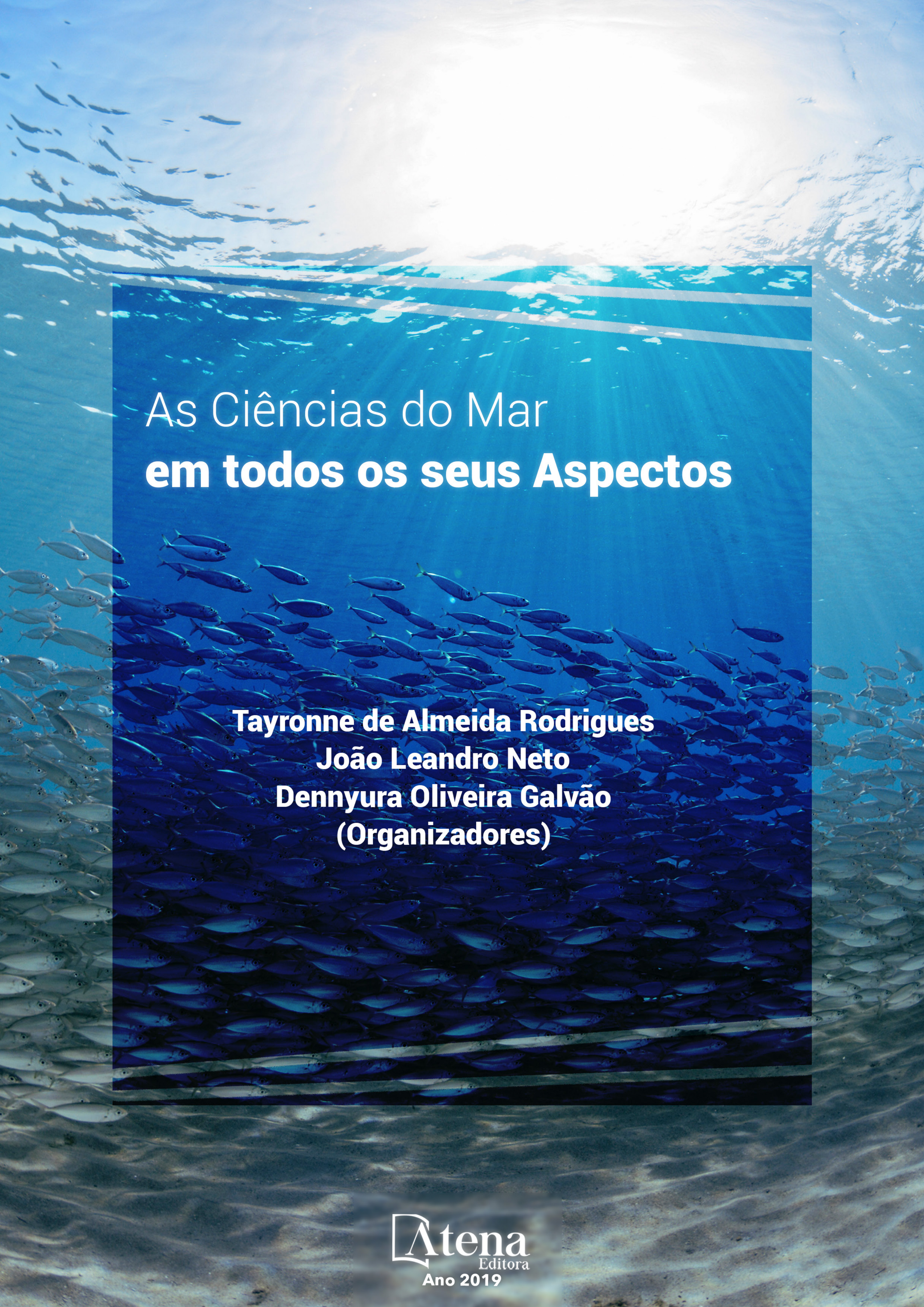

As Ciências do Mar
em todos os seus Aspectos

Tayronne de Almeida Rodrigues
João Leandro Neto
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(Organizadores)

Atena Editora
Ano 2019

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APRESENTAÇÃO

O mar representa para o Homem desde as mais antigas datas uma fonte de mistérios, um universo repleto de criaturas com formas fantásticas e inimagináveis e essa forma de pensar tornava mais restrita a exploração marinha e o aprofundamento de pesquisas. Em 380 A.C., o filósofo grego Aristóteles foi o primeiro a estudar os oceanos com um cunho mais científico.

As ciências do mar lidam diretamente com região costeira e a região oceânica, pois trabalha em seus diferentes aspectos, com a cultura, a função dinâmica dos sistemas e também a interação do homem com esses princípios, considerando os aspectos biológicos, físicos e químicos. A oceanografia se divide em cinco áreas: oceanografia física, oceanografia química, oceanografia biológica, oceanografia geológica e oceanografia social. Possui também subáreas: paleoceanografia, a biogeoquímica marinha, a ecotoxicologia marinha, podendo existir outras.

