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Short Communication

Effect of sudden changes in salinity on different stages of parthenogenetic *Artemia* sp.

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Abstract

Effect of sudden changes in salinity on different life history stages of the parthenogenetic Indian strain of *Artemia* from salinas of Thoothukkudi was studied. For the experiment, nine ranges of salinity (30 - 110 ppt) with an increase of 10 ppt at each range were prepared with 110 ppt as the control. Different life history stages of *Artemia* were suddenly introduced to lower salinities from 110 ppt and the survival rates were estimated during 5 days. The experiment showed that adults were more susceptible and nauplii more tolerant to sudden decline in salinity supported by significant two way ANOVA values.

Keywords: *Artemia*, life history stages, nauplii, effect of salinity

Introduction

Artemia has been found to be a suitable food for a diverse group of cultivable organisms (Sorgeloos 1979, 1980). Existing literature reveals that among the few species utilized as live food, the brine shrimp *Artemia* comes in for prime consideration (Patra and Mohamed 2003; Baxevanis *et al*., 2006). Kinne (1977) indicated that more than 85% of the marine animals cultivated so far have been fed with *Artemia*. For the successful rearing of larvae of marine fishes and crustaceans in hatcheries where intensive aquaculture is practiced, the availability of suitable food is an essential pre-requisite. The cyst of the brine shrimp *Artemia* forms one of the most important sources of food for the larvae. The demand for good quality *Artemia* cyst is far more than the present production level and the insufficient cyst supply sometimes is the major bottleneck in the proper functioning of hatcheries the world over (Schauer *et al*., 1979; Baxevanis *et al*., 2006).

Several workers (Kristenen and Hulscher-Emeis, 1972; Persoone and Sorgeloos, 1980; Ramamoorthi and Thangaraj, 1980; Scelzo and Voglar, 1980; Bhargava *et al*., 1987) have observed wide fluctuations in *Artemia* population in general and in different life history stages. The possible reason attributed for such fluctuations was the sudden decrease in salinity, which may be due to rainfall or dilution of water from other sources. All previous studies have given only circumstantial evidences to the above conclusion and these have not been supported by any experimental work (Clegg and Trotman, 2002). Therefore, it was felt appropriate to study the population variations by changing salinity conditions at short intervals and the present experiment was conducted in different life history stages of parthenogenetic Indian strain of *Artemia* (*Artemia* sp.) collected from salinas in Thoothukkudi (8° 46′52′′N lat.; 78° 09′25′′E long.), Tamil Nadu, India.

Material and Methods

Artemia population containing all developmental stages (nauplii, juvenile, pre-adult and adults - cyst bearing and nauplii bearing) collected at a particular time from a particular salinity range was introduced into a series of salinity ranges at the same time. This was done with an aim to see how the sudden changes in salinity actually affected the different life history stages. The experiments were carried out at Thoothukkudi Centre of Central Marine Fisheries Research Institute, Tamil Nadu, India.

For the experiment, 9 ranges of salinity (30 -110 ppt) with an increase of 10 ppt at each range were prepared by mixing salt collected from the salt pans with freshwater. The prepared media were kept separately in 3 L glass beakers. The different stages of *Artemia* were collected from the salina. During the time of sample collection, salinity at the site was 110 ppt. Each developmental stage was introduced separately into a salinity medium. A uniform stocking density of 50 nos. per beaker of each stage was maintained. All stages were uniformly fed twice a day with rice bran suspension and water in each beaker was renewed every day. Five replicates were maintained for each stage and salinity range. The available maximum salinity 110 ppt. was considered as control. Duration of the experiment was 5 days. Statistical analysis ANOVA (two-way) was conducted to observe the effect of salinity on the developmental stages.

Results and Discussion

The life-history stages of *Artemia* reacted differently to different salinity ranges. The mortality rates observed in each stage in the different salinity ranges are given in the Table 1. The experiment showed that the adults were more susceptible to the sudden changes in the salinities especially when they were transferred from the 110 ppt to 30 ppt (mortality rate 99.2%). The preadults were the next severely affected stage with a mortality rate of 76% followed by juveniles (60.8%) and nauplii (16%). The experiments showed that nauplii are the most tolerant to sudden decline in salinity with minimum mortality.

The result of two-way ANOVA is given in Table 2. The ANOVA test in relation to the ranges of salinity (30-110 ppt) *vs*. percentage of mortality of the total population was found to be highly significant (F=18.93; $p<0.01$). The mortality rates in different stages of *Artemia* in relation to salinity was also highly significant but at a lower level (F=9.07; $p<0.01$). The mean comparison in all ranges of salinities on the percentage mortality of the adult *vs*. pre-adult *vs*. juvenile *vs*. nauplii revealed that the salinity affected the survival of all stages of the population in a significant way. The mortality percentage of juveniles and nauplii were comparatively low compared to that of adults and pre-adults.

