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Selenium accumulation in the cockle *Anadara trapezia*.

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Abstract

An extensive study on Se accumulation in a population of *Anadara trapezia* from a marine lake is reported. The effects of organism mass, gender, reproductive cycle, and season on Se accumulation and tissue distribution were investigated. Analyses showed that gender and reproductive cycle had no significant effect on Se accumulation. *A. trapezia* showed a strong positive correlation between Se burden and tissue mass. Constant Se concentrations were observed within individual populations but varied spatially with sediment Se concentrations. Se concentrations in tissues decreased from gills > gonad/intestine > mantle > muscle > foot, which remained constant over twelve-months, however, significantly lower concentrations were observed in the summer compared to winter. *A. trapezia* is a good biomonitor for Se, as gender and size do not effect concentration, however, season of collection must be reported if changes in Se bioavailability are to be identified in short term studies, or during intersite comparisons.

Capsule. The marine bivalve *Anadara trapezia* has the attributes of a good bioindicator for marine selenium contamination, however, seasonal changes in Se concentration must be considered for short term studies and when results are compared to other studies.

Keywords: Selenium; *Anadara trapezia*; bioaccumulation; tissue distribution; effects - gender, mass and season.

1. Introduction

Selenium is a naturally occurring trace element that is an essential nutrient in biological systems. It is readily incorporated into amino acids, such as selenocysteine and selenomethione, through specific tRNA molecules (Lee et al., 1990; Hatfield et al., 1991), which are building blocks for various selenoproteins including glutathione peroxidase and selenoprotein P (Burk, 1991; Mizutani et al., 1991; Read et al., 1990). However, Se is toxic to organisms at elevated concentrations (Moxon and Olson, 1974), and high concentrations of selenium in fish tissues have been found to cause reproductive failure, teratogenic deformities and mortality (Lemly 1997; 1999; 2002).

Australian marine organisms are normally not exposed to Se and tend to have low Se concentrations (Maher et al., 1992, Baldwin and Maher, 1997, Maher and Batley, 1990). However, more than 80% of the Australian population is located on the coastal plains of the East (Yapp 1986), resulting in increasing industrial activity in major estuaries and on coastal foreshores. Anthropogenic sources of Se include oil combustion, fly-ash from coal-fired power stations, sewage effluent (Kirby et al., 2001a, Maher and Batley, 1990), and consumable products such as antidandruff shampoo and antifungal creams (Johnson, 1976; Alexander et al., 1988; Haygarth, 1994).

Maher et al. (1992) and Maher and Batley (1990) reviewed literature on Se in Australian organisms. Their reviews found that there is limited information on Se concentrations and factors influencing Se assimilation and accumulation in marine organisms, both in Australia and internationally. A decade after these reviews, the poor understanding of Se in marine systems was again highlighted by the release of the Australian and New Zealand Guidelines for Fresh and Marine Water Quality (ANZECC/ARMCANZ, 2000). Within these guidelines,

selenium values for the protection of aquatic species are only available for total Se in freshwater, as there is insufficient data within the literature to establish reliable values for marine sediment and water.

To assess the biological availability of Se, bioindicator species have been used. Bivalves are the traditional bioindicators because of their ability to accumulate and store metals for long periods (Lukyanova et al., 1993); equilibrate tissue metal concentrations with external media (Boyden, 1977); they are sessile; and they reflect spatial and temporal metal fluctuations (Ward et al., 1986). Previous studies in Lake Macquarie, NSW, Australia, have used indigenous populations of *Anadara trapezia* (Sydney cockle) to investigate biological tissue concentrations of Se in infauna from various sections of the lake (Batley, 1987; Maher et al., 1997; Peters et al., 1999a, Barwick and Maher, 2003). However, there is no information about *A. trapezia*'s intrinsic (size, gender, reproductive cycle) response to Se with respect to accumulation trends. Se is a unique essential metal because it is incorporated into proteins via seleno-aminoacids, as opposed to the post-translational incorporation of other metals into metalloproteins, and Se is not immobilised into the cysteine-rich metallothioneins which bind and sequester other metals. Therefore, it is possible that Se concentrations in biological tissues fluctuate differently concentrations of to other metals, and that biological Se concentration occur independently of changes in ambient conditions.

In this study we investigate the influence of gender and mass on Se accumulation using an indigenous population of *A. trapezia* from Lake Macquarie, Australia. The relationship of Se in different tissues of the cockle over a twelve-month study period is used to identify the seasonal accumulation of Se during one reproductive cycle.

