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Effect of Short Term Temperature on Physiological Body Indices of Two Estuarine Venerid Clams *Katelysiaopima* and *Meretrixmeretrix* (Mollusca: Bivalvia)

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Abstract: *In the present investigation, the physiological body indices like hepatopancreas index (HI), gonadal index (GI) and condition index (CI) of two estuarine clams (Katelysiaopima and Meretrixmeretrix) were estimated after exposing to three experimental temperatures (20, 25 and 35°C). In experimental design, both the clam species were exposed to selected temperatures for 192 hours (8 days) and during the exposures the body indices were determined at each 48 hours of interval. After exposing to experimental temperatures, in both the clams, all body indices like HI, GI and CI were considerably declined ($p<0.001$) after 192 hours of exposure. The highest decline in body indices has been noted particularly at high temperature (35°C) followed by low temperature like 20°C and 25°C. On the basis of obtained results, it confirms that, the gonadal index was closely related to hepatopancreas index and increment of condition index is directly associated with gonadal development. Relatively, M. meretrix species was more sensitive towards temperature change and it has been evident from the maximum decline in body indices than K. opima. Hence, the quantitative analysis of body indices among clams might be useful in assessing the probable effect of short-term change in water temperature on physiological process.*

Keywords: Physiological body indices, Clams, Hepatopancreas, Temperature.

Introduction

Coastal zones like estuaries, creeks, backwaters and lagoons are ecologically as well as economically important and are among those areas which strongly affected by global climate change (Laning *et al*., 2010). The environmental changes, affects the metabolic activities and biological performance of an organism, however the mechanism of action of environmental factors and their risks on marine ectothermal organisms that are not yet fully understood (Melzner *et al*., 2009; Portner, 2010).

Among environmental factors, temperature plays the crucial role in all facets of organisms life (Gillooly *et al*., 2001). To measure the environmental or temperature consequences, the behaviour and physiological alterations are considered as functional attributes to identify the stress. Generally, stress conditions cause energy limitation, which reduces the energy investment in to production hence, it will have the direct impact on the fitness of an organism (Laning *et al*., 2010).

Physiological fitness of an organism under various environmental stressor particularly temperature can be ascertained by assessing the physiological body condition indices like hepatopancreas index, gonad index and condition index. According to the literature, hepatopanceras and gonad are the major organs of invertebrate, which stored the reserve nutrients and these reserves are mobilized according to seasonal cycle (Galap *et al*., 1997; Lomovasky *et al*., 2004) or reserved

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nutrients of hepatopancreas are utilized for gonadal development (Sokolowicz *et al*., 2006). Condition index has been employed by various authors to determine the physiological activity like reproduction and growth (Li *et al*., 2009; Mladineo *et al*., 2007; Celik *et al*., 2012; Li *et al*., 2011). Literature reported that, the condition index among bivalve species fluctuates by change in water temperature, food availability and season (Flores-Vergara *et al,* 2004; Delgado *et al*., 2004; Ojea *et al*., 2004; Orban *et al*., 2006).

Earlier, few authors have reported the effect of temperature on the condition index and gonad maturity of bivalves (Newell *et al*., 1982; Mac Donald and Thompson, 1985; Martinez and Perez, 2003). In recent, Suja and Muthiah, (2009) studied the synergistic effect of starvation and temperature on hepatopancreas somatic index, gonad somatic index and condition index of Marcia opima from Tuticorin Bay. Matias *et al*., (2008) documented the effect of temperature on condition index of Ruditapes decussates.

This attempt has been ascertained to detect the effect of short-term exposure of temperature on hepatopancreas index, gonad index and condition index of two commercially important venerid clams (Katelysiaopima and Meretrixmeretrix) of Bhatye estuary, Ratnagiri coast. This study eventually evaluates the physiological fitness or the probable effects of both decline and elevated temperatures on sessile organism. Present study might be useful in understanding the nutritional value of commercially important clams at a different regime of temperature and this information may benefit from bivalve aquaculture as well as fishery point of view.

