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Selenium Occurrence, Distribution and Speciation in the Cockle *Anadara trapezia* and the Mullet *Mugil cephalus*

W. Maher, M. Deaker, D. Jolley, F. Krikowa and B. Roberts
University of Canberra, Faculty of Applied Science, PO Box 1, Belconnen, ACT 2616 Australia

Data on the factors affecting the accumulation of selenium in the cockle *Anadara trapezia* and mullet *Mugil cephalus* are presented, together with the distribution and speciation of selenium in tissues. Selenium concentration in whole cockles showed a small but significant decrease with weight. No further decrease in selenium concentration was apparent once an organism reached 0.25 g dry weight. Selenium concentration in cockles was not dependent on sex. The tissue distribution of selenium concentration in cockles was in the order gill > intestine > adductor > mantle > foot.

Selenium concentrations in liver tissues of mullet increased with the whole weight of the fish. In contrast, selenium concentrations in muscle, stomach, heart and kidney tissues were fairly low and constant in fish weighing less than 200 g (20 cm in length). Fish of greater weight and size (> 250 g and > 30 cm) had higher and more variable selenium concentrations. No differences in selenium concentration between male and female fish occurred; however, the sex of many of the fish could not be distinguished. The tissue distribution of selenium concentration in mullet was in the order liver > stomach > heart > muscle > kidney.

Most of the selenium recovered from both the cockle tissues and the mullet muscle tissues was found to be associated with proteins and to be present as selenocysteine. A conceptual model is presented for selenium transformations in marine organisms based on the formation of selenoamino-acids and subsequent incorporation into proteins.

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INTRODUCTION

In 1842, 25 years after Berzelius described selenium, Japha demonstrated that compounds of selenium were toxic to animals.[1] Diseases that caused malformations in human children and poultry, as well as loss of hair and fingernails, had been described in historical accounts by Marco Polo in 1295 and by Father Pedro Simon in 1560.[1] These are now attributed to toxic intakes of selenium.

A great deal of research has centred on trying to understand the significance of selenium's apparently paradoxical nature of both toxin and nutrient.[2–8] The late 1880s saw a period of endeavour into determining plant nutritional requirements for selenium. In the 1880s Cameron and Knop tested the effects of inorganic forms of selenium on plant growth[9–11] and found that selenium was toxic to plants. In the 1930s the link between various disorders in livestock and plants and seleniferous soils was recognized[10, 12] and selenium was identified as a toxic element.

Acute toxicity in domestic animals results in symptoms of diarrhoea, elevated temperature and pulse rate, abnormal motion and posture, and respiratory difficulties.[11] Two other well-defined diseases synonymous with chronically toxic levels of selenium in livestock are 'blind stag-

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gers' and 'alkali disease'. The former comes after stock have ingested seleniferous plants in limited amounts over extended time periods. The animals appear to have impaired vision, stumble, lose appetite and suffer paralysis.[9–11, 13] Respiratory failure may also develop.[13] Reproduction in many domestic animals is also affected; manifestations include decreased hatchings in chickens, and malformations in poultry, pigs, sheep, cattle and horses.[11] Cell division has been reported to be inhibited by the disruption of sulphur-dependent processes, and skeletal abnormalities have come about from chromosomal alterations during mitotic division.[10] The precise way in which toxic amounts of selenium affect an animal's physiology is not understood, but has been linked to the blocking of cell-energy production by removing sulphydryl groups essential to oxidative processes.[14]

Sublethal effects are also prevalent in wild animals exposed to high environmental levels from natural or human-induced sources. For fish these include oedema, reduced haematocrits and haemoglobin levels, swollen gill lamellae with extensive vacuolation, degeneration of ovarian follicles, and liver, myocardial and pericardial damage, and chromosomal aberrations.[15–19]

The essential nature of selenium in animal nutrition was not elucidated until 1957, when Schwarz and Foltz suggested that selenium was a factor protecting the liver from degenerative disorders.[1, 11, 13] Since that time, it has been established that selenium prevents a number of diseases from occurring in animals. These include: white muscle disease in sheep—lesions in liver, body cavity, myocardial and skeletal muscles;[10, 11, 13] muscular dystrophy in sheep, pigs and poultry—degeneration of muscle tissues;[10, 11] exudative diathesis in poultry—oedema or loss of fluid from body tissues with fluid accumulation under the skin and accompanied by anaemia;[10, 11] and hepatosis dietetica and dietetica microangiopathy in pigs—lesions in liver and blood vessels of the myocardia, respectively.[10, 11] Selenium is also required by fish to protect against damage to liver and to axon sheaths of nerve cords.[20–23]

Selenium deficiency in humans manifests itself as Keshan disease, or cardiomyopathy (heart disease in the form of tissue degeneration and scar tissue formation) which affects young women and children in particular, and Kaschin–Beck disease, an osteoarthropy (degenerative bone disease). Both diseases are endemic to regions where the selenium status of soils is very low.[24, 25]

In general, marine organisms exhibit higher selenium concentrations than terrestrial animals.[26] The biogeochemical cycling of selenium in the marine environment involves inorganic forms of the element and organometallic compounds such as dimethyl selenide[27] and probably selenium amino acids.[6, 28–30] Knowledge of the factors that affect accumulation, the site of accumulation and the chemical forms of this element within marine organisms is needed to allow the determination of the biochemical pathways that selenium follows and the roles this element plays in the function of an organism.

This paper presents the results of the occurrence, tissue distribution and speciation of selenium in two organisms, the cockle, *Anadara trapezia* and the fish, *Mugil cephalus*, from Lake Macquarie, an estuary chronically polluted with selenium.

