UNIVERSITY OF TECHNOLOGY, SYDNEY

METAL ACCUMULATION IN TOADFISH, *TETRACTENOS GLABER*, AND THEIR PREY ITEMS

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CERTIFICATE OF AUTHORSHIP

I certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree except as fully acknowledged within the text.

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ABSTRACT

Estuaries support productive and diverse ecosystems, based on the abundant food and diverse habitats. However with increasing human pressures, many estuaries along the coastline of Australia have become degraded. The primary objective of this study was to investigate the effects of estuarine metal contaminants on the physiology, and the subsequent accumulation, in the smooth toadfish, *Tetractenos glaber,* and their prey items in estuaries in the Sydney region, south eastern Australia.

Toadfish and sediments were collected during three seasonal sampling periods (June 2002, September 2002 and January 2003) in four estuaries in the Sydney region (Port Hacking River, Cowan Creek, Parramatta River and Lake Macquarie). Sediment and toadfish tissue metal concentrations varied both spatially and temporally, with highest concentrations exhibited in the metal contaminated estuaries (Lake Macquarie and Parramatta River) during spring 2002 and summer 2003. Toadfish accumulated metals in different tissue, however field experiments were limited in determining the main pathways of accumulation.

Controlled laboratory kinetic experiments using radioisotopes of ^{109}Cd and ^{75}Se, indicated that differences in the relative contributions of water and food in the accumulation of these metals are generally governed by the physiology of the toadfish and the type of prey eaten. The distribution of metals in fish organs is important for better understanding metal kinetics and their subsequent toxicity. Toadfish exposed to ^{109}Cd in both water or food showed a shift in distribution from gut lining at the end of the uptake phase to the excretory organs, such as liver, by the end of the loss phase, suggesting that the main uptake pathway for water exposure was via the gut and not the gills, due to fish drinking large amounts of water to maintain osmoregulation. There was no appreciable shift in the distribution of ^{75}Se from the uptake or loss phases, being mostly associated with the excretory organs (gills, liver and kidneys). Further investigation into the accumulation of metals in toadfish prey items revealed that differences in uptake and loss of ^{109}Cd and ^{75}Se were influenced by their aqueous speciation, as well as differences in animal physiology, sequestration, storage and excitation mechanisms. The uptake rates and CFs of ^{109}Cd and ^{75}Se were highest, and the biological half-lives the longest, in pygmy mussels, ghost shrimps and polychaetes relative to the semaphore and soldier crabs.

Elevated metal concentrations in certain toadfish tissues from the four estuaries were linked to reduced lipid concentrations and increased protein concentrations, which may be detrimental for growth, reproductive output and survivorship of the fish. A closer investigation into the nutritional value and metal concentrations of toadfish prey items in a selected metal contaminated estuary (Parramatta River) revealed that toadfish from the more contaminated sites within the estuary may grow faster due to higher nutritional value of prey items on a local scale, however on a larger spatial scale, this was not evident. Further investigation is required to determine whether larger toadfish size can be attributed to physiological acclimation or genetic resistance through generations of continuous metal exposure.

Many studies have assessed metal concentrations in water, sediments and biota in the field, however few studies have combined both field and laboratory experiments to examine the effects of metals on fish physiology and reproduction, and metal transfer pathways in aquatic biota. This study has contributed to a better understanding of metal accumulation and its physiological effects in estuarine biota and highlights the high spatial and temporal variability in responses of organisms to environmental metal pollution.

1.0 INTRODUCTION

This study involved the investigation of the effects of contaminants on the physiology, and subsequent transfer of contaminants in common estuarine biota, particularly the smooth toadfish. This investigation used a multi-disciplinary approach where ecological and ecotoxicological hypotheses were tested using field and laboratory techniques.

1.1 RATIONALE

1.1.1 Ecology and toxicology – the fundamental link

Ecology is the study of interactions between organisms and their surrounding environment including biotic processes, such as predator-prey interactions and competition, which may influence abundance, distribution, density and diversity of populations and communities (Bjornstad and Grenfell 2001; Brown *et al.* 2001; Jordan and Scheuring 2004). Not all species exert equal influences on a community, giving rise to the concept of a "keystone species" (Power *et al.* 1996). Keystone species exist in a number of trophic levels and are well known in many of the world's ecosystems (Power and Mills 1995). The addition or removal of a keystone species can have an overriding bottom-up or top-down effect on an ecosystem, causing a trophic cascade (Mengre 2000; Mills *et al.* 1993). Trophic cascades are dominated by biotic and/or abiotic forces acting on top-down control of community structure by predators, or bottom-up effect governed by primary producers or consumers (Jordan and Scheuring 2004). A study by Frank et al (2005) demonstrated a decline in top predator abundance (cod) by a collapse of the benthic fish community, a classic bottom-up trophic cascade.

Although biotic factors are crucial in understanding population and community dynamics, there are abiotic factors, such as physical and chemical stressors that can have an influence on ecosystem function (Bjornstad and Grenfell 2001; Chapman 2002b; Clements 1999; Hamilton 2002). Some of these abiotic factors include habitat modification and destruction, nutrient enrichment and introduction of foreign "toxic" chemicals, pollutants.

Toxicology is defined as the study of relative toxicities (lethal or sub-lethal effects) to organisms governed by changes in biochemical and physiological processes and responses (Chapman 2002b). Although toxicology and environmental toxicology use the concept of screening a variety of toxic chemicals on individual

biota to identify what chemicals are hazardous, it has little relevance to environmental processes (Chapman 2002b).

Ecotoxicology is the integration of ecology and toxicology, and forms a basis for understanding and predicting effects of chemicals on a realistic population and community level. Here, toxicology can be used to test effects of contaminants on population and community structure and function; hence, ecotoxicology can determine relationships between organisms and contaminants. For example, contaminants in estuaries may have a direct toxic effect on macroinvertebrates and their predators (e.g. fish), or an indirect effect on natural community structure by reducing prey item diversity, or reducing competition within a species, resulting in a trophic cascade (Chapman 2004; Fleeger *et al.* 2003). A reduction or elimination of an organisms primary food source due to contaminant exposure, will force the organism to switch to an alternative diet, known as functional redundancy (Fonseca and Ganade 2001; Petchey and Gaston 2002; Rosenfeld 2002), which may involve increased energetic costs of feeding, resulting in reduced fitness and growth efficiency (Chapman 2004; Fleeger *et al.* 2003). It is therefore important to investigate the effects of contaminants in a toxicological sense with the incorporation of ecological processes in order to produce a more relevant assessment of the environmental implications.

1.1.2 Metals in the environment

Although there are many different types of contaminants present in aquatic environments, including pesticides, metals, organochlorins, dioxins, petrochemicals and many other organic and inorganic compounds, this study focuses on metals and metalloids.

There is an extensive amount of literature available on metal contamination in sediment and water in Australia (Birch *et al.* 1999a; Irvine and Birch 1998; Peters *et al.* 1999a; Roy and Crawford 1984), and internationally (Amouroux *et al.* 2003; Besser *et al.* 1996; Nichols *et al.* 1986; Rios-Arana *et al.* 2003), as well as the subsequent metal transfer to biota (Bargagli *et al.* 1998; Borgmann 2000; Watras *et al.* 1998; Worthen *et al.* 2001). Sediments can act as sinks for contaminants, which may persist in the aquatic environment for decades, before biological, chemical and physical changes such as sediment infauna activity and water chemistry, allow these persistent contaminants to enter food chains, thus make their way into organisms (Birch and Taylor 1999; Kirby *et al.* 2001a; Kirby *et al.* 2001b).

Different mechanisms can influence the binding and release of metals in sediments (Chapman *et al.* 1998). Acid-volatile sulfides (AVS) are one such mechanism, where iron (FeS) and manganese (MnS) monosulfides, which contain highly soluble products, bind to dissolved metals by displacing Fe and Mn to form less soluble MeS (Ankley 1996). Particulate organic matter (POM) also has a high affinity to bind metals to sediments by attaching to the proton-dissociating sites of humic substances (Tipping and Hurley 1992). Metals can also directly bind to the hydroxyl group of iron and manganese oxyhydroxides (FeOOH and MnOOH), on organic matter, thus aiding in sediment binding (Tessier *et al.* 1996). Metals can also attach to organic and inorganic ligands in water (Chapman *et al.* 1998).

Although there is ample information on metal concentrations in sediments and water, this does not constitute evidence for biological significance. Hence, sediment quality guidelines (SQG) have been derived to make an initial assessment of toxicity to sediment dwelling biota, particularly macroinvertebrates (Birch and Taylor 2002b). The Australian and New Zealand Environment and Conservation Council (ANZECC) and the Agriculture and Resource Management Council of Australia and New Zealand (ARMCANZ) have derived Interim Sediment and Water Quality Guidelines (Table 1.1) as part of the National Water Quality Management Strategy (ANZECC/ARMCANZ 2000). The ISQG's were based on the guidelines set by the U.S. National Oceanic and Atmospheric Administration (NOOA), and take into account field data, laboratory based toxicity data and equilibrium partitioning of toxicants between sediments and interstitial waters (Birch and Taylor 2002b; Borgmann 2000; Mountouris *et al.* 2002; Simpson *et al.* 2002).

Estuarine and marine sediments and sediment-water interfaces are considered biologically important habitats (Morrissey *et al.* 2003; Rosenberg *et al.* 2003; Virgilio *et al.* 2003), however bioavailability (the extent of a contaminant in substrate, which is free for uptake by an organism) may be influenced by chemical speciation in the dissolved form (Newman 1998; Simpson *et al.* 2002). For example, metal cations compete for other cations, anions or molecules that form complexes (ligands) with other metals. Ligands combined with other metals include organic and inorganic compounds with functional groups such as the organic carboxylic and phenolic groups or the inorganic hydroxides (OH^-), carbonates (CO_3^-), chlorides (Cl^-), sulphates (SO_4^{2-}) and ammonium ions (NH_3) (Bushby 1998; He *et al.* 2001; Newman 1998). The extent of bioavailability to an organism can be estimated as a function of competing cation concentrations, ligand concentrations, pH, temperature and ionic strength (Newman 1998).

Table 1.1 Australian and New Zealand Interim Sediment Quality Guidelines (ISQG) for metals. Concentrations are in mg.kg^{-1} dry weight (ANZECC/ARMCANZ 2000).

Metal or metalloid	[a]ISQG - Low	[b]ISQG - High
Arsenic	20	70
Cadmium	1.5	10
Chromium	80	370
Copper	65	270
Lead	50	220
Nickel	21	52
Zinc	200	410

Values represent a [a]low or [b]high statistical probability of effect on organisms.

1.1.3 Metals in fishes

There are many different mechanisms associated with metal uptake in fishes (Figure 1.1), including uptake through the skin or dermis and gills or pulmonary surfaces associated with contaminated water (dissolved state) (Lyndon and Houlihan 1998) or dietary uptake via the gut through trophic transfer or ingestion of sediments (Chapman *et al.* 1998; Filion and Morin 2000; Liu *et al.* 2002; Newman 1998; Reinfelder *et al.* 1998).

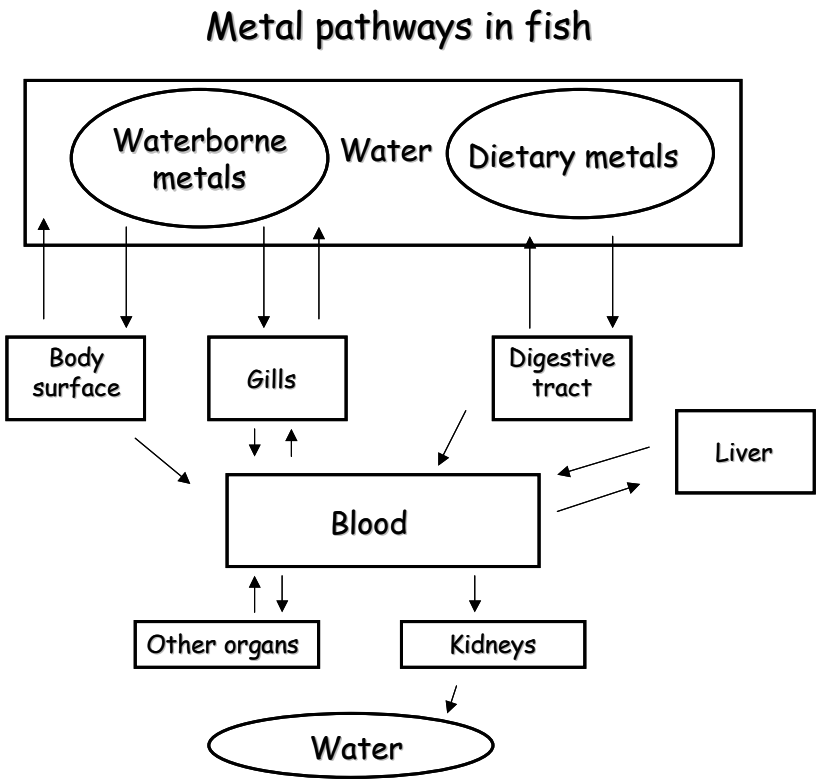

Figure 1.1 Conceptual model of metal pathways in fish tissues. Adapted from Jezierska and Witeska (2002).

Different organisms can eliminate or excrete, bind or sequester metals in a non-toxic form (Arts *et al.* 2004; Chapple *et al.* 1997; Newman 1998; Radlowska and Pempkowiak 2002; Traunspurger and Drews 1996). Metallothioneins are small proteins that help maintain homeostasis (the ability or tendency of an organism or cell to maintain internal equilibrium by adjusting its physiological processes). Metals can also be bound to inert calcareous exoskeleton or granular like structures to maintain cellular homeostasis. However when concentrations have exceeded the homeostatic capacity of the organism, toxicity can occur (Ahearn *et al.* 2004; Chapple *et al.* 1997; Hollis *et al.* 1999; Wang and Rainbow 2005). Effects on fish tissues can include skin lesions, excess mucous secretion of the gills, changes in blood circulation, changes in enzymatic activity in the gut lining, changes in histology, metallothionein induction and enzyme activity in the kidneys and liver, and disturbances in the neurotransmission of the nervous system (Jezierska and Witeska 2002).

As well as uptake and regulation mechanisms employed by different organisms, a host of other factors can affect metal bioaccumulation, including age, gender, size, genetics and organism behaviour (Borgmann 2000; Peakall and Burger 2003). For example, a study by Canli and Atli (2003) investigated the relationship between size and metal concentrations in six fish species, and found significant negative correlations between size/age and metal concentrations. Farkas et al. (2003) also found negative relationships between metal concentrations and the size and sex of bream. Although many toxicological studies have been conducted in the laboratory, few studies have incorporated field components, thus hindering an understanding of ecological significance. In the present study, sediments, macroinvertebrates and fish collected in the field were used to examine metals in biota and the environment.

1.1.4 Biological indicators

Laboratory based experiments, which are used to examine the link between contaminants in the water, sediments and aquatic biota, are limited and often ecologically irrelevant. However, many organisms have been used as biological indicators or bioindicators, to measure ecological significance (Hilty and Merenlender 2000; Xu *et al.* 2004). A bioindicator is an organism or group of organisms whose characteristics such as presence/absence, density or juvenile survivorship can be used to assess ecosystem health (Adams and Greeley 2000; Burger and Gochfeld 2001). Field based bioindicators can be used to define the possible causal links between environmental stressors and the ecologically relevant endpoints such as changes in molecular, individual, population or community integrity. Macroinvertebrates (Lindegarth and Hoskin 2001; MacFarlane *et al.* 2000; Pinel-Alloul *et al.* 1996) and fish (Guidetti *et al.* 2002; Soto-Galera *et al.* 1998; Taylor *et al.* 2004; Whitfield and Elliott 2002) have successfully been used as potential bioindicators in previous studies. The biological endpoint of bioindicator organisms can include changes at a sub-organism (physiological, biochemical and biomolecular), individual (reproduction, bioenergetics and histopathological), population or community level (population success, community structure and growth) (Adams and Greeley 2000). An organism or group of organisms should fulfil certain criteria to be defined as a potential bioindicator. These attributes include low natural variability, measurable response to change, widespread and easy to monitor, socially and/or commercially relevant with cost effective analyses (Burger and Gochfeld 2001; Whitfield and Elliott 2002). The smooth toadfish (Figure 1.2), *Tetractenos*

glaber, although not of commercial value, was selected as a relevant bioindicator because it was common in all estuaries examined, relatively easy to maintain in the laboratory, feeds on resident benthic macroinvertebrates associated with sediments and is typically site specific with relatively small homing ranges (~100-200m) (Booth and Schultz 1999).

Figure 1.2 The smooth toadfish *Tetractenos glaber,* common along the southeastern coast of Australia.

1.1.5 Sub-lethal effects of metals to biota

Many ecotoxicological and risk assessment studies have relied heavily on acute toxicity (LC_{50}) data, where the endpoint is death of the organism (Canli 2005; Hollis *et al.* 1999). The biological ligand model uses acute toxicity data to determine the extent of dissolved metal uptake by binding to organic ligands. It can be used to predict the binding efficiencies of metals in fish gill tissues and hence, predict the lethal toxicity to fish (Fisher and Hook 2002). However, the acute toxicity approach only determines metal uptake from the dissolved state, and sub-lethal or chronic effects are not considered (Fisher and Hook 2002). Sub-lethal effects can range from impared reproduction (Brooks *et al.* 1997; Sepúlveda *et al.* 2002), changes in predator avoidance behaviours (Jones and Reynolds 1997; Sloman *et al.* 2003) and impared development (Martinez *et al.* 2003; Sadler *et al.* 2001), which can lead to changes in population dynamics. Endpoints used to measure sub-lethal effects can include changes in essential lipid concentrations (Baker *et al.* 1998; Levesque *et al.* 2002; Roméo and Gnassia-Barelli 1997), induction of heat stress proteins (HSP's) (Radlowska and Pempkowiak 2002; Tedengren *et al.* 1999) and metallothioneins

(MT's) (Bordin *et al.* 1997; Seebaugh and Wallace 2004; Viarengo *et al.* 2000) , changes in genetic composition (Kirchhoff *et al.* 1999) and reduction of growth efficiencies (Farkas *et al.* 2003; Forrester *et al.* 2003). In this study, sub-lethal effects, such as growth, longevity, lipid and protein concentrations, were investigated in smooth toadfish to determine the effects on health and reproduction. Metals associated with toadfish macroinvertebrate prey items, and subsequent nutritional value was also examined in relation to toadfish growth and condition.

1.1.6 Metal kinetics and food webs

Organisms may accumulate metals through various environmental pathways, including water, diet and/or sediment (Boisson *et al.* 2003; Peters *et al.* 1999c; Selck and Forbes 2004; Wang and Fisher 1999). The relative importance of each pathway will influence the potential transfer of metals to higher order predators. Bio-energetic kinetic models have permitted a better understanding of the relative importance of the waterborne and dietary exposure pathways of metals in organisms (Fisher and Hook 2002). Different food webs contain varying potentials for metal bioaccumulation, and are governed by differences in animal metabolism and sequestration, regulation and excretion mechanisms (Blackmore and Wang 2004). Few studies have investigated the potential for trophic transfer of dissolved or dietary metals to higher order predators in a marine environment (Blackmore and Wang 2004; Reinfelder *et al.* 1998; Wang and Fisher 1999). In this study, toadfish guts were examined to identify macroinvertebrate prey items. Common prey items were used in controlled laboratory kinetics experiments to measure the uptake and loss of metals and subsequent trophic transfer to smooth toadfish.

1.2 STUDY LOCATIONS

Four estuaries (two metal contaminated and two minimally impacted estuaries) were selected in the Sydney region, in southeast Australia (Figure 1.7), based on *a prior* contaminant information (Birch and Taylor 1999; Birch *et al.* 1999b; Hatje 2002; Hatje *et al.* 2001; Irvine and Birch 1998; McCready *et al.* 2000).

1.2.1 Parramatta River

The Parramatta River has a catchment area of 130 km^2 and flows into Sydney Harbour (DLWC 2000). This river has been exposed to many anthropogenic inputs, due to a large industry base, over the past two centuries, including abattoirs, brick works, chemical production industries, a large petroleum refinery (Figure 1.3), and a

number of storm water and sewage drains that contribute to contaminants from urban and industrial practices (Birch and Taylor 1999; Birch *et al.* 1996; Hatje *et al.* 2001).

Extensive intertidal areas have been reclaimed for urban use and industrial waste disposal, resulting in leachate entering the waterways. There are also over 740 sewage discharge points in the Parramatta River contributing to the input of nutrients and bacteria (Birch and Taylor 1999). Furthermore, atmospheric deposition of metals has contributed to the Rivers' pollution loads, mainly through vehicular combustion (Irvine and Birch 1998). Metal contaminants are mainly contained in insoluble phases in the sediments and NSW Government policy has been to leave them undisturbed (Birch and Taylor 1999). However, surface sediments in many parts of the Parramatta River are often resuspended into the water column, thus contributing to pollution levels in the overlaying water column (Hatje *et al.* 2001).

Figure 1.3 Oil refinery close to the banks of the Parramatta River, near Site 2.

1.2.2 Lake Macquarie

Lake Macquarie is a large marine dominated barrier lake, situated 135 km north of Sydney and has a catchment area of 700 km^2 (DLWC 2000). A narrow entrance at Swansea separates the lake from the ocean and contributes to small tidal ranges (Kirby *et al.* 2001c). Lake Macquarie has an extensive amount of urban and industrial development. A metal smelter (Pasminco Metals Smelter; Figure 1.4a), commissioned in 1962, has contributed to elevated metal levels located on the northern section of the lake (Morrison 2003). On the southern end of Lake

Macquarie, there are three coal-fired power stations (Eraring, Vales Point; Figure 1.4b, & Wangi Wangi, the latter decommissioned in 1986), which contribute to fly ash, high in Se, Zn, Cu and Cd levels (Kirby *et al.* 2001c). Other industrial sites surrounding the shores of Lake Macquarie include a fertiliser plant, steel works and sewage treatment works (Barwick and Maher 2003; Kirby *et al.* 2001a; Peters *et al.* 1999b). Previous studies have indicated that both water and sediments contain elevated concentrations of a range of metals (Barwick and Maher 2003; Doyle *et al.* 2003; Peters *et al.* 1999b; Peters *et al.* 1999c).

Figure 1.4 (a) Pasminco Metals Smelter (northern Lake Macquarie) and (b) Eraring Power Station close to the banks of Lake Macquarie, close to Sites 1 & 2, respectively.

1.2.3 Port Hacking River

Port Hacking River is situated 35 km south of Sydney and has a catchment area of 180 km^2 (DLWC 2000). This river is considered relatively uncontaminated as it is surrounded by a National Park (Figure 1.5) with minimal anthropogenic inputs (Stark 1998). A small dredging event in 1942, caused local seagrass beds to deplete (Meehan and West 2002), however with low urbanisation and no industrial influence, Port Hacking River has minimal contaminant levels.

Figure 1.5 Port Hacking River surrounded by the Royal National Park.

1.2.4 Cowan Creek (tributary of the Hawkesbury River)

Cowan Creek is a tidally influenced tributary of the Hawkesbury River system situated 50 km north of Sydney, with a catchment area of 180 km^2 (DLWC 2000). The majority of the Cowan Creek catchment is comprised of national park and state forest (Figure 1.6) (Birch *et al.* 1998; MacFarlane and Booth 2001). The Hawkesbury River catchment supports a healthy agricultural industry, valued at $500AUD million, hosting a range of crops, animals and animal produce (Birch *et al.* 1998). The river also contains a large fisheries industry, estimated at $5AUD million, comprising of fish and prawns and is one of the larges oyster producing rivers in New South Wales (Birch *et al.* 1998). Effluent from over 40 sewage treatment plants enter the Hawkesbury River, which contributes to elevated nutrient concentrations causing frequent algal blooms (Arakel 1995). Although there are increasing environmental pressures from expanding urbanisation and development, the Hawkesbury River and tributaries can be relatively unpolluted.

Figure 1.6 Cowan Creek surrounded by the Ku-ring-gai Chase National Park.

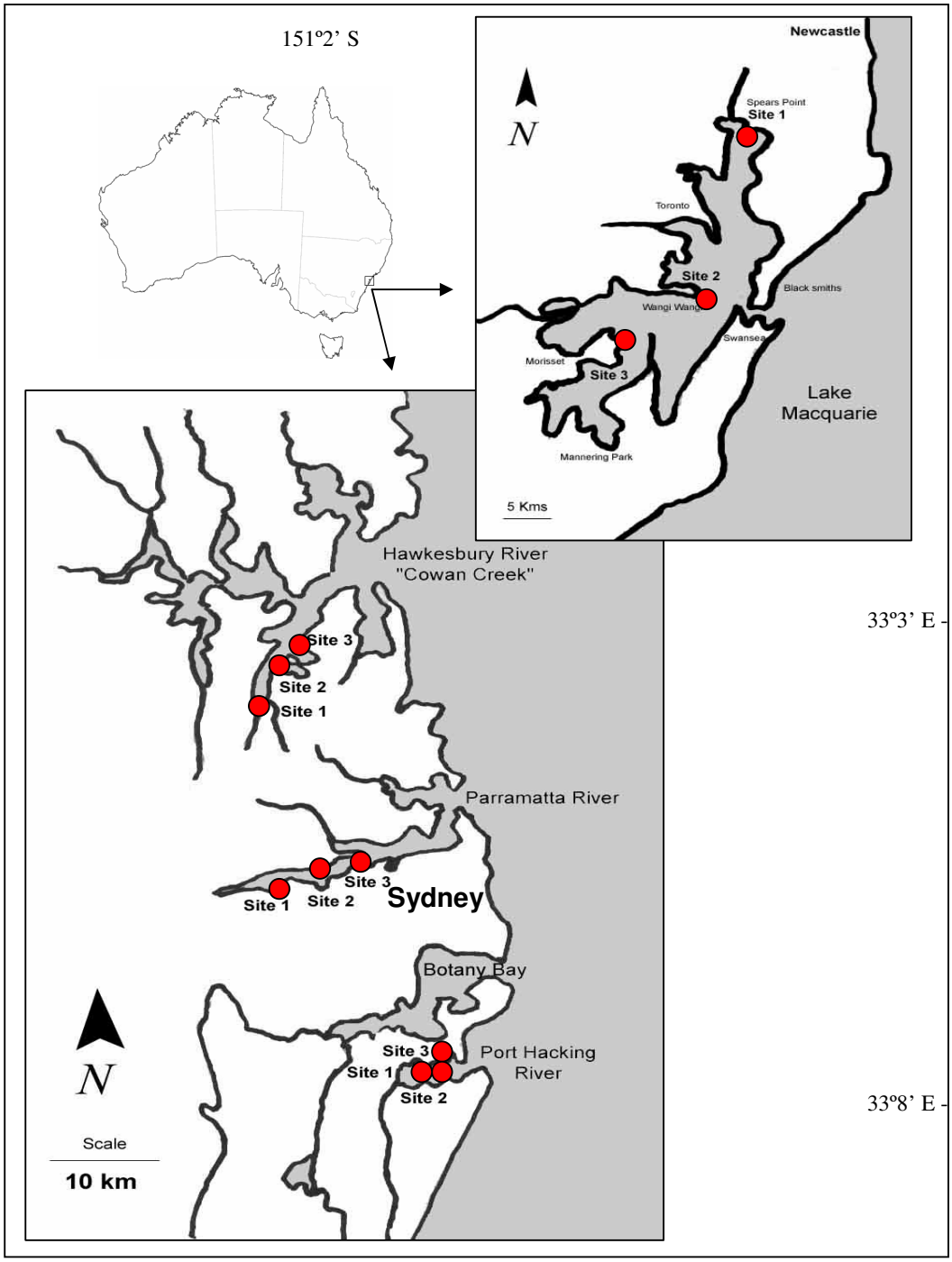

Figure 1.7 Locations of study sites in Lake Macquarie, Cowan Creek, Parramatta River and Port Hacking River. Inset: Location of sites on the southeastern corner of Australia.

1.3 STUDY OBJECTIVES

The purpose of this study was to investigate the physiological effects, and transfer of, contaminants in the smooth toadfish, *Tetractenos glaber*. The primary objectives were:

(a) To determine the metal concentrations in sediments and tissues of the smooth toadfish, *Tetractenos glaber,* in four estuaries (Parramatta River, Lake Macquarie, Port Hacking River and Cowan Creek), representative of varying metal contamination.

(b) To investigate potential links between metal concentrations in tissues of the smooth toadfish and growth, condition and reproductive output.

(c) To determine the common prey items of toadfish, examine their nutritional value and metal concentrations, and investigate their possible effects on toadfish growth.

(d) To compare and contrast the uptake and loss kinetics of two key metals in toadfish prey items.

(e) To compare the dietary and dissolved metal exposure pathways in smooth toadfish.

The following chapters address the physico-chemical characteristics of sediments and waters of the selected study estuaries (Chapter 2) and potential effects on the physiological condition and reproductive output of the smooth toadfish (Chapter 3). Metals, nutritional value and metal uptake strategies in toadfish prey items were investigated using field and controlled laboratory experiments (Chapters 4 & 5), followed by the examination of water and food exposure pathways in toadfish (Chapter 6). Chapter 7 evaluates the findings from the field and laboratory studies and addresses their implications for fisheries management. Radioecological experiments were carried out at the Institute for Environmental Research, ANSTO Laboratories, Lucas Heights. All other laboratory investigations were carried out at the Department of Environmental Sciences, University of Technology, Sydney.

2.0 METAL CONCENTRATIONS IN SEDIMENTS AND TISSUES OF THE SMOOTH TOADFISH, *TETRACTENOS GLABER,* IN SYDNEY ESTUARIES, SOUTH EASTERN AUSTRALIA

2.1 INTRODUCTION

The persistence of metals in aquatic systems has been recognised as potentially harmful to biota, however few studies have reported *in situ* metal uptake or accumulation in estuarine fishes. The research described in this chapter involved the characterisation of water and sediment in the study estuaries, with metal concentrations in sediments and tissues (gills, liver, muscle and gonads) of the common estuarine fish, *Tetractenos glaber* (smooth toadfish), also examined. These results were later used to investigate links between metal body burden and health and reproductive parameters (Chapter 3).

2.1.1 Estuarine sediments

The southeast coast of Australia has been subjected to high inputs of contaminants such as metals, dioxins, pesticides and petrochemicals over the last two hundred years, with most inputs contributed by the cities of Sydney and Wollongong (Birch and Taylor 1999; Birch 2000; Birch *et al.* 1996; Birch *et al.* 2001; He and Morrison 2001). Estuaries of the greater Sydney region have been contaminated by urban and industrial runoff from a range of sources, including power stations, metal smelters, steel works, refineries, sewage works and agriculture (Birch 1996; Irvine and Birch 1998; Kirby *et al.* 2001a; Kirby *et al.* 2001b; Peters *et al.* 1999a). These highly contaminated inputs are likely to be impacting resident fish populations and other biota (Newman 1998).

Sediments can act as sinks for contaminants, which can persist in the aquatic environment for decades (Birch *et al.* 1999a; Irvine and Birch 1998; Peters *et al.* 1999a; Roy and Crawford 1984). Biological, physical and chemical changes, such as alterations in sediment infauna activity and water chemistry, can allow these persistent contaminants to become more available and enter food chains, thus making their way into fish tissues (Birch and Taylor 2002a; Kirby *et al.* 2001a).

2.1.2 Metal accumulation by organisms

Organisms can take up metals from the environment through four main pathways: water uptake through the gills; trophic transfer via ingested food; drinking

water; and adsorption through the skin (Heath 1995). Several abiotic factors can also govern the amount of metal accumulation, including temperature, pH, water hardness, sediment and conductivity (Heath 1995; Newman 1998). Biotic factors such as intra and inter-specific competition and predation (Carrassón and Cartes 2002; Reinfelder *et al.* 1998; Smith and Weis 1997), gender, age and size of an organism can also affect metal accumulation (Canli and Atli 2003; Farkas *et al.* 2003).

2.1.3 Metals in estuarine fish

Fish have previously been successfully used as bioindicators of aquatic contaminants (Hellawell 1986). Metals can be accumulated by fish from the water column, sediment or diet, and further bioaccumulate through the food chain and potentially impact human health (Forstner and Wittmann 1983). Many studies have reported the impacts of metals on humans after consuming contaminated fish flesh (Chan *et al.* 2003; Rowat 1999; Suñer *et al.* 1999). However, little is known about accumulation or regulation of metals in fishes, particularly marine and estuarine species. Metal uptake pathways in fish may include metal exposure through diet (benthic prey items living among contaminated sediments), water (direct exposure of metals via the gills and/or drinking) and sediments (ingestion of sediments) (Bervoets and Blust 2003).

Metals in contaminated sediments may persist and impact upon estuaries for decades (Birch and Taylor 2002a; Birch *et al.* 1996). Although water quality is slowly improving in Sydney estuaries, aquatic organisms continue to accumulate persistent metal contaminants (Kirby *et al.* 2001c; Peters *et al.* 1999c). The smooth toadfish *Tetractenos glaber)*, although not of commercial value, is a common, site-specific fish that preys on resident benthic infauna and often burrows in the sediment (Booth and Schultz 1999; Kuiter 1997), making the toadfish a suitable bioindicator for the examination of the effects of metals in estuarine fish.

Few studies have determined the influence of gender on metal accumulation in marine/estuarine fish (Peakall and Burger 2003) with findings generally being inconsistent. Two studies have shown no significant difference in metal accumulation between males and females (Gaspic *et al.* 2002; Shen *et al.* 1998), whilst another study has reported that metal accumulation was higher in females relative to males (Al-Yousuf *et al.* 2000).

2.1.4 Experimental objectives

The overall objective of the research described in this chapter was to assess physical and chemical water and sediment parameters, and metal concentrations in sediments and determine their possible influence on metal concentrations in toadfish tissues. The following specific aims were to:

1. Evaluate spatial and temporal physical and chemical water and sediment parameters in two estuaries with minimal metal contamination (Port Hacking River and Cowan Creek) and two estuaries with high metal contamination (Lake Macquarie and Parramatta River);
2. Determine if metal concentrations in toadfish tissues in metal contaminated estuaries differed spatially and temporally from those in reference estuaries;
3. Investigate potential gender differences in toadfish metal uptake; and
4. Determine if toadfish metal concentrations reflect metal concentrations in their environment (sediments).

2.2 MATERIALS AND METHODS

2.2.1 Site selection

Three sites within each of the four estuaries (a total of 12 sites) were selected for investigation. In previous studies, these sites were found to exhibit similar surface water chemistry and sediment properties (Birch *et al.* 1998; Birch and Taylor 1999; Birch 1996, 2000; Hatje *et al.* 2001; Irvine and Birch 1998). Sites at the Parramatta River (Figure 2.1) included Kissing Point wharf in Putney (site 1), Ryde Bridge at Concord (site 2) and Exile Bay in Hen and Chicken Bay at Cabarita (site 3). Sites at Lake Macquarie (Figure 2.2) included Speers Point in Cockle Bay (site 1), Wangi Wangi Bay, adjacent to the Wangi Wangi power station outlet (site 2) and Wyee Bay in Mannering Park (site 3). Sites at the Port Hacking River (Figure 2.3) were Grays Point (site 1), Wants Beach at upper Audley (site 2) and Leg of Mutton Bay (site 3). Sites selected at Cowan Creek (Figure 2.4) were House Boat Bay, near Apple Tree Bay (site 1), Smiths Creek (site 2) and Coal and Candle Creek (site 3).

