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Sudden Cadmium Increases in the Digestive Gland of Scallop, *Nodipecten nodosus* L., Farmed in the Tropics

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Abstract Cadmium bioaccumulation in *Nodipecten nodosus* reared on a marine farm in a tropical bay was measured in muscle tissue and the digestive glands in animals ranging from 6 to 16 months. No bioaccumulation tendencies regarding Cd concentration were observed in muscle tissue (below 1 μg g^{-1}), while in the digestive gland concentrations remained below 5 μg g^{-1} until the organisms reached 10 months, and reached levels above 10 μg g^{-1} from 12 months onwards (reaching 27 μg g^{-1}). This is the same bioaccumulation pattern noted in animals transplanted to a different Cd exposure. Allometry and environmental factors cannot explain this sudden increase.

Keywords Heavy metal · Bioaccumulation · Marine bivalve · Biomonitoring

Cadmium bioaccumulation in Mytilidae and Ostreidae families has been useful in monitoring the presence and distribution of this element in aquatic ecosystems. Pectinidaes have also demonstrated high bioaccumulation potential, exemplified by the remarkable 600 μg g^{-1} of Cd found in the digestive gland of *Mizuhopecten yessoensis* (Evtushenko et al. 1990). Despite the evidence of high tolerance for metal bioaccumulation in this species and its worldwide distribution, only after the early 2000s was an increase in available scientific data regarding the features of heavy metal bioaccumulation in scallops observed. However, knowledge regarding metal homeostasis in tropical scallop species is still scarce. Previous studies conducted at New Caledonia with *Comptopallium radula* suggests its potential as a biomonitor for Cd exposure, since the differences observed in environmental Cd concentrations were reflected by the soft tissue Cd concentrations in this species (Metian et al. 2008). Data relative to the Australian Continent confirm the high Cd accumulation in the digestive gland (DG) of *Arnusiurn balloti,* even in remote areas (Francesconi et al. 1993). Known as “lion’s paw scallop” or “Coquille Saint-Jacques”, *Nodipecten nodosus* (Linnaeus, 1758) is the most farmed tropical scallop. However, to the best of our knowledge, no metal bioaccumulation data is available regarding *N. nodosus*. The purpose of this study was to access cadmium bioaccumulation by the *N. nodosus* scallop throughout the development of this organism.

Materials and Methods

We determined Cd concentrations in muscle – the main edible tissue of scallops – and the DG tissues of *N. nodosus* farmed at the pristine Ilha Grande Bay (IGB), located in Southeastern Brazil (044°–56 044° 40′W; 23°–23°40′S). Surveys began in February 2006. Specimens, numbering at least 30 individuals, with age differences of 2 months apart, were sampled, ranging from 6 (minimum size allowing dissection of muscle and digestive gland) to 12 months old (general commercial size). Subsequently, specimen samplings were conducted monthly, until the 16th month, measuring shell height, as well the total weight and the whole soft tissue wet weight (WW) of each specimen.

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Changes in metal accumulation rates at different environmental exposures were evaluated, comparing the bioaccumulation of transplanted organisms over a period of 3 months. A set of 200 individuals, aged twelve months, was transported from IGB to an area with historically high Cd contamination levels, Sepetiba Bay (SB) (043°34′–044°10′W; 22°54′–23°04′S). A subsequent 1 month depuration was conducted, returning the transplanted animals to IGB after 90 days of exposure in SB, in order to access depuration data. The control group for this transplant experiment was composed of 200 scallops, kept in a cage at IGB (set data of specimens from 12 to 16 months, grown at the IGB). Thirty animals were sampled monthly from both sites. No shell or cage cleaning were performed during the experiment.

The muscle tissue and the DG were dissected, washed with deionized water and pooled. Each pool group contained tissues from 5 to 6 animals, except for samples of 6 month olds animals, in which 12 animals were pooled. The pooled wet weight of these tissues was determined immediately after dissection, and the dry weight was obtained after drying at 80°C for 48 h. An aliquot of 0.2 g from the dried and homogeneized pool was taken for Cd concentrations determinations from 6, 8 and 10 month old organisms, by Graphite Furnace Atomic Absorbance (Analytical Jena GFAAS ZEEnit 60). Extraction was made by cold hard acid attack (2 mL HNO_3 65%) for 12 h, followed by 2 h in a hot water bath (80°C) (Dorneles et al. 2007). Cd concentrations in the remaining samples were determined by Flame Absorption Spectrometry (FAAS VARIAN AA-1475). Dry tissues samples were ashed at 430°C, followed by hard acid (HNO_3 65% and HCl 37%; 3:1) and oxidative (H_2O_2) extraction on a hot plate (90°C) (Lima et al. 1986).