Esta obra é de grande relevância, pois apresenta estudos pertinentes para a comunidade acadêmica que busca ampliar seus conhecimentos nos estudos sobre as Ciências do Mar. Apresentamos este volume em onze capítulos com abordagem em pesquisas científicas sobre os macroinvertebrados, biodiversidade algal, mudanças climáticas, moluscos marinhos, medicina popular, variabilidade genética, modelagem oceânica, oceanografia operacional e etnofarmacologia. Que estas contribuições possam refletir em futuros estudos para o crescimento das ciências do mar e todos os seus aspectos.

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SUMÁRIO

CAPÍTULO 8

METAL CORRELATIONS IN A RECIPROCAL MUSSELS TRANSPLANTATION: INDICATION OF PHYSIOLOGICAL RESPONSES AND BIOAVAILABILITY CONTRASTS

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RESUMO: Para testar a hipótese de que populações do mexilhão *Perna perna* respondem de maneira diferente às mesmas condições ambientais, foi utilizado um método de transplante recíproco. No primeiro transplante, populações de mexilhões provenientes de uma área pouco poluída (Arraial do Cabo, AC) e uma área altamente poluída (Baía da Guanabara, BG) foram transplantadas para um local na BG. Cinco meses depois, ambas foram transferidas para um local em AC, em um segundo transplante. As respostas das concentrações de metais dos tecidos foram avaliadas mensalmente. Os maiores níveis de Cd observados em mexilhões de AC foram depurados no primeiro transplante, voltando a aumentar no segundo transplante. Uma tendência inversa foi observada para o Mn. Estas tendências de Cd e Mn foram também observadas para mexilhões da BG no segundo transplante. As concentrações de Cu acompanharam o comportamento de Mn no segundo transplante. Outros metais traço (Ni, Pb e Zn) não foram suscetíveis à depuração ou processos de enriquecimento após o transplante. Diferentes correlações entre metais e mudanças de correlação após o transplante são propostas como evidências de diferenças adaptativas interpopulacionais sob a influência de ambientes costeiros contrastantes. Essas diferenças são explicadas como respostas do equilíbrio entre demandas nutricionais e para evitar toxicidade, além dos efeitos da biodisponibilidade de metais na bioacumulação desses elementos.

PALAVRAS-CHAVE: Transplante recíproco; incorporação; depuração; *Perna perna*.

ABSTRACT: To test the hypothesis that populations of mussels *Perna perna* respond differently to the same environmental conditions, a reciprocal transplant method was used. In the first transplant, populations of brown from a low-polluted area (Arraial do Cabo, AC) and a heavily-polluted area (Guanabara Bay, GB) were transplanted into the GB site. Five months lattes these populations were transferred to an AC site in a second transplant. Responses of soft tissues metal concentrations were assessed monthly. Higher Cd levels in AC mussels were depurated during the first transplant, increasing again after return to AC in the second one. An inverse trend was observed for Mn. Same tendency was observed for GB mussels during the second transplant for Cd and Mn. The Cu concentrations followed the Mn behavior in the second transplant. However, other trace metals (Ni, Pb and Zn) were not susceptible to metal depuration or metal enrichment processes after transplantation. Different metal correlations and correlation changes after transplanting are proposed as evidences of interpopulation adaptive differences under the influence of contrasting coastal environments. These differences are explained as responses of the balance between nutritional demands and toxicity avoidance, in addition to metal bioavailability effects on the bioaccumulation of these elements.

KEYWORDS: Reciprocal transplant; uptake; depuration; *Perna perna*.

INTRODUCTION

The "active" biomonitoring can be defined as the translocation of unstressed populations to polluted sites and quantification of their biochemical, physiological and/or organismal responses, providing indications of temporal and spatial variations of these responses for water quality monitoring (Roesijadi et al., 1984; Catharino et al., 2008). Transplantation experiments has several advantages in biomonitoring efforts, such as: i) these experiments facilitate the investigation of areas where the studied species is absent; ii) the use of a single population reduces the genetic variability, improving the homogeneity of the observed responses; and iii) similar size and reproductive stage of organisms avoids interpretation errors (Giarratano et al., 2010; Galgani et al., 2014). Therefore, bivalve transplantation studies have been useful to investigate metal uptake and depuration trends (Regoli; Orlando, 1994; Hédouin et al., 2011).