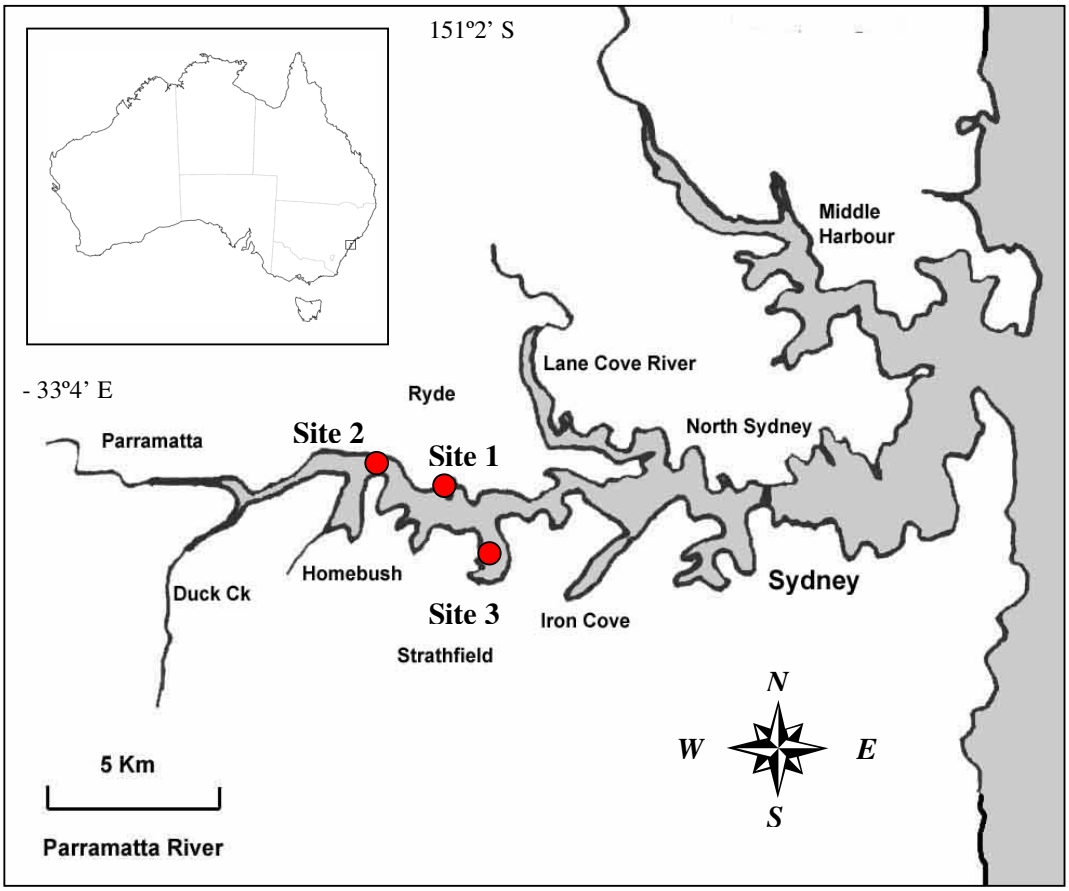

Figure 2.1 Study locations in the Parramatta River. Site 1, Kissing Point wharf (Putney); Site 2, Ryde Bridge (Concord); Site 3, Exile Bay (Cabarita).

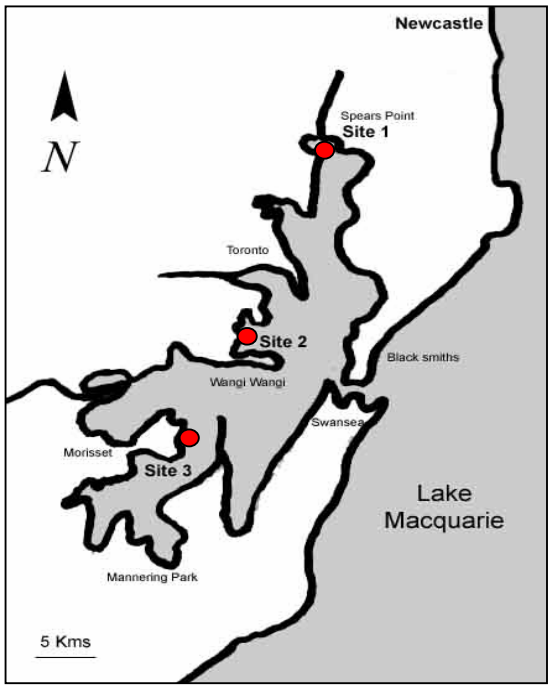

Figure 2.2. Study locations in Lake Macquarie. Site 1, Speers Point (Cockle Bay); Site 2, Wangi Wangi Bay (Wangi Wangi); Site 3, Wyee Bay (Mannering Park).

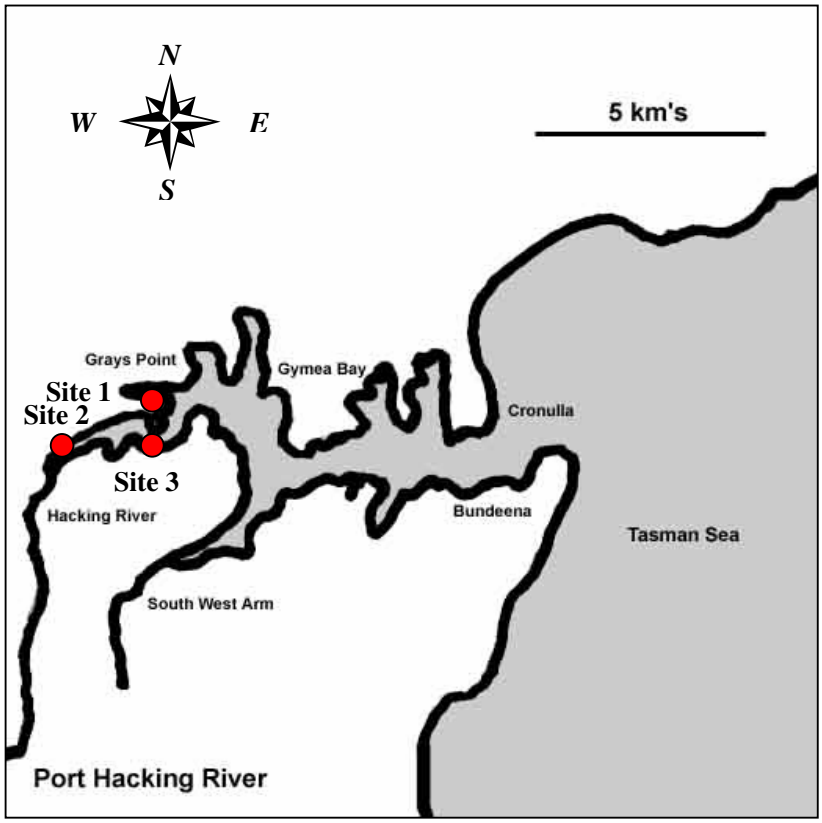

Figure 2.3. Study locations in Port Hacking River. Site 1, Grays Point (Grays Point); Site 2, Wants Beach (upper Audley); Site 3, Leg of Mutton Bay (Gundamaian).

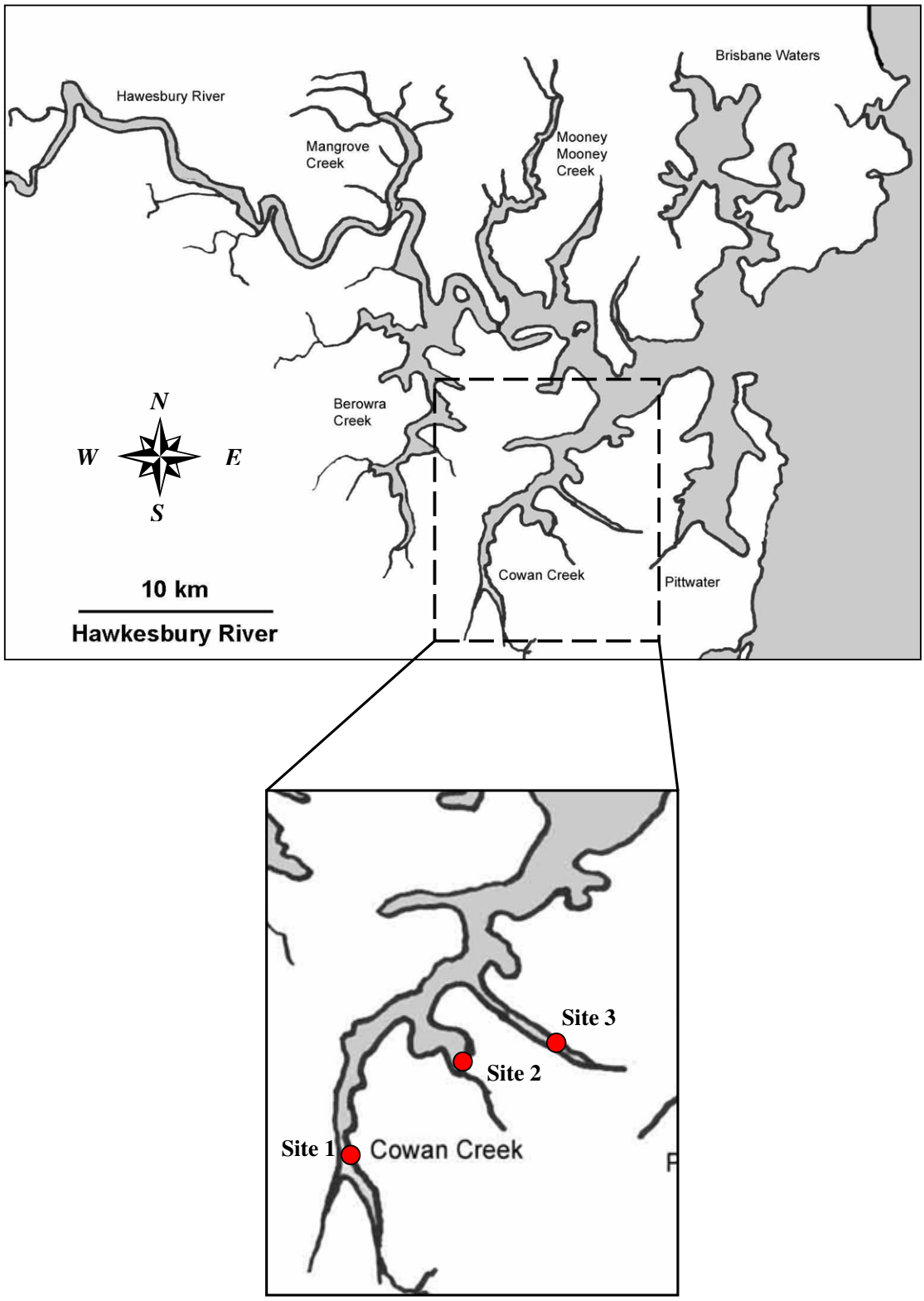

Figure 2.4. Study locations in the Hawkesbury River, insert: Cowan Creek. Site 1, House Boat Bay (Apple Tree Bay); Site 2, Smiths Creek (Cowan Creek); Site 3, Coal and Candle Creek (Cowan Creek).

2.2.2 Sediment and toadfish collection

All sediment sample collection devices and storage containers were acid washed in 5% nitric acid (AnalaR) and rinsed twice with deionsed water (Milli Q; 18MΩ/cm) prior to use. Salinity, conductivity, pH, temperature and dissolved oxygen were measured using a multiprobe water meter (YSI - 650MDS, YSI, Ohio, USA) at 1m below the water surface at each site.

Sampling was undertaken in June (winter), September 2002 (spring) and January 2003 (summer) to determine temporal/seasonal changes in sediment and toadfish metal concentrations. Sediment and fish sampling was conducted at low tide, to ensure sediment samples were representative of fish habitat. At each site, five surface sediment grab samples were randomly collected over an area of 50 m^2 during June and September 2002 and January 2003, placed in polyethylene containers, individually bagged to prevent contamination and transported back to the laboratory on ice for analysis (Birch and Taylor 2000; Mzimela *et al.* 2003).

In the laboratory, sediment samples were oven dried at 40°C to a constant measured weight and sieved to < 2 mm-particle size. The < 2mm particle size (non-gravel) was then used to determine the various sediment parameters. Organic content was measured as % loss on ignition (LOI) using a muffle furnace (Model H3BA Solid State Timer, Omron, Tateisi Electronics Co. Japan) at 550°C for 2 h (Barillé-Boyer *et al.* 2003; Mudroch *et al.* 2000). Sediment slurries in water (1:5 sediment:water) were prepared to measure salinity, determined as electrical conductivity (WTW Cond330i, WTW GmbH and Co. Kg, Germany), and pH (WTW pH330i, WTW GmbH and Co. Kg, Germany), as described by (Rayment and Higginson 1992).

At each site and sampling survey, 6-10 sexually mature toadfish of similar size (101 ± 18 mm,) and weight (27 ± 9 g) were collected using a beach seine net (15.5 x 1.75 m) with a mesh size of 16 mm (Figure 2.5). Fish were euthanased and frozen (-20℃), prior to being thawed and dissected into various tissues (liver, caudle-peduncle muscle, gill and gonad tissue). Fish gender was determined via gonad visual census (males-testis; females-ovaries). Toadfish tissues and sediments were oven dried at 40°C and digested in a sand bath at 109ºC using a hot mixture (2:1) of concentrated nitric acid and hydrogen peroxide, as per the method described by Krishnamurty *et al*. (1976).

Figure 2.5 Beach seine net used to collect toadfish. Dimensions are 15.5 m long x 1.75 m wide with a mesh size of 16 mm

2.2.3 Trace metal analysis

Concentrations of metals (Al, Fe, Zn, As, Cd, Co, Cr, Cu, Ni, Pb & Se) in the digested sediments and fish tissues were determined using either inductive coupled plasma mass spectrometry (ICP-MS, Hewlett Packard 4500) or inductive coupled plasma atomic emission spectrometry (ICP-AES, Varian Vista). Certified reference materials (Dogfish muscle - DORM-2; Dogfish Liver - DOLT-2, National Bureau of Standards, USA) and blanks were routinely included throughout the digestion and analysis protocols to ascertain the reliability of the methods and results. Metal recoveries for the standard reference materials ranged between 94 - 110%. See Appendix I for recovery and standard reference material data.

2.2.4 Data analysis

Differences in physicochemical variables, fish tissue metal concentrations and sediment metal concentrations among sites, estuaries, sampling periods and genders were analysed using one and two-way analysis of variance (ANOVA). Data were also tested for homogeneity of variance and normality, to satisfy the assumptions of ANOVA (Zar 1996). Significant differences were detected at the $P \leq 0.05$ level, however significance levels were reduced to $P \leq 0.01$ where data did not meet the criteria (O'Neill 2000; Underwood 1997). Following a significant difference, Bonferroni's multiple comparisons were performed among groups (Appendix III). Linear regression analysis was used to relate (a) metal concentrations in sediment

with metal concentrations in tissues and (b) metal concentrations in gills with other tissues, across estuaries. Significant regressions were tested at the $P \leq 0.01$ level. Metals associated with sediment particles (geochemical parameters; grain size) may not be bioavailable to organisms, however, metals associated with iron and manganese oxides attached to sediment particles may be readily bioavailable to organisms depending on factors like redox potential or salinity (Luoma and Bryan 1978), therefore, sediment metal regressions with toadfish tissues (Table 2.5) were corrected (eg. Cd / Fe in sediments) for iron sediment concentrations.

2.3 RESULTS

2.3.1 Surface water and sediment chemistry

There were no significant ($P > 0.05$) intra-estuarine (among sites within each estuary) differences in surface water and sediment physical and chemical parameters. Surface water conductivity, salinity and pH also did not differ ($P > 0.05$) significantly among estuaries or sampling periods (Table 2.1), with mean pH ranging from 8.4 to 8.6, mean salinity ranging from 29.3 to 31.7‰ and mean conductivity ranging between 45 to 52 mS/cm^2.

Significant differences ($P \leq 0.05$) were found in water temperatures among estuaries and sampling periods. Mean estuarine water temperatures ranged from 14.9 to 26.3°C, with lowest temperatures exhibited in June 2002 and highest temperatures in January 2003 (Table 2.1).

Dissolved oxygen (DO) showed the highest fluctuation across the estuaries (ranging from 4.5 to 12.4 mg/L) but did not differ significantly ($P > 0.05$) across Cowan Creek, Lake Macquarie or Parramatta River. Dissolved oxygen was significantly ($P \leq 0.05$) lower in the Port Hacking River. Significant ($P \leq 0.05$) differences in dissolved oxygen were also found among the sampling periods for all estuaries with no consistent patterns evident (Table 2.1).

Sediment pH did not differ significantly ($P > 0.05$) across the four estuaries (Table 2.2). However sediment pH varied significantly ($P \leq 0.05$) among sampling seasons within individual estuary, with higher pH rangers generally encounted in the January 2003 sampling period. Mean sediment conductivity and % organic content were both significantly ($P \leq 0.05$) higher in Lake Macquarie for most sampling periods compared to the other three estuaries (Table 2.2). Significant ($P \leq 0.05$) differences in conductivity and % organic matter were also observed for each sampling period within each estuary.

Table 2.1 Physicochemical parameters of surface waters for four estuaries. Values are mean ± standard error, n = 9 for each sampling period.

	Cowan Creek	Port Hacking River	Lake Macquarie	Parramatta River
Turbidity (NTU)				
June 02	1.1 ± 0.2	2.2 ± 1.3	8.5 ± 2.6	25 ± 17
September 02	0.6 ± 0.3	1.0 ± 0.2	15 ± 4.1	24 ± 14
January 03	2.0 ± 1.6	4.8 ± 2.3	7.0 ± 2.1	18 ± 8.6
Temperature (ºC)				
June 02	15 ± 0.7	17 ± 0.4	15 ± 1.2	15 ± 0.9
September 02	17 ± 0.9	17 ± 0.1	19 ± 1.4	18 ± 0.4
January 03	25 ± 0.2	22 ± 0.1	26 ± 1.9	25 ± 0.3
Conductivity (mS/cm)				
June 02	49 ± 0.3	48 ± 0.9	47 ± 1.4	45 ± 0.2
September 02	50 ± 0.1	52 ± 1.0	50 ± 1.4	48 ± 2.7
January 03	46 ± 7.8	54 ± 0.1	51 ± 4.8	52 ± 1.3
Salinity (‰)				
June 02	31.6 ± 0.2	31.7 ± 0.6	30.8 ± 0.9	29.4 ± 0.2
September 02	32.6 ± 0.1	33.9 ± 0.8	32.0 ± 1.0	31.7 ± 1.8
January 03	29.8 ± 5.6	35.5 ± 0.1	33.7 ± 3.5	34.3 ± 0.9
Dissolved oxygen (mg/L)				
June 02	12 ± 0.8	4.5 ± 1.9	9.3 ± 1.0	9.4 ± 4.2
September 02	11 ± 0.5	15 ± 1.2	12 ± 0.7	15 ± 3.3
January 03	4.8 ± 2.9	7.9 ± 0.4	9.9 ± 1.1	9.5 ± 4.5
pH				
June 02	8.5 ± 0.1	8.5 ± 0.1	8.6 ± 0.1	8.5 ± 0.1
September 02	8.6 ± 0.1	8.6 ± 0.1	8.8 ± 0.1	8.4 ± 0.1
January 03	8.5 ± 0.1	7.1 ± 2.2	8.7 ± 0.1	8.5 ± 0.1

Table 2.2 Physicochemical parameters of surface sediments for four estuaries. Values are mean ± standard error, n = 15 for each sampling period.

	Cowan Creek	Port Hacking River	Lake Macquarie	Parramatta River
pH				
June 02	7.2 ± 0.3	7.6 ± 0.2	6.9 ± 0.1	7.5 ± 0.1
September 02	7.5 ± 0.1	7.7 ± 0.1	7.5 ± 0.1	7.6 ± 0.1
January 03	8.6 ± 0.3	8.3 ± 0.2	8.0 ± 0.1	7.8 ± 0.5
Conductivity (mS/cm)				
June 02	4.7 ± 0.6	5.5 ± 0.5	8.2 ± 1.6	5.8 ± 1.1
September 02	6.7 ± 0.5	4.7 ± 0.4	11 ± 1.5	8.5 ± 1.1
January 03	5.6 ± 0.1	6.8 ± 2.2	5.7 ± 1.1	4.2 ± 0.5
Organic content (%LOI)				
June 02	1.8 ± 0.1	1.6 ± 0.3	4.2 ± 1.4	1.6 ± 0.2
September 02	3.8 ± 1.3	1.7 ± 0.1	4.2 ± 0.2	2.7 ± 0.1
January 03	0.6 ± 0.1	3.9 ± 1.6	3.0 ± 0.3	1.5 ± 0.2

2.3.2 Spatial and temporal sediment metal concentrations

Zinc, Cd, Cr, Cu & Pb sediment concentrations ($\mu g.g^{-1}$dry weight) varied significantly ($P \leq 0.05$) among the estuaries (Table 2.3). During all surveys, Zn, Cd and Pb concentrations were significantly ($P \leq 0.05$) elevated at Lake Macquarie, with Cd over 140-fold higher and Pb over 8-fold higher in Lake Macquarie than in the reference estuaries.

Chromium was significantly higher ($P \leq 0.05$) in the Parramatta River during all surveys with concentrations two-fold higher than other estuaries in June 2002 and up to 4 times higher during the warmer sampling periods (September 2002 and January 2003). During all surveys, sediment Cu and Pb concentrations were significantly ($P < 0.05$) higher in the Parramatta River and Lake Macquarie compared to Cowan Creek and Port Hacking River. Concentrations of As, Co, Ni and Se in sediments did not vary significantly ($P > 0.05$) among the estuaries during the June 2002 sampling period, however significantly ($P \leq 0.05$) elevated concentrations were observed in the latter two sampling periods in all estuaries.

Sediment metal concentrations differed significantly ($P \leq 0.05$) across sampling surveys (Table 2.3). Generally, sediment metal concentrations were significantly ($P \leq 0.05$) higher in September 2002 and January 2003 compared to June 2002. For example, As concentrations were over four-fold higher in sediments from Lake Macquarie and Parramatta River in September 2002 and January 2003 compared to June 2002. Sediment Cr concentrations were three-fold higher in the Parramatta River in September 2002 and January 2003 compared with June 2002. In September 2002 and January 2003, sediment Pb concentrations were also over twice as high in Lake Macquarie and Parramatta River compared to June 2002 (Table 2.3).

2.3.4 Spatial and temporal toadfish tissue metal concentrations

Metal concentrations in fish tissues showed no significant differences ($P > 0.05$) among sites within each estuary; therefore data for each estuary were pooled. The estuaries containing elevated concentrations of sediment metal, as expected, had the highest concentrations of metals in fish tissues. Toadfish from the Parramatta River and Lake Macquarie contained significantly ($P \leq 0.05$) higher concentrations of As, Cd, Co, Pb and Se in liver tissues (Table 2.4) compared to Cowan Creek and Port Hacking River. Lead and Zn gill tissue concentrations were significantly higher (up to 5 times) in toadfish from Lake Macquarie and Parramatta River compared with Cowan Creek and Port Hacking River (Table 2.5). Chromium concentrations varied significantly ($P \leq 0.05$) in toadfish muscle tissues, with

Parramatta River and Lake Macquarie muscle concentrations up to ten-times higher than the reference estuaries (Table 2.6). Nickel and Pb concentrations were also significantly elevated in muscle tissue of toadfish from the Parramatta River and Lake Macquarie. Lead concentrations were significantly higher in toadfish gonads from the metal contaminated estuaries compared to the minimally contaminated estuaries (Table 2.7).

Similarly to the sediment metal concentrations, toadfish tissue metal concentrations differed significantly ($P \leq 0.05$) across estuaries over time. Toadfish tissue metal concentrations were generally higher in most estuaries during the September 2002 and January 2003 sampling periods. For example, Cu muscle tissue concentrations in toadfish were up to two-fold higher in most estuaries in January 2003 compared to June 2002 and September 2003 (Table 2.6). Selenium concentrations in muscle tissues were also higher in September 2002 and January 2003 compared with June 2002 (Table 2.6). Gill Pb tissue concentrations in Lake Macquarie were up to seven-times higher in both September 2002 and January 2003 sampling period compared with June 2002 sampling period (Table 2.5). However, gill Cu tissue concentrations were significantly higher in all estuaries except Lake Macquarie in June 2002 compared to the other two sampling periods (Table 2.5). There were no clear patterns in metal concentrations in toadfish tissues among sampling dates or locations.

Table 2.3 Metal concentrations (µg.g^{-1} dry weight) in sediments among sample dates (June 02, September 02, January 03). Values are means ± SE (n =15).

	June 2002	September 2002	January 2003
Zn			
Cowan Creek	10 ± 0.4	n/a	n/a
Port Hacking	20 ± 10	n/a	n/a
Lake Macquarie	140 ± 70	n/a	n/a
Parramatta River	20 ± 10	n/a	n/a
As			
Cowan Creek	2.1 ± 0.7	1.1 ± 0.1	3.4 ± 0.4
Port Hacking	1.4 ± 0.1	3.1 ± 0.7	n/a
Lake Macquarie	1.2 ± 0.2	7.4 ± 1.2	9.5 ± 2.3
Parramatta River	1.7 ± 0.2	7.9 ±0.9	5.1 ± 0.6
Cd			
Cowan Creek	< 0.001	0.005 ± 0.01	0.006 ± 0.01
Port Hacking	0.04 ± 0.01	0.03 ± 0.01	n/a
Lake Macquarie	5.7 ± 2.2	4.0 ± 1.6	< 0.001
Parramatta River	0.04 ± 0.01	0.1 ± 0.03	1.0 ± 0.8
Co			
Cowan Creek	1.1 ± 0.5	0.2 ± 0.01	2.7 ± 1.1
Port Hacking	0.8 ± 0.3	0.6 ± 0.1	n/a
Lake Macquarie	0.7 ± 0.2	1.1 ± 0.1	3.4 ± 0.7
Parramatta River.	0.6 ± 0.1	1.4 ± 0.1	1.7 ± 0.05
Cr			
Cowan Creek	3.1 ± 1.2	1.6 ± 0.1	1.7 ± 0.05
Port Hacking	2.8 ± 0.6	3.5 ± 0.1	n/a
Lake Macquarie	3.3 ± 0.5	4.1 ± 0.1	11 ± 1.6
Parramatta River	6.0 ± 0.6	18 ± 2.6	18 ± 3.4
Cu			
Cowan Creek	3.3 ± 0.6	2.6 ± 0.3	4.8 ± 0.6
Port Hacking	3.4 ± 1.0	2.6 ± 0.5	n/a
Lake Macquarie	14 ± 6	37 ±22	40 ± 14
Parramatta River	15 ± 4	51 ± 18	37 ± 19
Ni			
Cowan Creek	1.18 ± 0.53	0.55 ± 0.03	1.1 ± 0.5
Port Hacking	1.92 ± 0.69	1.1 ± 0.2	n/a
Lake Macquarie	0.98 ± 0.23	1.3 ± 0.1	3.3 ± 0.3
Parramatta River	1.01 ± 0.16	2.4 ± 0.3	3.5 ± 0.2
Pb			
Cowan Creek	6.8 ± 2.3	3.5 ± 0.3	5.8 ± 2.2
Port Hacking	5.8 ± 1.4	7.0 ± 1.1	n/a
Lake Macquarie	52 ± 26	50 ± 14	18 ± 8
Parramatta River	23 ± 8	40 ± 6	30 ± 5
Se			
Cowan Creek	0.01 ± 0.01	0.05 ± 0.01	0.08 ± 0.01
Port Hacking	< 0.005	0.07 ± 0.01	n/a
Lake Macquarie	< 0.005	0.7 ± 0.1	1.0 ± 0.1
Parramatta River	< 0.005	0.07 ± 0.01	0.2 ± 0.1

Table 2.4 Metal concentrations (µg.g^{-1} dry weight) in toadfish liver tissues among sample dates (June 02, September 02, January 03). Values are means ± SE (n =30).

	June 2002	September 2002	January 2003
Zn			
Cowan Creek	0.15 ± 0.03	0.24 ± 0.05	0.13 ± 0.04
Port Hacking	0.06 ± 0.01	0.16 ± 0.06	0.22 ± 0.05
Lake Macquarie	0.10 ± 0.01	0.26 ± 0.03	0.11 ± 0.02
Parramatta River	0.13 ± 0.02	0.17 ± 0.02	0.16 ± 0.04
As			
Cowan Creek	3.86 ± 0.71	5.6 ± 1.0	3.1 ± 0.7
Port Hacking	2.98 ± 0.41	4.7 ± 1.2	10.9 ± 2.3
Lake Macquarie	4.79 ± 0.48	8.1 ± 0.5	6.4 ± 0.5
Parramatta River	4.76 ± 0.76	6.3 ± 0.8	4.3 ± 0.3
Cd			
Cowan Creek	0.03 ± 0.01	0.1 ± 0.04	0.1 ± 0.06
Port Hacking	< 0.001	0.2 ± 0.1	0.4 ± 0.1
Lake Macquarie	1.18 ± 0.24	1.8 ± 0.3	0.8 ± 0.2
Parramatta River	0.08 ± 0.03	0.3 ± 0.1	0.2 ±0.1
Co			
Cowan Creek	0.27 ± 0.09	0.3 ± 0.1	0.1 ± 0.04
Port Hacking	0.04 ± 0.02	0.3 ± 0.1	0.2 ± 0.07
Lake Macquarie	0.14 ± 0.03	0.2 ± 0.04	0.2 ± 0.08
Parramatta River.	0.56 ± 0.12	0.9 ± 0.1	0.2 ± 0.02
Cr			
Cowan Creek	0.09 ± 0.02	0.3 ± 0.1	0.12 ± 0.03
Port Hacking	0.06 ± 0.04	0.2 ± 0.1	0.27 ± 0.06
Lake Macquarie	0.07 ± 0.01	0.2 ± 0.1	0.14 ± 0.01
Parramatta River	0.15 ± 0.04	0.3 ± 0.03	0.17 ± 0.02
Cu			
Cowan Creek	5.9 ± 1.9	4.9 ± 0.9	4.2 ± 1.3
Port Hacking	4.0 ± 0.5	5.3 ± 0.8	5.3 ± 1.5
Lake Macquarie	10.5 ± 1.4	7.1 ± 0.6	6.7 ± 0.8
Parramatta River	6.9 ± 1.1	6.9 ± 1.3	5.1 ± 0.5
Ni			
Cowan Creek	2.2 ± 0.9	0.3 ± 0.1	1.6 ± 0.6
Port Hacking	0.3 ± 0.3	0.1 ± 0.1	2.2 ± 1.1
Lake Macquarie	1.5 ± 0.5	0.5 ± 0.2	1.2 ± 0.2
Parramatta River	1.5 ± 0.8	0.2 ± 0.07	1.8 ± 0.3
Pb			
Cowan Creek	0.7 ± 0.2	0.6 ± 0.1	0.3 ± 0.1
Port Hacking	0.2 ± 0.2	0.5 ± 0.3	0.6 ± 0.3
Lake Macquarie	2.1 ± 0.5	3.0 ± 1.2	1.0 ± 0.5
Parramatta River	2.6 ± 0.7	3.6 ± 0.4	1.3 ± 0.3
Se			
Cowan Creek	0.5 ± 0.2	2.3 ± 0.3	1.6 ± 0.5
Port Hacking	0.4 ± 0.2	1.9 ± 0.5	2.5 ± 0.6
Lake Macquarie	1.1 ± 0.2	3.9 ± 0.2	2.1 ± 0.3
Parramatta River	1.0 ± 0.2	2.6 ± 0.3	1.4 ± 0.2

Table 2.5 Metal concentrations (µg.g^{-1} dry weight) in toadfish gills among sample dates (June 02, September 02, January 03). Values are means ± SE (n =30).

	June 2002	September 2002	January 2003
Zn			
Cowan Creek	0.9 ± 0.1	1.5 ± 0.2	0.5 ± 0.1
Port Hacking	1.04 ± 0.09	0.8 ± 0.1	0.8 ± 0.2
Lake Macquarie	0.6 ± 0.05	0.7 ± 0.1	0.6 ± 0.1
Parramatta River	0.6 ± 0.04	0.7 ± 0.1	0.5 ± 0.1
As			
Cowan Creek	8.6 ± 2.1	6.1 ± 1.3	7 ± 2
Port Hacking	5.87 ± 1.4	12 ± 1	22 ± 3
Lake Macquarie	7.8 ± 0.9	15 ± 2	18 ± 2
Parramatta River	5.4 ± 0.4	6.0 ± 0.4	5.2 ± 0.4
Cd			
Cowan Creek	< 0.001	0.005 ± 0.001	0.02 ± 0.01
Port Hacking	< 0.001	< 0.001	0.3 ± 0.1
Lake Macquarie	0.33 ± 0.09	0.4 ± 0.1	0.2 ± 0.1
Parramatta River	0.01 ± 0.01	< 0.001	0.004 ± 0.001
Co			
Cowan Creek	< 0.001	0.004 ± 0.001	< 0.001
Port Hacking	< 0.001	0.03 ± 0.01	< 0.001
Lake Macquarie	< 0.001	< 0.001	< 0.001
Parramatta River.	0.06 ± 0.02	0.1 ± 0.05	0.007 ± 0.003
Cr			
Cowan Creek	0.6 ± 0.2	0.8 ± 0.2	0.6 ± 0.1
Port Hacking	0.5 ± 0.3	0.1 ± 0.03	0.8 ± 0.2
Lake Macquarie	0.07 ± 0.03	0.7 ± 0.2	0.6 ± 0.2
Parramatta River	2.1 ± 0.5	1.2 ± 0.3	1.7 ± 0.3
Cu			
Cowan Creek	18 ± 3	6.9 ± 0.8	12± 4
Port Hacking	11 ± 1	3.8 ± 1.3	3.7 ± 1.1
Lake Macquarie	4.3 ± 0.6	6.2 ± 1.9	6.6 ± 1.6
Parramatta River	13 ± 2	8.9 ± 1.8	6.1 ± 0.9
Ni			
Cowan Creek	0.5 ± 0.2	0.7 ± 0.3	1.6 ± 1.1
Port Hacking	12 ± 8	BDL	0.6 ± 0.2
Lake Macquarie	1.1 ± 0.5	2.3 ± 1.9	1.2 ± 0.6
Parramatta River	0.6 ± 0.2	0.4 ± 0.1	0.9 ± 0.4
Pb			
Cowan Creek	1.2 ± 0.6	1.0 ± 0.3	0.003 ± 0.002
Port Hacking	1.1 ± 0.6	0.4 ± 0.3	4.6 ± 1.1
Lake Macquarie	3.6 ± 1.3	10.7 ± 4.5	4.1 ± 3.3
Parramatta River	5.7 ± 1.0	7.9 ± 1.2	5.9 ± 1.1
Se			
Cowan Creek	0.3 ± 0.3	4.5 ± 0.6	3.1 ± 0.6
Port Hacking	< 0.01	1.6 ± 0.8	3.1 ± 0.8
Lake Macquarie	1.1 ± 0.4	7.7 ± 1.0	7.0 ± 1.8
Parramatta River	0.4 ± 0.2	3.4 ± 0.2	2.9 ± 0.3

Table 2.6 Metal concentrations (µg.g^{-1} dry weight) in toadfish muscle tissue among sample dates (June 02, September 02, January 03). Values are means ± SE (n =30).

