

Maintenance of Filtering Molluscs in Aquaria for Sub-Chronic Studies

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ABSTRACT

*This work determined the best survival conditions for the clam *Mytella guyanensis* and the mussel *Perna perna* in the estuary and sea aquaria respectively over at least 12 days, which could enable their use in the ecotoxicological studies. The aquaria were set up with the appropriate water and sedimen, and allowed to establish for a minimum of one month before adding the organisms. The best survival conditions for *M. guyanensis* required more time for the aquarium stabilization, addition of inocula and more frequent water changes than for *P. perna*. The organisms' lipid contents increased and their condition index was maintained indicating the good conditions of the aquaria, hence, their possible use in the sub-chronic studies.*

Key words: Condition index, lipid content, *Mytella guyanensis*, *Perna perna*

INTRODUCTION

According to Burger (2006), the environmental disturbance has increasingly been studied using the observations of the bioindicator organisms, thus aiding the sustainable use and management of the environment. To achieve meaningful answers, the bioindicators have to be maintained under the adequate conditions for a reasonable time period, such that any changes may be attributed to a stressing agent. Most of such studies utilise the biomonitoring of the organisms in a contaminated natural environment. The organisms and environmental samples are taken to the laboratory and then examined for different aspects, such as the embryo malformation (Klumpp et al., 2002) or quantitative determination of the pollutant agent in

the samples collected over a time period (UNEP/FAO/IOC/IAEA, 1992; Glynn et al., 1995; Morrison et al., 1995; Comba et al., 1996; Lauenstein and Cantillo, 1998; Furley and Oliveira F^o, 2000; Boonyatumanond et al., 2002; Metcalfe-Smith et al., 2002; Mwevura et al., 2002; Binelli and Provini, 2003; Gardner et al., 2003; Licata et al., 2003; Monirith et al., 2003; Muir et al., 2003; Ueno et al., 2003; Danis et al., 2004; Svensson and Förflin, 2004; Toro et al., 2004, among many others).

Artificial environments can be produced with the artificial water and sediments (Egeler et al., 1997). Then the organisms collected from the nature or farms are acclimatised and transferred to the small and simple systems for the studies over a short period of time (Gonzalez-Farias et al., 1997; Shin

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et al., 2002; Martel et al., 2003; Fowler et al., 2004) or are transplanted to other natural environments (Cheung et al., 2002; Roméo et al., 2003; Charissou et al., 2004; Amaral et al., 2005). However, certain studies need to have the chosen organisms in the equilibrated and stable aquaria for a longer period of time (Graaf, 1973). Studies on the dynamics of the biocontamination, for example, need good survival conditions for the organisms for the time periods higher than few hours. The aquaria must resemble the natural conditions and be relatively small due to the costs of treatment with the pollutants and the possibility of the contamination of the study laboratories. Hence, the studies on the influence of the pollutants on the biota require integration of the scientific experiments with the techniques of culturing organisms for the extended periods.

These studies are useful to identify "sentinels" that, according to Beeby (2001), are the bioindicator organisms which accumulate a pollutant without significant adverse effects and indicate the environmental pollution. The sentinels solve the problems of the bio-availability measurements and provide a summary of the contamination patterns. But, their habitat and feeding habits must be maintained so that the quantification of the pollutant bioavailability may be done without other additional pressures (Rice, 2003).

Amongst the bioindicator organisms, mollusc bivalves such as clams and mussels have been the main choice because they are sedentary and filter large amounts of the water, beyond to be naturally adapted to the dynamic environments as the estuaries and coasts (Fowler, 1997; Beeby, 2001; Shin et al., 2002; Roméo et al., 2003; Nicholson and Lam, 2005). They feed on the deposits or suspension of the substrates, and reflect the contamination of the sediment or the water column (Zulin et al., 2002). They occupy the lower levels of the food net and can indicate the potential hazard of the bioconcentration of the pollutant amongst these nets (Comba et al., 1996; Nendza et al., 1997; Shin et al., 2002).

This study described the set up and maintenance conditions of the estuary and sea aquaria for the bivalves *Mytella guyanensis* and *Perna perna*, being common in Brazil and fit the requirements of the bioindicators.

MATERIALS AND METHODS

Organisms, water and sediments

The specimens of *M. guyanensis* (Lamarck, 1819), water and estuary sediment, and the specimens of *P. perna* (Linnaeus, 1758), water and sea sediment were collected from the coast of São Paulo State (SP, Brazil), far from the potentially polluted areas in Ilha Comprida

(Nóbrega river, 24°58.55" S - 47°54.89" W) and in Ubatuba (Itaguá bay, 23°25'28.45" S / 45°02'54.23" W), respectively. The collected sediments were dried at the ambient temperature and sieved to 2 mm to remove the stones, large shell pieces and tree branches. Just before their use, they were analysed for the main physical-chemical characteristics. The water samples were stored in the dark in the laboratory for at least 15 days before being used, and the pH, salinity and nitrate presence were constantly analyzed, respectively by pH-indicator strips (Merck – pH 0-14, Germany), densitometer (Assistent – 1.000 to 1.060, Germany), and nitrate commercial kit with detection range between 0 and 50 ppm (Nitrate, Marine and Freshwater Test Lab, France).