Transplant experiments also facilitate the investigation of adaptive or compensatory mechanisms in native populations from chronically contaminated areas, which can limit the capacity of to discriminate different levels of environmental disturbance (Regoli; Principato, 1995; Giarratano et al., 2010). However, the use of transplanted bivalves is limited if the organisms are severely stressed, which could influence the rate and magnitude of contaminants uptake. In this case, the interpretation of differences in metal concentration between transplanted and native bivalves may be difficult (Cain; Luoma, 1985; Wallner-Kersanach et al., 2000).

In this context, how closely can transplanted mussels from different populations respond in relation to metal contaminants incorporation? This study is a contribution to answer this question, addressing reciprocal transplant experiments with two populations of the mussel *Perna perna* (Linnaeus, 1758). Considering that metal bioaccumulation may present interpopulation variability (e.g., Roesijadi et al., 1984; Wallner-Kersanach et al., 2000), the studied populations were exposed to polluted and non-polluted environments from Rio de Janeiro coast (Brazil). A first transplant aims to elucidate differences between populations associated with metal exposure in a polluted site. A second transplant was carried out to address possible inter-populational differences in the depuration of metals incorporated in the first transplant. Moreover, the applicability of metal intercorrelation trends was explored to evaluate potential effects of changes in physiological demands and toxicity avoidance, considering that these changes will affect metal relationships along cultivation periods.

The polluted site was located in Guanabara Bay (GB), which is the most economically important embayment and one of the most polluted sites of the Brazilian coast, receiving industrial and domestic effluents (Kjerfve et al., 1997; Soares-Gomes et al., 2016). Arraial do Cabo (AC) site was chosen as a nearly-pristine condition, showing no expressive contamination sources (Rezende; Lacerda, 1986; Amaral et al., 2005; Lino et al., 2016), maintaining oligotrophic conditions. However, boat traffic pollution can affect AC environmental quality (Toste et al., 2011; Galvão et al., 2012).

Previous research revealed that the metal-polluted GB can present comparatively low concentrations in organisms than the nearly-pristine AC coast, since GB eutrophication causes lower bioavailability, e.g. for Cd (Carvalho; Lacerda, 1992; Francione et al., 2004; Lino et al., 2016), Pb and Ni (Carvalho; Lacerda, 1992). An inverse trend was observed for Mn (Carvalho; Lacerda, 1992; Lino et al., 2016) and Cu (Carvalho; Lacerda, 1992; Francione et al., 2004; Lino et al., 2016). However, no previous work performed transplant experiments to test the hypothesis that populations adapted to these two contrasting conditions can present inverse responses. This study is an attempt to test this hypothesis in a reciprocal transplantation experiment, considering that for each area, some metals will be depurated, while other metals will be accumulated, which will be reflected by metal intercorrelation trends.

MATERIAL AND METHODS

The first transplant experiment (hereafter called as "T1") was performed from June 2015 to February 2016. Mussels sampled from the Feiticeira Rock, located within Guanabara Bay (population "GB"), and from a bivalve marine farm located at the Forno Beach, Arraial do Cabo (population "AC"), were transplanted to a longline located near the Praça Arariboia Ferry Boat Station (22º53'30"S, 43º07'36"W), Guanabara Bay (Figure 1). Three mussel meshes from each population were distributed in this longline.

Results from this transplant experiment are named as GB T1 and AC T1, respectively.

The second transplant (hereafter called as "T2") started in October 2015, lasting during the following 4 months. Nearly two mussel meshes from each population placed in Guanabara Bay were transferred to a mussel farm from the Marine Extractive Reserve of Arraial do Cabo, located in Forno Beach (22º58'4"S, 42º0'28"W). Results from this transplant experiment are named as "GB T2" and "AC T2". The third mussel mesh remaining in Guanabara Bay were used for the concurrent continuity of T1, until February 2016.