	June 2002	September 2002	January 2003
Zn			
Cowan Creek	0.03 ± 0.01	0.08 ± 0.02	0.09 ± 0.02
Port Hacking	0.02 ± 0.01	0.02 ± 0.01	0.07 ± 0.01
Lake Macquarie	0.04 ± 0.01	0.08 ± 0.03	0.09 ± 0.01
Parramatta River	0.05 ± 0.02	0.07 ± 0.01	0.1 ± 0.01
As			
Cowan Creek	16 ± 3	12 ± 2	8 ± 2
Port Hacking	8.1 ± 3.0	14 ± 1	25 ± 3
Lake Macquarie	27 ± 5	16 ± 2	17 ± 2
Parramatta River	5.2 ± 4.9	6.2 ± 0.5	4.7 ± 0.2
Cd			
Cowan Creek	< 0.001	0.1 ± 0.1	< 0.001
Port Hacking	< 0.001	< 0.001	0.1 ± 0.1
Lake Macquarie	0.3 ± 0.1	0.1 ± 0.1	0.3 ± 0.1
Parramatta River	0.01 ± 0.01	< 0.001	0.6 ± 0.3
Co			
Cowan Creek	< 0.001	0.01 ± 0.01	< 0.001
Port Hacking	< 0.001	0.03 ± 0.02	< 0.001
Lake Macquarie	< 0.001	< 0.001	< 0.001
Parramatta River.	1.3 ± 1.4	< 0.001	0.01 ± 0.01
Cr			
Cowan Creek	0.2 ± 0.1	0.4 ± 0.1	0.6 ± 0.1
Port Hacking	0.1 ± 0.1	0.1 ± 0.1	0.4 ± 0.1
Lake Macquarie	0.1 ± 0.04	0.8 ± 0.1	0.4 ± 0.1
Parramatta River	0.2 ± 0.1	1.0 ± 0.1	0.6 ± 0.1
Cu			
Cowan Creek	3.2 ± 0.9	5.5 ± 1.1	6.2 ± 1.2
Port Hacking	1.9 ± 1.5	2.8 ± 0.7	3.8 ± 0.7
Lake Macquarie	5.0 ± 1.0	3.5 ± 1.3	10 ± 3
Parramatta River	3.0 ± 0.6	2.4 ± 0.5	6.8 ± 0.9
Ni			
Cowan Creek	0.2 ± 0.1	2.6 ± 1.1	1.4 ± 0.3
Port Hacking	0.03 ± 0.03	0.8 ± 0.3	1.0 ± 0.3
Lake Macquarie	1.0 ± 0.4	3.3 ± 1.1	6.1 ± 3.1
Parramatta River	1.3 ± 0.9	2.1 ± 0.7	1.4 ± 0.4
Pb			
Cowan Creek	0.3 ± 0.3	< 0.01	0.03 ± 0.02
Port Hacking	< 0.01	< 0.01	0.4 ± 0.3
Lake Macquarie	1.4 ± 1.2	0.7 ± 0.5	< 0.01
Parramatta River	0.2 ± 0.1	< 0.01	5.4 ± 3.5
Se			
Cowan Creek	< 0.01	2.2 ± 0.2	2.1 ± 0.2
Port Hacking	< 0.01	3.0 ± 0.5	1.7 ± 0.2
Lake Macquarie	< 0.01	5.4 ± 0.4	4.5 ± 0.4
Parramatta River	0.2 ± 0.1	1.7 ± 0.2	2.2 ± 0.2

Table 2.7 Metal concentrations (µg.g^{-1} dry weight) in toadfish gonad tissue among sample dates (June 02, September 02, January 03). Values are means ± SE (n =30).

	June 2002	September 2002	January 2003
Zn			
Cowan Creek	0.1 ± 0.01	0.04 ± 0.01	0.3 ± 0.1
Port Hacking	0.2 ± 0.04	< 0.01	0.3 ± 0.1
Lake Macquarie	0.1 ± 0.01	0.7 ± 0.1	0.4 ± 0.1
Parramatta River	0.1 ± 0.01	0.2 ± 0.1	0.3 ± 0.1
As			
Cowan Creek	12 ± 4	25 ± 8	6.7 ± 1.6
Port Hacking	31 ± 13	5.8 ± 0.3	36 ± 8
Lake Macquarie	44 ± 12	12 ± 2	16 ± 2
Parramatta River	4.8 ± 0.8	3.5 ± 0.2	4.4 ± 1.2
Cd			
Cowan Creek	< 0.001	0.02 ± 0.01	< 0.001
Port Hacking	< 0.001	< 0.001	< 0.001
Lake Macquarie	0.1 ± 0.04	0.2 ± 0.1	5.7 ± 2.8
Parramatta River	< 0.001	< 0.001	0.5 ± 0.4
Co			
Cowan Creek	0.1 ± 0.02	0.04 ±0.02	0.02 ± 0.01
Port Hacking	< 0.001	< 0.001	0.01 ± 0.01
Lake Macquarie	< 0.001	0.03 ± 0.01	< 0.001
Parramatta River.	0.17 ± 0.04	0.2 ± 0.03	0.1 ± 0.05
Cr			
Cowan Creek	0.4 ± 0.1	0.3 ± 0.1	0.3 ± 0.1
Port Hacking	0.5 ± 0.2	0.3 ± 0.3	0.6 ± 0.2
Lake Macquarie	0.4 ± 0.04	0.2 ± 0.1	< 0.01
Parramatta River	0.4 ± 0.04	0.2 ± 0.1	0.3 ± 0.1
Cu			
Cowan Creek	3.8 ± 0.4	2.8 ± 0.4	9.9 ± 4.2
Port Hacking	3.4 ± 1.4	2.2 ± 0.5	3.3 ± 1.4
Lake Macquarie	4.3 ± 0.4	3.6 ± 0.9	20 ± 9
Parramatta River	4.4 ± 0.6	2.6 ± 0.2	6.3 ± 1.9
Ni			
Cowan Creek	1.1 ± 0.7	1.1 ± 0.6	14.7 ± 7.6
Port Hacking	5.7 ± 4.3	1.5 ± 0.1	4.9 ± 2.2
Lake Macquarie	2.1 ± 1.4	4.7 ± 1.9	8.8 ± 5.4
Parramatta River	3.8 ± 2.8	0.5 ± 0.2	5.2 ± 2.9
Pb			
Cowan Creek	0.1 ± 0.1	0.1 ± 0.05	4.1 ± 3.1
Port Hacking	0.1 ± 0.04	0.3 ± 0.1	2.3 ± 2.1
Lake Macquarie	0.3 ± 0.1	0.6 ± 0.3	1.5 ± 1.3
Parramatta River	0.2 ± 0.04	0.2 ± 0.03	1.0 ± 0.5
Se			
Cowan Creek	2.6 ± 0.5	3.6 ± 4.8	3.7 ± 1.4
Port Hacking	1.9 ± 0.7	1.9 ± 0.6	3.9 ± 0.8
Lake Macquarie	4.5 ± 0.4	4.3 ± 0.6	6.2 ± 3.1
Parramatta River	2.9 ± 0.3	2.3 ± 0.2	6.8 ± 1.6

2.3.5 Gender differences in fish tissue metal concentrations

Due to uneven male/female ratios encountered during the September 2002 and January 2003 sampling periods, only toadfish from June 2002 were used to compare metal accumulation among toadfish gender. For a full dataset of metal concentrations in male and female toadfish during the June 2002 sampling period, see Appendix II).

No significant differences ($P > 0.05$) in tissue metal concentrations for female toadfish were found among estuaries, with the exception of liver Cd and Pb (Figure 2.6 & 2.7). Liver Cd was significantly ($P \leq 0.05$) highest in female fish from Lake Macquarie, up to 75-times higher than reference estuaries, with Parramatta River liver Cd concentrations (0.05 µg/g) being also significantly ($P \leq 0.05$) higher than the two reference estuaries (Figure 2.6a). Lead liver concentrations were significantly ($P \leq 0.05$) highest in female fish from the Parramatta River and Lake Macquarie, relative to the reference estuaries (Figure 2.6b).

Concentrations of Fe, Zn, As, Cd and Co in liver varied significantly ($P \leq 0.05$) in male toadfish among the estuaries. Cadmium concentrations were significantly higher (up to 15-times) in liver tissue (Figure 2.6a) of male fish in Lake Macquarie compared to reference estuaries. Male toadfish in Lake Macquarie contained significantly highest concentrations of As (97.6 ± 17.6 $\mu g.g^{-1}$ gonad; 43.55 ± 9.2 $\mu g.g^{-1}$ muscle) and Cd (0.09±0.02 $\mu g.g^{-1}$ gonad; 0.25 ± 0.17 $\mu g.g^{-1}$ muscle). Zinc and Fe concentrations in male livers were significantly highest ($P \leq 0.05$) at Cowan Creek.

Gill metal concentrations in female toadfish exhibited the most variation among the estuaries, with significantly ($P \leq 0.05$) higher metal concentrations in Lake Macquarie and the Parramatta River (Figure 2.6c, d & e), except for Ni and Fe, which were higher in the reference estuaries, Port Hacking & Cowan Creek.

Concentrations of As and Fe in muscle tissue in male toadfish varied significantly ($P \leq 0.05$) among estuaries (Figure 2.6) with As concentrations highest in muscle and gonad tissue at Lake Macquarie (Figures 2.6f & 2.6h), and Fe concentrations significantly ($P \leq 0.05$) higher in male toadfish from the Parramatta River (Figure 2.6g). See Apendix III for multiple comparisons (*Post Hoc* tests).

Male and female gonad tissue exhibited significant differences ($P \leq 0.05$) among estuaries in As, Cr and Se concentrations. Arsenic concentrations were significantly ($P \leq 0.05$) higher in male testis from all estuaries (Figure 2.6h). Chromium concentrations were also significantly ($P \leq 0.05$) higher in male testis in all estuaries (Figure 2.7a), whilst Se concentrations in female ovaries were significantly

higher (up to 1.5 times) than male testis of toadfish in Cowan Creek, Port Hacking River and Lake Macquarie (Figure 2.7b).

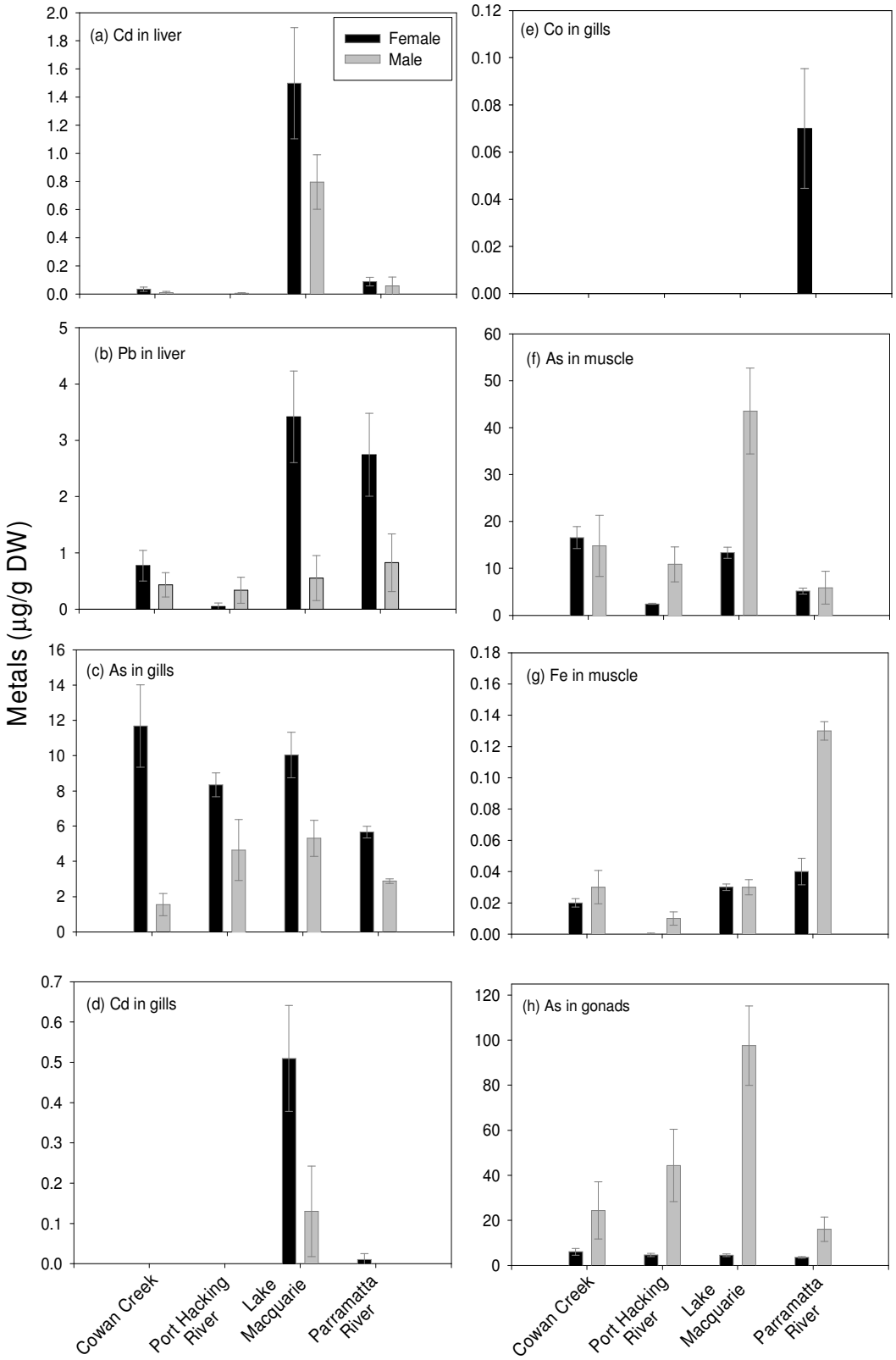

Figure 2.6 Sex differences in metal concentrations in liver, gill, muscle and gonad tissues of toadfish among different estuaries, n = 30.

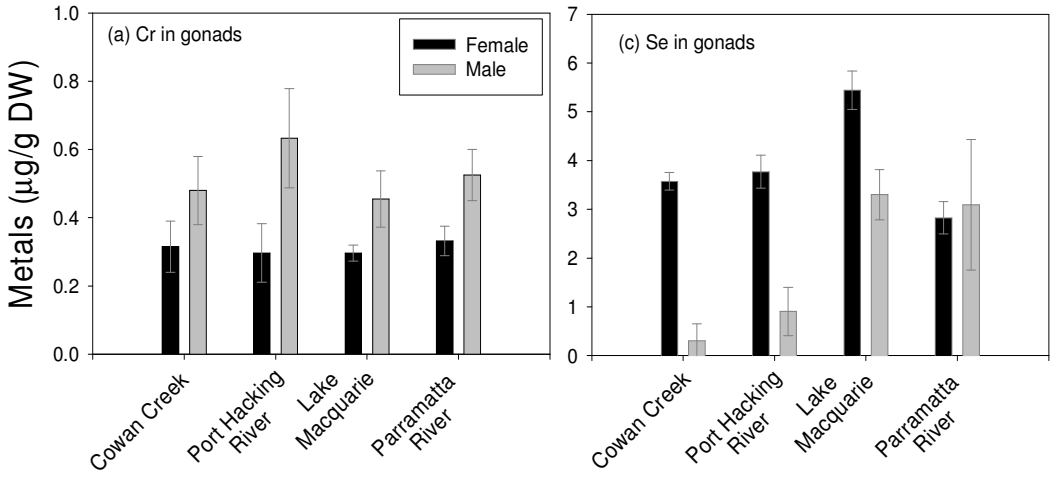

Figure 2.7 Sex differences in metal concentrations of gonad tissues in toadfish among different estuaries, n = 30.

2.3.6 Association between sediment and fish metal concentrations

Cadmium in female liver tissue was positively related to cadmium in sediment (Table 2.5), while male toadfish also exhibited significant positive linear relationships between Cd and Pb in sediment and liver and gonad tissue. Nickel concentrations in sediments were positively related to Ni concentrations in female muscle tissue (Table 2.8).

Table 2.8 Significant linear relationships (r^2; $P \leq 0.01$) between metal concentrations in fish tissues (muscle, liver and gonad) and sediments.

Regressions	Sediment Pb	Sediment Cd	Sediment Ni
Male liver tissue (Pb)	0.29	-	-
Male gonad tissue (Pb)	0.30	-	-
Male gonad tissue (Cd)	-	0.38	-
Female liver tissue (Cd)	-	0.14	-
Female muscle tissue (Ni)	-	-	0.17

Arsenic, Co, Cd and Pb concentrations in the muscle and liver tissue of female toadfish were positively related to those in the gills. Selenium concentrations in male gonad tissues were also positively associated with Se concentrations in gill tissues (Table 2.9).

Table 2.9 Significant linear relationships (r^2; $P \leq 0.01$) between metals in gills and other tissues (muscle, liver and gonad).

Regressions	Gill As	Gill Co	Gill Cd	Gill Pb	Gill Se
As in muscle tissue (females)	0.50	-	-	-	-
Co in liver tissue (females)	-	0.12	-	-	-
Cd in liver tissue (females)	-	-	0.25	-	-
Cd in muscle tissue (females)	-	-	0.20	-	-
Pb in liver tissue (females)	-	-	-	0.17	-
Se in gonad tissues (males)	-	-	-	-	0.17

2.4 DISCUSSION

2.4.1 Estuarine variation in sediment and water parameters

Sites and estuaries were selected with similar physico-chemical water and sediment parameters to minimise variability (Peakall and Burger 2003), however, although there were no significant differences in water and sediment physico chemical parameters, water and sediment parameters varied significantly among sampling dates (Table 2.1 & 2.2). Sediment bound metals and the exchange of metals between sediments and water interfaces can be strongly affected by physico-chemical parameters like redox potential, pH, temperature and organic matter (Hatje *et al.* 2003; Simpson *et al.* 2004; Simpson *et al.* 2002). Furthermore, biological influence, such as bioturbation and bioirrigation by sediment dwelling organisms can potentially have an influence on metal partitioning between the sediments and overlaying waters (Simpson *et al.* 2004; Simpson *et al.* 2002), thus making them more bioavailable to aquatic organism.

Sediment metal concentrations varied significantly among estuaries (Table 2.3). The elevated concentrations of Cd and Pb in sediments from Lake Macquarie may be attributed to a metal smelter on the northern part of Lake Macquarie, as well as power-generating, industrial and commercial activities (Kirby *et al.* 2001c). Lake Macquarie exceeded the Interim Sediment Quality Guideline lower trigger values for

Cd (5.7 µg/g) & Pb (52 µg/g) (ANZECC/ARMCANZ 2000) (Table 1.1). Although Se concentrations were not significantly elevated in Lake Macquarie sediments in June 2002, concentrations significantly higher than the other estuaries were found during September 2002 and January 2003. Elevated Se concentrations in Lake Macquarie have been found previously in other studies, and has been attributed to fly ash emissions from three power stations (Batley 1987; Kirby *et al.* 2001a; Peters *et al.* 1999a). Selenium may be available in the deeper sediments, as well as the examined surface sediments, as well as in benthic infauna (Peters *et al.* 1999b), which comprises the diet of toadfish (Booth and Schultz 1999; Kuiter 1997).

The Parramatta River has been subjected to high metallic inputs from both point and diffuse sources, such as leachate, atmospheric deposition, sewage outlets, urban and industrial inputs (Birch and Taylor 1999; Irvine and Birch 1998), which may account for elevated Co, Cr, Cu and Pb concentrations (Table 2.3) compared with reference estuaries. Studies have shown that elevated metal concentrations in the sediments and overlaying water of the Parramatta River are attributed to continuos suspended particle matter enrichment, thus metals are constantly been recycled to and from sediments and overlaying waters (Hatje *et al.* 2001).

In general, the Port Hacking River and Cowan Creek contained minimal metal concentrations compared to the two metal impacted estuaries. This may be due to the lower amount of urban and industrial development along the foreshores, reducing the impacts of pollutants into the surrounding waters (DLWC 2000), making them useful comparisons to the metal impacted estuaries.

2.4.2 Temporal variation in sediment and toadfish tissue metals

There was significant variation in metal concentrations of sediments and toadfish tissues among sampling surveys. Temporal changes in sediments and toadfish tissue metal concentrations can be influenced by a number of factors, such as metabolic activity, fluctuations in water and sediment chemistry, climate change (rainfall, flood events) and changes in point of discharge or diffusive sources (Daxboeck *et al.* 1982; Playle 1998).

Metal variability over a temporal scale is poorly understood (Eastwood and Couture 2002; Kraemer *et al.* 2006b; Mzimela *et al.* 2003; Zayed *et al.* 1994). In the present study, there were no consistent temporal patterns in all sediment or toadfish metal tissue concentrations. However, similar to the many metals in most of the estuarine sediment, toadfish tissue concentrations were generally elevated during September 2002 and January 2003 in comparison with June 2002 (Table 2.3 - 2.7).

This suggests that metal concentrations in toadfish tissues may be influenced by sediment concentrations. This seasonal pattern may be due to the mobilisation and redistribution of contamination in sediments as a result of increased rainfall and temperature, since September to February are generally wetter and hotter (Table 2.1) months of the year in Sydney compared to June (Lu and Yu 2002). Rainfall in Sydney was up to 2 times higher between September to February than March to August in 2002 (2.9 & 1.5mm average rainfall, respectively. Source: Bureau of Meteorology; http://www.bom.gov.au). Mzimela *et al.* (2003) found that increased metal concentrations in tissues of the Groovy Mullet, *Liza dumerelii*, corresponded with water and sediment concentrations, which appeared to be influenced by increased seasonal freshwater inflow from rainfall. Hatje *et al.* (2003) found that temperature was significantly correlated with elevated sediment metal concentrations in the Parramatta River attributed to changes in oxidation and reduction rates. Seasonal and/or temporal changes in prey item availability may also influence dietary metal uptake in toadfish. A study by Kraemer *et al*. (2006a) suggested that seasonal fluctuations in prey item availability influenced tissue metal concentrations in the Yellow Perch, *Perca flavescens*. Furthermore, changes in metabolic activity linked to reproductive cycles and/or metal regulation mechanisms, such as assimilation efficiencies and ingestion rates, may contribute to seasonal changes in tissue metal concentrations (Kraemer *et al.* 2006b).

2.4.3 Metal differences between toadfish tissue types

Metal concentrations in fish tissues varied significantly among estuaries, with highest concentrations found in toadfish from the contaminated estuaries, the Parramatta River and Lake Macquarie (Tables 2.4 - 2.7). Previous studies have reported similar trends in other fish species but lower tissue metal concentrations at study sites within the Parramatta River and Lake Macquarie (Kirby *et al.* 2001c; Lab 2001). Kirby *et al*. (2001a) found similar concentrations of Cd (0.3 ± 0.3 $\mu g.g^{-1}$ gill; 0.055 ± 0.049 $\mu g.g^{-1}$ muscle) and Se (5 ± 2 $\mu g.g^{-1}$ gill; 5 ± 4 $\mu g.g^{-1}$ muscle) in tissues of female mullet (*Mugil cepalus*) from the same location.

Metal concentrations also differed significantly among toadfish tissue types (Table 2.4 – 2.7). Metal concentrations were highest in gonads followed by muscle, gills and liver tissue. The gonads, liver and kidneys are storage organs and are the final repositories of metals (Bervoets and Blust 2003), which may explain the high metal concentrations in gonad tissue. Previous studies have found high concentrations of metals in the liver (Al-Yousuf *et al.* 2000; Farkas *et al.* 2003). In contrast, the present study found liver metal concentrations were lower than the other

studied organs (Table 2.4). The liver has been reported as the main organ used for metal homeostasis (Heath 1995), and is thought to have the ability to reduce metal toxicity and cellular damage, by binding metals to nuclear proteins, such as metallothioneins (Cherian and Nordberg 1983). Liver tissues contain a large number of parenchymal cells, or hepatocytes, lined with secretory and biosynthetic structures which are used to sequester, transport and/or excrete metals and other contaminants (Heath 1995). Metal concentrations were mainly higher in muscle tissue (Table 2.6) than liver tissue (Table 2.4), which contrasts with the findings of other studies on estuarine fish that reported muscle had some ability to regulate metals (Andres *et al.* 2000; Gaspic *et al.* 2002; Marcovecchio and Moreno 1993).

Other exposure pathways influencing metal uptake and regulation rates in fish are covered in Chapter 6.

2.4.4 Gender differences in toadfish metal accumulation

Tissue metal concentration patterns varied among male and female toadfish (Figures 2.6 & 2.7). Differences in metal concentrations between male and female toadfish could be due to dietary, behavioural, physiological or reproductive/hormonal variation (Heath, 1995), with the latter most likely. Male and female fish have different concentrations of sex steroid hormones including 17β-estradiol (E_2), 11-ketotestoserone (11-KT) and plasma concentrations of vitellogenin (VTG) (Sepúlveda *et al.* 2002). Vitellogenin is important for vitellogenisis in oocyte development (regulated by E_2 and 11-KT ratios), a developmental phase responsible for enormous oocyte growth, where nutritive as well as contaminant products, such as metals, are taken up and stored for future embryo development (Brooks *et al.* 1997). Metals can bind to different sex-linked proteins, such as lipoproteins and phosphoproteins, and lipids by binding to receptor-mediated uptake of VTG present in fish ovaries and sperm. Some metals such as Cd and Pb are lipophilic (Boullemant *et al.* 2004; Stanish and Monbouquette 2000), and may be transported through lipid membranes into the developing sperm or oocytes (Brooks *et al.* 1997), thus resulting in sex differences in metal uptake.

Several studies have found no gender differences in a variety of fish species. Gaspic *et al.* (2002) reported that red mullet and hake exhibited no gender differences in metal bioaccumulation, with Shen *et al.* (1998) also reporting no significant differences in metal tissue concentrations between male and female *Tilapia sp*. However, Al-Yousuf *et al.* (2000) found that metal concentrations in female tissues of *Lethrinus sp*. were higher than male tissue concentrations. Kirby et al. (2001b) reported lower concentrations of Cd (0.04 ± 0.09 $\mu g.g^{-1}$ muscle) in male

mullet compared with male toadfish from Lake Macquarie. In this study, concentrations of metals in the gills of female toadfish were higher than in males (Figure 2.6c, d & e), suggesting that metal uptake rates in females may be higher than in males due to higher metabolic processes (Daxboeck *et al.* 1982; Playle 1998).

2.4.6 Relationships between metals in sediments and toadfish tissues

Previous studies have documented that metal uptake pathways in fish may be direct (water via the gills) or indirect (sediment or diet, via the gut). High concentrations of water metals are often reflected in gill tissues of pelagic fishes (Baudin *et al.* 1994; Kraal *et al.* 1995). Alternatively, high metal concentrations are accumulated through dietary uptake (ingested food or sediment) in benthic fish (Baudin *et al.* 1994; Kraal *et al.* 1995), such as toadfish. Lead, Cd and Ni concentrations in the sediments were positively associated with the same metals in toadfish tissues (liver, gonads and muscle; Table 2.8). Cadmium in female liver tissue was positively associated with Cd in sediment (Table 2.8), which may indicate female toadfish may be accumulating cadmium directly from sediment or indirectly from dietary exposure through the food chain (benthic infauna), however, relationships among sediments and tissues were quite weak (up to 38%) and results should be interpreted with caution. Bervotes & Blust (2003) also found that Cd and Zn concentrations in the tissues of a small benthic fish accumulated via diet, reflected the concentrations of these metals in the sediments. Furthermore, a study by Altinda and Yigit (2005) found elevated levels of Pb in water, sediments and plankton samples corresponing with elevated tissue (gill and muscle) concentrations in mullet, carp and perch from a lake in Turkey. Conversely, a study by Zhou et al. (1998) found no significant relationships in Ni or Cd concentrations among sediments and tissues of the freshwater fish *Telapia* in an inland lake of Hong Kong. In Chapter 4, sediments and toadfish dietary items were investigated to assess the relationship between metals in dietary prey items, sediments and toadfish.

Arsenic, Co, Cd and Pb concentrations in gill tissues of toadfish were positively related to the same metals in other tissues (Table 2.9). Most waterborne toxicants are taken up by the gills of fish and transported to other target organs via blood bound to protein (Roesijadi and Robinson 1994), which may account for the association of metal concentrations in the gills of toadfish with other tissues (Table 2.9). In male toadfish, a positive relationship existed between concentrations of Se in the gills and gonads (Table 2.9), indicating that the mode of Se uptake may be from the water via the gills. The liver and kidney are target organs for the final deposition

of metals, and probably reflect uptake via a combination of dietary and water exposure (Bervoets *et al.* 2001), which may indicate that Se follows more than one pathway simultaneously as selenium was also high liver and gonads. Further biokinetic studies employing radioisotopes are needed to resolve contaminant pathways in toadfish. Therefore, water and dietary exposure pathways in toadfish were investigated using radioisotopes of ^{109}Cd and ^{75}Se to determine probable metal exposure routes (Chapter 6).

There may be other pathways, mechanisms or contaminants (e.g. PAH's, PCB's, pesticides, dioxins and chlorinated hydrocarbons) that may influence metal uptake, which were not investigated in this study. Fish size class was not investigated in this study, however other studies have shown size class differences in metal uptake between juvenile and adult fishes (Canli and Atli 2003; Farkas *et al.* 2003; Peakall and Burger 2003). In the present study, only mature adults were used.

2.5 SUMMARY

Water quality in Sydney estuaries has improved over the last 15 years, however elevated metal concentrations persist in sediments. Sediment metal concentrations varied temporally, with elevated concentrations in September 2002 and January 2003 in comparison with June 2002. Toadfish tissue metal concentrations were also elevated in September 2002 and January 2003 compared to June 2002 (Table 2.3), suggesting that toadfish tissue metal concentrations were to some extent related to sediment concentrations.

This chapter has demonstrated that toadfish accumulate metals from their environment in different tissues, with difference in accumulation patterns by different genders. Metals were also accumulated at different concentrations through various uptake pathways (waterborne contamination, sediment ingestion, metal bioaccumulation through ingested infauna). However, it is difficult to pinpoint which metals were taken up by which mechanism. In Chapter 6, radioisotopes were used to investigate metal uptake and loss kinetics using two separate pathways, dietary and water uptake. The following chapter describes the effects of metals in toadfish by investigating condition and reproductive output and their use as potential bioindicators.

3.0 EFFECTS OF METALS ON CONDITION AND REPRODUCTIVE OUTPUT OF THE SMOOTH TOADFISH

3.1 INTRODUCTION

Condition, a measure of health in fishes, may be influenced by a number of factors including diet and/or chemical contaminants. Reproduction is important for future sustainable fish populations, and survival of the species, and may also be influenced by chemical contaminants. The previous chapter examined tissue metal concentrations in toadfish in the four study estuaries. The research described in this chapter uses the results from the previous chapter to examine the effects of metals on size, condition and reproductive output. Furthermore, toadfish diet was investigated to determine possible links in size and condition among estuaries. Although different size classes were not investigated in the present study, toadfish age or longevity was also determined by otolith analysis and examined in relation to toadfish tissue metal concentrations.

3.1.1 Fish Condition (fitness)

Many studies have been conducted on the human health effects of the consumption of metal contaminated fish flesh (Chan *et al.* 2003; Rowat 1999; Suñer *et al.* 1999). Although there are many studies that have investigated effects of metals on macroinvertebrate and freshwater fish health using field and controlled laboratory experiments (Eastwood and Couture 2002; Farag *et al.* 1999; Farkas *et al.* 2003; Levesque *et al.* 2002; Nahmani and Rossi 2003), few studies have examined the effects on metals on marine teleosts in the field, including effects on condition and reproduction. Condition is a useful indicator of growth and survival, and can be influenced by diet (Booth and Alquezar 2002), reproductive status (Booth and Schultz 1999; Brooks *et al.* 1997; Heath 1995) and chemical pollutants (Forrester *et al.* 2003; Jones and Reynolds 1997; Weis *et al.* 2001). Lipid and protein content in tissues have previously been used as effective measures of condition in fish (Bierkens 2000; Saito *et al.* 1999; Suthers *et al.* 1992).

3.1.2 Fish reproduction

The success of individuals to reproduce can affect the ecology and community structure of an ecosystem (Heath 1995). Reproductive output in fish can

be affected by contaminants at a number of different levels, including the transfer of chemicals from parents to eggs, thus leading to reduced embryonic development (Miller 1993), direct uptake from the environment to eggs and physiological effects on newly born hatchlings (Von Westernhagen 1988). Contaminants may lead to yolk sac deformities, eye deformations, jaw anomalies, fin defects and malformations of the vertebral column and thus lead to reduced survivorship (Rosenthal and Alderdice 1976).

3.1.3 Otoliths

Otoliths, or ear stones, are balance organs located in the ear canals of the cranial cavities of all teleost fishes (Swearer *et al.* 2003). Otoliths are made up of calcium carbonate ($CaCO_3$) crystals in the form of aragonite embedded by a collagen based protein matrix (Morales-Nin 2000; Mosegaard and Morales-Nin 2000; Swearer *et al.* 2003). Otolith formation is governed by endogenous circadian rhythms of fish resulting in the deposition of a series of translucent and opaque rings known as growth increments, thus otoliths continually grow throughout the life history of the fish (Morales-Nin 2000). These growth increments or rings have been used in a number of studies as a tool for age estimation and growth of fishes (Butler and Folkvord 2000; Campana 2005; Campana *et al.* 2000; Labropoulou and Papaconstantinou 2000; Mc Dougall 2004; Morales-Nin 2000; Pilling *et al.* 2003; Withell and Wankowski 1988). Although the overall otolith growth rate is governed by metabolic control, there are a number of exogenous environmental cycles, fluctuations or events that influence otolith trace element composition, including changes in light, salinity gradient, temperature, water and food intake (Campana *et al.* 2000; Elsdon and Gillanders 2004).

As well as a tool for age estimation of fish, otoliths have also been used to track migratory patterns in teleosts (Gemperline *et al.* 2002) and assess origins of fish stocks on the basis of population modelling (Campana *et al.* 2000; De Pontual *et al.* 2000; Thresher 1999). Otoliths have also been used to investigate archival information of trace metals exposed throughout the life history of fish (Dove *et al.* 1996; Dove and Kingsford 1998; Milton and Chenery 2001).

3.1.4 Experimental objectives

The overall objective of this chapter was to determine the effects of metals on condition and reproduction of the smooth toadfish from two metal contaminated and

two reference, or minimally contaminated, estuaries in the Sydney region and to determine their use as potential bioindicators of estuarine health.

The specific aims were to:

- Examine toadfish prey items among estuaries;
- Investigate spatial and temporal variation in toadfish condition and reproductive output;
- Examine potential links between metals in toadfish tissues and condition;
- Investigate potential links between metals in fish tissues and reproductive output;
- Determine if metal accumulation has an effect on toadfish age/longevity.

3.2 MATERIALS AND METHODS

Sediment and fish sampling procedures, toadfish biology and metal analyses, are described in detail in Chapter 2. The methods described below detail the biochemical analysis of fish tissues, the examination of reproductive parameters, and the analysis of otoliths.