Materials and Methods

Animal Collection and Maintenance

In this study, experimental clams like Katelysiaopima (Gmelin, 1791) and Meretrix meretrix (Linnaeus, 1758) were collected from Bhatye estuary during low tide with the help of local fishers during April 2010. The collected clams brought to the laboratory, carefully cleaned to remove the adhered fine sediments. All clams were segregated according to their shell length, only mature/average size clams were selected for experimental purpose. The average shell length (38-42 mm) of K. opima and (45-50 mm) of M. meretrix has chosen for further experimentation. After segregation and selection, clams were acclimated in plastic container (3 × 2 ft) for 48 hours at room temperature (30 ± 1°C).

Experimental Design

Healthy clams were exposed to three experimental temperatures (20°C, 25°C and 35°C) along with a control group (30 ± 1°C) for 8 days i.e. 192 hours. The 20°C and 25°C experimental groups were considered as low temperatures, while 35°C group treated as a high temperature. The low temperatures (20°C and 25°C) were maintained by ice-cold water, while high temperature (35°C) was controlled by thermostat. In each group of experimental temperatures, thirty (30) clams were exposed. During experimental exposure, water from the container was changed after every six hours. Throughout the experimentation, 38 ppt sea water was used.

Experimental Analysis

During 192 hours of exposure, five individuals were removed after every 48 hours from experimental sets to assess physiological body indices like hepatopancreas index, gonad index and condition index. These individuals were sacrificed and flesh and shells were separated. The hepatopancreas and gonad were carefully separated from each other, and partially dried by blotting paper to remove the extra water. After blotting, tissues were weighed on monopan electronic digital balance. For drying, the flesh and shells were kept in oven at 60° C up to 72 hours to determine the constant dry meat and shell weight.

In experimental analysis, hepatopancreas index (HI) and Gonadal index (GI) was estimated as described by Giese (1959). The condition index (CI) was analyzed as suggested by Rainer and Mann (1992). Formulae for HI, GI and CI are as follows,

$$1.\ \text{Hepatopancreas index (HI)} = \frac{\text{Wet weight of hepatopancreas (g)}}{\text{Wet weight of meat (g)}} \times 100$$

$$2.\ \text{Gonadal index (GI)} = \frac{\text{Wet weight of gonad (g)}}{\text{Wet weight of meat (g)}} \times 100$$

$$3.\ \text{Condition index (CI)} = \frac{\text{Meat dry weight (g)}}{\text{Dry shell weight (g)}} \times 100$$

Statistical Analysis

All the results of HI, GI and CI were the mean of five separate analyses with ± SD. One-way ANOVA was used to test the significant difference between means of experimental exposure (20°C, 25°C and 35°C) with the means of control group (30 ± 1°C). All statistical difference was accepted at the 0.05 level of significance using the Graph Pad software version 5.04.

Results and Discussion

In the present investigation, physiological body indices like hepatopancreas index (HI), gonad index (GI) and condition index (CI) were studied in two clam species *Katelysiaopima* and *Meretrixmeretrix* under short-term exposure of temperatures (Table 1 and 2).In both the clam species, all body indices have been affected significantly after exposure to both increasing and decreasing temperatures.

Hepatopancreas Index (HI)

After 192 hours of temperature exposure, in *K. opima* the hepatopancreas index (HI) was reduced significantly (46%) at high temperature i.e. 35°C followed by low temperatures like 20°C (44%) and 25°C (33%), while there was (55%, 40% and 28%) reduction at 20°C, 25°C and 35°Cin *M. meretrix* species respectively. During exposure of 192 hours, at all temperature ranges (20°C, 25°C and 35°C) the *K. opima* clam showed decline ($p<0.01$) in HI at 48 hours, while 96 hours onwards it was declined slowly up to 192 hours. In *M. meretrix*, at 20°C, HI was decreased ($p>0.05$)at 48 hours, while it was declined ($p<0.05$)at 96 hours. The significant ($p<0.001$) decrease in HI was noticed at 192 hours, but less decline was recorded at 144 hours. At 25°C, the HI was declined ($p>0.05$) at 48, 96 hours, 144 hours, while ($p<0.001$) decline at 192 hours respectively. At high temperature (35°C), the HI was successively decreased from 48 to 192 hours of exposure. At 48 hours ($p<0.05$) and 96 hours ($p<0.01$) decline was recorded, however, it was reduced ($p<0.001$) 192 hours.

