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Physiological responses of *Perna. Sp.* (Various size, 20 to 50mm) towards alternations in Marine Temperature

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Abstract
The, *Perna sp.* is an important marine bivalve resource due to their dietary and medicinal properties. The increasing atmospheric CO_2, ocean warming and consequent acidic changes of the ocean waters adversely affected these sedentary organisms by directly influencing the physiological activities and their energy levels. Experiments were conducted in the present study to infer the influence of temperature on the physiological responses of *Perna sp.* collected off Kadiapattanam coast, Tamil Nadu, India. The mussels were acclimatized at different temperatures *viz*., 25.0 to 40.0 °C and the oxygen consumption levels were examined. The results indicated that the smaller size group *P. species.* (*viz*., 20 mm shell length) was more active at 35.0 °C compared to the larger size groups. The oxygen consumption of *P. indica* acclimated at 35.0 °C was higher with 0.65±0.05 ml/lit/g/h than those 25.0 to 34.0 °C and 36 to 40.0 °C. It was also observed that at 25 to 30.0 °C, the mussels started secreting new byssal threads and the numbers of threads formed were at the rate of 6±0.03 of 20mm shell size and 4±0.05 threads formed of 30 and 40mm shell size mussels at 25.0 to 28.0 °C respectively per hour. However, byssal threads were not produced by those specimens reared at the elevated temperature of 35.0 °C. The observations point out to the fact that temperature increase due to global warming and size variation could adversely affect physiological responses of the *Perna* sp. in its natural marine conditions.

Keywords: *Perna sp.,* physiology, temperature, byssal threads

1. Introduction
Perna species are commercially valuable species and amenable for large scale cultivation along with coastal areas. They are very important for marine ecology and for human diet, since they are an important source of nutrients. Consumption of these bivalve molluscs provides an inexpensive source of protein with a high biological value, essential minerals and vitamins (Astroga-Espana *et al*., 2007) [4]. Mussels are rich in omega-3, fatty acid which help to prevent the cardiovascular and heart disease. Zinc content of mussel promotes mental alertness and aids in proper brain function. In numerous studies the influence of the different environmental and nutritional conditions on the composition of mussels has been proven (Astorga-Espana *et al*., 2007; Khan *et al*., 2006) [4, 14]. On the other hand, temperature over 20 ˚C (Incze *et al*., 1980) [11] and variation in salinity (Bohle, 1972) [6] can decrease growth. Widespread mussel spat settlement occurs in the intertidal and sub tidal areas during the post monsoon period. Substantial spat also perish due to adverse ecological conditions (Appukuttan *et al*., 2001) [3]. Temperature is the most important environmental parameter for aquatic life. Rising temperature up to certain limit favors aquaculture by reducing the time required to produce marketable sized animal and produces more generation per year. On the contrary, temperature adversely affects the health of aquatic animal by increasing metabolic rates and subsequent oxygen demand, and assisting proliferation, invasiveness and virulence of bacteria and other pathogens that cause a variety of pathophysiological disturbances in the host (Wedemeyer *et al*., 1999) [21].
Currently the importance of temperature investigation was carried out to inter the effect of different marine temperatures (25 to 40.0 ˚C) on the physiological responses in various shell sizes (20,30,40 and 50 mm) of *Perna species* including O_2 consumption, Ammonia excretion, CO_2 content in mussels acclimatized water, as well as byssal thread formation.

2. Materials and Methods

2.1 Mussels

Mussels (*Perna* species) with shell lengths ranging from 20 to 50 mm were collected from the Kadiyapatanam coast, Kanyakumari district (Latitude 8^0 07' 57" N and Longitude 77^0 19' 27" E) and transported quickly to the laboratory in wet and aerated conditions. The mussels were detached carefully from their clumps by cutting their byssus threads with scissors, to avoid damaging the pedal apparatus which would impair their re-secretion. They were maintained in large wide mouth conical flasks (1 lit. capacity) in sea water with adequate air supply. The temperature of sea water was maintained at 25 to 40 ˚C in the holding flasks of different size groups such as 20, 30, 40 and 50mm.

