Effect of zinc and benzene on respiration and excretion of mussel larvae (*Perna perna*) (Linnaeus, 1758) (Mollusca; Bivalvia)

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(With 3 figures)

Abstract

The presence of pollutants in the ocean may affect different physiological parameters of animals. Oxygen consumption and ammonia excretion were evaluated in D-shaped larvae of mussels (*Perna perna*) exposed to zinc sulphate (ZnSO₄) and benzene (C₆H₆). When compared to the control group, both pollutants presented a significant reduction in oxygen consumption. A reduction in the ammonia excretion was also observed, both for ZnSO₄ and C₆H₆ and also in the oxygen consumption. The results indicate that anaerobic metabolism may occur at the beginning of *P. perna* mussels development, as observed in veliger larvae. The O:N ratio under experimental conditions showed low values indicating that catabolism in veliger larvae was predominantly proteic.

Keywords: larvae, *Perna perna*, bioenergetic, zinc, benzene.

1. Introduction

Introducing pollutants to the marine environment is a menace to the biota and may affect marine habitats by physical, chemical and biological interactions on different temporal and spatial scales (GESAMP, 1995). Among the pollutants that can contaminate, marine environment metals and hydrocarbon are common. The main external source of heavy metals is related to industrial dumping or waste dumped straight into the sea (Clark, 1986). Zinc sulphate is a non-biodegradable (chemically or biologically) type of metal, that may be converted to different compounds, sometimes with much increased toxicity over its original component and is easily incorporated into the ecosystem (Hood et al., 1971). Hydrocarbon sources could include leakages from ships, terminals or oil platforms and also incomplete combustion processes of fossil fuels and recent plankton biosynthesis (Clark and Brown, 1977; Connell and Miller, 1984; GESAMP, 1993).

The presence of these pollutants may induce physiological changes in animals, such as energetic demand. Respiration represents an important physiological index, because it reflects metabolism and has a vital meaning for cell energetic supplies (Verriopoulos et al., 1986) whilst excretion is essential in quantifying an organism’s energetic balance (Phan et al., 1993).

The aim of the present study is to determine oxygen consumption, ammonia-N excretion and O:N ratio of mussels D-shaped larvae (*P. perna*) when exposed to...
2. Material and Methods

2.1. Experimental animals

Mussels were collected on Taubaté’s coastal water (23° 54.34’ S and 45° 27.56’ W) of the southern point of São Sebastião, São Paulo, Brazil. Fouling was removed, by the byssus cut off and the animals washed in freshwater. Mussels were induced to release gametes by various stimuli: the removal of fouling, exposure to the air, temperature alternation, seawater filtered and sterilized by UV rays and by the presence of spontaneously liberate gametes. These mussel gametes were separated by sex and the ovocites were washed before spermatozoids. The washed ovocites complied to a net sequence going through different measures of mesh (100, 80, 70, 60, 50, 30 e 22 µm) and were re-suspended in a 1000 mL beaker, with UV sterilized and filtered seawater. The spermatozoids were recollected in a 50 mL beaker, in an ice bath, when only 100 and 80 µm mesh were used, and then were filtered and sterilized in seawater. The ovocyte number was determined by three counting, from an aliquote taken from the ovocyte suspension, under an optical microscope in a Sedgewick-Rafter chamber and was fertilized. Ovocytes were kept at a constant temperature chamber (24 ± 0.5 °C) for one hour. After this period, they were transferred to a 7 L container, where a 30 embryos/mL was not exceeded (ASTM, 1992). In this container, embryos stayed until they reached the D-shaped larva stage and the container was kept at a constant temperature (24 ± 0.5 °C) with weak aeration, sterilized and having filtered seawater as well as a photoperiod 12 hours light:12 hours dark. After 27 hours, the D-shaped larvae were washed in 100; 80 and 50 µm nets and recollected in a 250 mL beaker, according to the number of larvae in this fraction and 4500 larvae were used in each respirometer.