STUDY SITE

Lake Macquarie is a 125 km^2 barrier estuary located adjacent to the city of Newcastle, Australia (Fig. 1), and has a total catchment area of 66 000 ha. The estuary has an average depth of 6.7 m, a maximum depth of 11 m and is poorly flushed with a tidal range of about 6–15 cm because of its narrow ocean entrance.[31, 32]

The lake is divided into two sections by the projection of Wangi-Wangi Point and the sandy shallows off Swansea. The water movements in the two sections of the lake are essentially independent.[33] The catchment at the northern end of the lake is industrialized and urbanized with a lead–zinc smelter, a steel foundry a fertilizer plant, collieries and a number of sewage outflows. The catchment at the southern end of the lake contains bushland, collieries, coal-burning power stations and some residential development. The lake is contaminated with trace metals, including selenium.[31, 32] Selenium contributions from various point sources to Lake Macquarie have recently been reported as 400 kg^{-1} for Vales Point and Earing power stations; 200 kg y^{-1} for the lead–zinc smelter and 10 kg y^{-1} by sewage treatment works,[34, 35] thus making the power stations the largest contributor of selenium to the lake.

METHODS

Cockles were collected near Nords Wharf and mullet from Mannering Bay, Dora Creek, Wyee Bay, Wangi Seine and Wyuna Bay, locations at the southern end of the lake (Fig. 1).

Collection and dissection of tissues

Cockles
Organisms were collected by hand and purged in seawater for 24–48 h. Organisms were then either shucked and whole tissue stored at −10 °C, or dissected live and tissues stored at −10 °C until analysed.

Fish
Fish were caught using a gill net, transported to the laboratory on dry ice and stored at −10 °C until dissected. They were thawed in a cool room at approximately 5 °C to minimize cross-contamination between organs by decomposition. Only dissected tissues that could be identified and completely separated from other tissues were kept for analysis.

Selenium analysis

Samples were freeze-dried, finely chopped and digested with nitric acid using a low-volume microwave digestion procedure.[36] Selenium was determined by electrothermal atomic absorption

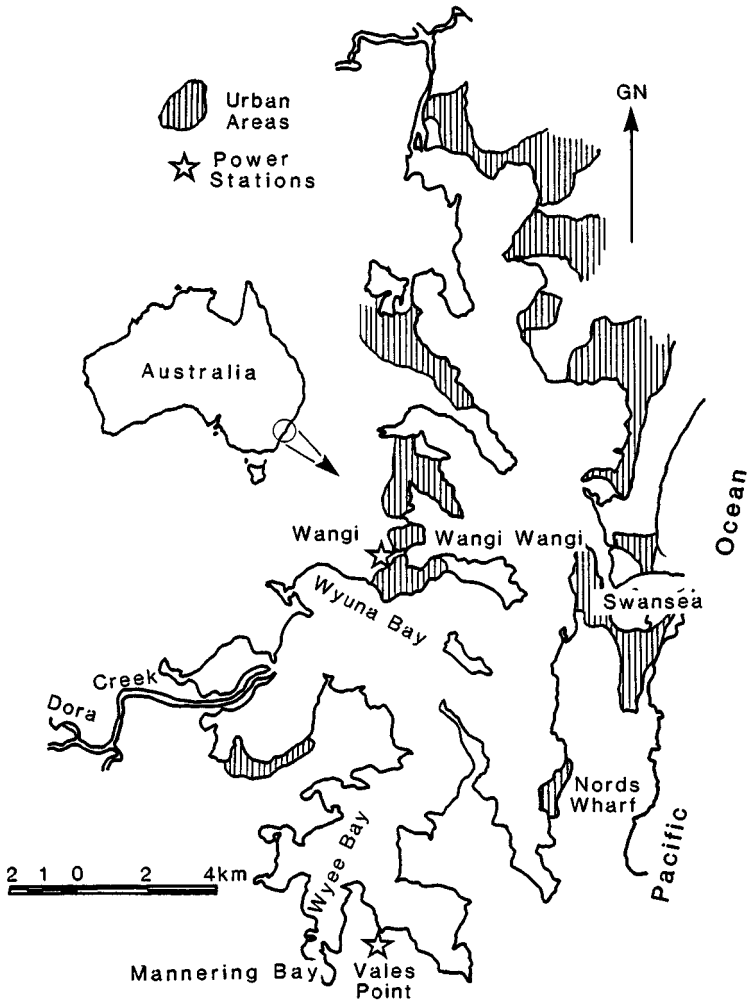

Figure 1 Location of sampling sites in Lake Macquarie.

spectroscopy using palladium and magnesium as matrix modifiers.[37] In this study reference materials NIST 1566a Oyster tissue and NRCC TORT-1 Lobster hepatopancreas were routinely run with each sample batch analysed. Recoveries of 2.2 ± 0.2 μg g^{-1} and 7.0 ± 0.3 μg g^{-1} respectively were in agreement with the certified values (2.08 ± 0.2 μg g^{-1} and 6.88 ± 0.4 μg g^{-1}).

Biochemical fractionation

Selenium was determined in selected biochemical fractions using a procedure of sequential solvent extractions as shown in Fig. 2.

Selenium speciation

The presence of selenium amino-acids in muscle tissues was determined by hydrolysing muscle tissues in 6 M hydrochloric acid under anaerobic conditions using microwave heating [150 °C, 60 psi (414 kPa), 40 min]. Hydrolysates were filtered (0.4 μm), freezed-dried and resuspended in loading buffer prior to analysis. Selenium species (selenocysteine, selenomethionine, selenate, selenite and trimethylselenonium) were separated by HPLC. Both an anion-exchange column (SGE SAX 5 μm) using 2 mM potassium dihydrogen phthalate in 20% methanol (pH 5) and a C18 reverse phase column (Applied