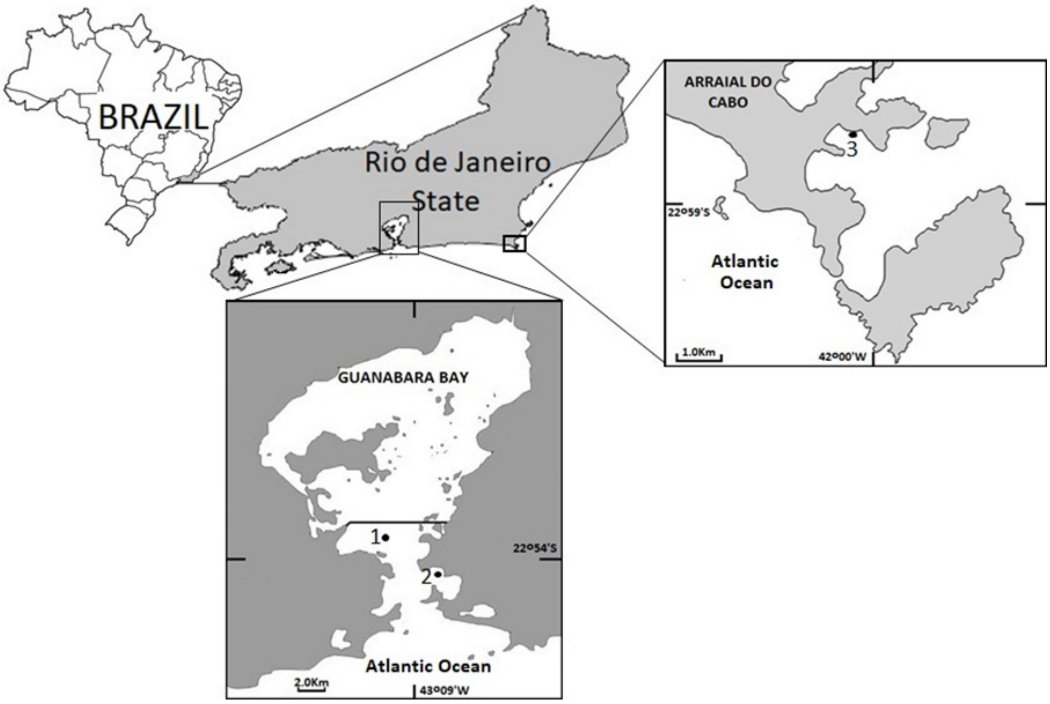

Figure 1 - Location of mussel sampling and transplant experiments sites. Site 1, Feiticeira Rock. Site 2, Guanabara Bay longline. Site 3, bivalve farm in Arraial do Cabo.

Fifteen individuals from each population were collected monthly, scrubbed clean of epifauna and kept in seawater from the sampling sites for 24 h to eliminate gut contents. Shell total length (TL) and mussel total weigh (TW) were monitored. The organisms were removed from their shells to obtain the fresh tissue weight (FTW) and, after lyophilization, the dry tissue weight (DTW). Three composed samples were obtained by homogenization of tissues from five animals, for each sampling time. The condition index (CI) was obtained by estimating the ratio of the FTW to TL, multiplied by 100 (Kagley et al., 2003).

About 0.3 g of dry tissue from each pooled sample triplicates and from a certified reference material (NIST CRM 1566b "Oyster Tissue"; n = 3) were submitted to a microwave-assisted digestion with 4 mL of concentrated HNO_3 and 2 mL of concentrated H_2O_2 in Teflon vials. After cooling, each sample was diluted to 15 mL with deionized water and metal contents were determined by ICP-MS. Reagent blanks were concurrently analyzed. Average recovery percentages of the certified reference material analysis are shown in Table 1.

Statistical analyzes were performed using the SPSS statistical package to address interpopulation differences in metal uptake and depuration trends. Mann-Whitney U test was used to evaluate metal concentrations differences. Pearson's correlation tests were performed to evaluate the relationships between heavy metals in mussel tissues. The accepted significance level was set at $p < 0.05$.

Metals	Certified concentrations	Obtained concentrations	Percentage of recovery
Cd	2.48 ± 0.08	1.77 ± 0.46	71.48
Cu	71.6 ± 1.6	72.3 ± 1.7	101.03
Fe	205.8 ± 6.8	217.5 ± 18.2	105.67
Mn	18.5 ± 0.2	15.3 ± 0.8	82.52
Ni	1.04 ± 0.09	0.67 ± 0.11	64.30
Pb	0.308 ± 0.009	0.237 ± 0.010	76.87
Zn	1424 ± 46	1395 ± 32	97.93

Table 1. Analysis of trace metal concentrations (mg kg^{-1} dry weight) and average recovery percentages of NIST SRM 1566b "Oyster Tissue" reference material (mean value ± SD, n = 3).

RESULTS

Biometric parameters

The Figure 2 shows the biometric parameters for GB and AC mussels during the transplant experiments. In T1, all parameters showed linear increase trends during the first 4 months, after which variable degrees of oscillation occurred, while oscillations were also observed during T2.

