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# The Effect of Temperature and Salinity on the Physiological Rates of the Mussel *Perna perna* (Linnaeus 1758)

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## ABSTRACT

*The aim of this work was to study the rates of respiration, clearance, excretion and absorption efficiency at different temperature and salinity under laboratory conditions for* Perna perna. *Results showed variations in physiological rates and in acclimatization capacity which, taken together, enabled to understand its behavior in the environment, as well as to estimate its scope for growth. All experiments were carried out in static conditions, in ten replicas with one mussel by flasks. Perna perna was capable of achieving acclimatization for both clearance and absorption efficiency (15 to 30 ºC), but it achieved only partial acclimatization for respiration and excretion under chronic temperature conditions. The clearance and respiration rates increased twofold as the mussel was submitted to temperature shock, which signified a response to metabolism activity. Acclimatization to salinity was clearly the best developed capability (20 to 40 ‰). Net growth efficiency reduced as the temperature increased, but remained constant in the 20 to 35 ‰ salinity range.*

**Key words:** Mussel, *Perna perna*; Physiological rates; Temperature; Salinity, Net growth efficiency

## INTRODUCTION

Among the factors in the marine environment, temperature and salinity are the most important and relevant variables in the study of physiology. These variables determine the metabolism rate of the organisms and consequently, the extent of distribution of the species (Vernberg and Vernberg, 1972). A knowledge of the influence of temperature and salinity on the physiological rates of mussels is essential for the interpretation of studies on production in natural environments. In these studies of production, the energy balance of the organisms is normally investigated, known as Scope for Growth, the physiology of which responds both to variations in environmental conditions and the adverse effects of the anthropogenic agents in contaminated environments (Smaal and Widdows, 1994).

Mussels have normally been used as models of production of coastal environments and as indicators of environmental quality. The ease of laboratory work, due to their sessile behavior, size, resistance to environmental alterations and economic importance, make these the most studied invertebrate in the world. The study of the physiology of mussels under the influence of temperature has been exhaustively investigated, particularly for the species *Mytilus edulis* (Bayne, 1973 and 1976; Gosling, 1992; Widdows, 1973 and Widdows and Bayne, 1971), which is used as a model for the physiology of bivalves. However, there has been little investigation on the influence of salinity on the physiological rates of these organisms.

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*Perna perna* is the most abundant Mytilidae on the Brazilian coast and the one which reaches the greatest size, which makes it the species of most commercial interest, and the most used in mitiliculture. There are several reports for its tolerance to variations in temperature (Bayne, 1967; Hicks and McMahon, 2002; van Erkom Schurink and Griffiths, 1992; Zuim, 1973 and 1976) and salinity (Salomão et al., 1980; Salomão and Lunetta, 1989; Zuim, 1973; Zuim and Mendes, 1980 and 1981). The aim of this work was to investigate and compare the physiological rates of the mussel *Perna perna* under acclimated conditions in a laboratory, to enable an understanding of its variations in reaction to temperature and salinity under constant conditions (chronic tests or routine metabolism) and thermal or saline shock (acute test or activity metabolism) investigating its acclimation capacity.

## MATERIALS AND METHODS

Specimens of the mussel *Perna perna* were obtained between 2000/2001 from the experimental mariculture station of CTTMar where they were previously washed and selected in for size (from 3.5 to 4.5 cm). The standard maintainance of the mussels consisted of acclimatizing the organisms to a salinity of 30 ‰ and a laboratory temperature of 20 ± 2 °C. Groups of 20 organisms were maintained in four-liter flasks with aeration and constant photoperiod (12 h light-dark cycle). Two liters of the maintenance water were renewed daily and food was inoculated (phytoplankton *Chaetocerus gracilis*) at a concentration of 500 Cells.mL$^{-1}$ (Resgalla Jr., 2004 and Resgalla Jr. et al., 2006) in order to establish a standard metabolism in the test organisms, eliminating seasonal variations in their metabolism.

All the groups of mussels were kept in filtered seawater (0.5 μm) for 24 h prior to the physiological tests, in order to clean the digestive tract and stimulate their metabolic rates. The rates of respiration (RR), clearance (CR), excretion (ER) and absorption efficiency (AE) were estimated under the following test conditions:

1-Chronic Temperature Test – the RR, CR, ER and AE were estimated for the mussels kept at 15, 20, 25 and 30 °C (constant salinity of 30 ‰) for a period of 15 to 20 days. The final temperature was obtained from the variation of 2.5 °C per day, based on an initial temperature of 20 °C.

2-The Acute Temperature Test – the mussels were maintained at 20 °C and 30 ‰ salinity for a period of 15 to 20 days. The RR and CR were estimated at 15, 25 and 30 °C under thermal shock 24 h before the tests.

3-The Chronic Salinity Test – the RR, CR, ER and AE were estimated for the mussels maintained in salinities of 15, 20, 25, 30, 35 and 40 ‰ (constant temperature of 20 °C) for a period of 15 to 20 days. The final salinity was obtained by the variation of 2.5 ‰ per day, based on an initial salinity of 30 ‰.

4-The Acute Salinity Test – the mussels were maintained at 20 °C and 30 ‰ salinity for a period of 15 to 20 days. The RR and CR were estimated at salinities of 15, 20, 35 and 40 ‰ under osmotic shock, 24 h before the tests.

