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Effects of Different Environmental Parameters on the Respiratory Metabolism of the Larvae of Malaysian Horseshoe Crab, *Tachypleus gigas* (Müller)

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ABSTRACT

Respiratory metabolism of the larvae of Malaysian horseshoe crab *Tachypleus gigas* (Müller) was studied under different salinities, pH, and temperature. The trend in oxygen consumption was uniform at all salinities, ranging from 10-40 ppt, indicating insignificant influence on the oxygen consumption by the larvae. Similarly, the correlation coefficient values showed that the relationship between oxygen consumption and salinity was not significant ($P > 0.05$; $r = 0.245$). During the first three hours, the oxygen consumption was 8.89, 10.72, 17.4, and 12.06% at 10, 20, 30, and 40 ppt salinities, respectively. Meanwhile, the maximum oxygen consumption was recorded after 12 hrs, i.e. at salinity 20 ppt. A sudden drop in oxygen consumption was recorded during 3-6 hours of the experiment. This was followed by a gradual increase in the consumption of oxygen up to 12 hours of experiment. A similar trend in the oxygen consumption was observed in different pH levels, ranging from 5 to 9. At pH 6 and 9, during the first six hour, a moderate consumption of oxygen was observed. However, at pH 6, 7 and 8, the rates of oxygen consumption were found to be relatively greater after six hours, indicating unfavourable conditions. The data were statistically tested and it was found that a high degree of correlations existed between pH and oxygen consumption ($r = 0.97$). The analysis of covariance showed a significant relationship between oxygen consumption and pH ($P < 0.05$). Meanwhile, minimal variation in oxygen consumption was recorded between 30 and 40°C, with a gradual decrease in dissolved oxygen concentration up to 12 hours of experimental time. At 50°C, almost all dissolved oxygen was consumed by the larvae. The rate of oxygen consumption between 30 and 40°C was low during the first 9 hours of the experiment but it was significantly increased at later hours. A sudden increase in the oxygen consumption was recorded at 50°C, suggesting that it might be the most unfavourable temperature condition. Meanwhile, a significant relationship was observed between temperature and oxygen consumption ($P < 0.05$; $r = 0.98$).

Keywords: Environmental parameters, respiratory metabolism, larvae, Malaysian horseshoe crabs

INTRODUCTION

Respiration is a process where marine organisms obtain sufficient quantity of oxygen and release carbon dioxide. It is also an important phenomenon in controlling different physiological activities of the animals, such as maintenance of the blood pH, proper oxygen tension of the blood and normal body temperature. The most frequently parameter used to assess crustacean respiratory metabolism

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is oxygen consumption by the animal. It is important to note that respiratory metabolism of marine organism is dependent on many environmental factors. Among other, salinity, temperature, and pH are the main external factors controlling the respiratory metabolism of the marine organisms (Wolvekamp and Waterman, 1960). In addition, there are also some other important intrinsic factors, like body size of the animal, that also play an important role in the organisms' metabolic rate (Gaudy and Sloane, 1981).

Horseshoe crabs have well-developed external gills in the form of appendages which are known as gill lamellae. These gill lamellae have well-developed circulating system with blood vessels that help the animal in respiration (Chatterji, 1994). Gases diffuse from the surrounding water through the moist epithelium of the gill and circulate these to the blood vessels. In the present study, the effects of salinity, pH and temperature on the respiratory metabolism of larvae of the Malaysian horseshoe crab, *Tachypleus gigas* (Müller) were assessed.

MATERIALS AND METHODS

For the purpose of this study, fertilized eggs of the Malaysian horseshoe crab (*T. gigas*) were directly collected from the nest made on the breeding beach of Balok in Kuantan, Pahang (Lat 3° 56.915' N; Long 103° 21.933' E). The experiments were carried out at Akuatrop Plankton Laboratory and Institute Oceanography (INOS) Biotech Laboratory, Universiti Malaysia Terengganu (UMT). The eggs were kept for incubation at a constant temperature of 27±1°C. The trilobite larvae were hatched between 42 and 45 days of incubation and this was followed by the first moulting after 75 days. The larvae were collected and divided into several groups immediately after the first moulting.