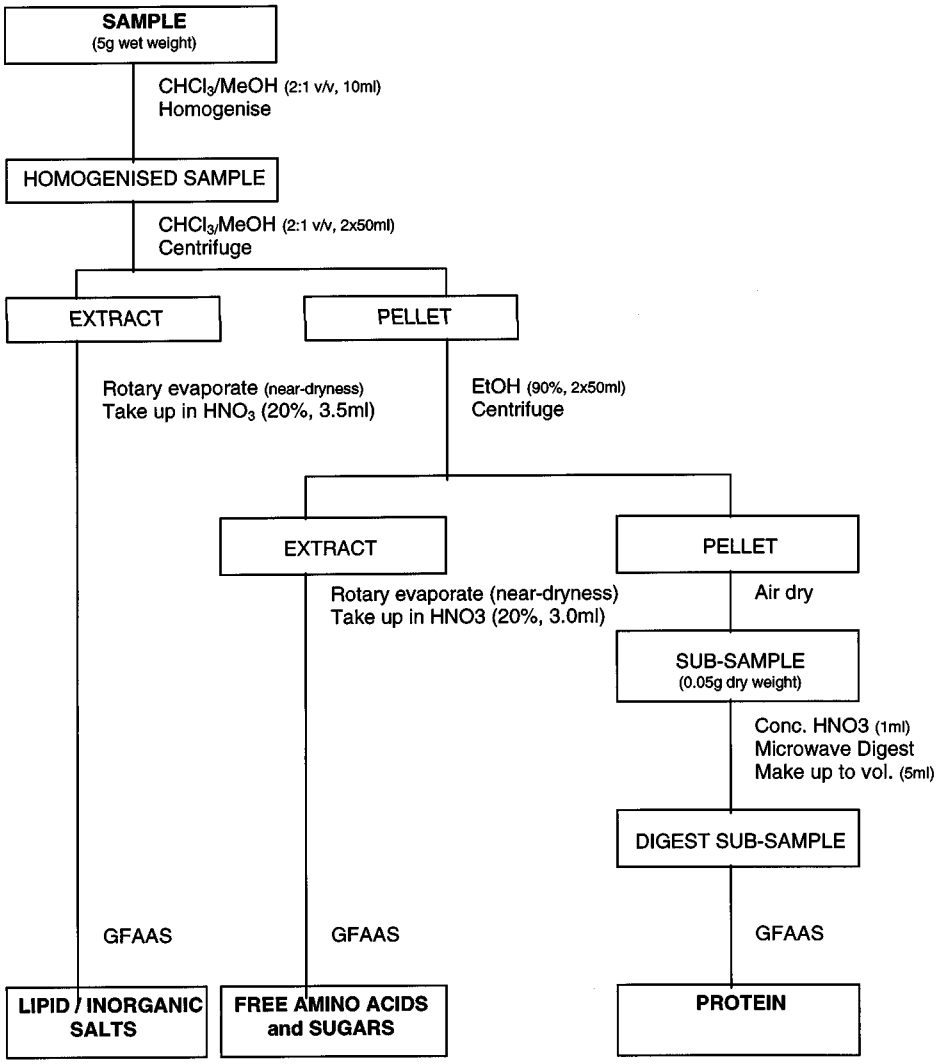

Figure 2 Biochemical fractionation scheme.

Biosystems, 5 μm) using 2 mM tetrabutyl ammonium dihydrogen phosphate in 50% methanol (pH 4) were used to separate the selenium compounds in standards and tissues. Fractions of the eluent (0.5 ml) were collected and analysed by graphite furnace atomic absorption spectroscopy.[37] The stability of selenium species during hydrolysis was confirmed by two procedures, the hydrolysis of standard solutions of selenium compounds (sodium selenate, sodium selenite, selenocysteine, selenomethionine, trimethylselenonium iodide) and spiked mullet tissues. Quantitative recoveries were obtained for the standard selenium compounds, while 65–75% of selenium in spiked muscle tissue was recovered. Most of the losses of selenium occurred during filtration prior to chromatography.

RESULTS AND DISCUSSION

Occurrence of selenium and its relationship to weight

Cockles

Cockles were collected shortly after spawning to minimize differences between male and female organisms. Concentrations of selenium in cockles [1.3–11.8 $\mu g\ g^{-1}$) are up to ten times the selenium concentrations found in molluscs from unpolluted areas in other parts of Australia.[30] The scatterplot of selenium concentration versus dry weight (Fig. 3) revealed a small but significant decrease of selenium concentration with weight ($r^2 = 0.5828$, $P = 0.001$, $n = 120$). Organisms heavier than 0.25 g dry weight showed no further decrease in selenium concentration with weight.

The trend of decreasing selenium concentration with increasing weight in molluscs has been found by other authors[38–43] and appears to be a general phenomenon for trace metals in marine molluscs.[44–49]

Short-term laboratory exposure studies have shown that, for *Macoma balthica* and *Mytilus galloprovincialis*, selenium uptake by smaller individuals was more rapid than uptake by larger organisms.[50, 51] It has been suggested that the trend of smaller molluscs having higher trace metal concentrations than the larger individuals is due to higher metabolic rates when the organism is smaller and younger.[50–53] It may also be due to regulatory uptake and excretion mechanisms operating in larger organisms.[44]

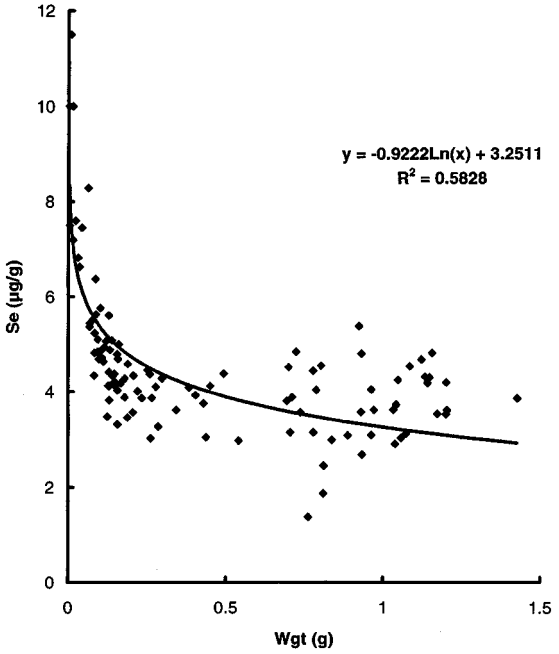

Figure 3 Relationship of selenium concentration and whole dry weight for *Anadara trapezia*.