**Clearance Rate (CR) = Pumping Rate**

The method used consisted of estimating the rate of removal of phytoplankton (*Chaetocerus gracilis*) cells by the mussels, in 1000 mL test flasks with 10 replicas (one mussel by flask) plus two control flasks (without mussel) under static conditions (Smaal and Widdows, 1994). For each flask, concentrations of 140 Cells.mL$^{-1}$ of phytoplankton and filtered seawater were inoculated for one hour. This concentration was initially established because it lower than 5 mg.L$^{-1}$, the lowest concentration at which the rejection or formation of pseudo-feces occurs (Resgalla Jr. et al., 2006, and Widdows et al., 1979). At the start and the end of the test, with an interval of 1 h, 50 mL of the seawater with phytoplankton was removed from each flask in order to determine the turbidity for absorbance in 750 and 230 nm through a spectrophotometer (Greenberg et al., 1992; Resgalla Jr. 2004 and Resgalla Jr. et al., 2006). The clearance rate was estimated through the application of the following equation:

$$CR = \frac{V}{N}\left\{\left[\frac{(Ln C_{t0} - Ln C_{t1})}{\Delta T}\right] - f\right\}$$

where:

CR = Clearance rate (L.h$^{-1}$)

$C_{t0}$ = Initial concentration of phytoplankton (in absorbance at 750 nm and/or 230 nm)
$C_{t1}$ = Concentration of phytoplankton (in absorbance at 750 nm and/or 230 nm) at the end of the experiment.
V = Volume of test flask (L)
N = Number of organisms per test flask
ΔT = Time interval of test (h)
*f* = phytoplankton correction factor (decantation rate) calculated using the same formula as for the control flasks.

**Respiration Rate (RR)**
The tests were carried out under the same conditions as previous experiments with two control flasks (without mussel) and ten test flasks with mussels, both incubated in filtered seawater without phytoplankton. The reduction in values of dissolved oxygen in the flasks was dosed using a digital oximeter (YSI Mod. 58) at the start and end of the test, and after an interval of 3 h. The respiration rate was estimated using the equation proposed by Widdows and Johnson (1988):

$$RR = \left[ (C_{t0} - C_{t1}) \times \frac{V}{\Delta T} \right] - f$$

where:
RR = Respiration Rate ($mLO_2.h^{-1}$).
$C_{t0}$ = Concentration of oxygen at time zero ($mLO_2.L^{-1}$).
$C_{t1}$ = Concentration of oxygen at the end of the experiment ($mLO_2.L^{-1}$).
V = Volume of test flask (L)
ΔT = Time interval of incubation (h)
*f* = Factor of correction in the control flasks obtained by the same equation.

**Absorption Efficiency (AE)**
The test was carried out under the same conditions as the clearance tests, but with a duration of 24 h. The estimate proposed by Conover (1966) compared the content of the organic material present in the phytoplankton offered, with the organic material eliminated in the feces collected from the bottom of the test flask and considers the inorganic content as unaltered. The estimates were carried out according to the following equation:

$$AE = \left[ \frac{(I - F)}{1 - F} \times I \right] \times 100\%$$

where:
AE = Absorption Efficiency (%)
I = Percentage of organic material in the food offered (phytoplankton) .
F = Percentage of organic material in the feces.

Both the phytoplankton and the feces produced were filtered in fiberglass filters (GF/F Whatman - 25 mm), previously calcined and weighed. After washing with distilled water to remove the salts, the dry weight was determined by drying in an oven at 60 °C for 24 h. The contents of the organic material were obtained by combustion of the filters in a muffle oven at 450 °C for two hours (Strickland and Parsons, 1972). To estimate the percentage of organic material of the feces produced by the mussels, each CF/F filter concentrated feces collected from two organisms was taken from the same treatment to ensure better precision in weighing.

**Excretion Rate (ER)**
The tests were carried out under the same conditions as the experiments for respiration rates. At the initial and final times and at intervals of 3 h, 15 mL samples of the incubation water were collected in the test flasks to determine the total amonical nitrogen, according to the colorimetric method of Strickland and Parsons (1972), modified by Baptista et al. (1987). The excretion rate was estimated according to the equation:

$$ER = \left[ (C_{t1} - C_{t0}) \times \frac{V}{\Delta T} \right] - f$$

where:
ER = Excretion Rate ($\mu g\ N\text{-}NH^{4+}.h^{-1}$).
$C_{t0}$ = Concentration of ammonium nitrogen at zero time.
$C_{t1}$ = Concentration of ammonium nitrogen at the end of the experiment.
V = Volume of test flask (L)
ΔT = Time interval of incubation (h)
*f* = Factor of correction in the control flasks obtained using the same formula.

After the tests, biomass data was obtained by dissecting the organisms and their shells and drying the tissue in an oven at 60 °C for 48 h. The specific rates for respiration, clearance and excretion were corrected for the standard weight of 1 g (Bayne et al., 1985) according the allometric

correlation coefficients between weight and rate, whose respective b values were 0.66; 0.48 and 0.91, according to the results obtained by Resgalla Jr. (2004) and Resgalla et al. (2006). The results of the physiological rate tests for temperature and salinity were compared in variance analysis (ANOVA) and by the analysis of the Tukey test after their standardization in $Log_{10}$ (Zar, 1996).