Seawater was collected from the marine hatchery of the Institute of Tropical Aquaculture and kept in a circular tank under the sun for evaporation to achieve a salinity of 40 ppt. Different salinities of water were prepared by diluting 40 ppt of seawater with sterilized freshwater. Meanwhile, a salinometer (Atago, S/Mill, Japan) was used to determine the salinity. A portable pH meter (Thermo Russel RL060P) was used to record the pH of the seawater after calibrating it with standard pH Buffers of 4.2, 7.0, and 9.2. The pH of seawater was adjusted from 5-7 by 1 N HCl and 7-9 by 1 N NaOH solutions with the help of a portable pH meter. A constant temperature water bath was used to control the temperatures of the experimental tanks ranging from 30-50°C. Oxygen consumption in larvae was studied in specially designed glass respiratory chambers of 2 L capacity. A small PVC tube was fitted at one end of the glass respiratory chamber to insert the oxygen probe. The oxygen concentration was recorded using a pinpoint dissolved oxygen monitor (YSI Hand Operated Oxygen: Temperature Meter Model 550 A).

In order to study the effect of salinity on respiratory metabolism, larvae that ranged in the weight from 0.63 to 0.89 g were initially acclimatized for one hour in 5 L seawater of different salinities, i.e. from 10, 20, 30, and 40 ppt. A batch consisting of 30 larvae each were then transferred from the acclimatization tank to the respective respiratory chamber. Dissolved oxygen concentrations were recorded immediately after transferring the larvae into the respiratory chambers. This was followed by sealing the surface of respiratory chamber with liquid paraffin so as to avoid any diffusion of oxygen from the air. The respiratory chamber was kept inside a constant temperature room, where the temperature was maintained at 24±1°C. Meanwhile, the pH of the water was maintained at 7.0 in each set of the experiment.

In the second set of the experiment, 30 larvae were acclimatized in 5 acclimatization tanks of 5L capacity and at a salinity of 30 ppt. The respiratory metabolism of larvae was studied under 5, 6, 7, 8, and 9 pH.

In the third set of experiment, the effects of temperature at 30, 40, and 50°C on the respiratory metabolism of the larvae were studied. About 10L of filtered seawater, at a salinity of 30 ppt and pH 7.0, was aerated for two hours at the beginning of the experiment. A batch of 30 larvae was then transferred into a respiratory chamber. The respiratory chamber was kept in a temperature-controlled water bath. The temperature of the water bath was slowly adjusted to the desired level to avoid any physical stress to the larva. Before sealing the surface of the respiratory chamber with liquid paraffin, dissolved oxygen concentration was recorded using an oxygen meter.

All the experiments were conducted in replicate and in all sets of the experiment; the values of dissolved oxygen were recorded at 3-h intervals for 12 hours. The decrease in dissolved oxygen level was considered as the amount of oxygen (mg/l per 3 hour) used by the larvae during the experimental period.

RESULTS

During the acclimatization and experimental period, no larval mortality indicating the sturdiness of the animal under different stress conditions was observed. The influence of salinity on the respiratory metabolism of the larva is presented graphically in *Fig. 1*. The trend in oxygen consumption was more or less uniformed at all salinities, ranging from 10-40 ppt indicating insignificant influence of salinity on the oxygen consumption by larvae. The correlation coefficient values showed that the relationship between oxygen consumption and salinity was not significant ($P > 0.05$; $r = 0.245$). During the first three hours of the experiment, the oxygen consumption by 30 larvae was 8.89, 10.72, 17.4, and 12.06% of the initial dissolved oxygen concentrations at 10, 20, 30 and 40 ppt, respectively (*Fig. 2*). The maximum oxygen consumption during the first three hours was recorded at 20 ppt. A sudden drop in the oxygen consumption was recorded at 3-6 hours of the experiment. This was followed by a gradual increase in the consumption of oxygen, while the maximum value of 26.29% was recorded at salinity 20 ppt after 12 hours of the experiment (*Fig. 2*).

