Orbital: The Electronic Journal of Chemistry
journal homepage: www.orbital.ufms.br
e-ISSN 1984-6428

| Vol 10 | | No. 4 | | Special Issue June 2018 |

FULL PAPER

Transplant Experiments as a Tool for Evaluating the Suitability of Sessile Organisms as Biomonitor Species in Tropical Coastal Waters: The Case of the Brown Mussel *Perna perna* (Linnaeus, 1758) in Rio de Janeiro State, Brazil

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Article history: Received: 31 August 2017; revised: 21 November 2017; accepted: 30 January 2018. Available online: 11 June 2018. DOI: http://dx.doi.org/10.17807/orbital.v10i4.1069

Abstract:

The present study evaluates mercury (Hg) toxicokinetics in *Perna perna* (L.) mussels from tropical bays through transplantation experiments. The mussels were transplanted from Guanabara Bay (GB) to Ilha Grande Bay (IGB). The experiments were carried out in December 2008 (experiment 1) and June 2009 (experiment 2). Both experiments lasted one month. In the experiment 2, the transplanted animals (77±8 $\mu g.kg^{-1}$) showed a significant increase in Hg concentrations and exhibited significantly higher Hg concentrations than the animals that remained in GB (41±4 $\mu g.kg^{-1}$). Despite this increase in mercury levels, the transplanted animals have not achieved the Hg concentrations of IGB resident animals (100±11 $\mu g.kg^{-1}$). These results suggest that individuals of this species rapidly incorporated Hg in tropical estuaries.

Keywords: bioavailability; biomonitoring; bivalve; metal; tropical bays

1. Introduction

The mussel *Perna perna* (Mytiloida: Bivalvia, Linnaeus, 1758) has a vast worldwide distribution, occupying several parts of Africa and America, in addition to southern Europe, South Africa, Arabian Peninsula and Sri Lanka [1]. *Perna perna* provides an important crop due to its suitability for mariculture [2]. In 2011, the production of *P. perna* for human consumption in Brazil was estimated to be around 3770 tons [3].

Among mollusks, the taxonomic family Mytilidae has been used in temperate areas to monitor levels of Hg in coastal waters; specifically, *Mytilus galloprovincialis* and *Mytilus edulis* [4-6]. Despite the wide use of mytilids for environmental monitoring, there is a lack of studies adopting this approach in tropical areas. Several authors have suggested the use of organisms for monitoring investigations, as the approach allows for direct evaluation of pollutant bioavailability. Therefore, monitoring studies using marine vertebrates [7-15], crustaceans [15] and bivalve mollusks [16-18] have been performed in tropical Brazilian estuaries.

Regarding investigations on Hg biomonitoring in Rio de Janeiro state (RJ) estuaries, three of them should be highlighted [15, 19, 20] due to higher Hg concentrations in fish from Ilha Grande Bay (IGB) than in those from Guanabara Bay (GB). Although GB has been reported as one of the most dramatic example of anthropogenic degradation along the Brazilian coast [7-12, 14, 21], it has been suggested that there is a higher

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bioavailability of mercury in IGB than in GB for a number of organisms [15, 19, 20].

Considering all the above-mentioned aspects, we have opted for evaluating Hg toxicokinetics in *P. perna* mussel at IGB and GB through transplantation experiments, inferring also on Hg bioavailability in these tropical estuaries.

2. Results and Discussion

Multiple studies around the world have used mytilid mussels in transplant experiments [5, 24]. However, studies using *P. perna* are hardly ever performed. This scarcity increases the importance of the present study and highlights the originality of the data generated herewith. In order to enrich the discussion, we have referred to studies that dealt with *Perna* spp. and *Mytilus* spp. In fact, the latter genus is frequently used in transplant experiments performed in temperate zones, not only for Hg but also for evaluating the bioavailability of other metals [5, 6].

In the 1st transplant period (from December to January) the mean (± SD) THg levels of resident mussels from GB and IGB was 47 (±11) and 60 (±10) $\mu g.kg^{-1}$, while in the 2nd transplant period (June to July) the levels were 44 (±6) and 80 (±20) $\mu g.kg^{-1}$, respectively. In the experiment performed in summer (December), there was no significant difference in THg levels between the transplanted (GB-IGB: 50 ± 8 $\mu g.kg^{-1}$) and IGB resident mussels (IGB-T1: 67 ± 8 $\mu g.kg^{-1}$) (U=51.00; p=0.22), as well as no significant difference was observed between the first group (GB-IGB) and the mussels kept at GB (GB-T1: 55 ± 7 $\mu g.kg^{-1}$) (U=51.00; p=0.23) (Figure 1a).

