II, figs. C–D*)

One specimen with carapace length of 4·0 mm. and width of 4·2 mm.; abdomen broadly circular unlike in male; walking legs and chelipeds elongate and slender, not hairy. Externally the only noticeable difference seen between soft-shelled males (Stage II) and this stage females is in the shape of the abdomen.

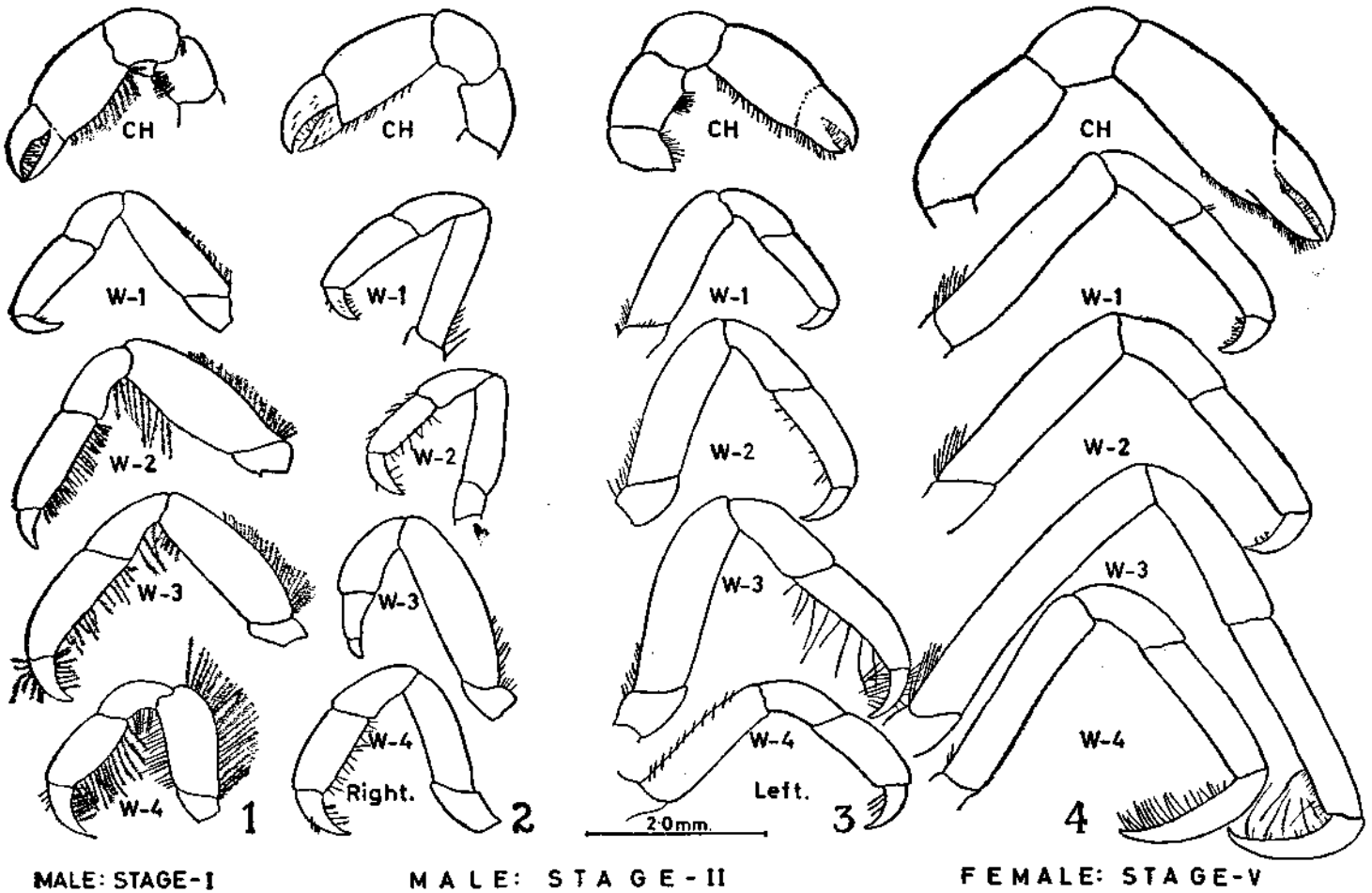
4. Female—Stages IV and V (Adult)

Six specimens of adult non-ovigerous females are present in the collection. Except for the non-attachment of the eggs there is hardly any difference between these specimens and the ovigerous females described above. The broad cupped 'petal-like' abdomen is very characteristic, and as wide as or slightly wider than the carapace. The differences between Stage IV and Stage V in the female are not great and it is likely that a few of the above-mentioned specimens may be border cases between the two stages. All ovigerous females represent State V.

5. Male—Stage I (Hard-shelled Stage) (*Pl. I, Figs. A, B*; *Text-Figs.* 1 : 3–5)

The carapace is orbicular, being very slightly broader than long, and with the margins curving downwards. The front projects out, is conspicuous and is slightly notched in the middle. The orbits and part of the antennae are seen when viewed from dorsally.

The merus-ischium of the outer maxilliped has the general shape as in the female. However, the dactylus of the propodus is short and slender and falls much short of the tip of the propodus. Unlike in the female, the dactylus is tipped with only two long silky hairs. In addition, the inner margin of the merus-ischium is implanted from base to distal margin with more numerous long setaceous hairs, and the outer margin is bordered with short stout hairs, similar hairs also being present along the outer border of the carpus. The hairs along the outer margin of the propodus are denser and those at the outer tip are relatively more elongate. Along the lateral sides of the merus-ischium are a number of short hairs.



TEXT-FIG. 2. *Pinnotheres* sp. Cheliped and walking legs in males and females: (1) Hard stage male (Stage I) (2 and 3) Soft stage male (Stage II) showing asymmetry of appendages on the left and right sides in the same individual. (4) Ovigerous female (Stage V).

As will be seen from Text-Fig. 2: 1, the chelipeds and the walking legs are relatively shorter, stouter, and bear more hairs, the disposition of which may be seen from the illustrations. The third pair of walking legs are the longest and the dactyli bear moderately elongate setae along the inner and outer margins. The dactyli of the first two legs are more or less equal in length and shorter than the third, which in turn is about three-fourths the length of the dactyi of the fourth leg. The hairy appendages are indicative of the free-swimming habit of the crab during this stage.

The male copulatory pleopod is shown in Text-Fig. 1: 5.

No conspicuous colour markings are seen except that the carapace which is hard is whitish.

6. MALE—STAGE II (SOFT-SHELLED STAGE) (*Pl. II*, FIGS. *A–B*; *Text-Fig.* 1: 6; *and* 2: 2-3)

A few males in which the carapace is soft as in the female were also encountered in the collection. The appendages of these specimens are also less hairy as in the females, suggesting that they represent a stage beyond the hard-shelled stage. The carapace is more or less subquadrate, and again as in the female, the chelipeds and the walking legs are slender and relatively elongate.

The distal half of the copulatory pleopod of a specimen measuring 4·0 and 4·3 mm. in carapace length and width respectively is shown in Text-Fig. 1: 6. There appears to be no noticeable difference in the structure of the copulatory pleopod of this stage and that of the hard-shelled stage.

In the same specimen, marked asymmetry in the size of the chelipeds and walking legs of either side of the body was seen and this is indicated in the scale drawings of the appendages in Text-Fig. 2: 2-3. Asymmetry to this extent was not noticeable in the other specimens, although in a few ovigerous females, the length of the legs in a pair were not exactly equal.

The soft shelled males were also examined for external and internal parasites to find out if any such infestation of the hard-shelled male could result in a soft-shelled male resembling the female. However, there were no indications of any parasites. Differences between these two stages in the shape of the carapace, the relatively slender and longer chelipeds and walking legs which are devoid of much of the hair seen in the hard-shelled stage, rule out the possibility that such soft-shelled condition could be the result of decalcification in the original preservative (5% formalin).

FECUNDITY

The eggs in the ovigerous females were found to vary slightly in diameter. In the preservative the eggs are 'light brown' to almost 'greyish' and it is felt that this may depend on the duration of the attachment of the eggs, the 'greyish' eggs being attached over a longer period and consequently showing more embryonic development. The sizes were also found to vary accordingly, the diameter of the 'light brown' eggs being slightly lesser than that of the 'greyish' eggs. In the following table (Table I) the egg counts for six ovigerous females are given along with other details. While teasing out the attached eggs, as a few are liable to be damaged, the counts when ending in less than a hundred have been rounded off to the next hundred; *e.g.*, 2468 has been rounded off as 2500, or 2736 to 2800. The actual counts excluding a few damaged eggs showed a range of 1688–2786 eggs (rounded off here as 1700 and 2800 respectively) in the six specimens examined.

TABLE I

	Length of carapace (mm.)	Width of carapace (mm.)	Total No. of eggs	Diameter of eggs (range in mm.)	Egg colour
	6·2	7·0	2,500	0·32–0·34	Light brown
	6·0	6·2	1,700	0·35–0·37	Greyish
	6·4	7·0	2,800	0·36–0·37	do.
	5·4	6·2	1,900	0·32–0·35	Light brown
	5·9	6·4	2,000	0·36–0·37	Greyish
	6·5	6·9	2,600	0·36–0·38	do.
Average	6·06	6·6	2,250	..	..

FREQUENCY OF OCCURRENCE OF *PINNOTHERES* sp. in *MERETRIX CASTA*

A total number of 379 clams out of the 450 were examined and it was found that 183 clams (48·3%) were infested with one or more pea-crabs. A further analysis of the frequency of occurrence of the pea-crabs in the infested clams showed the following position:

	No.	%
Number of infested clams out of 379 examined	183	..
Number of clams with single crab each	152	83·1
Number of clams with two crabs each	24	13·1
Number of clams with three crabs each	7	3·8

For sex-wise occurrence of crabs, stage of development, size, etc., 117 infested clams were specially examined. They had a total number of 136 crabs in the following stages of development and frequency of occurrence.

There were 82 females and 54 males as follows:

Female

	No.
Stage I (Hard-shelled stage) ..	1
Stage II (Soft-shelled stage) ..	1
Stage IV (Adult, non-ovigerous) ..	6
Stage V (Adult, ovigerous) ..	74
Total ..	82

Male

Stage I (Hard-shelled stage) ..	45
Stage II (Soft-shelled stage) ..	9
Total ..	54

The frequency of the single and multiple infestations is shown in the following table (Table II).

TABLE II

Infestation	No. of clams	Pea-crabs		Total No. of pea-crabs
		Female	Male	
1. Single infestation ..	101	65	36	101
2. Double infestation ..	13	13	13	26
3. Triple infestation ..	3	3	6	9
Total ..	117	81	55	136

A further analysis of the single and multiple infestations by the pea-crabs in the 117 infested clams examined shows the following:

Numbers

Single infestation—

Total number 101
Female—Stage I (Hard-shelled stage) .. 1
Female—Stage II (Soft-shelled stage) .. 1
Female—Stages IV–V (Adults) .. .. 64 (5+59)
Male—Stage I (Hard-shelled stage) 26
Male—Stage II (Soft-shelled stage) .. 9

Multiple infestation—

A. Double infestation:
Total number 13
Female—Stages IV-V (Adults) .. .. 13 (1+12)
Male—Stage I (Hard-shelled stage) 13

B. Triple infestation:
Total number 3
Females—Stage V 3
Males—Stage I (Hard-shelled stage) 6

In all cases of double infestation, a male and a female were present in each clam, and in the three instances of triple infestation, the ratio was one ovigerous female to two males (Stage I). In the three instances of triple infestation it was also seen that one of the males is much smaller than the other, the carapace measurements (mm.) being:

No.	Female		Male		Male	
	L.	W.	L.	W.	L.	W.
1.	6·2	6·7	4·4	4·8	3·5	4·7
2.	6·2	6·7	4·5	4·9	3·7	4·0
3.	6·4	7·0	4·3	4·4	3·8	4·0

The very low percentage of triple infestations combined with the differences in the sizes between the males lead us to believe that one of the males had entered the clam inadvertantly while seeking an uninfested clam or one infested only with a female.

The number of double infestations are also relatively low and here again it is possible that the males had entered inadvertently. This is possible as the females are all ovigerous and there is a much larger percentage of males showing single infestation. These appear to be plausible explanations for this manner of infestation seen.

As a matter of interest the location and disposition of the crabs inside the clam, namely whether they were found on the left or right side, positioned close to the gills or palps, etc., were noted. It is likely that some of the crabs could have moved to other positions at the time of preservation. However, the position was as follows: In the case of infestations with single crabs, out of 144 instances checked, 56 were found on the left side and 88 on the right side of the clam. In cases of multiple infestation, in some of the clams all the crabs were found on one side, either the right or the left with a few exceptions when they were seen on both sides. In all cases the crabs were seen inside the mantle cavity and the area of the gills was found to be by far the most haunted place, while a few were found in the anterior region of the foot, or near the labial palps.

EFFECT OF INFESTATION OF *Pinnotheres* SP. ON ITS HOST *Meretrix casta*

At the time of the removal of the crabs from the clam it was found that certain types of damages were present in the soft parts of the clams which are not likely to have been caused by any manner other than by the pea-crabs. A perusal of the literature showed that similar damages to soft parts of (bivalves) are known to be caused by some species of *Pinnotheres* (Strauber, 1945; Orton, 1921, 1931; McDermott, 1962; Berner, 1952; Sandoz and Hopkins, 1947, and others). A few of the non-infested clams we examined were also seen to show slight traces of damages and we presume that this may have been on account of earlier infestation of the clam by the hard-shelled stages of the crab which could have left the host, or could have been ejected by the latter.

Some workers have mentioned that the availability of food plays a role in the sexuality of oysters (Amemiya, 1929, 1935; Egami, 1935; Coe, 1934, and others). Heavy infestation of oysters resulting in hermaphroditic condition of the former has been reported by Awati and Rai (1931). In the present instance, as the clams were preserved, it was difficult to make any observations on the sexuality of the clams in relation to the infestation by *Pinnotheres* sp. In order to find out whether there was really any significant difference in the meat weight of infested and non-infested clams, 50 clams each of both categories were selected at random and the soft parts weighted to the nearest milligram. The weights graphically plotted (Text-Fig. 3) showed a wider spread and considerable overlap. An analysis of variance based on the meat weight of both categories indicated that the differences were not statistically significant. Since the data graphed showed wider spread, the weights were converted into logarithms but the result of the analysis given in (Table III) also shows the same results.

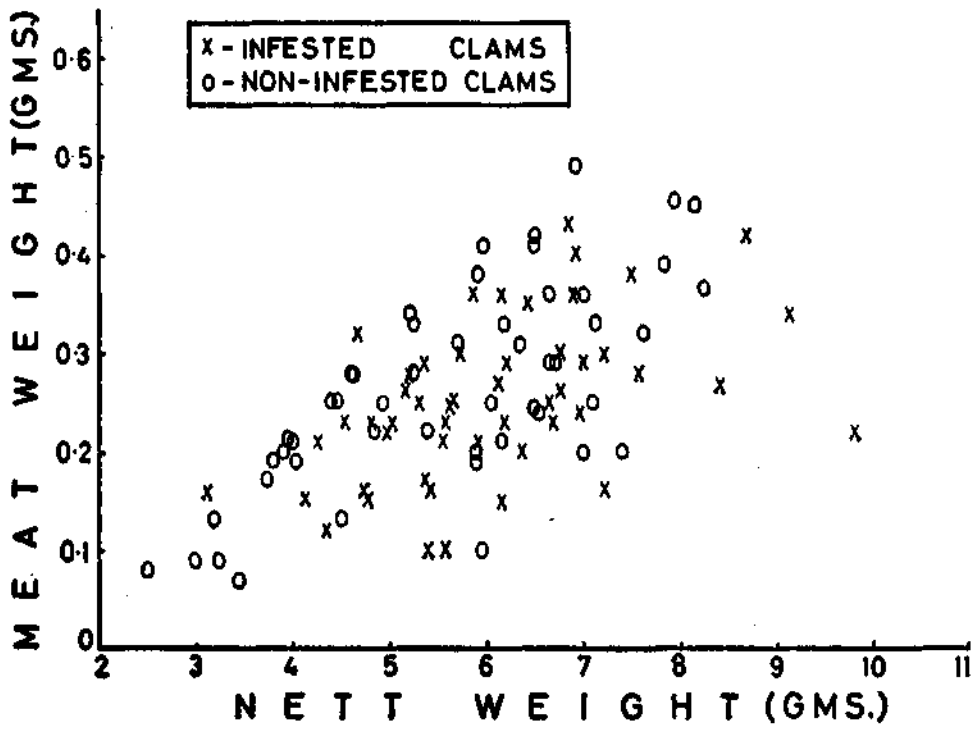
TABLE III

Analysis of covariance of linear regression of meat weight against shell weight to test the significance of differences among infested and non-infested Meretrix casta

Samples	D.F.	x^2	xy	y^2	b	D.F.	S.S.
Infested clams ..	49	0·4288	0·3604	1·4210	0·8405	48	1·1181
Non-infested clams	49	0·8245	0·9112	1·9567	1·1052	48	0·9587
Total ...	98	1·2533	1·2716	3·3867	1·0146	96	2·0768

Test of heterogeneity of regressions within the samples

Source of variation	D.F.	s.s.	M.S.	*F*
Deviation from individual regressions within samples	96	2·0768	0·0216	1·0964
Difference among samples	1	0·0197	0·0197	..
Deviations from average total regressions	97	2·0965	..	..



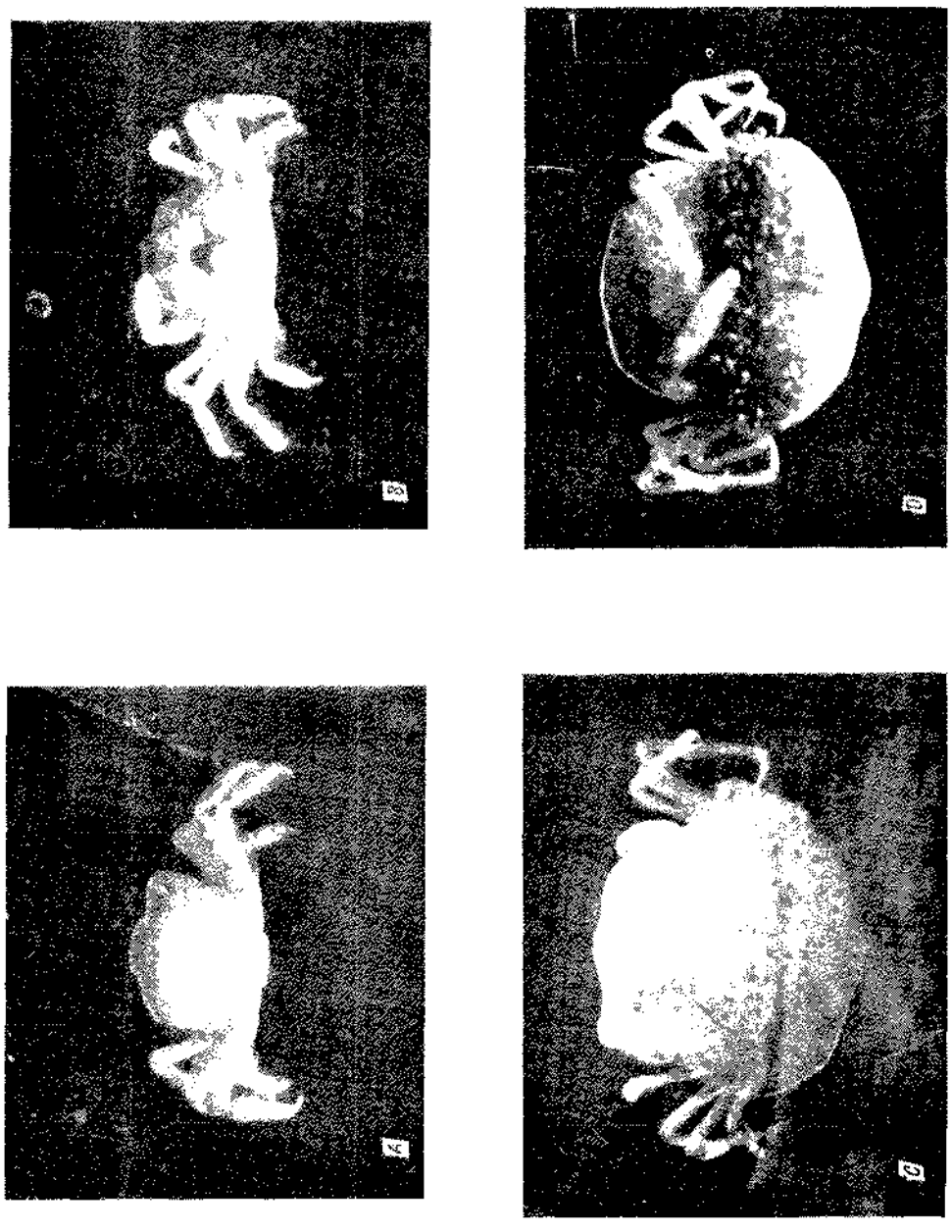
TEXT-FIG. 3. *Pinnotheres* sp. and *Meretrix casta*. Graph showing the meat weight–net weight relationship in two samples of *M. casta*, one infested and the other non-infested with *Pinnotheres* sp.

We are unable to give the reasons for this except presume that: (1) the damages noticed in the form of gill erosion, etc., to be presently discussed do not reflect in the weight to show any significant difference, or (2) the likelihood of clams once infested (earlier infested) being inadvertently included in the non-infested sample, or (3) the inclusion of infested clams which did not show any visual damage, in the sample studied. The above points arise as the selection of the clams for this study was made at random from infested and non-infested clams.

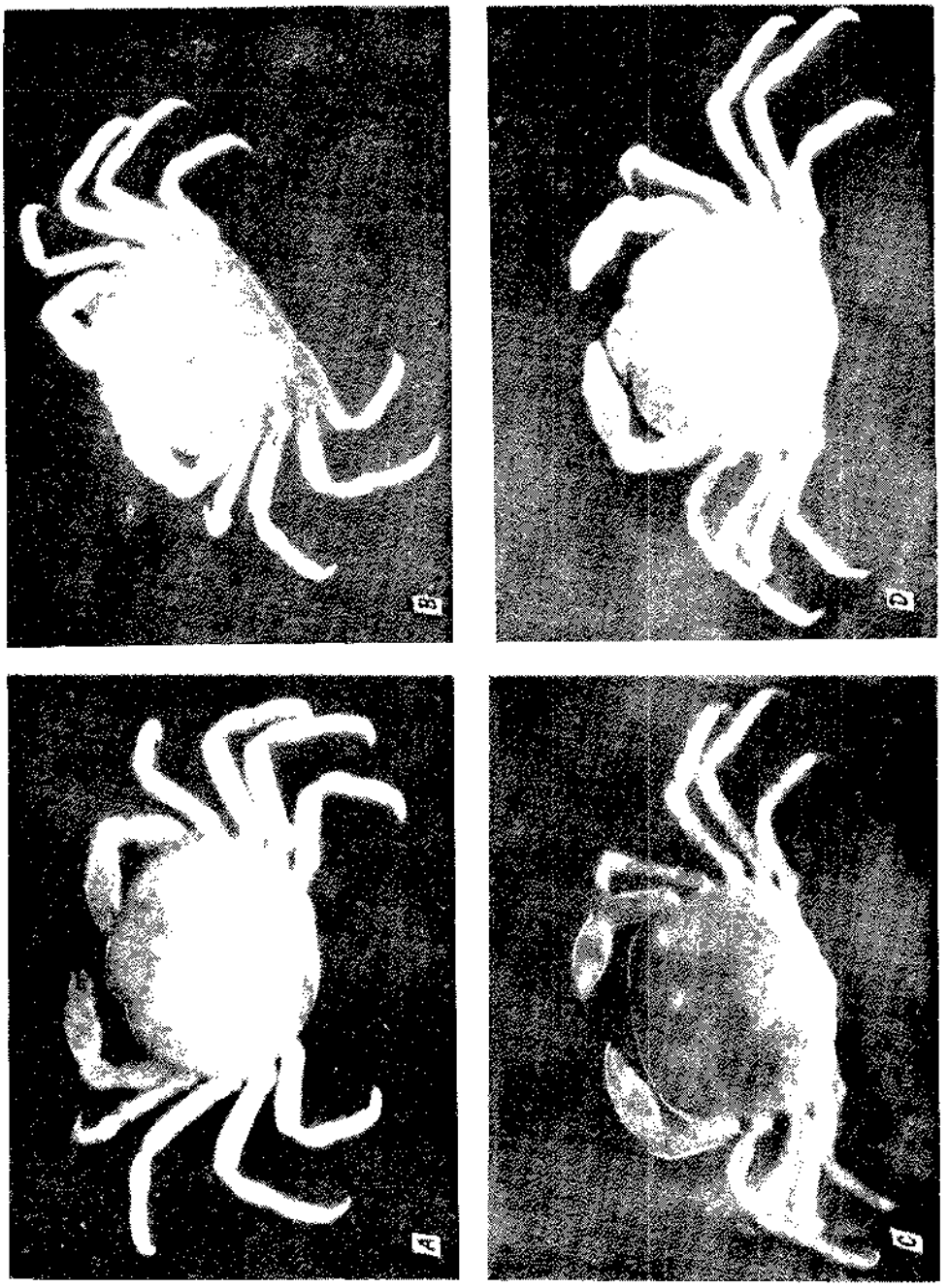
Injury to soft parts of the host:

The damages caused by the crab on the soft parts of the clam were noticeable in various organs such as the gills, the gonad, the digestive gland and the mantle. The nature and extent of the damages noticed are discussed below.