3.2.1 Size, biochemical analysis and reproductive output

Fish length-weight relationships have been investigated in the past (Bolger and Connelly 1989), however, fish morphology can have an effect on these relationships. Fish with deeper bodies vary in length-weight relationships to fish with more slender (streamline) bodies. Therefore it is important to investigate both length and weight in fishes. Toadfish total length (snout to caudle fin; mm) and wet weight (g) were determined before fish were dissected into the various tissues. Stomach contents were analysed to examine toadfish diet. Total lipids in muscle, liver and gonads tissues were determined using a chloroform: methanol extraction (Mann and Gallager 1985) and measured gravimetrically as a percentage of the fish dry body weight. Total proteins in the same tissues were determined using the Lowry method (Bovine albumin serum method) and expressed as a percentage of total dry body weight of the fish (Mann and Gallager 1985; Peterson 1977). Reproductive output was measured as gonad weight (g) of toadfish, oocyte density (a measure of fecundity; number of eggs per gram of ovaries) and oocyte diameter (mm).

3.2.2 Otolith analysis

Otoliths (fish earstones) were extracted, washed in deionised water (Milli Q; 18MΩ cm^{-1}), dried, embedded in crystal bond resin and polished using 200µm

followed by 50µm lapping paper. Otoliths were examined under an Olympus compound microscope (400X magnification) for age determination (Figure 3.1). Incremental rings were counted three times and recounts were performed if more than 10% error occurred between counts.

Age validation has been investigated in fish by correlating ring counts to fish age (Alquezar & Booth, unpublished data). In summary, toadfish were collected from a local site and maintained in the laboratory under controlled conditions (temperature, 18ºC; salinity, 28 ppt; dissolved oxygen, > 90% saturation; 12:12 hour light: dark photoperiod). Toadfish were immersed in a solution of Alizarin Comlexone (AC) and seawater (28ppt salinity) at a concentration of 60 $mg.L^{-1}$ for six hours. Fish were maintained in the laboratory for a further two weeks before they were immersed in another solution of AC and seawater of the same concentration. Following emersion, fish were maintained for a further 5 days before they were euthanased and their otoliths extracted and processed. Otoliths were viewed under a fluorescent microscope (Olympus compound microscope coupled to a fluorescent unit) at 100 x magnification and the rings between the two fluorescent lines (emersion dates with AC) were correlated to the number of days between emersions. For a description on other similar otolith validation techniques, see (Lewis and Mackie 2002; Pease *et al.* 2003; Smith and Walker 2003).

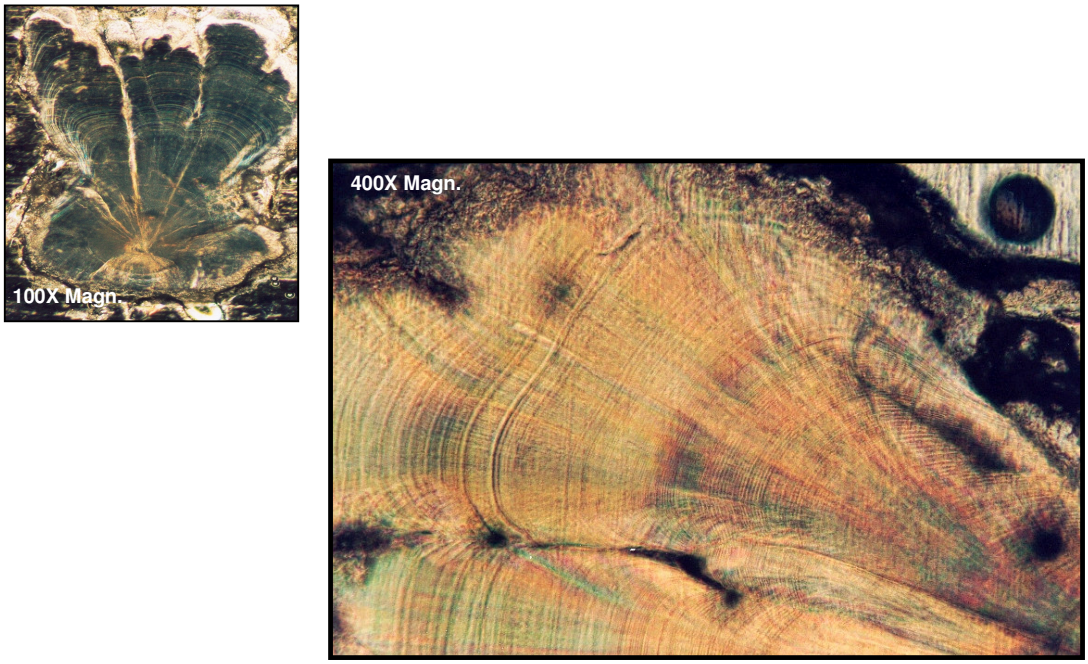

Figure 3.1 Otoliths from a toadfish at 100x and 400x magnification showing growth rings used to determine fish age, under light and dark phase microscopy.

3.2.3 Data analysis

Data analysis was performed using the Minitab statistical package (Ver. 13.1, Minitab Inc, 2002). One-way analysis of variance (ANOVA) was used to determine spatial and temporal differences in protein and lipid tissue concentrations and reproductive output in toadfish among estuaries. Data were tested for homogeneity of variance and normality, to satisfy the assumptions of ANOVA (Zar 1996). Significant differences were detected at the $P \leq 0.05$ level, however significance levels were reduced to $P \leq 0.01$ where data did not meet the criteria (O'Neill 2000; Underwood 1997). Linear regression analysis was used to compare significant ($P \leq 0.01$) metal concentrations in tissues with condition (total proteins and lipids) and reproductive output in toadfish. Chi-squared tests were used to determine differences in frequency of prey items found in toadfish gut contents to determine if toadfish gender, or toadfish from different estuaries, had different dietary preferences. Results were calculated as presence/absence data, and therefore were a qualitative measure of diet.

3.3 RESULTS

3.3.1 Toadfish gut contents

Crustaceans (prawns & semaphore crabs), black mussels and brown algae were the dominant food items in toadfish from all estuaries, comprising an average of 64% of the total food items found in their diet (Figure 3.2). There were no significant differences (chi-squared 12.5, $P > 0.05$) in prey selection between males and female toadfish, however, there were significant differences (chi-squared 28.4, $P \leq 0.05$) in prey types and selection for toadfish among estuaries (Figure 3.3). Twenty one percent of fish sampled had no food items in their guts (Figure 3.2), with the majority of those toadfish being from the Port Hacking River (Table 3.3). Toadfish from Parramatta River and Lake Macquarie consumed mainly prawns (23 & 41%, respectively; Figure 3.3). Black mussels were another common prey item, with the highest frequency (up to 31%) found in toadfish from the Parramatta River. Semaphore crabs were common prey items, with up to 17% found in toadfish from Cowan Creek.

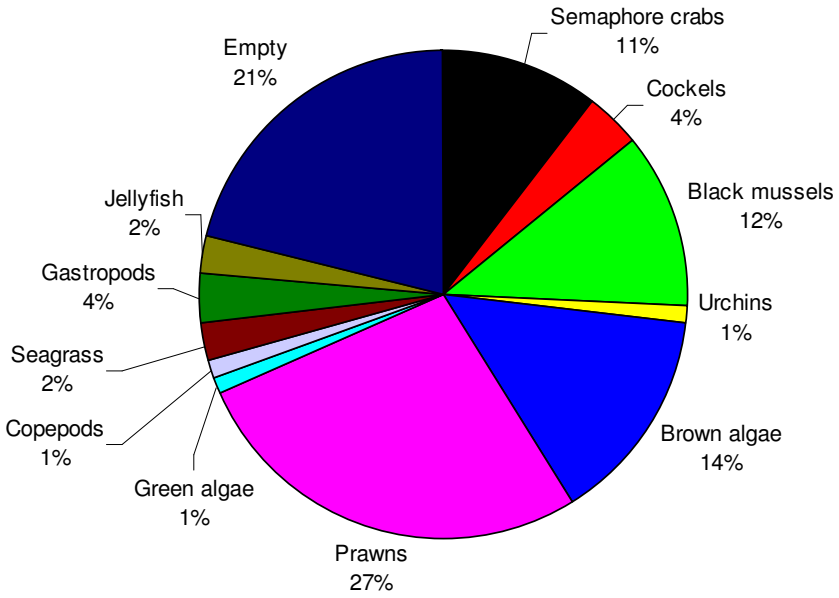

Figure 3.2 Frequency (%) of prey items found in gut contents of toadfish in four estuaries. Prey type calculated as a percentage of all prey items, n = 85.

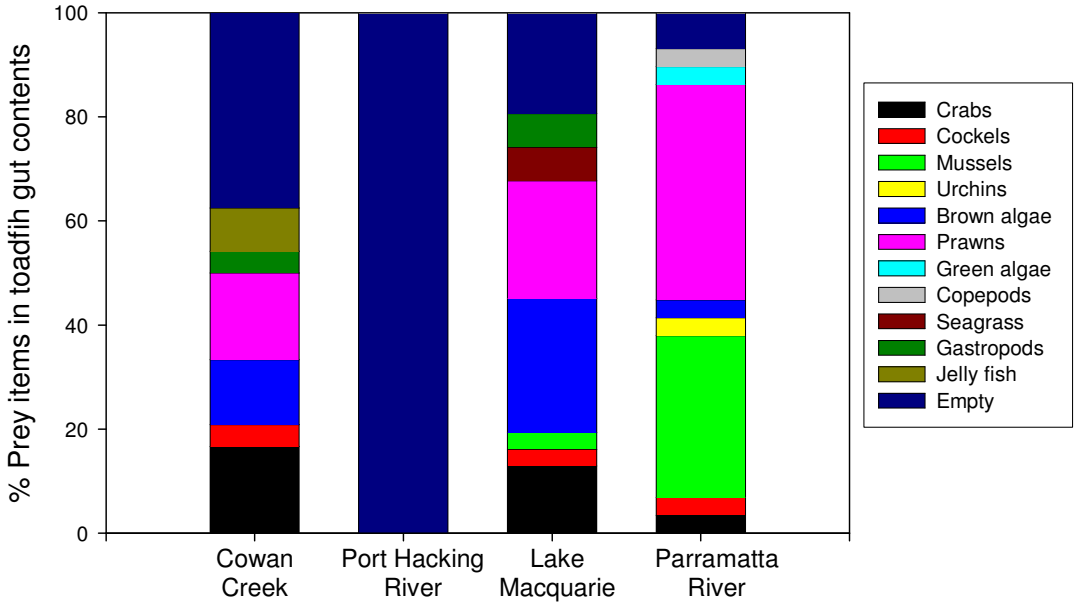

Figure 3.3 Frequency (%) of prey items found in gut contents of toadfish at each estuary. Prey type calculated as a percentage of all prey items, n = 85.

3.3.2 Toadfish condition

Due to inconsistent/uneven numbers of male and female toadfish encountered during the September 2002 and January 2003 sampling periods, only toadfish from June 2002 were used to compare condition and reproductive output among toadfish gender (Tables 3.1 to 3.3).

Female toadfish were significantly ($P \leq 0.05$) larger (wet weight & total length) than male toadfish (Figure 3.4a & b) in all estuaries. Female toadfish in reference estuaries were up to 1.5 times larger than female toadfish in metal contaminated estuaries. There were no significant differences ($P > 0.05$) in length or weight of male toadfish among contaminated or reference estuaries (Figure 3.4a & b). Otolith analysis indicated that both male and female toadfish from the reference estuaries were older (up to four months; $P \leq 0.05$) than those from the metal contaminated estuaries (Figure 3.4c).

Lipids in toadfish liver tissue significantly ($P \leq 0.05$) varied among estuaries and sampling surveys with highest lipid content exhibited in livers of toadfish from the Port Hacking River in June 2002 and Lake Macquarie in January 2003 (Table 3.1). However, there were no significant ($P > 0.05$) spatial or temporal differences in muscle or gonad lipid content of toadfish (Table 3.1). Toadfish from Port Hacking River contained higher liver lipid content than any other estuary (Figure 3.5a). Male toadfish contained a significantly ($P \leq 0.05$) higher percentage of total lipids in liver and muscle tissue than females in Cowan Creek and Parramatta River (Figure 3.5a & b), with female toadfish from Cowan Creek containing the lowest percentage of lipids in muscle tissue. Furthermore, female toadfish contained significantly ($P \leq 0.05$) higher lipid content in toadfish compared to males for all estuaries except the Parramatta River (Figure 3.5c).

Protein content in the liver tissue of toadfish varied significantly ($P < 0.05$) among estuaries and sampling dates (Table 3.1), with the highest protein content found in toadfish from the Parramatta River in September 2002. There were no significant ($P > 0.05$) differences in protein content in the muscle or gonad tissue of toadfish among estuaries or sampling periods (Table 3.1). Total proteins were significantly ($P \leq 0.05$) higher in female toadfish from metal contaminated estuaries, with the highest content in liver and gonad tissue in female toadfish from Parramatta River (Figure 3.5d & f) and muscle tissue in female toadfish from Lake Macquarie

(Figure 3.5e). Protein content in gonads was higher in females than in males for all estuaries, except Port Hacking River (Figure 3.5f).

Significant ($P \leq 0.01$) linear relationships between tissue metal concentrations and toadfish condition were exhibited (Table 3.3). Cobalt concentrations in liver and Co and Pb in gonads, were significantly ($P \leq 0.01$) inversely related to lipid concentrations in female toadfish. Nickel and Co concentrations in liver and muscle tissues were positively ($P \leq 0.01$) associated with protein content in female toadfish (Table 3.3).

Table 3.1 Spatial and temporal toadfish condition (Lipids and proteins; % mg.dry weight) in toadfish tissues (liver, muscle, gonads). Values are means ± SE for each sample survey (n = 20).

Date	Port Hacking River	Cowan Creek	Lake Macquarie	Parramatta River
% Liver Lipids				
June 2002	42 ± 0.8	34 ± 1.3	39 ± 0.6	35 ± 0.9
September 2002	32 ± 5.5	27 ± 2.4	30 ± 1.1	24 ± 0.9
January 2003	34 ± 1.9	39 ± 1.6	43 ± 2.2	38 ± 1.1
% Muscle Lipids				
June 2002	7.4 ± 0.3	6.7 ± 0.2	7.7 ± 0.2	7.1 ± 0.2
September 2002	7.9 ± 0.6	7.8 ± 0.4	8.7 ± 0.3	7.6 ± 0.2
January 2003	7.7 ± 0.4	7.8 ± 0.2	8.7 ± 0.4	8.9 ± 0.5
% Gonad Lipids				
June 2002	15 ± 2.1	15 ± 0.6	16 ± 0.7	17 ± 0.9
September 2002	18 ± 2.6	14 ± 0.8	15 ± 1.0	14 ± 0.8
January 2003	13 ± 2.6	13 ± 1.1	18 ± 1.7	15 ± 1.0
% Liver Proteins				
June 2002	13 ± 1.4	19 ± 2.1	15 ± 1.0	23 ± 1.6
September 2002	28 ± 10.4	32 ± 3.6	31 ± 2.4	40 ± 1.7
January 2003	6.8 ± 0.7	11 ± 1.8	6.3 ± 0.7	9.8 ± 1.4
% Muscle Proteins				
June 2002	68 ± 1.7	63 ± 1.3	74 ± 2.1	69 ± 1.0
September 2002	57 ± 2.2	57 ± 1.7	60 ± 1.2	57 ± 1.4
January 2003	55 ± 0.7	53 ± 1.2	59 ± 1.7	54 ± 1.1
% Muscle Lipids				
June 2002	41 ± 2.7	33 ± 1.9	33 ± 1.0	39 ± 1.7
September 2002	31 ± 9.1	36 ± 1.9	30 ± 2.8	36 ± 0.9
January 2003	51 ± 12.9	63 ± 20.6	36 ± 3.3	40 ± 1.8

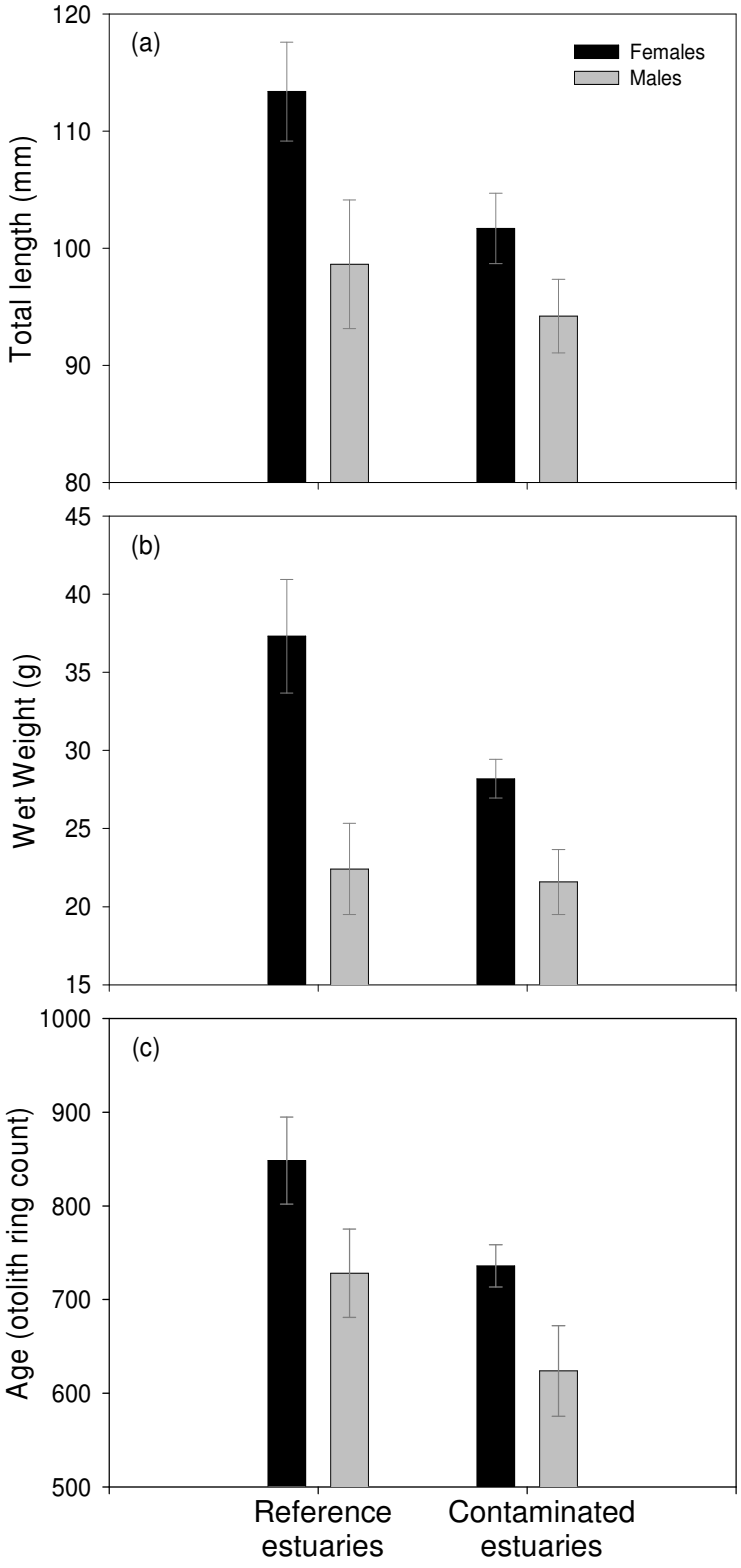

Figure 3.4 (a) Total length (mm), (b) wet weight (g) and (c) age (otolith ring count) of male and female toadfish from reference (Cowan Creek & Port Hacking River) and

metal contaminated (Lake Macquarie & Parramatta River) estuaries. Values are mean ± SE (n = 79).

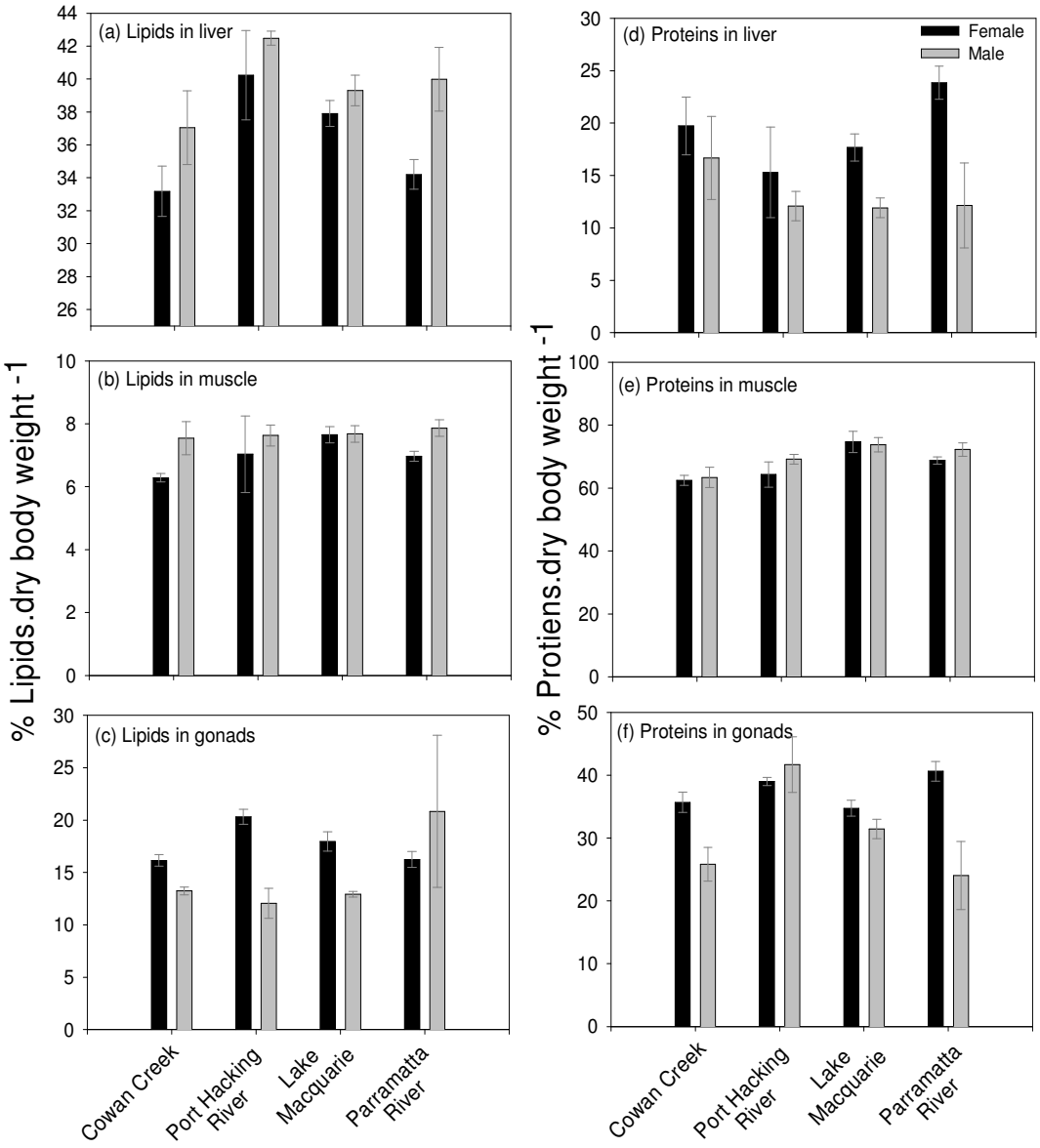

Figure 3.5 Lipid and protein content in liver, muscle and gonad tissue per dry body weight of male and female toadfish in four estuaries. Values are means ± SE (n = 79).

3.3.3 Reproductive output

Ovaries in females (Figure 3.6a) were heavier than testes in males (Figure 3.6b) for all estuaries. Ovaries from females at Cowan Creek were double the weight of those from females at other estuaries (Figure 3.7b).

The total number of oocytes significantly ($P < 0.05$) varied among estuaries and sampling surveys (Table 3.2), with highest numbers of oocytes found in female

toadfish from Cowan Creek in June 2002 and in the Parramatta River in September 2002. Total numbers of oocytes were generally highest during the June and September 2002 sampling period (Table 3.2).

Although no significant ($P > 0.05$) differences in oocyte diameter were found among estuaries, significant ($P \leq 0.05$) differences in mean oocyte diameter were found among sampling surveys (Table 3.2). Oocyte density did not differ significantly ($P > 0.05$) among estuaries or sampling dates (Table 3.2). Average oocyte diameter was similar in toadfish from all estuaries, except the Parramatta River, where toadfish had significantly smaller oocyte diameters (Figure 3.7a).

Oocyte diameter and density was lowest when Pb concentrations were highest. A significant ($P \leq 0.05$) inverse linear relationship was found between Cd and lipid content in male muscle tissue (Table 3.3). No significant ($P > 0.01$) relationships were found between Ni, As, Pb & Se concentrations and protein or lipid concentrations in gonads, liver or muscle tissue

Figure 3.6 (a) ovaries and (b) testis from gravid female and male toadfish.

Table 3.2 Spatial and temporal female toadfish reproductive output. (-) Indicates no females were available. Values are mean ± SE for each sampling period (n = 20).

Date	Port Hacking River	Cowan Creek	Lake Macquarie	Parramatta River
Total oocyte number				
June 2002	4990 ± 1550	5870 ± 1130	3880 ± 394	5050 ± 409
September 2002	2480 ± 991	4080 ± 992	4480 ± 537	6980 ± 785
January 2003	-	2760 ± 1010	186 ± 7	3150 ± 1170
Mean oocyte diameter (mm)				
June 2002	0.7 ± 0.1	0.6 ± 0.1	0.7 ± 0.1	0.5 ± 0.1
September 2002	0.5 ± 0.2	0.8 ± 0.1	0.7 ± 0.1	0.8 ± 0.1
January 2003	-	0.7 ± 0.1	0.9 ± 0.1	0.5 ± 0.1
Oocyte density (eggs/g)				
June 2002	7.4 ± 2.4	4.8 ± 1.4	4.1 ± 0.7	6.4 ± 0.7
September 2002	7.1 ± 2.9	4.2 ± 1.8	6.0 ± 1.3	4.6 ± 0.6
January 2003	-	4.1 ± 1.5	2.4 ± 0.5	5.0 ± 1.1

Table 3.3 Significant linear relationships (r^2) between metal concentrations in male and female tissues and condition. (+) indicates a positive linear relationship; (-) indicates an inverse linear relationship ($\alpha \leq 0.01$).

	Ni	Co	Cd	Pb
Lipids in liver (females)		$r^2 = 0.43$ (-) $p = 0.001$		
Lipids in gonads (females)		$r^2 = 0.46$ (-) $p = 0.000$		$r^2 = 0.27$ (-) $p = 0.048$
Lipids in muscle (males)			$r^2 = 0.38$ (-) $p = 0.050$	
Proteins in liver (females)		$r^2 = 0.50$ (+) $p = 0.000$		
Proteins in muscle (females)	$r^2 = 0.33$ (+) $p = 0.014$			
Oocyte diameter (females)				$r^2 = 0.32$ (-) $p = 0.0$
Oocyte density (females)				$r^2 = 0.26$ (-) $p = 0.049$

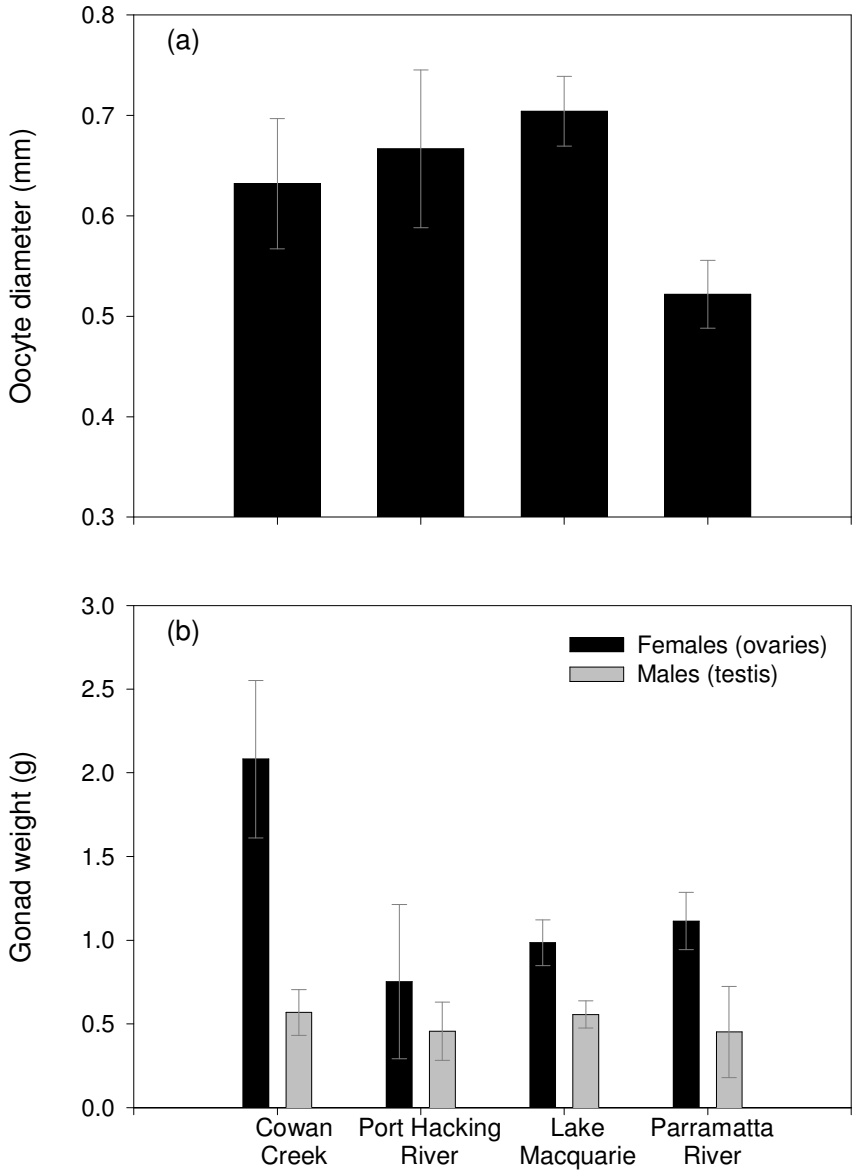

Figure 3.7 (a) Average oocyte diameter (mm) in female toadfish and (b) gonad weight (g) in male and female toadfish in four estuaries. Values are means ± SE (n = 79).

3.4 DISCUSSION

3.4.1 Toadfish growth

Female toadfish were larger (Figure 3.4a) and heavier (Figure 3.4b) than males in both metal contaminated and reference estuaries. Many fish species have shown sexual dimorphism, where fish size is related to gender-based physiological differences rather than the direct or indirect effects of metal contaminants on fish growth (Campbell *et al.* 2003). In the present study, no significant differences were found in male or female toadfish diet, which indicates that diet/prey selection did not account for the observed gender size differences.

Male toadfish size did not vary between metal contaminated and reference estuaries (Figures 3.4a, b). Female toadfish from the metal contaminated estuaries were significantly smaller than females from the reference estuaries (Figure 3.4a, b). This may be due to differences in the nutritional value of the available prey types / selection in the estuaries. Otolith age determination (Figure 3.4c) indicated that the smaller females from the metal contaminated estuaries were also younger, suggesting that survivorship may be reduced in toadfish from metal contaminated estuaries.

Prey items in the gut contents of female toadfish varied between the metal contaminated and reference estuaries, with more prawns and crabs in the gut contents of toadfish in the reference estuaries. Prey items may have different nutritional values, potentially influencing toadfish growth and condition. This may account for the difference in size of female toadfish from the reference and contaminated estuaries. Campbell *et al.* (2003) suggested that reduced growth in fish may be attributed to either metal toxicological effects and/or the lack of availability of preferred prey items. Sherwood *et al*. (2002) reported that yellow perch grew at slower rates and reached smaller sizes in relation to food ration, however food ration was linked to ecotoxicological effects of reduced prey item abundance and biodiversity.

Further research needs to resolve whether (a) the nutritional value of prey items can explain the smaller size and younger age of female toadfish in metal contaminated estuaries, (b) whether this is an indirect effect of the elevated metal concentrations on the abundance and diversity of prey items available to the toadfish or (c) other pollutants such as organics, PCB's, PAH, dioxins and/or pesticides are having an effect on toadfish nutrition, size and reproductive output. A detailed investigation into toadfish diet and nutritional value of prey items is described in Chapter 4.

3.4.2 Effects of metals on condition

There were significant differences in the lipid content of toadfish liver tissues among the reference and metal contaminated estuaries over the three sampling periods, however there were no significant differences in lipid content in toadfish muscle or gonads tissues (Table 3.1). Male toadfish generally showed a higher percentage of lipids in both liver and muscle tissue than female toadfish (Figure 3.5a, b), however female toadfish contained higher lipid content in the gonads (Figure 3.5c), which may be attributed to oocyte development in the gonads (Blaxter and Hempel 1963; Brooks *et al.* 1997).

Condition was associated with several tissue metal concentrations (Table 3.3). Increased tissue concentrations of Co, Cd & Pb corresponded with reduced lipid concentrations in both sexes. Lipids are used for energetic mechanisms associated with growth and survival as well as reproductive development; however, metals that bind to lipophilic molecules may cause toxicological damage (Brooks *et al.* 1997; Weis *et al.* 2001). Metal regulation across cell membranes involves both passive and active mechanisms and therefore requires energy (Connell 1995; Hudson 1998; Newman 1998; Phillips 1995). The main energy source from fish come from lipids (Heath 1995), which may suggest why lipids were lower in fish in estuaries with higher metal concentrations.

The protein content in liver and muscle tissue of female toadfish was significantly higher in the metal contaminated estuaries (Figure 3.5d & e). However, there were no significant differences in protein content in muscle or gonad tissues among the estuaries (Table 3.1). Conversely, although there were no significant differences among estuaries, there were significant differences in muscle and gonad protein content among the sampling dates, which may suggest that toadfish require different levels of proteins during reproductive development (Brooks *et al.* 1997; Heath 1995). In contrast to lipids, increased concentrations of Ni and Co in muscle and liver tissues in female toadfish corresponded with increased protein concentrations. Muscle and liver tissue have some homeostatic control with certain metals. However at elevated metal concentrations, homeostatic capacity is exceeded and direct toxic effects are more prevalent (Rajotte and Couture 2002).

Metabolic turnover rates for metal binding proteins or metallothioneins, may be increased for tissue repair mechanisms and metal regulation (Lam *et al.* 1998; Maracine and Segner 1998; Shen *et al.* 1998). Similar to the results of this study, Eastwood and Couture (2002) reported increased protein metabolism in the liver and muscle tissue of yellow perch exposed to elevated metals concentrations. This may suggest that toadfish are using protein for energy in metal regulation (Newman

1998), and not investing proteins into growth, which may account for the reduced growth rates and survivorship of female toadfish in the metal contaminated estuaries.

3.4.3 Effects of metals on reproductive output

Reproduction is an important process for maintaining the population size and survival of a species (Jones and Reynolds 1997; McHugh and Rouse 1998). Metals, as well as other pollutants, have the ability to reduce fish oocyte quantity and quality, and may result in malformations and impaired development (Brooks *et al.* 1997). Increased Pb and Co concentrations in the ovaries of toadfish were associated with reduced lipid concentrations (Table 3.3). Reductions in fish lipids, proteins and carbohydrates have been shown to affect egg quality and embryo survival (Carrillo *et al.* 1995; Harel *et al.* 1994; Washburn *et al.* 1990). Increased Pb concentrations in the gonads of toadfish also corresponded with a decrease in oocyte diameter and density (Table 3.3). Studies have shown that juvenile fish, hatched from smaller eggs, had a lower survival rate due to smaller yolk sacs, which led to reduced growth rate, and less ability in avoiding predators (Blaxter and Hempel 1963; Hinkley 1990; Moodie *et al.* 1989; Wootton 1994). Reduced lipid concentrations and smaller egg size and density in female toadfish may lead to disadvantages in growth and survival, thus affecting the sustainability of future local populations.