Other factors may also influence the uptake and retention of selenium by molluscs. Fowler and Benayoun[50] have shown that the speciation of selenium is important, as, for example, *Mytilus galloprovincialis* preferentially accumulates selenite over selenate. The speciation of selenium in inputs to Lake Macquarie are not known but Measures and Burton[54, 55] have shown that the oxidation state of selenium can vary in estuarine waters, while Cooke and Bruland[27] have isolated methylated selenium species from seawater samples. The route of selenium accumulation is also of importance. Luoma *et al.*[56] have shown that *Macoma balthica* assimilates 80% of selenium from diatoms, 22% of selenium from sediments and little selenium from water. The presence of metals may also influence the uptake and retention of selenium. Selenium has been found to be correlated with arsenic, mercury, cadmium and zinc in marine molluscs.[57–59] Pelletier[60] has shown that when *Mytilus edulis* is simultaneously exposed to selenium and mercury, an increase in the uptake of selenium occurs. In contrast, other authors[50, 58] have found that selenium uptake is not significantly affected by mercury. Watling and Watling[61] have shown that filtration rate and hence growth varies with selenium concentration. Filtration rates may also

be affected by salinity. These factors may vary with species, location and time, changing the relationship of selenium with dry weight found in this study.

Mullet

Selenium concentrations in mullet tissues (1.7–58.9 $\mu g\ g^{-1}$) are up to 39 times higher than those found in fish collected from unpolluted sites around Australia.[30] Selenium concentration in mullet tissues could not be correlated with the weight of individual tissues as it was not possible to separate all tissues completely. The scatterplots of selenium concentration in mullet tissues and whole dry weight of the fish (Figs 4a–f) revealed different trends. For liver tissue, selenium concentration increased with whole fish weight although considerable scatter occurred ($r^2 = 0.4875$, $P = 0.05$, $n = 30$). For heart, stomach, kidney and gonad tissues, there was no significant relationship between selenium concentration and whole weight of fish ($r^2 = 0.01254$–0.1502, $n = 11$–30). A clustering of data occurred for muscle, heart, stomach and kidney tissues. For mullet weighing below 200 g (20 cm in length) selenium concentrations were below 10 $\mu g\ g^{-1}$ dry wt and fairly constant. Mullet above 200 g (30 cm in length) had highly variable selenium concentrations.

Mullet of weight 200 g or less are juvenile fish and have spent their life within the estuary. Some of the mullet of greater weight would have left the estuary to spawn at sea.[62] The greater selenium concentration and scatter in tissues of older fish is probably a combination of some fish remaining in the estuary for longer periods and being exposed to more selenium, and some fish spawning at sea and losing accumulated selenium. It has been reported that selenium is concentrated in eggs during vitellogenesis.[63] In general, previous studies have not reported significant correlations between selenium concentrations in tissues and weight or length.[30, 64–66] A notable exception is the work of Mackay *et al.*,[46] who reported a significant correlation between selenium concentration and length, weight and girth in muscle and liver tissues of the black marlin *Makaira indica*. Many correlations between selenium concentration and other elements (e.g. copper, cadmium, zinc, iron, manganese, magnesium) in fish have been reported in the literature.[46, 67, 68] Thus, some accumulation of selenium may be occurring with the concurrent uptake of other elements.

Occurrence of selenium and the effect of sex

Cockles

There was no significant difference in selenium concentration between male and female cockles ($P > 0.95$) collected at different samples times (Fig. 5). We are only aware of one study, which measured trace metal concentrations in *Mytilus edulis*, that has published results indicating that male and female molluscs contain significantly different concentrations of selenium.[43]

Differences between gender for trace elements (manganese, zinc, cadmium, lead, bismuth, iron, magnesium, selenium, arsenic) have been reported for the molluscs *Donax trunculus*.[69] *Mytilus edulis*,[43, 70, 71] *Mytilus californianus*[72] and *Choromytilus meridionalis*.[73, 74] However, differences in metal concentration are generally not large, with females having elevated concentrations of trace elements in somatic tissues. It has also been reported that these differences may change with age.[70]

Mullet

There was no significant difference in selenium concentration between male and female fish ($P > 0.95$). Results for this fish should only be treated as preliminary as the data set contained only four males and seven females. Many of the larger mullet did not have distinguishable gonads, which may have been an effect of elevated metal concentrations in Lake Macquarie or an indication of immaturity. In other studies on the concentration of selenium in fish tissues[75, 76] no differences have been found between male and female fish.

These results are in contrast to a study in which selenium has been found to be distributed differently in male and female rats; this was attributed to differences in the selenium-containing enzyme glutathione peroxidase.[77] Some differences in the selenium concentrations of male and female marine organisms would be expected if the uptake of selenium was correlated with trace metals whose accumulation was gender-specific.

Tissue distribution

Cockles

The tissues of cockles of a similar size (between 4 and 5 cm) were analysed to minimize any

effects of weight. The regression between selenium concentration and dry weight in this range was not significant ($r^2 = 0.0013$, $P = 0.0731$, $n = 42$). This enabled the results to be interpreted knowing that the variations in concentration were not because of differences in cockle weight.

