



Effects of intrinsic and extrinsic factors on the haemocyte profile of the spiny lobster, *Panulirus homarus* (Linnaeus, 1758) under controlled conditions

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Abstract

The effect of intrinsic and extrinsic factors like disease, nutritional status, bilateral eyestalk ablation and different water exchange regimes on the total haemocyte count (THC) of the spiny lobster, *Panulirus homarus* was studied. The THC count reduced by 31.30, 50.34 and 72.01% in the lobsters infected by shell disease with 5-10, 10-20 and > 20% lesion cover respectively. The control healthy lobsters with 0% lesion showed the highest THC ($20.07 \pm 0.99 \times 10^6$ cells ml⁻¹) and the lowest was observed in the group displaying > 20% shell lesion cover ($5.59 \pm 0.49 \times 10^6$ cells ml⁻¹). The serum protein reduced by 16.19, 33.27 and 55.06% respectively in the groups displaying 5-10, 10-20 and > 20% lesion cover. Clotting time showed a significant ($p < 0.01$) negative correlation with THC and serum protein. Starved lobsters and those fed with pellet diet displayed significantly ($p < 0.05$) lower THC and serum protein compared to lobsters fed on green mussel (*Perna viridis*). Bilaterally eyestalk ablated lobsters showed a higher THC and serum protein content than their unablated counterparts. The lobsters grown in different water exchange regime (25, 50, and 75% exchange) had a comparatively lower (17.38, 12.92 and 8.73%) THC and the culture water had a higher (81.86, 48.80 and 21.84%) total heterotrophic bacterial (THB) and *Vibrio* sp. load (86.20, 77.83 and 18.40%) than the lobsters maintained at 90% water exchange regime. The haematological parameters like THC, serum protein content and clotting time which are altered by environmental and biological stress factors can thus be used as potential stress indicators for monitoring the health status of spiny lobsters.

Keywords: *Panulirus homarus*, total haemocyte count, serum protein, disease, nutritional status, clotting, extrinsic, intrinsic factors

Introduction

Circulating crustacean hemocytes have demonstrated several defense functions like coagulation, aggregation (Johnson, 1976; Newman and Feng, 1982), phagocytosis by encapsulation (Hose *et al.*, 1990) and subsequent annihilation of microbial invaders by the production of antimicrobial substances. In crustaceans, immunorecognition is mediated by the prophenoloxidase cascade present in the hemocytes which is triggered by the presence of non-self molecules inducing melanization reactions (Söderhäll and Smith, 1986; Söderhäll *et al.*, 1996; Cerenius *et al.*, 2008). Hence, haemograms/ total haemocyte counts (THC) is

considered as an indicator to monitor stress and/or health conditions in different crustaceans (Lorenzon *et al.*, 2001). Under captive conditions, the stress factors most frequently confronted by spiny lobsters include environmental (temperature, salinity, dissolved oxygen etc.), biological (nutritional, infections) and husbandry practices (stocking density, water exchange regimes etc.). These stress parameters bring about several homeostatic regulations in the host resulting in considerable changes in the host defense mechanism. Stress-related opportunistic infections reduce the haemocyte numbers in lobsters presumably by attrition (Stewart *et al.*, 1967; Stewart and Rabin, 1970; Jussila *et al.*, 1997; Lorenzon *et al.*, 2001; Cheng *et al.*, 2002).

In the western rock lobster, *Panulirus cygnus*, Jussila et al. (2001) found that clotting time could be used as an indicator of stress while alterations in the THC's affect the immune competence in the crayfish, *Pacifastacus leniusculus* (Persson et al., 1987).