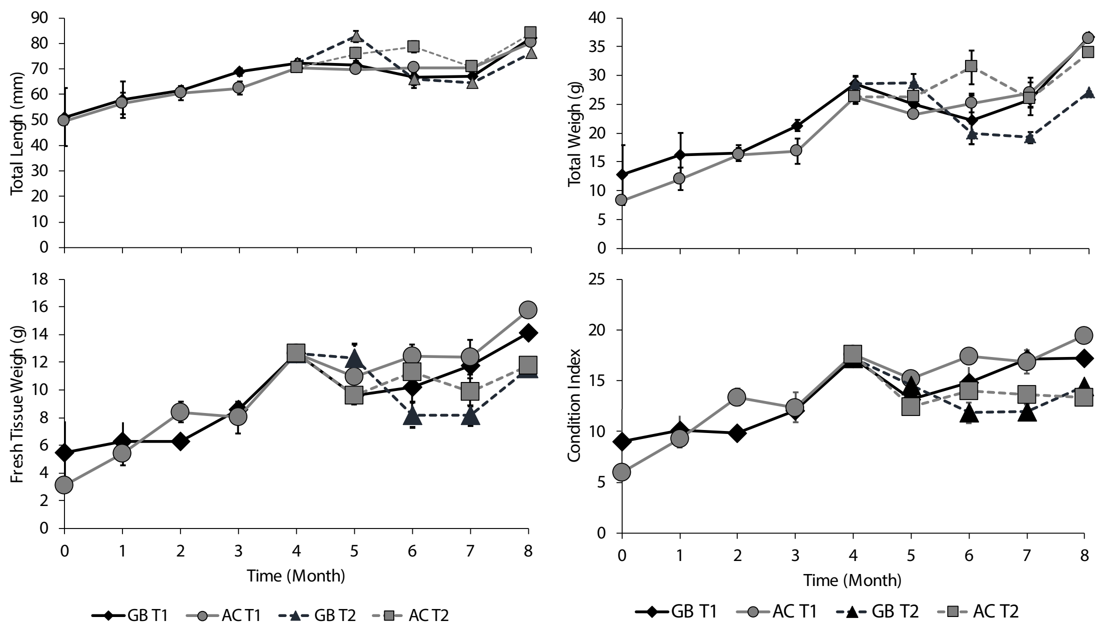

Figure 2 - Total Length (mm), Total Weigh (g) Fresh Tissue Weigh (g) and Condition Index from Guanabara Bay (GB) and Arraial do Cabo (AC) *Perna perna* mussels during the first transplant (T1) and second transplant (T2) experiments (symbols are mean values ± SE, n= 3)

Total length was the only parameter always with similar values in T1 and T2 ($p > 0.05$). TW presented resembling patterns, but GB T2 values fell below GB T1 ($p < 0.05$). After the mussels' transplant to Arraial do Cabo, CI and STW values declined to lower levels than observed in T1 ($p < 0.05$).

Interpopulation Trends Of Metal Concentrations

Metal concentrations in the mussels along the experiments are shown in Figure 3. The initial concentration (average ± SE) of Cd in mussels from AC (0.56±0.09 mg kg^{-1}) was one order of magnitude above those from GB (0.01±0.001 mg kg^{-1}). Differences of this magnitude were reported previously for mussels in the same areas (e.g., Francione et al., 2004; Lino et al., 2016). Mussels from AC exhibited a 6-times concentration decrease within the first month of T1 ($p < 0.05$), maintaining relatively constant values along the following T1 samplings. After the transplant to AC, mussels exhibited fast Cd accumulation, reaching values one order of magnitude higher than in GB ($p < 0.05$).

Manganese presented inverse concentration trends in relation to Cd (Figure 3). Initial Mn concentration was expected to be higher in Guanabara Bay than in Cabo Frio mussels (Carvalho; Lacerda 1992; Lino et al., 2016), as occurred. Within the first month of T1, Mn concentrations from both populations converged to similar intermediate values, after which an accumulation trend occurred in T1, while a depuration trend was observed in T2. This implied in significant differences between T1 and T2 for Mn in both populations ($p < 0.05$).

Along with T1, AC mussels generally maintained the concentrations of Ni and Zn more than twofold lower than the GB mussels ($p < 0.05$) during initial months, followed by periods of strong concentration oscillation for both T1 and T2, in which there was no significant differences between populations (Figure 3). For Fe and Pb, this general trend was also observed, but a shorter period of significantly higher values during T1 was found, observed only during the first two months (Figure 3). On the other hand, no significant differences were generally observed during T1 for Cu, though after the transplant to Arraial do Cabo, the Cu concentrations decreased significantly in both populations ($p < 0.05$).

Metal Correlations as Indicators of Interpopulation Differences

Significant correlation coefficients between metal concentrations in soft tissues are shown in Table 2. Mussels from Arraial do Cabo presented significant positive correlations between Cu and Zn (during T1 and T2), Cu and Mn (during T1), and Zn and Mn (during T2). During T1, GB mussels presented a significant negative correlation between Cd and Mn, whereas a positive significant correlation was found between Zn and Pb. For this population, a positive correlation was also found between Cd and Fe, during T2.