2.2 Estimation of Oxygen Consumption, Ammonia Excretion and CO_2

The rate of Oxygen consumption was measured using Respirometer fabricated indigenously by the modified Winklers method (APHA, 1998) [2]. The amount of CO_2 level and ammonia content were measured using the method of Adoni (1985) [1] and Solorzano (1969) [19] respectively. The following estimations were conducted, after the mussels were acclimated for 1 hr. at various temperatures (25 to 40.0 ˚C) in various shell sizes (30, 40, 50 and 60 mm). The experiment was repeated 5 times at each temperature for the different shell sizes.

2.3 Estimation of formation of Byssus threads

The mussels were placed for acclimatization for 1 h in a conical flask containing 1000 ml aerated, unfiltered seawater for a minimum 1 h. The temperatures of 25 to 40.0 ˚C were maintained in each respective group of conical flask. The different size group of mussels was introduced into the conical flask at the given temperature, without damaging the thread and the length was measured using a 0.05 mm precision caliper. The experiment was repeated 5 times in each temperature with each shell size group.

2.4 Statistical analysis

All the results were given with standardized mean ± SD values and were graphically represented. One-way ANOVA was used to detect the significant differences of the effect of temperature.

3. Results

At 25 to 26 ˚C (normal temperature) the O_2 level was 4.52±0.10ml/lit. The O_2 level was 18±0.14mg/lit and ammonia level was 4.32±10µg/lit. The 30 mm length group of mussels produced was noted an average 2±0.107 of threads; while 40mm of mussels produced 2±0.014 threads. No thread formation attained between 60 mm and 80 mm of mussels acclimatized in 1 h at the normal temperature of 25 to 26 ˚C.

The results of the experiments on O_2 consumption enhanced with the increasing temperature among the lowest shell size (20mm) group of mussels tested. At 25 ˚C, the O_2 consumption rate was higher with 0.65±0.01ml/lit/g/h among the 20 mm size group of mussels and low with 0.163±0.02 ml/lit/g/h along 50 mm of mussels. One-way ANOVA analysis showed a significant effect of temperature on oxygen consumption rate (F=12.2; P≤0.005) as could be noted from Fig.1

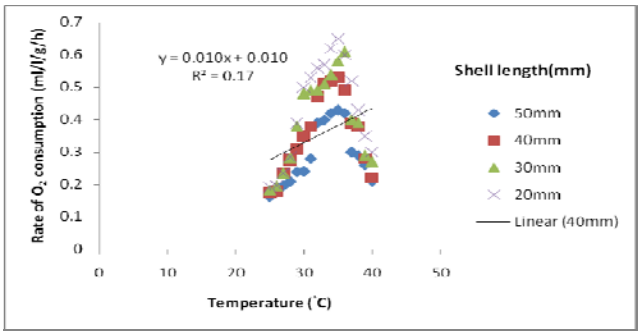

Fig 1: Rate of O_2 consumption of mussel *P. species* with different shell sizes at different temperatures

The results of experiment on CO_2 content in 1 hour after the various sizes (20, 30, 40 and 50mm) of mussels acclimatized with various temperature (25 to 40 ˚C) are presented in Fig.2. The CO_2 content increased at higher temperature among the smaller shell size group of mussels. The CO_2 level was highest with 23.9±0.01mg/lit at 35 ˚C in 20 mm size. But at the rate of CO_2 level was very low (18.6±0.03mg/lit.) among 50mm sizes of mussels acclimatized at 25 ˚C. The CO_2 content of brown mussel in acclimatized water increased significantly (F= 6.75 P≤0.1) as the temperature increased from 25 to 40 ˚C with 20 mm to 50 mm shell sizes.

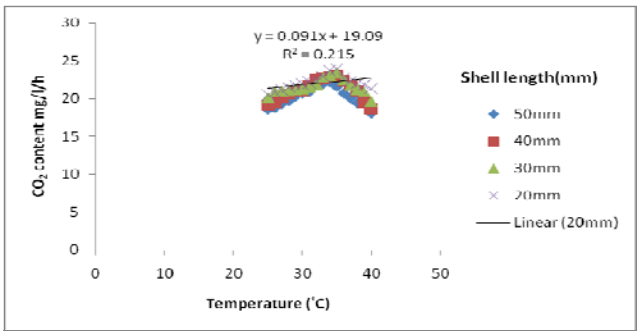

Fig 2: CO_2 content /h of mussel *Perna sp.* with various shell sizes after acclimation

The results of ammonia excretion of the brown mussel *P. indica* under different temperature conditions (25 to 40 ˚C) are presented in Fig.3. The ammonia excretion increased with increasing temperature among the smaller size groups. The ammonia excretion at 25 ˚C for the 50 mm shell size group was very low (4.82±0.3µg/lit). But the highest level at 35 ˚C (7.01 ±0.01µg/lit) among lower sizes 20mm was noted. The rate of ammonia excretion of brown mussel *P. indica* increased significantly (F=9.67; P≤0.01) as the acclimation temperature increased from 25 to 40 ˚C with 20 mm to 50 mm sizes of mussels (Fig. 3).

