Fishery Technology 50 (2013) : 294 - 300

Effect of Salinity Stress on the Biochemical and Nutritional Parameters of Tiger Shrimp *Penaeus monodon*

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Abstract

Biochemical and physiological changes in *Penaeus monodon* on exposure to salinity stress conditions were studied. Total haemocyte count (THC), total protein concentration (PC), phenoloxidase (PO) activity, respiratory burst and osmolality were measured in low (3‰) and high salinity (55‰) stressed shrimps in comparison to control shrimp samples maintained at 28‰ salinity. THC (18 ± 2 x 10^6 cells ml^{-1}) was significantly lower in the shrimps exposed to high salinity stress at one week interval when compared with the control group (38 ± 2 x 10^6 cells ml^{-1}) while no significant difference was observed in THC between the control and low salinity stressed shrimps at any time interval. Total protein concentration was maximum at 6 h interval under low salinity (195±10 mg ml^{-1}) and at 48 h under high salinity (219±15 mg ml^{-1}) conditions. Respiratory burst in the haemolymph was significantly high at 24 h in low salinity (3.71±0.01) and high salinity (0.905±0.01) stressed shrimps. Significant decrease in osmolality levels in low salinity stressed group was identified at 6 h whereas, in high salinity stressed group, maximum increase was at 48 h. Moisture content and lipid in shrimp muscle were significantly ($p<0.05$) higher in low salinity stress conditions than in high salinity conditions. Total ash content was significantly high at 55‰ salinity, whereas, no significant difference was observed in protein content in the muscle tissues of tiger shrimp maintained at 3‰ and 55‰ salinity conditions.

Keywords: *Penaeus monodon*, salinity stress, biochemical parameters, nutritional parameters

Received 16 August 2012; Revised 09 January 2013; Accepted 01 June 2013

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Introduction

Penaeid shrimps can survive in a wide range of salinities (3-50‰) and are cultured under a variety of conditions in many tropical and subtropical areas of the world. However, abiotic and biotic factors may cause stress to the animals during culture period. Cultured shrimps are exposed to climatic changes that affect the physico-chemical quality of water, of which, salinity and temperature have great impact on euryhaline penaeids, affecting their metabolism, growth, molting and survival (Staples & Heales, 1991; Chen et al., 1995). Changes in the physical (temperature), chemical (water quality) or biological factors (nutrition or infections) often reduce the immunity making them highly vulnerable to diseases such as WSSV (Annies & Rosamma, 2007). Effects of stress factors on shrimp can be assessed by measuring physiological and biochemical parameters. The innate immune system in crustaceans plays a primary role to recognize and respond to diseases. The three morphologically different haemocyte types in crustaceans: hyaline, semi-granular and granular cells carry out different functions, which are important in the host immune response including recognition, phagocytosis, cytotoxicity and cell–cell communication (Johansson et al., 2000). The haemocytes in crustaceans are also involved in melanization reaction which is another important innate immune function. Prophenoloxidase (proPO) the inactive form of phenoloxidase (terminal enzyme in the proPO system), is a complex system of proteinases, pattern recognition proteins and proteinase inhibitors (Soderhall & Cerenius, 1998). Osmotic and ionic regulation is an important mechanism of environmental adaptation in crustaceans (Péqueux, 1995). Haemolymph osmolality is one of the physiological biomarkers of stress in penaeids (Lignot et al., 2000). The effect of environmental stress factors such as ammonia (Chen

& Cheng, 1993), temperature (Qiu et al., 2011), salinity (Chen & Lin, 1994) and the ability of shrimps to osmoregulate, evaluated by the osmoregulatory capacity has been reported.

This study was aimed at measuring the effects of low salinity (3‰) and high salinity (55‰) on the biochemical and physiological parameters of *P. monodon*. For this purpose, parameters such as total haemocyte count (THC), phenoloxidase activity, respiratory burst (release of superoxide anion), osmolality and total protein concentration were examined as stress indicators in cultured shrimps.