The animals transplanted (GB-IGB) in June (77 ± 8 $\mu g.kg^{-1}$) demonstrated significantly higher THg concentrations than the mussels kept at the original site (GB-T1, 47 ± 7 $\mu g.kg^{-1}$) (U=0.00; p<0.001) (Figure 1b). Although the transplantation of mussels from GB to IGB caused a significant increase in Hg levels, they were not high enough to generate THg concentrations at the same level as those found in IGB resident mussels (IGB-T1: 100 ± 11 $\mu g.kg^{-1}$) in June. Our findings corroborate investigations performed at Kastela Bay, located on the central part of east Adriatic coast [5, 6], since a significant increase in THg concentration was found one month after the transplant.

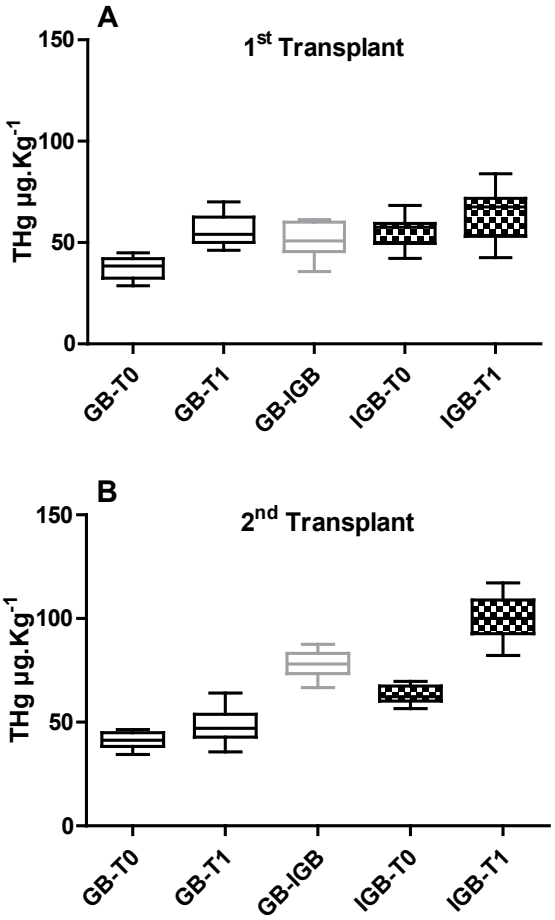

Figure 1. *Box Plot* of first (A) and second (B) transplants: THg concentrations in mussels, in $\mu g.kg^{-1}$. (A): GB-T0 and GB-T1, samples from Guanabara Bay collected in December 2008 and January 2009, respectively. GB-IGB, mussels transplanted to Ilha Grande Bay (IGB) and sampled in January 2009. IGB-T0 and IGB-T1, IGB resident mussels sampled in December 2008 and January 2009, respectively; (B): GB-T0 and GB-T1, samples from Guanabara Bay collected in June and July 2009, respectively. GB-IGB, mussels transplanted to IGB and sampled in July 2009; IGB-T0 and IGB-T1, IGB resident mussels sampled in June and July 2009, respectively. *GraphPad Software.*

In the first transplant period, mussels from GB (T0 and T1) exhibited a significant difference, *i.e.*, Hg levels were significantly lower in December (37 ± 5 $\mu g.kg^{-1}$) than in January (55 ± 8 $\mu g.kg^{-1}$) (U=0.02; p<0.05). The same pattern was not observed for the second transplant period (Table 1). No significant difference in THg concentration was observed between organisms

sampled in June and July in GB (U=50.00; p=0.20).

In the second transplant period at IGB, the mussels exhibited a significant difference in THg concentration between June (63 ± 4 μg.kg^{-1}) and July (100 ± 11 μg.kg^{-1}) (U=0.02; p<0.05). However, the same pattern was not observed for the first transplant period (Table 1).