3.5 SUMMARY

Elevated metal concentrations in certain toadfish tissues were linked to reduced lipid concentrations and increased protein concentrations, which may be detrimental for growth, reproductive development and survivorship of the fish. Increased protein concentrations and reduced lipid concentrations may be attributed to tissue repair and metal excretion mechanisms, suggesting that energy, in the form of lipids, is being invested into metal regulation rather than growth. Metals may also have indirect effects on toadfish. Toadfish may be ingesting infauna of lower nutritional value at metal contaminated sites, due to metal toxicity changing infauna abundance and biodiversity. This may result in reduced toadfish growth, and ultimately, reduced survivorship. It is important to examine the effects of metals at different organisational levels to understand the extent of metal impacts in ecosystems. The research described in Chapter 4 investigates links between toadfish diet and nutritional value in toadfish prey items in the Parramatta River.

4.0 ACCUMULATION OF METALS BY SMOOTH TOADFISH FROM SEDIMENT AND INFAUNA: ARE FISH WHAT THEY EAT?

4.1 INTRODUCTION

Metals may have direct and/or indirect effects on aquatic biota at different trophic levels. In the previous chapter (Chapter 3), it was shown that elevated metal concentrations in toadfish tissues were linked to reduced condition and reproductive output. However, it was not known whether this was due to toadfish using energy for metal regulation purposes (direct effect), or toadfish ingesting prey items of lower nutritional value (indirect effect). This chapter investigates the metal concentrations and nutritional value (protein and lipid concentrations) of sediment infauna (toadfish pre items) at four sites in the Paramatta River with varying sediment metal concentration, and how this potentially influences toadfish size/growth.

4.1.1 Macroinvertebrate food webs

Benthic macroinvertebrates are an important food source for higher order predators such as fish, and play a key role in the bioaccumulation and transfer of metal contaminants to higher trophic levels (Peters *et al.* 1999c). Elevated metal concentrations in estuaries may have a direct toxic effect on macroinvertebrates or have an indirect effect on natural community structure by reducing prey item diversity (negative effect) or reducing competition within a species (positive effect), resulting in a trophic cascade (Chapman 2004; Fleeger *et al.* 2003). Elevated metal concentrations have also been linked to increased concentrations of stress proteins and decreased lipid concentrations in benthic macroinvertebrates (Hamer *et al.* 2004; Panfoli *et al.* 2000), thus potentially affecting their nutritional value. Very few studies have reported on whether nutritional value, in the form of protein and lipid concentrations, in infauna influences prey selection by predators (Yearsley 2003). Nutritional value can be an important factor affecting organism growth, health and survival (Booth and Alquezar 2002; Britz and Hecht 1997; Knauer and Southgate 1996).

4.1.2 Contaminant transfer to fish

Fish may be exposed to metals via different pathways, including dietary trophic transfer or water uptake via the gills (Boisson *et al.* 2003; Selck and Forbes

2004; Wang and Fisher 1999). Metals may affect fish directly, via acute toxicity, or indirectly, by reducing condition and/or reproductive output (e.g. gonad weight; Chapter 3), thus potentially altering population dynamics (Heath 1995). Organisms have regulatory, excretory and/or detoxification mechanisms for controlling intracellular metal toxicity, however in certain situations of extreme bioavailability of toxic metals, there has been selection of genetic strains that can tolerate these high metal availabilities by one of/or combination of decreased uptake, increased excretion, increased detoxification (Klerks and Weis 1987). Such organisms may physiologically or genetically adapt to metal contaminated environments by increasing their ability to bind metals to inducible proteins such as cytosolic metallothioneins and/or granule-like structures (Mason and Jenkins 1995) Fish exposed to metal contaminated environments over multiple generations may also develop a genetic-based resistance to metals by coding for cellular physiology and behavioural mechanisms to avoid cellular damage (Belfiore and Anderson 2001; Klerks and Weis 1987).

4.1.3 Experimental objectives

This study examined the metal concentrations and nutritional value (lipid and protein content) of benthic macroinvertebrates consumed by the smooth toadfish at sites with varying metal contamination in the Parramatta River. The specific aims of this study were to:

1) Determine whether toadfish from metal contaminated sites had a reduced selection of dietary items in their gut;
2) Investigate the nutritional value of these dietary items, and whether they differed between taxa and sites;
3) Determine whether toadfish consumed similar proportions of dietary items among the study sites;
4) Investigate if metal concentrations in toadfish were positively associated with infauna and sediment metal concentrations; and
5) Determine whether toadfish size was influenced by metal concentrations and nutritional value in dietary items.

4.2 MATERIALS AND METHODS

4.2.1 Site description

Four study sites in the Parramatta River, southeastern Australia (Figure 4.1), were selected *a priori* with similar surface water physicochemistry and sediment composition (Melville *et al.* 2005), but with varying sediment metal concentrations (Birch *et al.* 1996; Hatje *et al.* 2001; Irvine and Birch 1998). Site 1 was located at Lovett Reserve (low/minimal metal contamination) located in the Lane Cove River, a tributary of the Parramatta River; site 2 was located at the mouth of Duck Creek (low-mid metal contamination), site 3 was situated at the mouth of Charity Creek (mid-high metal contamination) and site 4 was located at the mouth of Haslams Creek (high metal contamination).

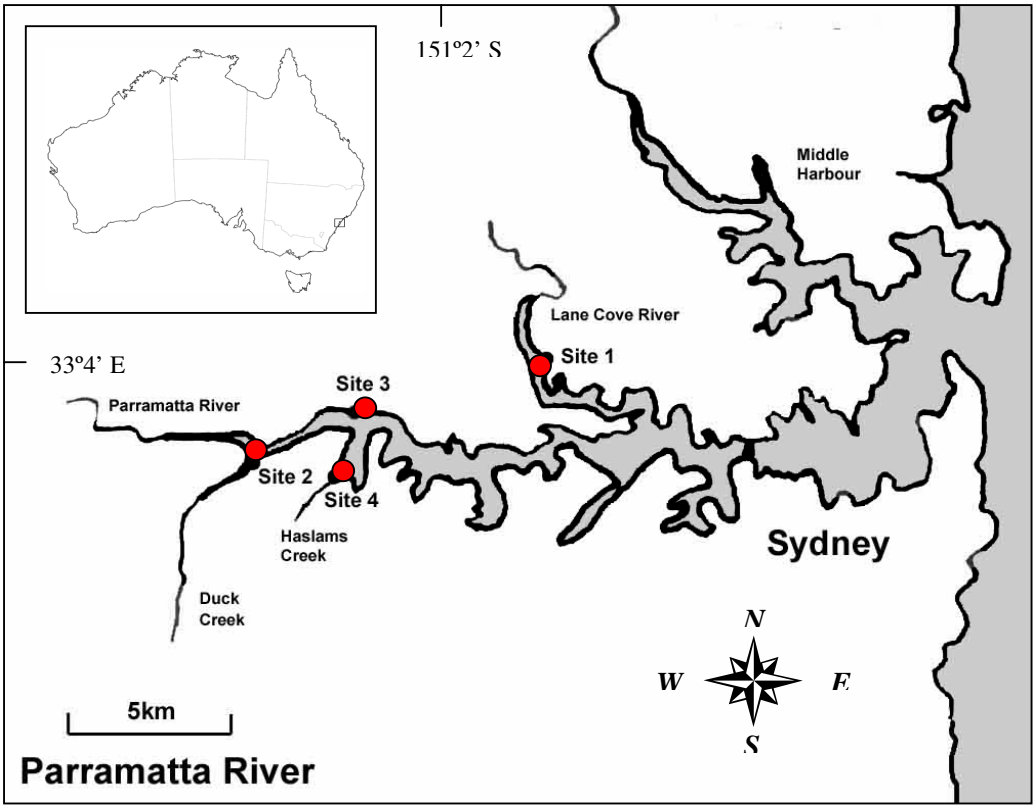

Figure 4.1 Location of study sites in the Parramatta River/Sydney Harbour. Site 1, Lovett Reserve; Site 2, Duck Creek; Site 3, Charity Creek; Site 4, Haslams Creek. Inset: Location of the Parramatta River on the southeast coast of Australia.

4.2.2 Sampling of fish, infauna and sediment

Fish, infauna and sediments were sampled at each site over a 30 x 20 m area in November 2003 at low tide. All samples were kept cool within insulated containers and transported to the laboratory within 6 h of collection and frozen (-20°C). All

sediment sample collection devices and storage containers were washed in 5% nitric acid (AnalaR) and then rinsed twice with deionised water (Milli Q; 18MΩ cm^{-1}).

At each site, 12-15 sexually mature toadfish were collected using a beach seine net (15.5 x 1.75 m) with a mesh size of 16 mm. Based on preliminary toadfish gut content analyses from each site, black mussels (*Xenostrobis securis*), semaphore crabs (*Heloecius cordiformis*) and polychaetes (*Marphysa* sp.) were selected as the main sediment infauna (prey items) consumed by toadfish. Approximately 30 individuals of each species of a predetermined size (semaphore crab carapace width, 13-20 mm; black mussel shell length, 11-16 mm; polychaete diameter ~ 2-3 mm), representative of toadfish gut contents, were collected from the sediment (0-5 cm depth) at each site. From the total pool of each species, 5-6 individuals were randomly selected for metal analyses and the same number for lipid and protein analyses.

At each site, five surface grab samples of sediment (0-5 cm depth) were randomly collected and stored in 500 ml polyethylene containers. After thawing, sediments were air-dried and sieved to determine general sediment composition (gravel, > 2 mm; % sand, 0.1-2 mm; % silt/clay, < 0.1 mm) and organic content (as percentage loss on ignition) using the methods described by (Allen 1989). Metal analyses were performed on the < 2 mm fraction.

4.2.3 Determination of fish size, age and gender

Once toadfish were thawed, standard length (snout to caudle fin; mm) and wet weight (g) were determined for each fish. The age of individual fish was determined using otolith ring counts (Section 3.2.2). Gut contents were removed and analysed to determine differences in prey selection preference (based on number of taxa in gut of each toadfish) at each site.

Fish were dissected into various tissues (liver, caudal-peduncle muscle, gill, gonad, kidney and gut lining) and rinsed in deionised water (Milli Q; 18MΩ/cm), prior to digestion for metal analysis. Fish gender was determined using a gonad visual census (males – testis; females – ovaries). Since the majority (95%) of sampled fish across all sites were female, only females were used for this study. Toadfish were not reproductively active (i.e. oocyte development was minimal) at the time of sampling. Otoliths of toadfish were extracted for determination of toadfish age (Section 3.2.2).

4.2.4 Trace metal analysis

Metal concentrations were determined (see section 2.2.4 for details), in toadfish tissues (liver, caudal-peduncle muscle, gill, gonad, kidney and gut lining) and macroinvertebrates (black mussel, semaphore crabs and polychaetes), from each site. To obtain a representative measure of metal concentrations in toadfish, the gut contents of infauna were not purged or removed.

4.2.5 Lipid and protein analysis

Five-six pool individuals of each macroinvertebrate species, black mussel, semaphore crabs and polychaetes, from each site were analysed for lipid and protein content (Section 3.2.2).

4.2.6 Data analysis

One and two-way analysis of variance was used to investigate differences in (a) protein and lipid percentages among infauna, and (b) metal concentrations in sediments, infauna and toadfish tissues, and (c) toadfish age among sites. Data were tested for homogeneity of variance and normality, to satisfy the assumptions of ANOVA (Zar 1996). Significant differences were detected at the $P \leq 0.05$ level, however significance levels were reduced to $P \leq 0.01$ where data did not meet the criteria (O'Neill 2000; Underwood 1997). Tukey's (HSD) pair wise multiple comparison tests were used to evaluate significant differences among means (Zar 1996). Simple linear regression analyses were used to investigate the relationships between (a) metal concentrations in infauna and sediment, (b) metal concentrations in toadfish and (i) infauna and (ii) sediment, and (c) metal concentrations in infauna and their proteins and lipid concentrations. All multiple comparisons were Bonferroni corrected.

4.3 RESULTS

4.3.1 Toadfish gut content analysis

Black mussels (22%) and semaphore crabs (17%) were the most common benthic infauna taxa present in the gut contents of 55 toadfish, sampled across the four study sites. Polychaetes, cockles, amphipods, prawns and brown algae were found at lower percentages. Toadfish gut also contained sediment (17%), likely to have been ingested when toadfish searched through sediments or when consuming benthic infauna. There were no significant differences ($P > 0.05$) in the number of infauna taxa present in the toadfish gut among the four study sites (Figure 4.2).

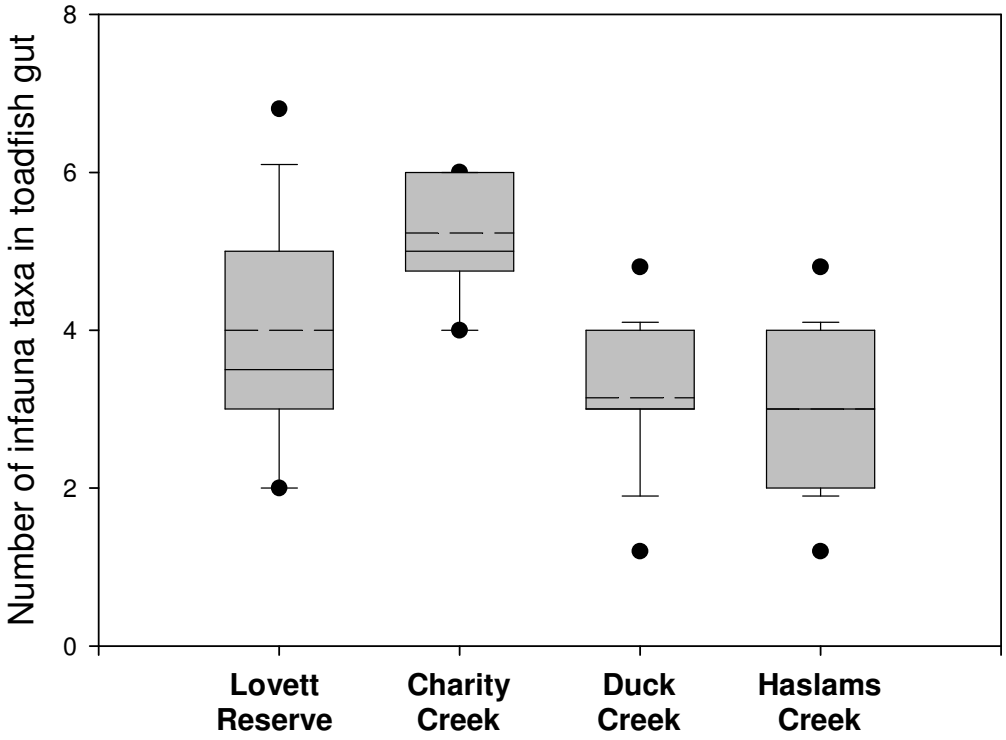

Figure 4.2 Box and whiskers plots showing the number of infauna taxa found within toadfish gut at each study site (n = 12). Boxes indicate interquartile range (50% of data), dashed lines indicate mean values, solid lines indicate median values, error bars represent the range (maximum and minimum) of values and solid dots indicate the 95% confidence interval.

4.3.2 Nutritional value of infauna

There were significant differences ($P \leq 0.05$) in the proteins or lipid content among infauna species, with polychaetes containing the highest protein (up to 6 times more than mussels) and lipid (up to 9 times more than mussels) concentrations, followed by semaphore crabs and black mussels (Table 4.1). In contrast, there were no significant differences ($P > 0.05$) in lipid or protein content in semaphore crabs or black mussels among sites except for polychaetes (Table 4.1), which contained significantly higher ($P \leq 0.05$) levels at the metal contaminated sites (Duck Creek and Haslams Creek; Table 4.1).

Table 4.1. Protein and lipid content (g dry weight^{-1}) of toadfish dietary items at each site. Values are means ± SE (n = 6).

Sites	Semaphore Crab	Black mussel	Polychaetes
Protein			
Lovett Reserve	18.8 ± 1.6	3.8 ± 0.3	19.3 ± 1.6
Charity Creek	17.2 ± 0.9	5.0 ± 0.5	24.1 ± 5.1
Duck Creek	16.9 ± 0.8	5.8 ± 0.9	28.3 ± 3.6
Haslams Creek	17.4 ± 0.4	3.5 ± 1.3	34.4 ± 1.8
Lipids			
Lovett Reserve	7.4 ± 1.2	1.0 ± 0.1	8.1 ± 1.1
Charity Creek	7.0 ± 1.2	1.2 ± 0.1	11.7 ± 2.0
Duck Creek	7.5 ± 1.1	1.0 ± 0.3	10.3 ± 1.3
Haslams Creek	7.5 ± 1.1	1.1 ± 0.3	12.0 ± 1.3

4.3.3 Metal concentrations in sediment and infauna

Sediment composition (% gravel, sand and silt/clay) was not significantly ($P > 0.05$) different among sites, with sand being the dominant fraction (83–88%), followed by silt/clay (9–12%) and gravel (3–6%) (Table 4.2). Sediment metal concentrations at Charity Creek, Duck Creek and Haslams Creek were higher than those from Lovett Reserve, with sediment from Haslams Creek containing the highest metal concentrations (Table 4.2). Arsenic, Cr, Ni and Pb concentrations in Haslams Creek sediments exceeded the low Interim Sediment Quality Guideline value (Table 4.2), while Zn exceeded the high Guideline value (ANZECC/ARMCANZ 2000).

In the toadfish prey items, metal concentrations were generally highest in polychaetes, with lower concentrations at similar levels in semaphore crabs and black mussels (Table 4.3). Overall, infauna metal concentrations (Zn, As, Cd, Co, Cr, Ni & Pb) were lower in Lovett Reserve (Table 4.3; Haslams Creek > Charity Creek ≈ Duck Creek > Lovett Reserve). In all prey items, concentrations of Pb were highest while Zn concentrations were lowest (Table 4.3).

Positive linear relationships were found between sediment and infauna metal concentrations (Table 4.4), indicating that infauna accumulate metals directly from the sediment. Sediment Zn, As, Cd, Co, Cr, Ni and Pb concentrations were positively associated with concentrations in black mussels (r^2 = 0.29-0.87), and polychaetes (r^2 = 0.43-0.87). Sediment Pb (r^2 = 0.30) and Se (r^2 = 0.50) were positively associated with semaphore crab concentrations (Table 4.4).

Zinc, As, Co, Cr & Pb concentrations in infauna were positively and linearly related ($P \leq 0.001$, $n = 60$) to their percentages of lipids ($r^2 = 0.26$-0.73) and proteins ($r^2 = 0.21$-0.59) (Table 4.5). Overall, polychaetes and semaphore crabs contained the highest metal concentrations (Table 4.3), in addition to the highest protein and lipid contents (Table 4.1).

Table 4.2 Sediment metal concentrations (µg.g^{-1}dry weight) at each site. Values are means ± SE (n = 4).

Metals	Lovett Reserve	Charity Creek	Duck Creek	Haslams Creek	ANZECC Sediment Guidelines	
Zn	54 ± 0.4	**150 ± 24**	22 ± 2	**434 ± 6**	[a]2001	[b]4102
As	5.0 ± 0.2	4.0 ± 0.2	**27 ± 0.5**	23 ± 0.8	20	70
Cd	0.06 ± 0.01	0.04 ± 0.01	0.11 ± 0.09	0.98 ± 0.06	1.5	10
Co	1.3 ± 0.1	7.4 ± 0.4	5.1 ± 0.1	12 ± 0.3	-	-
Cr	11 ± 0.3	37 ± 2.5	28 ± 0.7	**155 ± 1.3**	80	370
Ni	2.7 ± 0.4	**21 ± 1.7**	6.0 ± 0.5	**22 ± 0.9**	21	52
Pb	24 ± 0.6	**72 ± 2.9**	6.5 ± 1.6	174 ± 5.9	50	220
Se	0.16 ± 0.03	0.04 ± 0.03	0.52 ± 0.11	0.93 ± 0.03	-	-
% Gravel (> 2 mm) [c]	5 ± 2	3 ± 2	6 ± 2	5 ± 2		
% Sand (0.1 – 2 mm)	83 ± 5	88 ± 7	85 ± 7	85 ± 6		
% Silt (< 0.1 mm)	12 ± 3	9 ± 2	9 ± 3	10 ± 3		
% Organic content	13 ± 4	16 ± 4	15 ± 5	19 ± 5		

[a]Sediment quality guideline value below which there is low probability of biological effect (ANZECC/ARMCANZ 2000). Values shown in bold exceed (or equal) this guideline value (See Table 1.1 for guideline values).
[b]Sediment quality guideline value above which there is high probability of biological effect (ANZECC/ARMCANZ 2000). Values shown in red and bold exceed this guideline value.
[c]Sediment fractions and organic matter were measured as a proportion of total dry sediment weight.

Table 4.3 Metal concentrations (µg.g^{-1}dry weight) in toadfish dietary items at each site. Values are means ± SE (n = 6).

	Zn	*As*	*Cd*	*Co*	*Cr*	*Ni*	*Pb*	*Se*
Semaphore crabs								
Lovett Reserve	0.09 ± 0.003	9.5 ± 0.3	0.18 ± 0.006	0.32 ± 0.03	1.9 ± 0.11	1.2 ± 0.2	7.4 ± 0.5	3.0 ± 0.1
Charity Creek	0.12 ± 0.009	9.0 ± 0.6	0.41 ± 0.077	0.89 ± 0.05	6.3 ± 0.2	1.7 ± 0.1	15 ± 1.0	3.3 ± 0.1
Duck Creek	0.11 ± 0.004	8.4 ± 0.2	0.31 ± 0.032	1.4 ± 0.08	11 ± 1.0	2.0 ± 0.2	13 ± 1.0	3.6 ± 0.1
Haslams Creek	0.11 ± 0.004	12 ± 1.0	0.39 ± 0.037	0.95 ± 0.9	6.3 ± 0.4	1.0 ± 0.2	14 ± 1.0	3.6 ± 0.2
Black mussels								
Lovett Reserve	0.01 ± 0.001	1.0 ± 0.2	0.09 ± 0.02	0.56 ± 0.07	0.36 ± 0.12	2.2 ± 0.24	2.9 ± 0.42	3.1 ± 0.2
Charity Creek	0.02 ± 0.003	0.97 ± 0.15	0.29 ± 0.04	0.51 ± 0.09	1.5 ± 0.39	2.7 ± 0.15	7.6 ± 1.06	3.2 ± 0.1
Duck Creek	0.02 ± 0.002	0.92 ± 0.03	0.19 ± 0.01	0.44 ± 0.05	3.0 ± 0.42	2.7 ± 0.22	6.0 ± 0.43	3.1 ± 0.1
Haslams Creek	0.04 ± 0.005	1.9 ± 0.2	0.31 ± 0.03	1.2 ± 0.1	7.0 ± 0.26	3.3 ± 0.32	16 ± 1.1	2.8 ± 0.1
Polychaetes								
Lovett Reserve	0.09 ± 0.005	19 ± 3.3	0.20 ± 0.1	1.5 ± 0.2	7.5 ± 0.4	3.2 ± 0.6	24 ± 1.8	3.1 ± 0.2
Charity Creek	0.15 ± 0.005	9.4 ± 0.7	0.10 ± 0.1	3.3 ± 0.4	24 ± 2.8	7.3 ± 2.1	36 ± 1.9	3.2 ± 0.5
Duck Creek	0.11 ± 0.004	23 ± 2.3	0.4 ± 0.1	3.4 ± 0.4	18 ± 1.3	4.9 ± 1.0	46 ± 4.9	2.7 ± 0.2
Haslams Creek	0.20 ± 0.011	18 ± 0.9	1.5 ± 0.9	3.7 ± 0.3	34 ± 2.4	10 ± 3.7	91 ± 2.5	3.5 ± 0.3

Table 4.4 Significant (P ≤ 0.01) positive linear relationships (r^2) between metal concentrations (µg.g^{-1}dry weight) in sediment and toadfish dietary items, pooled for all sites (n = 12).

Metal	Semaphore crabs	Black mussels	Polychaetes
Zn	-	0.78	0.87
As	-	0.77	0.43
Cd	-	0.32	0.52
Co	-	0.58	0.67
Cr	-	0.89	0.75
Ni	-	0.32	0.58
Pb	0.3	0.87	0.74
Se	0.5	0.29	-

Table 4.5 Significant (P ≤ 0.01) positive linear relationships (r^2) between metal concentrations (µg.g^{-1}dry weight) and nutritional value (% lipids and proteins g dry weight^{-1}) of toadfish dietary items (infauna), pooled for all sites (n = 60).

Metal	Lipids	Proteins
Zn	0.64	0.55
As	0.73	0.59
Cd	-	-
Co	0.39	0.37
Cr	0.36	0.35
Ni	-	-
Pb	0.26	0.21
Se	-	-

4.3.4 Toadfish diet

Toadfish did not consume prey items in the same proportions at each site (Figure 4.3). Semaphore crabs were the dominant prey item (43-64%) of toadfish at three sites (Lovett Reserve, Duck Creek and Haslams Creek). Polychaetes were the dominant prey item (31%) at Charity Creek, formed a smaller component (7-17%) of toadfish diet at Duck Creek and Haslams Creek (Figure 4.3) and were absent from the toadfish diet at Lovetts Reserve. Black mussels formed a substantial component of the diet (17-31%) at all sites (Figure 4.3).

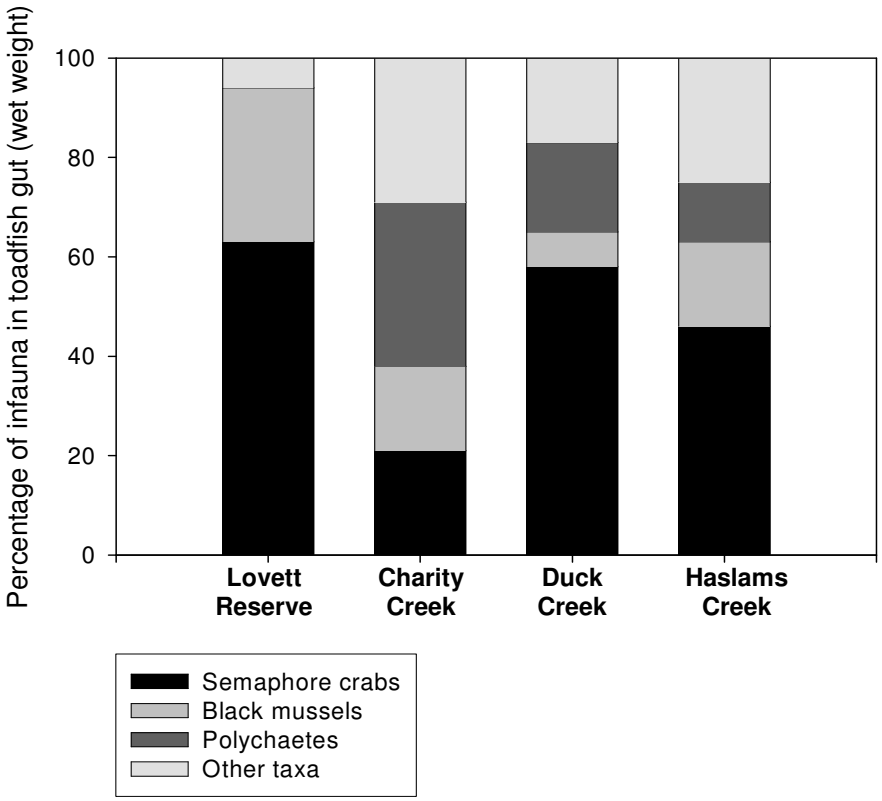

Figure 4.3 Proportion of key infauna, based on wet weight, present in toadfish gut at each site (n = 55).

4.3.6 Metal concentrations in toadfish tissues

Toadfish from Charity Creek, Duck Creek and Haslams Creek contained significantly ($P \leq 0.05$) higher concentrations of Cd, Co, Cr and Pb in their gut linings, livers and gills (Table 4.6) than the toadfish from Lovetts Reserve. Toadfish from the most contaminated site, Haslams Creek, contained higher tissue metal concentrations than toadfish from the least contaminated site, Lovett Reserve (Table 4.6). There were no significant differences ($P > 0.05$) in Zn liver; Zn, As, Cd, or Ni in the gut lining; As, Cd, Co, Cr, Ni or Pb in muscle; Zn, As, Cd, Cr, Ni, Pb and Se in gonads; Cd, Co and Pb in gills among the four study sites. Furthermore, significant linear relationships in metal concentrations were present in toad tissues among prey items and sediments (Table 4.7).

4.3.7 Toadfish size and age

Toadfish from the more contaminated sites, Haslams & Duck Creeks, were larger (~97 mm) and heavier (~33-36 g) than toadfish from the less contaminated sites, Lovett Reserve & Charity Creek (~86 mm, 25-30 g, Figure 4.4). There were no significant ($P > 0.05$) differences in toadfish ages among sites (Figure 4.4c).

Table 4.6 Metal concentrations (µg.g^{-1}dry weight) in toadfish tissues (gut lining, liver, muscle, gonads, kidneys, gills) at each site. Values are means ± SE (n = 12).

	Zn	As	Cd	Co	Cr	Ni	Pb	Se
Gut lining								
Lovett Reserve	230 ± 74	5.1 ± 3.2	0.019 ± 0.07	0.3 ± 0.3	0.7 ± 0.5	2.6 ± 2.1	3.2 ± 2.4	3.1 ± 1.3
Charity Creek	330 ± 171	6.4 ± 1.4	0.011 ± 0.02	0.6 ± 0.6	4.0 ± 3.4	1.3 ± 1.2	6.5 ± 3.8	3.8 ± 0.9
Duck Creek	289 ± 122	4.5 ± 1.1	0.018 ± 0.04	1.1 ± 0.7	3.4 ± 3.5	1.2 ± 1.1	3.7 ± 3.2	3.7 ± 0.6
Haslams Creek	286 ± 94	4.9 ± 1.4	0.070 ± 0.11	1.0 ± 0.5	1.8 ± 1.0	0.2 ± 0.1	3.7 ± 1.2	3.3 ± 0.4
Liver								
Lovett Reserve	184 ± 33	5.3 ± 1.5	0.18 ± 0.15	0.6 ± 0.3	0.09 ± 0.04	0.1 ± 0.1	0.4 ± 0.3	2.8 ± 1.2
Charity Creek	100 ± 16	4.6 ± 1.2	0.19 ± 0.15	0.9 ± 0.9	0.19 ± 0.07	0.1 ± 0.1	1.3 ± 0.5	2.7 ± 1.1
Duck Creek	148 ± 24	3.2 ± 0.8	0.63 ± 0.55	1.7 ± 1.5	0.69 ± 0.17	0.2 ± 0.1	3.9 ± 1.6	3.1 ± 1.8
Haslams Creek	171 ± 29	5.4 ± 2.0	1.1 ± 0.78	4.1 ± 2.8	0.40 ± 0.16	0.6 ± 0.2	5.4 ± 1.9	4.2 ± 1.8
Muscle								
Lovett Reserve	58 ± 19	5.8 ± 3.5	0.003 ± 0.01	0.01 ± 0.01	0.5 ± 0.4	1.0 ± 0.8	0.7 ± 0.5	2.8 ± 0.4
Charity Creek	65 ± 46	6.3 ± 1.3	0.002 ± 0.01	0.01 ± 0.01	0.2 ± 0.6	0.8 ± 0.6	0.5 ± 0.4	1.9 ± 0.3
Duck Creek	46 ± 23	6.6 ± 3.5	0.001 ± 0.01	0.06 ± 0.06	1.0 ± 0.8	0.7 ± 0.5	3.9 ± 3.0	2.4 ± 0.4
Haslams Creek	96 ± 54	7.6 ± 2.9	0.024 ± 0.01	0.07 ± 0.02	0.4 ± 0.3	1.1 ± 0.7	1.6 ± 1.0	2.3 ± 0.3
Gonads								
Lovett Reserve	221 ± 122	5.6 ± 2.4	< 0.001	0.2 ± 0.3	0.4 ± 0.3	2.8 ± 1.3	1.0 ± 0.6	2.0 ± 1.7
Charity Creek	247 ± 122	3.9 ± 2.2	0.003 ± 0.01	0.5 ± 0.4	0.5 ± 0.4	0.5 ± 0.4	0.3 ± 0.2	2.9 ± 0.8
Duck Creek	238 ± 89	4.5 ± 2.5	< 0.001	0.8 ± 0.6	0.5 ± 0.4	1.5 ± 1.2	0.9 ± 0.6	2.3 ± 1.2
Haslams Creek	214 ± 96	7.8 ± 3.8	0.001 ± 0.01	1.8 ± 0.4	0.3 ± 0.2	0.3 ± 0.2	0.4 ± 0.3	2.2 ± 1.5
Kidneys								
Lovett Reserve	8590 ± 2530	6.4 ± 2.4	< 0.01	0.09 ± 0.2	1.4 ± 0.8	5.4 ± 2.0	1.9 ± 1.8	8.4 ± 3.3
Charity Creek	6960 ± 2370	5.0 ± 1.6	< 0.01	0.41 ± 0.5	0.9 ± 0.2	1.4 ± 1.0	0.8 ± 0.3	7.3 ± 2.7
Duck Creek	9340 ± 4100	3.9 ± 1.0	< 0.01	1.00 ± 0.8	1.5 ± 0.5	1.6 ± 1.0	1.7 ± 1.3	10 ± 2.4
Haslams Creek	10,020 ± 2480	5.3 ± 1.5	< 0.01	2.2 ± 0.6	1.6 ± 0.4	1.6 ± 1.6	2.5 ± 1.5	10 ± 2.6
Gills								
Lovett Reserve	519 ± 153	6.3 ± 1.6	< 0.1	< 0.1	2.0 ± 0.5	3.0 ± 1.2	4.5 ± 1.6	3.9 ± 1.1
Charity Creek	353 ± 103	5.2 ± 1.1	< 0.1	< 0.1	1.9 ± 1.2	13 ± 4.0	4.8 ± 2.8	4.6 ± 1.4
Duck Creek	496 ± 167	3.9 ± 1.4	< 0.1	< 0.1	4.9 ± 2.8	1.2 ± 0.6	7.3 ± 5.9	3.9 ± 1.5
Haslams Creek	545 ± 187	5.2 ± 2.0	< 0.1	< 0.1	4.0 ± 2.0	7.3 ± 2.5	6.2 ± 2.5	5.0 ± 2.1

Table 4.7 Significant (P ≤ 0.01) positive linear relationships (r^2) between metal concentrations (μg.g^{-1}dry weight) in toadfish tissues (toadfish tissues were pooled), and their dietary items (infauna) and with sediment, n = 55.