In both the clams, maximum reduction of HI was recorded particularly at high temperature i.e. (35°C). Relatively, maximum decline in HI was observed in *M. meretrix* especially at high temperature however, from 20°C and 25°C ranges the maximum decline was noted in *K. opima* clam.

Among the environmental factors, temperature is one of the most vital and relevant abiotic factors, which controls all facets of organisms at biological and ecological levels (Heilmayer *et al.,* 2004; Resgalla Jr. *et al.,* 2007). Temperature regulates the extent of species distribution, physiological processes such as feeding, respiration, growth and reproduction (Davenport, 1979; Newell and Branch, 1980; Shumway, 1982). Therefore, in organism adaptation to varying environmental temperature is major challenge in evolutionary adaptation. The adaptation activity is totally relies on the large extent of the organisms ability to compensate metabolic rate under high influence of temperatures at both short-term and long-term exposures (Hochachka and Samero, 2002; Portner, 2002 a, b). According to literature, various body indices like hepatopancreas index, gonad index and condition index has been utilized to assess the physiological measure like reproductive output (Suja and Muthiah, 2009). According to Sokolowicz *et al.,* (2006) the reserved materials and their fluctuation in the hepatopancreas tissue is subjected to gonadal development.

In this study, after exposure to various temperature ranges the hepatopancreas index (HI) in both the clam species was maximally reduced at high temperature; about 46% and 55% decrease was recorded in *K. opima* and *M. meretrix* respectively. The hepatopancreas functionally plays intermediary role in metabolism and it also acts as a store house for fat bodies (Smith *et al.,* 1975; Bhide *et al.,* 2006).

It is generally accepted that, the nutrients are principally stored in the hepatopancreas tissue or digestive gland. These nutrients are utilized for growth and development, even in reproductive development the hepatopancreas supplies nutrients to gonad for gonadal development. In the present study, after exposure to various temperature maximum reductions in HI was occurred at high temperature. The metabolic rate increases, consequently the scope for growth reduces at high temperature (Widdows, 1978). In context of increased metabolic rate, clam might be utilized available nutrients to sustain at undesired condition instead of to use for growth and development. Therefore, in order to survive at increased temperature regime, the depletion in HI was occurred.

Sastry, (1968) reported that, the hepatopancreas index of scallops *Aequipectenirradians* was depleted at high temperature. He suggested that, the available reserves in scallops have been utilized for maintenance at high temperature regime. Suja and Muthiah (2009) observed considerable difference in digestive index of unfed treatment however, in fed treatment there was no significant change was recorded in HI at two rearing temperatures (23°C and 28°C). Sastry (1968) suggested that, the digestive gland index was maximum during vegetative and rearing stages.

Gonadal Index (GI)

Gonad index (GI) also decreased significantly in both the clams when exposed to both low and high temperatures. In *K. opima*, after 192 hours exposure, maximum decline (40%) in GI was occurred at high temperature like 35°C than