The spiny lobsters, *Panulirus homarus* and *P. ornatus*, have recently captured the attention of aquaculturists in India due to their high demand in international market (Vijayakumaran et al., 2009). As spiny lobster seed production from hatchery is still a distant possibility, the lobster aquaculture relies on wild caught undersized juveniles for fattening (Verghese et al., 2007; Vijayakumaran et al., 2009). Disease outbreaks in spiny lobster culture are rare but recent instances in Vietnam (Anon, 2004) and India (Jayagopal and Vijayakumaran, 2010) suggest that commercial ventures may confront water quality problems and disease outbreaks. Severe mortality of caged spiny lobsters occurred in 2001 in Xuan Tu village in Vietnam, when up to 50% of adult lobsters died due to possible bacterial (*Vibrio* sp.) infections (Anon, 2004), whereas gaffkemia infection was suspected to be the cause of mass mortality of spiny lobsters in an indoor rearing facility in India (Jayagopal et al., 2010). Therefore, adequate knowledge of factors which affect haematological parameters is essential. In this study, we evaluated the use of haematological parameters as health indicators by exposing *P. homarus* to situations such as disease, nutritional status (live feed vs. artificial pellet diet), bilateral eyestalk ablation and different water exchange regimes.

Materials and Methods

Animal maintenance and conditions: The sea water used for the maintenance of the lobsters in all the experiments was drawn through subsurface filters in the intertidal area. The water was filtered through sand filters, chlorinated and dechlorinated through charcoal filters and sterilized using an ultraviolet sterilizer. The rearing tanks were well aerated and provided with artificial hideouts made of PVC tubes or coral rocks. Water quality parameters monitored during the study were: Salinity 35 ± 2 psu, temperature $26 \pm 2^\circ\text{C}$, pH 7.8 - 8.5, dissolved oxygen ≥ 4.5 mg L⁻¹ and ammonia - 0.1 ± 0.0001 mg l⁻¹.

Room lighting was maintained at a 12:12 light /dark cycle. Approximately 80% of water was changed daily (unless otherwise stated) and the lobsters were fed *ad libitum* once in the evening with the meat of live green mussel, *Perna viridis*.

Effect of shell disease on the haematological parameters of lobsters: Juvenile *P. homarus*, ($n = 40$) with a mean carapace length of 51.25 ± 2.79 mm and mean body weight of 121.38 ± 2.56 g, maintained under captive conditions for long duration (8 months) and showing different degrees of black lesions on the shell (shell disease) were used for this experiment. The lobsters were assigned to four groups depending on the percentage cover of black spot lesions on exoskeleton (according to Spanoghe, 1996 and modified from Vogan et al., 1999): Group 1 - control healthy lobsters (0% lesion displaying a vigour index of 5); Group 2 -with 0-5% lesion cover; Group 3, 5-10% lesion cover; Group 4, >20% lesion cover. Each group comprised 10 lobsters in the intermoult stage with equal distribution of both the sexes. Individual lobsters were coded with different coloured water proof epoxy paints in the carapace. Recoding was done after carapace hardening of moulted lobsters. These lobsters were used to determine the total haemocyte count (Stewart et al., 1967), serum protein concentration (Lowry et al., 1951), haemolymph clotting time (Jussila et al., 2001), number of total heterotrophic bacteria and total *Vibrio* sp. in the haemolymph, and intermoult period. Haemolymph collection was done in the intermoult (C stage) to avoid moult related variation. The correlation between the above factors and degree of lesion cover was studied. The lobsters were maintained for 60 days in four separate 2 tonne Fibre Reinforced Plastic (FRP) tanks at a density of 5 animals/m³.

Effect of nutritional status on the haematological parameters of lobsters: Juvenile *P. homarus* used in this study were obtained from local fishermen at Kovalam, Chennai and acclimatized for two weeks to the controlled conditions in 2 tonne circular FRP tanks. Apparent healthy lobsters in the intermoult stage with a mean carapace length of 45.03 ± 4.51 mm and mean body weight of 83.24 ± 1.49 g were divided into two nutritional treatment

groups of 12 individuals each and a starvation group with 6 individuals, with equal distribution of both the sexes. Group 1 was fed *ad libitum* with green mussel (control) with protein content of 63% on dry weight basis; Group 2 was fed *ad libitum* with artificial pellet with a protein content of 40% on dry weight basis and Group 3 was starved for 45 days. Haemolymph was collected from intermoult (C stage) lobsters. Cessation of feeding was done 48 hours prior to sampling. The experiment was run for 120 days. Sampling for total haemocyte count (THC) and protein concentration in serum was done on day 0, 45 and 90.