Metal	Semaphore crabs	Black mussels	Polychaetes	Sediment
Zn	-	-	-	-
As	-	-	-	-
Cd	-	-	0.72	0.73
Co	-	0.5	-	0.49
Cr	0.27	-	-	-
Ni	-	-	-	-
Pb	-	-	-	0.32
Se	0.47	-	-	0.33

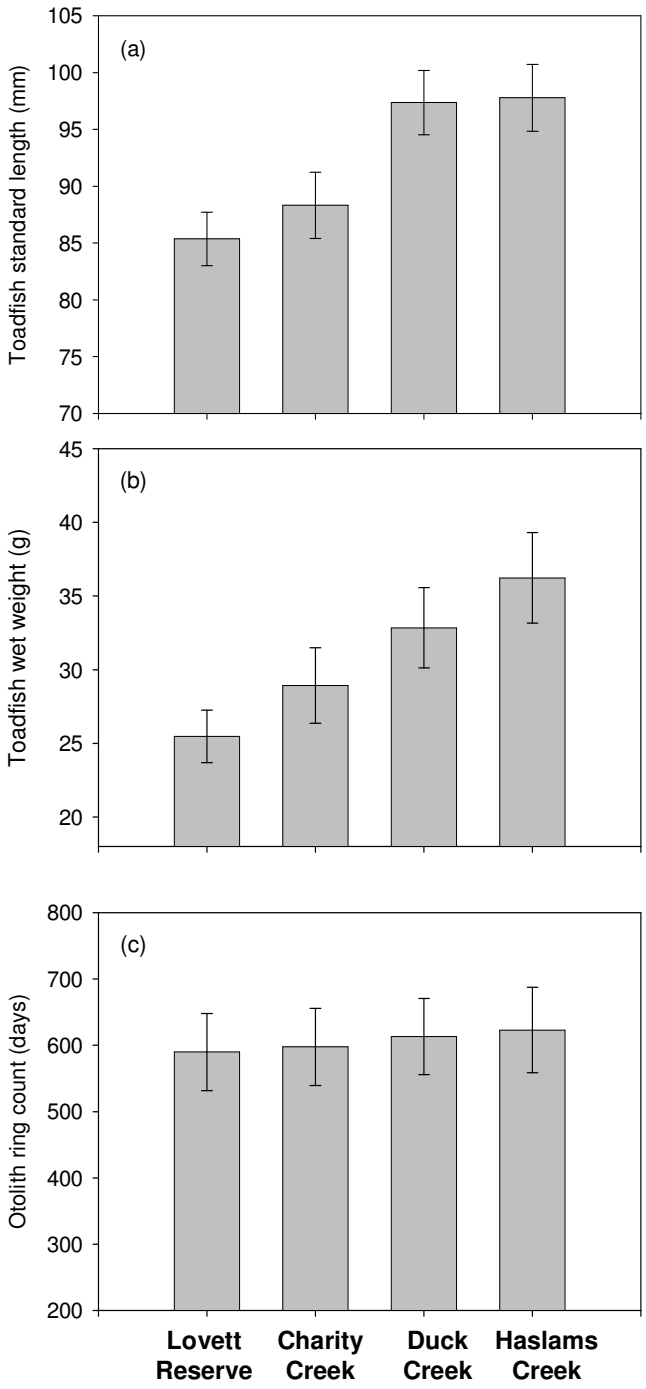

Figure 4.4 (a) Standard length (mm), (b) wet weight (g), and (c) age of toadfish at each site. Values are means ± SE (n = 55).

4.4 DISCUSSION

Although toadfish contained a number of different organisms in their gut, black mussels and semaphore crabs were frequently found. Toadfish from the different sites contained a similar number of organisms in their gut (Figure 4.2) indicating that a variety of prey taxa are available for consumption at each site.

Polychaetes from the most contaminated site, Haslams Creek, contained higher protein content than the polychaetes from all other sites, and higher lipid content than polychaetes from the least contaminated site, Lovetts Reserve (Table 4.1). This finding is in accord with that of Geracitano *et al*. (2004), who found that protein concentrations in polychaetes (*Laeonereis acuta*) from a metal contaminated site were markedly higher than those from a control site.

In contrast, there were no differences in lipid and protein content in semaphore crabs and black mussels from the various study sites. This result is similar to that of Pedersen *et al.* (1997), who reported protein concentrations in the gills of the shore crab, *Carcinus maenus*, did not differ significantly along a metal pollution gradient. However, Hamer *et al*. (2004) found protein concentrations were highest in mussels (*Mytilus galloprovincialis*) from sites with elevated metal concentrations, while Panfoli *et al.* (2000) found that phospholipase activity (which converts phospholipids into fatty acids) was reduced in mussels (*Mytilus galloprovincialis*) from metal contaminated sites.

Metal concentrations in infauna were associated with their nutritional value, or protein and lipid content. Increased concentrations of stress proteins used for control of cell damage, such as heat shock proteins, metallothioneins and multixenobiotic resistance transmembrane proteins, have been attributed to increased ambient metal concentrations (Barsyte *et al.* 1999; Hamer *et al.* 2004; van der Oost *et al.* 2003). These stress proteins have very low molecular weights and probably don't account for a measured effect on total protein levels (Basu *et al.* 2002), thus further controlled laboratory experiments are required to determine if increased stress proteins in fish are attributed to increased total lipid levels due to elevated metal body burden. Although some metals are lipophilic, metals have been reported as inhibiting lipid production, thus negatively impacting lipid content (Panfoli *et al.* 2000), however, this was not the case in the present study. Elevated lipid levels were related to elevated metal levels in infauna (Table 4.5), which may have come from infauna diet, however further studies are required to determine whether elevated metal levels have a true positive effect on lipid content.

Although specific nutrient concentrations in sediments were not determined as part of this study, previous studies have found that nitrogen, phosphorus and sulfur concentrations in surface sediments (0–5 cm) were very similar among several sites in the Parramatta River (including the selected sites in this study) with varying sediment metal concentrations (Melville *et al.* 2004). This study found that organic carbon content in sediments at the four selected sites was not significantly different (Table 4.2). Based on the available data, it is unlikely that differences in sediment nutrient concentrations contributed to the observed increase in the nutritional value (protein and lipid content) in polychaetes at sites with elevated metal concentrations.

Metals concentrations in sediments were positively associated with concentrations in infauna (Table 4.4). These findings are consistent with those from other studies with Peters *et al.* (1999) reporting that Se concentrations in the bivalve, *Spisula Trigonella,* and the polychaete, *Marphysa sanguinea,* were positively associated with sediment concentrations in Lake Macquarie, while Boisson *et al.* (2003) reported that ingestion of sediment was an important metal accumulation pathway in shrimp. In addition, Selck & Forbes (2004) reported that sediment was the main Cd uptake pathway of the deposit feeding polychaete, *Capitella* sp.

Sediment pore water metal concentrations were not investigated in this study. Although sediment pore water may be the most bioavailable fraction to sediment infauna, it may not be the primary exposure route (Chapman *et al.* 2002). Several studies have shown that the main exposure route of sediment dwelling infauna is generally through the direct ingestion of sediments (Forbes *et al.* 1998), or by sediment tube-dwelling organisms actively irrigating/bioturbating their tubes with overlaying water (McCall and Tevesz 1982; Wang *et al.* 2001). There are only a select few types of sediment infauna, such as oligochaete worms, which can tolerate low levels of oxygen and high levels of sulphides that are truly exposed to sediment pore waters (Chapman *et al.* 2002; Warren *et al.* 1998).

Metal concentrations were generally highest in the gills, kidneys and gut lining of toadfish (Table 4.6), although this varied slightly between metals. Metal concentrations were similar to those found in bream in a study by (Farkas *et al.* 2003). However, Canli and Atli (2003) found higher concentrations of Cd, Cr, Cu, Fe, Pb and Zn in muscle, gill and liver tissues were found in six species of Mediterranean fish.

Metal concentrations in toadfish tissues were positively associated with metals in infauna (Table 4.7). Barwick and Maher (2003) investigated the trophic transfer of a range of essential and non-essential metals in seagrass habitats in Lake Macquarie (Australia) and devised potential trophic transfer models through different

food chains (autotrophs, planktivores, herbivores, detritivores, omnivores and carnivores). They concluded that Se to a large extent, and Cu, Cd Zn & Pb to a lesser extent, were transferred and biomagnified to higher trophic concentrations (commercially important fishes) via diet.

Concentrations of Cd, Co, Pb and Se in toadfish tissues were also positively related (r^2 = 0.32-0.73) to those in sediment (Table 4.7). These results were similar to the results from chapter two, where Cd and Pb in sediments were related to toadfish tissue concentrations (section 2.3.6). Peters *et al.* (1999) also found that Se concentrations in the muscle tissues of three benthic-feeding fish species (*Mugil cephalus, Platycephalus fuscus, Acanthopagrus australis*) were positively related (*P* ≤ 0:05) with sediment metal concentrations. Toadfish gut contained mud/sediments (17%), which may explain why certain metals in sediments were positively related to toadfish tissues.

Concentrations of toadfish metals were positively associated with both sediment and prey items, suggesting multiple exposure pathways for toadfish; direct ingestion of sediments and/or ingestion of prey items that may have accumulated metals from sediments. Positive associations between toadfish and sediment metal concentrations were also observed in the study examining all four-study estuaries (Chapter 2), further reinforcing the concept of sediment as the main contaminant substrate of concern to toadfish.

Surface water is another potentially important metal exposure pathway for sediment infauna and toadfish. Although dissolved trace metal concentrations in surface waters were not determined as part of this study, available data (Hatje 2002; Hatje *et al.* 2003) indicates no significant differences in the dissolved concentrations of Cu, Zn, Cd, Mn and Ni amongst the four selected sites. These data reflect a vertically well-mixed estuary (Hatje *et al.* 2001), which is characterised by a low freshwater discharge and tidal turbulence (Revelante and Gilmartin 1978). Despite a lack of available data for some dissolved trace metals (e.g. As, Pb and Se) investigated in this study, it is unlikely that differences in dissolved surface water concentrations amongst sites are able to sufficiently account for the measured patterns of metal tissue concentrations in infauna and toadfish. Quantification of metal accumulation from surface and sediment pore water is ultimately required before ascertaining the importance of sediment as a metal uptake pathway in toadfish and their prey items.

Toadfish, although of similar age at all sites (Figure 4.4c), were 15% larger (Figure 4.4a) and 41% heavier (Figure 4.4b) from the most contaminated sites, thus, toadfish from the most contaminated sites (Haslams Creek and Duck Creek) had

higher growth rates compared to toadfish from the least contaminated sites (Lovett Reserve and Charity Creek). This contrasts with the results gained from the study examining all four estuaries (Chapter 3), where fish in the metal-contaminated estuaries were of a smaller size. Other studies have also reported reduced size of individual fish at metal contaminated sites (Canli and Atli 2003; Farkas *et al.* 2003).

In the sites examined in this chapter, toadfish from all sites were of a similar age (Figure 4.4c), thus it appears that toadfish from the more contaminated sites, Haslems and Duck Creek, had higher growth rates compared to the toadfish from the less contaminated sites, Lovett Reserve and Charity Creek. The higher lipid and protein content of polychaetes at Haslams Creek (Table 4.1) may be contributing to the larger toadfish size at this site. Alternatively, the toadfish from Haslams Creek may be larger than Lovett Reserve toadfish because they have better growth efficiency, stemming from a lower energy expenditure/metabolism which may be of genetic origin (Hawkins and Day 1996).

Toadfish from the most metal contaminated site may have genetically adapted and/or physiologically acclimated through the long-term exposure to elevated metal concentrations in sediments and associated prey items. Although toadfish were larger in metal elevated sites, metal toxicity can have an effect on reduced number of eggs and/or quality or longevity of offspring (Chapter 3), thus leading to detrimental changes in toadfish population dynamics (Chapman 2002a). This was demonstrated in a study by MacFarlane and Frazin (1978), where white suckerfish (*Catostomus commersoni*) from a metal contaminated lake had higher growth rates, but had reduced longevity, compared to white suckerfish from adjacent non-contaminated lakes. In Chapter 3, toadfish from contaminated estuaries had reduced longevity and were typically smaller in size, relative to toadfish from less-contaminated estuaries. Thus, metal toxicity and associated toadfish growth may be non-related and site specific, occurring on a local population level.

4.5 SUMMARY

Toadfish from the more contaminated sites within the Parramatta River may be larger in size from the ingestion of sediment infauna of a higher nutritional value on a local scale. Further investigation is required to determine whether larger toadfish size can be attributed to ingestion of higher nutritional diet in metal polluted sites as a result of physiological acclimation or acquired genetic resistance through generations' of continuous metal exposure. Furthermore, investigation into the

relative abundance and biodistribution of benthic infauna is required to assess the potential availability of prey items to toadfish.

In the following chapter (Chapter 5), common toadfish prey items were exposed to isotopes of cadmium (^{109}Cd) and selenium (^{75}Se), two metal contaminants commonly found in estuaries around the Sydney region (Chapter 2), to investigate the uptake and loss kinetics and potential transfer to toadfish.

5.0 UPTAKE AND LOSS OF DISSOLVED ^{109}Cd AND ^{75}Se IN ESTUARINE MACROINVERTEBRATES

5.1 INTRODUCTION

Semaphore crabs (*Heloecius cordiformis*), soldier crabs (*Mictyris platycheles*), ghost shrimps (*Trypaea australiensis*), pygmy mussels (*Xenostrobis securis*), and polychaetes (*Eunice sp.*), key benthic prey items of the smooth toadfish (Chapter 4), were exposed to dissolved ^{109}Cd and ^{75}Se for 385 h at 30 kBq/L (uptake phase), followed by exposure to radionuclide-free water for 189 h (loss phase). This chapter provides the first reported data for the biological half-lives of Se in estuarine decapod crustaceans. Moreover, it emphasises the importance of determining metal accumulation and loss kinetics in keystone prey items, which consequently influences their trophic transfer potential to higher order predators (toadfish). The information gathered from the research described in this chapter assists in the analysis of metal exposure pathways by the smooth toadfish, which is described in the following chapter (Chapter 6).

5.1.1 Food webs

Food web diversity is important in ecosystems. The higher the number of links or levels in a food web, the higher the number of niches organisms can occupy, which in turn creates higher species abundance and biodiversity (Bastolla *et al.* 2005; Post 2002; Worm and Duffy 2003). However, food webs are known to be vectors for contaminant transfer to higher order predators through bioaccumulation and biomagnification (Gary *et al.* 2002; Gaston *et al.* 1998; Lubetkin and Simenstad 2004; Seebaugh *et al.* 2005). Macroinvertebrates form an important component in the resource partitioning and food web energetics of estuarine ecosystems (Carrassón and Cartes 2002). They are an important food source for higher order predators, such as fish, and play a key role in bioaccumulation and transfer of metal contaminants to higher trophic concentrations (Peters *et al.* 1999c). Elevated metal concentrations in estuaries may have a direct toxic effect on macroinvertebrates and their predators, or have an indirect effect on natural community structure by reducing prey item diversity or reducing competition within a species, resulting in a trophic cascade (Chapman 2004; Fleeger *et al.* 2003).

5.1.2 Accumulation pathways

Macroinvertebrates may accumulate metals through various environmental pathways, including water, diet and/or sediment (Boisson *et al.* 2003; Peters *et al.* 1999c; Selck and Forbes 2004; Wang and Fisher 1999). The relative importance of each pathway, as well as the bioavailability of the source, will influence the potential transfer factors of metals to higher order predators, such as toadfish. Biokinetic models have permitted a better understanding of the relative importance of waterborne and dietary exposure pathways of metals in macroinvertebrates and are driven by physiological processes (Luoma and Rainbow 2005; Wang and Fisher 1999). These processes include assimilation efficiencies from ingested foods (dietary) and metal uptake and loss rate constants from the dissolved phase (water).

5.1.3 Experimental objectives

The aim of this study was to determine the uptake and loss of dissolved ^{109}Cd and ^{75}Se in five species of macroinvertebrates (semaphore crabs, soldier crabs, ghost shrimps, pygmy mussels and polychaetes) commonly consumed by the smooth toadfish (Chapters 3 & 4). Both Cd and Se are commonly found at elevated concentrations in sediments of urbanised estuaries in southeastern Australia (Barwick and Maher 2003; Irvine and Birch 1998), and were found at high concentrations in Lake Macquarie and Parramatta River sediments in this study (Section 2.3.2). These two metals have contrasting physicochemistry in water – Se occurs as an anion, whereas Cd occurs as a cation (Byrne 2002). Thus, a further aim of this study was to determine whether differences in physicochemistry, or speciation, might account for any differences in the accumulation of ^{109}Cd and ^{75}Se among macroinvertebrates.

5.2 MATERIALS AND METHODS

5.2.1 Test oganisms

Five macroinvertebrate species (Figure 5.1); semaphore crabs (*Heloecius cordiformis*), soldier crabs (*Mictyris platycheles*), ghost shrimps (*Trypaea australiensis*), pygmy mussels (*Xenostrobus securis*) and polychaetes (*Eunice sp.*) were collected at Towra Point Nature Reserve in Botany Bay (34°01' S, 151°10' E), 20 km south of Sydney, Australia. The Reserve is a relatively undisturbed sandy marine delta with mangrove forest and is minimally-impacted by metals and other contaminants (Spooner et al, 2003). Ten to fifteen individuals of each species, of a predetermined size (semaphore crab carapace width, 11.4–13.9 mm; soldier crab

carapace width, 11.1–12.1 mm; ghost shrimp body length, 34.2–35.2 mm; black mussel shell length, 26.1–27.6 mm, and polychaete body length, 60–65 mm), were manually collected from sediment (0–5 cm depth) at random over a 90 x 10 m intertidal area in November 2004. Animals were transported to the laboratory in insulated containers within 4 hours of collection.

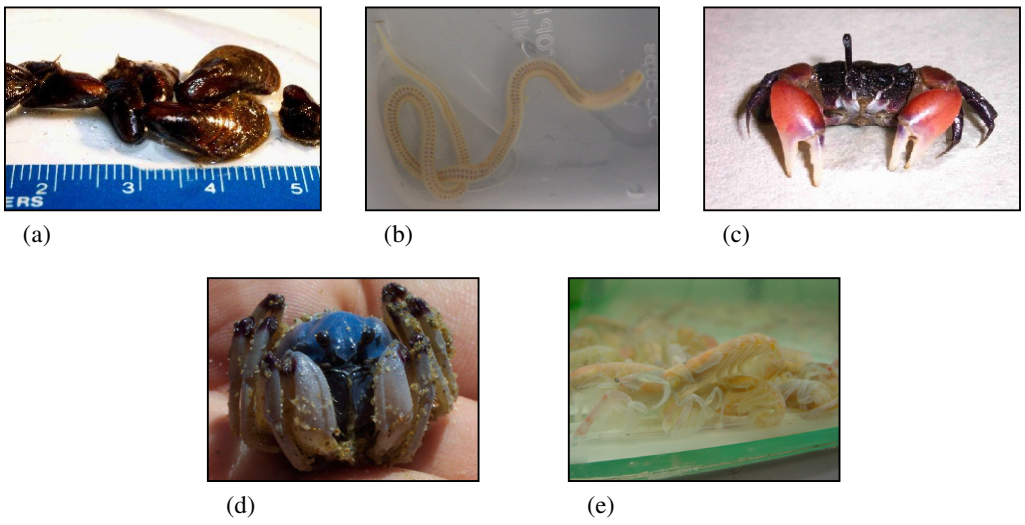

Figure 5.1 Macroinvertebrates used in experiments (a) black mussels, *X. securis*; (b) polychaetes (*Eunice sp.*); (c) semaphore crab, *H. cordiformis*; (d) soldier crab, *M. platycheles* and (e) ghost shrimps, *T. australiensis*.

5.2.2 Experimental system

Animals were acclimated to seawater under experimental conditions (pH 8.3 ± 0.1, temperature 18 ± 0.1 ºC, salinity 31 ± 1‰, dissolved oxygen 95–100% saturation and a 2 h light / 22 h dark photoperiod to mimic a natural photoperiod, where animals are exposed to sunlight outside of their burrows for approximately 2 h each day), similar to the conditions found in their environment, for five days prior to the start of experiments. Six individuals of each species were randomly selected and individually exposed to 0.8 L of filtered (< 5 µm) seawater collected from a natural minimally-impacted coastal area (Cronulla Fisheries Centre), 30 km south of Sydney and spiked with 30 kBq/L of ^{109}Cd ($T_{½}$ = 463 days) and ^{75}Se ($T_{½}$ = 120 days) for 385 h (16 days) in acid-washed polycarbonate containers (1.2 L) (Figure 5.2). There was no change in the measured pH of seawater after the radionuclide addition. Test waters were renewed daily under flowthrough conditions to maintain constant radionuclide concentrations (< 10% depletion) and minimise radionuclide recycling. Test waters

were aerated to maintain constant concentrations of dissolved oxygen (> 95% oxygen saturation) throughout the experimental exposure period. Natural concentrations of stable Cd and Se in filtered seawater were 2.8 ng/L (0.025 nM/L) and 70 ng/L (0.89 nM/L), respectively (Apte *et al.* 1998). High specific activities of ^{109}Cd (125 kBq/L) and ^{75}Se (613 kBq/L) were obtained from Isotope Product Laboratories (USA).

Figure 5.2 Experimental design (random design with replication) of macroinvertebrate kinetics experiments.

During the uptake phase, the six selected individuals of each species were independently sampled at 0 (control), 6, 12, 25, 50, 75, 100, 150, 200, 250, 310 and 385 h and live-counted for radionuclides (Section 5.2.3). Organisms were rinsed thoroughly in radionuclide-free seawater to remove any loosely adsorbed radionuclides, blotted dry and weighed before being placed in 5 ml glass vials prior to radionuclide analyses. Individuals were removed from the experimental waters for no more than five minutes at each sampling time.

At the end of the uptake phase, containers were thoroughly washed in 5% nitric acid (AnalaR, BDH) and then rinsed twice with deionised water (Milli Q; 18MΩ/cm) and animals re-introduced with radionuclide-free seawater. During the loss phase, the same six individuals of each species were sampled at 0 (control), 10, 25, 45, 70, 100, 140 and 189 h and live-counted for radionuclides. No mortalities occurred during the experimental exposures. Triplicate water samples (5 ml) were also taken for radionuclide analysis at each sampling time. Macroinvertebrates were not fed during the experiments, to remove the possibility of ingestion of radioactive particles. There

was no measured growth or observed moulting in any of the organisms during the uptake and loss experiments.

5.2.3 Radionuclide analysis

The radioactivities of ^{109}Cd (88 keV) and ^{75}Se (136 keV) in water and macroinvertebrates were counted for 90 seconds (coefficient of variation < 5%) using a high resolution gamma spectrometer, with an n-type closed end high purity germanium coaxial detector (30% relative efficiency) coupled to a multi-channel analyser (Ortec International). All samples were directly calibrated against mixed radionuclide standards with identical geometry and sample volume (water) and/or mass (whole organism). Additionally, the detector was calibrated over the full energy spectrum every seven days. The radioactivity in each sample was corrected for background counts and natural decay.

5.2.4 Speciation modelling

The speciation of Cd and Se was calculated using the HARPHRQ geochemical speciation code (Brown *et al.* 1991). The input parameters were based on measured physicochemical data (pH 8.3, pE 7.0, 18 ºC) and ion concentrations in standard seawater modified for a salinity of 31$^{o}/_{oo}$ (Pilson 1998). Stability constants were taken from (Markich and Brown 1999) and (Smith 2004). Only reliable and quality-assured data were used. The activity coefficients were corrected using a truncated Davies equation (Falck *et al.* 1996), which uses the Davies expression up to an ionic strength of 0.3 M and then fixes the charge up to ~0.70 M. Since most equilibrium constants used by HARPHRQ are standardised to 25^{o}C, the van't Hoff relationship (Smith 2004) was used to correct for the small reduction in temperature to 18^{o}C.

5.2.5 Data analysis

The uptake of ^{109}Cd and ^{75}Se from seawater was expressed as a change in concentration factors (CF = Bq/g in wet organism divided by the time-integrated Bq/g in seawater) over time. A single-component first-order kinetic model described uptake kinetics in whole animals:

$$CF_t = CF_{ss} (1 - e^{-\lambda t}) \quad (1)$$

and taking

$$CF_{ss} = K_e/\lambda \quad (2)$$

where CF_t and CF_{ss} represent the concentration factors at time t (h) and at steady state, respectively, K_e is the uptake rate constant (ml/g/h) and λ is the biological loss rate constant (h^{-1}) which controls the rate at which equilibrium is achieved (Boisson *et al.* 2003; Whicker and Schultz 1982). Alternatively, if individuals did not tend to reach a steady state over the period of experimental exposure, a simple linear regression model was used:

$$CF_t = k_u\, t \quad (3)$$

where k_u is the regression slope (i.e. rate of increase in CF, ml/g/h) over time (t). Linearity of the uptake kinetics expressed as CFs was tested using a linearity test (one-way analysis of variance) for regression with replication (Zar 1996).

Loss kinetics were expressed in terms of percentage of remaining radioactivity, i.e. radioactivity at time t divided by initial radioactivity (t_0), times 100, measured in whole organisms at the end of the uptake period. The kinetics were described by either a single-component exponential model:

$$A_t = A_0 e^{-\lambda t} \quad (4)$$

where A_t and A_0 are the remaining activities (%) at time t (h) and 0 h, respectively, and λ is the biological loss rate constant (h^{-1}), or a two compartment exponential model:

$$A_t = A_{0s} e^{-\lambda s t} + A_{0L} e^{-\lambda L t} \quad (5)$$

including a short-lived (s) and a long-lived (L) component, modified from (Boisson *et al.* 2003; Whicker and Schultz 1982). The determination of λ allows the calculation of the radionuclide biological half-life:

$$T_{b1/2} = \ln 2/\lambda. \quad (6)$$

The following model assumptions were made: (a) organisms were continually exposed to a constant radionuclide concentration, and (b) organisms were of similar size, with no observed growth over the experimental period.

One-way analysis of variance (ANOVA) was used to determine differences between concentrations of ^{75}Se and ^{109}Cd in macroinvertebrate species, as well as uptake and loss among species. Tukey's pair wise multiple comparison tests were used to evaluate significant ($P \leq 0.05$) differences among means (Zar 1996).

5.3 RESULTS

5.3.1 Speciation of Cd and Se

Both elements varied in the extent of their interactions with ligands in seawater, leading to substantial differences in their species distributions. Speciation calculations indicated that Cd occurs in the +2 oxidation state in oxic seawater and forms very strong complexes with chloride; the predominant species being $CdCl_2$ (42%), $CdCl^+$ (39%) and $CdCl_3^-$ (14%). The free cadmium ion (Cd^{2+}) forms only a very small (3.0%) component of dissolved Cd. In contrast to Cd, Se occurs predominantly in the +6 oxidation state (Se^{6+}) in oxic seawater as selenate (SeO_4^{2-}), an anion, with protonation characteristics very similar to sulfate (SO_4^{2-}).

5.3.2 Uptake of ^{109}Cd and ^{75}Se

The uptake kinetics of ^{109}Cd and ^{75}Se are shown in Figure 5.3, with fitted regressions based on either a simple linear analysis or a one-compartment model with first order kinetics. From a visual inspection of the uptake regressions, it is evident that equilibrium between the radionuclide concentrations in the organisms and water was not reached during the period of experimental exposure (385 h), except for ^{75}Se in soldier crabs (Figure 5.3a) and ^{109}Cd in semaphore crabs (Figure 5.3b).

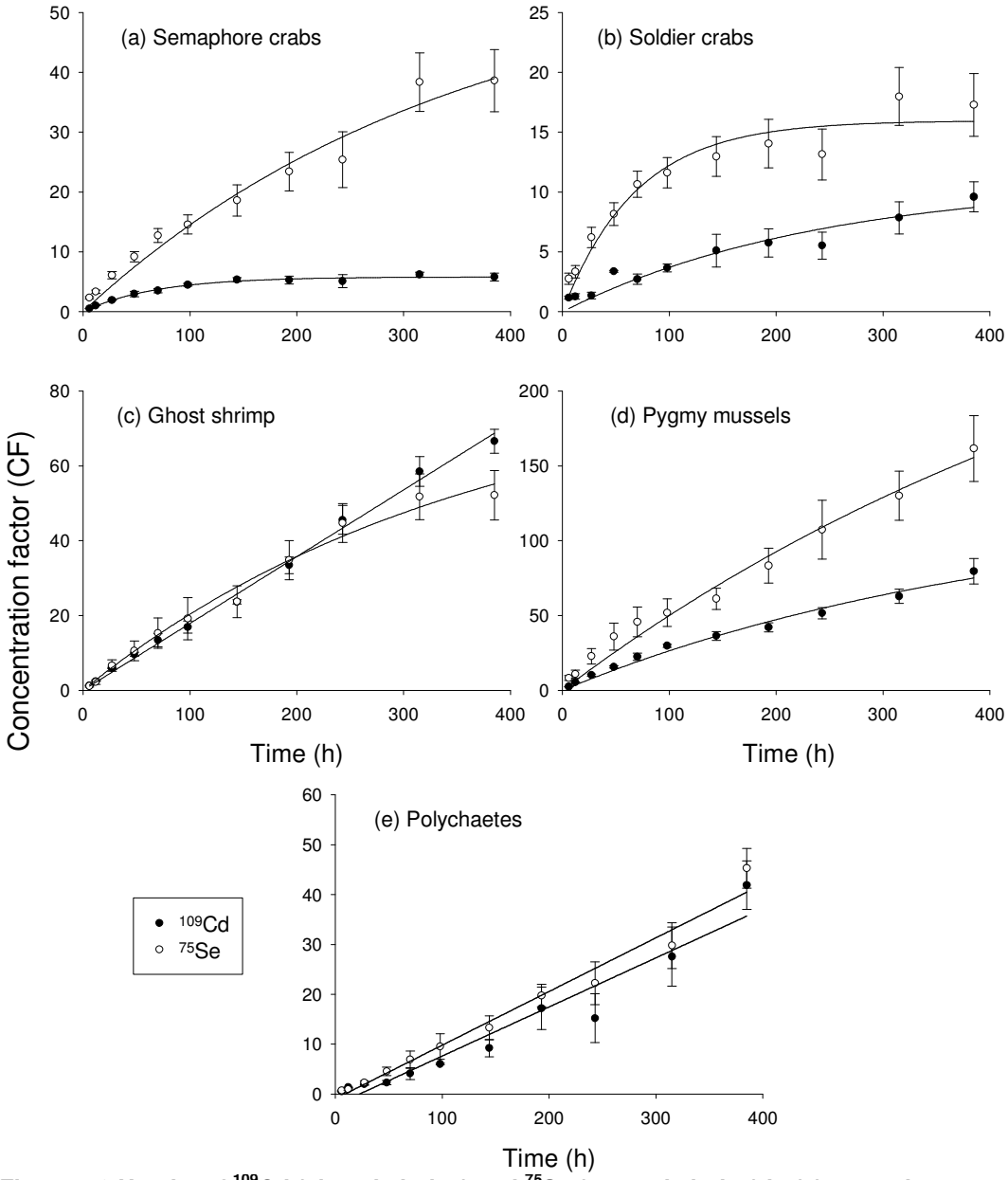

Figure 5.3 Uptake of ^{109}Cd (closed circles) and ^{75}Se (opened circles) in (a) semaphore crabs, (b) soldier crabs, (c) ghost shrimps, (d) pygmy mussels and (e) polychaetes, over 385 h. Each plotted point represents the mean ± SE (n = 6) of concentration factors (CFs) determined for six individuals at each sampling time.

The total concentrations of ^{75}Se in semaphore crabs, soldier crabs and pygmy mussels were significantly ($P \leq 0.05$) higher than ^{109}Cd at the end of the uptake phase (Table 5.1). The total concentrations of ^{75}Se were 7 times higher than ^{109}Cd in semaphore crabs and twice those in soldier crabs and pygmy mussels. There were no significant ($P > 0.05$) differences between the concentrations of ^{75}Se and ^{109}Cd for ghost shrimps and polychaetes (Table 5.1).

The concentrations of ^{109}Cd were highest in pygmy mussels (2.44 kBq/g) and lowest in semaphore crabs (0.17 kBq/g), a factor of 14 difference (Table 1). Similarly, the concentrations of ^{75}Se were highest in pygmy mussels (4.85 kBq/g) and lowest in soldier crabs (0.52 kBq/g), a factor of nine difference (Table 5.1). In general, the concentrations of both radionuclides were lowest for both crab species. The CFs reflects the above data, where pygmy mussels had the highest CFs for both ^{75}Se (162) and ^{109}Cd (81) (Table 5.1, Figure 5.3).

The uptake rate constants for ^{109}Cd and ^{75}Se are given in Table 5.2. Pygmy mussels had the highest uptake rates (K_u) for both ^{109}Cd and ^{75}Se, consistent with the trends described above, being six times higher than soldier crabs for ^{109}Cd, and six times higher than polychaetes for ^{75}Se (Table 5.2).

Table 5.1 Concentrations of ^{109}Cd and ^{75}Se in macroinvertebrates (kBq/g wet weight) at the end of the uptake (385 h) and loss (189 h) phases.

	^{109}Cd		^{75}Se	
	Uptake[a]	Loss[b]	Uptake[a]	Loss[b]
Semaphore crabs	0.17 ± 0.10 (5.7)	0.1 ± 0.03 (59)	1.2 ± 0.10 (39)	0.33 ± 0.05 (28)
Soldier crabs	0.29 ± 0.10 (9.7)	0.19 ± 0.02 (66)	0.52 ± 0.08 (17)	0.22 ± 0.04 (42)
Ghost shrimps	2.0 ± 0.23 (68)	1.4 ± 0.075 (69)	1.6 ± 0.25 (54)	1.5 ± 0.75 (91)
Pygmy mussels	2.4 ± 0.51 (81)	1.9 ± 0.25 (77)	4.9 ± 0.38 (162)	3.5 ± 0.31 (71)
Polychaetes	1.2 ± 0.22 (41)	0.76 ± 0.10 (62)	1.3 ± 0.13 (45)	0.95 ± 0.23 (71)

[a]Values represent the mean ± 95% confidence limits (n = 6), with concentration factors (calculated by dividing the mean radionuclide organism (kBq/g wet weight) by the mean radionuclide water concentration (0.03 kBq/g)) in parentheses.
[b]Values represent the mean ± 95% confidence limits (n = 6), with the percentage of radionuclide remaining in the organism (calculated by dividing the radionuclide concentration in an organism at the end of the loss phase by their radionuclide concentration at the end of the uptake phase x 100) in parentheses.

Table 5.2 Uptake and loss rate constants for ^{109}Cd and ^{75}Se in macroinvertebrates.

	^{109}Cd			^{75}Se		
	Uptake		Loss	Uptake		Loss
	K_u[a]	K_e[b]	λ[c]	K_u[a]	K_e[b]	λ[c]
Semaphore crabs	0.068	0.0029	0.00051	0.16	0.17	0.0043
Soldier crabs	0.044	0.0027	0.00028	0.18	0.036	0.0021
Ghost shrimps	0.18	0.018	0.00027	0.19	0.025	0.00046
Pygmy mussels	0.26	0.026	0.00032	0.49	0.24	0.0015
Polychaetes	0.096	0.0049	0.00012	0.078	0.054	0.0012

[a] K_u values were calculated using a linear equation ($CF_t = K_u t$) and are expressed as ml/g/h.
[b] K_e values were calculated using an exponential rise equation ($CF_t = CF_{SS}(1 - e^{-\lambda t})$ where $CF_{SS} = K_e/\lambda$ and are expressed as ml/g/h.
[c] λ values were calculated using an exponential decay equation ($A_t = A_0 e^{-\lambda t}$) and are expressed as h^{-1}.