**Growth efficiency**

The estimates for assimilated energy, consumed energy and net growth efficiency ($K_2$) were carried out using the average values for the physiological rates obtained in the chronic temperature and salinity tests, using the caloric equivalents proposed by Bayne et al. (1985) for comparisons of physiological balance in view of the variables tested. The caloric equivalents used were:

Weight or organic material of the food = 23.5 $J.mg^{-1}$

Assimilated Energy (A) = Clearance rate (CR) x Absorption efficiency (AE) x Concentration of organic material of the food in joules ($J.L^{-1}$).

Respiration rate (RR) = 20,33 J per mL $O_2$ breathed.

Excretion rate (ER) = 0,0249 J per mg $N\text{-}NH_4$ excreted.

Energy consumed = RR + ER.

Net Growth Efficiency ($K_2$):

$$K_2 = \frac{A - (RR + ER)}{A}$$

## RESULTS

### Temperature

**Respiration rate**

*P. perna* showed an increase in respiration rates as the temperature increased. For the chronic tests, it was observed that the rates at extreme temperatures of 15 and 30 °C were significantly different (ANOVA, F = 140.22), with acclimation only between 20 and 25 °C (Fig. 1A). Under acute conditions or thermal shock, the respiration rates showed a more pronounced variation (Fig 1B), in which the rates were significantly different (ANOVA, F= 81.96).

**Clearance rate**

For the clearance rate under chronic conditions, *P. perna* showed complete acclimation, since not all the rates showed significant differences (ANOVA, F = 2.17) (Fig. 2A). A tendency was observed for the rate to increase with temperatures up to 25 °C, after which they decreased. This behavior was evident in the thermal shock test (Fig. 2B), in which the clearance rate was significantly higher at a temperature of 25°C (ANOVA, F = 36.96) and with inhibition at 30 °C.

**Excretion rate**

The excretion rate presented behavior similar to that of the respiration rate, i.e., acclimation was only observed at temperatures of 20 and 25 °C at which *P. perna* showed high excretion values for 30 °C (Fig. 3A) and significantly higher values than for the other temperatures (ANOVA, F = 38.01).

**Absorption efficiency**

The variation in absorption efficiency as a result of temperature showed a total acclimation for the temperatures tested, i.e., this rate was independent of temperature since its values were significantly equal between 15 and 30 °C (Fig. 3B) (ANOVA, F = 2.443).

### Salinity

**Respiration Rate**

Under chronic conditions, the respiration rate presented acclimation under a wide interval of salinity (from 20 to 40 ‰), with a significant reduction in salinity of 15 ‰ (ANOVA, F = 14.73) (Fig. 4A). Under acute conditions, the salinity interval for acclimation was reduced to 30 to 40 ‰ (Fig. 4B), and a variation at low salinities, with a minimum of 15 ‰ and a maximum of 20 ‰ (ANOVA, F = 203.82).

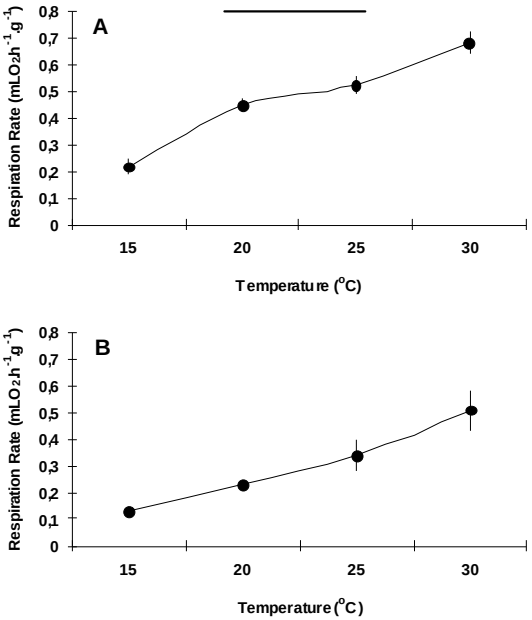


**Figure 1 –** Specific rates for respiration (mL $O_2.h^{-1}.g^{-1}$) at different temperatures. (A) Chronic test (constant temperature) and (B) Acute test (thermal shock after acclimation at 20 ºC) for mussel the *Perna perna*. Average values and confidence interval of 95%. The bars indicate the rates significantly similar according to the Tukey test.