Materials and Methods

Intermoult stage *P. monodon* shrimps (10-15 g) were collected from shrimp farms located at Chennai, India and maintained at Muttukadu experimental station of the Institute (Central Institute of Brackishwater Aquaculture, Chennai) for salinity stress induction experiments for a period of two weeks under standard conditions (28°C, pH 7.8 and 28‰ salinity). The experiment was conducted in two separate batches of shrimps. Each batch of the shrimps was divided into two groups. In one group, the salinity of sea water (28‰) was decreased gradually by 2‰ per day by addition of fresh water until the salinity reached 3‰. In the other group, the salinity was increased by 2‰ per day until it reached 55‰ by addition of brine solution. Two separate control groups (shrimps at 28‰) were maintained in two different batches at different times for both the batches of experiments. Biochemical analysis was carried out at 6 h, 24 h, 48 h, one week and two weeks intervals using six shrimps at each time point from control and test (low and high salinity) groups. Shrimp haemolymph was collected from the first abdominal segment in equal volume of the anticoagulant solution (0.45 M sodium chloride, 0.1 M glucose, 30 mM sodium citrate, 26 mM citric acid, 10 mM EDTA, pH 7.0). Haemolymph samples were collected at different time intervals (6 h, 24 h, 48 h, one week and two weeks) post salinity stress.

THC was assessed using Neubaeur haemocytometer and values were expressed as the total number of cells ml^{-1} of haemolymph (Le Moullac et al., 1997). Phenoloxidase activity was measured using spectrophotometer (Model U-2000, Hitachi, Tokyo, Japan) by the method of Hernandez-Lopez et al. (1996). Production of superoxide anion was quantified by reduction of nitroblue tetrazolium (NBT) to formazan following the method of Song & Hsieh (1994). The total protein concentration was measured spectrophotometrically at OD_{590} (Lowry et al., 1951). Osmolality of the haemolymph samples (10 µl) and the medium was measured in Wescor 5520 vapour pressure osmometer (Wescor, USA) and expressed in milliosmols per kilogram (mOsm kg^{-1}).

Shrimp muscle tissues were collected in triplicates for proximate analysis. The analysis was carried out as per standard protocols of AOAC (1990). Moisture content was analyzed by drying samples overnight at 105°C. Protein content was measured by estimating the nitrogen content and multiplying with factor 6.25. Ether extract (lipid) was analyzed by using petroleum ether. Total ash was analyzed by igniting the sample in muffle furnace.

Statistical analysis was performed using SPSS software and the values were expressed as mean ± SE for six shrimps in each group. The statistical significance was determined by one-way analysis of variance (ANOVA). Significant difference was calculated between control and stressed ones in the same time duration and the values with $p<0.05$ were considered significant.

Results and Discussion

No significant difference in the THC between the control and low salinity stressed (3‰) group of shrimps at all time intervals of sampling was observed (Fig.1a). However, THC (18×10^6 cells ml^{-1}) was significantly lower in the shrimps exposed to high salinity stress (55‰) at one week time interval when compared with the control group (38×10^6 cells ml^{-1}) which was maintained at 28‰ (Fig. 1b). This decrease in THC levels by 52% is in agreement with results reported by other workers (Perazzolo et al., 2002; Wang & Chen, 2005, 2006). Significant increase by 11% in THC has been reported for the shrimps transferred from 25‰ to 35‰ (Wang & Chen, 2005). Duration of at least 48 h was found to be essential for shrimps (*P. monodon*) to adjust to new salinity conditions and regulate normally by stabilizing osmolality concentration of the hemolymph (Diwan et al., 1989). Similar observations of increase in haemocyte number (about 20%) in *P. paulensis* exposed to high salinity (34‰) in comparison to low salinity (22 and 13‰) has been reported by Le Moullac & Haffner (2000). These changes in THC levels may be due to factors such as cell proliferation, cell movement from tissues into circulation or the water osmosis between