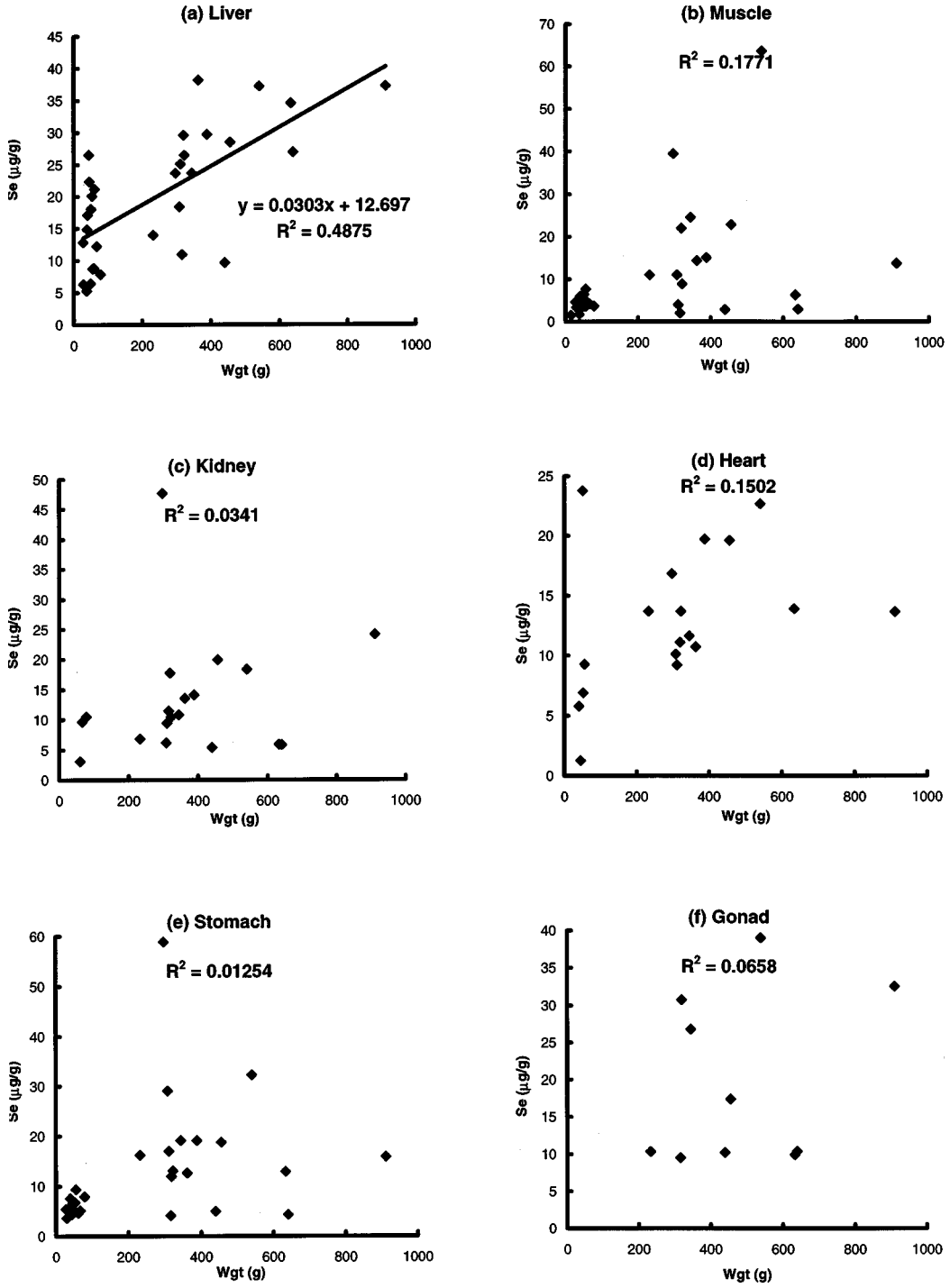

Figure 4 Relationship of selenium concentration in tissues of *Mugil cephalus* and whole dry weight.

There was a significant difference between selenium concentrations ($P = 0.001$) in the various tissues of *Anadara trapezia* (Fig. 6). A Tukey–Kramer multiple comparison test revealed that selenium concentrations were in the following order: gill > intestine > adductor > mantle > foot.

These results are consistent with trace element distributions found in bivalves from other locations[43, 45, 78, 79] and it appears to be a general phenomenon that metals accumulate within gills and digestive system tissues in molluscs.[80, 81]