2. Study Site

Lake Macquarie is a 125 km² barrier estuary south of the city of Newcastle on the central coast of New South Wales (King, 1986). The lake catchment has an area of approximately 622 km². It is shallow with a maximum depth of about 11 m east of Pulbah Island, and an average depth of about 6.7 m (Batley, 1987; King, 1986). The estuary extends 22 km in a north-south direction with a maximum width of 9 km and is the largest coastal lake in eastern Australia (Whitehead et al., 1991). The lakes' narrow entrance results in poor tidal flushing and an intertidal range of approximately 6-15 cm, however, the lake is characteristically marine because of the minimal freshwater contribution from the two main fluvial inflows.

The lake is naturally divided into two sections through the easterly projection of Wangi-Wangi Point and the sandy shallows west of Swansea causing limited mixing between the northern and southern sections. There is significant urbanisation on the foreshores of Lake Macquarie, particularly to the north. Industrialisation in combination with this urbanisation has resulted in a significant increase in the concentration of metals in the lake water and sediment (Peters et al., 1999a; Batley, 1991). Industrial sources of metal contamination in the northern section of the lake include the discharges from the lead-zinc smelter (commenced operation in 1897), a steel foundry, collieries and sewage-treatment works. The southern section of the lake includes two coal-fired power plants at Eraring and Vales Point (Roy and Crawford, 1984; Batley, 1987). Overflow from ash dams and stack emissions from coal-fired power stations produce enormous amounts of seleniferous fly-ash (50-300 µgSe.g⁻¹, Lemly, 1999), and in Lake Macquarie the power stations are known to be contributing selenium to the lake (Peters et al., 1999a; Kirby et al., 2001b). As a result of the poor tidal flushing most metals entering the system are expected to accumulate in the lake. Samples were collected

from Nord's Wharf, located in the south-east section of the lake, as shown in Figure 1. The area is relatively isolated from industry, and has minor urbanisation.

3. Methods

3.1 Field collections and sample preparation.

The cockles *Anadara trapezia* (Deshayes) were collected from Nord's Wharf, Lake Macquarie. Organisms were obtained from the sediment amongst the *Zostera capricornia* seagrass bed and were generally located between 1-10 mm below the sediment's surface. Organisms were placed in a mobile insulated container filled with clean lake water and fitted with an aerator, and left to depurate for 48 h. The length along the anterior-posterior axis was recorded to the nearest 0.1 mm.

For the gender and mass study, all *A. trapezia* were collected on a single day in October 1995. A total of 240 individuals (123 female and 117 male) were collected, ranging between 18.0 and 59.5 mm in length. For the 12-month study, 5 females and 5 males were collected each month for a twelve-month period from August to July (with the exception of November). Individuals in the range of 40 to 48 mm in length were selected to eliminate any mass related Se variations.

Shell exteriors were carefully washed prior to opening to prevent sample contamination by epiphytes or sediment. During dissection males and females were identified based on the colour of the gonad tissue (female gonads were orange and male gonads were white). For the gender and mass study, organisms were dissected into gonad (gonads and small section of intestinal tissue) and somatic (balance of tissue mass) tissue, and a blood sample was

1 collected. In *A. trapezia*, the gonad tissue is spread across the umbo region, and it is not
2 possible to quantitatively separate these tissues during routine dissection. For the temporal
3 study, organisms were dissected into adductor muscle, foot, gills, gonad/intestine and mantle.
4 A blood sample was also collected. Samples were stored at -20°C prior to freeze-drying at -
5 80°C for 48 h.

6
7 All field and laboratory equipment for dissolved metal analysis was detergent washed for 24
8 h, soaked in 10% (v/v) HNO₃ (Analytical Reagent grade) for a minimum of 24 h followed by
9 soaking and rinsing three times with high purity milli-Q water (millipore corp, 18 MΩ/cm
10 resistivity). Plastic ware was dried in a Class-100 laminar flow cabinet.

12 **3.2 Digestion and Analysis.**

13 Trace metal grade acids (Trace Pur, Merck) were used for metal determinations. All other
14 acids and chemicals were Analytical Reagent grade. Solutions were always prepared with
15 milli-Q water.

16
17 Samples were digested in nitric acid using a low volume microwave digestion technique
18 (Baldwin et al., 1994; Deaker and Maher, 1997). Digests were analysed for Se using a Perkin-
19 Elmer 5100 PC Electrothermal Atomic Absorption Spectrometer equipped with Zeeman
20 background correction, HGA-600 graphite furnace and AS-60 auto-sampler. A palladium and
21 magnesium matrix modifier was used (Deaker and Maher, 1995).