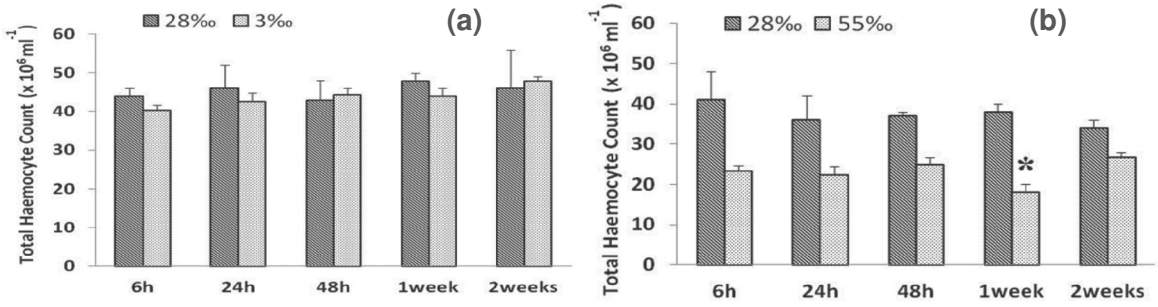

Fig. 1. Effect of salinity stress on the total haemocyte count of shrimps at different time intervals (a) Low salinity stress (b) High salinity stress

*Mean values revealing significant difference ($p<0.05$)

haemolymph and medium for osmotic regulation which needs further clarification (Wang & Chen, 2006).

Significant increase in the protein levels was observed in shrimps that were exposed to 3‰ salinity conditions at 6 h (53 %) and at 24 h (50%) (Fig. 2a). Significant increase in the protein levels was also observed between control and high salinity stressed group (55‰) by 72% at 24 h, 87% at 48 h and 58% at two weeks intervals (Fig. 2b). The mean total protein concentration in the low salinity group (183 ± 1 mg ml^{-1}) was observed to be less than the mean total protein concentration in the high salinity group (186 ± 9 mg ml^{-1}). Similar observations of low mean value (119±40 mg ml^{-1}) for total serum protein concentration has been reported in shrimps *F. paulensis* (20.0± 2.5 g) maintained in lower salinity (13‰) when compared to the shrimps maintained at higher (34‰) salinity (146 ± 30 mg ml^{-1}) (Perazzolo et al., 2002). Initial observations revealed that the shrimps at higher salinity had a lower serum protein concentration than that of the shrimps in lower salinity which increased in the subsequent weeks. Considerable decrease in protein concentration was observed in shrimps kept for one week at low salinity (Perazzolo et al., 2002). In the present study, we also observed a decrease in the protein concentration both in low (168 ± 0.9 mg ml^{-1}) and high salinity (160 ± 15 mg ml^{-1}) shrimp groups at one week interval which increased in the subsequent week to 183 ± 9 mg ml^{-1} and 186 ± 9 mg ml^{-1} in low and high salinity groups respectively.

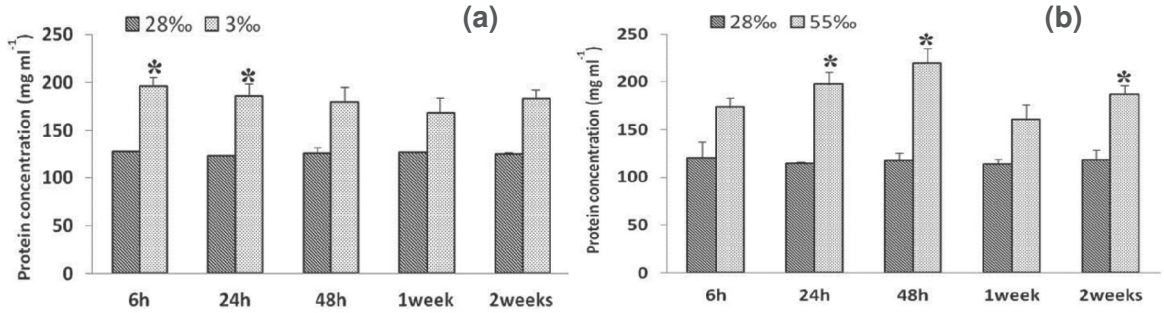

Fig. 2. Effect of salinity stress on the total protein concentration of shrimps at different time intervals (a) Low salinity stress (b) High salinity stress

*Mean values revealing significant difference ($p<0.05$)

However, the effect of salinity on plasma protein concentration in juvenile yellow leg shrimp *(Penaeus californiensis*) when acclimated at different salinities (28, 32, 36, 40 and 44‰) for a period of 20 days, revealed no change in total protein levels (Vargas-Albores et al., 1998).