Experimental aquaria

Four-compartment aquaria (Fig. 1) were specially constructed for the study, containing the external boxes and a PVC system (bioballs) for the mechanical filtration and a compartment for the sedimentation of the filtered material. These aquaria were filled with 7.7 kg or 11 kg of estuary and sea sediments, respectively, to give a 5 cm deep layer; approximately 300 g of the dead *Artemia* biomass as organic matter supply for the sediments' microbiota, and 50 L of estuary and sea waters. As *P. perna* was a water-column specie, a stone (granite) support was also set up at approximately 10 cm above the sediment for encrusting and support of the organisms in the sea aquarium.

After the aquaria were set up, they were left with continuous water circulation to stabilize for at least one month. The organisms collected in the clean areas were transported to the laboratory, either in the natural sediment (*M. guyanensis*) or water (*P. perna*) of the sampling sites. After cleaning the encrusting from the *P. perna* shells and separating the *M. guyanensis* from the roots a total of 65 and

55 organisms (length 4.0 cm to 5.0 cm) of each species were introduced into their respective aquarium. The *M. guyuanensis* together with their byssus were placed almost completely buried in the sediment and the *P. perna* on the stone

support. The survival was observed and periodical samplings were done after 12 and 34 days for determinations of the lipid amounts and condition index of the organisms.

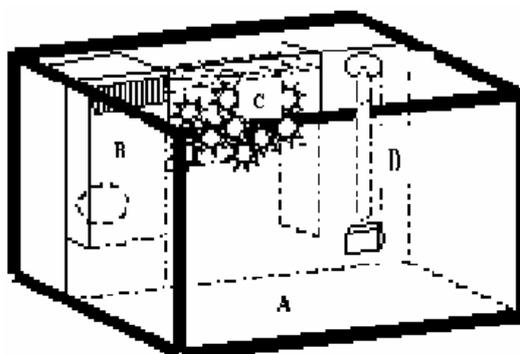


Figure 1 - Aquarium for maintenance of the bivalves. Compartment A: containing the sediment, water, organisms and a comb-device for water filtration IIIIII. Compartment B: for water circulation from the aquarium to the Compartment C with “bioballs”  for water filtration. Compartment D: filtered water circulation to the compartment A, by means of a motor  and a tube .

Approximately 10^7 cells of 13 species of marine and estuarine microalgae were added to each aquarium one day before the organisms' placement and daily thereafter as the food. The algae cultures: *Skeletonema costatum*, *Chaetoceros calcitrans*, *C. gracilis*, *Pavlova lutheri*, *Isochrysis galbana*, *Phaedactylum tricornutum*, *Minutocellus polymorhus*, *Thalassiosira pseudonana*, *T. ocean*, *T. C16*, *Dunaliella tertiolecta*, *Tetraselmis chuii* and *T. tetrathele* were provided by the Mariculture Laboratory of the Fishery Institute (Santos, SP). Every two days, 30 L of the water of each aquarium was replaced by the stored clean water and the feeding resumed after the change.

Condition Index

Seven organisms of each aquarium were sampled every two days for the calculation of the condition index according to Zulin et al. (2002), Binnelli and Provini (2003) and Roméo et al. (2003), by the ratio between the weight of soft tissues and the total weight of each bivalve, multiplied by 100:

$$\text{Condition index} = \frac{\text{Weight of the soft tissues (mg)}}{\text{total weight (mg)}} \times 100$$

Lipid extraction

The soft tissues of the collected organisms were mixed with the anhydrous Na_2SO_4 (1:3 w/w) and the mixture was homogenized (Sorvall, Omni Mixer) at high speed for 3 minutes. The samples were divided into three sub-samples that were extracted with 100 mL of hexane in the Soxhlet (Fowler et al., 1978; UNEP/FAO/IOC/IAEA, 1986; Villeneuve and Cattini, 1986; Brito et al., 2002; Carvalho et al., 2002) for 16 hours.