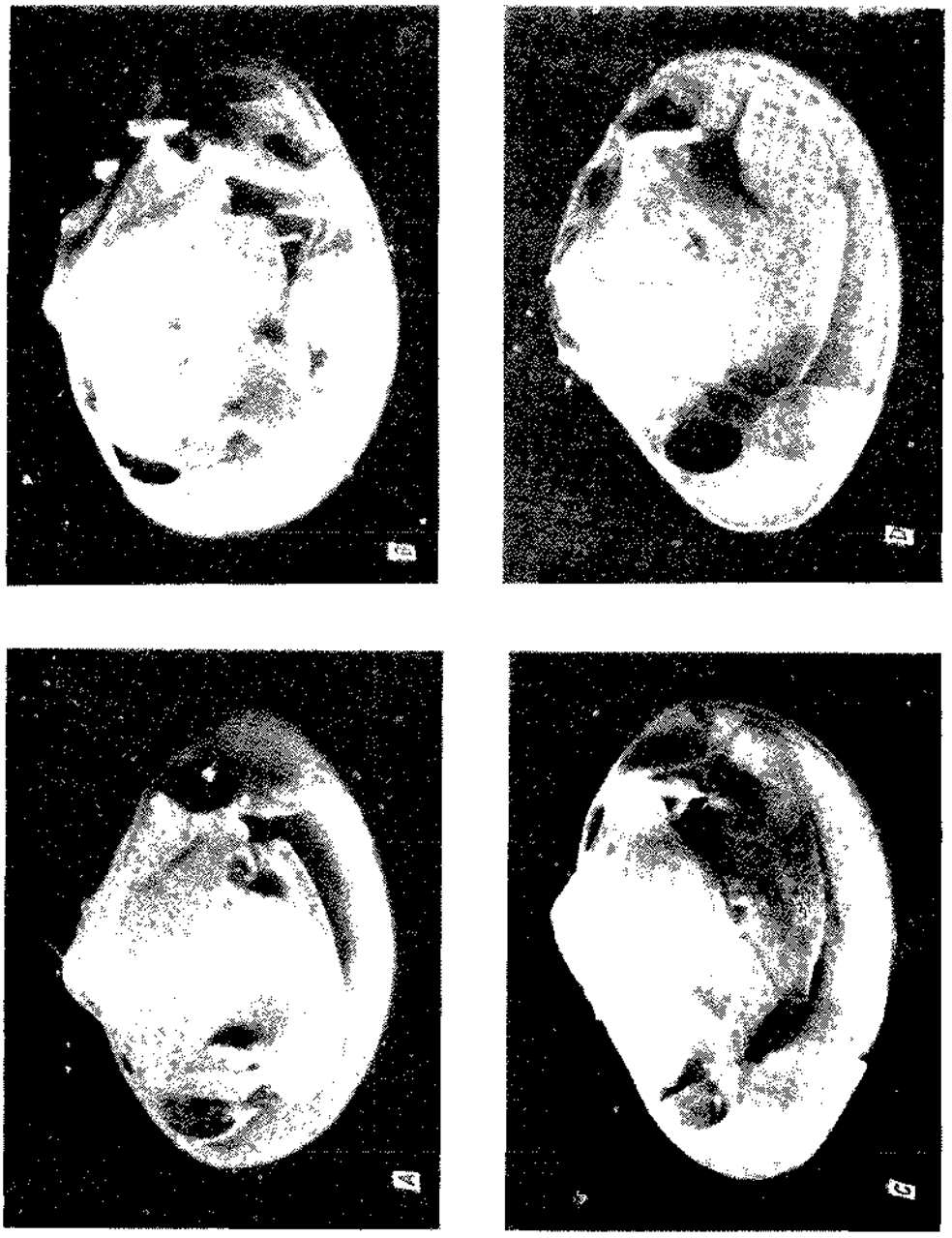
1. *Gills* (*Plate III, Figs. A–D*).—The gills appear to be the organ most affected by the cralb and various types of gill erosion and malformation were noticed. The lower margin of the gill,



Pinnotheres sp.
(A) Dorsal view of hard-stage male (Stage IV). (B) Ventral view of same. (C) Dorsal view of ovigerous female (Stage V). (D) Ventral view of same.



Pinnotheres sp.
(A) Dorsal view of soft-shelled male (Stage II). (B) Ventral view of the same. (C) Dorsal view of soft-shelled female (Stage II). (D) Ventral view of the same.



A-D. Four different types of gill erosion in *Mercierix casta* (Chernin) caused by *Pinnathecus* sp. (see Text)

laminae in many of the specimens were distorted and unevenly curled. In most cases the affected portion of the gill laminae is distinctly shorter than the unaffected portion and the functional part of the gills is restricted leaving a distal papery or membranous laminar portion (Pl. III, Fig. C). Whether the latter portion represents an area of regeneration or not is difficult to say. In some instances the erosion of the gills as a result of nipping or pinching of the margins by the crab is clearly noticeable (Pl. III, Figs. A–B). In a few cases the crab was found between the viscera and the gills with the carapace resting against the gonad of the clam. Whether this is its natural position or brought about during its struggle at the time of preservation is not understood. In some clams the margins of the gill laminae are wavy and often thickened in places suggesting malformation or healing. In an interesting case the distal half of the gill lamina was found to have been pinched off throughout its length with no apparent regeneration in evidence. In an extreme case it was found that the anterior halves of the gill laminae are completely damaged leaving a thin papery structure which is lying flapped on the functional posterior part of the lamina. A large lesion in the visceral mass is also noticeable (Pl. III, Fig. D).

2. *Digestive gland and gonads.*—In some of the infested clams, the digestive gland as well as the gonad are flacid with the outer skin loose. Often due to the lodging of the crab lateral to the gonad or the digestive gland, conspicuous depressions are seen, no doubt brought about by the pressure exerted by the crabs. How far this would affect the normal functioning of the gonads is not known.

GENERAL REMARKS

To our knowledge there is hardly any reference to Indian species of *Pinnotheres* the activities of which have been definitely reported to the parasitic in nature. Awati and Rai's (1931) remarks on *Pinnotheres* sp. affecting the sexuality in *Ostrea cucullata* is suggestive. Hence the present observations showing *Pinnotheres* sp. infesting *Meretrix casta* at Malpe more as a parasite than as a commensal should be of interest. Unfortunately, as already mentioned, neither subsequent collections to the one conducted, nor observations on live clams and the pea-crabs were possible. It is now known through the works of Strauber (1945), Christensen and McDermott (1958), and others that pea-crabs, the activities of which are definitely known to be parasitic could at times lead a commensal existence. This may depend to a great degree on fluctuations in the numbers of the pea-crab population in the oyster and mussel beds, the tendency towards parasitism being more pronounced with an increase in the crab population. It is likely that the single and multiple infestations of *Meretrix casta* by *Pinnotheres* sp. that we have noticed may be due to such a phenomenon, namely an abundance of the crabs in the area during November 1960. Such fluctuations in the occurrence or the non-occurrence of pea-crabs where bivalves occur are possible. Abraham (1954) reporting on the biology of *Meretrix casta* from Madras, does not mention anything about the occurrence of pea-crabs in the clams in that area. Dr. V. S. Durve of the Central Marine Fisheries Research Institute who is working on *M. casta* and with whom we had occasion to discuss this aspect informs us that he has not come across pea-crabs in the large samples of clams he has examined from Athankarai and the C.M.F.R.I. fish farm (Palk Bay).

In the case of *Pinnotheres ostreum*, parasitic on the American oyster *Crassostrea virginica* it has been reported that a good amount of damage is also caused by the multiple infestation of the host by the hard-shelled stages of the crab. The young pea-crabs are more likely to nip the gills and other tissues of the host. In addition, the hard unyielding carapace, due to friction may cause lesions in the soft parts of the host. In this connection also our observations seem to be of interest as we found gill erosion more commonly in the clams harbouring the hard-shelled stage of the male (Stage I) as well as this stage of the male when present with the ovigerous female.

In the course of carrying out this work we found that there was a great need to synthesise the available informations on the biology, ecology, and ethology of pea-crabs of the genus *Pinnotheres*,

especially to draw attention on the investigations successfully carried out in these fields in different parts of the world and at the same time highlight the several gaps that need filling. These are dealt with in the ensuing part.

II. REVIEW OF THE WORK ON THE ECOLOGY, BIOLOGY AND ETHOLOGY OF THE PEA-CRABS OF THE GENUS *PINNOTHERES*

The early Greeks as exemplified from the writings of Aristotle seem to have been aware of the association between pea-crabs and bivalves which they regarded as an example of friendship. General accounts of pea-crab associations are dealt with by various writers (Calman, 1909; Freame, 1943; Thompson, 1835; MacGinite and MacGinite, 1949; Mansueti, 1955; Ricketts and Calvin, 1952; Haven, 1957; Young, 1960, and others). Bashford Dean (1892) appears to be one of the first to observe that the activities of the pea-crab *P. ostreum* in its host were parasitic in nature, as the crabs affected the palps of the oyster which show thickened outgrowths, or are malformed or stunted. However, the great majority of pea-crabs, especially those of the genus *Pinnotheres*, are known to occur as commensals in bivalves, ascidians, holothurians, tubes of polychaetes, and even in brachiopods. Proven cases of *Pinnotheres* being a facultative parasite of bivalves are few, the notable instances being *P. ostreum* in the American oyster *Crassostrea virginica*; *P. pisum* in the mussel *Mytilus edulis* and *P. sinensis* in the Japanese oyster *Ostrea gigas*. We have herein reported an additional instance of *Pinnotheres* sp. in the backwater clam *Meretrix casta*.

LIFE-HISTORY

1. Egg Deposition and Hatching

Information on ovigerous females is available for a few species. However, very little is known about the duration between egg deposition and hatching, nor is any information available about the duration individual females carry their eggs under natural conditions, but laboratory observations are available for some species.

Hart (1935) found that the eggs of an ovigerous *P. taylori* brought to the laboratory on 16 March 1933 did not hatch until the first week of May the same year.

Atkins (1955) has observed egg deposition and hatching in six specimens of *P. pisum* and found the egg bearing period to vary between 35 and 59 days. She attributed temperature differences to be an important factor responsible for the considerable delay in the duration between deposition and hatching noticed. In the case of a *P. pinnotheres* brought to the laboratory with eggs in the early stage, hatching took place after 40 days.

The seasons and stages during which egg deposition takes place in *P. ostreum* is given by Christensen and McDermott (1958). They also found that the egg carrying duration (in nature 3-5 weeks) to be much shorter in the laboratory. This is in agreement with the observations of Sandoz and Hopkins (1947) who obtained zoea from a *P. ostreum* carrying a light orange egg mass after 12 days in the laboratory.

2. Development of Species of *Pinnotheres* (Life-history Stages)

Out of well over a hundred species of *Pinnotheres* described in literature, the complete or almost complete developmental stages are known for only two species, namely *P. ostreum* and *P. pisum*. Stray accounts of descriptions of one or more developmental stages are available for a few species and by far nothing is known about the great majority of species.

Differentiating characters for distinguishing the early larval stages from allied genera of Pinnotheridae are not many. As for the family, Hyman (1924) found that the minute size of the antennae in the larvae of Pinnotheridae is an important differentiating character for the larvae since the telson in some species may be normally forked and the carapace with all spines developed. As for *Pinnotheres*, Lebour (1928) has shown that the rudimentary antennae may not be an important diagnostic character (even for Pinnotheridae) as *Ebalia* (*E. tuberosa*) also posseses such rudimentary antenna. According to her, larvae of British species of *Pinnotheres* (*P. pisum* and *P. veterum*) are distinguished by the telson which has three lobes.

Briefly the available information on the developmental stages of *P. latissimus*, *P. maculatus*, *P. moseri*, *P. novaezealandiae*, *P. ostreum*, *P. pisum*, *P. ridgewayi*, *P. sinensis*, *P. taylori* and *P. veterum* are given below.

Pinnotheres latissimus Burger

Miyake (1935) has described the zoeal stages of this species.

Pinnotheres novaezealandiae Filhol

Bennett (1964) has illustrated the first zoea of this species.

Pinnotheres maculatus Say

Smith, S. I. (1879) gave some notes on the larvae of *P. maculatus*.

Fish (1926) remarked that young males of *P. maculatus* were frequently seen swimming, and young stages of the species were most abundant in the plankton of the Woods Hole Region from July 6 to November 1, 1922, but subsequently in 1922 and 1923 the larvae were not present in the plankton.

According to Christensen and McDermott (1958) the figure given on Plate I, Fig. 2 as the hard stage of *P. ostreum* by Smith, S. I. (1874) should in fact refer to *P. maculatus*.

Reference is also invited to Hyman (1924) for the early stages of this species.

Pinnotheres moseri Rathbun

Goodbody (1960) reports on the greatly abbreviated development of *P. moseri* found in the atrial cavity of the simple ascidian *Ascidia nigra*. For two batches of eggs, the time between hatching and megalopa stage was found to be 24 and 36 hours and such rapid development has not been reported for other species of *Pinnotheres*. Goodbody remarks that "the significance of such abbreviation cannot be at present assessed; but it is pertinent to point out that *Ascidia nigra* is confined in its distribution to sheltered water such as enclosed bays and lagoons and is absent from communities of animals in more open areas. If *P. moseri* is an obligate commensal in *A. nigra* and cannot live in other hosts, an abbreviated larval development might be advantageous in preventing the larvae from dispersing too far away from the concentrations of its host population."

Pinnotheres ostreum Say

Birge (1882) described the first zoea of *P. ostreum*.

Hyman's (1924) description of the larval (first and second zoeas) of this species shows the trilobed condition of the telson, while the carapace is seen to be devoid of spines.

Strauber (1945) has given lengthy descriptions of the five crab stages of *P. ostreum* parasitic in *Crassostrea virginica*.

Sandoz and Hopkins (1947) based on larvae collected from the plankton in the Delaware Bay, described the development of *P. ostreum* from the egg to the first crab instar (the stages being: the first, second, third, and fourth zoea, megalopa and the first crab stage).

Christensen and McDermott (1958) have remarked on the first zoea and some of the subsequent stages of this species from Chesapeake Bay. They have classified the developmental stage of the female from the first crab stage as:

A. First crab stage (Invasive stage).
B. Pre-hard stage.
C. Hard stage: Stage I.
D. Immature female: Stage II (soft stage)
E. Immature female: Stage III („)
F. Immature female: Stage IV („)
G. Mature female: Stage V

These authors have also shown that *P. ostreum* invades *C. virginica* in the first crab stage and not in the hard-shelled stage as generally believed. Theirs is the first record of the first crab stage from inside the oyster, which stage is otherwise known to be planktonic. They have also given the descriptive accounts, the habits and data onthe range of carapace width of the pre-hard stages, hard stages and post-hard stages of this species, at the same time comparing the stages with those described by other workers (Strauber, 1945, Sandoz and Hopkins, 1947). They conclude that the "hard stage" earlier believed to be the invasive stage is in fact a specialised stage which "... primarily serves the purpose of uniting the two sexes for copulatory purposes. The males leave their hosts in this stage to search for females in other oysters, but this free-swimming period is only a phase in the otherwise parasitic life of the crab."

Reference has already been made elsewhere to host change associated with growth in this species reported on by McDermott (1961).

Pinnotheres pisum (Pennant)

Thompson (1835) described the first-hard shell stage of the male of *P. pisum* and this in all probability represents the earliest description of a young stage of any species of the genus *Pinnotheres*.

In a series of papers Atkins (1926, 1954 *b* and 1955 *b*) has touched upon several aspects of the development of *P. pisum*. She has shown in 1926 that the female of *P. pisum* occurs under two forms, the young female being almost indistinguishable from the male (♀ Stage-I), while the next and subsequent stages are entirely different in appearance and may be considered typically female in form. She pointed out to what is now generally agreed that the change of form between Stage I and Stage II relates to change in mode of life " though it would seem that it can only take place after copulation. One exception has been found among the material so far examined; a Stage I female more nearly resembling the female form than the male though with empty spermathecae " Later she (Atkins, 1954 *b*) gave an account of the disposition of the thoracic appendages in the megalopa of *P. pisum* when swimming. The post-embryonic development of *P. pisum* is described by her in 1955 *b*.

Lebour (1928) described and figured the prezoea and the first zoea stages of *P. pisum*.

Christensen (1959) has illustrated the first crab stage, and the pre-hard growth stage of *P. pisum*, while also discussing these and other stages of development in relation to the free-swimming habits, copulation, and host change with growth.

Pinnotheres ridgewayi Southwell

Prasad and Tampi (1959) have described and figured the zoea of this species, remarking that the zoea resembled in its general shape, telson, etc., the zoea of *P. ostreum* described by Sandoz and Hopkins (1947), but at the same time showing differences in many other respects.

Pinnotheres sinensis Shen

The developmental stages are not known; but the following information may seem pertinent. Suguira *et al.* (1960) found in *P. sinensis* the optimum hatching season to be from September to October and from the next May to July many young crabs were found in the host and they in turn became berried in autumn. According to them "... the biological minimum size was 5·3 mm. in carapace length."

Pinnotheres taylori Rathbun

Hart (1935) described two zoeal stages, one megalopa stage, and the first young crab stage of *P. taylori.* The last said stage was found to emerge four weeks after hatching in the laboratory. She found that the megalopa of this species is easily separable from that of *P. veterum* by the presence of a small rostrum, a dorsal spine and the rudimentary sixth abdominal segment. She has suggested that when the zoea described by Aikawa (1933) as "Dissodactylozoea" and "Pinnozoea" are assigned to their correct parental species, some will be found probably to be larvae of *Pinnotheres*, and also felt that Aikawa's *Grapsizoea brevispinosa* showed superficial resemblance to *Pinnotheres taylori.*

Pinnotheres veterum Bosc

Lebour (1928) has described the first zoea, second zoea and megalopa of *P. veterum* Bosc. She has also drawn attention to an error in Gourret's (1884) work wherein figure 3 of plate 2 should refer to *Pinnotheres veterum* and figures 4 and 5 should be *Pisa.*

Atkins (1954 *b*) has given an account of leg disposition while swimming, in the megalopa of *P. pinnotheres* (= *P. veterum*). The post-embryonic developmental stages of this species [*P. pinnotheres* (= *P. veterum*)] are also given by her (1955 *b*).

3. Growth in Relation to Moulting

Unlike most brachyurans, moulting between copulation and egg deposition (at least four intermediate moultings in the female) occurs in *Pinnotheres* as copulation takes place when the female is in the hard shell stage. The position will be very clear from Passano's (1960) remarks, apparently based on the works of Christensen and McDermott (1958), and others. According to him "Strong metamorphic changes are relatively rare in crustacean post larval development, although comparatively minor alterations in relative growth such as the increased size of brachyuran chela... or abdomen ... may be secondary sexual characters linked to the reproductive maturation." Such changes may occur abruptly at one ecdysis or more gradually during several. However, recent work has disclosed a very interesting exception in the oyster crab *Pinnotheres ostreum.* This parasite has a hard shell and appendages adapted for a free-swimming existence during the first post-larval instar, the first crab or invasive stage. After invading its host it metamorphoses into a soft-shelled stage with modified appendages. Following several moults both sexes metamorphose into a hard stage. This is characterised not only by the hardened exoskeleton, but also by a reinforced endophragmal skeleton and by appendages specialised again for swimming. The male then leaves the host, seeks out and copulates with a hard stage female in another oyster. This is the last and only sexually mature stage in the male. Fertilized female *Pinnotheres* then resumes a series of soft-shelled moults until it is fully grown.

Christensen and McDermott (1958) have given the size increases of the invasive stage, pre-hard stage, hard stage, and the post-hard stages (4 stages) of *P. ostreum*. They have also shown that growth moultings with little or no morphological changes may occur in all stages of *P. ostreum* with the exception of the invasive stage and probably also the hard stage. Nothing is known of the physiological mechanism controlling the termination of moulting of male *Pinnotheres*.

4. Longevity

Upto now there is no definite information on the longevity of pea-crabs. What is known is that in a species, *P. ostreum*, the males do not develop beyond the hard stage and disappear shortly after copulation with one or more females. The observations in the laboratory carried out by Christensen and McDermott (1958) showed that all hard stage males did not survive long. However, these do not throw light on the life-span.

SEXUAL DIMORPHISM, DIMORPHIC FEMALES AND SOFT-SHELLED MALES

Sexual dimorphism is conspicuous in relation to the sizes of the respective sexes in many species of *Pinnotheres*. The males which are active and free swimming for most part aregenerally much smaller than the adult females which live in the host. However, in some species such as *P. pinnotheres* (Linnaeus) (= *vetrum* Bosc) no sexual dimorphism is seen and the young adult female is also capable of swimming. Dimorphism as pertaining to differences in the shape of the abdomen in the two sexes in crabs is well known. Descriptive accounts of males and females given by various workers give information on size differences.

In the early growth stages, especially in Stage I, differentiation between sexes at sight may be difficult unless the genital openings and abdominal appendages are examined. The resemblance at this stage between the sexes may be so great that in the case of *P. pisum*, Orton (1921) was misled at first to suspect protandry to be the case until he was able to obtain a female form, presumed to be a male, in which examination showed the full complement of abdominal appendages of the female. Such male-like females are recorded by Rathbun (1917) in the American species *P. maculatus*, *P. margarita* and *P. taylori*. Remarking on this and the condition obtaining in *P. pisum*, Orton (1921) observes that "These hairy male-like females are probably at first free-swimming, but after a time enter a mussel when copulation takes place."

When the male and female of *P. pisum* are seen as a pair in a host, their occurrence in relation to size and age appears to be haphazarded as young males may be found with large adult females or *vice versa*, or when young pairs are present, there may be hardly any difference between the sizes of the males and the females.

Males resembling females in consistency and lack of colour have been reported by Rathbun (1918) in *P. maculatus* and Strauber (1945) in *P. ostreum*. The soft-shelled second stage males reported in the first part of this paper partly come under this category.

In the case of *P. pisum*, for a long time it was believed that males occur as one form (Atkins, 1926 *a*). However, in 1958 Atkins found that the male of this species occurred in two main forms, one of which approaches the young State II female in appearance. In the south-west coast of England, the latter which is seasonal in occurrence may be found from June to August. Such occurrence is also said to coincide with the occurrence of moulting stages of males and when young crabs occur in mussels and hardly ever at other times. This, as Atkins remarks, would suggest the occurrence of males in thin-shelled form during periods of rapid growth and these thin-shelled males have the normal male abdomen.

The soft-shelled male in *P. ostreum* is generally larger in size than the hard stage male and Atkins (1933), Mercier and Poisson (1929), Christensen and McDermott (1958) and others have

commented on this. Atkins has refuted a statement by Mercier and Poisson that such an unusual condition could be caused by the infestation of the crab by parasitic isopods. In the soft-shelled stage of the male (Stage II) of *Pinnotheres* sp. that we have examined there is no evidence of any parasites on the crab. According to Christensen and McDermott (1958) one plausible avenue for further investigation on this problem is whether a tendency towards protandric hermaphroditism on the part of the male crab may be involved resulting in the moulting of the hard stage male into a soft-shelled stage much resembling the second stage female. The problem may remain unsolved until an examination of the gonads of freshly caught specimens could be made.

REPRODUCTION

Some aspects of reproduction in *Pinnotheres* have been dealt with in the sections "Sexual dimorphism, Dimorphic females, soft-shelled males; Ethology—Mating behaviour," etc.

Fecundity is not known for most of the species, but available information indicates that the total number of eggs although varying within the species may also show differences from species to species. In the case of *P. ostreum*, Christensen and McDermott (1958) found ovigers measuring 9·4 and 10·8 mm. in width carrying 7957 and 9456 eggs respectively. An ovigerous female of *P. pisum* measuring 10·4 mm. in width was found by them to carry 5800 eggs. This has led them to doubt Berner's (1952)statement that *P. pisum* deposits only about 100 eggs. In the case of *Pinnotheres* sp. we have described in the earlier part of this paper six ovigerous females examined for the number of eggs have 1,700–2,800 (average 2,250) eggs.

Christensen and McDermott (1958) found that only a single batch of eggs is produced with the first spawning season in *P. ostreum*. They opine that the possibility that the crab may spawn twice during the second and twice during the third year cannot be ruled out. Thus it will be seen that there is a paucity of information in this field.

SEX RATIO

Out of 20 live *Placuna placenta* examined by them from the Gulf of Kutch, India, Hornell and Southwell (1909) found the pea-crab *P. placunae* occurring as follows:

10	individuals	contained	1 female each
2	,,	,,	1 female and 2 males each
3	,,	,,	1 female and 1 male each
1	,,	,,	12 males
1	,,	,,	11 males
1	,,	,,	3 males
2	,,	,,	2 males each.

Southwell (1910) mentions that of 28 specimens of *Mytilus* sp. examined, 24 had one female *P. margaritiferae* each; 2 had one male and one female each; and 2 had no crabs. Ohshima (1927) remarks of a case of multiple infestation by *P. lattissimus* where 7 males were found within the shell of a *Paphia* sp. In the material (holothurians) he had examined, Chopra (1931) mentions 41 females and 3 males of *P. villosissimus*; and 11 females and one male of *P. deccanensis*.