5.3.3 Loss of ^{109}Cd and ^{75}Se

The loss kinetics of ^{109}Cd and ^{75}Se are shown in Figure 5.4, with fitted regressions based on a two-compartment model with first order loss kinetics. The total loss of ^{109}Cd over the experimental period (189 h) was lowest in pygmy mussels (77% retained in the whole body; i.e. 23% lost) and highest in semaphore crabs (59% retained; i.e. 41% lost) (Table 5.1, Figure 5.4). In contrast, the total loss of ^{75}Se was lowest in ghost shrimps (91% retained; i.e. 9% lost) and highest in semaphore crabs (28% retained; i.e. 72% lost) (Table 5.1; Figure 5.4).

The loss rate constants (λ) for ^{109}Cd and ^{75}Se are given in Table 5.2. Semaphore crabs had the highest loss rate for ^{109}Cd, whilst polychaetes had the lowest - a factor of four difference. Similarly, semaphore crabs had the highest loss rate for ^{75}Se, with ghost shrimps having the lowest loss rate - a factor of nine difference. The loss rates of ^{109}Cd in all species were lower than those for ^{75}Se; polychaetes were ten times higher and ghost shrimps double.

The biological half-lives of ^{109}Cd and ^{75}Se in macroinvertebrates are presented in Table 5.3. The loss of ^{109}Cd and ^{75}Se in all species was best described by a two-compartment model (i.e. a short and a longer-lived compartment). In the short-lived, or rapidly exchanging, compartment, the biological half-lives of ^{75}Se (16–39 h) were about three times greater than those of ^{109}Cd (5–12 h) (Table 5.3). In contrast, the biological half-lives of ^{109}Cd in the longer-lived, or slowly exchanging compartment(s), were typically greater (1370–5950 h) than those of ^{75}Se (161–1500 h). Semaphore crabs had the shortest biological half lives of both radionuclides in the long-lived compartment, whereas polychaetes has the greatest biological half life for ^{109}Cd

(5950 h), and ghost shrimps had the greatest biological half life for ^{75}Se (1500 h) (Table 5.3).

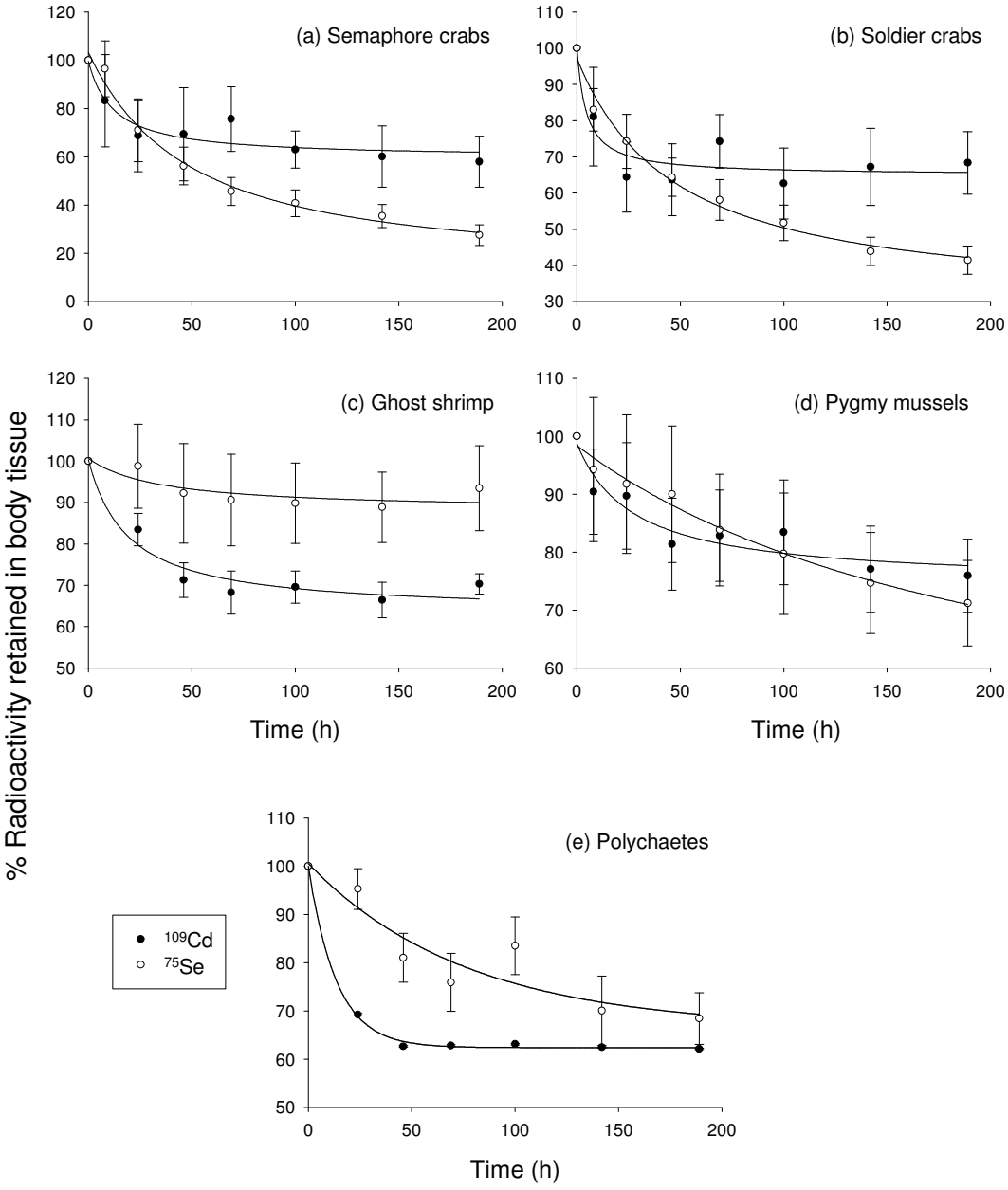

Figure 5.4 Percentage loss of ^{109}Cd (closed circles) and ^{75}Se (opened circles) in (a) semaphore crabs, (b) soldier crabs, (c) ghost shrimps, (d) pygmy mussels and (e) polychaetes, over 189 h. Each plotted point represents the mean ± standard error of the percentage of retained radioactivity, relative to original radioactivity, for six individuals at sampling time.

Table 5.3 Biological half-lives (h) of ^{109}Cd and ^{75}Se in macroinvertebrates. Values for each compartment were estimated based on the patterns of loss over 189 h.

	^{109}Cd (1st) [a]	^{109}Cd (2nd)	^{75}Se (1st)	^{75}Se (2nd)
Semaphore crabs	7	1370	23	161
Soldier crabs	5	2510	22	330
Ghost shrimps	12	2590	16	1500
Pygmy mussels	11	2140	39	490
Polychaetes	11	5950	34	555

[a]The biological half-life is the time (h) taken for half of the radioactivity in an organism to be lost. Macroinvertebrates exhibited two compartments [1st (short-lived) and 2nd (longer-lived)] in the loss phase.

5.4 DISCUSSION

Differences in metal accumulation among macroinvertebrates may be attributed to a number of factors, including differences in feeding habit, general physiology and geochemical effects on bioavailability (Luoma and Rainbow 2005; Rainbow 2002; Wang and Fisher 1999). Differences between Cd and Se uptake among organisms may be ascribed to different uptake pathways at the cell surface. Cadmium, a non-essential element, is taken up as an analogue of Ca, via Ca transporters such as Ca^{2+}-ATPase at the cell surface (Bondgaard and Bjerregaard 2005; Rainbow and Black 2005), since Cd^{2+} and Ca^{2+} ions are physicochemically similar (Frausto da Silva and Williams 2001). As Ca^{2+}-ATPase is under the physiological control of the organism, the rate of Cd uptake may vary among different species, based on the organisms' underlying physiology (e.g. number and strength of binding sites/transporters). In this study, the rate of ^{109}Cd uptake was greatest in pygmy mussels, being six times higher than soldier crabs (Table 5.2). The small percentage of Cd^{2+} (3%) in the test waters suggests that the general bioavailability of Cd was low. Moreover, there is no evidence to suggest that Cd-chloride complexes, which comprise the major percentage (96%) of dissolved Cd in the test waters, are bioavailable.

In contrast to Cd, Se is a naturally occurring essential element required for normal growth and development. It may occur in different physiocochemical (oxidation) forms in estuarine waters – as selenate (Se^{6+}; SeO_4^{2-}), selenite (Se^{4+}; SeO_3^{2-}) or organoselenium (Se^{2-}; e.g. selenomethioneine), the latter produced by biological reduction of selenite. Each physicochemical form differs in its bioavailability (Hamilton 2004; Sappington 2002), with the organic forms being more bioavailable than the inorganic forms (Fournier *et al.* 2005; Wang and Lovell 1997). Selenate (SeO_4^{2-}), the predominant form of Se in surface waters, is taken up as an analogue of sulfate (SO_4^{2-}), via sulfate transporters, since these two oxyanions are chemically similar (Frausto da Silva and Williams 2001; Ogle and Knight 1996). Since sulfate

transport is under the physiological control of the organism, the rate of Se uptake may vary naturally among macroinvertebrates. In this study, the rate of ^{75}Se uptake was greatest in pygmy mussels, being six times higher than polychaetes (Table 5.2). Speciation modelling of Se in surface waters should be exercised with caution, as Se^{4+} and Se^{6+} are rarely in true thermodynamic equilibrium because of biologically-mediated reduction reactions (e.g. formation of organoselenium) and the slow kinetics of Se^{4+} oxidation (USEPA 2004). Thus, the actual percentages of selinite and organoselenium are typically higher than predicted by equilibrium speciation modelling (Cutter 1989).

Some macroinvertebrates excrete metabolically-active metals at the same rate as they are accumulated, whilst others accumulate metals and sequester them in granules or metallothioneins, which may act as long-term sinks (Amiard *et al.* 2006; Rainbow 2002). In addition, some macroinvertebrates may accumulate and sequester metals and excrete the sequestered forms rather than the metabolically-active forms (Rainbow 2002). This is of particular relevance to the trophic transfer potential (TTP) of dissolved metals accumulated by the prey organisms studied here. The concentration factor (CF) concept either assumes, or extrapolates to, equilibrium conditions when applying first order kinetics, or represents the factor prevailing at sample time for non-equilibrium states, as observed herein or as typically applied in field-based assessments. In first order kinetics, the CF is given as the ratio of the uptake and loss rate constants. Hence an organism with slow uptake and loss may have a similar CF to one that has fast uptake and loss rates. However, the organism with the lower loss rate (longer biological half-time) will have the greater TTP as it will retain the accumulated metals longer. Assuming similar assimilation rates, this observation is true, however the CF is estimated. However, as discussed further later, other factors including the uptake mechanism (via gills or digestion) are also crucial to this potential.

Metal uptake from the dissolved phase is a potentially significant source for the overall accumulation of metals in bivalves because these animals filter substantial volumes of water through their gills. Filter-feeding bivalves typically accumulate high concentrations of metals from solution and sequester them in shells, granular deposits or metallothioneins, which serve as long-lived repositories over the lifetime of the animal (Amiard *et al.* 2006; Wang and Rainbow 2005). This is consistent with pygmy mussels accumulating the highest concentrations of ^{109}Cd and ^{75}Se (Table 5.1, Figure 5.3d).

The dissolved phase is the predominant source of Cd (55–95%) in filter-feeding marine bivalves (Borchardt 1983; Cossa 1988; Riisgård *et al.* 1987; Wang *et al.*

1996). The uptake rate constants (K_u) for Cd and Se in the pygmy mussel, *X. securis* (Table 2), were 4–30 times and 3–6 times lower, respectively, than those reported in previous studies for filter-feeding bivalves (Blackmore and Wang 2002; Chong and Wang 2001; Ke and Wang 2001; Lee *et al.* 1998; Shi and Wang 2004; Wang *et al.* 1996). The reduced Cd uptake rates in this study may reflect the low algal (food) ration in the filtered seawater (Riisgård *et al.* 1987), but may also be influenced by differences in species-specific physiological attributes such as assimilation rate, gill surface area or general permeability of the cell membrane to metal ions (Lee *et al.* 1998; Wang 2001). In contrast to Cd, the dissolved phase generally contributes only a minor proportion (5–40%) of Se uptake in filter-feeding bivalves, with the predominant source being ingested Se (Ke and Wang 2001; Wang *et al.* 1996).

The 23% loss of ^{109}Cd in *X. securis* (Table 5.1) after 189 h (~8 days) was consistent with previous studies (Blackmore and Wang 2002; Chong and Wang 2001; Ke and Wang 2001; Lee *et al.* 1998; Reinfelder *et al.* 1997; Shi and Wang 2004; Wang *et al.* 1996), who found similarly small percentage losses (20–35%) for several marine bivalve species over a similar loss phase (6–8 days). The biological half life of Cd in the long-lived or slowly exchanging compartment (89 days; Table 5.3) was of a similar magnitude to those reported by Okazaki and Panietz (1981) for *Crassostrea virginica* (84 days), Wang *et al*. (1996) for *Mytilis edulis* (78 days), Reinfelder *et al.* (1997) for three bivalve species (~70 days) and Chong and Wang (2001) for *Ruditapes philippinarum* (69 days). Once accumulated, Cd is slowly exchanged (second compartment) and strongly associated with insoluble metallothioneins and granular structures (Amiard *et al.* 2006; Wang and Rainbow 2005).

The 29% loss of ^{75}Se in *X. securis* (Table 5.1) after 8 days was consistent (19–45%) with previous studies (Ke and Wang 2001; Reinfelder *et al.* 1997; Wang *et al.* 1996) for several species of marine bivalves. The biological half life of Se in the long-lived compartment (20 days; Table 5.3) was similar to that reported by Wang *et al.* (1996) for *Mytilis edulis* (28 days), Reinfelder *et al.* (1997) for three bivalve species (10–35 days) and (Ke and Wang 2001) for *Crassostrea rivularis* (20 days). Wang *et al.* (1996) reported that that the duration of exposure and the uptake pathway did not significantly affect loss rate constants in marine bivalves. Furthermore, Fisher *et al.* (1996) reported that loss rates of metal in bivalves maintained in the laboratory were directly comparable to those transplanted into the field.

The polychaete, *Eunice sp.* is a surface deposit feeder, so the relative importance of ^{109}Cd and ^{75}Se uptake from the dissolved phase is thought to be minor relative to

dietary sources (Selck and Forbes 2004; Wang *et al.* 1999). However, polychaetes may also accumulate dissolved metals through respiratory holes, or nephridiopores, which account for a large dermal surface area in individuals, thus allowing them to potentially absorb high concentrations of dissolved metals (Dallinger and Rainbow 1991). The bioaccumulation pattern of *Eunice sp.* may be explained as uptake of metabolically available ^{109}Cd and ^{75}Se, that were sequestered and stored as metallothioneins or insoluble granules (Berthot *et al.* 2003; Geffard *et al.* 2005; Mouneyrac *et al.* 2003), with some excretion from the stored form resulting in the small decrease in radionuclide concentrations after 8 days of loss.

The similar uptake rate constants (K_u) for Cd and Se in *Eunice sp* (Table 5.2), were 2–15 times and 3 times lower, respectively, than those reported in previous studies for other species of deposit feeding polychaetes (Geffard *et al.* 2005; Ray *et al.* 1980; Wang *et al.* 1999; Zhou *et al.* 2003). Wang *et al.* (1999) also reported similar K_u values for Cd and Se in the polychaete, *Nereis succinea*.

The 38% loss of ^{109}Cd in *Eunice sp* (Table 5.1) after 8 days was consistent with previous studies (Geffard *et al.* 2005; Redeker *et al.* 2004) who found similarly small percentage losses (33–35%) for several marine worms over a similar loss phase (8–10 days). The biological half life of Cd in the long-lived compartment of *Eunice* sp. (248 days; Table 3) was similar to that reported by Geffard *et al.* (2005) for *Nereis diversicolor* (224 days), but typically lower than those re-calculated from (Bernds *et al.* 1998) for four species of deposit feeding polychaetes (280–430 days).

With all decapod crustaceans, including ghost shrimps, semaphore crabs and soldier crabs, the gills are the major site of Cd and Se uptake from the dissolved phase, with most of the metal transported to the hepatopancreas for storage (Rainbow 1997). Their bioaccumulation pattern may be explained as uptake of metabolically available ^{109}Cd and ^{75}Se, that were sequestered and stored as metallothioneins or insoluble granules (Rainbow 1997, 2000) with metal-dependent excretion from the stored form. The decapods have mostly impermeable surfaces with restricted gill areas and might be expected to have amongst the lowest uptake rate constants of the crustaceans (Marsden and Rainbow 2004; Rainbow 1997). This study provides the first reported data for loss rate constants/biological half lives for Se in decapod crustaceans.

The ghost shrimp, *T. australiensis* is a (sub-) surface deposit feeder (Boon *et al.* 1997), so the relative importance of ^{109}Cd and ^{75}Se uptake from dietary sources may exceed that from the dissolved phase. The uptake rate constant (K_u) for Cd in *T. australiensis* (Table 5.2) was very similar (within a factor of two) to that of the shrimp, *Palenaeus elegans* (Nugegoda and Rainbow 1995; Rainbow and White 1989; White

and Rainbow 1986) and consistent with that of the shrimp, *Penaeus indicus* (Nuñez-Nogueira and Rainbow 2005) after correction for salinity effects. The loss of ^{109}Cd (31%) was substantially greater than ^{75}Se (9%) in *T. australiensis*, indicating that Se exchanged slower from its stored form than Cd. The biological half-life of Cd in the long-lived compartment of *T. australiensis* (108 days; Table 5.3) was similar to that re-calculated (~120 days) from results by (Nuñez-Nogueira and Rainbow 2005) for *P. indicus*.

Semaphore crabs (*H. cordiformis*) and soldier crabs *(M. platycheles*) are both deposit feeders (Maitland 1990; Takeda 2005), so the relative importance of ^{109}Cd and ^{75}Se uptake from the dissolved phase should be minor relative to dietary sources (Bjerregaard *et al.* 2005). Amongst the macroinvertebrates tested in this study, the two crabs typically had the lowest uptake rates and the highest loss rates (or lowest biological half-lives). Whilst crabs are known to store metal(loids) as soluble metallothioneins, they tend to be more rapidly exchanged and effectively eliminated via urine (MacFarlane *et al.* 1999; Rainbow 1990). The uptake rate constants (K_u) for Cd in both crabs (Table 2) were 2–4 times lower than those reported for the shore crab, *Carcinus maenas* (Jenkins and Wheatley 1998; Jennings and Rainbow 1979; Rainbow and Black 2005; Roast *et al.* 2002; Wright and Brewer 1979). The biological half-lives of Cd in the short-lived compartment of *H. cordiformis* (7 days) and *M. platycheles* (5 days) were similar to those reported for *C. maenas* (5–8 days) (Chan *et al.* 1992; Martin and Rainbow 1998b). This represents the rapid exchange of Cd with haemocyanin in the haemolymph of these crabs (Martin and Rainbow 1998a). The biological half lives of Cd in the longer-lived compartments of *H. cordiformis* (57 days) and *M. platycheles* (105 days), most likely represent binding to metallothioneins (Amiard *et al.* 2006) and inorganic granules (Nott and Nicolaidou 1994) where ^{109}Cd is more tightly bound to the internal organs. No other studies have reported on multi-compartment loss of Cd in crabs where water has been the source of Cd uptake.

Pygmy mussels and ghost shrimps have a high TTP to higher order predators, reflecting their higher uptake and lower loss rates of dissolved ^{109}Cd and ^{75}Se, relative to those of semaphore and soldier crabs. Since the degree of metal transfer in food webs may vary between the extremes of bioreduction and biomagnification, understanding the mechanisms that control TTP have been problematic (Seebaugh *et al.* 2005). Differences in the TTP of metal(loids) from prey to consumers may be influenced by the pathways of metal uptake (water vs diet/sediment) of the prey and their assimilation efficiencies by predators (Wang 2002; Wang and Fisher 1999). This study only considered metal accumulation in the selected macroinvertebrate

species from the dissolved phase. The metal exposure pathway in prey species may influence potential assimilation by the predator (Wang and Fisher, 1999). Gills are the major pathway for dissolved metal uptake and distribution to body fluids, and hence other internal tissues. In filter-feeding bivalves, >90% of Cd is accumulated from the dissolved phase via the gills (Riisgård *et al.* 1987). In contrast, Cd is accumulated predominantly from the diet in deposit feeding crustaceans and polychaetes (Bjerregaard *et al.* 2005; Wang *et al.* 1999). Thus, the relative proportions of prey items consumed by predators will influence their degree of metal accumulation and any estimates of TTP based on the dissolved phase for the polychaete and three crustacean species may be an underestimate.

The partitioning of metals between tissues, and the chemical form of the stored metals in these tissues, may influence their assimilation efficiency by predators (Wang 2002; Wang and Fisher 1999). If the stored metals can be broken down by the digestive processes of the predator, then the metals may be transferred along the food chain. Several studies have shown that metals associated with organelles and cytosolic proteins (e.g metallothioneins) in some prey have a higher bioavailability to predators than metals bound to insoluble components (e.g. granules) (Wallace *et al.* 1998; Wallace and Luoma 2003). In addition, TTP may be influenced by the nutrient extraction efficiency of prey items. For example, a prey item with a hard exterior (eg. mussel shell or crab carapace) will be harder to digest by a predator than one with a soft exterior (eg. chitinous body of a polychaete). Models designed to estimate TTP should consider differences in internal metal partitioning between trophic concentrations, in addition to multiple routes of exposure and abiotic factors (Reinfelder *et al.* 1998; Wang and Fisher 1999).

5.5 SUMMARY

Differences in the uptake and loss of ^{109}Cd and ^{75}Se in the selected estuarine macroinvertebrates were influenced by differences in their aqueous speciation, as well as differences in animal physiology, sequestration, storage and excretion mechanisms. The uptake rates and CFs of ^{109}Cd and ^{75}Se were highest, and the biological half-lives the longest, in pygmy mussels, ghost shrimps and polychaetes relative to the semaphore and soldier crabs. This study provides the first reported data for biological half-lives of Se in estuarine decapod crustaceans. It is recommended that other metal uptake pathways, such as diet (food) and/or particulates (sediments) should be investigated in individual prey species in order to

determine the TTP of metals to higher order predators and evaluate the relative importance of each pathway.

The following chapter (Chapter 6) describes the exposure of toadfish to isotopes of cadmium (^{109}Cd) and selenium (^{75}Se), through various pathways (dietary and dissolved) to examine exposure routes of contaminants.

6.0 COMPARATIVE ACCUMULATION OF ^{109}Cd AND ^{75}Se FROM WATER AND FOOD BY THE SMOOTH TOADFISH

6.1 INTRODUCTION

Few data are available on the comparative accumulation of metals from water and food in estuarine or marine fish. In separate experiments, smooth toadfish were exposed to radiolabeled water (14 kBq/L of ^{109}Cd and 24 kBq/L of ^{75}Se) and food (ghost shrimps exposed to 200 Bq/L of ^{109}Cd and ^{75}Se for five days) for 25 days (uptake phase), followed by exposure to radionuclide-free water and food for 30 days (loss phase). Radionuclides were measured in water, food, food waste, whole fish and selected organs (gills, gonads, gut lining, kidney, liver and muscle). This chapter emphasises the importance of differentiating accumulation pathways to better understand metal transfer dynamics and subsequent toxicity to aquatic biota, as demonstrated in Chapters 2, 4 and 5.

6.1.1 Estuarine bioindicators

Estuaries have been subject to increased anthropogenic pressure from rising population growth, urban development and the alteration of catchment processes, resulting in elevated concentrations of contaminants, such as metals, which impact upon resident biota (GESAMP 2001; Kennish 2002; Luoma 1996). There is a growing emphasis on selecting aquatic biota from different trophic levels within a food web as key indicators of ecological conditions, and thus estuarine health (Hirst 2004). Fish may serve as good indicators of ecological conditions, since they are long-lived and mobile, forage at different trophic levels, integrate effects of lower trophic concentrations, and are reasonably easy to identify in the field (van der Oost *et al.* 2003; Whitfield and Elliott 2002) (Chapter 1.1.4).

6.1.2 Contaminant transfer through food webs

Metals transferred through aquatic food webs to fish, humans and other piscivorous animals, are of environmental and human health concern. There is increasing recognition of the quantitative importance of metal accumulation from dietary (food) pathways in estuarine and marine food webs (Wang 2002). Biokinetic models have permitted a better understanding of the relative importance of food and water exposure pathways of metals in aquatic biota. These models are governed by physiological processes, including assimilation efficiencies from ingested foods and

metal uptake and loss rate constants from the dissolved (water) phase (Luoma and Rainbow 2005; Wang 2002; Wang *et al.* 1996). Metals can be accumulated in different organs at different concentrations depending on the exposure pathway, and can thus cause toxicity at different concentrations depending on the metal pathway (Fisher and Hook 2002). It is therefore important to differentiate between metal pathways in fish to better understand metal transfer dynamics and subsequent toxicity. However, a limited number of studies have quantified the comparative accumulation of metals in estuarine or marine fish from water or food exposures (Milner 1982; Willis and Sunda 1984; Xu and Wang 2002; Zhang and Wang 2005).

6.1.3 Cadmium and Se as toxicants

Cadmium (Cd) and selenium (Se) are common metals found in anthropogenically degraded aquatic environments (Barwick and Maher 2003; Irvine and Birch 1998) and are known to be toxic to aquatic biota at elevated concentrations (Hamilton 2004; Hollis *et al.* 1999). Cadmium is a non-essential element, which is highly toxic to selected fishes, especially salmonoids, and effects can include damage to fish gills, reproductive impairment, skeletal deformities and disruption of calcium balance mechanisms (De Conto Cinier *et al.* 1999; Hollis *et al.* 1999). In contrast, Se is an essential element required for normal metabolic purposes, including growth and development; however, at elevated concentrations, Se may also become toxic to fishes (Hamilton 2004). Selenium uptake in aquatic organisms can be through the gills or epidermis, however, many studies have shown that the major pathway of Se uptake is via food exposure in aquatic food chains (Baines and Fisher 2002; Hamilton 2004; Luoma *et al.* 1992; Sappington 2002).

6.1.4 Experimental objectives

The aim of this study was to compare the uptake and loss of ^{109}Cd and ^{75}Se (two common toxicants found in sediments, prey items and tissues of the smooth toadfish; Chapters 2 & 4) from water and food in the smooth toadfish and the subsequent partitioning and accumulation in selected organs (gills, gonads, gut lining, kidney, liver and muscle). Organs were selected on the basis of exposure route (gills and gut lining), excretory (kidney and liver) and reproductive (gonads) function, and consequent human exposure through ingestion of flesh (muscle).

6.2 MATERIALS AND METHODS

For a detailed description of the biology and habit of the smooth toadfish, refer to "Fish Species" Chapter 1.1.4

6.2.1 Collection and maintenance of fish

Seventy five sexually mature toadfish of similar size (total length: 110 ± 3 mm; mean ± standard error) and wet weight (28 ± 2 g) were collected using a beach seine net (15.5 x 1.75 m; 16 mm mesh size) from Hen and Chicken Bay (33o51' S, 151o07' E), Sydney, south-eastern Australia. The study site is an integral part of Sydney Harbour and is characterised by elevated levels of metal(loid)s, including Cd and Se, in sediments (Irvine and Birch, 1998) (Chapter 2). Toadfish were maintained in aerated site (sea) water (31‰ salinity) within two 50 L acid-washed polyethylene containers and transported to the laboratory within 2 h of collection. Natural seawater was used for all experiments and was collected from a minimally-impacted coastal area (Cronulla Fisheries Centre), 30 km south of Sydney.

Two separate experiments were designed to investigate potential differences between dissolved (water) and dietary (food) uptake and loss of ^{109}Cd (T½ = 463 days) and ^{75}Se (T½ = 120 days) by T. glaber (Figure 6.1). In each experiment, one fish was placed in each of sixty six 10 L acid-washed glass tanks (33 for water exposure study and 33 for food exposure study) filled with seawater (8 L) and acclimated to experimental conditions (18 ± 0.2ºC, 30 ± 2‰ salinity, dissolved oxygen 8.2 ± 0.2 mg/L, pH 8.3 ± 0.1 and 12:12 h light/dark regime) for seven days prior to commencing the uptake experiments. Seawater in each tank was filtered (<5 µm), aerated to maintain constant oxygen levels (> 90% saturation) and renewed daily via a flow- though system. Natural concentrations of stable Cd and Se in filtered seawater are 2.8 ng/L (0.025 nM/L) and 70 ng/L (0.89 nM/L), respectively (Apte et al., 1998). High specific activities of ^{109}Cd (125 kBq/L) and ^{75}Se (613 kBq/L) were obtained from Isotope Product Laboratories (USA).

Ghost shrimps (*Trypaea australiensis*) were selected as a food source for toadfish in both experiments, since they are a known benthic prey item (consistent and abundant in toadfish gut contents; Chapters 3 and 4) and have similar uptake rates and concentration factors of ^{109}Cd and ^{75}Se from water (Chapter 5). Ghost shrimps were collected from a minimally-impacted region of the Port Hacking estuary, 20 km south and adjacent to Sydney Harbour. Toadfish were fed one live ghost shrimp (2–3 g wet weight) at 10 am daily during the acclimation period, with any uneaten food (typically < 1% of ghost shrimp wet weight) removed after 2 h.

Toadfish were fed at a rate of 7–10% of body weight per day, which typically exceeded the normal range of 2–5% for fish (Pauly 1989).

6.2.2 Dissolved (water) exposure: Experiment 1

Toadfish were exposed to ^{109}Cd (14 kBq/L; corresponding to 0.015 µg/L or 0.13 nM/L) and ^{75}Se (24 kBq/L; corresponding to 0.01 µg/L or 0.13 nM/L) in seawater for 25 days (uptake phase), followed by 30 days to radioactive-free seawater (loss phase). Test waters were renewed daily as part of a flow-through design to maintain constant activities of both radionuclides. Radionuclides were equilibrated with natural stable metals in filtered seawater (header tanks) for at least 24 h before the uptake experiments. There was no change in the measured pH of seawater (pH 8.3 ± 0.1) after radionuclide addition. Each toadfish was fed one live, non-radioactive, ghost shrimp (2.3 ± 0.1 g wet weight; mean ± standard error) at 10 am daily. Any uneaten food (typically < 1% of ghost shrimp wet weight) was removed after 2 h. Activity in the food was checked and found to be negligible.

Six replicate 5 mL water samples were collected daily and gamma counted (see below). To assess radionuclide biokinetics, three fish were randomly sampled at each time throughout the uptake (0, 2, 5, 10, 16, 20 and 25 days) and loss (0, 5, 10, 20 and 30 days) phases of the experiment, plus three controls not exposed to radioactivity over both experimental phases. Toadfish growth during the uptake and loss experiments was negligible.

Figure 6.1 Experimental setup for toadfish water and food exposure experiments.

6.2.3 Dietary (food) exposure: Experiment 2

Each toadfish was fed a radioactive ghost shrimp (2.4 ± 0.1 g wet weight; mean ± standard error) at 10 am daily for 25 days (uptake phase), followed by a non-radioactive ghost shrimp for a further 30 days (loss phase) in radioactive-free seawater (Figure 6.2). Any uneaten food (typically < 1% of ghost shrimp wet weight) was removed 2 h later to minimise any extraneous radioactivity in the experimental system. Test waters were renewed daily as part of a flow-through system. Ghost shrimps were previously exposed to 50 KBq/L of ^{109}Cd and ^{75}Se in seawater for five days, resulting in a radioactive body burden of 875 ± 42 Bq/g wet weight (mean ± standard error) for ^{109}Cd and 1130 ± 56 Bq/g wet weight for 75Se. Ghost shrimps fed to toadfish were rinsed thoroughly in radioactive-free seawater to remove any loosely adsorbed radionuclides.

To assess radionuclide biokinetics, three fish were randomly sampled at each time throughout the uptake (0, 2, 5, 10, 20 and 25 days) and loss (0, 5, 20 and 30 days) phases of the experiment, plus three controls not exposed to radioactivity over both experimental phases. Faeces were regularly removed from the test containers to minimise the potential for radionuclide recycling. Fish were not fed 24 h prior to each sampling time, ensuring that no undigested food was present in the gut, and thus minimising any overestimate of net accumulation. Toadfish growth during the uptake and loss experiments was negligible.

Figure 6.2 Toadfish ingesting a radioactive prey item.

6.2.4 Preparation of fish and Radionuclide analysis

At each sampling time, fish were quickly euthanased with 50 mg/L of MS222 (tricaine methane sulphonate) for 2 to 3 min, rinsed thoroughly in radionuclide-free seawater to remove any loosely adsorbed radionuclides, blotted dry, weighed and gamma counted whole before being dissected into various organs (gills, gonads, gut lining, kidney, liver and muscle). There was no measured growth or mortalities during either experiment.

The radioactivities of ^{109}Cd (88 keV) and ^{75}Se (136 keV) were counted for 90 seconds (coefficient of variation < 5%) in ghost shrimps (food), seawater and wastes (uneaten food and faeces), and 600 seconds (coefficient of variation < 5%) in whole toadfish and organs using a high resolution gamma spectrometer, with a p-type closed end high purity germanium coaxial detector (30% relative efficiency) coupled to a multi-channel analyser (Ortec International). All samples were directly calibrated against mixed radionuclide standards with identical geometry and sample volume (water) and/or mass (whole fish or organs). Additionally, the detector was calibrated

over the full energy spectrum every seven days. The radioactivity in each sample was corrected for background counts and natural decay to the start of the experiments.

Toadfish whole body and organ radioactivity was corrected for wet weight, and the time integrated concentration factor (CF, mL/g wet weight) was determined, defined by the radioactivity in whole toadfish divided by the radioactivity in (i) exposure water or (ii) net food items consumed, at time of sampling.

Speciation modelling was carried out prior to the testing and is outlined in Section 5.2.4.

6.2.5 Data analysis

Net uptake of ^{109}Cd and ^{75}Se from seawater or food (ghost shrimp) was expressed as a change in concentration factors (CF = Bq/g wet weight of whole fish divided by the time-integrated Bq/g seawater or food) over time. Uptake in whole fish was described by a single-component first-order kinetic model:

$$CF_t = CF_{ss} (1- e^{-\lambda t}) \quad (1)$$

and taking

$$CF_{ss} = K_e/\lambda \quad (2)$$

where CF_t and CF_{ss} represent the concentration factors at time t (days) and at steady state, respectively, K_e is the uptake rate constant (ml/g/day for water and mg/g/day for food) and λ is the biological loss rate constant (day^{-1}) which controls the rate at which equilibrium is achieved (Whicker and Schultz, 1982; Boisson et al., 2003). Alternatively, if individuals did not tend to reach a steady state over the period of experimental exposure, a simple linear regression model was used:

$$CF_t = k_u\, t \quad (3)$$

where k_u is the regression slope (i.e. rate of increase in CF, (ml/g/day for water and mg/g/day for food) over time (t). Linearity of the uptake was tested using a one-way analysis of variance for regression with replication (Zar, 1996).