No significant difference was observed in the phenoloxidase activity levels between the control and salinity stressed group of shrimps at any of the time intervals (Fig. 3a and 3b) which may be attributed to the different salinity range adopted. As decrease in phenoloxidase activity after 12 h, by 48%, 44% and 24% has been observed in the shrimps (*P. monodon)* (16-23 g) held in 25‰ and then transferred to low salinity (5‰, 15‰) and high salinity (35‰) levels respectively (Wang & Chen, 2006). Significant decrease was also observed after 96 h in phenoloxidase activity (40% and 27%) at low salinity of 5‰ and 15‰ respectively (Wang & Chen, 2006). Similar observations of decrease in the phenoloxidase activity by 67 and 33% after 12 h , 41 and 18% after 24 h in the shrimp in *L. vannamei* (12 to 13 g), transferred from 25‰ to 5‰ and 15‰ respectively has been reported (Wang & Chen, 2005).

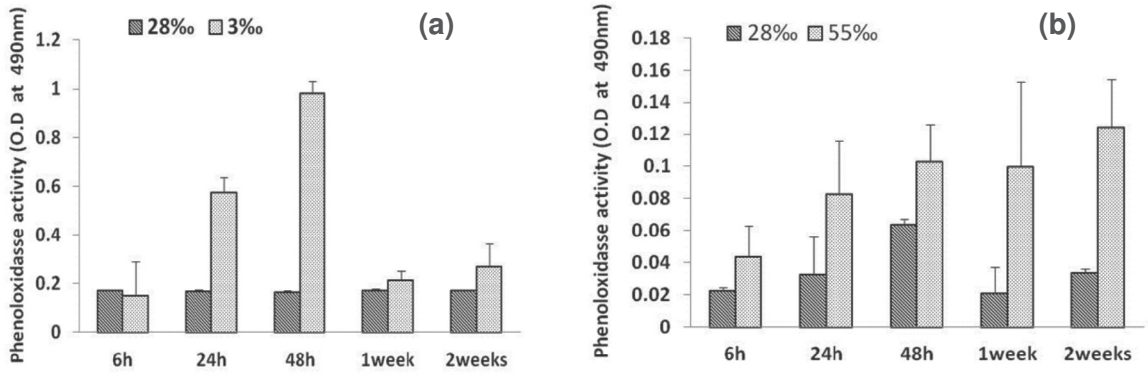

Fig. 3. Effect of salinity stress on the total phenoloxidase activity of shrimps at different time intervals (a) Low salinity stress (b) High salinity stress

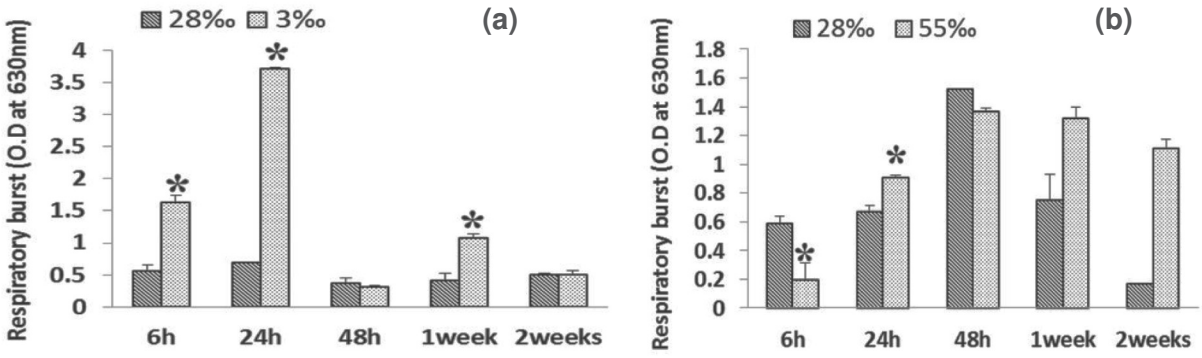

Fig. 4. Effect of salinity stress on the respiratory burst activity of shrimps at different time intervals (a) Low salinity stress (b) High salinity stress

*Mean values revealing significant difference ($p<0.05$)

Significant increase in respiratory burst in low salinity stressed group (3‰) was observed at 6 h, 24 h and one week intervals after which the respiratory burst activity levels came back to that of normal values in the subsequent week (Fig. 4a). The significant increase in the respiratory burst activity levels by 35% in high salinity stressed group (55‰) observed after 24 h interval (Fig. 4b) is in agreement with 16% increase in the respiratory burst in the shrimps transferred from 25‰ to 35‰ (Wang & Chen, 2005). Increase in phenoloxidase activity and respiratory burst in *L. vannamei* were a consequence of increase in the THC (Wang & Chen, 2005).