A sudden change in salinity from 110 to 30 ppt had affected the survival of all stages of *Artemia* with

Table 1. Mortality rates of different stages of *Artemia* when introduced suddenly into different ranges of salinity

Treatment	Salinity decrease (ppt)	Salinity suddenly reduced by ppt	Percentages of mortality for a period of 5 days: Adult (T1)	Pre-adult (T2)	Juvenile (T3)	Nauplii (T4)	Mean (T) (%)
R1	110 to 30	80	99.2	76.0	60.8	16.0	63.0
R2	110 to 40	70	76.4	53.6	18.0	5.2	38.3
R3	110 to 50	60	64.0	35.2	9.6	4.4	28.3 NS
R4	110 to 60	50	55.6	31.2	6.8	0.0	23.4 NS
R5	110 to 70	40	42.8	19.2	3.2	0.0	16.3 NS
R6	110 to 80	30	33.6	13.2	2.8	0.0	12.4 NS
R7	110 to 90	20	24.0	8.8	3.6	0.0	9.09
R8	110 to 100	10	9.6	7.2	2.4	0.0	4.8
Control	110 to 110	0	2.8	2.4	2.0	0.0	1.8
Mean (R)			45.33	27.42	12.13	2.84	

NS-Mean comparisons not significant at 1% level between treatments; pair-wise comparisons in rest of the cases significant at 1% level

T-Population

Table 2. Two-way ANOVA - salinity *vs.* stages of population

Source	DF	Sum Sq.	Mean Sq.	F	Remarks
Population	3	9343.024	3114.341	18.93	p<0.01
Salinity	8	11932.960	1491.620	9.07	p<0.01
Error	24	3948.817	164.534		

high mean mortality rate (63%) in comparison to the other salinity ranges. The mean mortality rate decreased to almost 50% at 40 ppt (38.3%). In salinity ranges of 50 and 60 ppt, the mean mortality rates of the total population were still low but more or less the same (28.3% and 23.4% respectively). The mean mortality rate further declined with reduced salinity decrease showing the least value of 1.8% in control (no change in 110 ppt salinity). Pair-wise comparison of mean mortality rates between different treatments were statistically significant (p<0.01) except between treatments R_3 and R_4; and R_5 and R_6 (Table 1, 3).

Artemia is reported to be the best osmoregulator in the animal kingdom; adults are found even in the final crystallizing salt ponds, where the brine specific gravity is around 1.22 at 30°C, but brine of salinity over 300 ppt has been proved to be lethal to the animals (Clegg and Trotmann, 2002). Normally the egg clutch in adults upto 110 ppt salinity follow ovoviviparous path and produce nauplii larvae, but with increasing salinity (usually above 110 ppt) in the salt lakes, the developing eggs follow the oviparous path, resulting in encysted gastrula embryos (cysts) that enter diapause, a state of obligate dormancy in the hypersaline environment. The leaf-like structures (metepipodites) on the swimming appendages (phyllopodia), gut and maxillary glands of adults containing very high levels of ion-regulatory enzymes such as Na/K-ATPase actively secrete ions to the environment, thus helping these animals to maintain an internal osmotic medium, in extreme hyperosmotic conditions (Garcia-Saez *et al.*, 1997). The level of Na/K-ATPase increases dramatically from nauplius to the adult stage and its highest activity has been reported in adults (Clegg and Trotmann, 2002).

Table 3. Comparison of specific populations

Mean comparisons	Remarks
T1- T2	Sig.
T1- T3	Sig.
T1- T4	Sig.
T2- T3	Sig.
T2- T4	Sig.
T3- T4	NS

Sudden drop in salinity due to rainfall or dilution of environment was found to be detrimental to *Artemia* population (Bhargava *et al.*, 1987). Generally the cysts hatch out and nauplii survive in low saline conditions. Conte *et al*. (1980) and Conte (1984) established that the nauplii survive better at reduced salinity. The haemolymph of nauplii is maintained better at concentrations and compositions that are considerably lower and qualitatively different from the hyperosmotic medium compared to the adults, with the help of the Na/K-ATPase secreted by the larval 'neck organ'(salt gland). Ahl and Brown (1991) reported high fluctuations of Na/K-ATPase activity in *Artemia* nauplii with the changing saline conditions. Compared to the adults, rapid increase in the synthesis of ornithine carboxylase (ODC) – an enzyme essential for various basic cellular processes including altered cellular osmolarity was reported when nauplii reared in seawater (32 ppt) was rapidly transferred to 4 and 12 ppt (Watts *et al.*, 1996). All these indicate that the nauplii are better adapted to saline conditions and this may be the main reason for the survival of nauplii and high mortality of adults and pre-adults when salinity was suddenly decreased in the present experiment.

References

Ahl, J. S. B. and J. J. Brown. 1991. The effect of juvenile hormone III, methyl farnesoate and methoprene on Na/K-ATPase activity in larvae of the brine shrimp, *Artemia*. *Comp. Biochem. Physiol., A,* 100: 155-158.