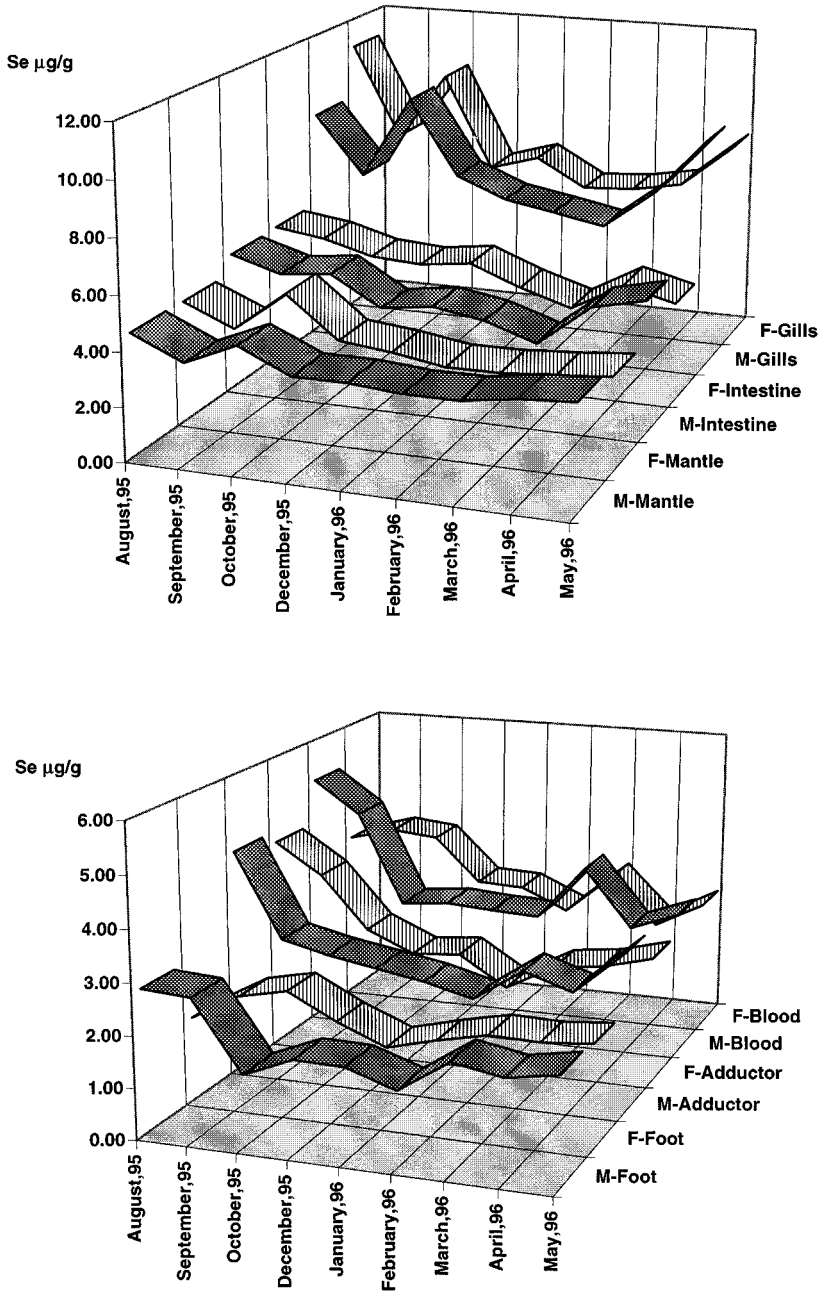

Figure 5 Selenium concentrations in male (M) and female (F) *Anadara trapezia* tissues ($n = 5$).

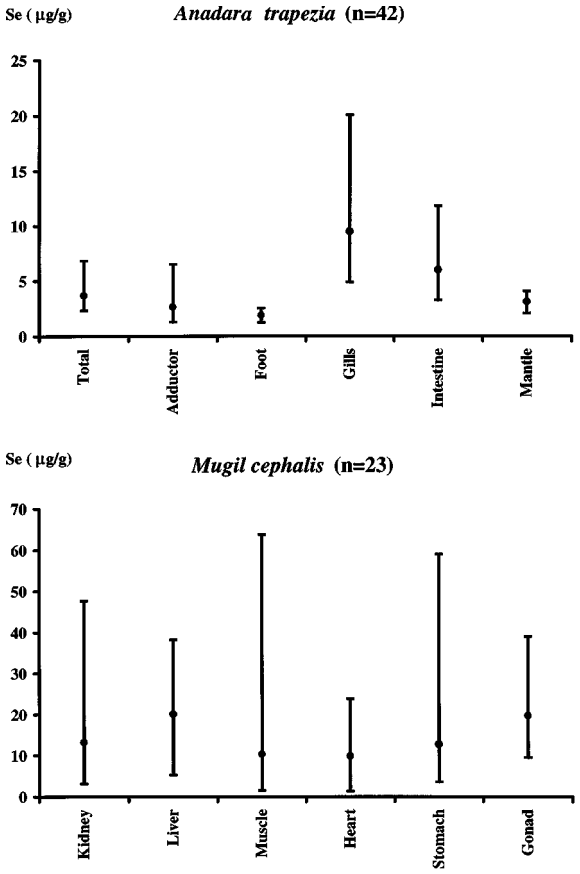

Figure 6 Selenium concentrations in tissues of *Anadara trapezia* and *Mugil cephalus*: the points (●) are mean values, with the ranges indicated by the bars.

However, many bivalves show different patterns of metal distribution[82, 83] and patterns differ between species, location and metals type.[81, 84, 85] Diversity exists in elemental uptake, metabolism, and storage and excretion mechanisms between species; therefore variations in patterns of selenium distribution with different species may occur.

It is interesting to note that although tissue concentrations of selenium are significantly different, no individual tissue exhibited a high concentration of selenium as occurs in some bivalves for other metals, e.g. zinc in the kidney of *Mytilus edulis*.[86]

Scanes,[87] who transplanted cockles from areas of high to low sediment metal concentration and vice versa, showed that sediment trace metal concentration had little influence on the metal concentration of cockles; thus cockles are suspension feeders, not deposit feeders.

The high concentrations of selenium found in the gills and intestines indicates that selenium is being accumulated from both seawater and food.[50, 56, 85, 88] Fowler and Benayoun[50] showed that, for *Mytilus edulis*, ^{75}Se taken up from seawater accumulated in gills while ^{75}Se taken up in food sources accumulated in digestive tissues. Zhang *et al.*[85] also showed that the clam *Chaetoceros mellena* accumulated selenium in gills when taken up from water and in the digestive tissues if taken up from food (phytoplankton).

It should be noted that other studies have shown that the distribution of metals within tissues may change with time. Orren *et al.*[74] have shown that short-term accumulation of trace metals results in high concentrations in gills while longer term trace-metal accumulation

Table 1 Distribution of selenium in marine animal tissues

Species/Tissue	Total selenium[a] ($\mu g\ g^{-1}$ dry wt)	Selenium[c] (%) MeOH/$CHCl_3$	EtOH/H_2O	Residue
Anadara trapezia[a]				
Adductor	3.7±0.8	21±14	2±3	80±16
Foot	2.7±0.9	6±1	0.4±0.3	94±2
Gills	10±3	30±18	2±1	68±18
Intestines	6±3	9±3	0.5±0.3	90±3
Mantle	3.1±0.5	6±2	0.7±0.4	93±1
Blood	3.6±0.8	4±2	1.3±0.3	95±2
Mugil cephalus[b]				
Muscle	10±4	0.22±0.12	0.1±0.1	78±27