In order to assay Cd concentration in the sediment as an estimate of the environmental exposure level, Ekman dredges were used to collect bottom sediments from both sites at the 4th sampling month. Three surveys were performed, with the aim of developing one composite sample composed of the top 10 cm of sediment. Three composite samples were taken from each site. The sediments were sieved (pore size <74 µm) with water collected from the sampling station, dried (80°C/48 h) and grounded on porcelain plates. A two-step extraction was performed. The first step is an assay of the Cd weakly bound to the sediment, cold 0.1 M HCl for 16 h, and the second is the total sediment matrix opening (hard extraction) with HNO_3:HF (5:4) in a closed system (120°C/12 h) (Fiszman et al. 1984). Each sample was analyzed in triplicate. The detection limit for the biological samples in FAAS was of 0.01 µg g^{-1} and in the GFAAS was of 0.7 µg kg^{-1}. Reagent blanks and certified reference materials (TORT-2, NIST2976 and IAEA405) were processed at the same time as the environmental samples. Recovery efficiencies ranged between 88 and 108% for biological samples and 116% for the analyzed sediment.

Results and Discussion

We had access to a large number of animals (totaling 360 analyzed individuals) of known ages, provided by a mariculture laboratory, by artificial fertilization, which can reduce the inter-individual variation regarding growth rates (Hawkins and Day 1996) and, ultimately, metal accumulation (Pan and Wang 2009) as discussed ahead. Additionally, the animal's age corresponds to the exposure period to environmental contaminants. This scenario reduces complex interpretations.

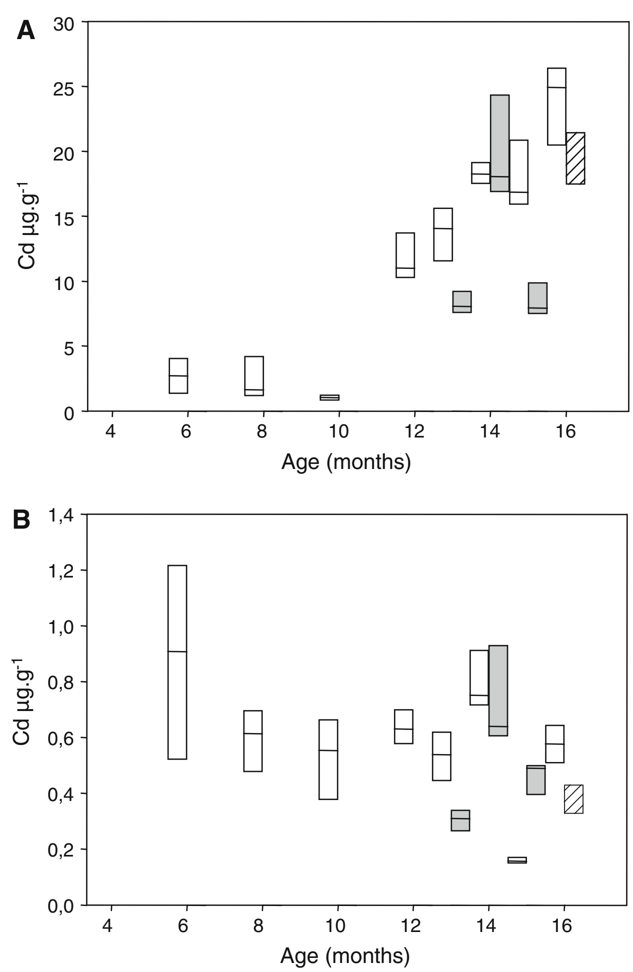

Fig. 1 Cd concentrations in µg g^{-1} (dry weight) in the Digestive Gland (**a**) and in the Adductor Muscule (**b**) of *N. nodosus* farmed in a pristine site (*empty bars*), and of animals transplanted to a polluted site (*filled bars*) for 3 months and then returned to the pristine site (hachured bars). Box plots represent 25th–75th percentile data range. The horizontal bar inside the box represents the median value

The Cd sediment assay confirmed the farming area as a pristine site. Analyses regarding weakly bound Cd revealed low concentrations (0.1 μg g^{-1}), and the remaining Cd content extracted by hard acid extraction was below the detection limit (<3 ng g^{-1}). At the polluted SB site, the Cd sediment results were of 1.7 and 0.1 μg g^{-1}, resulting from the weak and hard extractions, respectively.