22
23 The accuracy of the electrothermal atomic absorption technique was previously assessed using
24 certified reference materials (Deaker and Maher, 1995). In the current studies the certified

reference materials NIST 1566a (Oyster tissue) and NBS SRM 1577a (Bovine Liver) were routinely run in each sample batch. For the mass and gender study, selenium analyses of these reference materials produced recoveries of $2.06 \pm 0.5 \mu\text{g.g}^{-1}$ (n=12) and $0.65 \pm 0.04 \mu\text{g.g}^{-1}$ (n=21), which were in agreement with the certified values ($2.08 \pm 0.2 \mu\text{g.g}^{-1}$ and $0.71 \pm 0.07 \mu\text{g.g}^{-1}$, respectively). For the 12-month study, NIST 1566a recoveries of $2.03 \pm 0.05 \mu\text{g.g}^{-1}$ (n=25) were achieved, which were also in agreement with the certified value.

3.3 Statistical analysis.

For the effect of mass and gender study, a series of correlations based on the Spearman's rho were used to investigate the relations of Se concentration with dry tissue mass (SPSS, 1998). A Mann-Whitney T-test was used to investigate the differences in Se concentration between genders (SPSS, 1998). Because of the high number of comparisons in the size/gender study (2 sexes, 4 tissues, 240 individuals) the significance level was lowered from $\alpha = 0.05$ to $\alpha^1 = 0.013$ in accordance with the Dunn-Sidak method for the correction of multiple comparisons (Sokal and Rohlf, 1995).

For the 12-month study, a factorial ANOVA was used to investigate the difference in Se concentration between individual tissues. A separate analysis was performed for each month and for the entire year. Significant effects detected were followed up with a Partial Model multiple comparison test using Scheffe adjust for type 1 error (Sokal and Rolf, 1995). Effects for each factor were compared after the removal of the other factor, using the LSMEANS option in the SAS® procedure GLM (SAS®, 1988). Because of the high number of comparisons in the study (11 months, 2 sexes, 7 tissues) the significance level was once again lowered from $\alpha = 0.05$ to $\alpha^2 = 0.0073$ (Sokal and Rohlf, 1995).

4. Results and Discussion

4.1 The effect of gender on Se accumulation.

Selenium concentrations and burdens in male and female *A. trapezia* were not significantly different ($P > 0.05$) for any of the tissues at any time of the year. Other studies have reported differences in selenium concentrations between male and female bivalves (Lobel et al., 1991a, Watling and Watling, 1976). These results demonstrate that the effect of gender on selenium accumulation in bivalves is species specific, and can not be generalized.

4.2 The effect of mass on Se accumulation.

Se concentrations and burdens for individual tissues and the entire organism are shown in Figure 2 as a function of dry tissue mass. The concentration of Se ($\mu\text{g.g}^{-1}$) had a poor negative correlation with tissue mass (Figure 2 A-D) indicating that Se concentration is independent of organism size. There was a highly significant positive correlation between Se body burden and dry tissue mass ($P < 0.0005$, Figure 2 E-H) showing that as the organism increases in size it maintains an almost constant concentration of Se within its' tissues.

In the majority of published studies (Baldwin and Maher 1997; Fowler and Benayoun 1976; Johns et al., 1988; Lytle and Lytle, 1982; Cossa et al., 1979, 1980; Lobel et al., 1991a), trends of decreasing metal concentration with increasing mass have been reported, and were primarily attributed to increased metabolic rates in smaller organisms. Increased metabolic rates, and subsequent growth rates, in juveniles would explain the greater variability in Se concentration for *A. trapezia* with a dry mass below 0.5 g (Figure 2A-D).

1 In this study, the near constant Se concentrations observed in the range of organisms (18-60
2 mm in length, 1 – 4 years old (Nell et al., 1994)) indicates that age has no effect on Se
3 concentration in *A. trapezia*. This is in contrast to some other bivalve species which were
4 found to contain higher metal concentrations with age including: Cd, Fe and Zn (not Cu or
5 Hg) in *Anodonta anatina* (Tessier et al., 1994); Zn in *Mytilus edulis* (Lobel, 1987); and Cu
6 and Zn in *Crassostrea virginica* (Phelps and Hetzel, 1987). Mackay and co-workers (1975)
7 found that the oyster *Crassostrea commercialis* decreased in Cu, Cd, and Zn concentration
8 with age. The differences in accumulation trends between Se and other metals may be related
9 to their affinity for metallothionein, whereas selenium is metabolically incorporated into
10 proteins (Maher et al., 1997; Peters et al., 1999b).