Effect of bilateral eyestalk ablation on the haematological parameters of lobsters: Wild collected juvenile *P. homarus* were acclimatized for two weeks to laboratory conditions in 2 tonne circular FRP tanks. Apparently healthy lobsters in the intermoult stage with a mean carapace length of 46.91 ± 6.91 mm and mean body weight of 77.05 ± 4.48 g were divided into two groups with equal distribution of sexes. Group 1 lobsters underwent bilateral eyestalk ablation ($n = 12$) and Group 2 was the control lobsters (unablated; $n = 12$). Ablation was done at the intermoult stage with the help of a pair of heat-sterilized dissection scissors and forceps. Individual lobsters were coded as described above. Only intermoult lobsters were sampled for THC and serum protein.

Effect of water exchange regime on the haematological parameters of lobsters: Apparently healthy lobsters in the intermoult stage (48 nos), with a carapace length of 53.35 ± 3.49 mm and average body weight of 144.58 ± 2.89 g were divided into four groups of 12 individuals each with equal distribution of sexes and were reared in four 1 ton FRP tanks. Group 1, 2, 3 and 4 lobsters were maintained with a daily water exchange of 25, 50, 75 and 90%, respectively for 30 days. Sampling for THC was done in the intermoult stage. The total heterotrophic bacterial (THB) and *Vibrio* sp. load of culture water were also observed during the experimental period.

Sampling for haemolymph bacterial load: First, the ventral side of the lobster was sterilized with 96% ethanol and the haemolymph was extracted

from the base of fifth walking leg with a sterile disposable insulin syringe. Aliquots, 0.1ml of undiluted to 10^{-3} dilution, of haemolymph were spread plated in triplicate on Zobell Marine Agar (ZMA-2216 Himedia Ltd, India) for enumerating total aerobic heterotrophic bacteria and in thiosulphate citrate bile salt sucrose agar media (TCBS-M189, Himedia, Bombay, India). The diluent blanks were prepared with sterile saline.

Bacterial load of culture water: Total aerobic heterotrophic bacterial (THB) load of culture water was estimated by the dilution plate method in which 0.1 ml aliquots of undiluted to 10^{-3} dilution of culture water samples were spread plated in triplicate on Zobell Marine Agar and incubated at 37°C for 24h prior to enumeration. The diluent blanks were prepared with 50% aged sterile (autoclaved at 121°C , 15psi, 20 minutes) sea water.

For the estimation of total *Vibrio* sp. 0.1ml aliquots of undiluted to 10^{-3} dilution of culture water samples were spread plated in triplicate on TCBS (M189, Himedia, Bombay, India) and incubated at 37°C for 24h prior to enumeration.

Total haemocyte count (THC): For THC determination, 0.9ml of lobster hemolymph was drawn from the ventral abdominal sinus into a 1 ml syringe preloaded with 0.1ml of cooled (4°C) 10 % v/v formalin in sterile sea water and mixed well (Stewart *et al.*, 1967). The haemolymph sample was stored on ice for analysis. For each sample, haemocytes were counted in triplicate under phase contrast Nikon microscope (Eclipse E600, Nikon Japan) using an improved Neubauers haemocytometer and the THC was reported as the number of cell.ml⁻¹ of haemolymph (Jussila *et al.*, 1997).

Another haemolymph sample of 200 μ l was taken and placed in an eppendorf tube on ice and clotting time was determined following Jussila *et al.* (2001). The samples that did not clot within 120 seconds were also noted.

Protein concentration of serum: The preparation of lobster serum was done according to Stewart *et al.* (1966). The protein concentration of serum was determined using the method of Lowry *et al.* (1951) with bovine serum albumin (BSA) as standard.