A similar trend in oxygen consumption was observed at different pH ranges of 5 to 9. At pH 6 and 9 during the first six hours, a moderate consumption of oxygen was observed (*Fig.3*). After 6 hours, however, at pH 6, 7 and 8, the rates of oxygen consumption were suddenly increased and this indicated the most unfavourable conditions (*Fig. 4*). The consumption rate suddenly dropped after 9 hours at pH 8, whereas it continued to increase at pH 6 and 7 after 9 hours and up to 12 hours. The data were tested statistically and it was found that a high degree of correlations existed between pH and oxygen consumption ($r = 0.97$). The analysis of covariance showed a significant relationship between oxygen consumption and pH ($P < 0.05$).

The effects of temperature on the oxygen consumption are presented in *Fig. 5*. The variation in the consumption of oxygen was minimal, i.e. between 30 and 40°C, whereby a gradual decrease in the dissolved oxygen value was recorded up to 12 hours of the experimental time. At 50°C, almost all dissolved oxygen was consumed by the larvae. Initially at 30 and 50°C, the rate of oxygen consumption was low, but it was considerably increased after 9 hours of experiment (*Fig. 6*). A sudden increase in the oxygen consumption was recorded at 50°C after 6 hours, and this showed the most unfavourable conditions. The data were tested statistically using the analysis of variance (ANOVA), where a significant relationship was observed between temperature with oxygen consumption ($P < 0.05$; $r = 0.98$).

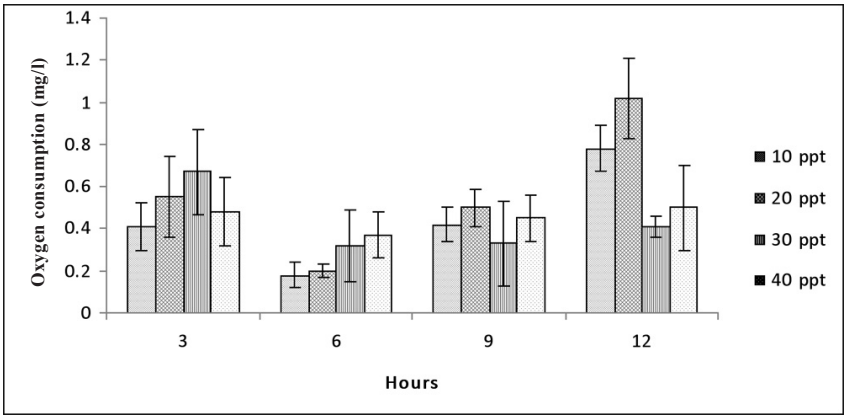


Fig. 1: Oxygen consumption (mg/l) by the larvae of T. gigas *at different salinities*