The higher Cd concentrations (27.2 μg g^{-1}) were observed in the DG tissue in 16 month old samples (Fig. 1a). In the adductor muscle the highest Cd concentration (about 1 μg g^{-1} – Fig. 1b) was approximately 20-fold lower than the highest concentrations noted 2in the DG, and did not show any accumulation tendency over time. Cd concentrations in the DG remained below 5 μg g^{-1} until the 10th growth month (exposure/age), but doubled in the 12th month, and then rose to 27.2 μg g^{-1} by the end of the 16th month (Fig. 1a). The animals transplanted to the site with higher Cd environmental levels (SB), and then returned to the farming site (IGB), also presented no Cd bioaccumulation tendency in muscle tissue, remaining below 1 μg g^{-1} (Fig. 1b) but Cd concentrations in DG rose to about 20 μg g^{-1} (Fig. 1a). No extra Cd contamination source or event (accident, new industrial activity, new waste discharge, etc.) that could lead to misleading conclusions about the bioaccumulation patterns was reported during the study period. Leading us to assume that the Cd exposure was always continuous.

The Japanese Scallop, *Mizuhopecten yessoensis,* also presented relatively high increases in DG Cd concentrations in animals larger than 10 cm (shell height), however muscle Cd concentration showed no tendency for accumulation with size increases (Evtushenko et al. 1990). However, when we consider data of one study that sampled the Variegated Scallop, *Chlamys varia,* of a similar shell height range than the organisms analyzed in the present study (20–50 mm), a different pattern of Cd bioaccumulation in the DG is observed. The authors demonstrated a regular increase in Cd concentrations, reaching Cd concentrations of approximately 50 μg g^{-1} in larger animals (Bustamante and Miramand 2005).

It is stated that Cd possesses a long biological half-life time in bivalves (100–120 days) (Brown et al. 1996),