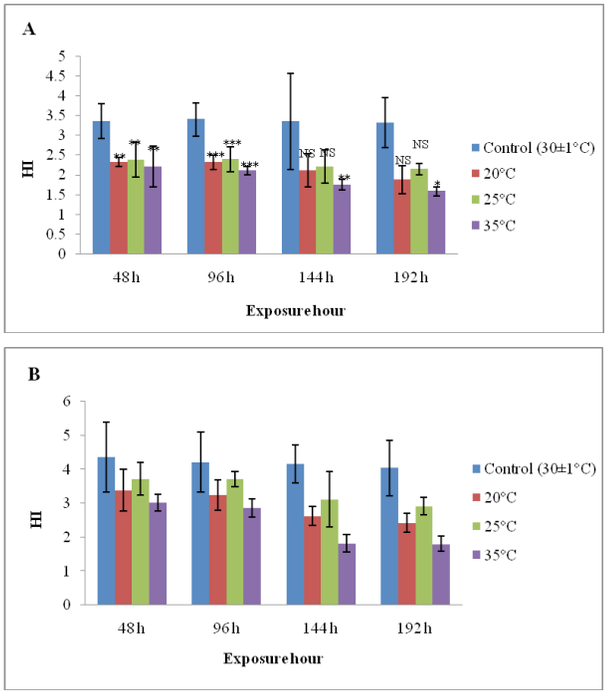

Fig. 1 Effect of temperature on hepatopancreas index (HI) of (A) *K. opima* and (B) *M. meretrix.*

(27% and 24%) at 20 and 25°C. In *M. mere-trix* clam also recorded similar trend of decline GI, the maximum decline (41%) in GI was noted at high temperature (35°C) followed by (36%) at 20°C and (31%) at 25°C respectively.

During 192 hours of exposure, in *K. opima*, the GI was declined significantly ($p<0.01$) in *K. opima* at 48 hours of exposure, while it was ($p<0.05$) declined in *M. meretrix* clam. However, 96 hour onwards the GI was declined successively up to 192 hours in both the clams species. Overall, after 192 hours exposure, the highest decline in GI was detected in clam *M. meretrix* than *K. opima*.

Condition Index (CI)

Condition index (CI) in both the clams has been declined considerably after exposing to both low and high temperatures. Condition index of *K. opima* was decreased significantly at all experimental temperatures after 192 hours of exposure. After 8th day of exposure, the maximum reduction in CI was recorded

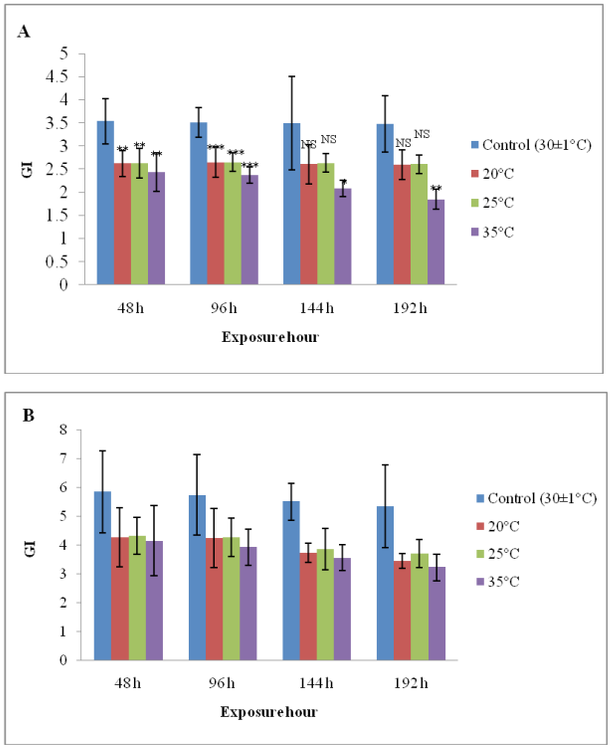

Fig. 2 Effect of temperature on gonadal index (GI) of (A) K. opima and (B) M. meretrix.