Strauber (1945) found multiple infestation involving various combinations of crab stages of *P. ostreum* in *C. virginica*, but in no case did he find oysters with more than a single specimen of either the 3rd, 4th or 5th stage of crabs. First stage males and females were in great abundance, but the data on frequency of occurrence for 1941 and 1942 in two oyster beds he has tabulated is wanting in a sex-wise analysis of the 'Stage I crabs'. Chhapgar (1957) remarks that of 10 shells

of *Placuna placenta* opened, 4 contained a male as well as a female; 5 contained a female each and one had no crabs.

The few instances cited above indicate that the abundance of males over females may be seasonal for the species as the disparity appears most marked during the first crab stage. Besides this, on the basis of what is known no definite conclusion seems possible at this stage.

DIFFERENTIAL GROWTH IN *PINNOTHERES*

Huxley (1931, 1932) has discussed this problem in relation to the pea-crab *P. pisum*. In dealing with growth-gradients in the different regions of the body he has shown that in the abdomen of *P. pisum* the growth centre is not necessarily confined to the penultimate segment as it would appear on casual inspection, but to the terminal segment (telson) which is so small in the young female that for it to "achieve the only moderate and definitive size it must, and does, contain the growth centre."

Inspired by Huxley's preliminary observations, Williams and Needham (1935) made a study of the growth of the abdomen in *P. pisum*. The graph relating width of abdominal segment to carapace size was fitted by two successive straight lines on arithmetic graph paper, and by three such lines on log-log paper. However, instead of providing a simple method of analysis of temporal and spatial variations in the differential growth of the abdomen " ..they contradicted the manifest smoothly continuous change in growth rate of abdomen relative to the carapace." They found that the growth constant was not constant even over restricted periods of growth but was a continuously variable function of carapace size giving a temporally disjointed growth picture as well as spatially disjointed into seven discrete width-measurements.

In a further study of the form transformation of the abdomen of the female *P. pisum*, Needham (1950) has elaborated on the earlier work (Williams and Needham, 1938) enabling him to give an algebraical definition of the form of the abdomen, continuous both spatially and temporally and proposes the use of the term "algebraical form—Cinematograph" for form change. These studies led him to conclude that the equation "form = shape + size," would seem to be a useful convention.

SIZE OF PEA-CRAB *VERSUS* SIZE OF HOST

The information given by some authors suggest that the crabs grow towards maturity concurrently with their hosts (Hornell and Southwell, 1909; Atkins, 1926 *a*; Christensen and McDermott, 1958, and others). In the case of *P. pisum* and its host *Mytilus edulis* Atkins (1926 *a*) has shown that a rough relationship in size between the female crabs and its host exists, the larger crabs being found in the larger mussels (*e.g.*, crab 4·0 mm. in carapace width in mussel 41 mm. long and so on to crab 15·0 mm. in carapace width in mussel 104 mm. long). Recently, Houghton (1963) has confirmed this in his studies on *P. pisum* and *Mytilus edulis*.

Hornell and Southwell (1909) remark that immature shells of *Placuna placenta* less frequently reveal the presence of the pea-crab *Pinnotheres placuna* and in such instances the crabs were mostly immature. They opine that in this case the crabs grow towards maturity concurrently with their hosts.

Christensen and McDermott (1958) have also shown that the growth rate of *P. ostreum* from the invasive to the mature stage is positively correlated with the growth rate of the host. However, the development of the crab is not retarded in slow-growing oysters to the same extent as the rate of growth as is seen from the marked size variations of female crabs moulting into mature stage (4·4 to about 9 mm. in carapace width).

ECOLOGY

Sugiura, Kihara and Sugita (1960) found seasonal variations in the occurrence of *P. gordoni* in *Tapes japonicus*. Very low infestation was noticed from August to November when water temperature ranges from 16°–29° C., but infestation was considerably high from December–July when the water temperature ranged from 10°–26° C. In the case of *P. sinensis* infecting *Tapes japonicus*, Sugiura *et al.* (1960) found that the optimum hatching season for the species was September–October, when the water temperature ranged from 24°–19° C.

Flower and McDermott (1952–53) reporting on the heavy infestation of the Delaware Bay oysters (*C. virginica*) by *P. ostreum* remark that the crab may have less tolerance for low salinity water than the oysters.

Houghton (1963) investigated the relationship between tidal level and the occurrence of *P. pisum* in *Mytilus edulis*. The crabs were found to occur more frequentlyin larger mussels which were found in greater numbers lower in the shore. He found the percentage of infection to differ from one locality to another at the same tidal level and it increases from the middle shore to the sublittoral zone for any given size group. As elsewhere mentioned, he also found very low incidence of infection by *P. pisum* of mussels taken from the rafts suggesting that those near the surface of the water on floating structures are generally not infected by *P. pisum*. Houghton also found double infestation to occur in the same manner in relation to tidal level.

Depth of occurrence of some of the species of *Pinnotheres* is available from literature (Alcock, 1900; Dell, 1960; Heller, 1965; Tesch, 1918,and others). Most of the species are shallow water inhabitants found in bivalves, holothurians and ascidians. An exception seems to be *P. abyssicola* Alcock, collected from 430 fathoms from the south-west coast of India (off Travancore coast), from *Lima indica*.

Experimenting with *P. ostreum* (females of Stage V) collected from the oyster *Crassostrea virginaca* close to Glouster point, U.S.A., Nagabhushanam (1965) found that the crabs lived in salinities between 0·7 and 1·9% NaCl, while in freshwater they died within 24 hrs. and in salinities between 0·3 and 0·6% they survived for a maximum period of 3–4 days. Thus the lethal salinity was found to lie between 0·6 and 0·7%. On the basis of this Nagabhushanam (1965) has suggested that the oysters could be protected from attacks by *P. ostreum*, if exposed to salinities of 0·6% or below for 4 or 5 days, the oysters not being affected as they can survive in salinities as low as 0·3% for as much as 15 days.

INCIDENCE OF INFESTATION BY *PINNOTHERES*

Strauber (1945) drew attention to thehigh percentage of incidence of infestation of *P. ostraeum* in *Crassostrea virginica*, but was unable to explain the reaosns for the unusually very high increase in the numbers of the pea-crabs in the Oysters. More than 10 small crabs per oyster were found to be present while in one oyster measuring 85 × 46 mm. as many as 262 crabs were present. For the same species collected from two localities, during different periods, Christensen and McDermott (1958) found the incidence of infestation to vary from 11·7% to 72·8%. In the sample of *Meretrix casta* examined by us as reported earlier in this paper, we found *Pinnotheres* sp. present in 48.3% of the clams. Sugiura *et al.* (1960 *a*, 1960 *b*) have given information of the incidence of infestation of *P. sinensis* and *P. gordoni* in *Tapes japonicus*.

ETHOLOGY

Ethology is defined as ' the science of character' and would broadly imply an approach in the study of animal behaviour. Thorpe (1958) speaks of three scientific ways of probing into

the behaviour of animals, namely ".... the way of the naturalist, the way of the psychologist, the way of the physiologist." In the case of the pea-crabs of the genus *Pinnotheres*, what little information available on the subject of ethology is to be found under the first and the last categories mentioned above. A resume of this is given below under suitable subheadings.

1. Physiology of Commensalism with Special Reference to Pea-Crabs

Recent years have found more emphasis placed on studies relating to the physiology of commensalism, especially the role of specific chemical factors controlling host commensal relationships, which is an important aspect of ethological studies. Some notable contributions touching on this field—physiology of commensalism—are by Bartel and Davenport (1956), Caullery (1952), Dale (1957), Davenport (1950, 1953 *a*, 1953 *b*, 1955, 1960), Davenport, Camongis and Hickock (1960), Davenport and Hickock (1951), Hickock and Davenport (1957), Hodgson (1955), Johnson (1952), Mansueti (1963), Sastry and Menzel (1962), Thorape and Jones (1937), Welsh (1930, 1931), and others. All these works do not directly pertain to studies on behaviour of pea-crabs, but touch on important aspects of such studies which may also have to be extended to pea-crabs for a proper understanding of their behaviour patterns.

In a series of papers mentioned above, Davenport and collaborators have tried to demonstrate in host-commensal associations to be found in the marine environment, whether or not there is any chemical bond existing between host and the partner. Although excellent biological studies on species of *Pinnotheres* and their host species are available, hardly anything is known about the factors which attract these crabs to their hosts. In the case of pea-crabs this is very significant as the crab enters a host during the invasive stage, when there is a host change during growth, and when the male moves about from host to host in search of the female of the species for mating. It is not known that if there be any chemical attractant to attract the commensal to its host, whether this would be present throughout the adult part of the life-span of the host or restricted to seasons or to only a particular time during its adult life-span. Whether the commensal evinces any 'territorial behaviour' namely the capability to drive away any intruders or whether the host could expel the commensals at will is not well understood. Whether the reported cases of parasitism by the pea-crabs in oysters, mussels, and clams take place at a time when already the host is not "in condition" being affected by other parasites such as heavy infestation of oysters by cercariae is not known. This is of importance where mortality of the pea-crab infested host takes place.

In a species of an allied genus of *Pinnotheres*, namely *Dissodactylus*, Johnson (1952) has demonstrated by the use of Y-tube choice apparatus that water from an aquarium containing the host sand dollar *Mellitia* has a strong attraction for the crabs, and as pointed out by Devenport (1955), that this is the first time that such a chemotaxis has been demonstrated in Pinnotherids, though one has long suspected as a result of their habit that such a response could be shown. The specific factors for bringing about this response in *Dissodactylus* is not known. Johnson's work on two other pea-crabs commensal with the polychaete *Chaetopterus* and the oyster crab commensal with *Ostrea*, however, did not result in the crabs demonstrating any positive response as in the case of *Dissodactylus*. Whether closer contact with the host could have elicited any recognition response was not demonstrated.

In this connection the work of Sastry and Menzel (1962) dealing with host recognition of *Pinnotheres maculatus* already referred to under the section 'Host specificity' needs special mention. They found that *P. maculatus* is capable of recognising its hosts *Aequipecten irradians concentricus*, and *Atrina rigida* under experimental conditions described by them. They deduce that some unknown attractant from the hosts is responsible for the active movements of the crabs in the presence of the hosts, and such stimulated movements depended on the proximity of the hosts. They opine that the decreased attraction could result either from a gradient or the highly diffusable nature of the attractant. That the attractant could emanate only from the soft parts of the scallop is evident from their experiments as the crabs showed no attraction or response towards empty shells

attached with epizooites. Another interesting result of their work was the finding that both males and females of *P. maculatus* were equally responsive to their host scallops.

On the whole it will be seen that even the fringe of this problem has not been tackled as innumerable associations involving pea-crabs and their host bivalves, holothurians, ascidians, etc. remain yet to the investigated. Problems such as how from the free living early stage the pea-crab changes to a commensal habit may remain unanswered for a long time to come. However, the finding that *Pinnotheres* is hardy enough to survive in the laboratory for experimentation for physiological and behaviour studies is heartening.

2. Phototropism of Larvae of *Pinnotheres* under Laboratory Conditions

The first zoeal stage of *P. ostreum* was found to exhibit a distinct positive phototropism in the laboratory (Christensen and McDermott, 1958). This is in agreement of Lebour's (1928) observations on newly hatched larvae of *P. pisum* which were found at first to rise to the surface, but soon descend to the bottom when they feed. Welsch (1932) reported zoea of *P. maculatus* seeking the bottom three to five days after hatching the Miyaki (1935) found that zoea of *P. latissimus* doing likewise even one or two days after hatching.

In this connection it may be mentioned that Lebour (1928) has suggested that the form of the telson in the zoea of *Pinnotheres* may be an adaptive feature enabling the zoea to descend and remain close to the bottom. The telson at this stage is flat, plate-like and not forked and possesses only short spines and this condition may help the larva to curl up its body. The similar habit of curling up the abdomen under the body so as to form a ball is also reported in the case of *P. ridgewayi* by Prasad and Tampi (1959).

3. Colour Change in *Pinnotheres*

Atkins (1926 *b*) reports of some observations she made on colour change in two specimens of *P. veterum* from the branchial chamber of *Ascidia mentula*. At night, activity in the dark was accompanied in the male by the loss of colour. The female which had no definite colour pattern showed no appreciable change. "The male was golden brown, shaded with dark brown, the colour being due to orange and dark brown chromatophores, now fully expanded and their pigment diffused. In the dark it became pallid and transparent, some faint yellow diffuse pigment only being visible; the gut contents showed black, the testes white. This loss of colour is due to the reaction of the pigment in the chromatophores induced by the onset of darkness. Of the two pigments the orange had the quicker rate of flow and is probably lodged in a smaller cell." Atkins also found that when the crabs were placed in the dark during day, they sometimes reacted as at night taking 40–60 minutes to do so. When uncovered, the males took about the same time to recover.

4. Effects of Temperature and Light on Movements of Larvae of *Pinnotheres*

Welsh (1932) found that temperature and light were important factors in determining the locomotions of larvae of *P. maculatus*. He observed velocities over the range 13·4°–27·0° C. in the directional light of constant intensity and also over a range of intensities from 0·093–93·0 meter candles at constant temperatures indicating that the velocity of progression was related to the light intensity according to the expression:

$$\log V = K \log I - C.$$

This treatment yielded rectilinear graphs which were convenient for comparing effects of temperature and intensity of illumination.

5. Reaction of *Pinnotheres* to Anaerobic Conditions in the Host

In the course of his work on *Pinnotheres pisum*, Berner (1952) found that mussels subjected to prolonged desiccation at the upper tidal limits were devoid of pea-crabs. He felt that the anaerobic conditions within the mantle cavity of such mussels during their exposure could be a possible explanation. Other factors, such as depth of water in such beds during high tide, flow of water (currents in the beds), frequency and duration of exposures all have a bearing as to whether or not the invasive stage will be able to settle and invade the host. As discussed elsewhere (*vide infra*) the feeding behaviour of the crabs may also preclude to a great extent crabs infesting oysters in such exposed beds.

In this connection, the general non-occurrence of pea-crabs in mussels attached to submerged floating objects (rafts) drawn attention to by Houghton (1963) perhaps needs some other explanation for which the behaviour of the crab at the invasive and post-invasive stages may also have to be taken into account.

6. Feeding Behaviour

Orton (1921), Strauber (1945), and others have made direct observations on the feeding behaviour of species of *Pinnotheres* within mantle cavity of the host bivalves. MacGinitie and Mac Ginitie (1949) by putting a glass window in the shell of a mussel were able to watch the activities of the pea-crab *Pinnotheres concharum* (= should be *Fabia subquadrata*, closely allied to *Pinnotheres*). The crab was found to obtain its food by eating some of the mucous strings by means of which the mussel carried its food to its mouth.

Orton (1921) found *P. pisum* pick up food strings from the margins of the gills of the host with the chelipeds. Strauber (1945) has given details of the mode of feeding of *P. ostreum* in *Crassostrea virginica* and the disposition or orientation of the crab within the oyster at the time of feeding. He found considerable similarities in the feeding of *P. pisum* in *Mytilus edulis* reported by Orton, and his own observations.

There is strong evidence that some species of *Pinnotheres*, especially those living in the excurrent region of the atrial cavity of tunicates such as *P. pugettensis*, *P. taylori*, *P. pinnotheres*, etc., resort to filter feeding (Welsh, 1940). This mode of feeding is also likely to occur in the earlier post-invasive stages. This is evident as large numbers of immature *P. ostreum* found in the mantle cavity of *Crassostrea virginica* appear not to cause any damage to the host. The pre-invasive stages depend on filter feeding. White (1937) remarks that the stomach of *P. pisum* has been found to contain diatoms and other food substances of the mussel. Coupin (1894) found this species to feed on food filtered from the water by the host mussel.

The mode of feeding thus is likely to change with growth, and this may partly account for damages seen in the tissues of the host when it harbours larger crabs. It is also possible that the smaller pea-crabs resort to both filter feeding and picking up food particles from the mucous strings formed along the margins of the gills of the host. Thus, the feeding behaviour at the different stages may restrict the number of crabs in a host as they have to depend on the amount of food brought into the oyster per time unit, and the young which may resort to also filter feeding more so. These considerations have led Christensen and McDermott (1958) to suggest the possibility that differences in survival of oyster crabs invading spat and older oysters may be due to difference in the amount of water pumped by the host animal. This may also thus account for the absence of crabs in oysters found in beds liable to be frequently exposed.

7. Mating Behaviour

Atkins (1926 *a*) has shown that *P. pisum* is peculiar in copulating precociously at an extremely early stage, and suggests that in all probability the sperm from the first copulation is sufficient to

fertilise several batches of eggs. However, the occurrence of occasional adult females with empty spermathecae suggests the possibility that copulation may take place more than once. Earlier, Orton (1921) found that in *P. pisum* copulation normally took place inside the host, and males visit mussels in search of females. He also found a hard stage female with its spermathecae filled with mature sperms indicating thereby the early stage at which the crabs copulate.

Strauber (1945) doubted the possibility of copulation of males and females differing too much in size in *P. ostreum*. Conclusive evidence is wanting as to the copulation of hard stage males with Stage V females in *P. pisum*, although reported on by Berner (1952). The possibility that crabs which became ovigerous in the laboratory tanks may already have had sperm in the spermathecae when they were collected cannot be overlooked in this case although Berner observed the male and female together. In the laboratory tanks, Christensen and McDermott (1958) have also observed a hard stage male with its pleopods extended enclosed under the abdomen of a Stage V female. However, they are not sure whether actual copulation took place or not.

As reported in the first part of this paper, in the case of *Pinnotheres* sp. infesting *Meretrix casta* we have found a number of instances of hard stage males with Stage V ovigerous females and one possible explanation may be that the males in search of hard stage females had entered the clams with the ovigerous females. The occurrence of several hard stage males and ovigerous females singly in clams may add weight to this explanation.

The problem thus revolves round the question whether the hard stage males effectively copulate with the Stage V females or only with the hard stage females. While the latter condition is the rule, the former is suspected in species evincing disparity in sizes of the sexes. Nothing appears to be known about this in species in which adult males and females are of about the same size.

8. Free-living Habit of Adult Female *Pinnotheres*

Adult females (Stage V) are not known to occur outside their hosts, and there is no definite information about their moving from host to host, especially as the body form is not suited for free-living. In this connection, the work of Sugiura *et al.* (1960) is of interest as they found that female *Pinnotheres sinensis* could live free from its host *Tapes japonicus* for as much as four to six months in the laboratory aquaria.

9. Behaviour of Pea-Crabs in Presence of Anemones

Plessis (1954) has noted the behaviour of *P. pisum* kept in an aquarium along with anemones.

HOST SPECIFICITY

A large number of works dealing with the original descriptions of species or mere distributional records of pea-crabs of the genus *Pinnotheres* unfortunately do not throw light on the specific identity of the host species other than mention the host as a bivalve, a holothurian, an ascidian or give only the generic identification (*e.g.*, *Donas* sp.). This is an unsatisfactory state of affairs which will not permit even a gross appraisal as to whether species or species groups show any preference to particular host or host groups. However, in the case of a few species, such as *P. ostreum*, *P. pisum*, *P. gordoni*, etc., we have more definite host identifications. The last said species from Japan is said to occur in six species of bivalves [*Tapes japonicus*, *Mytilus edulis*, *Ostrea* (*Crassostrea*) *gigas*, *Meretrix lusoria*, *Mactra veneriformis* and *Chlamys nipponensis*) and one gastropod [*Umbonium* (*Suchium*) *moniliferum*] (Sugiura *et al.*, 1961). It should be mentioned here that the occurrence of a pea-crab in a gastropod is most unusual.

At the same time, a species showing a more extensive distribution as *P. pisum* in the Atlantic and Mediterranean is known from fewer hosts, all bivalves. A careful scrutiny of the known host records of the different species of *Pinnotheres* indicates that generally a species commensal in a bivalve is not met with also in a holothurian or an ascidian. As to what extent this would reflect our ignorance of all the natural hosts of the known speices of pea-crabs is difficult to say at this stage. Similarly the correct identification of the crab species itself may stand in the way of a proper assessment. We have mentioned this to draw attention to the lacunae which needs filling.

Experimental studies on host specificity is a relatively new field of investigation and in the case of pea-crabs we have at least one instance in the work of Sastry and Menzel (1962) wherein they have shown that *Pinnotheres maculatus* commensal on the bay scallop *Aequipecten irradians concentricus* and the pen-shell *Atrina rigida* (from Alligator Harbour, Franklin County, Florida) did not show preference for one host over the other. In fact, the crabs removed from the scallops and experimented appeared to be non-host specific.

Similarly, in places where more than one species of bivalve is present in an area, it is not known whether and if so to what extent the pea-crab could evince host specificity. The collections of the same species of *Pinnotheres* in a particular area from more than one host species no doubt indicates the non-host specific nature of the crab. However, before arriving hastily at this conclusion one factor dealt with below has to be thought about. Such an interesting situation while studying host specificity arises in instances where host change occurs at different stages of growth of the crab. One such instance of definite host change in relation to the growth stage of the pea-crab is reported by Christensen (1959) in the case of *P. pisum*. He obtained the different progressive growth stages from two hosts from the same area, namely *Spisula solida* harbouring the invasive and first crab stage and *Modiolus modiolus* the hard-shelled stage and subsequent stages. Examining specimens of both the host species from the same dredge haul he remarks that "...it was found that 21 *Modiolus* contained 10 hard-shelled stage, 2 Stage IV and 5 Stage V crabs but no invasive or small soft-shelled specimens, while 38 *Spisula* contained 5 first crab stages varying in size from about 0·70 to about 0·75 mm. and 5 hard-shelled crabs (Stage I)."

A parallel instance is that of *P. ostreum* drawn attention to by McDermott (1961). This species matures and becomes ovigerous in *Anomia simplex* whereas in *Mytilus edulis* they develop only to hard swimming stage. According to him, in the Jersey area of the east coast of U.S.A., *P. ostreum* leaves the mussels at the attainment of the hard swimming stage during the late fall apparently in search of some other host and mature females have not been found in mussels.

In instances such as those mentioned above where host change of the crab occur with growth and consequently two host species are needed for the successful completion of the life-history of the commensal species it will not be wrong to say that the crab shows host specificity to two species. The possibility that the pea-crab could be host specific at one phase of its growth, but not at another cannot also be ruled out. The problem needs special attention.

Among the Indian species of *Pinnotheres* one species, *P. placuane*, has a markedly dorso-ventrally flattened body with a more or less squarish carapace to enable it to live in its host, the greatly flattened window-pane oyster *Placuna placenta*. In view of the adaptive modifications seen in this pea-crab, it can be safely stated that it is host specific.

EFFECT OF INFESTATION BY THE PEA-CRAB ON THE HOST

The species of the genus *Pinnotheres* have a wide variety of hosts, but up to now the activities of the crabs bordering on parasitism or actually being parasitic has been reported only in the case of bivalves harbouring them. Hale (1927) cities a statement that *Pinnotheres subglobosa*

(= *Ostracotheres subglobosa* ?) from South Australia known to infect at least three bivalves, *Chlamys bifrons, Spondylus tenellus* and *Modiolaria australis* is apparently "deeper sunk in parasitism than other members of the genus." In the case of at least one species it has been shown that the physical damages it causes to the host tissues may lead to mortality of the host. Indirect effects on the host, such as hermaphroditism have also been attributed to the pea-crab and these are dealt with below.

1. "Index of Condition" of Infected Oysters

Sandoz and Hopkins (1947) found *Crassostrea virginica* to show poor condition when infested with *P. ostreum*, than when uninfested. However, exceptions of crab infested oysters showing excellent condition were also seen by them. They found that mortality of oysters on account of pea-crab infection did not occur in Virginia as in New Jersey (Strauber, 1945), although incidence of crabs was noticed. They remark that "Miss Alice Elizabeth Overcash, in an unpublished thesis, 1946... has studied the "Index of Condition" of Virginia oysters, including many with oyster crabs. She reports that this index:

$$\frac{(1000 \times \text{dry weight of meat in gm.})}{\text{Volume of shell cavity in milliliters}}$$

averaged only 82·3 for the crab-infested oysters in New York River, compared to 90·6 for oysters without crabs. In Rappahannock River she found the mean index of oysters with crabs to be only 71·7, while the entire sample averaged 90·0. Both samples show significantly poor condition in infested oysters.'

Christensen and McDermott (1958) found that oysters showing extreme cases of gill erosion were found to be in very poor condition. However, they feel that there is no conclusive evidence of possible differences in shell thickness or weight of living tissue in relation to infestation. This led them to conclude that only the presence of mature crab over a longer period of time will noticeably affect the growth of the host under normal environmental conditions.