The pollutants used were zinc sulphate (ZnSO₄) in sublethal nominal concentration of 0.47 mg.L⁻¹ and benzene (C₆H₆) in sublethal nominal concentration of 5.69 µL.L⁻¹. Controls with no pollutant were used for comparison (n = 5). The larvae were dried at 80 °C (±1.0 °C) for 48 hours, and weighed on an analytical balance (Mettler Toledo) with 0.1 mg precision.

2.2. Oxygen consumption, ammonia-N excretion and O:N ratio

The concentration of oxygen was measured by the Winkler’s micro-method which is meant for small samples (Fox and Wingfiled, 1938) and modified by Lemos and Phan (2001) for larval stages of aquatic animals. The acclimation time, as well as incubation time was 5 hours. The experimental total time was determined so that the final oxygen concentration would be approximately 75% of the initial concentration, thus avoiding the hypoxic effects on the metabolic processes. Initial and final measures were taken in each group, in the breathing period, for dosage solved oxygen and ammonia-N.

The difference between initial and final concentrations represented oxygen consumption and ammonia-N excretion, corresponding to this experimental condition. Closed system respirometers were used and they contained water which was not changed or re-circulated. An acclimation period preceded the experiment, where larvae were kept in respirometers covered by a 50 µm net, and maintained in a container with filtered and sterilized seawater, having weak aeration under a constant temperature (24 ± 0.5 °C) of 5 hours. Then, the respirometers were tightly closed and remained for another 5 hours period in a constant temperature chamber, when part of the respirometer volume was transferred to a glass syringe where proper reagents were added to determine oxygen consumption.

To determine ammonia-N excretion, the used technique was based on the colorimetric method described by Koroleff (1983) and modified by Lemos and Phan (2001) for larval stages of aquatic animals. After the oxygen dosing in a sample, another aliquot was taken (10 mL) for dose ammonia-N. Following a specific reagent addition, the sample was read in a spectrophotometer with 630 nm absorbance.

The O:N ratio was calculated by the oxygen and nitrogen atomic equivalent obtained from oxygen consumption and ammonia-N excretion values of the experiments, therefore indicating the relation between oxygen consumption and excretion nitrogen (Mayzaud and Conover, 1988).

2.3. Statistical analysis

The non-parametric Kolmogorov-Smirnov test (K-S) (Zar, 1984) was applied to check data normality. The non paired test t was able to find differences considered significant (p <0.05) and was used to analyse all substances. Software used to do the analysis was GraphPad Prism® (v. 2.00).

3. Results

The statistic analysis showed data normality for both oxygen consumption and ammonia excretion. Animal mortality did not occur since the concentrations used were sublethal, as previously determined.

Each D-shaped larvae, with 27 hours, presented 98.45 (±8.66) ng of dry weight and 82.05 (±6.67) µm in length.

Oxygen consumption in the presence of ZnSO₄ presented a reduction in relation to the control group [Control: 1.81124 (±0.25283) nLO₃·h⁻¹ vs. ZnSO₄: 0.71297 (±0.33546) nLO₃·h⁻¹; p <0.05] and it was statistically significant. For C₆H₆ a reduction in oxygen consumption was also observed [Control: 1.09321 (±0.32986) nLO₃·h⁻¹ vs. C₆H₆: 0.49511 (±0.35660) nLO₃·h⁻¹; p <0.05] and so was statistically significant (Figure 1).
Ammonia excretion was also significantly reduced by both pollutants when compared to the control group [Control: 0.02349 ±0.00344 ngat.h⁻¹ vs. ZnSO₄: 0.01482 (±0.00534) ngat.h⁻¹; p <0.05 and Control: 0.02983 (±0.00827) ngat.h⁻¹ vs. C₆H₆: 0.01334 (±0.00332) ngat.h⁻¹; p <0.05] (Figure 2).

The mean values for the O:N ratio obtained for controls of zinc sulphate were 4.0838 and were 3.8813 with pollutant. Benzene obtained values in controls of 2.5289 and 2.8736 with pollutant (Figure 3).