Determination of the lipid contents and percentage

The amount and percentage of the lipids in the extracts were measured gravimetrically according to UNEP/FAO/IOC/IAEA (1986) and Lauenstein and Cantillo (1998), respectively.

$$\text{Amount (mg/g) of lipids} = \frac{\text{Weight of the residue (mg)} \times \text{volume of the extract (mL)} \times 10^3}{\text{Volume evaporated (\mu L)} \times \text{weight of the extracted tissue (g)}}$$

$$\text{Percentage (\% of lipids)} = \frac{\text{TV}}{\text{AV}} \times \frac{\text{LW}}{\text{SW}} \times 100$$

where, TV is the total volume of the extract (mL), AV is the volume of the aliquot (mL), LW is the weight of the lipids (g) and SW is the weight of the tissue sample (g).

Statistical analysis

Results were statistically analyzed by the comparison of the condition index mean values using the *t* distribution test with $P \leq 0.05$.

RESULTS

The salinity of the collected waters measured on arrival at the laboratory varied from 30 to 36 ‰ for the sea water and from 21 to 23 ‰ for the estuary water. Their temperatures varied from 20 to 23° C and the pH of stored waters was always 7.0. Nitrate was never detected in the sea aquarium. However, in the stabilization period of the estuary aquarium containing the sediment, the pH decreased to 6.0. Both the organisms acclimatized very well the feeding conditions.

Survival of *Mytella guyanensis*

The mortality of the clams *M. guyanensis* even under the natural conditions was frequently observed by the open and empty shells in all the collected samples. The closed living organisms were selected by the size and carefully placed with their byssus buried into the sediment. In the first attempts with a short period of stabilization and periodical additions of the dead *Artemia* as the food, complete mortality occurred in just 5 to 10 days. After the additions of the biomass of *Artemia* in the water, an oily cover on the water surface was observed, possibly derived from fatty acids (Sorgeloos et al., 1986). This cover hampered the gas change between the water and air, causing a strong smell, probably by anoxia and methane production, and consequent mortality of all organisms. Thus, in the other settings, the 300 g of dead biomass of *Artemia* were mixed with the 7.7 kg of estuary sediment, before the addition of the water. The aquarium was left to stabilize for one month and a specimen of the mussel *P. perna* without the shell was buried into the sediment at 15, 10 and 3 days before the *M. guyanensis*' placement, as the inocula to the sediment nitrifying microorganisms (Graaf, 1973), and so enrich the sediment's microbiota. These inocula were left for 24 h and then removed to avoid the excessive putrefaction.

In the first attempts and before the clams' placement, the pH decreased to 3.0 during the aquarium maturation. The aquarium was treated with 20 g of Na₂CO₃ dissolved in 1.5 L of theseparated aquarium water, which readjusted the pH to 6.0.

This aquarium was left to stabilize for two months in the final set up. During this time, the water acquired the typical yellowish colour of the natural estuary environment and a rusty 2.0 mm cover was developed on the sediment, indicating ferrous oxidation as observable also in the natural environment. After the introduction of the 55 organisms, 7 specimens were collected each two days over 15 days. Despite the 30 L water changes each two days, the nitrate levels increased up to 20 mg L⁻¹ between the changes. Then, the water change was done in 24 h interval. In these conditions, despite a detected 16.4% mortality, healthy specimens were sampled till the 12th day after their placement, and the water was maintained at pH 6.0. The condition index and the amount of lipids were determined in these healthy sampled organisms (Fig. 2). Their condition index values were constant ($P > 0.05$) during all the experimental time and the percentage of the lipids varied from $1.91 \pm 0.35\%$ in the time 0 to maximum $18.95 \pm 4.28\%$ at 10th day after, decreasing till $14.08 \pm 2.47\%$ on the 12th day.

Survival of *Perna perna*

Unlike *M. guyanensis*, no *P. perna* died during the experimental period. After a month of the sea aquarium stabilization, the 65 specimens of *P. perna* were placed on the stone support where the byssus formation and motion of the organisms were clearly observable. A maximum of 5.0 mg L⁻¹ nitrate was only detected when the population was of up to 51 specimens and in the days between the water changes. The null values were restored after the 30 L water changes every two days.

The seven mussel specimens were sampled for the analysis over 15 days, but as some were left over, they were maintained in the aquarium up to 34 days. The condition index values (Fig. 3) were nearly constant, but with an increasing trend ($P = 0.0185$ and 0.0002 , respectively at 4 and 34 days after the introduction into the aquarium). The percentage of the lipids varied from $1.75 \pm 0.01\%$ at time 0 to $14.2 \pm 2.42\%$ at 14th day but diminished to $6.83 \pm 0.80\%$ at 34th day.