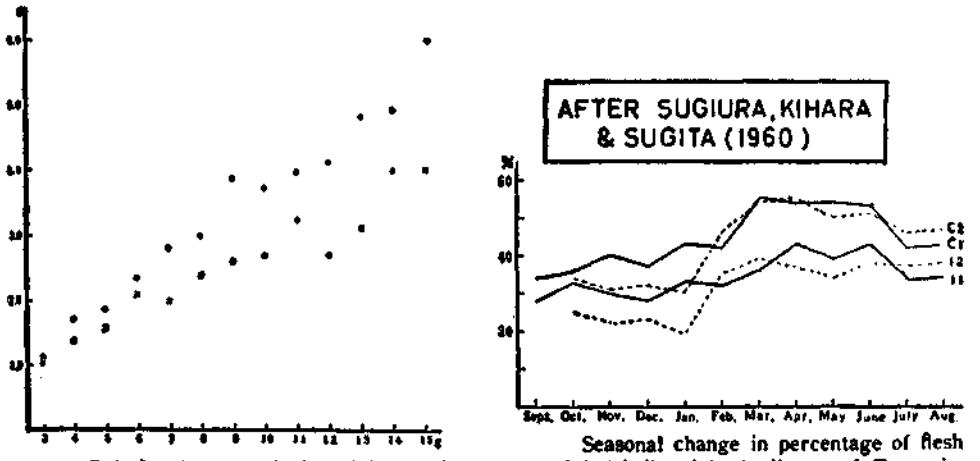
Sugiura, Sugita and Kihara (1960) found that *Tapes japonicus* infested with *Pinnotheres sinensis* was lean without regard to their size or season. In the case of *P. gordoni* found also infesting the same host, they (Sugiura, Kihara and Sujita, 1960) found certain amount of damages caused by the pea-crabs. Using the formula $W \times 100/sw$, where W = total weight of flesh of clam; and sw = shell weight of it, uninfected clams gave a value of $49{\cdot}8 \pm 12{\cdot}1\%$ and infested clams $41{\cdot}6 \pm 12{\cdot}6\%$. This as well as the graphs they have given for flesh weight/shell weight of *Tapes japonicus* infested and uninfested with *P. sinensis* and *P. gordoni* have been reproduced here to facilitate reference and to clearly show the effect of the infestation (Text-Fig. 4).

In our observations on *Pinnotheres* sp., infesting *Meretrix casta* we found no significant difference in the meat weight/total weight ratio and have given possible reasons for this.

2. Damages Caused to the Gills of the Host

Strauber (1945) described two types of gill damages caused by *P. ostreum* to *C. virginica*; "the small-crab type with a local, sharply delimited erosion of one or more demibranchs, and the large-crab type where an extensive shortening of one or more demibranchs may be seen reaching from the anterior end of the gills to a point usually ventral to the adductor muscles." Christensen and McDermott (1958) found in *C. virginica* infested by *P. ostreum* after examining 1502 oysters that nearly all oysters infested showed some gill damage: 50% had light gill damage, about 40% had moderate gill damage, and about 9% had heavy gill damage and only 1% had no discernible gill damage. This led them to conclude that gill damage or gill erosion gradually develops from the

first type to the next. They also found that among older oysters a few extreme cases of heavy gill damage where there was hardly anything left of the gills were present, and that these were in very poor condition. Strauber (1945) has also remarked on instances of gill regeneration. He is of the opinion that the gill damaged oysters lose their crabs at times or they eject the dead crabs. Regeneration of the essential gill structure may take place after this, but the scar mark will be still evident at the place of original damage. There is hardly any study on the histological aspects of the healing process in once parasitised oysters or other bivalves.



Relation between flesh weight (ordinate) and shell (abscissa) weight (g) on average in *Tapes japonica* infested with *Pinnotheres sinensis* (♀). ×, infested; ●, control.

Seasonal change in percentage of flesh weight/shell weight (ordinate) of *Tapes japonica* infested with *Pinnotheres sinensis* or *Pinnotheres gordoni*. Abscissa, Months. ——, 1st year; ····, 2nd year. I_1, I_2, infested; C_1, C_2, control.

TEXT-FIG. 4. Relation between meat weight and shell weight in the Japanese clam *Tapes japonica* infested with *Pinnotheres sinensis* and *P. gordoni* and in control samples (after Sugiura, Kihara and Sugita, 1960).

McDermott (1962) found gill damage in *Mytilus* caused by *P. maculatus* and *P. ostreum*, but the latter species when found in *Anomia simplex* did very little damage to the gills of this species.

Sandoz and Hopkins (1947), and Flower and McDermott (1952-53) have shown that heavy infestation of the oysters by *P. ostreum* produced gill erosions of the host in varying degrees. We have elsewhere in this paper shown the various types of gill erosion seen in *Meretrix casta* caused by *Pinnotheres* sp.

3. Damages Caused to the Palps of the Host Bivalves

Strauber (1945), Christensen and McDermott (1958) and others have mentioned that pea-crabs cause damage to the palps of the host also. McDermott (1962) found palp erosion in *Mytilus* caused by *P. maculatus* and *P. ostreum* and pointed out that the palp abnormalities in *Mytilus* reported by Atkins (1931) might also have been caused by pea-crabs.

4. Effects on Reproduction of Host Bivalves—Hermaphroditism and Other Anomalies

Awati and Rai (1931) attributed the change of sex of the Bombay oyster (*O. cucullata*) partly due to the infestation of a pea-crab, *Pinnotheres* sp. In this connection the following details are given by them;

Sex of animals harbouring pea-crabs	Numbers examined	Percentage
Male ..	71	82·56
Female ..	9	10·46
Hermaphrodite ..	6	6·97
Total ..	86	

They remark that "The very large proportion of males against females amongst oysters harbouring the pea-crab is remarkable. Ordinarily the proportion of males and females is almost equal, while the existence of the hermaphroditic individuals is almost negligible amongst oysters without the pea-crabs..." This they deduce from the data they have given which is reproduced below:

Sex	Numbers counted	Percentage
Male ..	326	41·7
Female ..	445	56·4
Hermaphrodite ..	23	2·9
Total ..	794	

No information is available from their work as to the stages of the pea-crabs they found infesting the oysters, the sex-wise ratio, etc. It is interesting that Durve (1960) studying the Bombay oyster *Crassostrea gryphoides* found only 5 out of 3,000 examined by him containing one each of a pea-crab (*Pinnotheres* sp.).

Amemiya (1929) and Coe (1934) have shown that *Crassostrea* has a strong tendency towards protandric hermaphroditism and the sex ratio in this oyster is definitely influenced by environmental conditions. Experimental works on removal of parts of the gill tissue in *Crassostrea gigas* carried out by Amemiya (1935), and Egami (1953) have shown the increase in the proportion of males to females during the breeding season when the operations are performed "...no later than the previous October." To elaborate, Egami's (1953) studies on the sexuality of this Japanese oyster after removal of gills indicate that animals in a state of undistinguishable sexuality (those which have passed one winter) from which gills were removed in October 1951 (October 21 and 22) and January 1952 showed the following results: the Proportion of males in the breeding season is definitely larger in the group from which the gill tissue had been removed in the October batch than in the control. However, no appreciable changes in the sex ratio between the controls and those operated on in January were noticeable. The results indicate the role played by environmental conditions in controlling the sex-phase of the Japanese oyster. This we mention here especially to lay stress on the significance of gill erosion in oysters and other bivalves caused by the pea-crabs and the indirect effect this could have on the sex-phase of the host.

In the case of the mussel, *Mytilus edulis*, Berner (1952) found that the gonads of individuals parasitised by large specimens of *P. pisum* are functionally inhibited without showing visible signs of atrophy. He found on examining over 300 mussels thus infected that those having crabs measuring 10 mm. or more in carapace width showed partial or even complete cessation in the production of sexual products, while those harbouring smaller pea-crabs were hardly affected. His

interpretation was that the crabs which caused these changes in the host were in their second and third year. This finds favour with Christensen and McDermott (1958) who suggest that the reproductive system in *Crassostrea virginica* may similarly be affected by *P. ostreum* "···atleast in the second spawning season in which it harbours the same crab, *i.e.*, yearling oysters are probably never affected."

5. Other Effects on Host Bivalves

White (1937) speaking of the 'apparently harmless' association between *P. pisum* and its host *Mytilus edulis* remarks that larger crabs may exert pressure on various organs of the host, especially the mantle and subscribes to the view that the damaged mantle by its pathogenic secretions may cause the wearing away of the nacreous layer of the shell.

Young (1960) mentions that the picking off of mucous laden strings of food by the pea-crab would affect both the food and feeding efficiency of the host, although the pea-crab may not cause any deliberate damage to the host tissues.

Some of the damage caused by the pea-crab to its host has been attributed to the friction caused by the hard and unyielding exoskeleton of the first stage crab on the soft parts of the host.

A characteristic irritating flavour in the Madagascar oyster, *Ostrea vitrefacta* was linked with the presence in this oyster of a species of *Pinnotheres*, by Poisson (1946). Further he associated the presence of *Sertularia* often found on the shells of oysters containing *Pinnotheres* and the attacks of nettlerash of people consuming oysters with such association. According to Korringa (1955), this information corresponds to the belief in Holland that *P. pisum* is responsible for bringing about nettlerash, in those who consume the mussel, *Mytilus edulis*. He concludes that no special flavour has been noticed in mussels containing *Pinnotheres* and there is no justification for assuming that *Pinnotheres* has anything to do with the allergic reaction in some people seen after eating mussels. However, it would be "interesting to hear about possible associations of *Pinnotheres*, irritating flavour and nettlerash in other species of oysters." To our knowledge such information is wanting.

PARASITES OF SPECIES OF THE GENUS *PINNOTHERES*

In his account on "Challenging problems in shellfish biology" Loosanoff (1958) mentions that the successful control of oyster enemies such as starfish, gastropods, crabs, etc., 'is extremely difficult and often impossible'. Biological control is a possibility, but has not been tried, nor will it be in the forseable future. Through the excellent works of Atkins and others it is known that *Pinnotheres* itself may be subjected to attacks by isopod parasites, certain saprolegniaceae, and rhizocephalans, some of which may cause death to the host crab.

1. Isopod Parasites of *Pinnotheres*

Giard and Bonnier (1899) reported briefly with illustrations a new genus and species of Entoniscidae, *Pinnotherion vermiformae* which they found infesting a *Pinnotheres* inhabiting *Modiolus modiolus* at Dimereaux (Belgium). They remarked that the host pea-crab was not *Pinnotheres veterum*. Bonnier (1900) later gave the host identification as *P. pisum* without adding further details to the description of *Pinnotherion vermiformae*. Mercier and Poisson (1929) found this parasite infecting *P. pisum* at Luc-sur-mer. Atkins (1933) found it on *P. pisum* inhabiting *Mytilus* in the estuary of Camel, North Cornwall. She discovered that the male of *P. vermiformae* may occur as an internal parasite and also showed that the percentage of infestation with this species (*P. vermiformae*) was greater in female *P. pisum* (31·8% or 129 out of 415 specimens examined and 3·45% or 2 out of 58 in males examined). 28·69% of *P. pisum* were found to be infected

with one or more specimens of *P. vermiformae* and on the whole male *P. vermiformae* were found to be more common in occurrence (26·85%) than the female parasites. Mercier and Poisson (1929) found that *Pinnotherion* infected 9% of females and 1·5% of males of *Pinnotheres*. Such occurrence was explained even earlier by Bonnier (1900) who noticed this frequency of occurrence and by Mercier and Poisson (1929) as being due to fewer males than females of *P. pisum* being present in their host *Mytilus*. Atkins (1933) has drawn attention to another possibility, namely the relative thickness of the carapace of the male and female *P. pisum* as an important factor affecting the incidence of infestation in both sexes. Bonnier (1900) has shown that infestation with epicarid isopods appears to be heavier in some years than in others. According to Atkins (1933) the presence of adult *Pinnotherion vermiformae* in *P. pisum* would seem to cause partial or complete atrophy of the gonads.

Giard and Bonnier (1887) have noted for other Entoniscidae as well as for *P. vermiformae* (in 1889), that the hepatopancreas (liver) of the host *P. pisum* are much reduced, the parasite occupying almost all the available space.

Whether female *Pinnotherion* prevents moulting of the host *P. pisum* is not known, but Atkins (1933) opines that this seems probable.

The presence of dead or degenerating male *P. vermiformae* have been found in different parts of the body of the host attached to the lobes of the hepatopancreas, or embedded in the gonads, thorax, or abdomen as small yellowish oval masses. Atkins (1933) presumes that these males would have died in an attempt to moult as the position of the abdomen suggest "...except in one case when there seems to be an attempt on the part of the host to surround the male."

Hale (1927) reports that one specimen of *Pinnotheres subglobosa* (Baker) (= *Ostracotheres subglobosa*?) taken from the bivalve *Chlamys* had a large parasitic isopod (Epicarid) beneath its abdomen.

2. Sacculation in *Pinnotheres*

Boschma (1953) described a new rhizocephalan, *Sacculina pertenuis* from a species of *Pinnotheres* taken at Gulf of Suez.

Sacculinization and sex differentiation were reported on by Semitu (1944) in *Pinnotheres cyclinus* and *P. sinensis*. According to him, the secondary male characters of these species turn female-like as a result of sacculinization, but the female secondary sexual characters and the ovary are scarcely affected. The actual changes in the parasitised male are the appearance in the middle part of the testes pseudo-egg cells derived from the primordial germ cells. The latter are accompanied by a few follicle cells, but are devoid of yolk and reduction division is not seen. The spermatocytes are located round the pseudo-egg cells which occur as groups or clusters in the spermatogonium stage. As the change of the secondary male characters is always brought about even if the testes is completely degenerated by sacculinization, Semitu (1944) is of the opinion that some other phenomenon which cannot be explained endocrinologically is at work in addition to the function of the sexual hormone.

3. Barnacles and Other Organisms Fastened to *Pinnotheres*

Strictly speaking the information given here will not come under parasitism, but this appears to be an appropriate place for mentioning this. Ryder (1882) remarking on Protozoa and Protophytes as direct source of food of fish mentions of an instance of multiple parasitism involving also a *Pinnotheres* as "On another occasion I found something like *Poteriodendron* on the *Zoothannium* which covered a *Pinnotheres* inhabiting an oyster; but the chain of parasitism did not stop here, for on the monad, as well as on the bell-animal, there were rod-like bodies attached which were

presumably bacteroid..." A similar observation by Hale (1927) bears citing here. He "...on removing a pea-crab from *Spondylus*, discovered that a parasitic barnacle (Rhizocephalid) was attached to the pleon of the crab, and that a parasitic isopod (Epicarid) was fastened to the barnacle. In this remarkable case we have a tiny, malformed isopod feeding upon a degenerate barnacle, which in turn is drawing its nourishment from the soft-shelled little crab which relies upon a mollusc for shelter and food."

Hale (1927) also mentions of a specimen of parasitic barnacle taken from beneath the abdomen of the pea-crab *P. holothuriensis* (= *Ostracotheres holothuriensis*?) from Edithburg, South Australia.

4. Infection of Species of *Pinnotheres* by Marine Fungi (Saprolegniaceae)

Three species of marine fungi, *Leptolegnia marina*, *Plectospira dubia* and *Pythium thalassium*, have been described by Atkins (1929, 1954 *a*, 1954 *c*, 1955 *a*) from *P. pisum* and the first mentioned species also doubtfully from *P. pinnotheres* (= *veterum*), from British waters.

Leptolegnia marina was described from *P. pisum* taken from *Mytilus edulis*. The fungus penetrates deep into the body of the pea-crab surrounding the organs, and generally the gills, and may also extend into the appendages, mouthparts and even eye-stalks. Atkins (1954 *a*) found that the duration from the onset of the infection to the death of the crab was highly variable, and under laboratory conditions this was found to vary from 8–57 days and in one exception the crab died on the 78th day. More females than males of *P. pisum* were found to be infected with *L. marina* but this could have been due to the fact that more female crabs than males were examined. The infection was also seen in the eggs as well as embryos of *P. pisum*.

External growth of the fungus was not seen in dead infected crabs left in the sea-water. Regarding this Atkins (1929) says that the apparent inability of this fungus "...to form a growth on dead crabs may seem to argue in favour of its truly parasitic nature, but putrefaction of *Pinnotheres* is somewhat rapid and it may be that the number of bacteria, etc., present, check the growth of the fungus and kill it." Atkins (1929) concluded that *P. pisum* infected with this fungus always dies, but definite evidence as to whether the fungus is pathogenic or only invades tissues which have been destroyed by parasitic bacteria is wanting. The data she gives of mortality of pea-crabs infected with fungus when kept together in bowls, and individual crabs kept in separate bowls indicate low mortality rate for the latter while very high mortality rate was seen in the former kept in batches varying from 5-21 specimens. This would suggest that the disease is extremely infectious.

As regards the lone instance of the occurrence of *L. marine* on *Pinnotheres pinnotheres*, Atkins (1954 *a*) remarks that this was found on a tiny male (2·1 mm. in carapace width) "...obtained on 15th March 1928 from Teignmoth and found dead on 4th May with the fungus visible on the walking legs. It most probably became infected in the laboratory."

Plectospira dubia was described by Atkins (1954 *c*) from the eggs of *P. pisum*. The infected eggs showed a greater development of the space between the inner and outer egg membranes caused by the growth of the fungus. Such eggs showed a greater diameter (about 0·5 mm.) than the healthy eggs (0·33 mm.). The gills of the pea-crabs were found to be unaffected.

Pythium thalassium was also described by Atkins (1955 *a*) infecting the eggs of *P. pisum* and other crustaceans.

REACTIONS OF THE HOST BIVALVES TO INVASION BY PEA-CRABS

In a review of this nature it is equally important to consider the reaction by the host animal to invasion by the commensal. At the outset it should be mentioned that the available information is scanty, but we have given below the relevant observations we are able to glean from literature.

It is generally believed that the crab being a commensal the partnership is one of mutual concord. However, a few instances have been reported where this is not the case. Strauber (1945) found that invasion of older oysters (*C. virginica*) by the hard-stage crabs of *P. ostreum* is always not easily accomplished. The larger oysters may expel a number of tiny invasive stages of the crab by enveloping them in mucous and by passing them out by ciliary action and clamping the valves.

Atkins (1955) observed the expulsion of megalopa of *Pinnotheres pinnotheres* inserted in *Mytilus edulis*. Some observations made by Strauber (1945) on the mode of infestation of *C. virginica* by *P. ostreum* indicates that the pea-crabs which have not effected complete entry are liable to be crushed or trapped due to the clapping of the valves. He agrees with Orton's (1921) view that although the hard flat shell of the invasive first stage of the pea-crab has an adaptive value in allowing the crab to slip within the valves of the mollusc and also in avoiding crushing by the closing valves, experiments carried out indicated that successful invasion did not always occur.

In *P. pisum*, Orton (1921) remarks that "male crabs have been found with their legs or bodies trapped by the mussel closing its shell before the crab could get inside. These crabs survive the rough treatment by reason of their extraordinary strong carapaces and creep inside the mussel later when it must perforce relax and open its shell in order to breath."

Woodward (1886) remarked on a very interesting instance of a male *Pinnotheres* being encysted in the nacreous layer of the pearl oyster *Meleagrina margaritifera* suggesting that the animal had "... intruded at an unfortunate time, when no female of his kind happened to be in, and that having penetrated too far beneath the mantle in the ardour of his search was made prisoner before he could escape." This instance is also cited by Cooke (1959).

III. TAXONOMY, NOMENCLATURE, SPATIAL DISTRIBUTION AND HOST RECORDS OF SPECIES OF THE GENUS *PINNOTHERES*

TAXONOMY AND NOMENCLATURE

Alcock (1900) provisionally subdivided the family Pinnotheridae into four subfamilies which are recognised by later workers (Tesch, 1918; Sakai, 1935). These subdivisions are, subfamily Pinnotherinae Alcock with the following genera recognised by Tesch (1918) under it—*Ostracotheres* H. Milne-Edwards (1853); *Dissodactylus* Smith, S. I. (1870); *Cryptophyrs* Rathbun (1893), *Xanthista* White (1846); *Durckheimia* (Ruppel) de Man (1889); *Scleroplax* Rathbun (1893); *Raphonotus* Rathbun (1897); and *Pinnotheres* Latreille (1802). Under subfamily Pinnotherelinae Alcock, Tesch (1918) treated the genera *Pinnotherelia* H. Milne-Edwards and Lucas (1843); *Pseudopinnixa* Ortmann (1894); *Pinnixa* White (1846); and *Tetrias* Rathbun (1899). The subfamily Xenophthalminae Alcock is known from a single genus *Xenophthalmus* White (1846). The fourth subfamily Asthenognathinae Stimpson embraces the genera *Asthenognathus* Stimpson (1858); *Mortensenella* Rathbun (1909); *Tritodynamia* Ortmann (1894); *Hapalonotus* Rathbun (1897) [To replace *Malacosoma* de Man (1879) which is preoccupied]; *Opisthopus* Rathbun (1893); *Chasmocarcinops* Alcock (1900); *Voeltzkowia* Lenz (1905); and *Aphinodactylus* Tesch (1918).

In the course of the present study we have experienced the same difficulties as other recent workers who have dealt with members of the family Pinnotheridae, especially the genus *Pinnotheres* in that the taxonomy of this genus itself is in a nebulous state badly needing a critical review on a global basis.

As for the generic name *Pinnotheres*, Alcock (1900) felt that *Pinnoteres* being the correct transliteration of the Greek word, which was also used by Rumph in 1705, 'no apology is necessary for reverting to it'. Tesch (1918) disagreeing with Alcock (1900) advocates using *Pinnotheres* drawing

attention to the ambiguity surrounding *Pinnoteres* in early Greek literature and sums up his argument with the statement that "Apart from such arguments it seems preferable not to cling too firmly to Aristotle or even Rumphus, but to return simply to authors using the regular Linnean nomenclature and I see no reason to follow Alcock in his orthography."

The authorship of *Pinnotheres* ascribed to Latreille (1802-03) has been disputed and Direction 45 (1956) of the International Commission on Zoological Nomenclature was for the substitution of *Pinnotheres* Bosc (1801-02) for *Pinnotheres* Latreille (1802-03). (*See* also Monod Th. 1956.)

Neave's "Nomenclator Zoologicus" gives the following:

Pinnoteres (pro-theres Latreille, 1802) Leach, 1816.

Pinnothera (pro-res Latreille, 1802) Dana, 1851.

Pinnotheres Latreille (1802), *Hist. Crust. Ins.*, 3, 25-Crust.

Hardly any emphasis has been given up to now for the study of the species of *Pinnotheres* taking into account the possibility of polytypic species occurring in the genus; the host relationships and spatial distributional patterns of the species, etc., for a proper understanding of the species complexes in this genus. Difficulty in the taxonomy of this genus will be experienced by a reviewer as stable or dependable characters for distinguishing the species are not many and even so some of them have been proven to be affected during growth. In this connection, the need for taking into account the phenomenon of asymmetry in pea-crabs, when present, has been stressed by Gordon (1936). In view of these, the sanest course to follow in *Pinnotheres* taxonomy at present would be to refrain from describing new species on very ill-defined and variable characters, or merely on grounds of a new host record. These considerations have probably led some recent authors (Poisson, 1946; Barnard, 1950; Jones, 1950; Monod, Th., 1956; Dell, 1960) to describe or denote their finds as merely *Pinnotheres* sp., a course which we have also followed in the present study.

One of the notable works on *Pinnotheres* during the close of the 19th century is that by Burger (1895) who described as many as 30 new species from the Philippines and nearby islands. As already mentioned, Alcock's (1900) contribution has been towards a proper classification of the family Pinnotheridae itself, and in attempting this he has recorded 5 species of *Pinnotheres* from the Indian Seas. Other useful works dealing with Indian species of the genus *Pinnotheres* are those by Hornell and Southwell (1909), Southwell (1910), Chopra (1931), and Chhapgar (1957). The most important work to appear on Indo-Pacific Pinnotheridae is the provisional review of the group by Tesch (1918) wherein 57 species of the genus *Pinnotheres* are keyed and 65 species from the Indo-Pacific (excluding species from Eastern Pacific—the western coast of North and South America) are check-listed. Some criticism has been expressed by later workers on the usefulness of the key characters used by Tesch (1918) for separating the species of the genus *Pinnotheres*. This again draws attention to the urgent need for a taxonomic review of the genus based on a study of the type material and wherever possible additional series of specimens of the different species, preferably of both sexes.

Since 1918 when Tesch check-listed or referred to a total number of 76 species of the genus *Pinnotheres* from the Indo-Pacific (including Eastern Pacific), at least 21 new species have been described from this area up to 1963 and in addition about a dozen have been described or denoted merely as *Pinnotheres* sp. These do not include about 13 species reported at one time or other from the Atlantic and the Mediterranean. Before giving a list of these with host records and distributional details, we shall consider here two other aspects having a bearing on the taxonomy of the genus *Pinnotheres*, namely larval characters and their role in taxonomy and phylogeny, and asymmetry in *Pinnotheres*.

1. Larval Characters and Their Role in Taxonomy and Phylogeny

In this connection the remarks of Gurney (1939) appear pertinent. He has drawn attention to the fact that in spite of increase in our knowledge of decapod larvae in recent years, the larval

characters have not been used in the classification of the crabs. The considerable differences in the larvae of closely allied species of even a single genus and the difficulties of identification of the larvae if not linked with the adult are drawn attention to. As an example he cites the case of the differences between the species of *Pinnotheres* and allied genera with reference to telson and carapace and remarks that "···it is difficult to believe that the systematics of the adult can be correct. If typical zoeas of Pinnotherid, Ebaliid, and Hymenostomatid crabs are taken they are found to have so many points of agreement that they must be supposed to be related; and yet each is now placed in a group widely separated from the others." His further observations on the larval and adult characters of certain species of *Pinnotheres* and allied genera such as *Dissodactylus*, *Pinnixa*, *Chaetopterina*, *Ostracotheres* and *Tridacnae* have led him to conclude that the larval characters point to a relationship between Pinnotheridae, Leucosiidae, and Hymenosomidae and also suggest that the genus *Pinnotheres* may have to be subdivided. Since 1939 the larval stages of a few more species of *Pinnotheres* are known and for a natural classification within the genus as well as at the higher category levels it may also be necessary to take into account salient larval characters.