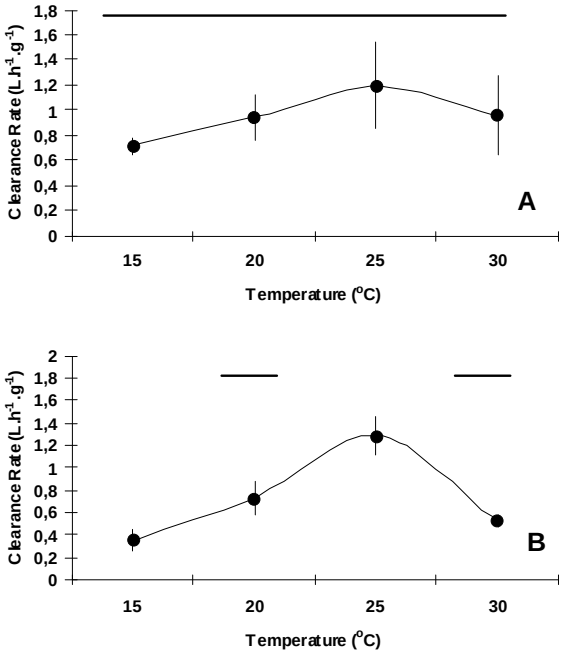


**Figure 2 -** Specific rates for clearance ($L.h^{-1}.g^{-1}$) at different temperatures. (A) Chronic test (constant temperature) and (B) Acute test (thermal shock after acclimation at 20 ºC) for mussel the *Perna perna*. Average values and confidence interval of 95%. The bars indicate the rates significantly similar according to the Tukey test.

**Clearance rate**
Similar to the respiration rate, the clearance for *P. perna* showed a wide band of acclimation to salinity (from 20 to 40 ‰) with a significant inhibition at 15 ‰ (ANOVA, F= 15.99) (Fig. 5A). Under osmotic shock (Fig. 5B), the acclimation interval was reduced to 20 to 35 ‰ with significant inhibitions at 15 and 40 ‰ (ANOVA, F = 50.66).

**Excretion rate**
For the excretion rate, *P. perna* presented two separate acclimation intervals under acute conditions (ANOVA, F = 13.05). The first was characterized by low rates, or inhibition of excretion between salinities of 15 to 25 ‰ and the second by higher rates of between 30 and 40 ‰ (Fig. 6A).

**Absorption efficiency**
In acute conditions, the absorption efficiency presented a wide band of acclimation, which included salinities of 20 to 35 ‰ (Fig. 6B), with significant inhibition for the extreme salinities of 15 and 40 ‰ (ANOVA, F = 10.80).

## DISCUSSION

In this work, the existence of compensation capacity of *P. perna* was determined to variations in temperature and salinity. Initially, the acclimation capacity was evaluated after 15 days, the time required to enter a state of routine metabolism (Resgalla Jr. et al., 2006) and demonstrate physiological compensations to the variations in temperature and salinity, i.e. to present the same routine metabolism, irrespective of the treatment administered.

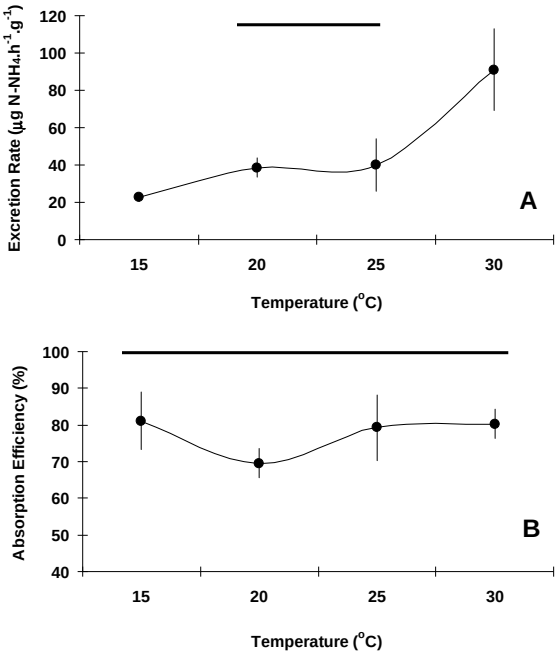


**Figure 3 –** (A) Specific excretion rates (mg N-NH4.$h^{-1}$.$g^{-1}$) and (B) absorption efficiencies (%) in chronic tests (constant temperature) for the mussel *Perna perna*. Average values and confidence interval of 95%. The bars indicate the rates significantly similar according to the Tukey test.

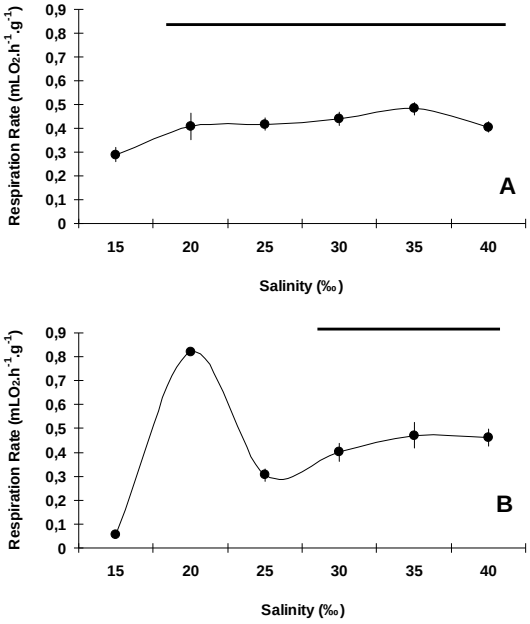


**Figure 4 –** Specific rates of respiration (mL O2.h-1.g-1) at different salinities. (A) Chronic test (constant salinity) and (B) Acute test (osmotic shock after acclimation at 30 ‰) for the mussel Perna perna. Average values and confidence interval of 95%. The bars indicate the rates significantly similar according to the Tukey test.