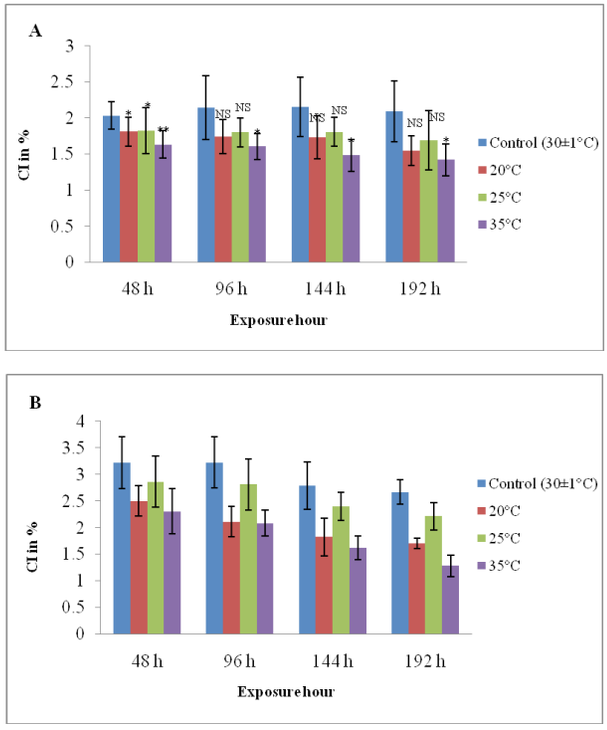

Fig. 3 Effect of temperature on condition index (CI) of (A) *K. opima* and (B) *M. meretrix*

All results are the mean of five observations and bar indicates the ± standard deviations.

($p< 0.001$) = ***, ($p< 0.01$) = **, ($p< 0.05$) = * and ($p>0.05$) = NS.

at high temperature (35°C) than lower ranges (20°C and 25°C). At high temperature, CI was reduced to 32%, while 26% and 19% reduction was recorded at 20°C and 25°C temperature groups respectively. In this clam, at 20 and 25°C the CI was declined ($p>0.05$) during exposure i.e. from 96-144 hours however, at 48 and 192 hours CI was noticed with less ($p<0.05$) reduction. In case of high temperature (35°C), CI was slightly ($p<0.05$) reduced from 96 to 192 hours, but significant ($p<0.01$) reduction was noticed at 48 hours.

In *M. meretrix* clam maximum reduction in CI was noticed at high temperature than low temperatures. After 8th day of exposure, 39% reduction was recorded at 35°C, while at 20°C, 36% reduction and at 25°C, 17% reduction was recorded respectively. In all temperature ranges, CI was suddenly declined at 48 hours however, later i.e. from 96 to 192 hours, there was slow decline in CI. During 192 hours exposure of both high and low temperatures, the CI was considerably declined. At high temperature (35°C), the CI was declined slightly ($p<0.05$) at 48 hours, however significantly ($p<0.01$) at 96-120 hour and ($p<0.001$) declined at 144-192 hours respectively. At low temperature like (20°C), the CI was non-significantly ($p>0.05$) declined at 48 hours, while ($p<0.01$) at 96, 144 hours and ($p<0.001$) reduction was recorded at 192 hours respectively. However, at 25°C the CI was decreased ($p>0.05$) from 48-144 hour, while ($p<0.05$) reduction at 192 hours.

In recent past, several authors well documented the studies on physiological body indices in bivalves and they have reported a strict relationship between the condition index increment and the gonadal development (Hamida *et al.,* 2004; Ojea *et al.,* 2004; Mladineo *et al.,* 2007). Similarly, Abraham (1996) and Suja and Muthiah

Table 1 Effect of temperature on HI, GI and CI of *Katleysia opima* clam.

Exposure hours	Temperature ranges											
	Control (30±1°C)			20°C			25°C			35°C		
	HI	GI	CI	HI	GI	CI	HI	GI	CI	HI	GI	CI
48 h	3.36 ±0.44	3.54 ±0.48	2.03 ±0.18	2.32 ±0.11***	2.62 ±0.28**	1.81 ±0.10*	2.38 ±0.44***	2.62 ±0.31**	1.82 ±0.31*	2.20 ±0.52***	2.44 ±0.42**	1.632 ±0.388**
96 h	3.40 ±0.42	3.51 ±0.31	2.14 ±0.44	2.31 ±0.18***	2.65 ±0.33***	1.74 ±0.33NS	2.38 ±0.31***	2.65 ±0.20***	1.80 ±0.20 NS	2.10 0.11***	2.37 ±0.18***	1.606 ±0.180*
144 h	3.34 ±1.42	3.48 ±1.01	2.15 ±0.41	2.11 ±0.42 NS	2.60 ±0.42 NS	1.72 ±0.30 NS	2.21 ±0.42 NS	2.60 ±0.20 NS	1.80 ±0.20 NS	1.75 ±0.14 *	2.08 ±0.18 **	1.480 ±0.220 *
192 h	3.32 ±0.62	3.48 ±0.60	2.08 ±0.42	1.87 ±0.36***	2.59 ±0.31**	1.54 ±0.20 NS	2.14 ±0.14***	2.60 ±0.20**	1.69 ±0.41 NS	1.58 ±0.11***	1.85 ±0.22***	1.42 ±0.220*