2. Asymmetry in Pea-Crabs

De Man, one of the first to notice asymmetry in the length of the appendages of *Pinnotheres* at first considered this phenomenon as being due to the cross breeding between two species inhabiting the same molluscan host. However, in 1929 (p. 16) he considered this improbable. Gordon (1936) while discussing this phenomenon in greater detail has also enumerated some of the immediate problems in connection with this as: "(1) How many species of the large genus *Pinnotheres* are markedly asymmetrical; (2) whether it is characteristic of both sexes, or only the females; (3) whether or not the position of the female within the mollusc shell (should the asymmetry prove to be restricted to females of species inhabiting Mollusca) is constant and/such that growth of the legs on one side might be rather hampered, and (4) at what stage in the development of the Pinnotherids the asymmetry first becomes apparent." The taxonomist should give due consideration to these facts.

SPECIES OF THE GENUS *PINNOTHERES* LATREILLE

Pinnotheres abyssicola Alcock and Anderson (1899)

Distribution: Travancore coast (S.-W. Coast of India) from 430 fathoms.

Host: Lima indica.

References: Alcock and Anderson (1899), Alcock (1899, 1900), Hornell and Southwell (1909), and Tesch (1918).

Pinnotheres afinis Burger (1895)

Distribution: Ubay, Philippines; Gulf of Siam; Shantung (Coast of China).

Hosts: Pinna sp.; *Pecten hastatus* (?).

References: Burger (1895), Rathbun (1910), Tesch (1918) and Tu (1938).

Remarks: This species is different from *Ostracotheres affinis* H. Milne-Edwards (1853) described from Mauritius and later reported from the Red Sea by Nobili (1906). This clarification is necessary as some species of *Pinnotheres* have been placed under the genus *Ostracotheres* H. Milne-Edwards or *vice versa* by some workers.

Pinnotheres alcocki Rathbun (1910)

Distribution: Mergui Archipelago, Burma; Padang; Noordwachter Island near Batavia, Indonesia; Burias, Philippines.

Host: *Cytherea* sp., *Mytilus* sp.

References: de Man (1881 *a*, 1888 *b*), Burger (1895), Alcock (1900), Rathbun (1910), Tesch (1918), Sakai (1935) and Gordon (1946).

Remarks: Rathbun (1910) proposed a new name for the material described as *Pinnotheres* (—*teres*) *parvulus* by de Man, Burger and Alcock (*nec P. parvulus* Stimpson, 1858).

***Pinnotheres angelicus* Lockington (1876)**

Distribution: Vera Cruz, Coast of California.

Host: ?

References: Lockington (1876), Miers (1880) and Tesch (1918).

***Pinnotheres arcophilus* Burger (1895)**

Distribution: Ubay, Philippines.

Host: *Arca* sp.

References: Burger (1985), and Tesch (1918).

***Pinnotheres ascidicola* Hesse (1871)**

Distribution: Coast of France.

Hosts: *Ascidia canina, Ascidia intestinalis.*

Reference: Hesse (1871).

***Pinnotheres barbatus* Burger (1895)**

Distribution: Aibukit, Philippines.

Host: *Donax* sp.

References: Burger (1895), and Tesch (1918).

***Pinnotheres bidentatus* Sakai (1939)**

Distribution: Wakayama Prefecture, Japan.

Host: Unknown.

Reference: Sakai (1939).

***Pinnotheres bipunctatum* Nicolet (1849)**

Distribution: Chile, W. Coast of S. America.

Host: ?

References: Nicolet (1849), and Tesch (1918).

***Pinnotheres boninensis* Stimpson (1858)**

Distribution: Bonin Island.

Host: "Small oysters."

References: Stimpson (1858, 1907), Tesch (1918), and Sakai (1935).

***Pinnotheres borradailei* Nobili (1906 *a*)**

Distribution: Red Sea; Minicoy Island, Laccadive Archipelago.

Hosts: *Mya* sp. (?); *Pinna* sp.

References: Borradaile (1903), Nobili (1906 *a*), Paulson (1875), and Tesch (1918).

Remarks: *P. borradailei* was proposed by Nobili (1906 *a*) for *P. tenuipes* Borradaile, 1903 (*nec P. tenuipes* Burger, 1895), and for *P. rouxi* Paulson, 1875 (*nec P. rouxi* H. Milne-Edwards, 1863).

***Pinnotheres burgeri* Rathbun (1920)**

Distribution: Gulf of Siam.

Host: Uuknown.

References: Rathbun (1910), and Tesch (1918).

***Pinnotheres cardii* Burger (1895)**

Distribution: Burias, Philippines; Gulf of Siam; Japan.

Host: *Cardium unedo.*

References: Burger (1895), Rathbun (1910, 1924), Tesch (1918), and Sakai (1935).

***Pinnotheres coarcatus* Burger (1895)**

Distribution: Zamboanga, Philippines (brackish water).

Host: ?

References: Burger (1895), and Tesch (1918).

***Pinnotheres consors* Burger (1895)**

Distribution: Palos Island; Siboga Exped. Station No. 277 off Dammer Island, N.E. of Timor.

Hosts: *Circe* sp.; *Arca* sp.

References: Burger (1895), and Tesch (1918).

Remarks: Tesch (1918) provisionally placed one female obtained at Siboga Exped. St. 277 under this species, at the same time drawing attention to the several characters in which the specimen differed from Burger's description of *P. consors.*

***Pinnotheres corbiculae* Sakai (1939)**

Distribution: Nagasaki; Kagoshima Prefecture, Japan.

Host: *Corbicula japonica*

Reference: Sakai (1939).

***Pinnotheres coutieri* Nobili (1905)**

Distribution: Red Sea.

Host: ?

References: Nobili (1905, 1906), and Tesch (1918).

***Pinnotheres cyclinus* Shen (1932)**

Distribution: China Seas.

Host: Corbicula japonica.

Reference: Shen (1932).

***Pinnotheres deccanensis* Chopra (1931)**

Distribution: Coast of Madras Presidency (S.E. Coast of India).

Host: Holothuria scabra.

Reference: Chopra (1931).

Remarks: Chopra (1931) has drawn attention to the affinities of this species to *P. ortmanni* Burger (1895) from Aibukit, Philippines.

***Pinnotheres dilatatus* Shen (1932)**

Distribution: China Seas.

Host: ?

Reference: Shen (1932).

***Pinnotheres dofleini* Lenz (1914)**

Distribution: Cape of Good Hope, Simons Bay, Algoa Bay, St. James, False Bay, Simonstown (Union of South Africa).

Hosts: Ascidians *Phallusia canaliculata* and *Pyura stolonifera*; also unidentified ascidians attached to ships; *Pinna* sp.

References: Lenz (1914), Tesch (1918), and Barnard (1950).

***Pinnotheres edwardsi* de Man (1888 *b*)**

Distribution: King Island Bay, Mergui Archipelago, Burma; Siboga Exped. Stn. 258 off Tual, Kei Island; Siglap, Singapore; Abrolhos.

Hosts: Ostrea sp.; *Pinna* sp.

References: de Man (1888 *b*), Alcock (1900), Hornell and Southwell (1909), Tesch (1918), Montgomery (1931) and Gordon (1936).

***Pinnotheres exiguus* Burger (1895)**

Distribution: Palapa and Samar Islands, Philippines.

Host: Unknown.

References: Burger (1895), Tesch (1918).

***Pinnotheres flavus* Nauck (1880)**

Distribution: Zamboanga, Phillippines.

Host: Holothurian.

References: Nauck (1880), de Man (1887), Burger (1895), and Tesch (1918).

***Pinnotheres glaber* Burger (1895)**

Distribution: Palaos Islands.

Host: *Tapes turgida.*

References: Burger (1895), and Tesch (1918).

***Pinnotheres glaberrimus* Burger (1895)**

Distribution: Zamboanga, Ubay, Philippines; Gulf of Siam (brackish water).

Hosts: *Arca* sp.; *Lima divaricata.*

References: Burger (1895), Rathbun (1910), and Tesch (1918).

***Pinnotheres globosus* Jacquinot and Lucas (1853)**

Distribution: Singapore; New Caledonia; Mozambique.

Host : ?

References: Jacquinot and Lucas (1853), H. Milne-Edwards (1853), Hilgendorf (1878), Tesch (1918) and Barnard (1950).

Remarks: After comparing the types of *P. globosus* Jacquinot and Lucas, and *P. obesus* Dana, H. Milne-Edwards (1853) considered the former to be a synonym of the latter. Tesch (1918: footnote on p. 257) did not agree with this treatment of *P. globosus*, but at the same time did not include this species in the list of species considered valid by him, nor as a synonym of any species.

Hornell and Southwell (1909) apparently following H. Milne-Edwards (1853) have considered *P. globosus* to be conspecific with *P. obesus* Dana. In their list of recorded species of *Pinnotheres* they have given *Meroe quadrata* and *Cytherea* sp., as hosts of *P. globosus* and mentioned "China Seas" as the distribution of this species. This is no doubt incorrect.

On the basis of Hilgendorf's (1878) record of this species from Mozambique, Barnard (1950) has listed *P. globosus* in his treatise on Decapod Crustacea of South Africa, and he has also mentioned this species in the key to the identification of South African species of *Pinnotheres* given by him.

***Pinnotheres gordoni* Shen (1932)**

Distribution: China Seas; Kisarazu Coast, Chiba Prefecture, Japan.

Hosts: Sugiura *et al.* (1960) record this species from: *Tapes japonica, Mytilus edulis, Ostrea* (*Crassostrea*) *gigas, Meretrix lusoria, Mactra veneriformis, Chlamys nipponensis* and *Umbonium* (*Suchium*) *moniliferum.*

References: Shen (1932), Sugiura, Kihsra and Sugitta (1960).

***Pinnotheres gracilis* Burger (1895)**

Distribution: Ubay, Philippines; Gulf of Siam.

Host: *Solen* sp.

References: Burger (1895), Rathbun (1910), and Tesch (1918).

***Pinnotheres haiyangensis* Shen (1932)**

Distribution: China Seas.

Host: ?

Reference: Shen (1932).

***Pinnotheres holothuriae* Semper (1880)**

Distribution: Zamboanga, Philippines.

Host: From cloaca of holothurian *Stichopus variegatus.*

References: Semper (1880), Burger (1895), and Tesch (1918).

***Pinnotheres holothuriensis* (Baker) (1908)**

Distribution: St. Vincent Gulf, Edithburgh, South Australia.

Hosts: Holothurians and ascidians.

References: Baker (1908), Tesch (1918), and Hale (1927).

Remarks: This species was first described as *Ostracotheres* (?) ("*Pinnotheres*") *holothuriensis* by Baker (1908). Hale (1927) considers this a species of *Pinnotheres* and remarks that a variety of *P. holothuriensis* living in ascidians is very much smaller in size. We have provisionally placed this species under the genus *Pinnotheres* and additional comments are given under 'Remarks' for a similar problematic species *P. subglobosa* (Baker).

***Pinnotheres impressus* Burger (1895)**

Distribution: Aibukit, Philippines.

Host: Unknown.

References: Burger (1895), and Tesch (1918).

***Pinnotheres jamesi* Rathbun (1923)**

Distribution: Pichilinque Bay, Lower California. (By electric light.)

Host: ?

Reference: Rathbun (1923).

***Pinnotheres kamensis* Rathbun (1910)**

Distribution: Gulf of Siam.

Host: ?

References: Rathbun (1910), and Tesch (1918).

***Pinnotheres kutensis* Rathbun (1910)**

Distribution: Gulf of Siam.

Host: ?

References: Rathbun (1910), and Tesch (1918).

***Pinnotheres lacquei* Sakai (1962)**

Distribution: Rocky bottom at Amadaiba, off coast of Hayama, Japan.

Host: From body cavity of brachiopod, *Laqueus rubellus*.

References: Sakai (1962).

***Pinnotheres laevis* Burger (1895)**

Distribution: Palaos Islands.

Host: *Coralliophaga* sp.

References: Burger (1895), and Tesch (1918).

***Pinnotheres lanensis* Rathbun (1910)**

Distribution: Gulf of Siam.

Host: ?

References: Rathbun (1910), and Tesch (1918).

***Pinnotheres latissimus* Burger (1895)**

Distribution: Manila, Philippines.

Host: *Paphia* sp.

References: Miers (1880), Burger (1895), Tesch (1918), Ohshima (1927), and Gordon (1936).

Remarks: Gordon (1936) considers *P. obesus* Miers (1880) (*nec* Dana, 1851) as a synonym of this species.

***Pinnotheres latus* Burger (1895)**

Distribution: Burias, Philippines; Palaos Island; Siboga Exped., Stn. 53, Nangamessi Bay, Sumba; Stn. 291, off Ambon.

Host: *Pinna* sp., *Pinna nigrina*.

References: Burger (1895) and Tesch (1918).

Remarks: Tesch (1918) draws attention to some differences between his Siboga Expedition material provisionally placed under this species, and Burger's account of *P. latus*.

***Pinnotheres lithodomi* Smith (1870)**

Distribution: Pearl Island, Coast of Panama, and vicinity.

Host: ?

References: Smith (1870), and Tesch (1918).

***Pinnotheres longipes* Burger (1895)**

Distribution: Aibukit, Philippines.

Host: Unknown.

References: Burger (1895), and Tesch (1918).

***Pinnotheres lutescens* Nobili (1905)**

Distribution: Red Sea.

Host: ?

References: Nobili (1905, 1906 *a*), and Tesch (1918).

***Pinnotheres mactricolus* Alcock (1900)**

Distribution: Mouth of Hooghly River, Bengal, India.

Host: Mactra violacea.

References: Alcock (1900, 1903), Hornell and Southwell (1909), and Tesch (1918).

Remarks: The species was originally described as *Pinnotheres mactricola* by Alcock (1900) who found it to show affinities to *P. cardii* Burger.

***Pinnotheres maculatus* Say (1818)**

Distribution: From several localities from East Coast of North America.

Hosts: Aequipecten irradians concentricus; *Atrina rigida*; *Anomia simplex*; *Pinna* sp.; *Pecten magellanicus*; *P. gibbus*; *Modiolus modiolus*; *Mytilus edulis*; also known to occur in tubes of annelid *Chaetopterus variopedatus.*

References: Say (1818); Smith (1874, 1891), Sumner *et al.* (1913), Hay and Shore (1918), Fish (1926), MacGinite and MacGinite (1949), Christensen and McDermott (1958), McDermott (1961), and Sastry and Menzel (1962).

***Pinnotheres maindroni* Nobili (1905)**

Distribution: Red Sea.

Host: ?

References: Nobili (1905, 1906 *a*), and Tesch (1918).

***Pinnotheres major* (Ortmann) (1894)**

Distribution: Japan.

Host: ?

References: Ortmann (1894), and Tesch (1918).

Remarks: This species was originally described by Ortmann as *Pinnaxodes major.*

***Pinnotheres malaguena* Garth (1948)**

Distribution: Malaga Bay, Columbia.

Host: Unknown.

Reference: Garth (1948).

***Pinnotheres margarita* Smith (1870 ?)**

Distribution: Coast of California; Coast of Panama; and adjacent regions (W. Coast of S. America).

Host: ?

References: Smith (1870?), and Rathbun (1911).

Remarks: A redescription of the species was given by Smith in 1870.

***Pinnotheres margaritiferae* Laurie (1906)**

Distribution: Kondatchi Paar, Pearl Banks off Ceylon, Gulf of Mannar.

Hosts: *Pinctada margaritifera*, *P. vulgaris*, *Mytilus* sp.

References: Laurie (1906), Hornell and Southwell (1909), Southwell (1910), and Tesch (1918).

Remarks: The species was originally described as *Pinnoteres margaritiferae*.

***Pinnotheres marioni* Gourret (1886)**

Distribution: ?

Host: ?

References: Gourett (1886).

***Pinnotheres modiolicolus* Burger (1895)**

Distribution: Philippines.

Host: *Modiola philippinarum*.

References: Burger (1895), and Tesch (1918).

***Pinnotheres moseri* Rathbun**

Distribution: Jamaica, West Indies.

Host: *Ascidia nigra*.

References: Goodbody (1960).

***Pinnotheres mytilorum* (Herbst) (1782–1804)**

Distribution: French Coast.

Host: ?

References: Herbst (1782–1804), and Lucas (1881).

***Pinnotheres nigrans* Rathbun (1910)**

Distribution: Gulf of Siam.

Host: ?

References: Rathbun (1910), and Tesch (1918).

***Pinnotheres novae-zealandiae* Filhol (1886)**

Distribution: Punipet, Auckland, and several other localities from New Zealand.

Host: ?

References: Filhol (1886), Heller (1865), Lenz (1901), Tesch (1918), Powell (1947), and Scott (1961).

Remarks: Scott (1961) has shown that records of *P. pisum* from New Zealand by Heller (1865) and others (*nec P. pisum* Pennant) refer to only variations of *P. novae-zealandiae*, which she con-

siders to be a polytypic species and the sole representative of the genus in New Zealand. Thus in all probability *P. schauinslandi* Lenz (1901) should also be a synonym of this species.

***Pinnotheres nudifrons* Burger (1895)**

Distribution: Lapinig, Philippines.

Host: Unknown.

References: Burger (1895), and Tesch (1918).

***Pinnotheres nudus* Holmes (1895)**

Distribution: Santa Cruz, Monterey Bay, Coast of California.

Host: ?

References: Holmes (1895, 1900), Weymouth (1910), and Tesch (1918).

***Pinnotheres obesus* Dana (1851)**

Distribution: Fiji Islands; Borneo; Gulf of Siam; Siboga Expedition Stn. 174 off Waru Bay, North Coast of Ceram.

Hosts: *Arca* sp.

References: Dana (1851, 1852), Rathbun (1910), Hornell and Southwell (1909), and Tesch (1918).

Remarks: Tesch (1918) considers *P. siamensis* Rathbun (1910) from Gulf of Siam as a synonym of this species. We have elsewhere drawn attention to the work of Hornell and Southwell (1909) (p. 1199) wherein these authors consider *P. globosus* Jacquinot and Lucas (1853) as a synonym of this species, which is not correct. Gordon (1936) has shown that *P. obesus* Miers (1880) (*nec* Dana, 1851) given as a synonym under this species by Tesch (1918) is in fact a synonym of *P. latissimus* Burger.

***Pinnotheres obscurus* Stimpson (1858)**

Distribution: Hong Kong.

Host: ?

References: Stimpson (1858, 1907), and Tesch (1918).

***Pinnotheres onychodactylus* Tesch (1918)**

Distribution: Siboga Expedition Stn. 172, between Gisser and Ceram Laut.

Host: ? (unknown).

Reference: Tesch (1918).

Remarks: Tesch (1918) remarks that this species shows close affinities to *P. rhombifer* Burger, *P. coarctatus* Burger, *P. tenuipes* Burger, and *P. borradailei* Nobili.

Pinnotheres orcutti

Distribution: W. Coast of North America.

Host: ?

Reference: Glassel (1938)

***Pinnotheres ortmanni* Burger (1895)**

Distribution: Aibukit, Philippines.

Host: Unknown.

References: Burger (1895), and Tesch (1918).

***Pinnotheres ostrearius* Rathbun (1918)**

Distribution: Delagoa Bay, St. James, and Cape Peninsula, Union of South Africa (Stebbing, 1920).

Hosts: From *Modiola* sp. shell and ascidian (Stebbing, 1920).

References: Rathbun (1918), and Stebbing (1920).

***Pinnotheres ostreum* Say (1817)**

Distribution: From several localities along the East Coast of North America.

Hosts: Crassostrea virginica, Anomia simplex, Mytilus edulis, Pecten sp.

References: Say (1817), De Kay (1844), Stimpson (1859), Rathbun (1884), Paulmier (1905), Hay and Shore (1918), Fish (1926), Christensen and McDermott (1958), McDermott (1961), and several other authors.

Remarks: This species is definitely known to be parasitic on the American oyster *Crassostrea virginica*, as reports by several workers (Strauber, 1955; Christensen and McDermott, 1958; and others) would indicate.

***Pinnotheres palaensis* Burger (1895)**

Distribution: Palaos Islands.

Hosts: Arca scapha, Arca sp., *Placuna sella, Byssoarca* sp.

References: Burger (1895), and Tesch (1918).

***Pinnotheres parvulus* Stimpson (1858)**

Distribution: China Sea; Japan; Gulf of Siam.

Hosts: Meroe quadrata.

References: Stimpson (1858, 1907), Ortmann (1894), Rathbun (1910), Tesch (1918), Hornell and Southwell (1909), Makai (1931), and Sakai (1935).

Remarks: Reference may be made to our comments given under *P. alcocki* Rathbun.

***Pinnotheres pectinicolus* Burger (1895)**

Distribution: Fundordt, Ubay, Philippines; Red Sea.

Hosts: Pecten radula.

References: Burger (1895), Nobili (1906 *a*), and Tesch (1918).

***Pinnotheres pectenculi* Hesse (1871)**

Distribution: Coast of France.

Host: Paetenculus flammelatus.

Reference: Hesse (1871).

***Pinnotheres perezi* Nobili (1905)**

Distribution: Persian Gulf.

Host: ?

References: Nobili (1905, 1906 *b*), and Tesch (1958).

***Pinnotheres pernicolus* Burger (1895)**

Distribution: Ubay, Philippines; New Guinea; Red Sea.

Host: *Perna* sp.

References: Burger (1895), Nobili (1899, 1906 *a*), and Tesch (1918).

***Pinnotheres pholadis* de Haan (1835)**

Distribution: Japan.

Host: *Pholadis japonicae.*

References: de Haan (1835), Ortmann (1894), Adensamer (1897), Tesch (1918), and Sakai (1935).

Remarks: Tesch (1918), and Sakai (1935) consider *P. pisoides* Ortmann (1894) as a synonym of *P. pholadis* de Haan.

***Pinnotheres pichilinquei* Rathbun (1923)**

Distribution: Pichilinque Bay, Lower California. (By electric light.)

Host: ?

References: Rathbun (1923).

***Pinnotheres pinnotheres* (Linnaeus)**

Distribution: Coast of Camaron and Gabon, W. Africa; Puget Sound, Washington State, N.-W Coast of America.

Hosts: *Pinna* sp.; excurrent region of atrial cavity of tunicates (Wells, 1940).

References: Balss (1922 *b*), Wells (1940), Christensen and McDermott (1958) and other authors.

***Pinnotheres pilumnoides* Nobili (1905)**

Distribution: Red Sea.

Hosts: In sponges and holothurians.

References: Nobili (1905, 1906 *a*), Laurie (1915), and Tesch (1918).

***Pinnotheres pisoides* Ortmann (1894)**

Distribution: Japan.

Host: ?

References: Ortmann (1894), Tesch (1918), and Sakai (1935).

Remarks: Tesch (1918), and Sakai (1935) consider this species a synonym of *P. pholaides* de Haan.

***Pinnotheres pisum* (Pennant) (1777)**

Distribution: From several places along the coast of Europe (France, Belgium, Denmark, Sweden, etc.) and from the British coast (England, Wales).

Hosts: *Mytilus edulis*, *Cardium norwegicum*, *Spisula solida*, *S. elliptica*, *S. subtruncata*, *Modiolus modiolus*, and *Modiola modiolaria*.

References: Pennant (1777), Hesse (1871), Lucas (1881), Bouvier (1940), Gils (1947), Poulson (1949), Smith and Weldon (1958), Christensen (1959, 1962), Houghton (1963), and several others.

Remarks: The records of *P. pisum* from New Zealand waters have been shown to refer to *P. novae-zealandiae* by Scott (1961). Tesch (1918) gives the authorship of this species as: '*P. pisum* (Linné) Latreille'.

***Pinnotheres placunae* Hornell and Southwell (1909)**

Distribution: Balapur and Rann Bays, Beyt Island, all in the Gulf of Kutch, India; Tampalkam Bay near Trincomalee, Ceylon.

Host: *Placuna placenta*.

References: Hornell and Southwell (1909), Tesch (1918), and Chhapgar (1957).

***Pinnotheres politus* (Smith)**

Distribution: Chile.

Hosts: ?

References: Lenz (1902), Rathbun (1911), Tesch (1918), and Garth (1957).

Remarks: This species was originally described under the genus *Ostracotheres*. Tesch (1918) considers this as a species of *Ostracotheres* with the remarks that it resembles *O. cynthiae* Nobili by the last pair of the legs being longer than the preceding legs, but differing in the distinctly broader than long carapace, the more slender last pair of legs and the host being a lamellibranch instead of an ascidian. However, Garth (1957) considers this a species of the genus *Pinnotheres*.

***Pinnotheres pugettensis* Holmes (1900)**

Distribution: Coast of California; Puget Sound, Coast of Washington State, N.-W. Coast of U.S.A.

Hosts: From excurrent region of atrial cavity of tunicate *Cynthia* sp., and *Tethyum aurantium*.

References: Holmes (1900), Way (1917) and Tesch (1918).

***Pinnotheres purpureus* Alcock (1900)**

Distribution: Andaman Islands; Felidu Atoll, Maldive Archipelago; Red Sea.

Host: *Ostrea* sp.

References: Alcock (1900, 1903), Borradaile (1903), Nobili (1906), and Tesch (1918).

Remarks: This species is said to show affinities to *P. palaensis* Burger by Alcock (1900).

***Pinnotheres quadratus* Rathbun (1910)**

Distribution: Gulf of Siam; Siboga Expedition Stn. 34, Labuan Pandan, East coast of Lambok; Stn. 152, Wuncoh Bay, N.-W. Coast of Waigau Island.