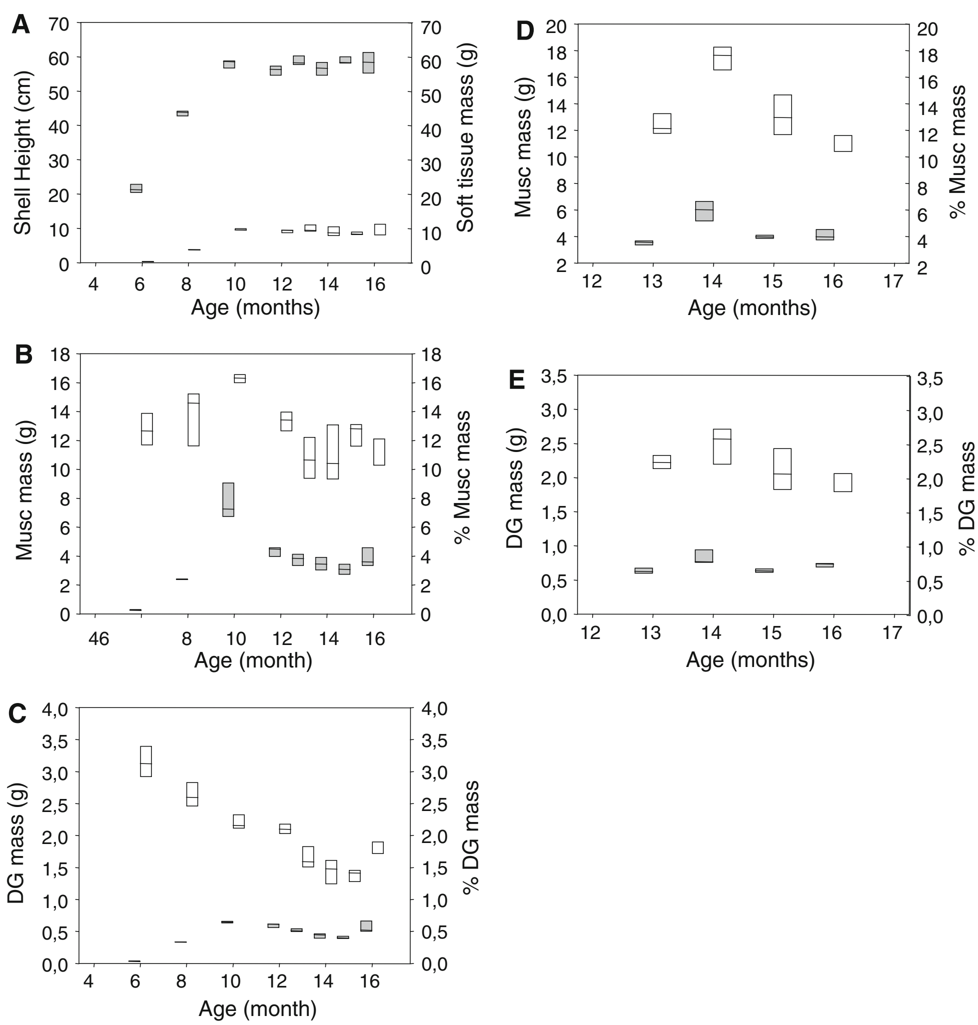

Fig. 2 : Biometrics parameters. (a) to (**c**): data from *N. nodosus* farmed in a pristine site. (**d**) and (**e**): *N. nodosus* transplanted to a polluted site and returned to the pristine site (16th month). (**a**) Shell height in cm (filled bars) and total wet mass of soft tissue in g (empty bars). (**b**) to (**e**): Average of Adductor Muscle and Digestive Gland mass (wet weight) in each analyzed pool (filled bars) and the percentage of Adductor Muscle and Digestive Gland mass in relation to the total wet weight (empty bars) of *N. nodosus*. Box plots represent 25th–75th percentile data range. The horizontal bar inside the box represent median value

which could favor an early bioaccumulation in the scallops‘ life stages. Nevertheless, considering the changes in shell height growth rates and total soft tissue mass (Fig. 2a) it is possible to identify the 10th month old specimens as an important shift point in *N. nodosus* development. Both these parameters stop increasing at this stage. The same shell height growth pattern observed in *N. nodosus* was also observed in the *Nodipecten subnodosus* (Sowerby 1835) (Barrios-Ruiz et al. 2003), suggesting that relevant physiological changes takes place in these animals in this development step. The biodillution effect on heavy metal concentrations in bivalves has been discussed as a possible phenomenon explaining contaminant decrease in larger animals (Reinfelder et al. 1998). Less attention has been given to the same effect considering the each organ's growth separately. If one organ grows more slowly than the whole body, a bioconcentration effect could take place. The increase of the absolute muscle mass (Fig. 2b) follows the same general tendency observed with regard to shell growth, and the percentage of muscle mass in relation the whole soft tissue remained between 10 and 14% over time (Fig. 2b). Otherwise, since the DG absolute mass also presented the same observed tendency to absolute muscle mass tissue over time, the percentage of DG mass in relation to whole soft tissue represented 3% at the beginning of the exposure and dropped to about half at the end (Fig. 2c). This general tendency was also observed in the specimenstransplanted to the polluted site (Fig. 2d, e). Even though Cd bioconcentration could be higher in the DG due to the (50%) reduction of relative mass in relation to the whole body, this fact alone is not enough to explain the expressive increase of Cd concentration (1,000%) observed in the DG.

Cd concentration in muscle (up to 0.16 $\mu g\ g^{-1}$ – WW), which is the main edible tissue of *N. nodosus*, was lower than the accepted value instated by the "Codex Alimentarius", of 1 $\mu g\ g^{-1}$ for mollusks (FAO and WHO 2004). In conclusion, in this first evaluation of Cd bioaccumulation by the tropical scallop *N. nodosus* we found evidence of a sudden shift in Cd concentration in the DG. This sudden increase cannot be explained by a variance of environmental factors or allometry.

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