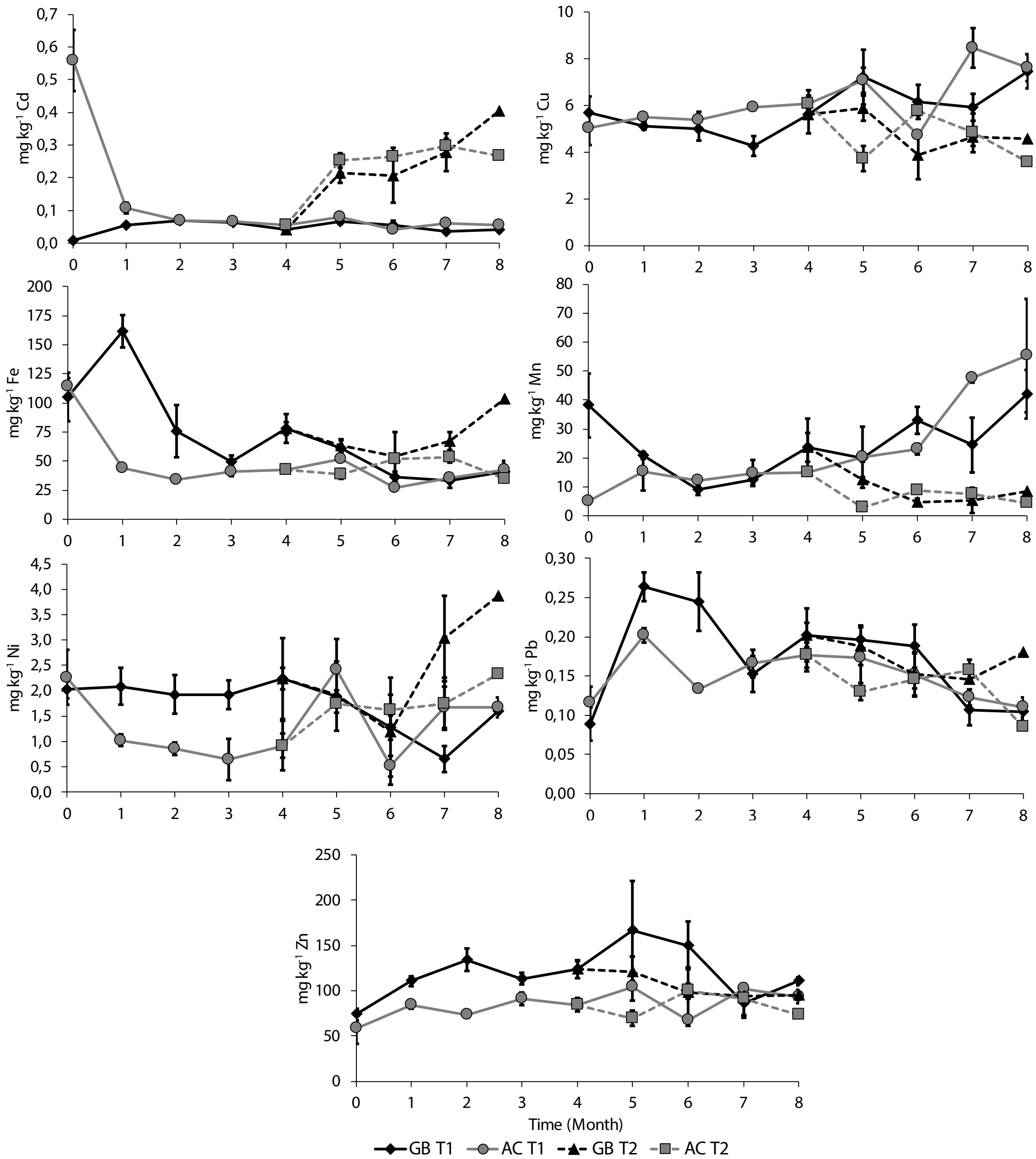

Figure 3 - Heavy metal concentrations (mg kg^{-1} dry weight) in *Perna perna* mussels from Guanabara Bay (GB) and Arraial do Cabo (AC) during the first transplant (T1) and second transplant (T2) experiments (symbols are mean values ± SE, n= 3)

Guanabara Bay	First Transplant	Second Transplant
Cd[a]	(-)DTW*, (-)CI*, (+)Pb*, (-)Mn*, (+)Zn*	(+)Fe*
Cu	-----	-----
Fe	-----	(+)Cd*
Mn[a]	(+)TL*, (+)DTW**, (+)CI*, (-)Cd*	(+)TL*
Ni	-----	-----
Pb	(+)Cd*	-----
Zn	(+)Cd*	-----

Arraial do Cabo	First Transplant	Second Transplant
Cd[a]	(-)DTW*, (-)CI*	-----
Cu	(+)Zn**, (+)Mn**	(+)Zn*
Fe	-----	-----
Mn	(+)TL*, (+)TW*, (+)DTW*, (+)CI*, (+)Cu**	(+)Zn*
Ni	-----	-----
Pb	-----	-----
Zn	(+)Cu**	(+)Mn*, (+)Cu*

Table 2. Significant Pearson's correlations between metals and total length (TL), total weight (TW), dry tissue weight (DTW) and condition index (CI) for mussel populations from Guanabara Bay and Arraial do Cabo, during the first and second transplant.

a Correlations performed without using the initial concentration for the T1 experiment, considered as a outlier (see Supplementary Materials for details and scatterplots view). * $p < 0.05$ level; ** $p < 0.01$ level.