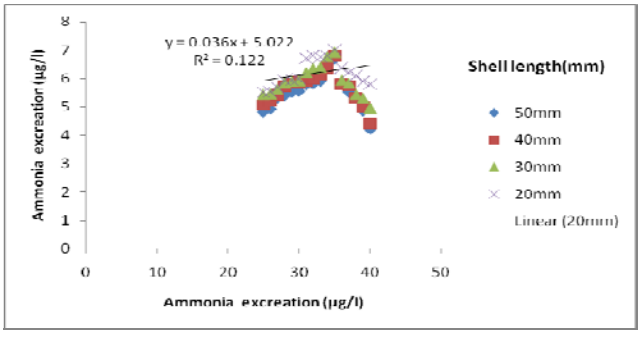

Fig 3: Ammonia excretion among *Perna sp.* with various shell sizes at different temperatures

Byssus thread formation gradually increased with the increase in ambient temperature in lowest shell sizes. Maximum number was 6±0.01 at 28 to 30.0 ˚C among the 20 mm size group. The lowest thread formation was at 25 to 28 ˚C and with highest shell size of 30 and 50 mm groups. Interestingly, no thread formation occurred at 35 ˚C among all the group of shell sizes (Fig. 4). One-way ANOVA analysis showed a significant effect (F=9.16; P≤0.01) of temperature (25 to 40 ˚C) with in different sizes 20 to 50 mm in the formation of threads.

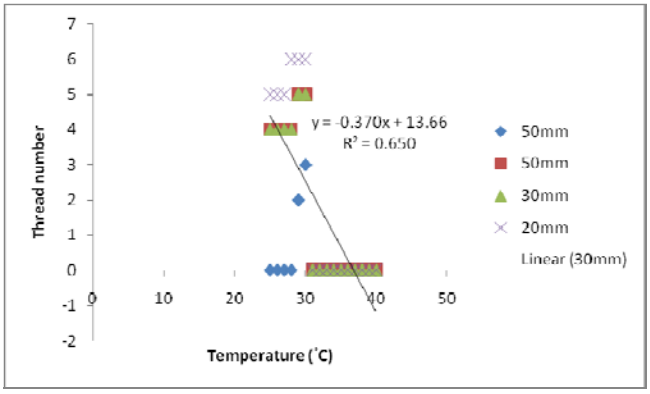

Fig 4: Byssal thread formation within different size groups of *Perna sp.* after exposure in different temperature

The highest thread length of 12.5±0.1 mm was noted in 50 mm shell size at 30 ˚C. But the lowest thread length was measured at 25.0 and 26.0 ˚C in 30 mm shell size (Fig. 5). One-way ANOVA analysis showed a significant influence (F=14.40; P≤0.1) of temperatures (25 to 40 ˚C) on the formation of new thread in mussels with various shell sizes (20 to 50 mm).

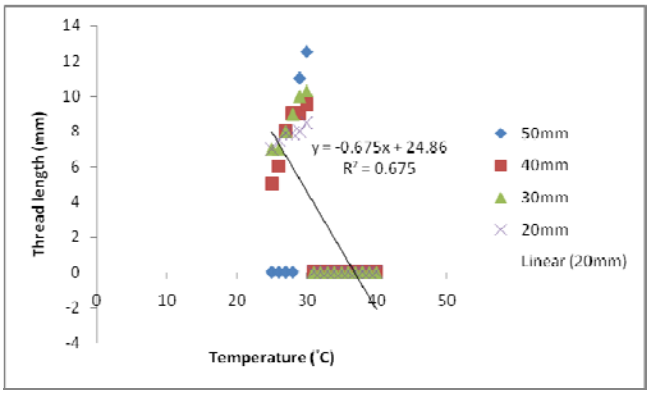

Fig 5: Thread length in mussels with different shell sizes at different temperatures