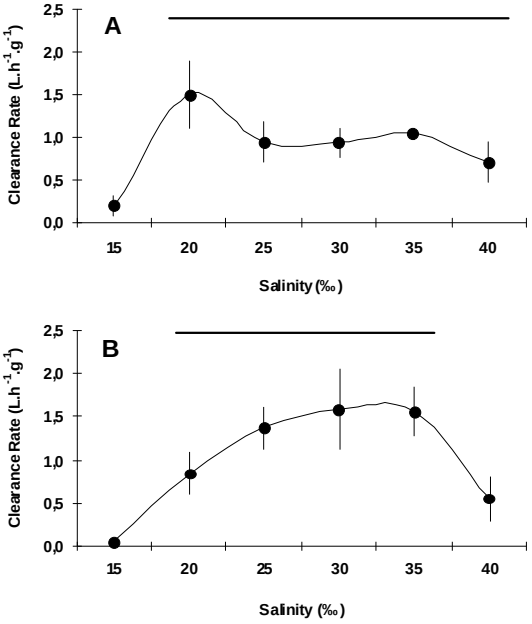


**Figure 5 –** Specific rates for clearance ($L.h^{-1}.g^{-1}$) at different salinities. (A) Chronic test (constant salinity) and (B) Acute test (osmotic shock after acclimation at 30 ‰) for the mussel *Perna perna*. Average values and confidence interval of 95%. The bars indicate the rates significantly similar according to the Tukey test.

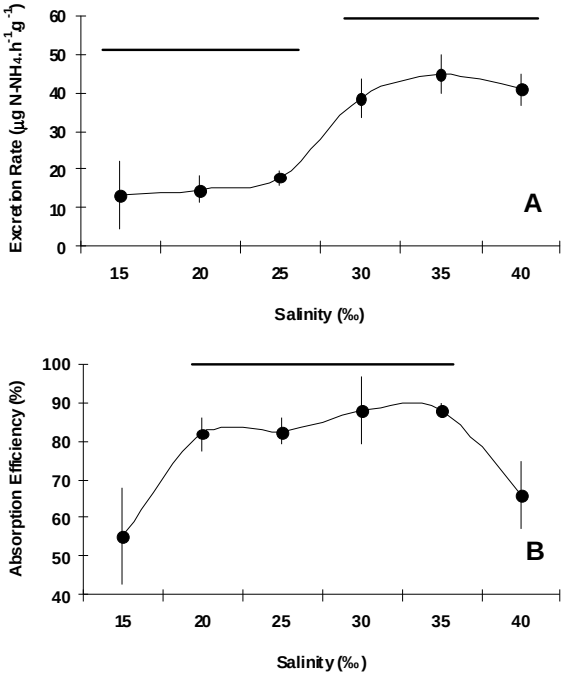


**Figure 6 –** (A) Specific excretion rates (mg $N\text{-}NH_4.h^{-1}.g^{-1}$) and (B) absorption efficiencies (%) in chronic tests (constant salinity) for the mussel *Perna perna*. Average values and confidence interval of 95%. The bars indicate the rates significantly similar according to the Tukey test.

Subsequently, the potential activity of the organisms submitted to acute shocks was investigated, following an acclimation period, with the aim of causing the metabolism to leave routine state and enter a state of activity or standard metabolism, depending on the occurrence of increase or decrease in the physiological rates tested (Bayne, 1973 and Widdows, 1973). With this, it is possible to determine the level at which the activity of the organism can reach, since the activity metabolism cannot be maintained for a long period of time, before returning to the routine metabolism (Bayne et al., 1973).

**Temperature**

Temperature is recognized as one of the major factors influencing the respiration rates and energy balance of poikilothermal marine organisms. Various authors have highlighted the fact that the respiration rates of bivalves are dependant on temperature, be they in acute or chronic conditions (Bayne and Newell, 1985; Griffiths and Griffiths, 1987 and Widdows, 1973, 1976, 1978). Some compensatory adjustment is to be expected in organisms in terms of energy balance, to minimize the losses. In fact, acclimation of the organisms occurs, but its amplitude can vary as a result of the species and geographical distribution. Widdows (1973) found that a temperature of 20 °C was the limit of tolerance of *Mytilus edulis*, with an independent metabolism at temperatures below this and dependence above this limit (Bayne and Thompson, 1970 and Bayne et al., 1973).

Under acute conditions, *P. perna* presented a partial acclimatization of respiration rates between 20 and 25 °C. Between 15 and 30°C, *P. perna* had not demonstrate this compensation capacity, whether in chronic or acute conditions.

For acute conditions, *P. perna* changed its routine metabolism to the activity metabolism between 20 and 30 ºC, with a 2.22-fold increase in respiration rates. This meants $Q_{10}$ values higher than 2 obtained for this interval (Table 1). Other authors presented different $Q_{10}$ values for *P. perna* in different maintenance conditions (Table 1), in which the thermo-independence ($Q_{10} < 2$) varied greatly as a result of the interval considered. The results obtained in this work was close to those

obtained by Hicks and McMahon (2002). Some authors highlighted that the inhibition of the respiration rate based on a maximum temperature was known as the lethal temperature. According to Zuim (1973), the lethal temperature of the species from the coast of São Paulo was 40 ºC. Hicks and McMahon (2002) showed that the lethal temperature of *P.perna* from the Gulf of México was 30 ºC. In this work, despite the fact that no inhibition in respiration rates was observed, it was observed that the organisms acclimated to 30 ºC did not present the capacity to form byssus, a characteristic which was used as an indicator of stress in mussels (Bayne, 1976).