DISCUSSION

Changes in environmental conditions can lead to bivalve environmental stress (Dailianis, 2011), which may partly explain different responses between T1 and T2. However, the influence of the environment on mussel growth is complex and the controlling factors may be difficult to be evaluated (Bergström; Lindegarth, 2016). The interaction of environmental factors can be responsible for differences in physiological responses (Bergström; Lindegarth, 2016), as respiration and clearance rates, and the quality of food may have a direct reflection on the energy balance, consequently affecting survival, growth and reproduction (Resgalla et al., 2007). Besides seasonal changes in weight due to a storage and utilization of food reserves associated to interactions of food availability and environmental factors (Karayücel; Karayücel, 2000), the weight variability observed between the studied populations may be influenced by differences in reproductive periods.

This study corroborates previous observations of lower Cd bioaccumulation under the eutrophicated conditions of Guanabara Bay, where Cd is associated to organic matter and precipitates as cadmium sulfide, decreasing its bioavailability (Carvalho et al., 1991; Carvalho; Lacerda, 1992). In oligotrophic environments, like Arraial do Cabo, the distribution of Cd between the particles and solution is strongly affected by formation of cadmium chloro-complexes, favoring Cd maintenance in solution (Comans; Van Dijk, 1988). In addition, it is possible that more saline upwelling waters reaching Arraial do Cabo are also responsible for a high Cd bioavailability (Francione et al., 2004).

The contrasting trends between Mn and Cd may be partly explained by the strong Cd affinity to sulfides, while Mn does not tend to form stable metal sulfide compounds. These trends can result in inverse tendencies of stronger release of Mn from the sediments, while Cd is retained (Gobeil et al., 1997). This appears to be important for the bioavailability of this metal to GB mussels.

The results are also consistent with previously reported higher values of Cu in GB than in AC (Carvalho; Lacerda, 1992; Francione et al., 2004; Lino et al., 2016), evidencing larger Cu loads in sewage-contaminated sites and depuration under oligotrophic conditions. Moreover, other studies have reported that bivalves' concentrations of Cu are higher in areas of extensive use of antifouling paints (Wallner-Kersanach et al., 2000; O'Connor; Lauenstein, 2005). Antifouling paints are used in both areas (Fernandez et al., 2005; Toste et al., 2011), which possibly have higher importance in GB, due to extensive and multiple nautical, shipping and harboring activities.

The contents of Ni, Zn, Fe and Pb in AC mussels did not show a significant increase during the first transplant, which is probably related to the history of metal exposure of this populations. Organisms from a pristine area transplanted to a polluted site can respond to the environmental conditions at an earlier life stage (Wepener, 2008), whereas whole life exposure to high metal concentrations may have more efficient concentration of metals in their tissues as non-toxic forms and reflects past contamination (Hédouin et al., 2011). The same trend of bioaccumulation was observed for other species during transplant experiments (e.g. Roesijadi et al., 1984; Wallner-Kersanach et al., 2000; Amaral et al., 2005; Hédouin et al., 2011).

In relation to metal input variability, possible seasonal differences in the metal loadings may have affected GB results. For example, while metal input from industrial and sewage sources can be concentrated during the river discharges from dry season, the urban runoff promoted by rainfall may result in high metal loading to this bay in the wet season. Many other areas of GB have been affected by these sources of metals (Fonseca et al., 2013; Silveira et al., 2013; Baptista Neto et al., 2016), which have stronger rainfall influence from January to March (Kjerfve et al., 1997). In the case of areas influenced by large seasonal variability of human occupation, Avelar et al. (2000) detected the highest values Pb in *P. perna* in a nearby region (Ubatuba Bay, SE Brazil), during the vacation seasons (January and July), when this region attracts larger numbers of tourists. Though AC region also present the same seasonal tendency of human occupation, the Pb concentration variability reported by Avelar et al. (2000) was not reproduced in this study area.

Correlation tests have been usually performed to evaluate the effects of body size and other allometric variables on metal contents (Ahn et al., 2001; Zhong et al., 2013) and possible associations between metal concentrations in bivalve tissues (Szefer et al., 1998, 2000, 2004). Alternatively, differences in correlation trends between metals are explored here as an additional form of to evaluate interpopulation variability in metal accumulation trends.