Statistical analysis: Data presented are the mean \pm SEM. Statistical analyses were done using one way analysis of variance (ANOVA) followed by Tukey – Kramer HSD tests for post – hoc comparison. Logistic correlation analysis of different parameters was also done. SPSS ver.17 was used for analysis. Significant levels for all analyses were set at $p < 0.05$.

Results

Effect of shell disease: The THC profile of *P. homarus* displays different percentages of shell lesion cover (Fig. 1). The control healthy lobsters with 0% lesion showed the highest THC ($20.074 \pm 0.991 \times 10^6$ cells ml⁻¹) and the lowest THC was observed in the group displaying >20% lesion cover ($5.598 \pm 0.487 \times 10^6$ cells ml⁻¹). There was 31.30, 50.34

percentage of shell lesions (expressed as percentage lesion cover) in diseased lobsters (Table 2). Haemolymph did not clot in 5 of the lobsters in the > 20% lesion cover group of lobsters.

Table 1. Intermoult period and clotting in diseased *Panulirus homarus*; values are mean \pm SEM. Means with different superscripts are significantly different (one way analysis of variance and Tukey test, $p < 0.05$, n = 10)

Lesion cover	Moult interval (days)	Clotting time (s)
0%	29.9 \pm 0.835 ^{a,b}	102 ^{a,b}
5-10%	35.0 \pm 0.835 ^{a,b}	123.5 ^{a,b}
10-20%	37.1 \pm 1.1 ^{a,b}	124 ^{a,b}
>20%	59.9 \pm 0.632 ^{a,b}	149.2 ^{a,b} (n = 5)

Table 2. Correlation matrix of parameters in diseased lobsters *Panulirus homarus* expressed as Pearson correlation coefficient and significance (* $p < 0.01$, N=40)

Parameters	THC	Serum protein content	THB
Serum protein content (mg/ml)	0.874*		-0.877*
Clotting time (s) (n=35)	-0.745*	-0.794*	0.837*
THC ($\times 10^6$ cells/ml)		0.874*	-0.920*
Intermoultperiod (days) (n=35)	-0.755*	-0.755*	0.823*
THB Mean Log ₁₀ CFU/ml	-0.920*	-0.877*	
Percentage lesion cover	-0.935*	-0.932*	0.941*

and 72.011% reduction in the THC count in the group displaying 5-10, 10-20 and > 20% lesion cover, respectively. The reductions were significantly lower ($p < 0.05$) from the control group (healthy lobsters with 0% lesion). The total heterotrophic bacterial load was below detectable level in the haemolymph of control (healthy) lobsters. The THB load in the haemolymph of different diseased group of lobsters ranged from 2.66 ± 0.08 log₁₀ CFU ml⁻¹ to 5.18 ± 0.27 log₁₀ CFU.ml⁻¹. The shortest intermoult period was observed in control (healthy) lobsters (Table 1). Five lobsters in the > 20% lesion cover group did not moult during the experimental period. The intermoult period correlated negatively with THC and haemolymph serum protein content (Table 2). In contrast there was a significant ($p < 0.01$) positive correlation between intermoult period and THB. Clotting time correlated negatively with the THC and severity

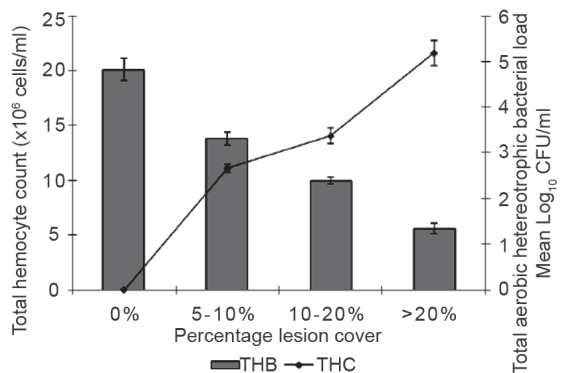


Fig. 1. Total haemocyte count and total aerobic heterotrophic bacterial load of haemolymph in the different shell disease groups of *Panulirus homarus*. Values are mean \pm SEM

Fig. 2 shows the serum protein content in *P. homarus* with different percentage shell lesion cover. There was a significant positive correlation between

serum protein content and THC ($p < 0.01$, Table 2). The highest serum protein content was observed in the control (healthy) lobsters. In contrast there was a significant ($p < 0.01$) negative correlation between serum protein content and THB.