11
12 The ability for a single population of *A. trapezia* to maintain almost constant internal Se
13 concentrations through reduced uptake or increased excretion, means that Se accumulation is
14 being metabolically controlled, that is, *A. trapezia* are Se regulators. However, Se
15 concentrations within individual populations of *A. trapezia* co-vary with sediment Se, as
16 demonstrated by Se concentrations of 4.4, 6, 5.2 and 14 $\mu\text{g.g}^{-1}$ dry mass of *A. trapezia* from
17 sediment concentrations of 0.7 (Nord's Wharf), 3.4 (Wyee Bay), 6 (Vales Point), and 9
18 (Mannering Bay) $\mu\text{g.g}^{-1}$ dry mass, respectively (Peters et al., 1999; Barwick et al, 2003).
19 Hence, higher Se concentrations are observed in cockle populations in regions of elevated
20 sediment Se. Further investigations of Se uptake by *A. trapezia* in our laboratory, where
21 animals from a relatively pristine environment (Se concentration of 2.5 $\mu\text{g.g}^{-1}$ dry mass) were
22 transplanted to Lake Macquarie, showed that Se concentrations approached those of
23 indigenous animals after three months (Se concentration of 4-5 $\mu\text{g.g}^{-1}$ dry mass). Hence, *A.*
24 *trapezia* have the ability to regulate internal concentrations of Se, and these concentrations
25 within individual populations appear to be in equilibrium with the surrounding environment.

The ability for the species to regulate selenium at extremely high concentrations is still undetermined.

4.3 Selenium distribution between tissues.

Significantly higher Se concentrations ($P < 0.0005$) were found in the gonads than the somatic tissues (Figure 2) indicating preferential accumulation of Se in the reproductive tissue. There was a positive correlation observed in Se burden between the somatic and gonad tissues ($R^2 = 0.764$), demonstrating that although Se concentrations were higher in the gonads, the proportion of Se in the tissues remained constant as the tissue mass increased.

There are no data available for the distribution of Se between gonad and somatic tissues in any bivalve species. The trace metals Zn, Ni, Cu and Cd in the *Mytilus edulis* were found to be at much higher concentrations in the somatic tissues than the gonads (Lobel and Wright, 1982; Latouche and Mix, 1982). Unlike Se, many trace metals are immobilised as granules in the somatic tissues such as the liver and kidney of bivalves (Lobel et al., 1991b), which results in much higher concentrations and burdens of metals other than Se in these tissues.

Although the concentration of Se in the individual tissues of *A. trapezia* fluctuated throughout the year (Figure 3), the distribution of the metal between tissues remained fairly constant (Figure 4). The tissues decreased in Se concentration from gills > gonad/intestine > mantle > adductor muscle > foot.

Se distribution in *A. trapezia* tissues was similar to Se studies in other bivalves, such as *Anadara granosa* tissues in India, mantle > gill > visceral mass > podium > adductor muscle (Chatterjee et al., 2001); *Mytilus edulis* tissues, gill >> kidney > gonad/intestine > mantle >

foot (Lobel et al., 1991a); and the clam *Puditapes philippinaru*, viscera > gills > gonads > mantle > muscle (Zhang et al., 1990). Fowler and Benayoun (1976b) also found increased uptake of ^{75}Se in the visceral mass, gills, and muscle of *Mytilus galloprovincialis*, with lower concentrations in the mantle. Wrench (1979) found similar results for the oyster *Ostrea edulis* from Hampshire, England; the digestive gland of the oyster contained the greatest Se concentration, while the adductor muscle, blood, and foot contained the lowest.

4.4 Changes in selenium concentration of tissues over a 12 month period.

Changes in Se concentration, Se burden and dry mass for all tissues and the combined soft tissue mass over the 12-month period are shown in Figure 3.

A highly significant variation in Se concentration over the 12 months occurred for the intestinal tissue ($P < 0.0001$), which encases the gonadal mass. Decreases in Se concentration in the gonad/intestine were accompanied by increases in tissue mass (Figure 3E), indicating a relatively constant tissue load of Se which was unaffected by changes in tissue mass.

Although there were fluctuations in dry mass for the remaining tissues and the whole tissue mass, these had no significant effect ($P > 0.5$) on Se concentration.

The changes in Se concentration were significant for all tissues and the whole soft tissue mass ($P < 0.001$), with the exception of the foot ($P < 0.009$) whose insignificance was marginal based on an adjusted $\alpha^2 = 0.0073$. A Spearman's Rank (SAS®, 1988) of whole organism Se concentrations for individual months revealed 2 distinct groups. Group A contained summer and autumn (December to April) and group B encompassed winter and spring (May to October). The Se concentrations were significantly greater in all tissues during the winter and

spring months of group B and significantly lower during the summer and autumn months of group A ($P < 0.007$). The concentrations of Se decreased from August to February by 58% in the muscle, 43% in the blood, 27% in the foot, 42% in the gills, 29% in the intestine/gonads, 27% in the mantle, and 35% in the whole soft tissue mass, as shown in Figure 4.