Haemolymph osmolality of *P. monodon* was linearly related to external osmolality. The isosmotic point computed from the linear regression was 707 mOsm kg^{-1}. The osmoregulatory capacity of shrimps are presented as haemolymph osmolality (Fig. 5a) in relation to osmolality of the external medium. Significant decrease in the osmolality levels in low salinity stressed group (3‰) was identified at 6 h (337.66 ±100 mOsm kg^{-1}) intervals when compared with the control group (594.75 ± 4.3 mOsm kg^{-1}) (Fig. 5b). Significant increase in osmolality levels in high salinity stressed group (55‰) was identified at 6 h (1175 ± 67 mOsm kg^{-1}), 24 h (1111 ± 75 mOsm kg^{-1}), 48 h (1241 ± 98 mOsm kg^{-1}) and one week (1115 ± 37mOsm kg^{-1}) intervals when compared with the control group (757 ± 22 mOsm kg^{-1}) (Fig. 5c). Haemolymph osmolality increased directly with salinity and exhibits a close relationship between the salinity tolerance and osmoregulatory capacity. A similar finding was also reported in decapod *Crangon crangon* (Ude Cieluch et al., 2005). *P. monodon* may regulate haemolymph osmolality towards a chronic change in salinity from normal (28‰) to low (3‰) or high (55‰) and exhibit