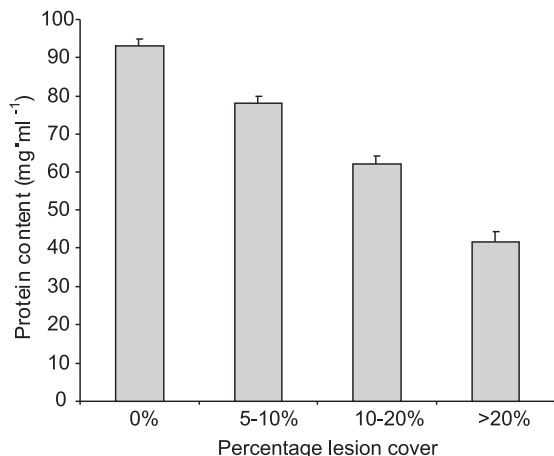


Fig. 2. Serum protein content in different shell disease groups of *Panulirus homarus*. Values are mean \pm SEM

Effect of nutritional status: After 45 days of starvation there was a significant ($p < 0.01$) lowering of THC in the starvation group when compared to the groups fed with any one of the diets (Fig. 3). There was wide variation in the THC of the three groups, which ranged from $7.23 \pm 0.931 \times 10^6$ cells ml⁻¹ in the starvation group to $19.04 \pm 0.161 \times 10^6$ cells ml⁻¹ in the group fed with green mussel. Initially

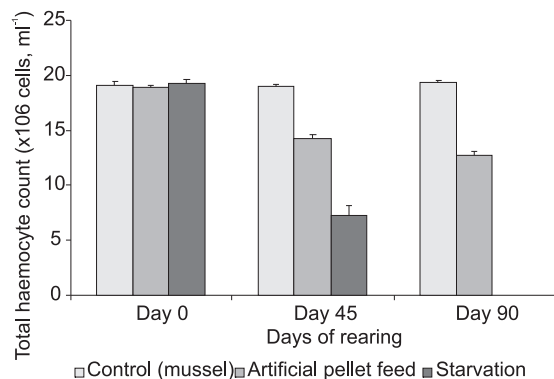


Fig. 3. Total haemocyte count (THC) in *Panulirus homarus* with different nutritional status. Values are mean \pm SEM

there was no significant ($p > 0.05$) variation in the THC of the three groups. However, after 45 days, there was significant ($p < 0.05$) reduction in the THC in the starvation group (62.01%) and the group fed on artificial diet (25.20%) compared with the group fed on green mussel. During the 90th day sampling THC in the group fed with artificial pellet feed was significantly ($p < 0.05$; 35.25%) lower than the group fed with green mussels. There was no significant variation in the THC between the three sampling periods in the group fed with mussels. However there was significant ($p < 0.05$) reduction in the THC in the group fed on artificial pellet diet throughout the experimental period.

The serum protein content of lobsters fed on artificial pellet diet was significantly ($p < 0.05$) lower than the lobsters feeding on green mussels during the 45th day and the 90th day of sampling (Fig. 4). The serum protein content of the lobsters starved for 45 days dropped from 95.24 mg.ml^{-1} to 44.73 mg.ml^{-1} .