In an inverse situation, under negative thermal shock (from 20 to 15 ºC), *P. perna* switched from routine to standard metabolism, with an approximately two-fold reduction in respiration rates, both in acute and chronic conditions. Thompson and Bayne (1972) and Widdows, (1973) highlighted the existence of a respiration rate in which no pumping of water by the organism occurred, which was considered to be the standard rate of metabolism. This rate was obtained by the interpolation of a regression between these two variables and the estimated value for *P. perna* was 0.099 mL $O_2.h^{-1}.g^{-1}$, i.e., close to the respiration rate at 15 °C.

For the clearance rates, the results were similar to those obtained by Widdows (1973 and 1976) for *M. edulis*. For this species, Widdows (1978) highlighted that at temperatures of above 20 ºC, the clearance rate suffered inhibition. Griffiths and Griffiths (1987) emphasized that the maximum clearance rate was known as the optimum rate and was dependant on the acclimation temperature of the organism. According to these authors, the optimum rate for the oyster *Ostrea edulis* occurred at 5 ºC above the acclimation temperature. *P. perna* presented this inhibition only in acute conditions and the maximum rate was also 5 ºC above the acclimatization temperature. But in chronic conditions, its acclimatization was total, despite showing the same tendency to vary with temperature.

The excretion rate has been used as an indicator of stress of the organism (Smaal and Widdows, 1994), and it increases with temperature due to the metabolic energy demand in *M. edulis* (Griffiths and Griffiths, 1987 and Widdows, 1978). In this work, the excretion rate presented acclimation only for the 20 to 25 °C interval, with maximum at 30 and minimum at 15 °C.

The relation between absorption efficiency and temperature presents conflicting results among authors. According to Griffiths and Griffiths (1987), filtrating bivalves can show from a relation of independence through to an increase or decrease in efficiency with increase in temperature. For Widdows (1978), the absorption efficiency of *Mytilus edulis* was independent of temperature, but even for this species, this relation can vary from one author to another (Griffiths and Griffiths, 1987).

**Table 1 -** $Q_{10}$ Values obtained for *Perna perna* based on its respiration rates in different temperatures obtained from different authors.

| | **Temperature Interval** | **This work** | **Bayne (1967)** | **Berry and Schleyer (1983)** | **van Erkom and Griffiths (1992)** | **Hicks and McMahon (2002)** |
|---|---|---|---|---|---|---|
| Chronic | 15-20 | 4.29 | | | | |
| | 20-25 | 1.37 | | | | |
| | 25-30 | 1.69 | | | | |
| Acute | 10-15 | | | | 1.56 | 3.55 |
| | 15-20 | 3.12 | 1.28 | 2.04 | 2.37 | 1.31 |
| | 20-25 | 2.16 | 1.20 | 1.74 | | 2.24 |
| | 25-30 | 2.20 | 1.49 | | | 1.43 |
| | 30-35 | | 2.64 | | | |

In this work, *P. perna* showed independence of temperature for the interval between 15 and 30 °C. According to Bayne and Newell (1983), the absorption efficiency depends on the length of time the food remains in the digestive tract and the rate of intake. In other words, there is a balance

between clearance rate and absorption efficiency. This highlights the independence of clearance and absorption rates in relation to acclimation temperature (Figs. 5A and 6B).

**Salinity**

Little information is known about the variation of physiological rates of mussels under conditions of different salinities. The common reaction of bivalves to alterations in salinity is a reduction in functional activity (Berger and Kharazova, 1997) and a closing of the valves, which enables a low rate of loss of salts to the perivisceral fluid and survival for up to two days (Salomão et al., 1980). It is generally known that the respiration rate decreases with the decrease in salinity (Griffiths and Griffiths, 1987). This was also observed by Bayne (1967) for *P. perna*, and was classified as stenohaline. Zuim (1973) and Zuim and Mendes (1981), *P. perna* presented low respiration rates in salinities below 24 ‰. In this work, *P. perna* showed ample acclimatization to different salinities in the chronic tests, presenting inhibition of respiration rates only for salinities of 15‰. In acute situations, the oscillations in respiration rates probably reflect compensatory mechanisms which the organism is able to release in situations of hyposmotic shock, in an attempt to keep the blood hyperosmotic (Gilles, 1982).

Just as observed for the respiration rates, *P. perna* showed ample acclimation capacity for the clearance rate and for the absorption efficiency in different salinities, showing inhibition only at the extremes of 15 and 40 ‰. Bayne (1976) found an inhibition in clearance rates in high salinities for *M. edulis*, while Navarro (1988) observed the same inhibition but in low salinities for Mytilidae *Cloromytilus clorus* as well as independence of absorption efficiency from salinity. Navarro and Gonzalez (1998), in a study with the scallop *Argopecten purpuratus*, observed similar results to those obtained in this work for the clearance rate and absorption efficiency.