Hosts: *Arca* sp.

References: Rathbun (1910), and Tesch (1918).

Remarks: Tesch (1918) has provisionally placed a male and a female pea-crab from the Siboga Expedition collections under this species and at the same time drawn attention to the differences between his specimens and the description of the species given by Rathbun.

***Pinnotheres rhombifer* Burger (1895)**

Distribution: Ubay, Philippines.

Host: *Pectenculus aurifluus*.

References: Burger (1895), and Tesch (1918).

Remarks: Tesch (1918) remarks that *P. rhombifer* Burger could be identical with *P. palaensis* Burger and that his species *P. onychodactylus* shows affinities to both these species.

***Pinnotheres ridgewayi* Southwell (1910)**

Distribution: Kondatchi Paar, Ceylon Pearl Banks, Gulf of Mannar; Kundugal Point, Pamban Island, Gulf of Mannar.

Hosts: *Pinna bullata* ; *Pinna aequillatera*.

References: Southwell (1910), Tesch (1918), Gravely (1927), Prasad and Tampi (1959), and Sankarankutty (1965).

***Pinnotheres rotundatus* Burger (1895)**

Distribution: Burias, Philippines.

Host: *Circe* sp.

References: Burger (1895), and Tesch (1918).

***Pinnotheres rouxi* H. Milne-Edwards (1853)**

Distribution: Indian Ocean.

Hosts: ?

References: H. Milne-Edwards (1853), Tesch (1918).

Remarks: *P. rouxi* Paulson, 1875 (*nec* H. Milne-Edwards) has been considered as a synonym of *P. borradailei* Nobili.

***Pinnotheres rouxi* Rossignol (1957)**

Distribution: Pointe-Noire, French Congo.

Host: *Donax* spp.

References: Rossignol (1957 , 1962).

Remarks: Rossignol (1957) has described the species as *Pinnoteres rouxi*. As the original description is not available to us for reference, we have provisionally placed this species separately. The specific name *P. rouxi* is preoccupied and if Rossignol's species is meant to represent a species different from *Pinnotheres rouxii* H. Milne-Edwards, then a new name will have to be proposed of this West African species.

***Pinnotheres sanguinolariae* Pillai (1951)**

Distribution: Ashtamudi Lake, Travancore Coast (S.-W. Coast of India).

Host: *Sanguinolaria diphos.*

Reference: Pillai (1951).

Remarks: This species is said to be allied to *P. gracillis* Burger. The generic name is given as *Pinnotherus* by Pillai (1951).

***Pinnotheres schauinslandi* Lenz (1901)**

Distribution: New Zealand.

Host: *Mytilus* sp.

References: Lenz (1901), and Tesch (1918).

Remarks: Scott (1961) considers all species of *Pinnotheres* recorded from New Zealand as being referable to a single polytypic species, *P. novae-zelandiae.*

***Pinnotheres semperi* Burger (1895)**

Distribution: Java, Indonesia.

Host: From Cloaca of *Holothuria fusco-cinerea.*

References: Burger (1895), Tesch (1918), and Gordon (1934).

***Pinnotheres serrignathus* Shen (1932)**

Distribution: China Sea.

Host: ?

Reference: Shen (1932).

***Pinnotheres setnai* Chopra (1931)**

Distribution: Viper Island and Dunda's Point, Port Blair, Andamans.

Host: From main respiratory system of an unidentified holothurian.

Reference: Chopra (1931).

Remarks: Chopra (1931) remarks that his species is closely allied to *P. semperi* Burger.

***Pinnotheres siamensis* Rathbun (1910)**

Distribution: Gulf of Siam.

Host: ?

References: Rathbun (1910), and Tesch (1918).

Remarks: Tesch (1918) considers this species a synonym of *P. obesus* Dana (1851).

***Pinnotheres silvestrii* Nobili (1901)**

Distribution: West coast of S. America.

Host: ?

References: Nobili (1901), and Tesch (1918).

***Pinnotheres similis* Burger (1895)**

Distribution: Ubay, Philippines.

Host: ?

References: Burger (1895), Tesch (1918), and Gordon (1936).

Remarks: Gordon (1936) has provisionally placed one ovigerous female collected from *Placuna placenta* at Siglap, Singapore, under this species "... until re-examination of the type for the nature of the walking legs."

***Pinnotheres sinensis* Shen (1932)**

Distribution: China Sea.

Host: ?

Reference: Shen (1932).

***Pinnotheres sinensis atrinae* Sakai (1939)**

Distribution: Kisarazu Coast, Chiba Prefecture, Japan.

Hosts: Tapes japonica, Mytilus edulis.

References: Sakai (1939), and Sugiura, Sugita and Kihara (1960).

Remarks: We have provisionally placed *P. sinensis* from Japan mentioned by Sugiura *et al.*, (1960) under this subspecies, as the forma typica (*P. sinensis* Shen) is known from the China Sea.

***Pinnotheres socius* Lanchester (1901)**

Distribution: Pulau Bidan, Penang, Malayasia.

Host: A Bivalve.

References: Lanchester (1901), and Tesch (1918).

***Pinnotheres spinidactylus* Gordon (1936)**

Distribution: Siglap, Singapore.

Host: Modiolus philippinarium.

Reference: Gordon (1936).

Remarks: This species is said to be closely related to *P. parvulus* Stimpson.

***Pinnotheres subglobosa* (Baker) (1908)**

Distribution: St. Vincent Gulf, South Australia.

Hosts: Chlamys bifrons, Spondylus tenellus, Modiolaria australis.

References: Baker (1908), Tesch (1918), and Hale (1927).

Remarks: This species was originally described by Baker (1908) as *Ostracotheres* (?) ("*Pinnotheres*") *subglobosus.* According to Tesch (1918) this species, as well as *P. holothuriensis* de-

scribed by Baker from South Australia do not belong to *Pinnotheres* as the dactylus of the external maxillipeds are absent in both. He remarks that "···as the propodus of these maxillipeds is much widened distally, especially in *P. holothuriensis*, there is perhaps a greater affinity to *Cryptophrys* than to *Ostracotheres*, though the distal margin of this propodus is not obliquely-truncate, but rounded, as in the latter genus." Hale (1927) considers this a species of *Pinnotheres*.

***Pinnotheres taylori* Rathbun**

Distribution: Puget Sound, Washington State, N.-W. Coast of U.S.A.

Host: From excurrent region of atrial cavity of tunicates.

***Pinnotheres tenuipes* Burger (1895)**

Distribution: Ubay, Philippines.

Hosts: Holothurians.

References: Burger (1895), and Tesch (1918).

Remarks: *P. tenuipes* Borradaile (1903) (*nec* Burger) was given a new name, *P. borradailei* by Nobili (1905).

***Pinnotheres tivelae* Gordon (1936)**

Distribution: Muscat, Arabia.

Host: *Tivela ponderosa.*

Reference: Gordon (1936).

***Pinnotheres trapeziformis* (Nauck) (1888)**

Distribution: ?; Mazatalan, West Coast of Mexico.

Hosts: *Holothuria maxima, H. inornata.*

References: Nauck (1888), Burger (1895), and Tesch (1918). Also see deMan (1887, p. 721).

Remarks: Originally described as *Holothuriophilus trapeziformis* Nauck.

***Pinnotheres trichopus* Tesch (1918)**

Distribution: Siboga Expedition Stn. 281, Great Kei Island.

Host: *Meleagrina* sp.

Reference: Tesch (1918).

Remarks: The description is based on a single male associated with young females of *P. villosulus* Guerin. According to Tesch, "I should certainly declare it to be the male of this species were it not for a few differences, which perhaps are not sexual." He also draws attention to the similarities in the shape of the carapace and the resemblance in the minute dactylus of the external maxillipeds, and the walking legs in the two species.

***Pinnotheres tsingtaoensis* Shen (1932)**

Distribution: China Sea.

Host: ?

Reference: Shen (1932).

***Pinnotheres veterum* Bosc.**

Distribution: Coast of Europe and England.

Hosts: *Pinna squamosa*; also from branchial chambers of *Ascidia mentula*.

References: Bosc (1801–2), Heller (1865), and Atkins (1926 *a*).

***Pinnotheres vicajii* Chhapgar (1958)**

Distribution: Bombay, India.

Host: *Paphia malabarica*.

References : Chhapgar (1957, 1958).

Remarks: The description of the new species appeared one year later (Chhapgar, 1958) than a brief diagnosis of it published by the same author in 1957.

***Pinnotheres villosissimus* Doflein (1904 *a*)**

Distribution: Padang; also several localities from Andaman Islands.

Hosts: Holothurians *Muelleria lacanora, Actinopyga mauritiana*.

References: Doflein (1904 *a*), Tesch (1918), and Chopra (1931).

***Pinnotheres villosulus* M. Guerin (1830)**

Distribution: Timor; Torres Straits; Zamboanga, Ubay, Philippines; Siboga Expedition Stn. 261, Elat, Great Kei Island.

Hosts: *Meleagrina* sp., *Pinna chemnitzii*, and *Meleagrina margaritifera*.

References: M. Guerin (1830, 1844), Miers (1886), Burger (1895), Tesch (1918), and Rathbun (1924).

Remarks: Tesch (1918) has assigned one juvenile female collected from Siboga Expedition Stn. 261 from *Meleagrina* sp., to this species. In a foot note (p. 251) he refers to the use of the specific name *P. villosulus* as follows: "This species is referred by H. Milne-Edwards and Burger under the name *villosus*. I could not consult Guerin's original description in Voy. "Coquille," t. 2, 1830, p. 13, but in his subsequent work (Iconogr. Regne An., Crust. p. 7, pl. 4, f. 4) the species is named *villosulus*, which term is used by Miers. "Since we have also not referred to the original description of the species, the spelling as given by Tesch (1918) is followed here. Hornell and Southwell (1909) have listed two species "*P. villosus* Guir." and "*P. villasulus* Guerin and Minivelle" which no doubt refer to one and the same species *P. villosulus* M. Guerin.

***Pinnotheres winckworthi* Gordon (1936)**

Distribution: Penang, Malaysia.

Host: *Paphia gallus*.

Reference: Gordon (1936).

***Pinnotheres* sp. Doflein (1904 *a*)**

Distribution: Algoa Bay, Union of South Africa.

Host: ?

Reference: Doflein (1904 *a*).

***Pinnotheres* sp. Szüts (1921)**

Distribution: Adriatic.

Host: Ascidian.

Reference: Szüts (1921).

***Pinnotheres* sp. Awati and Rai (1931)**

Distribution: Bombay, India.

Host: *Ostrea cucullata.*

Reference: Awati and Rai (1931).

***Pinnotheres* sp. Poisson (1946)**

Distribution: Madagascar.

Host: *Ostrea vitrefacta.*

Reference: Poisson (1946).

***Pinnotheres* sp. Jones (1950)**

Distribution: Trivandrum to Cape Comorin (S.-W. Coast of India) and beyond as far as Tinnevelly Coast, Gulf of Mannar.

Host: Brown mussel *Mytilus* sp.

Reference: Jones (1950).

***Pinnotheres* sp. Barnard (1950)**

Distribution: Mossel Bay and Delagoa Bay.

Hosts: Pearl Oyster *Avicula*; *Modiola* sp.

Reference: Barnard (1950).

Remarks: This is based on Stebbing's 1920 specimens described under the names *P. pisum* (?) Stebbing 1920 (*nec* Linnaeus); and *P. ostrearius* in part (Stebbing, 1920—"the Delagoa Bay specimen"). According to Barnard, the elongate dactyli of the 4th and 5th legs distinguish this from the European *P. pisum.*

***Pinnotheres* sp. *A.* Monod (1956)**

Distribution: West Africa.

Host: ?

Reference: Monod (1956).

***Pinnotheres* sp. *B.* Monod (1956)**

Distribution: West Africa.

Host: ?

Reference: Monod (1956).

***Pinnotheres* sp. *C.* Monod (1956)**

Distribution: West Africa.

Host: ?

Reference: Monod (1956).

***Pinnotheres* sp. D. Monod (1956)**

Distribution: Pointe-Noire, French Congo, West Africa (sub-littoral).

Hosts: ?

References: Monod (1956), and Rossignol (1962).

Remarks: Rossignol (1962) uses *Pinnoteres* sp. for this species.

***Pinnotheres* sp. Dell (1960)**

Distribution: Petre Bay, Chatham Islands (43° 57′ S: 176° 47′ W).

Host: Nemocardium pulchellum.

Reference: Dell (1960).

***Pinnotheres* sp. Silas and Alagarswami (1965)**

Distribution: Malpe, North of Mangalore, West Coast of India.

Host: Meretrix casta.

Reference: Description given in the present account.

IV. ANNOTATED BIBLIOGRAPHY ON THE GENUS *PINNOTHERES* LATREILLE

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———— 1906 *a*. Faune Carcinologique de la Mer Rouge. Decapodes et Stomatopodes. *Ann. Sci. Nat. Zool.* **4**(9): 1–347, text-figs. 1–12, pls. 1–11 [*Pinnotheres borradailei* n. sp., p. 306—substitute name for *P. tenuipes* Borradaile (*nec* Burger), *P. coutieri* Nobili, p. 305, fig. 10; *P. lutescens* Nobili, p. 304; *P. maindroni*, p. 306, pl. 8, fig. 8, text-fig. 11; *P. pectenicolus* Burger, p. 303; *P. pernicolus* Burger, p. 303; *P. purpureus* Alcock, p. 303; *L. pilumnoides* Nobili, p. 307, fig. 12; all from the Red Sea].

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PLESSIS, Y. 1954. Compartement de *Pinnotheres pisum* Penn. en aquarium. *Bull. Mus. Hist. Nat. Paris*, (2), **25** (6): 1954, 562 (Behaviour of *Pinnotheres pisum* in the presence of anemones).

POISSON, H. 1946. Huitres et Ostreiculture a Madagascar. Etude zoologique et economique. *Trav. Sect. Oceanogr. Appl. Soc. Amis. Parc. Bot. Zool., Tananariva*, **3**: 1–37 (*Pinnotheres* sp. from *Ostrea vitrefacta*—affecting flavour of oyster meat).

POULSON, E. M. 1949. On the distribution of the Brachyura (Crustacea Decapoda) in the Danish Waters. *Vidensk. Medd. fra Dansk naturh. Foren.*, Bd. **111**: 111–130 (Records *Pinnotheres pisum* L. from 7 localities from the hosts *Sipsula subtruncata* and *Modiola modiolaria*).

POWELL, A. W. B. 1947. *Native Animals of New Zealand*, Auckland, 1947, 96 pp. figs. (Crust. pp. 35–42, *P. novaezelandiae* included).

PRASAD, R. R. AND P. R. S. TAMPI 1957. Notes on some Decapod larvae. *J. Zool. Soc. India*, **9**: 22–39 (Zoea of *Pinnotheres ridgewayi* Southwell described and figured, some notes on behaviour, study based on eggs hatched from ovigers kept in aquarium).

RATHBUN, R. 1884. Natural History of the economic crustaceans of the United States. *The Fisheries and Fishery Industries of the United States*, Sec. 1, pt. 5, 763–829. (pp. 765–766—*Pinnotheres ostreum* Say and *P. maculatus* Say, mentioned.)

RATHBUN, M. J. 1910. The Danish Expedition to Siam 1899–1900 V. Brachyura. *Kjohenhavn Vid. Selks. Skr.* 7, *Raekke* 5, No. 4, 303–367, figs. 1–44, pls. 1, 2, Map 1. (*Pinnotheres affinis*, p. 330; *P. burgeri* n. sp., p. 331, fig. 12; *P. cardii* Burger, p. 330, pl. 2, fig. 8; *P. glaberrimus* Burger, p. 330; *P. gracilis* Burger, p. 330; *P. kamensis* n. sp., p. 355, fig. 18; *P. kutensis* n. sp., p. 335, fig. 19; *P. lanensis* p. 332, fig. 4; *P. nigrans*, n. sp., p. 334, figs. 16, 17; *P. parvulus* Stimpson, p. 331, pl. 2, fig. 9; *P. quadratus* n. sp., p. 333, fig. 15 *P. siamensis* sp. nov. ; all species are reported from the Gulf of Siam.)

——— 1911. *Proc. U.S. Nat. Mus.*, **38**: 578–688. (Records *Pinnotheres margarita* Smith, S. I.; *Pinnotheres silvestrii* Nobili; and *P. bipunctatum* Nicolet.)

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——— 1923. *Bull. Amer. Mus. Nat. Hist.*, **48**: (*Pinnotheres jamesi* n. sp., p. 625, pl. 29, text-fig. 1, 2; *P. pichilinquei*, n. sp., p. 627, pl. 30, text-figs. 3–4; both species from Lower California).

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——— 1962. Catalogue des Crustaces Decapodes Brachyoures, Anomoures et Macroures littoraux en collection au centre d' Oceanographique de Pointe-Noire. *Travaux de centre Oceanogr. Pointe-Noire* (*O.R.S.T.O.M.*) No. 2 (5): 111–138. (*Pinnotheres rouxi* Rossignol, 1957 and *Pinnotheres* sp., from Pointe-Noire (French Congo) recorded).

RYDER, J. A. 1881. *Rept. Maryland Fish. Comm. for* 1880., p. 24 (Commensalism, food relationship between Oyster and pea-crab).

RYDER, J.A. 1882. The Protozoa and Protophytes considered as the primary or indirect source of the food of fishes. *Bull. U.S. Fish Comm.*, **1** (1881): 236–251. (An instance of multiple parasitism: remarks on finding 'something like *Poteriodendron* on *Zoothamnium* which covered a *Pinnotheres* inhabiting an oyster....')

SAKAI, T. 1934. Brachyura from the coast of Kyushu, Japan. *Sc. Rep. Tokyo Bunrika Daigaku, Secct.*, B, **1**: No. 25 (p. 316—*Pinnotheres pholadis* de Haan.)

———— 1935. *Crabs of Japan* (69 plates in life colours with descriptions). Sanseido Co., Ltd., Tokyo, 1–239 and 27 (*Pinnotheridae*, pp. 194–199—Descriptions, figures and distribution of: *Pinnotheres parvulus* Stimpson, fig. 101, *a, b*; *P. alcocki* Rathbun, pl. 56, fig. 4; *P. boninensis* Stimpson, fig. 102, pl. 56, fig. 1; *P. pholadis* de Haan, pl. 56, fig. 2; and *P. cardii* Burger, pl. 56, fig. 3.)

———— 1939. *Studies on the Crabs of Japan* IV. *Brachygnatha, Brachyrhyncha.* Ookendo Ltd., Tokyo, pp. 365–471, text-figs. 1–129, pls. 1–70. (Descriptions, key to Japanese species, coloured figures and photos, two new species and one new subspecies described: *Pinnotheres sinensis atrinae* subsp. nov. p. 585, *P. corbiculae*, n. sp., 591; *P. bidentatus*, n. sp., pp. 592.)

———— 1962. New species of Japanese crabs from the collection of His Majesty the Emperor of Japan. *Crustaceana*, 3: 131–150 (pp. 145–146, figs. 3 *a, b*: description of *Pinnotheres lacquei* n. sp. from brachiopod *Laqueus rubellus*, based on 10 females with carapace length less than 4 mm., from 65–85 meters deep at Amadaiba (rocky bottoms), off coast of Hayama, Japan.)

SANDOZ, M. AND S. H. HOPKINS 1947. Early life-history of the oyster crab, *Pinnotheres ostreum* (Say). *Biol. Bull.*, **93**: 250–258. (Description of first zoea, second zoea, third zoea, fourth zoea, magalopa and first crab stage of *P. ostreum* figured.)

SASTRY, A. N. AND R. W. MENZEL 1962. Influence of host on the behaviour of commensal crab *Pinnotheres maculatus* Say. *Biol. Bull.*, **123**: 388–395 (Experimental work indicates non-host specificity of *P. maculatus* occurring in *Aequipecten irradians concentricus* and *Atrina rigida*.)

SAY, THOS. 1817. An account of the Crustacea of the United States. *J. Acad. Nat. Sci. Philad.*, **1** (1): 65–80. (p. 67 —*Pinnotheres ostreum* n. sp.).

———— 1918. *Id., Ibid.*, **1** (2): Appendix pp. 445–458 (p. 450—*Pinnotheres maculatum* n. sp.).

SCHELLENBERG, A. 8928. Krebstiere order Crustacea. 2. Decapoda Zehnfusser. In: *Friedrich Dhal, Die Tierwelt Deutschlands. Jena*: Fischer. 1–146. (*Pinnotheres pisum* probably deprives mussel of food and poisons it by CO_2 and waste products.)

SCHWABE, G. H. 1936. Investigactiones sobore *Loxechinus albus* Mol. y. *Pinnotheres chilensis* Edw. *Bot. Soc. Biol. Concepcion*, **10**: 125–136 (Commensalism: *Pinnotheres* in *Loxechinus*).

———— 1941. Uber Mariscos und Mariscofischerei (Mariscos and Mariscos fishing). *Zeitschr. Fisch. v. Hilfsw.*, **39** (3): 313–347. [*Pinnotheres chilensis* found in a great numbers of *Loxechinus albus* (sea urchin) in the intestine, only one female per host and male active seeking sea urchins with symbiotic females].

SCOTT, M. 1961. A review of the New Zealand Pinnotheridae. *Trans. Roy. Soc. New Zealand Zool.*, **1** (22): 303–309. (Considers all previous records of *Pinnotheres pisum* from New Zealand to be erroneous as the family is represented in New Zealand by a single polymorphic species *Pinnotheres novae-zealandiae*, the latter species and the typical *P. pisum* are briefly compared.)

SEMITU, K. 1944. Sacculinization, with a note on the mechanism of sexual differentiation in the host. *Annot. Zool. Japonenses*, **22**: 173–184 (Secondary male characters of *Pinnotheres cyclinus* and *P. sinensis* turn female-like by sacculinization, while the secondary sexual characters and ovary of female are scarcely affected by sacculinization; since even if the testes is degenerated entirely by sacculinization the change of the secondary male characters always results due to some other phenomena which cannot be explained endocrinologically; this leads him to suggest that there is the function of a certain essential mechanism besides the function of the sexual harmone).

SEMPER, C. 1880. Die natürlichen Existenzbedingungen der Thiere, Leipzig, **1**: 98 and 99 (*Pinnotheres holothuriae* sp nov.).

SEMPER, K. G. 1881. *Animal Life as Affected by Natural Conditions of Existence.* Appleton, N.Y., 1–472 (suggested that pea-crab invades host in the zoeal stage which is now proven to be errnoneous).

SHEN, C. J. 1932. The Brachyuran crustacea of North China. *Zoologica Sinica, Peiping*, Ser. A9, Fasc. 1: 1–320. 10 pls., map and 171 figs. (Seven new species of *Pinnotheres* described: *P. sinensis* n. sp., p. 131; *P. cyclinus* n. sp., p. 135; *P. dilatatus* n. sp., p. 140; *P. serrignathus* n. sp., p. 142; *P. haiyangensis* n. sp., p. 145; *P. tsingtaoensis* n. sp., p. 149; *P. gordoni* n. sp., p. 152).

———— 1935. On some new and rare crabs of the families Pinnotheridae, Grapsidae and Ocypodidae. *Chin. Jour. Zool.*, **1**: 19–40.

SHERBORN, C. D. 1933. *Index Animalium.* Part 32. *Index to Trivialia under Genera*, pp. 655–873 (1801–50), London, (p. 843—*Pinnotheres* sensu lato): 31 species names mentioned some of which are at present considered species of allied genera, such as *Pinnixa, Ostracotheres*, etc.)

SMITH, G. AND W. F. R. WELDON 1958. Crustacea in *The Cambridge Natural History*. **4**. Ed. by S. F. Harmer and A. E. Shipley, 4, 1–217 (pp. 195–196: *Pinnotheres pisum* from the mantle cavity of *Cardium norwegicum* off the English coast).

SMITH, H. M. 1891. The giant scallop fishery of Maine. *Bull. U.S. Fish. Comm.*, **9**: (1889): 313–35. (p. 318—*Pinnotheres maculatus* in *Pecten magellanicus*).

SMITH, S. I. 1870 (*Pinnotheres margarita* n. sp.).

———— 1870. Notes on American Crustacea. *Trans. Conn. Acad.*, **2**: 113–374. (*Pinnotheres lithodomi* n. sp., p. 169, from Pearl Island; and *P. margarita* Smith, redescribed, p. 166.)

———— 1874. In Verril, A. E. and S. I. Smith: Report on the Invertebrate animals of Vineyard Sound and the adjacent waters. *Rept. U.S. Fish. Comm., for* 1871–72, 295–778, pls. 1–38 [p. 367—*Pinnotheres ostreum*; Christensen and McDermott (1958) remark that pl. 1, fig. 2 referred to as *P. ostreum* is in fact *P. maculatus*].

———— 1879. The stalk-eyed crustaceans of the Atlantic coast of North America, north of Cape Cod. *Trans. Conn. Acad. Arts and Sci.*, **5**: 27–136 [*Pinnotheres maculatus* (Say): note on its larva, p. 265].

SOUTHWELL, T. 1910. Description of a new species of *Pinnotheres* and of the female of *P. margaritiferae* Laurie. *Ceylon Mar. Biol. Rept.*, **19**: Part 5, 226–227. (Descriptions and figures of *Pinnotheres ridgewayi* n.sp., and redescription of *P. margaritiferae* Laurie from Ceylon pearl banks, Gulf of Mannar.)