[a] Mean±standard deviation, $n=42$
[b] Mean±standard deviation, $n=23$
[c] Mean±standard deviation, $n=5$

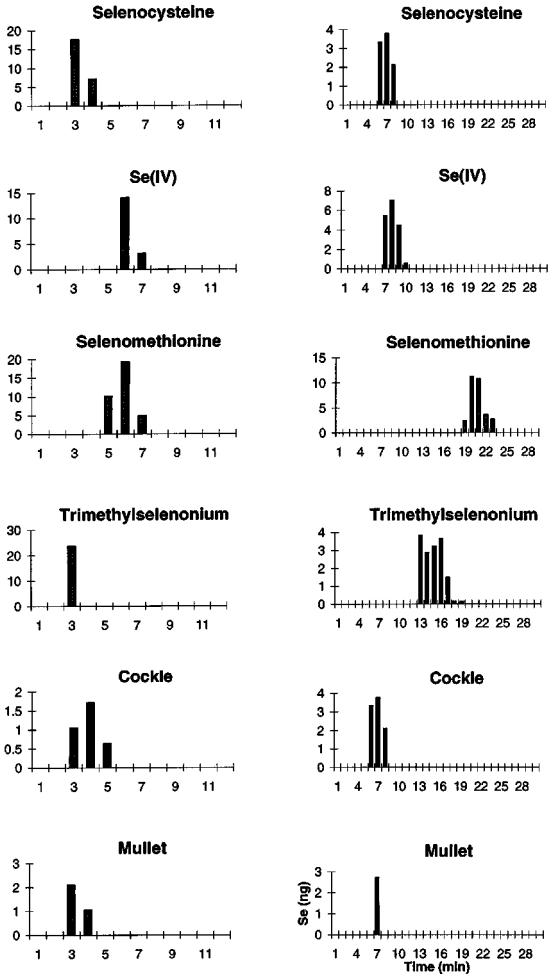

Figure 7 HPLC of selenium standards and selenium compounds in hydrolysed cockle and mullet muscle tissue. The left- and right-hand plots are the SAX and C18 columns respectively.

occurs in the kidney. Latouche and Mix[70] have shown that distribution of metals in *Mytilus edulis* changes with age as the organism becomes sexually mature. Manganese concentrations are higher in somatic tissues of smaller organisms while nickel, copper and cadmium concentrations in somatic tissues are higher in older organisms.

Mullet

The tissues of mullet of a similar size (< 30 cm) were analysed to minimize the effects of weight. The regression between selenium concentration and weight was not significant ($r^2 = 0.1456$, $P = 0.2361$, $n = 22$). There was a significant difference between selenium concentrations ($P < 0.001$) in various tissues (Fig. 6). A Tukey–Kramer multiple comparison test revealed that selenium concentrations were in the following order: liver > gonad > kidney > stomach > heart = muscle. Other studies on fish have reported similar results.[30, 89–91] Patterns of accumulation may change if fish are exposed to high concentrations of other elements e.g. cadmium.[92] Accumulation of selenium in the liver appears to be a general phenomenon in marine fish, cetaceans and other mammals.[46, 93, 94] Sorensen postulates that selenium concentrations in fish livers are significantly elevated because of internal cycling. Generally, the liver accumulates most of its selenium following acute or chronic exposure,[95–97] which suggests that fish from Lake Macquarie have been chronically exposed to high levels of selenium.

Food is considered by many authors to be the most important source of selenium to fish.[91, 92] However, selenium may also be accumulated through the gills and the site of accumulation is dependent on the species of selenium present in

Table 2 Selenium compounds isolated from organisms exposed to selenium under laboratory conditions

Compound	Structure	Organism
Selenomethionine	$CH_3SeCH_2CH_2CH(NH_2)COOH$	Marine algae
Selenomethionine selenoxide	$CH_3Se(O)CH_2CH_2CH(NH_2)COOH$	Oysters
Selenocysteine	$HSeCH_2CH(NH_2)COOH$	Marine algae
Selenohomocysteine	$HSeCH_2CH_2CH(NH_2)COOH$	Oysters
Selenocysteic acid	$HOSe(O)CH_2CH(NH_2)COOH$	Oysters, marine algae
Se-methyl selenomethionine	$(CH_3)_2Se^+CH_2CH_2CH(NH_2)COOH$	Marine algae
Se-methylselenocysteine	$CH_3SeCH_2CH(NH_2)COOH$	Marine algae
Se-methylselenocysteine selenoxide	$CH_3Se(O)CH_2CH(NH_2)COOH$	Marine algae
Se-methylselenocysteine selenone	$(CH_3)Se(O_2)CH_2CH(NH_2)COOH$	Marine algae