The fact that a significant difference between transplanted mussels (GB-IGB) and those *P. perna* that were kept in the original site (GB-T1) has occurred only in austral winter might be explained by pluviometry at the IGB region and hence suspended particulate matter (SPM) input to IGB. The southern coast of the Rio de Janeiro state littoral, where IGB is located, is one of the areas of highest pluviometric index [20] of the region known as the Central South Brazil Bight, which extends from Cabo Frio (23ºS) to Cabo de Santa Marta (28ºS), comprising the states of Rio de Janeiro, São Paulo, Paraná and Santa Catarina [9]. The austral summer is the rainy season and the highest pluviometric index in this period of the year increases the SPM input to IGB [20]. Giving Hg affinity to SPM, the highest burden of this material received by the IGB in austral summer could reduce the bioavailability of this metal in the rainy season (austral summer) in comparison to the dry one (austral winter) [15, 20]. It is important mentioning that any trends of possible differences between summer and winter suggested by the data from the present study should be seen with great caution, as evaluating seasonality was not the aim of this investigation and our experimental design was not conceived for this objective.

Table 1. Variation of total mercury concentrations in the whole soft tissues of resident mussels during first and second transplant periods. Mean total mercury concentrations (THg) in μg.kg^{-1} (± SD). Guanabara Bay (GB); Ilha Grande Bay (IGB).

	1st Transplant		2nd Transplant	
	December	January	June	July
GB	37 ± 5	55 ± 8	41 ± 4	47 ± 5
IGB	55 ± 7	67 ± 8	63 ± 4	100 ± 11

Some studies have also reported a rapid increase in concentrations of mercury and other metals in mussels transplanted to areas where there is greater bioavailability [5, 6, 22-24]. For example, Catharino et al. [22] and Seo et al. [23] performed studies in São Paulo state littoral (Southeast Brazil) using *P. perna* in transplant experiments. These two investigations transplanted mussels from a control marine farm to areas under higher anthropogenic pressure. Catharino and co-authors [22] revealed an increase in mercury concentrations in *P. perna* mussels during the winter period, after the transplant, from a value of 24 μg.kg^{-1} in organisms from the control area (Cocanha Beach, São Paulo) to 41 μg.kg^{-1} in one site with high domestic discharges (Ilhabela, São Paulo) [22]. Calculating from the mean values, it is possible to verify that the data obtained by the latter investigation [24] represent a 71% increase in Hg concentrations, while those from the present study constitute a 64% increase for the same parameter and season (austral winter).

The mussel species *Perna viridis* has also been used in experiments comprising transplants between low and high contaminated waters in the straits of Johore, Iran [24]. In that study, there was a significant increase in THg concentrations two weeks after transplants to a highly contaminated area (6.7 μg.kg^{-1} to 29.3 μg.kg^{-1}), while there was a decrease in THg after transplant to a less contaminated area (6.7 μg.kg^{-1} to 4.9 μg.kg^{-1}) [24]. Similar results were observed for *M. galloprovincialis* when samples of the species were transplanted from non-contaminated to contaminated areas [5, 6]. In those studies, there was a significant increase in mussel THg concentrations after a one-month transplant. The same trend was found for other metals, such as iron, manganese and, lead [4].

Catharino et al. [22] have also found a seasonal variation for the Hg burden bioaccumulated by *P. perna*, with higher tissue concentrations in winter. Therefore, our results for the IGB resident mussels corroborate the findings of Catharino et al. [22] because higher THg concentrations were found in June-July (Figure 1b) than in January-December (Figure 1a) in the present study.

These temporal variations were found in mussels from other studies [25, 26]. This variation might be related to oscillations in the

quality and quantity of available food, as well as to algal blooms [27]. The increase in the Dissolved Organic Carbon (DOC) input is another factor that may be related to the monthly variations in THg concentrations found in the present study. Some investigations have shown an inverse relationship between Hg accumulation by marine invertebrates and DOC [28, 29], which may be related to a decrease in Hg availability due to binding of the metal to chelating agents present in DOC. However, a positive correlation between DOC and Hg bioaccumulation has been identified in fly larvae (Diptera, Simuliidae) collected in ponds, which have a varied concentration of DOC [30].

3. Material and Methods

Two coastal bays from the Rio de Janeiro state (Southeast Brazilian region), *i.e.*, GB and IGB, were selected for the present study (Figure 2). These two bays represent distinct degrees of anthropogenic pressure. As mentioned, GB is regarded as the most intense example of man-made degradation of the Brazilian littoral [7-12, 14, 21], while IGB constitutes a relatively preserved environment [15].

In GB, sampling was performed in the marine farm of Jurujuba (22°55'59''S; 046°06'40''W) (Figure 2), which had a production of 130 and 65 tons of *P. perna* in 2005 and 2006, respectively [31]. There is no record of other marine farm in GB that produces mussels on a commercial scale and in a systematic way. Sampling was carried out with the help of a local marine farmer, who provided transportation on the boat for the researchers.