For the excretion rates, however, the results obtained in this work were not in agreement with the majority of works on other species of filtrating bivalves. Normally, excretion rates increase with the decrease in salinity (Navarro and Gonzalez, 1998 and Griffiths and Griffiths, 1987) and these are also used as indicators of metabolic stress. In this work, the excretion rates were inhibited in low salinities (< 25 ‰). Mollusks are osmoconformers, i.e. the blood is in osmotic equilibrium with the external fluid. This capacity is provided by inorganic and organic osmotic effectors (Gilles, 1982). The inorganic effectors have been studied by Salomão and Lunetta (1989), who showed that the osmotic equilibrium of the hemolimph was established at 72 h with the highest concentration of K and Ca ions. But the most important effectors are organic, particularly free amino acids, whose concentration is regulated by the enzyme aminopeptidase-1 (Newell, 1989). According to Griffiths and Griffiths (1987), the highest excretion rates observed in mussels exposed to low salinities (or hyposmotic shocks) are the result of elimination and breaking down of these amino acids by the cells (Bayne and Newell, 1983; Navarro and Gonzalez, 1998 and Newell, 1989). However, Gilles (1981) highlights that a second destination for these amino acids would be the formation of proteic material in the blood and that they may present a lesser effect on osmolality, perhaps serving as a source of amino acids in case of hyperosmotic shocks. This process would be more economic for the organism, since it requires only 16% of the energy of the basal metabolism (Hawkins, 1985) and would make raw material available for the proteic catabolism. On the other hand, Navarro (1988) obtained inhibition in excretion rates for the mussel *Choromutilus chorus*, justifying its results as an product of the closure of the valves and inhibition of excretion by an accumulation of ammonium in the visceral liquid.

**O:N Ratio**

The O:N ratios were compared as an indicator of the nutritional conditions of the organisms. According to Bayne and Thompson (1970), a prevalence of catabolism of carbohydrates and lipids results in values higher than 30, while a protein catabolism (conditions of alimentary deficiency) results in values of less than 30. Initially, all conditions of maintenance showed a prevalence of protein catabolism (Fig. 7) due to maintenance conditions in which the quantity of food was not sufficient for the needs of the organism (Resgalla Jr. 2004 and Resgalla Jr. et al. in press). However, for the temperature tests, the values for the O:N ratio were significantly higher at 25 °C (ANOVA, F = 25.652) than at the other temperatures, and close to 30, indicating an optimum maintenance temperature.

On the other hand, for the tests in low salinities,(between 15 and 25 ‰), the values of the

ratio were higher than 30 and significantly different from the other salinities (ANOVA, F= 24.051).

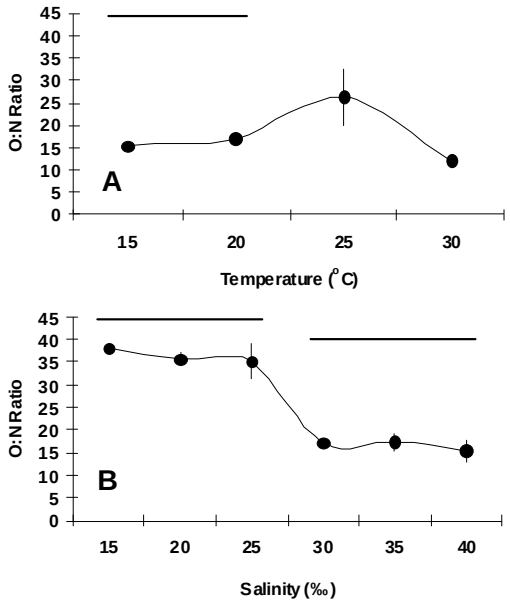


**Figure 7 –** O:N Ratios (A) at different temperatures and (B) at different salinities obtained in chronic tests for the mussel *Perna perna.* Average values and confidence interval of 95 %. The bars indicate the rates significantly similar according to the Tukey test.