Differences in growth patterns, sexual maturation and physiological responses to metal exposure can affect the patterns of metal uptake and elimination by bivalves (e.g., Cain; Luoma, 1985; Paez-Osuna et al., 1995). This can contribute to explain negative correlations between essential and non-essential metals (e.g., as observed for Mn and Cd; Szefer et al., 1997). However, though previous studies have not considered the differences in metal bioavailability for explaining metal correlation trends in mussels, these differences are possible factors affecting the observed results.

An inverse variability of Cd and Mn bioavailability, involving different processes of retention and release by sediments (as discussed above; e.g., Gobeil et al., 1997), may contribute to explain their negative correlation in GB population, along T1. Moreover, mussels from both areas presented Mn concentrations correlated positively with all (AC population) or almost all (GB population) allometric parameters during T1, whereas the GB population presented a positive relationship between Mn and TL during both transplant experiments (Table 2). This agrees with the expected accumulation of essential elements along with mussel development. On the other hand, Cd presented negative correlations with DTW and CI during T1, suggesting excretion and/or concentration dilution while body weight increases. These results may be net effects of physiological responses (e.g., different adaptive traits of populations) and environmental factors (e.g., water quality effect on metal bioavailability). For other elements, a positive relationship between essential metals has been found (e.g., for Cu and Zn; Cunningham 1979; Rigoli et al., 1998; Szefer et al., 2004).

The importance of a careful selection of the geographical origin of bivalves to be used in biomonitoring efforts is known, since this origin may imply in different bioaccumulation efficiencies (Hédouin et al., 2011), whereas contrasting metal depuration trends have been reported for the same elements (Roesijadi et al., 1984; Yap et al., 2004). Variable metal concentration trends have given support for the use of bivalves in monitoring metal concentrations and bioavailability in the aquatic environment (Amaral et al., 2005; Birch; Hogg, 2011) and on the factors affecting the net metal uptake and safe use of bivalves for human consumption (Anacleto et al., 2015; Wang; Lu, 2017).

The examination of metal correlations differences and changes in these correlations may contribute for assessing interpopulation physiological responses and/or effects of metal bioavailability on metal concentrations. The involved physiological requirements may include the support to nutritional demands and toxicity avoidance (Anacleto et al., 2015), whose combined effects determine the proportions between net bioaccumulation and net depuration trends.

Metal incorporation by bivalves may involve dissolved and particulate matter forms that are not easily traced in the environment (e.g., Araujo et al., 2017), but there are substantial evidences in the literature that metal elimination and bivalve growth are critical for determining bioaccumulation (Wang; Lu, 2017). On the other hand, correlation trends between metals and the changes in these trends in bivalve tissues may be assessed in future efforts to elucidate the strategies for balancing nutritional demands and toxicity avoidance, coupled with evaluations of bioavailability effects on bioaccumulation and bivalve growth.

The results showed above evidenced that (1) there were no coincident correlations between metals for the different mussel populations exposed to the same environmental conditions and (2) the changes in significant correlations between T1 and T2 were also not coincident for these populations (Table 2). These findings are not attributable to effects of metal bioavailability in the coastal environment itself, considering that both populations were exposed to similar conditions within each site. While the negative relationship between Cd and Mn was partly associated to growth requirements of Mn and dilution and/or excretion of Cd, the other metals did not have significant correlations with mussel allometric measures, indicating a comparatively low importance of growth. For Cu, Ni, Pb and Zn, the balance between metabolic demands (in the case of micronutrients) and toxicity avoidance seems to be a dominant influence on the results.

CONCLUSIONS

The reciprocal transplant experiments demonstrated that the changes in environmental conditions (e.g., eutrophic, heavily-polluted vs. oligotrophic, low-polluted) can promote significant differences not only in the bioaccumulation trends, but also in trace metals intercorrelation trends. Though the results demonstrate that mussel populations from different regions of Rio de Janeiro coast can exhibit different capacities of metal uptake and/or depuration, the metal bioavailability differences in response to contrasting environments seems to contribute for determining some observed results (at least for Cd and Mn). Clearly inverse behaviors displayed by Cd and Mn may be associated to opposite bioavailability trends and inverse associations to allometric measures. The mussel growth variability seems to be less important for other metals. GB mussels presented Cu concentrations following the Mn behavior along the second transplant. In the low-polluted environment, fast depuration of Mn and Cu was observed for mussels transplanted from the eutrophic site. Other trace metals presented less pronounced changes, suggesting low variability in both physiological requirements (Ni and Zn) and/or bioavailability (Ni, Pb and Zn). This low susceptibility to be affected by depuration processes contrasts with previous studies. Different metal correlations and correlation changes after transplanting suggested the applicability correlation tests to assess interpopulation variability in metal bioaccumulation and depuration responses to physiological requirements (i.e., nutritional demand and toxicity avoidance).

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