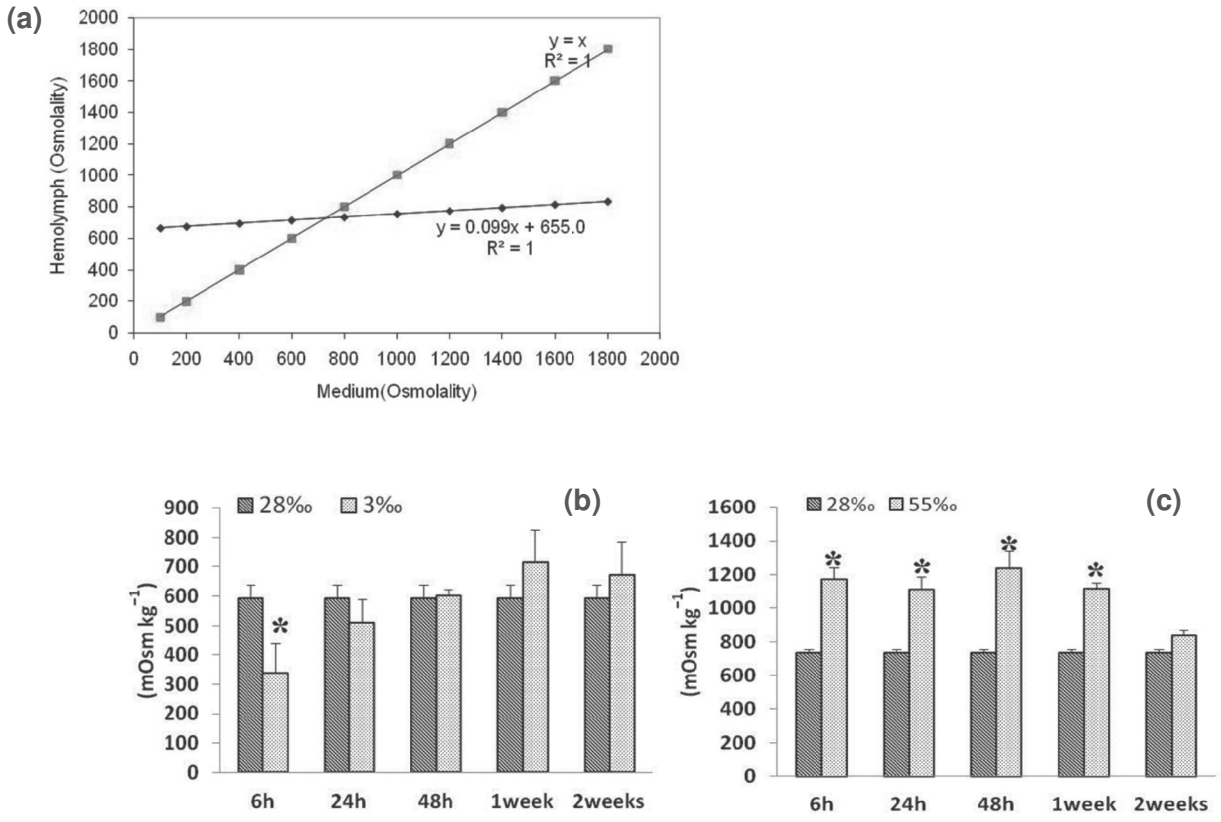

Fig. 5. (a) Relationship between haemolymph osmolality of *Penaeus monodon* and external osmolality (b) Effect of low salinity stress on the osmolality of shrimps at different time points (c) Effect of high salinity stress on the osmolality of shrimps at different time points

*Mean values revealing significant difference ($p<0.05$)

Table 1. Proximate composition of shrimps subjected to low and high salinity stress conditions

Sample Collection	Moisture		Crude Protein		Lipid		Total Ash	
	Low (3%)	High (55%)	Low (3%)	High (55%)	Low (3%)	High (55%)	Low (3%)	High (55%)
6 h	77.32	76.23	82.54	79.61	2.59	2.36	6.56	7.62
24 h	75.24	77.64	83.34	83.62	2.77	2.05	6.45	6.61
48 h	79.75	75.74	82.13	83.93	2.79	1.73	6.00	7.56
1 week	78.93	77.02	83.19	83.80	2.42	1.91	5.96	7.36
2 week	77.78	75.58	84.21	84.82	2.47	1.71	5.90	7.30
Control	77.61	77.67	82.87	86.90	2.28	2.41	6.61	6.05
Mean* ± SE	77.77[a] ±0.63	76.65[b] ±0.38	83.05[a] ±0.29	83.78[a] ±0.97	2.55[a] ±0.08	2.02[b] ±0.12	6.24[a] ±0.13	7.08[b] ±0.25

*Mean values bearing different superscripts with in a column (parameter) differ significantly ($p<0.05$).

hyper-osmotic or hyper-ionic regulation at low salinity condition and hypo-osmotic or hypo-ionic regulation at high salinity condition. The present study showing the hyper-osmotic behaviour of *P.monodon* in the hypo-osmotic medium and hypo-osmotic behavior in hyper-osmotic medium were in consensus with Diwan et al. (1989) and Chen & Lin (1994).

Changes in proximate composition of shrimp subjected to salinity stress are given in Table 1.

In low salinity stress conditions, the moisture content in muscle was significantly ($p<0.05$) higher than in high salinity conditions. Similar results of inverse relationship of moisture with rearing salinity have been observed in *Penaeus chinensis* (Chen et al., 1995) in tail muscle of *Penaeus latisulcatus* (Sang & Fotedar, 2004) and in *Litopenaeus vannamei* (Huang et al., 2004; Velaquez et al., 2007). Significantly ($p<0.05$) higher lipid values were observed in low salinity stress conditions compared to high salinity in the present study (Table 1). Similar results were reported in *L. vannamei* maintained at 3‰ and 32‰ salinities (Li et al., 2007). The total ash content in muscle of tiger shrimp reared at 55‰ was significantly ($p<0.05$) higher and this may be due to the role of the ions in osmoregulation and the macro minerals like calcium, magnesium, potassium and sodium that are easily accessible to the shrimp from water (Davis & Gatlin III, 1996).

In conclusion, the study reveal adaptation of *P. monodon* to varying salinity changes (3‰ or 55‰) by exhibiting significant difference in the levels of total haemocyte count, total protein and respiratory burst. The changes in these biochemical and physiological parameters may be used as stress indicators in cultured shrimps in response to biotic and abiotic stress.

Acknowledgement

The authors greatly acknowledge NAIP (ICAR) for providing the funding support under the project "Bioprospecting of genes and allele mining for abiotic stress tolerance".

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