4. Discussion

The information on relationship between water temperature with shell size and oxygen consumption is not only useful for comparative physiological aspects, but also for aquaculture, transporting of stocks, harvests as well as, mussel fishing management options along the coastal areas. The present study revealed that the lower size group *P. indica* with average length of 20 mm showed higher rate of O_2 consumption at the tested elevated temperature of 35 ˚C. This aspect relates their sensitivity to temperature. Similar reports were revealed by a few researchers indicating the relationship between metabolic rates of bivalve molluscs and temperature. Metabolic rates of bivalve molluscs usually increase directly with the ambient temperature, up to an optimum limit beyond which they rapidly decrease (Shumway 1982; Yang *et al*., 1998; Wang *et al*., 2002 and Yukihira *et al.*, 2000) [17, 23, 20, 24]. This peak and decline in metabolic rates could be related to the balance between two opposite effects at temperature increases to speed up chemical reactions versus greater denaturation of the enzymes that catalyze them as suggested by Yukihira *et al.*, (2000) [24]. Effect of seasonal temperature on O_2 consumption in relation to body size of fresh-water fish, the flying barb, *Esomus dandicus* (Ham.) was documented by Bhattacharya and Suba (2006) [5].

Ammonia excretion is known to be affected by factors such as species, body weight, water temperature, feeding and ration size (Yager and Summerfelt, 1993) [22]. Results of this study are in agreement with earlier reports on the weight specific rate of excretion. Ammonia induces detrimental changes in tissue structure, cell function, blood chemistry, osmoregulation, disease resistance, growth and reproductive capacity (Jeney *et al*., 1992) [13]. Ammonia may affect gill structure (Smart, 1976) [18], respiratory function (Chen and Lai, 1992) [8] in aquatic animals. The increase in fasting ammonia excretion rate at higher temperatures indicates that the higher metabolic demands at elevated temperature are partially met via deamination of amino acids (Forsberg and Summerfelt, 1992) [9]. Krishnamoorthy *et al.*, (2008) [15] reported that ammonia excretion enhanced with increasing temperature in *Alepes djidaba* fingerlings indicating that degradation of protein for energy was more at higher temperature as also observed in the present experimental study at different temperature range. The results indicated that the ammonia excretion also increased with the increase in temperature suggesting degradation of protein for energy was more at elevated temperature. Ammonia excretion decrease with increasing body weight and increase with increasing water temperature were reported in fasting organisms. Thus it could be inferred that the degradation of protein was more at elevated temperature as evidenced from higher ammonia excretion in *Perna* sp.

Coastal zones are ecologically and economically important and are among those areas that will be strongly affected by global climate change. It is commonly accepted that increases in anthropogenic emissions of carbon dioxide (CO_2) in the atmosphere are mainly responsible for Global Climatic Changes. Increasing atmospheric CO_2 concentrations is expected to increase mean temperatures and a higher frequency of thermal extremes as well as to ocean acidification. Anthropogenic climate change poses a serious threat to biodiversity. In marine environments, the important abiotic changes are likely to be increased water temperature and elevated carbon dioxide concentration (Harley CDG *et al*., 2006) [10]. In marine environments, multiple climate variables, including temperature and CO_2 concentration are changing simultaneously. Although temperature has well-documented ecological effects, and many heavily calcified marine organisms experience reduced growth with increased CO_2, little is known about the combined effects of temperature and CO_2 as indicated by Rebecca *et al*. (2009) [16]. Global climate changes are predicted to occur in the next hundred years through increases in temperature, water acidification and changes in sea water salinity (IPCC, 2007) [12]. This study also indicated that, experimental shells of lower size group of *Perna* sp. when acclimatized to higher water temperature excreted more carbon dioxide content (21.9±0.03mg/lit.) while comparison with increased shell size mussel. It also revealed that lower water temperature indicated lower carbon dioxide content.

According to Brodsky *et al.,* (2011) [7], the mussels *Geukensia demissa*, *Mytella charruana*, had lowered their byssal thread production capabilities at the colder tested temperature of 10 °C and 13 °C than at the control temperature of 23 °C. However, based on the results from our current study, temperature would act as a barrier to where in *Perna* sp. could not establish and itself due to non production of byssal thread, at elevated water temperature of 35.0 °C during the extended period of experimental duration. These observations point out to the fact that the rise in temperature as a consequence of global warming could adversely affect physiological responses of the brown mussel *Perna* sp. in its natural marine conditions.

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