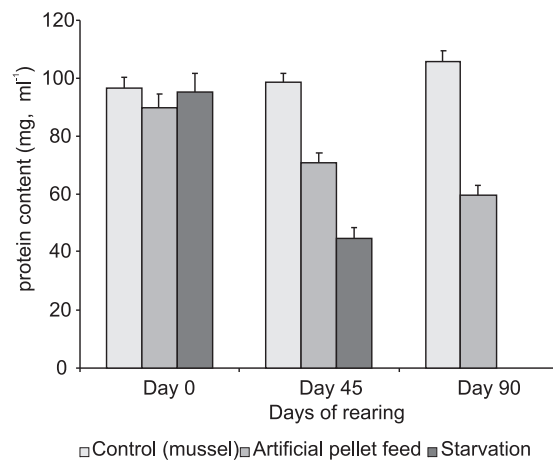


Fig. 4. Serum protein content in *Panulirus homarus* with different nutritional status. Values are mean \pm SEM

Effect of bilateral ablation: Bilateral ablation resulted in a significantly ($p < 0.05$) higher THC level 30-35 days post ablation when compared to unablated (control) lobsters (Table 3). Similarly the serum protein content of the bilaterally ablated lobsters was significantly ($p < 0.05$) higher than that of the control lobsters (Table 3).

Table 3. THC and serum protein content in bilateral ablated and unablated (control) group of *Panulirus homarus* lobsters. Values are mean \pm SEM. Means with different superscripts are significantly different (One way analysis of variance; $p < 0.05$, $n = 12$)

Group	THC ($\times 10^6$ cells.ml ⁻¹)	Serum protein
Bilateral ablation	20.69 \pm 0.621 ^a	105.63 \pm 6.147 ^b
Unablated	19.57 \pm 0.728 ^a	149.2 \pm 14.474 ^b

Effect of different water exchange regime: The influence of water exchange regime on the THC and the bacterial load of culture water are presented in Fig. 5. The lobsters in different daily water exchange regimes (25, 50 and 75%) for 30 days had a comparatively lower THC (17.38, 12.92, and 8.73%). There was significant difference ($p < 0.05$) in the THC between the lobsters maintained at 25, 50% water exchange regime and control group maintained at 90% water exchange regime. However there was no significant ($p > 0.05$) difference in THC in the lobsters maintained at 75% water exchange regime and 90% water exchange regime. The culture water of lobsters grown in different water exchange regime (25, 50 and 75%) had a THB (81.86, 48.80 and 21.84%) and *Vibrio* sp. load (86.20, 77.83 and 18.40%) higher than the culture water of lobsters maintained at 90% water exchange regime.

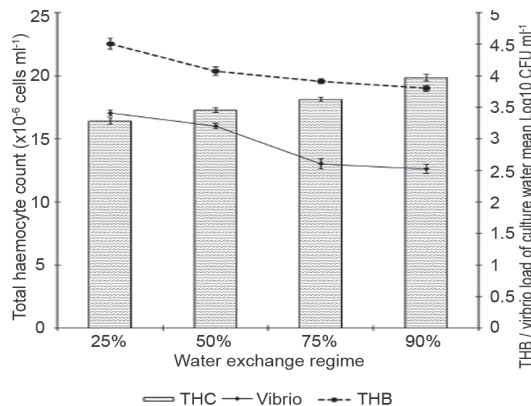


Fig. 5. Total haemocyte content in the *Panulirus homarus* maintained in culture waters with different water exchange regimes and total aerobic heterotrophic bacteria, *Vibrio* load of culture water. Values are mean \pm SEM