4. Discussion

Xenobiotics affect the organisms’ respiration processes, inducing the animals to use other energy sources that may be employed in detoxicating reactions and metabolism patterns (Vargas et al., 1991). For mussels, zinc is an essential element and necessary for a variety of biological molecules, including enzymes and structural protein (Nolan and Dahlgaard, 1991). However, in concentrations higher than necessary, it can be deleterious for some of these functions. For instance, in high metal concentrations bivalves keep their shells closed for a long period, reducing byssus production and heart beat (Kraak et al., 1997). The closed shell can be observed in the presence of other pollutants and can induce some physiological responses. For sublethal hydrocarbon concentrations, they may cause physiological disturbance and also, development alterations, resulting in the premature death of individuals (Clark, 1986).

There is a direct correlation between the shell closing and respiration rates. When important respiration changes occur, there is a sublethal shift in the capacity of animals to fulfill their physiological processes or to adapt, and, as a result, changes in survival time, growth and reproductive potential may occur, and the respiratory response is highly affected by the concentration of pollutants (Verriopoulos et al., 1986). Some of these changes can be observed for ammonia excretion or oxygen consumption in adults of amphipod Gammarus salinus and mussel Mytilus edulis (Carr and Linden, 1984; Tedengren and Kautsky, 1987), shrimp Marsupenaeus japonicus (Chen and Chen, 1997), mysid Neomysis integer (Laughlin and Lindén, 1983), and crabs Ucides cordatus (Toledo, 1999) and Uca marionis (Yeragy and Koli, 2000).

When exposed to different pollutants, alterations in the physiology of animals may occur such as anaerobic metabolism. In mussels, exposed to long desiccation periods, anaerobic metabolism is used, e.g., in low tide in adults or extreme salinity variations. For larvae, this response may happen for other reasons. The fact that these substances can alter the physiological parameters making the environment inhospitable and inadequate, the mussel larvae are induced to close their valve, preserving homeostasis. In such a situation, the altered metabolism enables animals to use an anaerobic metabolism which is optional for aerobic or a blend of both. This behavior has been observed for some substances, such as high concentrations of ammonia, for adults and juvenile clams Mercenaria mercenaria and adult oysters Crassostrea virginica (Epifanio and Sma, 1975); heavy metals for Crassostrea virginica larvae (Calabrese et al., 1973); and organic compounds (benzene, ethyleneglycol, formaldehyde, methanol, phenol, oil and oil dispersant) for adult Mytilus edulis (Borseth et al., 1995).

Anaerobic metabolism has been thoroughly studied in adult mussels (Thompson and Bayne, 1972; Bayne,
1973; Labarta et al., 1997; Sadok et al., 1999; Babarro et al., 2000). However, the present results suggest that this response has been observed since the beginning of the development of mussels in the D-shaped larvae stage.

An additional factor to consider is the affinity that certain pollutants have towards certain chemical and structure compounds of the organism. For instance, hydrocarbons are lipophilic and zinc has a great affinity with calcium. During the development and planktonic mussel’s life, this could affect the breathing metabolism and O:N ratio consequently; but this is yet to be a confirmed hypothesis.

The O:N ratio values found in mussel’s larvae exposed to different xenobiotics in the present assays were lower than 16, which indicate protein metabolism preponderancy. This index could be directly related to a stress condition, as suggested by Widdows (1978), Carr and Linden (1984), Widdows, (1985) and Vargas et al. (1991), when O:N ratio was found under 7. However, additional experiments are needed to confirm such a hypothesis, such as the obtained values in the absence of pollutants.

For the D-shaped larvae of *P. perna*, it was shown that the oxygen consumption for both pollutants, when compared to the control group, showed a significant decrease. A decrease in ammonia-N excretion was also observed in zinc sulphate and benzene and the oxygen consumption was significantly low. These results show that anaerobic metabolism can be present in the development of the *P. perna* mussel, as observed in the D-shaped larvae. The O:N ratio showed low values, thus indicating that the D-shaped larvae catabolism was predominantly protein in the present studied conditions.

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