In IGB, sampling was performed in the experimental marine farm of the POMAR Project (*Programa de Repovoamento Marinho*), of the *Instituto de Ecodesenvolvimento* of Ilha Grande Bay (IEDIGB). This farm cultivates the scallop, *Nodipecten nodosus*; however, the natural and frequent fouling of *P. perna* mussels on the cultivation structures (long lines) allowed the sampling for the present study. The marine farm is in the Biscaia Inlet (23°01'38''S; 044°14'14''W) (Figure 2). The fieldwork was conducted with the help of the IEDIGB workers.

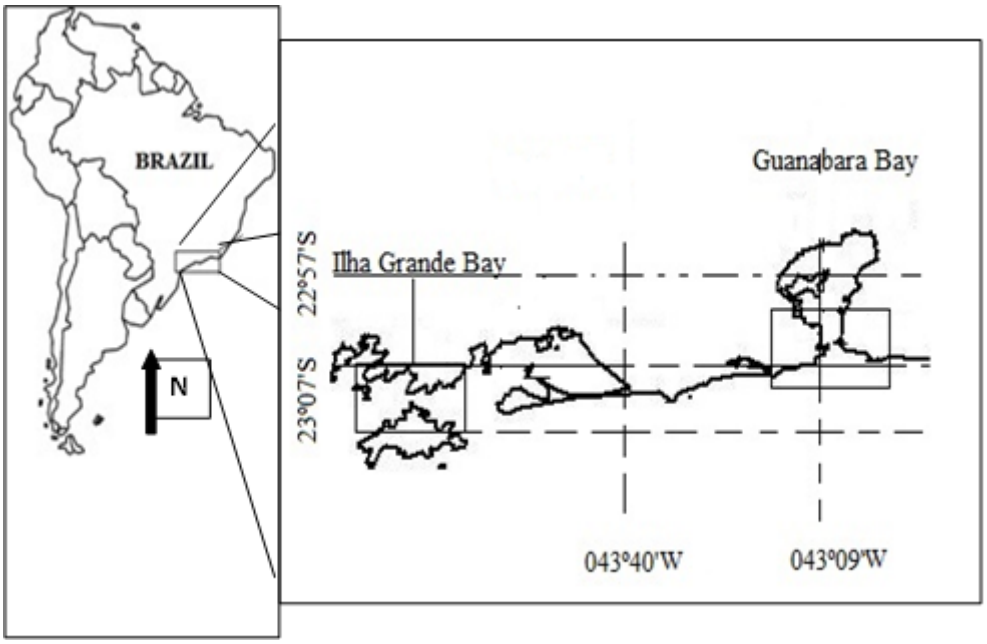

Figure 2. South America map stressing Brazil and amplifying the region of the Rio de Janeiro State coast (SE-Brazil) where there is Guanabara Bay (GB) and Ilha Grande Bay (IGB), which are highlighted. The two main poles of marine bivalve production are indicated by arrows.

Two transplanting experiments were performed. The first one initiated in December-

2008 and the second in June-2009 - both lasted 30 days. The experiments were performed during austral summer and winter, respectively. In both experiments, mussels cultivated in GB were transported to IGB, where the organisms would supposedly be more exposed to environmental levels of Hg [15]. Additionally, one mussel long line was kept in the origin estuary (GB) in both, the 1st and the 2nd experiment (1ºTr or 2ºTr). In December 2008 and June 2009, a mussel long line was transported from GB to IGB and fixed in the IEDIGB cultivation system. In addition, 30 samples were collected from the mussel long line that stayed in GB, representing the initial condition of the experiment, *i.e.*, the time zero (GB-T0). The same number (30) of resident mussels was collected from IGB at the beginning of the experiment, as the time zero of this bay (IGB-T0). Thirty days after the beginning of the experiment, 30 samples were collected from the mussel long line that was kept in GB (GB-T1), as well as 30 mussels were taken from mussel long line that was transported to IGB (GB-IGB). The long lines were located at the 7-m isobath approximately. They were composed of buoys and ropes, attached to two stone anchors at their extremities. The mussels were placed at, approximately, 2-meters depth in the water column. In order to measure the Hg levels of resident mussels from IGB, 30 organisms were also collected at the end of the experiment (IGB-T1), *i.e.*, in January and July 2009.