HI= Hepatopancreas index, GI= Gonadal index, CI= Condition index. All results are the mean of five observations with ± SD.
(p< 0.001) = ***, (p< 0.01) = **, (p< 0.05) = * and (p>0.05) = NS.

Table 2 Effect of temperature on HI, GI and CI of Meretrixmeretrix clam.

Exposure hours	Temperature ranges											
	Control(30±1°C)			20°C			25°C			35°C		
	HI	GI	CI	HI	GI	CI	HI	GI	CI	HI	GI	CI
48 h	4.36 ±1.03	5.83 ±1.42	3.21 ±0.48	3.38 ±0.62 NS	4.26 ±1.02*	2.49 ±0.28 NS	3.71 ±0.47 NS	4.30 ±0.64*	2.86 ±0.48 NS	3.02 ±0.24*	4.14 ±1.22*	2.30 ±0.42*
96 h	4.21 ±0.88	5.73 ±1.40	3.22 ±0.47	3.24 ±0.44*	4.23 ±1.03 NS	2.11 ±0.28**	3.70 ±0.22 NS	4.26 ±0.66 NS	2.81 ±0.48 NS	2.86 ±0.26**	3.92 ±0.62*	2.08 ±0.24**
144 h	4.15 ±0.56	5.50 ±0.64	2.99 ±0.44	2.62 ±0.28**	3.72 ±0.33***	1.82 ±0.35**	3.11 ±0.82*	3.85 ±0.72**	2.40 ±0.26 NS	1.82 ±0.26***	3.55 ±0.44***	1.86 ±0.22***
192 h	4.04 ±0.82	5.33 ±1.44	2.90 ±0.23	2.42 ±0.28***	3.44 ±0.26**	1.70 ±0.10***	2.92 ±0.26**	3.70 ±0.48*	2.21 ±0.26*	1.80 ±0.22***	3.22 ±0.46**	1.79 ±0.20***

HI= Hepatopancreas index, GI= Gonadal index, CI= Condition index. All results are the mean of five observations with ± SD.
(p< 0.001) = ***, (p< 0.01) = **, (p< 0.05) = * and(p>0.05) = NS.

(2009) observed direct relationship between condition index and gonadosomatic index.

In the present study, both clam species showed considerable decrease in condition index (CI) at high temperature. It clearly indicates that, the CI of clams is closely associated with the gonad index. At stress condition, there was a limitation of both oxygen and food hence, the organism suppressed their oxygen consumption and ceasing all activity in order to conserve the energy and maintenance at undesired environment (Laing *et al.,* 1987). The probable reason for declining the gonadal and condition index might be the availability of food at different temperature regime. Because insufficient quantity of nutrients may suppressed the gonadal development and maturation (Suja and Muthiah, 2009) ultimately the physiological condition of clams may affected. Recently, Yap and Al-Barwani, (2012) recorded decline in condition index when mussel *Perna-viridis* subjected to stress condition.

Finally we concluded that, the physiological measures like hepatopancreas index, gonadal index and condition index amongst both the clam species were significantly declined when exposed to three experimental temperatures (20, 25 and 35°C). At all experimental temperatures,

the physiological body indices were declined significantly however, highest decline in body indices has been noticed at high temperature (35°C) followed by 20°C and 25°C. Relatively, maximum decline in the indices was recorded in *M. meretrix* species than *K. opima*. Based on this observation, it assumed that, both the clam species are very sensitive to changing temperature particularly to high temperature (35°C) and comparatively *M. meretrix* species is more sensitive towards temperature change than *K. opima*.

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