Discussion

Shell disease caused by chitinolytic microbes invading crustacean exoskeleton is reported from stressful environment like intense aquaculture systems (Sindermann and Lightner, 1988) impounded population (Therault *et al.*, 2008) or polluted natural environments (Ziskowski *et al.*, 1996). Similar to our studies reduction in THC associated with bacterial infections was reported by Abraham *et al.* (1996) and Manjula *et al.* (1999) in this same species; in the American lobster, *Homarus americanus* by Stewart *et al.* (1983) and in *P. cygnus* kept under post harvest stress by Jussila *et al.* (1997). As lowered THC is an indicator of stress in crustacea (Verghese *et al.*, 2007), a sharp decline in the THC recorded in this study might indicate lowering of immunocompetence as also seen in lobsters associated with various stressors such as bacterial infections (Söderhäll *et al.*, 1988) or environmental stressors (Smith *et al.*, 1995). Total haemocyte count observed for the control healthy lobsters during the study were comparable to the THC for wild collected *P. homarus* (Manjula *et al.*, 1997) and the American lobster, *H. americanus* (Cornick and Stewart, 1978). There was a significant positive ($p < 0.01$) correlation between the serum protein content and THC in *P. homarus*. Similar observations were earlier reported in the lobster, *H. americanus* (Stewart *et al.*, 1969) and the crab, *Cancer pagurus* (Vogan *et al.*, 2001).

The respiratory pigment, haemocyanin, constitutes approximately 90–95% of the serum protein content in crustaceans (Uglow, 1969; Hagerman, 1983) and the reduction in the serum protein of stressed crustaceans like shrimp or lobsters, as in this study, may be mostly due to decrease of haemocyanin and specific immune proteins (Persazzola *et al.*, 2002). One of the important consequences of lowered serum protein in the European lobster, *Homarus gammarus* was the concomitant lowering of haemocyanin and reduced oxygen transport to the muscles during active period (Hagerman, 1983). Lowered haemocyanin may also result in significant increase in clotting time (Glavind, 1948). In *P. homarus*, the clotting time showed a significant negative correlation with serum protein content and the haemolymph of

lobsters with very low serum protein and THC did not clot. Clotting defects in diseased lobsters may be due to decline in granulocyte numbers, increasing number of damaged haemocytes or consumption of the plasma coagulogen in late infection due to an intravascular clotting or to its lack of renewal (Durliat and Vranckx, 1983). Newman and Feng, (1982) also reported significant change in the clotting time in the presence of bacteria in the rock crab *Cancer irroratus*. In *P. homarus*, there was a positive correlation between the bacterial load and clotting time, endorsing the findings in other crustaceans.

Moulting, which is indispensable for growth and replacement of damaged shell (Laufer *et al.*, 2003) was normal in control healthy lobsters and in diseased lobsters with mild shell lesion, though it was slightly delayed in the latter group (Table 1). However, lobsters with a very low THC and non-clotting haemolymph rarely moulted and had an extended intermoult period (Table 1). Moulting inhibiting hormone (MIH) is a neuropeptide, which among other functions regulates glucose metabolism (Liu *et al.* 1997). During stress, levels of Crustacean Hyperglycemic Hormone (CHH) can increase and inhibit moulting (Chang *et al.*, 1998). This might have been the probable reason for the inhibition of moulting in severely diseased lobsters. Additionally presence of bacteria in the haemolymph and progress of wound healing process might have caused delayed moulting in *P. homarus* as reported in the crayfish, *Procambarus clarkii* (Vafopoulou *et al.*, 2007).

Total haemocyte count in *P. homarus* starved for 45 days declined sharply compared to lobsters fed to satiation with green mussel or pellet feed. Decreases in THC after starvation may be an indication that the haemocytes are influenced by nutritional condition as observed by Cheng (1981) in bivalves. Apart from their cellular defense function, the haemocytes are also involved in nutrient digestion and transportation (Feng *et al.*, 1977). The haemocytes may thus absorb nutrients from the digestive gland and pass them directly to other tissues (Cheng, 1996). However, if the organism is subjected to long-term starvation, as in this study, this function may be reversed and a reversible mobilization from hemolymph into soft tissues will take place in order