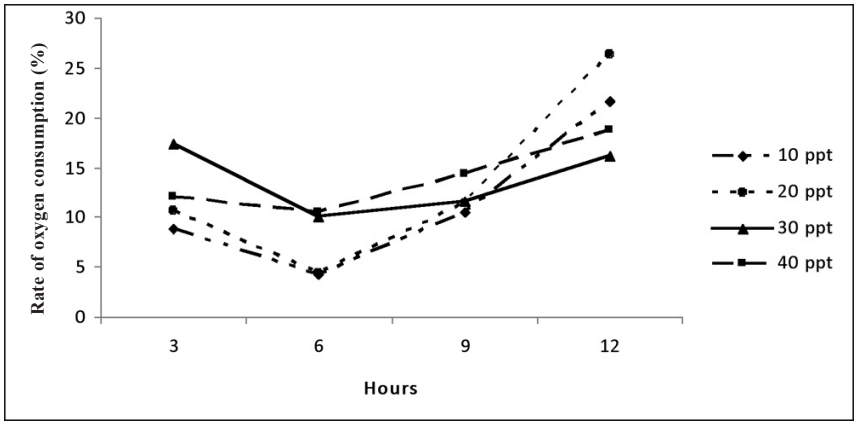


Fig. 2: The rate of oxygen consumption (%) by the larvae in different salinities

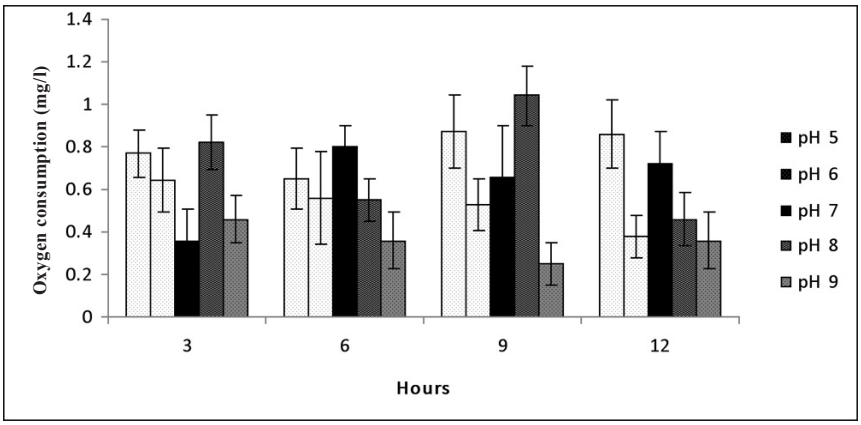


Fig. 3: Oxygen consumption (mg/l) by the larvae of T. gigas *at different pH levels*

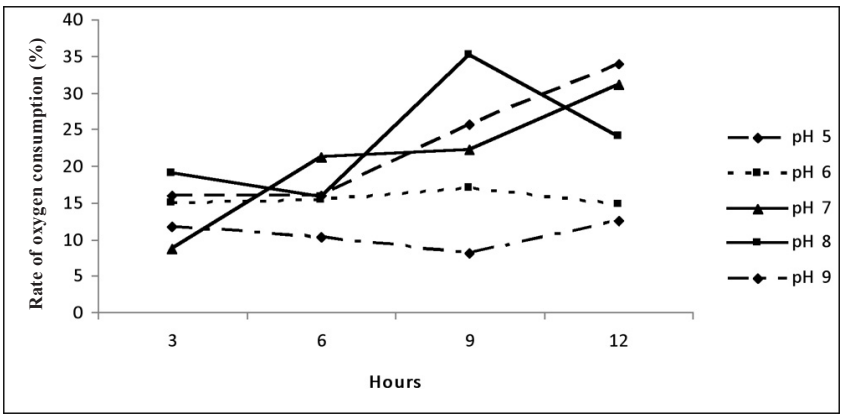


Fig. 4: Rate of oxygen consumption (%) by the larvae in different pH

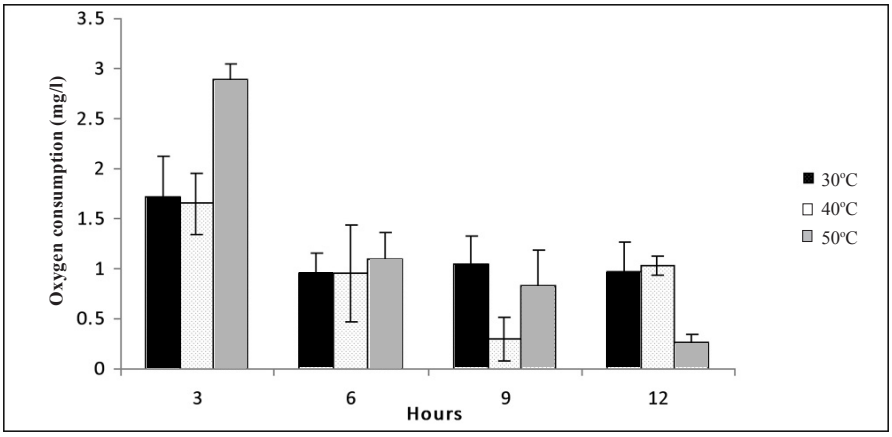


Fig. 5: The oxygen consumption (mg/l) by the larvae of T. gigas *at different temperature*