STRAUBER, L. A. 1942. The oyster crab, *Pinnotheres ostreum*, parasitic on the oyster. *Anat. Rec.*, **84** (Suppl.): 45–46 (Abstract).

———— 1945. *Pinnotheres ostreum*, parasite on the American oyster, *Ostrea* (*Gryphaea*) *virginica*. *Biol. Bull.*, **88**: 269–291. [Life-history review; morphology of various stages of male and female crabs; figures; descriptions of first, second, third, fourth and fifth stage (adult) females and the second stage males; abundance of crabs in oysters; mode of feeding of crab; injury to oyster gills and gill regeneration; other effects of pea-crab on host oyster; invasion of oysters by pea-crab].

STEBBING, T. R. R. 1910. General catalogue of South African Crustacea (Part V of S. A. Crustacea, for the Marine Investigations of South Africa). *Ann. S. Afr. Mus.*, **6**: 281–599 (Genus *Pinnotheres* Bosc.; synonyms; *Pinnotheres* sp. Doflein recorded).

———— 1920. South African Crustacea (Part X of South African Crustacea for the Marine Investigations of South Africa). *Ibid.*, **17**: 231–272 [Records *Pinnotheres pisum* (?) (Linn.); and *P. ostrearius* Rathbun].

STIMPSON, W. 1858. Prodromus descriptionis animalium.... Johanne Rodgers Ducibus, observavit et descripsit. Crustacea. 1857 (1858), Pars V. Crustacea Ocypodoidea. *Proc. Acad. Nat. Sci., Philad*, 93–110. (Descriptions of three new species; *Pinnotheres boninensis* n. sp., p. 108 from Bonin Islands in "small oyster"; *P. obscurus* n. sp., p. 108 from Hongkong; *P. parvulus* n. sp., p. 108 from China Sea.)

———— 1859. Notes on North American Crustacea. No. 1. *Ann. Lyceum Nat. Hist. N.Y.*, **7**: 49–93, pl. 1. (p. 67—*Pinnotheres ostreum* and *P. maculatus* recorded).

———— 1907. *Smithson. Inst. Misc. Coll.*, **49**: 142 (Lists *Pinnotheres boninensis, P. obscurus* and *P. parvulus*).

SUGIURA, Y., A. SUGITA AND M. KIHARA 1960. The ecology of Pinnotherid crabs as pests in culture of *Tapes japonicas*. I. *Pinnotheres sinensis* living in *Tapes japonica* and the influence of the crab on the weight of the host's flesh (in Japanese with English summary). *Bull. Japanese Soc. Sci. Fish.*, **26** (2): 89–94 (*Pinnotheres sinensis* parasitic on *Tapes japonicus*; female ovigers found from July to October; size of berried crab varied with period in season; female pea-crab also capable of living free from host for 4–5 months as rearing in aquariun showed).

SUGIURA, Y., M. KIHARA AND A. SUGITA 1960. The ecology of Pinnotherid crabs pest in culture of *Tapes japonicus* —II. *Pinnotheres gordoni* living in *Tapes japonica* and the influence of the crab on the weight of the hosts flesh. *Ibid.*, **26** (2): 565–569 (*Pinnotheres gordoni* parasitic on *Tapes japonicus*).

SUMNER, F. B., R. C. OSBURN AND L. J. COLE 1913. A biological survey of the waters of Woods Hole and vicinity. Section I. Physical and Zoological. 8. Decapoda. *Bull. Bur. Fisheries*, **31** (1911): 137–42, chart 119, p. 337 (*Pinnotheres maculatus* commensal in *Modiolus modiolus, Mytilus edulis* and *Pecten gibbus*; the distribution of the pea-crab is said to be dependent on that of the hosts.)

———— 1913 *b*. A biological survey of the waters of Woods Hole and vicinity. Section III. A catalogue of the Marine fauna. *Ibid.*, **31** (1911): 663–675. (674—*Pinnotheres ostreum* and *P. maculatus*).

SZÜTS, A. 1921. Az Adria tizlabu rakjai (The crustacean Decapoda of the Adriatic). *Allatt. Kozlem. Budapest*, **18**: (1921): 13–22 (p. 47—*Pinnotheres* in ascidian).

TESCH, J. J. 1918. The Decapoda Brachyura of the Siboga Expedition II. Goneplacidae and Pinnotheridae. *Siboga Expedition*, **39** *C*1: 295 pp., 18 pls. (Two new species described and figured: *P. trichopus* n. sp., pp. 256–257, pl. 17, fig. 6; and *P. onychodactylus* n. sp., p. 259, pl. 17, fig. 5; Notes and figures of six other species—*P. villosulus*; *P. obesus*; *P. edwardsi*; *P. latus*; *P. consors*; and *P. quadratus*; Key to Indo-Pacific species of *Pinnotheres*; checklist of known species from Indo-Pacific.)

THOMPSON, J. V. 1835. Memoirs on the metamorphosis and natural history of the *Pinnotheres* or pea-crabs. *Entomol. Mag.*, 3: 85–90. (First zoea of *Pinnotheres pisum* illustrated).

THORPE, W. H. 1958. Ethology as a new branch of biology. In *Perspectives in Marine Biology*. Edited by A. A. Buzzati-Traverso. Part III, pp. 411–428, Univ. Calif. Press.

THORPE, W. H. AND F. G. W. JONES 1937. Olfactory conditioning in a parasitic insect and its relation to the problem of host selection. *Proc. Roy. Soc. London*, Ser. B, 124: 56–81.

TU, T. J. 1938. Ueber einige in Polychaetenrohren und Muscheln gefundene Dekapoden an cler kuste von Schantung. *Zool. Anat. Leipzig*, 122: 177–186. (*Pinnotheres affinis* and other species of the genus commensal with *Pecten, Ostrea*, etc., on the North China Coast.)

URITA, T. 1926. A checklist of Brachyura found in Kagosima Prefecture, Japan (p. 18—*Pinnotheres pholadis* listed).

VERRILL, A. E. 1869. On the parasitic habits of Crustacea. *Amer. Nat.*, 3: 239–250 (*P. ostreum* as a parasite for shelter).

VERRILL, A. E. AND S. I. SMITH 1874 (*see* Smith, S. I., 1874) (description of female; colour; distribution; host tunicate *Tethyum aurantium*).

WAY, E. Brachyura and Crab-like anomura of Fridry Harbour, Washington. *Pub. P. S. Biol. Sta.*, 1: 349–352 (pp. 360–361: *P. pugettensis*, and *P. concharum*).

WELLS, W. W. 1928. Pinnotheridae of the Puget Sound. *Publ. P. S. Biol. Sta.*, 6: 283–314 [pp. 285–86: Genus *Pinnotheres* Latreille described; *P. pugetteensis* Holmes, synonyms, figures (figs. 1–3].

———— 1940. Ecological studies on the Pinnotherid crabs of Puget Sound. *Bull. Univ. Washington Publ. Oceanogr.*, 2: 19–50. (*Pinnotheres pugettensis, P. taylori* and *P. pinnotheres* from excurrent region of atrial cavity of tunicates and species of allied genus *Fabia subquadrata* in *Modiolus modiolus*.)

WELSH, J. H. 1932. Temperature and light as factors influencing the rate of swimming of larvae of the mussel crab, *Pinnotheres maculatus* Say. *Biol. Bull.*, 63: 310–326. (Temperature and light were found to be important factors determining the rate of locomotion of larvae of *P. maculatus*. The velocity of progression was related to the light intensity according to the expression $\log V = K \log I - C$, this treatment yielding rectilinear graphs which facilitated comparing effects of temperature and intensity of illumination.)

WEYMOUTH, 1910. True crabs of Monterey Bay. *Leland Stanford Jr. Univ. Publ.*, No. 4, 53–54. (Family Pinnotheredae and Key to six genera of the family found in Monterey Bay area; pp. 53–54—*Pinnotheres nudus* Holmes records one female '.. probably from Monterey Bay .....'; specimen figured).

WHITE, K. M. 1937. *L.M.B.C. Memoirs*, 31, *Mytilus*. The University Press of Liverpool, pp. 1–177.

WILLIAMS, G. AND A. E. NEEDHAM 1938. On relative growth in *Pinnotheres pisum*. *Proc. Zool. Soc. London*, 108 A: 539–556 (Differential growth in *P. pisum*).

WOLVEKAMP, H. P. AND T. H. WATERMAN 1960. Respiration. In *The Physiology of Crustacea*. Edited by T. H. Waterman, Ch. 2: 35–100 (Reduction of gills to 3 pairs in *Pinnotheres* an adaptive feature).

WOODWARD, H. 1886. Commensals and parasites of *Meleagrina margaritifera*. *Proc. Zool. Soc., London*, p. 176 (Records an interesting case in which a male Pinnotherid crab is enclosed in a cyst of pearl).

YOKOYA, Y. 1928. Report on the biological survey of Mutsu Bay, 10, Brachyura and crab-shaped Anomura. In *Sci. Repts. Tohoku Imp. Univ.*, Ser. 4, 3: 773– . [*Pinnotheres pholadis* de Haan, recorded (p. 773)].

———— 1933. On the distribution of Decapod crustaceans inhabiting the continental shelf around Japan, chiefly based on materials collected by S.S. "*Soyomaru*", during the years 1923–30. *J. College Agric. Tokyo Imp. Univ.*, 12 (1): 208 (p. 208—*Pinnotheres pholadis* de Haan).

YOUNG, C. M. 1960. *Oysters*. Collins, St. James Place, London, 1–118.

DISCUSSION

Dr. C. V. Kurian: Can you call the pea-crab a parasite?

Dr. E. G. Silas: The definition of a parasite is still in an unsettled state and in the absence of a term which can fit in here precisely, we have thought it better to call this as a case of parasitisation due to the damages caused to the soft parts of the clam.

Dr. N. Krishna Pillai: Quite a large number of groups of invertebrates harbour crustaceans such as Copepods. It requires a lot of study to say if a particular animal is a parasite or not. With the present study I would suggest that the pea-crab is termed as an 'associate' of the clam and leave it until a fuller study is made.

Dr. N. Balakrishnan Nair: Can we not call this as a case of predation than parasitisation?

Dr. E. G. Silas: The clear damages caused in this particular case and from the instances available in the literature recording even mortality of oysters make us feel that this is a case of parasitisation and we do not want to coin any new term for this association.

Dr. B. Patel: In *Anadora granosa* and *A. rhombia* harbouring pea-crabs we have observed that the amount of haemoglobin present was lower than in the non-infested ones. From our observations we have found that the population of *A. granosa* from the east coast was heavily infested with pea-crabs whereas from the west coast only *A. rhombia* was found infested. Can you explain this?

Dr. E. G. Silas: This is the same as the case where pea-crabs have not been found in the Mandapam region in the clams such as *Meretrix casta* while they have been collected from the Malpe clams. The occurrence, whether seasonal or not, and distribution of the species are not yet clearly understood.

ADDENDUM

For making the references up to date, the following papers dealing with *Pinnotheres* which have appeared latter than 1965 are given below:

COSTLOW, J. D. JR. AND C. G. BOOKHOUT 1966. Larval stages of the crab, *Pinnotheres maculatus*, under laboratory conditions. *Chesapeake Science*, 7 (3): 157–163 (Larvae of *P. maculatus* obtained from crab commensal in scallop *Aequipecten irradianus* reared successfully in laboratory from hatching to crab stage; five zoeal stages (1–5) and one megalopa stage described and figured; comparisons of larvae of *P. maculatus* with known larval stages of *P. pisum* and *P. veterum*).

JONES, S. AND S. MAHADEVAN 1967. Notes on animal associations. 5. The pea-crab *Pinnotheres deccanensis* Chopra inside the respiratory tree of the sea cucumber, *Holothuria scabra* Jager. *J. Mar. biol. Ass. India*, 7 (2): 377–380.

SHANKARANKUTTY, C. 1966. On Decapoda Brachyura from the Gulf of Mannar and Palk Bay. *Proceedings of the Symposium on Crustacea*, Marine Biological Association of India, 1965, Part I: 341–362 (Records *P. deccanensis* Chopra from host *Holothuria scabra* from Gulf of Mannar, and *P. ridgewayi* from host *Pinna* sp. from Palk Bay and Gulf of Mannar).

COMMENSAL CRUSTACEA

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ABSTRACT

A commensal is here defined as a symbiotic organism which derives its energy from sources other than host tissues. The incidence of commensalism among the various crustacean groups is reviewed. Typical commensal associations involve a smaller commensal, semi-permanently associated with a larger host, and can be subdivided into three categories: (1) those commensals that feed independently of the host; (2) those that share the host's food; and (3) those that feed on substances associated with the host, such as mucus shed by the host or debris that settles on it. Various adaptations shown by commensals to their way of life are discussed as are the relationships between commensal and host and between commensals of the same and different species.

INTRODUCTION

THE term symbiosis was originated by de Bary (1879) and was used by him to refer to the living together of unlike organisms (Hertig *et al.*, 1937). It has often been used since then in a restricted sense implying mutual benefit, but the original broad meaning is also widely used and will be employed here. In a symbiotic association there is typically a larger organism called the host and a smaller one called the symbiont which always benefits from the relationship. Typically, also, the association involves some degree of permanence. Especially in insects there are certain associations which are on the border-line between symbiosis and predation. Perhaps these could be decided by the duration of the association and thus parasitic Hymenoptera could be considered as larval symbionts, while mosquitos would be regarded as micro-predators, since they are only briefly associated with an individual host.

Symbiotic associations are generally divided into three categories; parasitism, commensalism and mutualism, but, while mutualism has an accepted meaning as an association involving mutual benefit, the first two terms have been subject to a variety of definitions and interpretations with each author defining the terms as seems reasonable to him. There have been many recent discussions of the situation (Lincicombe, 1963; Noble and Noble, 1964; Rogers, 1962; Smythe, 1962; Sprent, 1963). The often used criterion of harmfulness to the host is now generally regarded as a poor basis for definitions, since with a given species the extent of harm done to the host may vary from none to considerable, depending on relative factors such as the number of symbionts present and the general health of the host.

I shall define *parasitism* as an association in which the symbiont derives its source of energy from host tissues either by feeding on them or by absorbing from them. The term has been widely used in this sense by many authors. *Commensalism*, then, would include all other associations in which the symbiont does not obtain its energy source from living host tissues. *Mutualism* would include any symbiotic association, be it commensal or parasitic, in which the host is benefited. Although the typical mutualistic organisms such as those in the intestine of the termite and in the digestive tract of herbivores are commensals, Lincicombe (1963) gives several examples of parasites which may be of benefit to their host.

Recognizing that all definitions have a certain arbitrary element, the ones given above do have the advantages of (1) having an exact meaning, and (2) dealing with factors which, potentially at least, can be determined for every association. They have the further lesser advantage of not requiring a large-scale shifting of generally known organisms into new categories. In some cases

a given organism would be partially parasitic and partially commensal, but in any case the use of the terms would say something precise about what is felt to be the relationship between the symbiont and its host.

The Crustacea are one of the groups most frequently involved in commensalism and a large number of associations have been reported. Balss (1956) gives an excellent review of commensal decapods and Arndt (1933) deals with the Crustacea associated with Porifera. The following recent books and review articles deal in part with commensal Crustacea: Caullery (1952), Dales (1957), Davenport (1956), Green (1961), Hyman (1940, 1955), MacGinitie and MacGinitie (1949), and Nicol (1960).

The purpose of the present paper is to draw attention to some of the recent work on commensal Crustacea and to discuss briefly various aspects of their associations and some of the interesting problems which they present. An attempt has been made to include material from all the curstacean groups, although due to a greater familiarity with the decapods, this group has doubtless been favored.

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The Commensals

The following brief list is obviously not at all comprehensive, but is intended to supply a few examples and references and give some idea of the occurrence of commensalism in the various crustacean groups. The classification scheme used is that of Waterman and Chace (1960).

Class Crustacea.

Subclass Cephalocarida—none.

Subclass Branchiopoda—none.

Subclass Ostracoda—Although this is largely a free-living order, a number of species of the family Entocytheridae occur on fresh-water crayfish, and scattered other associations have been reported, such as from a sponge (Pearse, 1934) and from the gills of *Limnoria* (de Vos and Stock, 1956).

Subclass Mystacocarida—none.

Subclass Copepoda—There are a large number of copepods associated with marine invertebrates, and there are many recent papers identifying and describing these animals. Bocquet and Stock (1963) will serve as an entrance into the sizeable literature of this field. As has been pointed out by Gooding (1957) and others, the biology of many of these animals is not well enough known to be able to say whether they are commensal or parasitic. There are, however, several very interesting accounts of the biology of individual commensal species (Gotto, 1957; Humes and Cressey, 1960).

Order Calanoida—entirely free living.

Order Cyclopoida—Representatives of this order have been found associated with a wide variety of marine Coelenterata, Annelida, Mollusca, Crustacea, Echinodermata and protochordates and possibly may be found with every group that is investigated thoroughly. The majority of those found with ascidians were once placed in the Order Notodelphyoidea, but authorities now doubt the validity of this category. Gotto (1960) has listed the ascidicolous copepods of

Western Europe and Illg (1958) has monographed the North American members of the family Notodelphyidae.

Order Harpacticoida.—There are a smaller number of associates in this group than in the preceding one and I think that a greater proportion of them may be true commensals.

The remaining three orders of copepods are exclusively parasitic.

Subclass Branchiura.—All parasitic.

Subclass Cirripedia.—Many species have been found on other animals, for example, *see* Pilsbry (1907, 1916). A number of these, however, may simply be examples of the barnacle settling on whatever substrate was available. A few of the cases which evidently involve at least some specificity are listed below.

Order Thoracica.

Suborder Lepadomopha—*Octolasmis* and related species have been found on various Crustacea (Newman, 1961 *a*, 1961 *b*). *Conchoderma auritum* is found on the humpback whale and *Alepas* is attached to medusae. *Anelasma squalicola* has roots penetrating into its shark host and is thought to be mainly parasitic, although food has been reported in the gut (Hickling, 1963).

Suborder Verrucomorpha—Several possible commensals, since many have been found on living substrates.

Suborder Balanomorpha—The species of *Balanus* (*Conopea*) are obligate commensals of gorgonians and *Millepora*. *Pyrgoma* is found embedded in corals and *Acastia* is sponges and corals. *Chelonibia* occurs on turtles, crabs and Sirenia. *Coronula* is restricted to Cetacea and other genera occur on various marine vertebrates.

Order Acrothoracica—This group of boring barnacles burrows into corals and mollusc shells and even into barnacle plates (Utinomi, 1957).

The remaining orders of Cirripedia are composed entirely of parasites.

Subclass Malacostraca.

Orders Nebaliacea, Anaspidacea, Thermosbaenacea and Spelaeogriphacea are all free living.

Order Mysidacea—Mysids are mostly actively swimming animals but forms are known to be associated with sea anemones, hermit crabs, an ophiuroid and a freshwater sponge (Tattersall, 1962; Arndt, 1933).

Orders Cumacea and Tanaidacea are free living.

Order Isopoda—Although there are many parasitic isopods, this large group contains relatively few commensals. Some examples are *Aega spongiophila* in sponges (Pearse, 1934), *Phyllodurus abdominalis*, which lies across the abdomen of the mud shrimp *Upogebia pugettensis* (MacGinitie and MacGinitie, 1949) and *Colidotea rostrata* among the spines of sea urchins (MacGinitie and MacGinitie, 1949). Brooks (1942) and Mathes and Strouhal (1954) discuss the interesting mutualistic association between the land isopod *Platyarthus hoffmannseggii* and colonies of ants.

Order Amphipoda.

Suborder Gammaridea—The moderate number of associations reported seem to be scattered among various families. However, Anrdt (1933) reports that the Lysianassidae and to a lesser degree also the Leucothoidae are especially well represented in sponges and the Stenothoidae seem to be chief among the commensals of coelenterates. Ruffo (1957) lists the

amphipods found on echinoderms at Banyuls. A most interesting association is that of *Polycheria osborni* which makes a cavity for itself in a colonial tunicate but does not feed on host tissues (Skogsberg and Vansell, 1928).

Suborder Hyperiidea—This group of pelagic amphipods contains several symbiotic members. Dahl (1959) has shown that the well-known association between *Hyperia galba* and the medusa *Cyanea capillata* is a parasitic one, by finding the nematocysts of *Cyanea* in the gut of the amphipod. Hyperiids are also found in salps, but the zooid containing the amphipod is usually dead, implying a parasitic association (Green, 1961). Laval (1963) has observed the larva of *Vibilia* eating the tissues of its salp host.

Suborder Caprellidea—Members of the family Cyamidae are found on the skin of whales. Members of the other family, the Caprellidae, are frequently found on hydroids. MacGinitie and MacGinitie (1949) think that they use the hydroid as a platform from which to wait for prey animals, but the possibility of their browsing on the polyps has not been eliminated.

Order Euphausiacea—Entirely free living.

Order Decapoda—This large order contains a host of commensal associations, many of which are discussed by Balss (1956).

Suborder Natantia.

Section Penaeidea—Entirely free living.

Section Caridea—Holthuis (1955) recognizes 22 families and 174 genera in this large group. Most of the commensals are found in the three largest families. In the family Palaemonidae, Holthuis (1952) lists 84 species of the subfamily Pontoniinae which have been found with various marine invertebrates. In the family Hippolytidae, species are found on crinoids (Potts, 1915*a*; Nouvel, 1953), on a gorgonian (Voss, 1956) and on corals and sea anemones (Kemp, 1916; Patton, 1966). Several species of the Alpheidae are commensal with bottom invertebrates. Species of *Synalpheus* are common in sponges and occur on crinoids (Pearse, 1934; Potts, 1915), while *Alpheus ventrosus* is a well-known and widespread obligate commensal of branching corals. The small family of the Gnathophyllidae contains *Gnathophylloides minerii*, commensal of sea urchins (Lewis, 1956).

Section Stenopodidea—This small group contains several commensals of sponges, the most famous of which is *Spongicola venusta* which lives in pairs in the glass sponge, *Euplectella* (Arndt, 1933).

Suborder Reptantia.

Section Macrura—These large crustaceans are all free living.

Section Anomura.

Superfamily Thalassinidea—These burrowing crustaceans include several commensals of sponges (Arndt, 1933), and are themselves hosts to many commensals.

Superfamily Galatheidea—Of the two main families in this group, the Galatheidae contains several commensals, the most spectacular of which are those found on crinoids (Potts, 1915*a*) The Porcellanidae on the other hand contains many species commensal with various invertebrates (Haig, 1960; Johnson, 1962).

Superfamily Paguridea—All free living. Several hermit crabs of the family Paguridae carry sea anemones on their shells (Hedgpeth, in Dales, 1957) and this association is usually by no means a casual one. The colonial sea anemone *Epizoanthus* and colonial hydroids *Hydractinia* and *Podocoryne* are also often found on hermit crab shells.

Superfamily Hippidea—All free living.

Section Brachyura.

Subsection Gymnopleaura—All free living.

Subsection Dromiacea—Almost all free living. Hornell (1922) found a *Dromia* living in slits in colonies of the alcyonarian *Spongodes*. Members of the family Dromiidae, the sponge crabs, typically carry a sponge or some other material on their backs.

Subsection Oxystomata—All free living. Certain species of *Dorippe* which carry fragments of shell on their backs, prefer pieces of shell containing sea anemones (Uchida, 1960).

Subsection Brachygnatha.

Superfamily Brachyrhyncha—This group includes the majority of the crabs, most of which are free living. There are however, some very interesting examples of commensalism. The swimming crabs, family Portunidae, are mostly active free living animals, but there is one subfamily, the Caphyrinae, composed of commensals (Stephenson and Campbell, 1960). The Xanthidae are common littoral crabs and contain several commensals including the spectacular small crab *Lybia tessalata* which carries a sea anemone in each cheliped. One subfamily, the Tetraliinae or Trapeziinae, consists entirely of specialized commensals of coelenterates. All members of the family Pinnotheridae are commensal, chiefly with pelecypods, polychaetes and echinoderms. Most live inside the host, or its tube or burrow, but species of *Dissodactylus* are external commensals of sand dollars. The family Hapalocarcinidae contains the gall crabs of madrepore corals. The female of *Hapalocarcinus* lives in branching corals of the family Pocilloporidae and causes the coral to form a gall around it, while the other genera live on more solid corals. This amazing group has recently been monographed by Fize and Serene (1957).

Superfamily Oxyrhyncha—This group contains the spider crabs and their allies. The Parthenopidae contains the subfamily Eumedoninae, all members of which are commensals of echinoderms (Johnson, 1962). The true spider crabs, the Majidae, contain a few commensals of coelenterates and many species which place various parts of their environment on their backs.

Order Stomatopoda.—All predatory and almost all free living. An *Acanthosquilla* lives in tubes of a polychaete and a hemichordate (Coutiere, 1905).

Types of Commensalism

(A) The great majority of cases of crustacean commensalism consist of a smaller commensal in more or less permanent association with a larger host. These are the typical commensals and will be the main subject of the rest of this paper. They can be divided into three groups based on their feeding habits. These are discussed below and a few examples of each are given.