to compensate for the lack of nutrients (Oubella *et al.*, 1993). A significant decrease in THC and serum protein was also observed in lobsters fed with artificial diet compared to control lobsters on green mussel diet. Juvenile spiny lobsters waste a significant quantity of pellet through external fragmentation before sweeping the particles into their mouth. Up to 50% of pellet food is wasted due to poor consumption efficiency and inefficient feeding behaviour of lobsters (Sheppard *et al.*, 2002). Hence, long term feeding of artificial pellets results in lowered nutritional status in lobsters. Stewart *et al.* (1967) have also shown nutritional status to affect hemocyte level and hemolymph serum protein in the American lobster *H. americanus*. They suggested the use of these two parameters for evaluating the nutritional adequacy of a diet. Similarly, Castell and Covey (1976) registered a decrease in serum protein and THC when American lobsters were fed on a non saponifiable sterol deficient diet. Haemocyte activities including circulating haemocyte frequency and phagocytic oxidative activities were also reported to be severely affected by nutritionally deficient diet in Pacific oyster *Crassostrea gigas* and the clam *Ruditapes philippinarum* (Delaporte *et al.*, 2003).

A marked reduction in THC (43.7%) after unilateral eyestalk ablation was reported in the shrimp, *Farfantepenaeus paulensis* by Persazzola *et al.* (2002) whereas Maggioni *et al.* (2004) did not find any significant difference in THC in the female shrimp, *Litopenaeus vannamei* 14 days post unilateral ablation. In contrast, bilaterally eye stalk ablated *P. homarus* had a significantly higher THC and serum protein than their unablated counterparts 30-35 days post ablation. This variation in THC response to ablation may be partly attributed to the difference in the resistance and response of shrimp from the lobsters to ablation. In decapod crustaceans, Crustacean Hyperglycemic Hormone release is a potential indicator of stress. Crustacean eyestalk is a pivotal neuroendocrine centre that produces several neurohormones including the CHH (Fingerman, 1997). Chang *et al.* (1999) have reported a significantly lower level closer to resting level of CHH in bilaterally ablated American lobster, *H. americanus*. Hence, it is possible that the magnitude

of stress caused by ablation on the haematological parameters is manifested to a lesser extent in lobsters than in shrimps.

Environmental stress results in a decline in haemocyte count, phenoloxidase activity and other enzyme activities relevant to immunity, and an increase in susceptibility to pathogens in shrimps and lobsters (Gomez-Jimenez *et al.*, 2000; Cheng and Chen, 2000; Wang and Chen, 2006). *Panulirus homarus* maintained in 25% and 50% water exchange regimes had a lower THC compared to the lobsters maintained in 75% and 90% water exchange regime. During this study, the lobsters in different water exchange regimes were fed with whole green mussels with crushed shells. Ammonia and other toxic wastes accumulate in the culture water originating from the uneaten feed, lobster excretory products and microorganisms surviving in the waste. Concomitantly, there was a very high THB and *Vibrio* sp. load in the culture water of these two groups (with 25% and 50% water exchange). Hu *et al.* (2009) have reported alteration in immune parameters including THC, DHC, phenoloxidase activity and phagocytic activity of haemocytes, bacteriolytic and antibacterial activity in the haemolymph of *L. vannamei* exposed to hypoxia of 3.0 and 1.5 mg/L. In addition to this, environmental stressors may also lead to fluctuations in the concentrations of noradrenaline, dopamine and serotonin in crustacean haemolymph (Zatta, 1987). Hence, it may be inferred that environmental stressors like hypoxia and or high ammonia may result in neuroendocrine response and changes of neurotransmitter concentration in the haemolymph and concomitantly makes the animals more susceptible to pathogens.

In conclusion, alteration in the culture conditions whether biological or environmental, affects haematological parameters of *P. homarus* depending on the magnitude of stress. Among the haematological parameters evaluated, the total haemocyte count (THC), total protein concentration of serum and clotting time were prominent indications to predict the health of lobsters. A prolonged reduction in THC in cultured lobsters may lead to reduced immunocompetence and make

them more susceptible to opportunistic pathogens. Therefore disease prevention measures should aim at providing nutritionally ideal diet, better water quality management practices and optimal culture conditions.

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