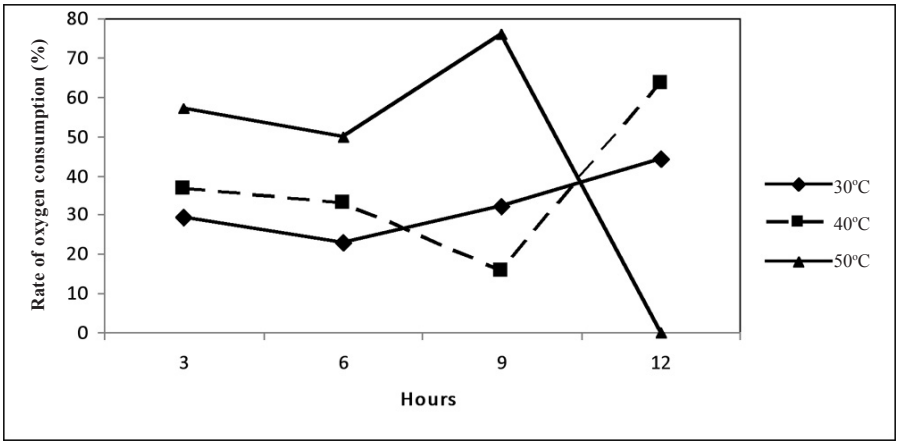


Fig. 6: The oxygen consumption rate (%) by the larvae in different temperatures

DISCUSSION

It is important to note that oxygen requirements and oxygen consumption rates are dependent on both biotic and abiotic factors (Brett, 1987). The most important abiotic factors affecting the oxygen consumption in aquatic organisms are temperature and salinity. Meanwhile, temperature directly affects the rate of all biological processes, whereas salinity influences osmoregulatory demand by the organisms. Both the factors have effects on the oxygen content of the medium. However, the interaction between salinity and temperature can be rather complex, with one variable acting as a modulating factor on the effects of the other (Vernberg, 1983).

When a marine organism is exposed to unfavourable temperature or salinity conditions, it must accommodate in some ways with this physiological stress. Short-term adjustments require additional expenditure of metabolic energy which can be measured by an increase in respiration. On the other hand, some organisms respond to sub-optimal conditions by reducing their metabolic rates, and this is either by withdrawing into a shell or as a result of lowered enzymatic activity (Gaudy and Sloane, 1981). Marine organisms can respond to the changes in salinities as osmoregulators or osmoconformers. Regulation is an active process which usually results in an increase in metabolic rate.

Meanwhile, metabolic rate varies with the changes in salinity, temperature and pH. In addition to these parameters, other factors such as the size of the animal, light condition and the amount of oxygen present in the environment also play significant roles in controlling the oxygen consumption in aquatic animals. Several studies have demonstrated that the oxygen consumption varies in juveniles and post-larvae of the penaeid prawns in relation to changes in salinity (Kutty *et al*., 1971; Gaudy and Sloane, 1981, Janakiraman *et al.*, 1985; Diwan *et al*., 1989), temperature (Kutty *et al*., 1971; Yagi *et al*., 1990; Villarreal and Rivera, 1993) and body weight (Kurmaly *et al*., 1989). Similarly, for *P. monodon*, the influence of salinity on the oxygen consumption has not been found to be significant (Gaudy and Sloane, 1981; Lei *et al*., 1989). In *P. stylirostris*, however, an increase in the oxygen consumption was observed at low salinities (Gaudy and Sloane, 1981).

In the present study, salinity was found to have no significant effect on the oxygen consumption in larvae of *T. gigas*. This indicates that the animal can thrive well in wide range of salinity changes. Nonetheless, salinity was found to affect the metabolism of decapod crustaceans, and this has been well studied through the measurement of oxygen consumption (Lemos *et al*., 2001). Similarly, the rate of oxygen consumption by *Amphibola crenata* has been reported to be unaffected by the salinity of the external medium in the range 0-125% seawater (Shumway and Sandra, 1981). However, *A. crenata* showed an increase in respiratory rate during exposure to anaerobic conditions, and a declining oxygen trend at higher salinity revealed almost no ability to regulate the rate of oxygen consumption (Shumway and Sandra, 1981). Therefore, when the animal has been exposed to both salinity and anoxic stress conditions, they can reach the maximum degree of oxygen dependence (Shumway and Sandra, 1981).