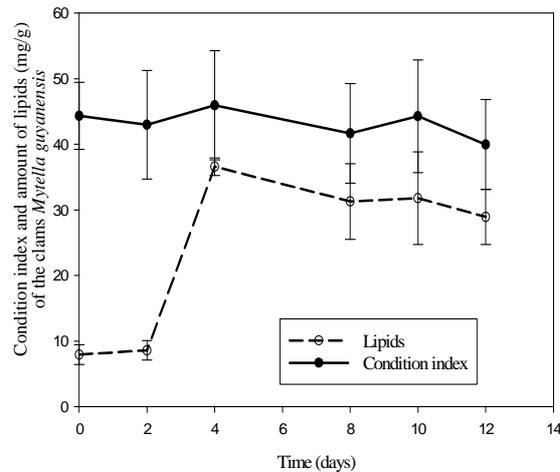


Figure 2 - Condition index and amount of lipids of the clams *Mytella guyanensis* maintained in the aquarium over 12 days.

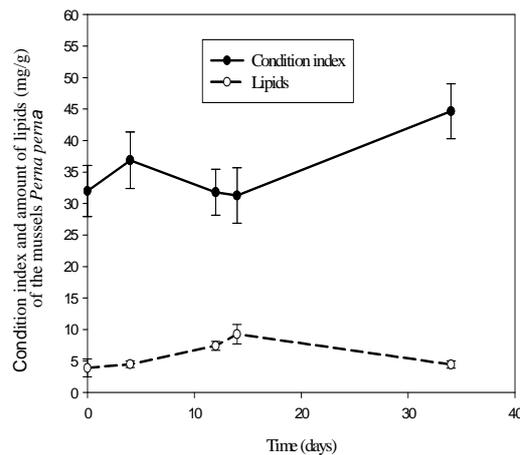


Figure 3 - Condition index and amount of lipids of the mussels *Perna perna* maintained in the aquarium over 34 days.

DISCUSSION

The drop in the pH values seen in the estuary aquarium was likely to occur in such reducing environments because of the acidic characteristics of the sediment (Table 1). As the clams lived buried into the sediment and survived by feeding on the sediment and filtering the water (INFORMEBIO, 2006; INBio, 2006), they needed an enriched and well stabilized environment which

was achieved by mixing the dead biomass of *Artemia* before the addition of the water and the addition of the soft tissues of *P. perna* as the inocula. The intense metabolism of the microbial organisms and clams increased the nitrate levels (Graaf, 1973), but the more frequent water changes probably simulated the environment renewal provided by the tidal action. But, even under these good conditions, there was mortality of nearly 16%.

Table 1 - Main physical-chemical characteristics of the estuary and sea sediments.

Sediment	Sand	Silt	Clay	Organic Matter	pH
	(g kg ⁻¹)	(g kg ⁻¹)			
Estuary	650	100	250	49	3.8
Sea	920	20	60	16	6.4

These conditions allowed the samplings up to 15 days, but although the amount of the lipids increased initially, both the condition index and amount of the lipids tended to decrease with the time. This indicated that a period of 12 days could be used for the maintenance of *M. guyanensis* in the aquaria, but should not be exceeded because the organisms could be stressed and the results masked.

Good survival conditions were more easily achieved for the sea aquarium and mussels. The organisms survived longer and all other parameters did not present significant changes up to 34 days. However, although the organisms were maintained over 34 days, as proved by the condition indices being almost constant, the 15-day period was actually safer for the studies because less lipids were detected at time 34 days, indicating a higher consumption of the nutritional reserves to support the growth.

CONCLUSIONS

The 12-day period was safe for the maintenance of good conditions for 55 and 65 specimens of *Mytella guyanensis* and *Perna perna*, respectively in 50 L capacity aquaria (near 3.2 g and 2.9 g L⁻¹, respectively), which allowed medium-term studies. The healthy conditions of both the species were sustained for two and one months in the previously stabilized aquaria with closed but constant circulation of the estuary and sea waters, respectively.

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RESUMO

Estudos sobre a dinâmica de contaminação de organismos marinhos devem ser feitos sob condições controladas pelo tempo necessário para que os organismos possam responder à presença do agente contaminante. No entanto, a manutenção de organismos em aquários por determinado período pode ser difícil porque todas as outras variáveis do ambiente precisam ser próximas às condições naturais. Este trabalho determinou as melhores condições de sobrevivência do marisco *Mytella guyanensis* e do mexilhão *Perna perna*, respectivamente em aquários de estuário e de mar, por período de até 12 dias. Os aquários foram montados com água e sedimento de estuário ou de mar e estabilizados por, no mínimo, um mês antes da colocação dos respectivos organismos. As melhores condições de sobrevivência de *M. guyanensis* requisitaram mais tempo de estabilização do aquário, adição de inóculos e trocas de água mais frequentes do que para os *P. perna*. Os conteúdos de lipídios aumentaram com o tempo e o índice de condição dos organismos foi mantido, indicando as boas condições dos aquários e, conseqüentemente, a possibilidade de uso em pesquisas ecotoxicológicas.

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