Mussels, whose size varied between 50 and 80mm, were manually collected from the mussel long lines. The animals were cooled up to the arrival at the lab, where they were frozen (-20 °C). After defrosting, the animals were weighed (to identify the water mass percentage) and distributed into six groups of pooled samples, with five individual samples within each group. In total, there were 60 pooled mussel samples. Subsequently, samples were frozen (-80 ºC) and freeze-dried. The dried samples were ground in a homogenizer containing stainless steel vessel and blades (Marconi®/MA-345H). To perform its decontamination before the next sample, the vessel was cleaned with detergent and alcohol after the homogenization of each dried sample in the abovementioned laboratory mill. The samples were stored in screw-capped glass flasks. Four sampling campaigns (2 for each study bay) were performed.

The analytical procedure followed a previously described method [32]. Aliquots of whole soft tissues of approximately 0.05 g of dry tissue were used. The digestion procedure started with the addition of 1 mL of 30% H_2O_2 (Merck® p.a.) and 3 mL of concentrated sulfonitric mixture (H_2SO_4:HNO_3 1:1 v/v) (Merck® p.a.). The vessel was then heated to 60 ºC for 2 h in a water bath. After cooling for 15 minutes, 5 mL of $KMnO_4$ (5%) was added to each sample. Then, the extracts went through a step of heating in the water bath (60 ºC) for 15 minutes and left to cool overnight. The following day, 1 mL of 12% hydroxylamine hydrochloride ($NH_2OH.HCl$) was added to each sample, with subsequent homogenization of the extract. The final extract was made up to 12 mL with high purity deionized water (18.2 MΩ cm) from a Milli-Q system. Total mercury determination was performed by Cold Vapor Atomic Absorption Spectrophotometry (CV-AAS), using a Flow Injection Mercury System (FIMS-400, Perkin Elmer) equipped with an AS-90 auto sampler [31].

All the glassware used during sampling and analytical procedures had been previously decontaminated in two steps, *i.e.*, in two different baths with (1) detergent and (2) acid solutions. In both cases, the flasks were kept immersed for 24 hours. The baths consisted of (1) a 5% neutral detergent solution (Detertex®) and (2) a 5% HNO_3 solution. Between each bath and after the second bath, the flasks were rinsed with distilled and deionized water. Then, all the glassware was dried in the stove (100 ºC).

The determination of THg concentrations in mussels was performed in duplicates. Blanks were carried through the procedure in the same way as the samples, being three analytical blanks for each batch of twenty samples. Only results presenting a variation coefficient between duplicate samples ≤15% were considered. Analyses of aliquots of the certified reference material (CRM) were performed in the same way as the samples. The CRM used was NIST-2976 (mussel soft tissues) and our recoveries were between 85 and 114%. The detection limit of the method (DLm) was 1.8 $ng.g^{-1}$. DLm was calculated by multiplying the detection limit of the instrument (DLi, in ng/mL) by the final volume of the extracts (in mL) and dividing this result by the mean value of the sample mass (in grams). DLi was determined based on three times the

standard deviation of 10 runs of the blank solution, divided by the slope (α) of the calibration curve (DLi=SD*3/α).

The results were grouped by study area. The *Shapiro-Wilk's W* test was used in order to test for normality of the data. For non-normal data, the *Mann-Whitney U* test was applied for investigating possible differences. The data treatment was performed using the statistical package Graphpad Prism 5.0 (*GraphPad Software*) and the significance level adopted was 5%

4. Conclusions

Confirming our hypothesis, this study suggests that IGB presents higher Hg bioavailability than GB, as well as that *P. perna* mussels quickly accumulate the Hg in their tissues. However, further studies evaluating the transplant of animals for a longer period are needed for a better understanding of Hg toxicokinetics in coastal environments.

Acknowledgments

We give thanks to the shellfishermen from Jurujuba beach, to the Instituto de Ecodesenvolvimento in Ilha Grande Bay (Project POMAR). This work was supported by the Brazilian National Council for Scientific and Technological Development (CNPq) through a Universal Call CNPq-Project from PRD (proc. 456614/2014-1), as well as through a scientific cooperation established between CNPq (proc. 490279/2013-9) and FNRS (Fonds de la Recherche Scientifique, from Belgium), in which a PDE (proc. 203074/2014-9) grant was included for the post-doctoral investigations of PRD, at University of Liege (Belgium) in 2015. OM and PRD have research grants from CNPq (PQ-1A proc. 306703/2014-9 and PQ-2 proc. 306847/2016-7, respectively).

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