Not much work has been carried out on the respiratory metabolism of crustaceans under different pH conditions. In the present study, the larvae of *T. gigas* were found to be influenced by the changes in pH of seawater, whereby a wide fluctuation in the oxygen consumption was observed. The normal oxygen consumption was found at pH 6 and 9 up to six hours, but this was shown to have a sudden increase in the consumption rate at pH 6, 7 and 8. This revealed that up to 6 hours, the respiration of the larvae was normal, but they underwent a stress condition which resulted in a high consumption of oxygen after 6 hours.

Meanwhile, temperature was found to show a profound effect on the metabolic rate of aquatic animals. Endothermic animals expend extra energy to maintain their body temperature as the environmental temperature increases or decreases (Villarreal and Rivera, 1993). Nevertheless,

exothermic organisms can not alter their internal temperature and they usually show an increased metabolism as temperature rises, within a zone of tolerance. Outside of the tolerance zone, however, their metabolism falls as enzymatic process can no longer work properly.

Pagurus longicarpus or mussels (*Mytilus edulis*) are common intertidal animals and they are capable of surviving with fluctuations in temperature and salinity. Hermit crabs respond to stress by withdrawing into their shell. Several attempts have been made by several researchers to demonstrate the effects of temperature on the survival of the larvae of marine animals (Costlow *et al*., 1960: 1962: 1966; Choudhury, 1971; Young and Hazlett, 1978; Moreira *et al*., 1980). However, in adult penaeid prawns, the respiratory metabolism was found to be moderate between the temperatures of 20 and 30°C (Dall, 1986; Liao and Murai, 1986). In the present study, the oxygen consumption was the maximum at 40 and 50°C after 9 hours. These conditions are not suitable for the larvae of *T. gigas*. Therefore, the present study has demonstrated that temperature plays a significant role in influencing the respiratory metabolism, specifically at higher temperatures (>40°C).

There has always been a controversy to interrelate the respiratory metabolism with osmotic regulation in marine animals (Gross, 1957; Panikkar, 1969). However, most of the work carried out on the energy spent during osmotic regulation is based on the consumption of oxygen by the animals in a particular environment (Rao, 1968). Hence, the respiratory metabolism is considered to be an important biological process, especially for the aquatic cultivable animals which help in assessing the organism's oxygen requirement under different environmental conditions. It is also the best indicator to precisely evaluate the energetic expenditure in the osmotic regulation process of any aquatic organism.

Horseshoe crabs have a relatively high tolerance for a wide range of salinity. Normally, salinities for larvae and adults range from 8 ppt to natural seawater (36 ppt). According to Humberto and Jorge (1993), salinity and temperature are the most important external parameters but dissolved oxygen (DO) is the major limiting factor since biochemical reactions of an organism are controlled by the level of available oxygen, which is essential for aerobic metabolism.

CONCLUSIONS

The estimation of oxygen consumption during a brief experimental period does not seem to be a reliable index of respiratory metabolism as compared to natural condition which is primarily a culture system for extended periods. However, oxygen is an important environmental constituent and the scarcity of oxygen in the environment results in various phenotypic and physiological changes in the animals for their survival in a particular environment due to stress which is caused by depletion of oxygen. In the present study, the respiratory metabolism of the larvae of Malaysian horseshoe crab *Tachypleus gigas* (Müller) was investigated under different salinities (10-40 ppt), pH (5-9) and temperature (30-50°C). The oxygen consumption was found to be normal under different salinities for the first three hours, but the maximum oxygen consumption rate was recorded at salinity 20 ppt after 12 hrs. Meanwhile, a moderate consumption of oxygen was observed at pH 5-9, i.e. during the first six hours, and this was suddenly increased at pH 6, 7 and 8 after 6 hours, indicating unfavourable conditions. A gradual decrease in dissolved oxygen value was recorded up to 12 hours of the experimental time at all temperatures and almost all dissolved oxygen was consumed by the larvae of *T. gigas* at 50°C. The present study has proven that temperature (>40°C) and pH are the limiting factors influencing the oxygen consumption rates in the larvae of *T. gigas*.

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