Baxevanis, A. D., I. Kappas and T. J. Abatzopoulos. 2006. Molecular phylogenetics and asexuality in the brine shrimp *Artemia*. *Mol. Phylogenet. Evol.,* 40: 724-738.

Bhargava, S. C., G. R. Jakher, M. M. Saxena and R. K. Sinha. 1987. Ecology of *Artemia* in Didwana Salt Lake (India) *In*: P. Sorgeloos, D. A. Bengston, W. Decleir, E. Jaspers (Eds.) *Artemia Research and its Application*, Vol. 3, Universia Press, Wetteren, Belgium. 535 pp.

Clegg, J. S. and C. N. A. Trotmann. 2002. Physiological and biochemical aspects of *Artemia* ecology. *In*: J. Abatzopoulos Th, J. A. Beardmore, J. S. Clegg and P. Sorgeloos (Eds.) *Artemia: Basic and Applied Biology*. Kluwer Academic Publishers, Dordrecht, The Netherlands. p. 129-170.

Conte, F. P. 1984. Structure and function of the crustacean larval salt gland. *Int. Rev. Cytol.*, 91: 45-106.

Conte, F. P., J. Lowry, J. Carpenter, A. Edwards, R. Smith and R. D. Ewing. 1980. Aerobic and anaerobic metabolism of *Artemia* nauplii as a function of salinity. *In*: G. Persoone, P. Sorgeloos, O. Roels and E. Jaspers (Eds.). *The Brine Shrimp Artemia*. Vol. 2, Universia Press, Wetteren, Belgium. p. 125-136.

Garcia – Saez, A., R. Perona and L. Sastre. 1997. Polymorphism and structure of the gene coding for the a-1 subunit of the *Artemia franciscana* Na/K-ATPase. *Biochem. J.*, 321: 509-518.

Kinne, O. 1977. Cultivation of animals. *In*: O. Kinne (Ed.) *Marine Ecology*, Vol. III *Cultivation, Part 2*, Wiley London. p. 579-1293.

Kristenen, I., and T. M. Hulscher-Emeis. 1972. Factors affecting *Artemia* populations in Antillean salines. *Stud. Fauna Curaco, Carribean Islands*, 39: 87-111.

Patra, S. K. and K. S. Mohamed. 2003. Enrichment of *Artemia* nauplii with the probiotic yeast *Saccharomyces boulardii* with its resistance against pathogenic *Vibrio*. *Aquacult. Int.*, 11: 505-514.

Persoone, G. and P. Sorgeloos. 1980. General aspects of the ecology and biogeography of *Artemia*. *In*: G. Persoone, P. Sorgeloos, O. A. Roels and E. Jaspers (Eds.) *The Brine Shrimp Artemia*. Vol. 3. *Ecology Culturing, Use in Aquaculture*. Universia Press, Wetteren, Belgium. 428 pp.

Ramamoorthi, K. and G. S. Thangaraj. 1980. Ecology of *Artemia* in salt pans of Tuticorin, South India. *In*: G. Persoone, P. Sorgeloos, O. A. Roels and E. Jaspers (Eds.) *The Brine Shrimp Artemia*. Vol. 3. *Ecology Culturing, Use in Aquaculture*. Universia Press, Wetteren, Belgium. 428 pp.

Scelzo, M. A. and J. F. Voglar. 1980. Ecological study of the *Artemia* populations in Boca Chica Salt Lake, Margarita Island, Venezuela. *In*: G. Persoone, P. Sorgeloos, O. A. Roels and E. Jaspers (Eds.) *The Brine Shrimp Artemia*. Vol. 3. *Ecology Culturing, Use in Aquaculture*. Universia Press, Wetteren, Belgium. 428 pp.

Schauer, P. S., L. M. Richardson, and K. L. Simpson. 1979. Nutritional status of marine larval and juvenile *Menidia menidia* reared on *Artemia salina* and various artificial diets: Cultivation of fish fry and its live food. *In*: E. Styczynskajure Wicz, T. Backiel, E. Jaspers and G. Persoone (Eds.) *EMS Spl. Publ. No.4*. Institute for Marine Scientific Research, Bredene, Belgium. 534 pp.

Sorgeloos, P. 1979. The brine shrimp *Artemia* in aquaculture. *In*: T. V. R. Pillay and W. A. Dill (Eds.) *FAO Technical Conference on Aquaculture, Kyoto, Japan. 1976*. Fishing News Books Ltd., Farnham, England. 653 pp.

Sorgeloos, P. 1980. The use of brine shrimp *Artemia* in Aquaculture. *In*: G. Persoone, P. Sorgeloos, O. A. Roels and E. Jaspers (Eds.) *The Brine Shrimp Artemia*. Vol. 3. *Ecology Culturing, Use in Aquaculture*. Universia Press, Wetteren, Belgium. 428 pp.

Watts, S. A., E. W. Yeh and R. P. Henry. 1996. Hypo-osmotic stimulation of ornithine decarboxylase activity in the brine shrimp *Artemia franciscana*. *J. Exp. Zool.*, 274: 15-22.

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