1. *Filter feeding commensals*

(*a*) *Those independent of host water currents.*—With this group, the host provides only a substrate and the feeding of the commensal bears little relation to the activities of the host. The barnacles, of course, provide the most examples of this type of association with many being found on living substrates. The coral gall crab *Hapalocarcinus* (Potts, 1915 *b*) belongs here, as does the amphipod, *Polycheria osborni* which makes a cavity for itself in the colonial tunicate *Amaroucium* (Enequist, 1949). Porcelain crabs of the genus *Porcellanella* are all commensals on sea fans (Johnson, 1962) and presumably these also filter their food from the passing water. Although anthozoan coelenterates are particularly common hosts, almost any organism that is openly exposed to free flowing water could serve as a host for this type of association.

(*b*) *Those dependent on a water current created by host.*—A number of filter feeders live in the cavities of various animals or in tubes produced by them, habitats which provide considerable

safety from predation. It is apparent that the only hosts available for this type of commensalism are those which build tubes or possess cavities and have some mechanism for sending a water current through these spaces. Filter feeding porcelain crabs are found in polychaete tubes, and in the siphons of the clam *Aspergillum* (Pearse, 1913; Johnson, 1962). Among the pinnotherid crabs, *Pinnixa chaetopterana* living in *Chaetopterus* tubes and *Pinnaxodes floridanus* living in the respiratory trees of holothurians are filter feeders as are several other species (Pearse, 1913; Wells and Wells, 1961). Stalked barnacles of *Octolasmis* and related genera typically live attached to the gill filaments of decapods and filter their food from the respiratory current of the host. Several species of these barnacles, however, live attached to the mouthparts of the host, and have cirri which appear to be poorly adapted for filter feeding. These barnacles evidently share at least some of the food eaten by the host and thus have evolved into the next grouping (Newman, 1961 *a*).

2. *Commensals which share the food of the host*

Favorite hosts for this group of commensals are the so-called ciliary-mucoid feeders, those animals that create a feeding current by means of cilia and trap small particles from this current with mucus. The chains of food-containing mucus so produced are utilized by a number of commensal crustacea. The pea crabs *Pinnotheres pisum* and *P. ostreum* have been shown to feed on the mucus chains produced by their pelecypod hosts (Orton, 1920; Christensen and McDermott, 1958). The copepod *Ascidicola rosea* feeds entirely on the food chains of its host ascidian (Gotto, 1957), and many ascidicolous copepods doubtless do likewise. Crinoids of the family Comatulidae have a heavy burden of commensals including many Crustacea (Potts, 1915 *a*). One of the reasons for this may be that they are not able to close off their ambulacral grooves and the food in these grooves is therefore available to commensals. Members of other families of crinoids have plates that can be closed down over these grooves (Hyman, 1955). I have seen the shrimp *Neopontonides beaufortensis* remove material from the polyps of its host gorgonian. I assume that this material was host food and expect that other commensals may have similar habits.

3. *Commensals which feed on host associated materials*

(A) Commensals in this category are found on a diversity of hosts and feed on a number of things other than host tissues, food passing by in the water or host food. The caprellid amphipod *Pariambus typicus* feeds on the mucus produced by its host starfish, *Asterias rubens* (Durham, 1888). I believe that many of the decapods present on branching corals feed on silt containing mucus shed by the host (Patton, 1960). The mysid *Heteromysis actinae* does not attempt to feed on fresh food given to the host anemone but feeds actively on the material ejected by the anemone (Clarke, 1955). *Heteromysis harpax* lives in hermit crab shells and presumably feeds on host feces (Tattersall, 1962). The isopod *Platyarthus hoffmanseggii* eats the feces of its ant hosts and also the "honeydew" of their aphids (Mathes and Strouhal, 1954). A most interesting case is that of the harpacticoid copepod *Balaenophilus unisetus* that feeds on small algae which it scraps from the baleen plates of its whale host (Vervoort and Trantor, 1961). The crab *Quadrella nitida* which inhabits the gorgonian *Muricea misca*, evidently eats the polychaetes and ophiuroids which are its fellow commensals (Crane, 1937).

(B) There are several additional types of association which do not fit neatly into the category of a smaller commensal semipermanently associated with a larger host. *Lybia tesselata* carries small anemones in its chelae and brandishes them at any intruder. It evidently also takes a good portion of its food from that collected by the anemones (Duerden, 1905). The shrimp *Alpheus djiboutensis* generally has a fish (Gobiidae) living in its burrow. The shrimp retreats into the burrow when the fish does and thus perhaps profits from the fish's awareness of danger (Luther, 1958). The spider crab *Stenorhynchus* often lives in the shelter of a sea anemone, but leaves it to search for food (Thompson, 1923). This is just a modification of the free living condition and it is interesting that this type of association has not been reported more often. *Dromia personata* generally carries a neatly shaped sponge on its back. The behavior of the crab in cutting out and fitting this sponge has been well described by Dembowska (1926).

It is well known that many hermit crabs carry anemones on their shells (Dales, 1957; Balss, 1956). The association is evidently beneficial to both partners, the anemone obtaining some scraps of the hermit's food and the crab obtaining a certain degree of protection from predators. In one case, a hermit crab has been shown to be remarkably immune to the nematocyst toxin of its own anemone, but for another species this does not hold true (Rey, 1940). In some cases the hermit crab actively places the anemone on its shell and in other cases does not (Ross and Sutton, 1961; Caullery, 1952). The anemone *Adamsia palliata* grows as the crab does and relieves the crab of the necessity of having to find a larger shell. A similar situation occurs with the colonial anemone *Epizoanthus* and has also been reported in shells covered with bryzoans (MacGinitie and MacGinitie, 1949). This is obviously of great advantage to the hermit crab and insures that the association will last the life of at least one individual.

(C) There are several associations involving Crustacea that resemble commensalism but cannot be considered as such. For example, many brachyurans but especially the spider crabs (Majidae) place materials on their backs, thus disguising themselves to a degree. There is little if any specificity here and the crabs usually cover themselves with whatever is available in their particular environment, be it hydroids, brozoans, algae or pieces of shell. Many Dromiacea and Oxystomata have modified legs which aid in carrying things on their back. Limbaugh *et al.* (1961) have described the very interesting case of six species of shrimp from three families which remove ectoparasites from various species of fish. The cleaner shrimp is only briefly associated with a given host and thus should perhaps be regarded as a special type of predator. It is also interesting to realize that although its gastropod shell is not living, the hermit crab itself is absolutely dependent on it, is somewhat specific as to shell species, is morphologically adapted to use it and has behavior patterns which bring the association about (Reese, 1963).

ADAPTATIONS

As a result of evolution all organisms possess certain features which fit them to the environment in which they live. This is, of course, true of commensals and due to the fact that their environments, namely the various hosts, differ in many respects from those of free-living animals, a variety of different and seemingly amazing adaptations have evolved. The many and varied adaptation shown by commensal Crustacea have fascinated generations of biologists. These are discussed below under several general headings.

A. *Structure*

The commensal Crustacea possess a host of structural modifications most of which are quite evidently of selective value. Thus the hapalocarcinid crabs (*Cryptocheirus* s.l.) which live in cylindrical cavities in stony corals have a cylindrical shape and have the front part of the carapace bent over ventrally, forming an operculum which neatly fills the opening of the cavity. Ectocommensals often have well-developed hold-fast structures. The barnacle *Balanus* (*Conopea*) *galeatus* lives on the gorgonian *Leptogorgia virgulata* and has plates which grow through the host coenenchyme and fasten to the axial skeleton. The amphipod *Parapleustes commensalis* on the spiny lobster has the periopods modified for grasping (Shoemaker, 1952) while many of the decapods commensal with branching corals have the typical sharply pointed dactyl of the walking legs modified into various types of blunted structure more suitable for an unyielding substrate. In the whale barnacles of the coronulid series, the borders of the mantle form a hood over the cirri (Pilsbry, 1916). It appears possible that this hood may enable the barnacle to feed even when it is being pulled through the water at considerable speed. *Pinnixa longipes* which lives in the quite narrow tubes of the polychaete *Clymenella* is nearly three times wider than long and has all but one pair of legs reduced in size (MacGinitie and MacGinitie, 1949).

Many of the commensals found in sheltered situations have a stout and clumsy form often accompanied by an increased egg-carrying capacity in the female. Another common feature of

these commensals is some degree of softening of the exoskeleton. The adaptive significance of this is not certain, but it must reduce both the efficiency of movement and the protective value of the exoskeleton and so would be rapidly eliminated in free-living forms. A hard exoskeleton might conceivably irritate the host enough to reduce its food intake or even bring about its early death and thus a soft exoskeleton could have selective advantage.

B. *Color*

Many instances of color correspondence between host and ecto-commensals have been reported. The color of many of these commensals seems fairly constant, but cases are known where different colors occur in commensals whose hosts vary in color. Thus colonies of the gorgonian *Leptogorgia virgulata* vary in color from white, which is rare, to various shades of yellow to shades of orange to purple. Females of the commensal shrimp, *Neopontonides beaufortensis*, have a number of yellow and deep red chromatophores in their bodies and I have collected yellow shrimp from yellow gorgonians, orange shrimp from orange hosts, and purple shrimp from purple goregonians (Patton, 1963). This animal seems almost unbelievably well adapted to its host. Similarly, a given colony of the gorgonian *Muricea misca* is also constant in color, but in this case the color of a colony may be all white, or white with the tips of the branches a shade of orange, gold or scarlet. Crane (1937) found that the commensal crab *Quadrella nitida* was all white or white with the chelipeds a shade of yellow, orange or red. The crabs were usually found on correspondingly colored corals and those with colored chelipeds even clung in such a way that these appendages were among the colored tips of the branches. Voss (1956) found that shrimp of the species *Tozeuma carolinensis* were purple when collected from purple gorgonians and green when collected from the turtle grass *Thalassia testudinum*.

Potts (1915 *a*) found that not all the individuals of *Synalpheus brucei* collected from crinoids were adaptively colored and concluded that perhaps this species is so protected that selection with regard to color does not occur. Some of the decapods on branching stony corals are quite conspicuously colored and here also, color may be selectively neutral.

C. *Behavioral adaptations*

Behavior patterns are of course adaptive and subject to evolution, and animals with specialized habitats such as commensals may possess specialized types of behavior. Reese (1964) suggests that there is a basic behavior pattern for a given group of animals and thus comparisons of behavior in groups containing commensal and free-living forms might offer interesting insights into the evolution of behavior. A comprehensive review of crustacean behavior is given by Schöne (1961).

Probably all the mobile commensal Crustacea possess a strong thigmotropism. In some species this may be extremely strong as in the case of the many external commensals which can hardly be made to leave their host regardless of how much they are disturbed. While most of the decapods found on branching corals will evade capture in an aquarium, the shrimp *Coralliocaris graminae* will actually grasp one's fingers and remain attached, even when the hand is removed from the water. Potts (1915 *a*) observed a similar situation with the commensal of crinoids *Synalpheus brucei*. These animals even grasp each other if nothing else is present. Most commensals are undoubtedly positively geotropic, but perhaps no more so than most benthic Crustacea. A negative phototropism is probably a general trait especially in the commensals not normally exposed to light. Pearse (1913) found that the two commensals of the tube worm *Chaetopterus*, *Polyonyx macrocheles* and *Pinnixa chaetopterana* were active in bright light but stopped moving when a shadow passed over them. This type of behavior obviously serves to keep the commensal inside the host's tube. A response to the mere presence of a water current (rheotaxis) might be expected in some commensals, although Davenport *et al.* (1960) failed to find it in *Pinnixa*. A positive response to humidity is found in the myrmecophilous isopod *Platyarthus hoffmannseggi* but also occurs in free-living species (Brooks, 1942).

In recent years there have been several studies on the response of crustacean commensals to substances liberated by their hosts. *Platyarthus* is attracted to the formic acid produced by its ant hosts (Brooks, 1942), while *Pinnotheres maculatus* moves toward a water current coming from either of its two hosts, *Aequipecten irradians* or *Atrina rigida* (Sastry and Menzel, 1962). The ecto-commensal crab *Dissodactylus mellitae* is similarly attracted to substances produced by its host sand dollar, *Mellita quinquiesperforata* (Johnson, 1952). Davenport *et al.* (1960) found that *Pinnixa chaetopterana* showed an increased rate of random turning in the presence of water coming from either of its two possible hosts, *Chaetopterus* or *Amphitrite*, but that it did not move toward the source of the host factor. Larval and juvenile commensals must locate their appropriate host and it seems highly probable that chemical attraction plays an important role in this process. Recent studies on the larvae of benthic marine invertebrates have shown that in most species there is a shorter or longer period of pelagic life, after which they reach an exploratory stage which investigates the bottom. At this stage, the presence of the preferred habitat or of some molecule diffusing from this habitat will accelerate metamorphosis (Scheltema, 1961; reviews by Wilson, 1958 and Reese, 1964). It is likely that the larvae of some commensals behave in a similar manner, being induced to metamorphose by some host substance or, as Davenport (1956) has suggested, by some factor in the normal environment of the host.

D. *Ability to modify host*

A few commensals modify their host in such a way as to provide living space for themselves. The most famous example is female of the gall crab *Hapalocarcinus* which at an early stage settles between two branches of a madrepore coral of the family Pocilloporidae. Branches of the coral grow up eventually forming a gall which encloses her. The crab feeds on plankton drawn through the small openings in the gall. *Cryptocheirus* (s.l.) mentioned above, lives in cavities formed when a solid coral grows around it (Potts, 1915 *b*; Balss, 1956).

Pinnaxodes chilensis lives in the periproct of the sea urchin *Strongylocentrotus* and distorts the test of its host (Garth, 1946 *a*). A crab of the genus *Dromia* lives in slits which it may cause to form in its alcyonarian host *Spongodes*, and an alpheid may cause branches of its gorgonian host *Solenocaulon* to become grooved and tubular and thus offer shelter for the commensal (Hornell, 1922). The female acrothoracican barnacle *Berndtia purpurea* is not able to burrow until the host coral grows up around it (Utinomi, 1961).

E. *Reproductive adaptations*

Many female endo-commensal decapods have enlarged abdomens and therefore a larger egg-carrying capacity than would free-living relatives of a similar size. Balss (1956) reports that alpheids commensal with sponges often have larger eggs with shorter larval development than do their free-living relatives. Goodbody (1960) found that *Pinnotheres moseri* had a larval development of only one or two days which was much briefer than that reported for other species of the genus. Gotto (1957) observed an interesting adaptation in the ascidicolous copepod *Ascidiella rosea*. The nauplii are released inside sheaths which protect them during their voyage through the host intestine, but rupture when they meet the current in the exhalent siphon. In an article on the relation between egg number and the hazards of larval life in commensal and parasitic copepods, Gotto (1962) gives an excellent discussion of general and important questions relating to the problem of reproductive adaptations.

Relationships between Commensal and Host

The commensal and the host are in long term intimate association and may interact in many ways. Some of the aspects of this relationship are discussed below.

A. *Effects of the commensal on its host*

Although there is little precise information on the subject it seems likely that many of the typical commensals do not harm their host very greatly. No case of a crustacean commensal benefiting the host has been proven, but it is possible that certain ectocommensals may be of some benefit by removing silt and small organisms that settle on the host. With regard to the harmful effects of commensals, burrowing barnacles might weaken their coral hosts and free-swimming animals must be slowed down to some degree by their attached barnacles. The alimentary canal may be deformed or displaced in the zooids of colonial tunicates which contain copepods (Gotto, 1960), and as mentioned above some commensals are dependent on the changes which they induce in the growth of their host. In the case of some *Pinnotheres* the host is damaged by the mere presence of the crab. Christensen and McDermott (1958) found some gill damage in almost all the oysters that contained *P. ostreum*, while in a few older oysters the gills were very much reduced. Sastry and Menzel (1962) also found gill damage in scallops containing *P. maculatus*. It is possible that certain commensals could take enough of the host's food to retard its normal growth. Berner (1952) found a temporary inactivity of the gonads in *Mytilus edulis* infected by larger (10–13 mm.) females of *P. pisum*. He attributed this to a loss of nourishment resulting from the amount of food taken by the crab, since when the crab was removed normal gamete production was restored. A similar phenomenon has been reported in ascidians containing copepods (Gotto, 1960).

Presumably most animals have some defense mechanism which prevents other organisms from settling on them or from wandering around inside them. A commensal then is a fairly specialized animal which is either immune to host reactions or which fails to set them off. Davenport (1962) found that the facultative commensal crab *Hyas araneus* does not elicit normal feeding behavior when it is dropped on its host anemone, *Tealia felina*. However, when a *Hyas* and a non-commensal crab *Carcinus* are dropped simultaneously, both are eaten by the anemone.

B. *Variations in host infection*

While certain commensals doubtless inhabit their host throughout the year and throughout the host's range, there are examples of changes in host infection with time of the year, with stage of development and with geographic region. Seasonal changes in the relations between host and commensal do not seem to be too common, or at least have not yet been shown to be common, although Humes and Cressey (1960) found striking seasonal fluctuations in the infection of the clam *Tagelus* with the copepod *Myocheres major*. Gotto (1961) observed that young and non-ovigerous females of the ascidicolous copeod *Enteropsis sphinx* live in the stomach of the host while ovigerous females live in the pharynx. In studies in Puget Sound, MacGinitie and MacGinitie (1949) found that the pinnotherid crab *Pinnixa eburna* only inhabited those tubes of *Arenicola claperedi* which occurred in sand and never those found in adjacent mud flats. Several cases of geographic variation in host preference and host infection have been found, for example see Gotto (1961), Gray (1961) and Williams and Needham (1939). Gotto (1962) discusses the fact that hosts are often most heavily infected under sheltered conditions. Research on such variations could be of great help in understanding the host-commensal relationships.

C. *Host specificity*

Host specificity is one of the main problems underlying all studies of symbiotic associations. While more collecting has to be done to determine the precise ranges of specificity for many individual species, it is apparent that some commensals occur only in one host, many occur on related hosts and some may inhabit quite unrelated hosts. Gotto (1960) reports some ascidicolous copepods from one ascidian host and others from as many as fifteen. Christensen and McDermott (1958) report that many species of *Pinnotheres* have been taken in half a dozen or so different bivalves, while one species occurs only in one and a few species occur in hosts as different as tunicates and bivalves or bivalves and *Chaetopterus* tubes. The reasons for these variations in specificity are an interesting subject for research and speculation. Some commensals have been

shown to have a preferred host but will occupy others when this one is not available. Gotto (1961) reports that the ascidian *Clavelina* contains few ascidicolous copepods in an area where other ascidians are abundant but is heavily infected in a region where it is the only flourishing ascidian. In all Great Barrier Reef localities, crabs of the genus *Trapezia* occur commonly on corals of the family Pocilloporidae but are extremely rare on *Acropora*, yet at a locality to the south of the reef where there are no pocilloporid corals, two species of *Trapezia* are fairly common on *Acropora* (Patton, 1966).

Davenport (1956) and Bocquet and Stock (1963) think that a response to a specific host is a primary factor in a commensal relationship and that if an individual commensal should under unusual circumstances get on a "wrong" host, olefactory conditioning to the new host might serve to isolate it from the parent stock and allow a new species to evolve. I agree with the above authors that this is an exciting field for experimentation. A problem with this model of speciation without geographic isolation is however, that if the accident happens once, it can happen again, thus allowing gene flow between the two populations and preventing a new species from being formed. This matter is much more fully discussed by Mayr (1963).

Relationships between Commensals of the Same Species

Although in many commensal Crustacea several individuals of both sexes are found together on the same host, there are quite a few decapods in which only a single male and a single female are found on a given host individual. A famous example of this phenomenon is the pair of shrimps *Spongicola venusta* which enter their glass sponge host as larvae and grow up together into imprisoned adults. There are numerous other examples of pairing in commensal decapods, several of which are given by Balss (1956). In addition certain free-living alpheid shrimp and mud shrimp (Thalassnidea) live in pairs in tubes and burrows. All these cases of pairing have certain features in common: the animals involved are of fair size in relation to that of the space which they occupy (burrow, host), they are sedentary and they enjoy considerable protection from potential predators. The selective advantage of pairing in eliminating competition for a limited resource is apparent, but it can also be seen that pairing will be selected against if there is a reasonable likelihood that one member of the pair will suddenly disappear. Support for this line of reasoning is found in the decapods commensal with branching corals (Patton, 1960) where those species which live in the peripheral branches occur in groups while those living near the base of the coral colony are more likely to occur in pairs. In some of the latter species such as the shrimp *Alpheus ventrosus* and the crab *Trapezia ferruginea* f. m *areolata* pairing is quite strict and regardless of whether the colony is small or very large, only a single pair will be present. It is probable that, in these and other cases of pairing, the existing pair destroys or, which seems less likely, drives off the young stages of their own species that attempt to settle. Evidence for this theory is provided by the case of the crab, *Tetralia*, of which usually only a single pair is found per coral colony. Yet on one occasion in a very tightly branched flat colony which allowed very little freedom of movement for the crabs, I found eleven pairs to a small species of *Tetralia*, arranged at intervals about three inches apart around the edge of the colony.

In the coral shrimp *Coralliocaris graminea* there are usually about twice as many males as females (Patton, 1960). Although this situation would appear to be of selective advantage, it does not seem to be very common, at least in the decapods, where most of the species which occur in groups contain the two sexes in roughly equal proportions.

In addition to the commensals occurring in groups and in pairs there are many examples among decapod and copepod commensals where only the female is found with the host. This situation is particularly common in ascidicolous copepods, indicating that the males are either mainly free-living or else are able to escape from the host before it is examined (Illg, 1958; Gotto, 1960). The males have been proved to be free-living in a number of cases, as for example, the coral gall-crab *Hapalocarcinus marsupialis*, where the male fertilizes the female before the gall completely closes

(Potts, 1915 *b*). In many instances however, the males are unknown and merely assumed to be free living. Certain pinnotherid crabs have been thought to have free-living males, but for two of these species this has recently been shown not to be true. Christensen and McDermott (1958) found that both sexes of *Pinnotheres ostreum* go through a period of commensal development as juveniles and then molt to a hard shell copulatory stage. Males leave the host at this stage in search of females in other oysters. Most males die a short time later, while the females go through several soft shelled stages the last of which becomes ovigerous. A similar situation occurs in the pinnotherid *Fabia subquadrata* commensal with the mussel *Modiolus modiolus* (Pearce, 1962). Here however, both sexes leave the host in the hard shell stage and copulation takes pace. The female then returns to a mussel and after several molts becomes ovigerous. Pearce believes that this situation represents an adaptation toward a symbiotic existence and that the presence of an adult male and an adult female might be too much of a drain on the host. I certainly agree with this interpretation. In view of the rather serious effects which a single adult female pinnotherid can have on its host, it seems certain that any mechanism which results in non-commensal males would have great selective advantage.

In studies on copepods in the ascidian *Corella parallelogramma*, Gotto (1957) found that in *Ascidiella rosea* which lives in the small niche of the host's esophagus there is usually only one female per host, while in *Notodelphys allmani* inhabiting the much larger pharynx of the same host, several specimens are found. This example supports those given above indicating that a mechanism for regulating the number of commensals will be selected for most strongly in the species which inhabit the smallest niches.

Relationships between Commensals of Different Species on the Same Host

There are several cases known where the activities of one commensal interfere with the activities of another commensal species. In an extreme and perhaps rare case already mentioned, Crane (1937) found that stomachs of the commensal crab, *Quadrella nitida*, contained remains of the worms and brittle stars commensal on the same host. Kemp (1922) found that pairs of the shrimps *Anchistus custos* and *Conchodytes biunguiculatus* occurred in the same species of *Pinna* but never in the same host individual. Similarly pairs of *Pinnixa* and *Polyonyx* are rarely found in the same *Chaetopterus* tube (Gray, 1961). Evidently the adults of one species prevent both the young of their own species and the young of the second species from developing in their host.

There are many cases however where several commensals live together on the same host in apparent harmony. On theoretical grounds it might be expected that no two of these commensals would occupy exactly the same niche. If they did competition between them would lead either to the elimination of one of the species or to strong selection in one or both favoring non-competing individuals and resulting in species with different niches. A second result of this competition could be selection for mechanisms which prevent the two species from occurring simultaneously and examples of this have just been cited. In most cases however, co-existing commensals do indeed seem to occupy different niches. Gotto (1957) found one species of copepod in the esophagus of the ascidian *Corella parallelogramma* and another in the pharynx. The various commensals of the gorgonian *Leptogorgia virgulata* also seem to have different niches (Patton, 1963). With regard to the commensals inhabiting burrows of the echiuroid, *Urechis caupo*, the facultative commensal goby *Clevelandia ios* leaves the burrow to feed, the pinnotherid crab *Scleroplax granulata* is a filter feeder and eats particles of flesh that fall into the burrow, while the scale worm *Hesperonoe adventor* eats small animals swept into the host or particles entrapped in the host's mucus tube (MacGinitie and MacGinitie, 1949).

An interesting case of one commensal benefiting another is the relationship between the snail *Neosimnia uniplicata* and the barnacle *Balanus galeatus* on the gorgonian *Leptogorgia virgulata* (Patton, 1963). The snail lays egg cases around the stem of the gorgonian and young barnacles are often found attached to remains of these egg cases. The plates of the barnacle grow down

through the egg case and host coenenchyme and attach to the axis of the gorgonian. The egg case thus facilitates attachment and often brings several barnacles together in one place allowing eventual cross-fertilization.

Conclusions

Careful observation of potential hosts will doubtless reveal many new and interesting associations. In addition there is a great need for detailed study of individual commensal associations such as those of Christiensen and McDermott (1958), Gotto (1957) and Humes and Cressey (1960). The problems raised by commensal Crustacea are so varied that a zoologist of whatever interest will find a chance to exercise his speciallity.

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