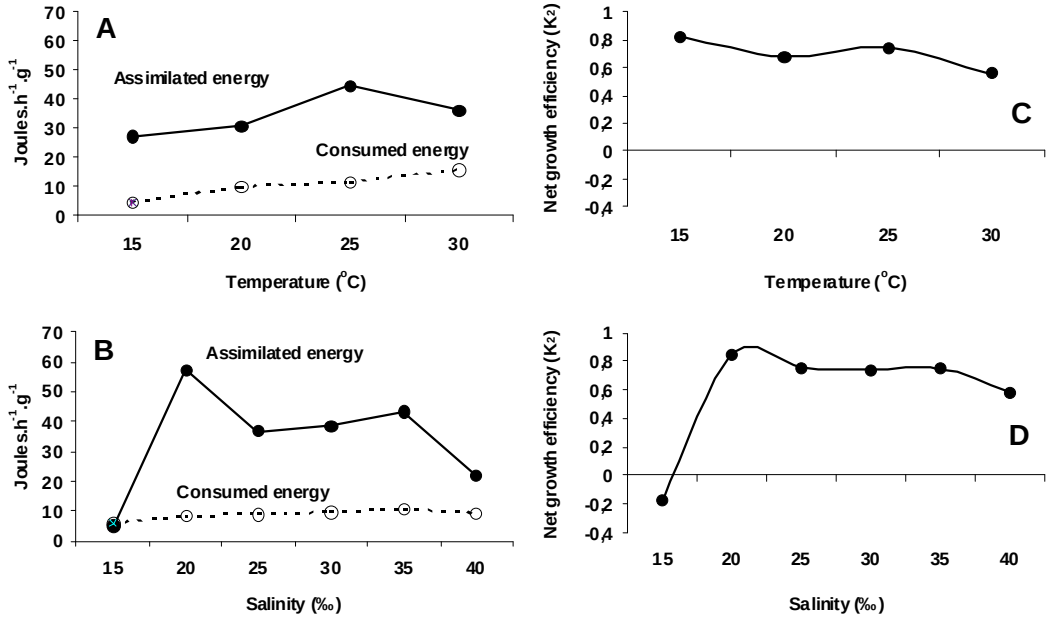


**Figure 8 –** Variations in assimilated energy, consumed energy ($J.h^{-1}.g^{-1}$) (A e B) and net growth efficiency ($K_2$) (C and D) of the mussel *Perna perna* at different temperatures and salinities of acclimation (chronic tests).

In this case, the O:N ratio presented false results and loses its power to be used as an indicator of stress since in low salinities the inhibition of excretion rates occured.

**Energy balance and net growth efficiency.**

*P. perna* presents acclimatization for the interval of 20 to 25 °C, with 25 °C being the optimum temperature due to the higher clearance rates in acute conditions (obtainance of energy for metabolism) (Fig. 8). At 15 °C *P. perna* presented a partial, but acceptable, acclimation capacity, since the positive balance of energy was possible due to the low respiration and excretion rates. At 30 °C, the mussel would be in conditions of stress, which could jeopardize its survival due to the higher respiration and excretion rates, resulting in a higher energy expense. According Zuim (1976), the medium lethal time ($LT_{50}$) of *P. perna* submitted to thermic shock at 30 °C would be 2.2 days. The survival of *P.perna* in a laboratory at 30 °C was due to its slow acclimation (2.5 °C.day$^{-1}$), which enables the development of Heat Shock Proteins (HSP), given that the sensitivity of their expression is dependant on the thermal history of the organism (Feder and Hofmann, 1999). The sign which triggers the production of HSP is the concentration of abnormal proteins in the cell, occasioned, among other factors, by variations in temperature (Hofmann et al., 2002). This fact gives the organisms resistance to the maintenance conditions at temperature extremes. Thus, there was a tendency towards a decrease in net growth efficiency of *P. perna* with the increase in temperature caused primarily by the increase in energy expenditure including biochemical compensatory mechanisms.

For salinity, the amplitude of acclimation was higher (from 20 to 35 ‰), the net growth rate being negative for 15 ‰ and reduced for 40 ‰ (Fig. 8). Contrary to temperature, these negative growth are due not to energy expenditure, but to the inhibition of energy assimilated by the organism.

In general, *P. perna* presented a higher acclimation capacity for salinity than for temperature variations, both for chronic and acute conditions. There is no doubt that *P. perna* is a euryhaline and eurythermic species. Temperature strongly influences the respiration rates (direct relation) and the excretion rates above 25 °C. Salinity proved to have a strong influence on clearance rates, which are inhibited at low salinities (< 25 ‰).

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## RESUMO

O molusco bivalve *Perna perna* é o Mytilidae de maior tamanho e o mais abundante na costa brasileira. Apresenta uma grande importância sócio-econômica devido ao seu uso na mitilicultura, disponibilizando para o consumo humano uma fonte protéica barata proporcionado pelos sistemas de cultivos costeiros. Entretanto, existe uma carência de estudos fisiológicos da espécie, que poderiam ser úteis na avaliação de novas áreas de cultivo, assim como no monitoramento de ambientes contaminados. Neste trabalho foram realizadas testes fisiológicos para determinar as taxas de respiração, clareamento, excreção e eficiência de absorção em laboratório, sob condições crônicas e agudas, em diferentes temperaturas e salinidades. Desta forma, foi possível determinar as oscilações e a capacidade de aclimatação da espécie que servem como base para o entendimento do organismo no ambiente natural, além de gerar conhecimento básico para estimativas do seu potencial de crescimento. Todos os experimentos foram realizados em condições estáticas com dez réplicas e com um mexilhão por frasco teste. A taxa de respiração foi estimada pela diminuição do oxigênio dissolvido na água de incubação, a taxa de clareamento pela diminuição da concentração do fitoplâncton oferecido como alimento, a taxa de excreção pelo aumento da concentração do N-$NH^{4+}$ e a eficiência de absorção pela diferença entre os conteúdos de matéria orgânica do alimento e das fezes produzidas. *Perna perna* apresentou capacidade de aclimatação total para as taxas de clareamento e para a eficiência de absorção (15 a 30 ºC) e parcial para as taxas de respiração e excreção sob

condições crônicas de temperatura. Em condições de choque térmico, as taxas de clareamento e respiração dobraram em magnitude como resposta ao metabolismo de atividade. Para a salinidade, *P. perna* apresentou uma maior capacidade de aclimatação (20 a 40 ‰). Para o crescimento líquido, *P. perna* apresentou uma diminuição de sua eficiência com o aumento da temperatura e foi constante entre as salinidades de 20 a 35 ‰.

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