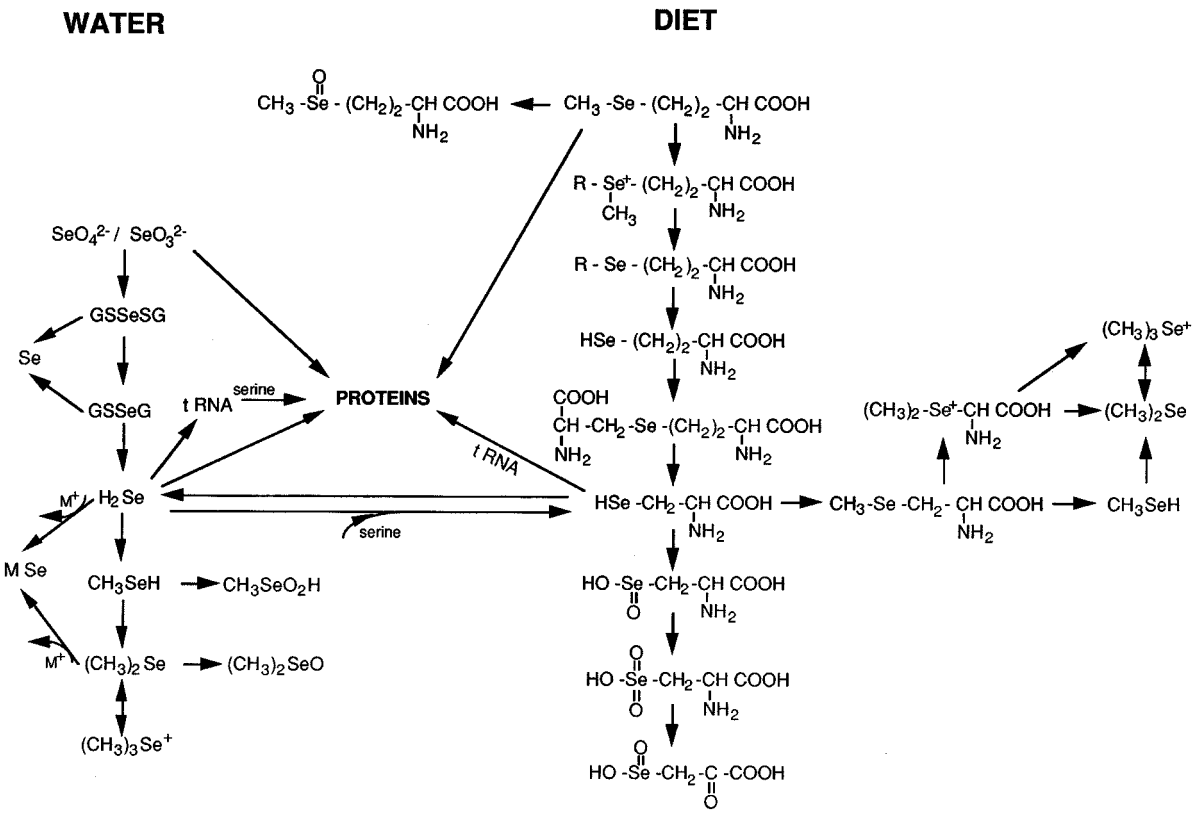

Figure 8 Pathways of selenium transformations in marine organisms.

solution.[90, 98] Selenite accumulates in the liver while selenomethionine accumulates in the muscle.[99] It is not known if the high levels of selenium in fish livers in this study are due to direct uptake of selenite/selenate from solution, selenium being taken up in food and internally cycled, or a combination of both.

Biochemical fractionation

Most of the selenium recovered from cockle tissues and mullet muscle tissues (Table 1) was found to be associated with the protein fraction. For most samples, this represented more than 80% of the selenium content. Previous studies have also isolated selenium in protein extracts of marine tissues.[3, 100, 101] Subsequent attempts to extract selenium from protein residues were only successful when a phosphate buffer solution incorporating a detergent (i.e. sodium dodecyl sulphate) was used. This indicates that selenium is either membrane-bound or associated with lipoprotein material.[102]

Speciation

Selenium isolated from hydrolysed mullet and cockle muscle tissue was found to be at the specific location of selenocysteine (Fig. 7) using both HPLC separation methods. After being left to stand for 24 h, if samples were rechromatographed on the C_{18} column a peak shift to 13–14 min was observed. Selenocysteine combined with cysteine to form a selenosulphide which eluted at this time, confirming that selenium was present as selenocysteine. Only 65–75% of selenium in the muscle tissues was recovered as selenocysteine; the loss of selenium occurred during filtration to remove particlulates prior to chromatography. Thus we cannot say that other selenium species are not present in muscle tissues. It has been shown that selenium can readily form selenotrisulphides with thiols such as cysteine or glutathione,[103, 104] and that these can be incorporated and stabilized within protein structures.[105] A purified protein sample obtained from the mullet muscle tissue by extraction with phosphate buffer yielded 100%

selenocysteine on hydrolysis indicating that selenium incorporated into protein was selenocysteine and not present as a non-protein moiety.

Selenium transformations—conceptual model

The results above (Table 1, Fig. 7) indicate that selenium in marine tissues is probably predominantly incorporated in proteins as selenocysteine. Other studies have isolated selenium in protein extracts from the muscle and liver tissues of marine animals.[3, 101, 106, 107] Additional evidence for the presence of selenium in combined and free analogues of sulphur containing amino–acids comes from the laboratory uptake studies performed by Wrench[28, 29] and Bottino *et al.*[6] The compounds identified are shown in Table 2. Enzymic hydrolysis with Pronase was used to release selenoamino-acids from tissues in these studies, but no indication of selenium recoveries was given. Selenium-dependent glutathione peroxidase has been isolated from the Black Sea bass *Centropristis striata*,[100] further indicating that at least some selenium is present in marine fish as selenocysteine. Selenocysteine is a normal component of glutathione peroxidase.[21, 108] Cooke and Bruland[27] have isolated several methylated selenium species from seawater (DMSe, $DMSe^{+}-R$) and have proposed that these are produced by the decomposition of selenomethionine released from biological material.

Probable transformations of selenium in marine animals are shown in Fig. 8. If selenium is following sulphur pathways, it is likely that selenomethionine in the diet is converted to selenocysteine as occurs in terrestrial organisms. The model also incorporates a pathway for the formation of M–Se (M = metal) compounds. Mercury–selenium associations (1:1 molar ratio) have been reported in marine mammals.[109] The trimethylselenonium ion appears to be the major excretion metabolite in terrestrial organisms[110, 111] and is likely to be the major excretion metabolite of marine organisms.

CONCLUSIONS

1. Selenium accumulation in the cockle and mullet is dependent on weight (age?) but not on sex.
2. Selenium distribution in cockle and mullet tissues is significantly different, with the highest selenium concentrations in the gill and digestive system of the cockle and in the liver of the mullet.
3. Most of the selenium in cockle tissues and mullet muscle tissue is associated with proteins.
4. Selenium was identified in the muscle tissue of both the cockle and the mullet as selenocysteine.

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