Chatterjee et al. (2001) also found seasonal variations in Se concentrations in *Anadara granosa* from India, where higher concentrations occurred in the monsoon, then decreased from post-monsoon to pre-monsoon. Various other studies also found higher trace metal concentrations in bivalves in winter and spring, including Se in the brackish water clam *Macoma balthica* (Johns et al., 1988); Zn, Cu and Ag in the clam *Macoma balthica* (Cain and Luoma, 1986 and 1990); Zn in *Mytilus edulis* (Lobel, 1987); Co, Cu, Fe, Mn, Ni, Pb and Zn in the scallops *Pecten maximus* (L.) and *Chlamys opercularis* (L.) (Bryan, 1973); Cd, Co, Cu, Fe, Pb, Ni and Zn in the oyster *Crassostrea gigas* (Boyden and Phillips, 1981); and Co, Ni and Pb in *Saccostrea iridescense* (Páez-Osuna and Marmolejo-Rivas, 1990).

The study of mass effects on Se accumulation in *A. trapezia* showed that Se concentrations were not related to organism mass when the population was sampled at the same time. However, the 12-month study showed that the entire population experienced a significant seasonal fluctuation in both Se concentration and mass for the same population. Our results show that Se concentrations decreased as tissue mass increased, particularly in the summer months. Many researchers have attributed seasonal fluctuations in metal concentrations to food availability during the summer, corresponding to an increase in tissue mass and a dilution of metal concentration (Bryan, 1973; Orren et al., 1980). Although phytoplankton was not monitored in Lake Macquarie during this study, phytoplankton productivity is high when water temperatures are at a maximum (summer and autumn) (Baron, 1992; Hadfield and

Anderson, 1988; Jeffrey and Carpenter, 1974; Bryan, 1973). However, an increase in food supply may not be the only factor causing a decrease in organism metal concentration in warmer waters. Tissue metal concentrations may also decrease in summer because of the general increases in organism metabolism (ventilation, metal uptake and excretion rates) associated with heat (Bryan 1973; Orren et al., 1980; Phillips and Rainbow, 1993; Hare, 1992; Baron, 1992). Alternatively, metabolic changes associated with gametogenesis may induce an increase in trace metal concentration because of increased sequestration during particular stages (Orren et al., 1980).

The effect of gametogenesis on Se concentrations could explain the significant variation in Se over 12 months that occurred in the intestine/gonad tissue (Figure 3E). During this study, the number of mature oocytes (hence, the reproductive cycle) in *A. trapezia* was monitored over the 12-month period (Jolley, 2000). Two spawning events were observed, the first between January and February, and the second between April and May. No relationship was established between oocyte maturation and Se concentration. It is possible that the reproductive cycle effected Se concentrations, contributing to the lower concentrations during the summer and autumn, without being directly associated with it. That is, the biochemical systems responsible for altering the proportions of proteins, lipids and carbohydrates during gametogenesis may also affect the affinity of Se to these biomolecules (Cossa et al., 1979, 1980). This may subsequently alter the load and distribution of Se in the organism.

It is possible that the changes in Se observed in the intestine/gonads are because of selenium's role in the formation, nourishment and storage of gametes within the gonads of males and females, rather than being lost with gametes on spawning, resulting in no gender differences. The Se that was lost from *A. trapezia* during spawning events (in February and April, Figure

3G) was probably a decrease in tissue mass (gametes) that contained average Se concentrations. Therefore, the reproductive cycle does not have a large influence on whole Se concentrations in *A. trapezia*.

5. Conclusions

Selenium accumulation in *Anadara trapezia* is not effected by gender at any stage of the year (reproductive cycle). The concentration of Se within the whole soft tissue mass of the cockle was not dependent on mass or size (18 – 60 mm), as the organism maintains a constant tissue concentration. Therefore, the biological parameters mass, size and gender do not need to be considered when using *A. trapezia* as a biomonitor for Se.

The concentration of Se within the individual tissues of *A. trapezia* decreased from gills > gonad/intestine > mantle > adductor muscle > foot. This tissue distribution remained constant throughout the year, however, the Se concentrations in all of these tissues were significantly lower in the summer. Hence, when considering *A. trapezia* for Se biomonitoring studies, the season of collection is an important parameter that needs to be standardized, and analyzing the whole soft tissue mass is recommended because it has the median amount of temporal variability and is representative of the overall variation across the individual tissues.

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