Loss was expressed in terms of percentage of remaining radioactivity (i.e. radioactivity at time t divided by initial radioactivity (t_0) measured in whole fish at the

end of the uptake period, times 100). The losses were described by either a single-component exponential model:

$$A_t = A_0 e^{-\lambda t} \quad (4)$$

where A_t and A_0 are the remaining activities (%) at time t (days) and 0 days, respectively, and λ is the biological loss rate constant ($days^{-1}$), or a two compartment exponential model:

$$A_t = A_{0s} e^{-\lambda s t} + A_{0L} e^{-\lambda L t} \quad (5)$$

including a short-lived (s) and a long-lived (L) component (modified from Whicker and Schultz, 1982; Boisson et al., 2003). The determination of λ allows the calculation of the radionuclide biological half-life:

$$T_{b1/2} = \ln 2/\lambda. \quad (6)$$

Rate constants were corrected for the specific activity of each radionuclide

The trophic (food chain) transfer factor (TTF), or the ratio of radionuclide concentration in fish to radionuclide concentration in prey (ghost shrimp), can be calculated as:

$$TTF = K_{eU}/K_{eL} \quad (7)$$

Where K_{eU} is the uptake rate constant, and K_{eL} is the loss rate constant, of a radionuclide from food, assuming a constant ingestion rate of food. The TTF is analogous to the CF (water). A value of TTF > 1 would indicate the possibility of biomagnification (over lower trophic levels in the food chain), whereas a value of < 1 would indicate a biodiminuation when the radionuclide is transferred from the lower trophic levels to the higher trophic level (Wang, 2002). The following model assumptions were made: (a) toadfish were continually exposed to a constant radionuclide activity (water or food), and (b) toadfish were of similar size, with no observed growth over the experimental period (i.e. no mass dilution of the acquired activity).

The assimilation efficiency, defined as the fraction of ingested metal(loid)s remaining in toadfish after emptying undigested material from the gut (waste), was calculated using the y-intercept method (Wang and Fisher, 1999):

$$y = AEe^{-\lambda t} \quad (8)$$

where y is the percentage of remaining radioactivity retained in toadfish after 48 h, AE is the assimilation efficiency, which was calculated as the y-intercept of the linear regression between the natural log ($\log_e$) of y and the time of loss (t) and λ is the biological loss rate constant ($days^{-1}$)

Differences amongst uptake and/or loss rate constants (regression slopes) for toadfish exposed to ^{109}Cd or ^{75}Se in water or food were tested using the generalised *F*-test (Ratkowsky, 1990). Where differences amongst slopes were significant, differences between slopes were then tested using *t*- tests among all pairs. All data were tested for normality (Shapiro-Wilks test) and homogeneity of variance (Levene's test), with model adequacy being met in all cases (Zar, 1996). Significance was tested at the P = 0.05 level.

6.3 RESULTS

The results of the speciation modeling analyses are outlined in Section 5.3.1.

6.3.1 Uptake of ^{109}Cd and ^{75}Se by whole toadfish

The uptake kinetics of 109Cd and 75Se from water or food by whole toadfish are shown in Figure 6.3, with fitted regressions based on single compartment model with first order kinetics. The shape of the uptake curves for whole toadfish indicate that equilibrium CFs were approximated over the course of the experiment (25 days) for both ^{109}Cd and ^{75}Se with water or food exposures. The linear (K_u) and exponential (K_e) uptake rate constants for ^{109}Cd and ^{75}Se in whole toadfish from water or food are given in Table 6.1. The uptake rate of ^{109}Cd by toadfish was six times higher ($P \leq 0.05$) from water than from food (Table 6.1, Figure 6.3a). There were no significant ($P > 0.05$) differences between the uptake rates of ^{109}Cd and ^{75}Se by toadfish from water. However, the uptake rate of ^{75}Se from food was nearly six times higher than ^{109}Cd, when compared on a molar basis.

Table 6.1 Uptake and loss rate constants for ^{109}Cd and ^{75}Se in whole toadfish from water or food.

	^{109}Cd			^{75}Se		
	Uptake		Loss	Uptake		Loss
	K_u[a]	K_e[b]	λ[c]	K_u[a]	K_e[b]	λ[c]
Water	0.17	0.028	0.017	0.15	0.027	0.015
Food	0.028	0.005	0.018	0.11	0.026	0.032

[a] Values represent the mean ± 95% confidence limits (n = 5–6).

[b] Ku values were calculated using a linear equation ($CF_t = K_u t$). Water is expressed as mL/g/day and food is expressed as mg/g/day.

[c] Ke values were calculated using an exponential rise equation ($CF_t = CF_{ss} (1- e^{-\lambda t})$ where CFss = K_e/λ . Water is expressed as mL/g/day and food is expressed as mg/g/day.

[d] λ values were calculated using an exponential decay equation ($A_t = A_0 e^{-\lambda t}$) and are expressed as day^{-1}.

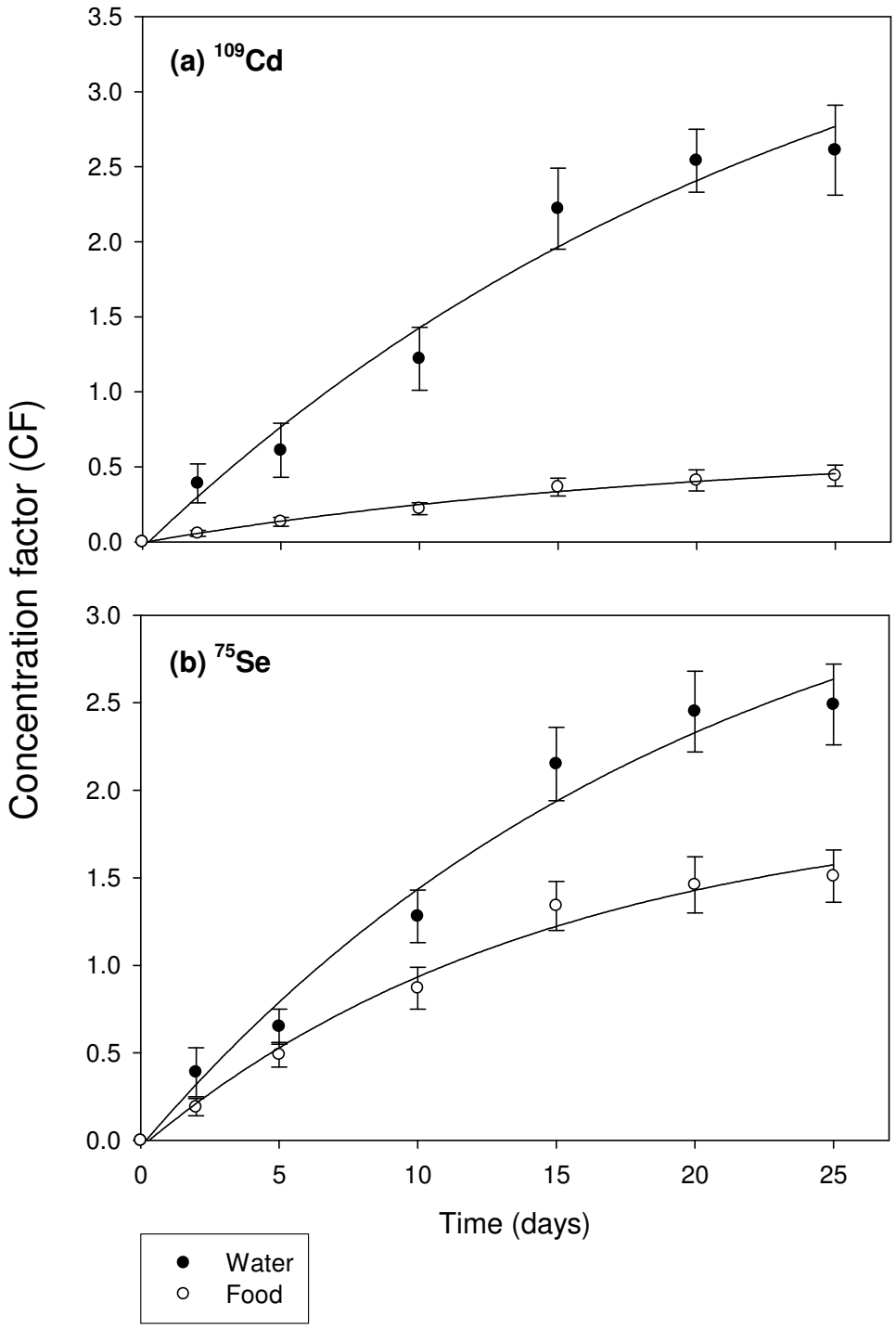

Figure 6.3 Uptake of (a) ^{109}Cd and (b) ^{75}Se from water (closed circles) and food (open circles) over 25 days. Each plotted point represents the mean ± standard error of concentration factors (CFs) determined in whole toadfish (n = 3) at each sampling time.

6.3.2 Loss of ^{109}Cd and ^{75}Se by whole toadfish

The loss kinetics of ^{109}Cd and ^{75}Se from water or food by whole toadfish are shown in Figure 6.4, with fitted regressions based on a two compartment model with

first order loss kinetics. The loss of ^{109}Cd and ^{75}Se from whole toadfish exposed to water or food was incomplete after 30 days. Toadfish exposed to ^{109}Cd in water had the greatest (73%) total loss (i.e. 27% remaining) after 30 days, whereas toadfish exposed to ^{75}Se in water had the lowest (52%) total loss (i.e. 48% remaining) (Figure 6.4). Toadfish exposed to ^{75}Se in food (67% loss: 33% remaining) and ^{109}Cd in food (55% loss; 45% remaining) were intermediate (Figure 6.4).

The loss rate constants (λ) for ^{109}Cd and ^{75}Se in whole toadfish from water or food are given in Table 6.1. There were no significant ($P > 0.05$) differences between the loss rates of ^{109}Cd by toadfish from water or food (Table 6.1, Figure 6.4a). Conversely, the loss rate of ^{75}Se from food was two times higher than from water, when compared on a molar basis (Table 6.1, Figure 4.6b). Furthermore, there were no significant ($P > 0.05$) differences between the loss rates of ^{109}Cd and ^{75}Se by toadfish from water; however, the loss rate of ^{75}Se from food was 2.5 times higher than ^{109}Cd, when compared on a molar basis (Table 6.1, Figure 6.4).

The loss of ^{109}Cd and ^{75}Se in whole toadfish was best described by a two compartment model (i.e. a short and a longer-lived compartment, Table 6.2). The biological half-lives of both radionuclides were similar in each compartment, but varied between compartments. In the short-lived, or rapidly exchanging, compartment, the biological half-lives of ^{109}Cd and ^{75}Se were 4–7 days and 3–11 days, respectively. In the longer-lived, or slowly exchanging compartment(s), the biological half-lives of ^{109}Cd and ^{75}Se were 39–41 days and 22–46 days, respectively (Table 6.2). The trends shown for the biological half-lives are an inverse of the loss rate constants.

Table 6.2. Biological half-lives (days) of ^{109}Cd and ^{75}Se in whole toadfish from water or food. Values for each compartment were estimated based on the patterns of loss over 30 days.

	^{109}Cd (1st) [a]	^{109}Cd (2nd)	^{75}Se (1st)	^{75}Se (2nd)
Water	4(3-5)	41(36-47)	3(2-4)	46(41-53)
Food	7(6-8)	39(34-44)	11(10-12)	22(19-25)

[a] Values represent the mean ± 95% confidence interval (n = 5–6). The biological half-life is the time (h) taken for half of the radioactivity in an organism to be lost. Macroinvertebrates exhibited two compartments [1st (short-lived) and 2nd (longer-lived)] in the loss phase.

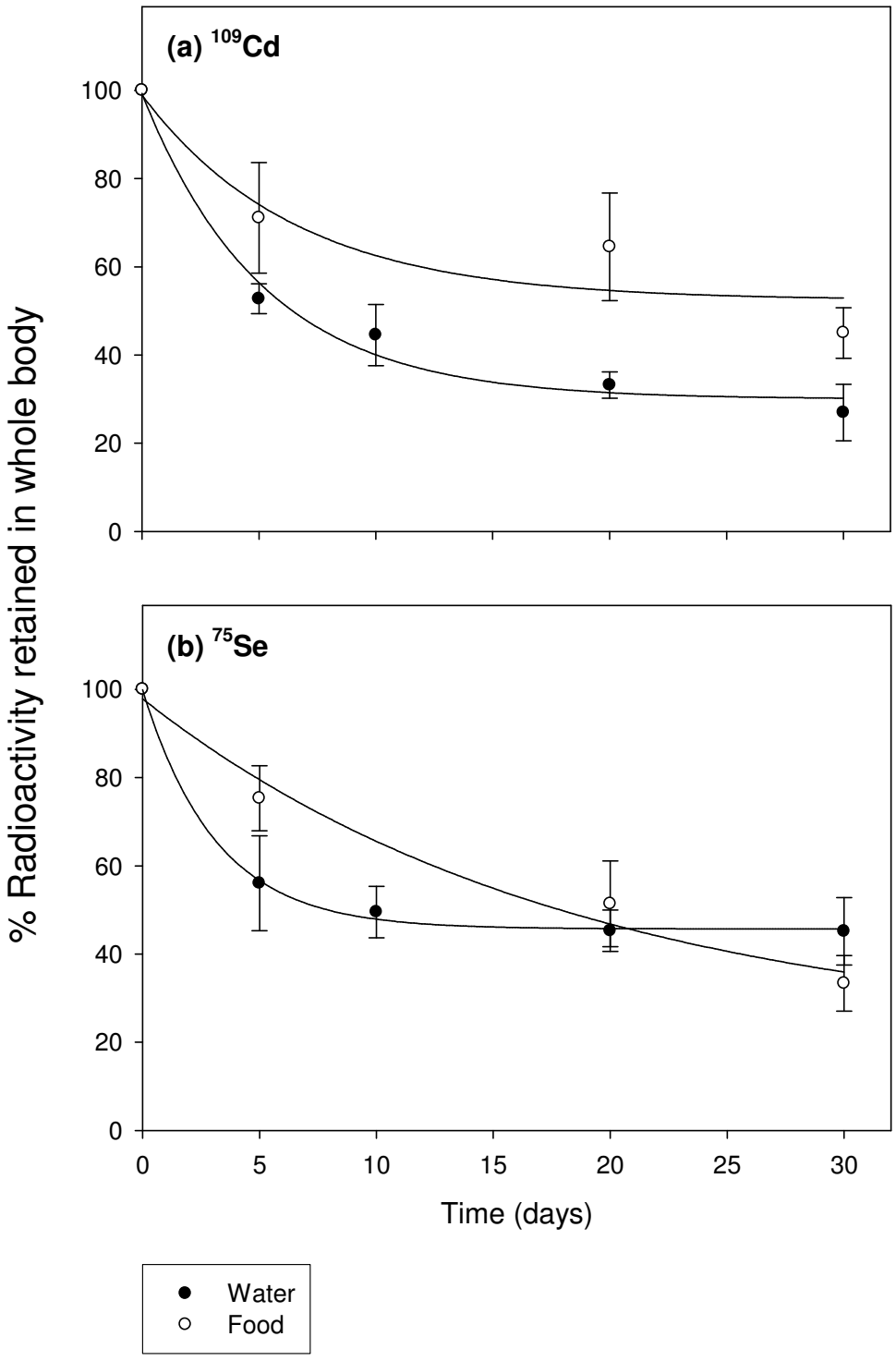

Figure 6.4 Percentage loss of (a) ^{109}Cd and (b) ^{75}Se over 30 days from water (opened circles) and food (open circles) exposures. Each plotted point represents the mean ± standard error of the percentage of retained radioactivity, relative to original radioactivity, in whole toadfish (n = 3) for each sampling time.

6.3.3 Distribution of ^{109}Cd and ^{75}Se in toadfish organs

The mean percentage distribution of ^{109}Cd (Figure 6.5) and ^{75}Se (Figure 6.6) in gut lining, liver, kidneys, gills, gonads and muscle from toadfish exposed to water or food, was plotted over time for both the uptake and loss phases. For both the water and food exposures, ^{109}Cd was predominantly located in the gut lining (60–75%) at the end of the uptake phase (Table 6.3, Figure 6.5). By the end of the loss phase, ^{109}Cd had predominantly shifted to the excretory organs - the liver (81%) in toadfish exposed to food and in the liver, gills and kidney (82% combined) of toadfish exposed to water (Table 6.3, Figure 6.5). The patterns of ^{75}Se distribution in toadfish organs were generally different to those observed for ^{109}Cd. For both the water and food exposures, ^{75}Se was predominantly located in the excretory organs (gills, kidneys and liver; 66–76% combined) at the end of the uptake phase, with minimal change in the percentage distribution (76–83%) throughout the loss phase (Table 6.3, Figure 6.6). Of the toadfish organs selected, the percentage of ^{109}Cd (1.6%) and ^{75}Se (4.3–5.7%) in toadfish muscle was lowest for both water and food exposures, with no apparent change in the mean percentage distribution of either radionuclide from the end of the uptake phase to the end of the loss phase (Table 6.3, Figure 6.6).

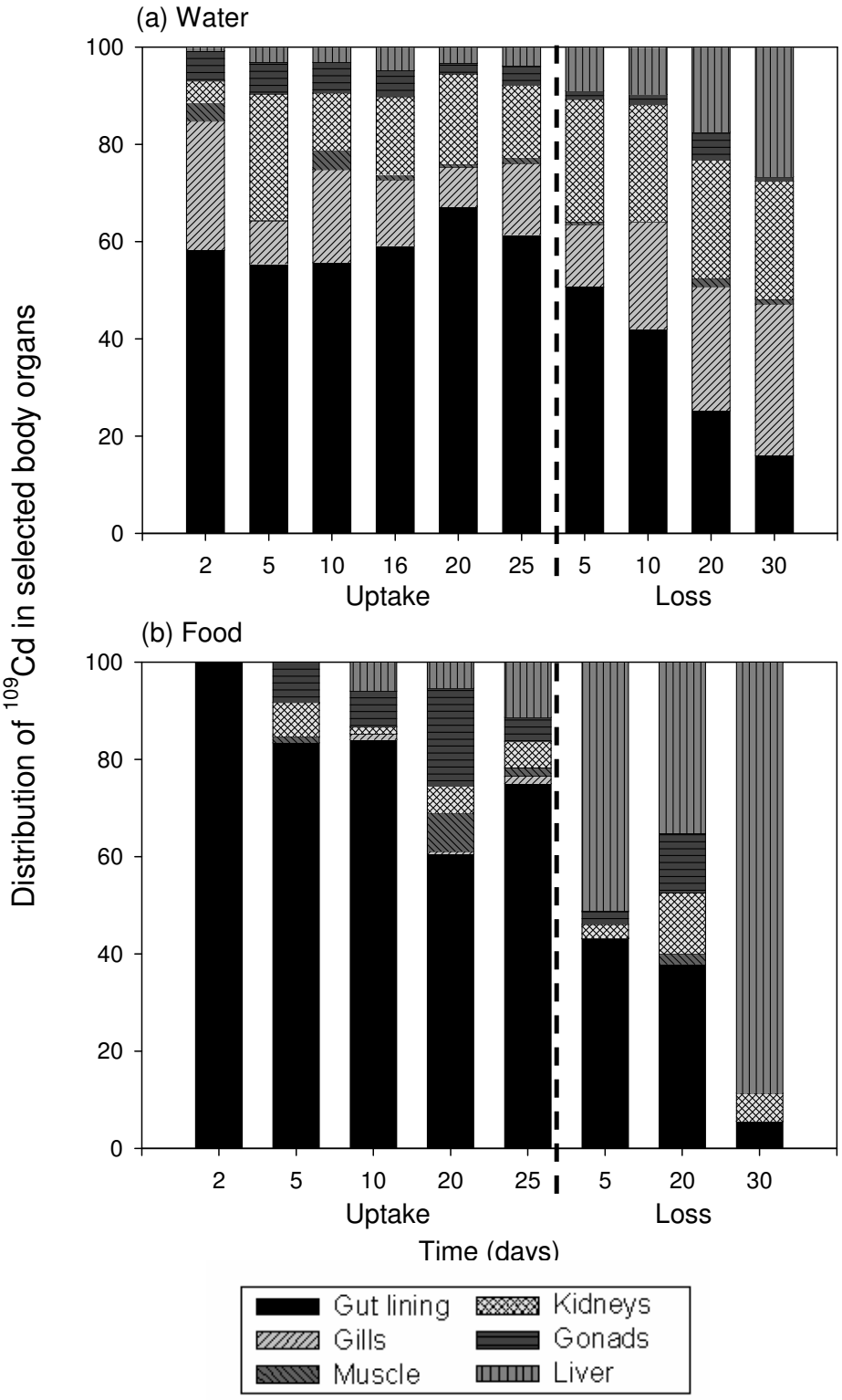

Figure 6.5 Mean percentage distribution of ^{109}Cd in selected toadfish organs (n = 3) from (a) water and (b) food exposures over the uptake (25 days) and loss (30 days) phases of the experiment. Vertical dashed line indicates transition between uptake and loss phases.

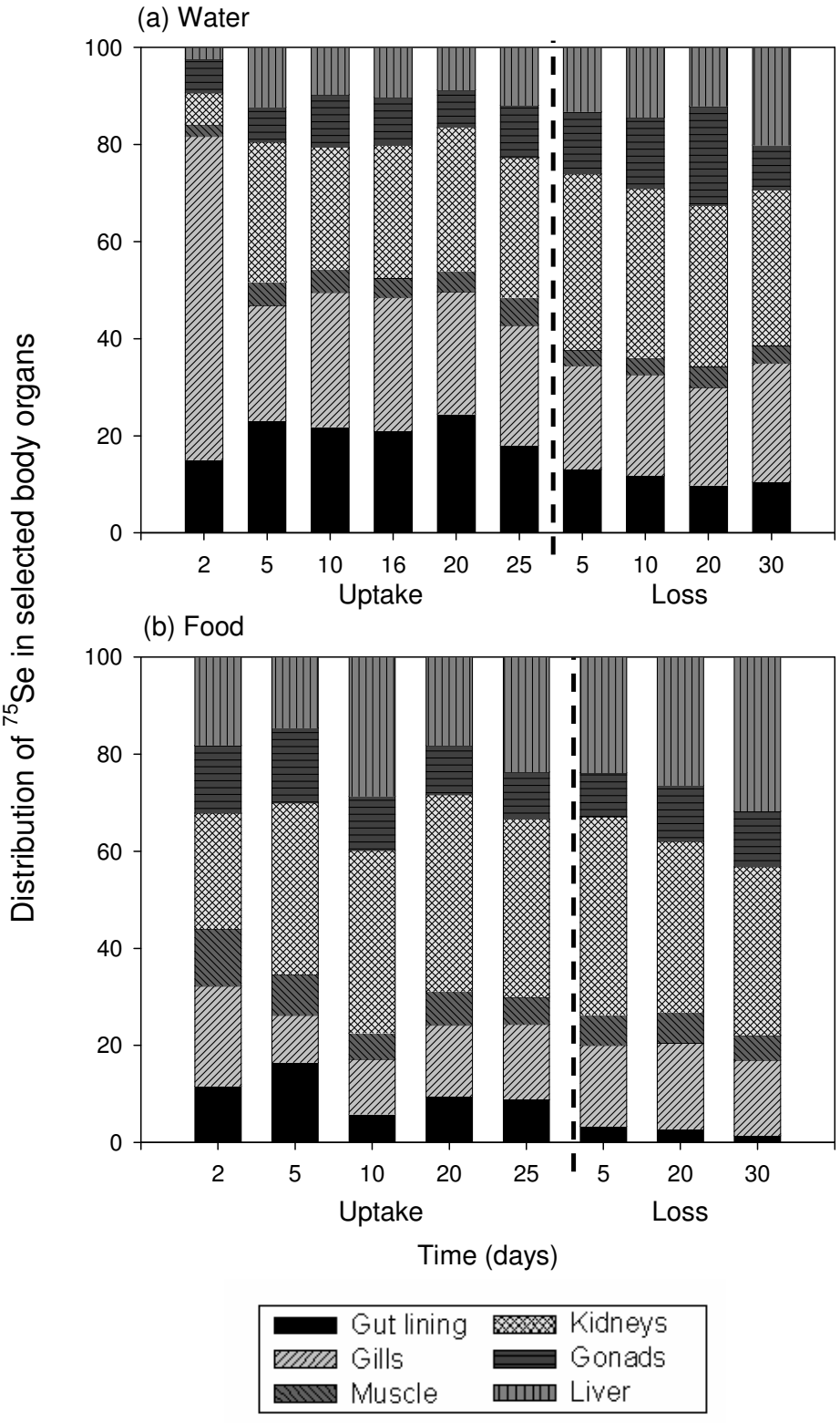

Figure 6.6 Mean percentage distribution of ^{75}Se in selected toadfish organs (n = 3) from (a) water and (b) food exposures over the uptake (25 days) and loss (30 days) phases of the experiment. Vertical dashed line indicates transition between uptake and loss phases.

Table 6.3 Mean percentage (%) activities of ^{109}Cd and ^{75}Se (kBq/g, wet weight) in toadfish organs at the end of the uptake and loss phases, following exposure to water or food.

	Gut Lining	Gills	Muscle	Kidneys	Gonads	Liver
Water ^{109}Cd						
End of uptake	61 ± 13	15 ± 3.3	1.6 ± 0.4	15 ± 3.1	4.2 ± 1.1	4.2 ± 1.1
End of loss	15 ± 3.1	29 ± 6.8	1.6 ± 0.5	24 ± 4.3	1.5 ± 0.4	29 ± 7.1
Food ^{109}Cd						
End of uptake	75 ± 18	1.6 ± 0.5	1.6 ± 0.5	5.4 ± 1.2	4.7 ± 1.1	12 ± 2.7
End of loss	6.8 ± 1.9	1.6 ± 0.6	1.6 ± 0.6	6.8 ± 1.5	1.6 ± 0.6	81 ± 19
Water ^{75}Se						
End of uptake	18 ± 3.4	25 ± 6.1	5.7 ± 1.4	29 ± 5.6	10 ± 3.3	12 ± 2.5
End of loss	11 ± 2.3	24 ± 4.2	4.3 ± 1.0	33 ± 5.1	8.7 ± 2.7	19 ± 4.5
Food ^{75}Se						
End of uptake	8.8 ± 2.1	15 ± 2.8	5.6 ± 1.3	37 ± 6.9	9.6 ± 2.0	24 ± 4.2
End of loss	1.0 ± 0.5	16 ± 3.4	5.2 ± 1.3	35 ± 7.2	11 ± 2.1	32 ± 6.7

[a] Values represent the mean ± standard error (n = 3).

6.4 DISCUSSION

The CF of ^{109}Cd in toadfish exposed to water (CF = 2.6) was nearly twice higher than reported by Jeffree *et al.* (2006) for spotted dogfish (*Scyliorhinus canicula*; CF = 1.6) and turbot (*Psetta maxima*; CF = 1.5), similar to that reported by Zhang and Wang (2005) for black bream (*Acanthopagrus schlegeli*) (CF ~ 2.5), and twice lower than that reported by Xu and Wang (2002) for the mangrove snapper (*Lutijanus argentimaculatus*) (CF = 5). The CF of ^{109}Cd in toadfish exposed to food (CF = 0.4) was consistent with that reported by Zhang and Wang (2005) for black bream (CF ~0.4). Very few data are available for Se. The CF of ^{75}Se in toadfish exposed to water (CF = 2.5) was slightly lower than that reported by Xu and Wang (2002) for the mangrove snapper (CF = 3.0).

The uptake rate of ^{109}Cd by toadfish from water (K_u = 0.17 mL/g/day; Table 6.1) was similar to turbot (K_u = 0.13), but 2–3 times lower than spotted dogfish (K_u = 0.45) (Jeffree *et al.* 2006), 15 times lower than the grunt (*Terapon jarbua*) (K_u ~2.5) (Zhang and Wang, 2005) and black bream (Long and Wang 2005), and 30 times lower than

the mangrove snapper (K_u ~5.1) (Xu and Wang, 2002). The uptake rate of ^{109}Cd in toadfish from food (K_u = 0.028 mg/g/day; Table 6.1) was about 80 times lower than black bream (K_u ~2.2) (Zhang and Wang, 2005). In the only other study that has determined the uptake rate of ^{75}Se in estuarine/marine fish, Xu and Wang (2002) found that the mangrove snapper had an uptake rate about five times higher (K_u = 0.8) than toadfish (K_u = 0.15) from water. Xu and Wang (2002) found that uptake rate constants were relatively independent of the metal(loid) concentration in the exposure medium. The variation in uptake rates may be explained by differences in (a) the drinking and/or gill ventilation rates amongst these fishes (Perrot et al., 1992), (b) the bioavailability of metal(loid)s stored in the prey eaten (Wang, 2002) or (c) prey ingestion rates.

Biphasic loss, indicating a short-lived and a longer-lived compartment, was observed for ^{109}Cd and ^{75}Se in toadfish for both water and food exposures (Table 6.2). The loss rate constants of ^{109}Cd and ^{75}Se in toadfish were inversely related to their biological half-lives, so further discussion will address the latter only for the longer-lived or slowly exchanging compartment. The biological half-life of ^{109}Cd in toadfish exposed to water (42 days; Table 6.2) was similar to that of turbot (35 days; Jeffree *et al.*, 2006) and twice lower than that of dogfish (86 days: Jeffree *et al.,* 2006). The biological half-life of ^{109}Cd in toadfish exposed to food (39 days; Table 6.2) was 2–3 times higher than that of mangrove snapper (15 days: Xu and Wang, 2002). The biological half-life of ^{75}Se in toadfish exposed to water (46 days; Table 6.2) was twice higher than that of mangrove snapper (26 days; Xu and Wang, 2002). The biological half-life of ^{75}Se in toadfish exposed to food (22 days; Table 6.2) was identical to that of mangrove snapper (22 days: Xu and Wang, 2002). Measured differences in the loss rates, or biological half-lives, of metal(loid)s may be largely attributed to differences in the distribution dynamics of metal(loid)s in individual organs after exposure.

A limited number of studies have investigated the comparative accumulation of metals from water and food in estuarine or marine fish (Milner 1982; Willis and Sunda 1984; Xu and Wang 2002; Zhang and Wang 2005). In toadfish, the uptake rates of ^{109}Cd from water and food were almost identical (Table 6.1). This trend was also evident for the loss rates (Table 6.1), indicating an equal contribution of water (50%) and food (50%) exposures to the overall accumulation of ^{109}Cd by toadfish. Zhang and Wang (2005) found that ^{109}Cd accumulated in black bream (*Acanthopagrus schlegeli*) and grunt (*Terapon jarbua*) was predominantly (75–89%) from water (11–25% from food). Xu and Wang (2002) found that the relative contribution of water and food as a source of ^{109}Cd in the predatory mangrove

snapper (*Lutjanus argentimaculatus*) was dependent on the type of food (prey). When zooplankton were the main prey item, > 70% of ^{109}Cd was accumulated from food (< 30% from water), whereas when planktivorous fish were the main prey item, <20% of ^{109}Cd was accumulated from food (> 80% from water).

In contrast to the results for ^{109}Cd, the uptake rates of ^{75}Se in toadfish from water and food were markedly different, with the uptake rate of ^{75}Se from water being 11 times higher than food (Table 6.1). With the loss rates of ^{75}Se in food being twice that in water, it is apparent that water is the predominant source (> 90%) of ^{75}Se in toadfish. This finding is in contrast to that of Xu and Wang (2002), who reported that food was the predominant (> 90%) source of ^{75}Se accumulation in the mangrove snapper. Milner (1982) reported that up to 50% of Zn accumulated in plaice (*Pleuronectes platessa*) was from water, whereas Willis and Sunda (1984) found that Zn was accumulated in mosquitofish (*Gambusia affinis*) and spot (*Leiostomus xanthurus*) predominantly (80%) from their prey (food). Given that the speciation, and hence potential bioavailability, of Cd and Se in oxic seawater is relatively constant, observed differences in the relative contributions of water and food in the accumlation of these metal(loids) are governed largely by the physiology of the fish and the type of prey eaten (Wang 2002; Xu and Wang 2002).

The CF of ^{109}Cd in toadfish exposed to water (CF = 2.3) was slightly higher than those reported by Jeffree *et al.* (2006) for spotted dogfish (*Scyliorhinus canicula*; CF = 1.6) and turbot (*Psetta maxima*; CF = 1.5), similar to that reported by Zhang and Wang (2005) for black bream (CF ~ 2.5), and about half that reported by Xu and Wang (2002) for the mangrove snapper (CF = 5). The CF of ^{109}Cd in toadfish exposed to food (CF = 2.2) was about six times higher (CF ~0.4) than that reported by Zhang and Wang (2005) for black bream. Very few data are available for Se. The CF of ^{75}Se in toadfish exposed to water (CF = 2.9) was almost identical to that reported by Xu and Wang (2002) for the mangrove snapper (CF = 3.0).

A limited number of studies have investigated the comparative accumulation of metal(loid)s, including Cd and Se, from water and food in estuarine/marine fish (Xu and Wang, 2002; Zhang and Wang, 2005). Toadfish accumulated ^{109}Cd predominantly from water (85%), with only a minor contribution from food (15%) (Figure 6.3a). This finding is consistent with that of Zhang and Wang (2005) who also found that ^{109}Cd was accumulated predominantly from water (75–89%) in black bream and grunt, with only a small contribution from food (11–25%). Xu and Wang (2002) found that the relative contribution of water and food as a source of ^{109}Cd in the predatory mangrove snapper was dependent on the type of food (prey). When zooplankton were the main prey item, > 70% of ^{109}Cd was accumulated from food (<

30% from water), whereas when planktivorous fish were the main prey item, <20% of ^{109}Cd was accumulated from food (> 80% from water). Toadfish accumulated ^{75}Se predominantly from water (58%), but food (42%) comprised an appreciable, but smaller contribution. This finding is in contrast to that of Xu and Wang (2002), who reported that food was the predominant (> 90%) source of ^{75}Se accumulated in the mangrove snapper, as well as studies with other estuarine/marine biota (Reinfelder *et al.,* 1998). Was the uptake of ^{75}Se from (i) ghost shrimp lower or (ii) water higher, than expected? While no definitive explanations can be given here without further investigation, a few empirical observations can be made, and hypotheses proposed.

Given that the uptake rate and CF of ^{75}Se in toadfish from water is not elevated compared to previous work, and that the speciation, and hence potential bioavailability, of Cd and Se in oxic seawater is relatively constant (Byrne, 2002; Hamilton, 2004), it may be that ^{75}Se absorption from food was lower (about a factor of two) than expected. In comparison to other predatory estuarine/marine fishes, such a snapper, black bream and grunt, the gut length in toadfish is 2–5 times shorter, lacking a pronounced foregut and hindgut (Alquezar, unpublished data). This may explain, in part, the lower assimilation efficiencies of ^{109}Cd (4.8%) and ^{75}Se (20%) in toadfish compared to other predatory estuarine/marine fishes (^{109}Cd, 6.2–20%, Xu and Wang, 2002; Long and Wang, 2005; Zhang and Wang, 2005: ^{75}Se, 32–62%, Baines and Fisher, 2002; Xu and Wang, 2002).

The bioavailability, or chemical form, of stored metal(loid)s in the organs of prey also influences metal(loid) assimilation efficiency by predators (Wang and Fisher, 1999a; Wang, 2002. Metal(loid)s associated with organelles and cytosolic proteins (e.g. metallothioneins) in some prey have a higher bioavailability to consumers than metal(loid)s bound to insoluble components (e.g. granules) (Reinfelder and Fisher, 1994; Wallace and Luoma, 2003). Xu and Wang (2002) found that the assimilation efficiency of ^{75}Se was twice as high for two species of molluscs as it was for two species of crustaceans. Hence, it may be possible that Cd and Se in ghost shrimps (crustaceans) used in the present study were assimilated in a manner that may not be representative of other key toadfish prey items. This hypothesis could be assessed in further studies by measuring the uptake and loss kinetics of 3–4 other key species of toadfish prey, such as bivalves, crabs and polychaetes (Chapter 5). It may be also possible that a daily feeding rate of 7–10% toadfish body weight, which typically exceeds the maintenance requirements of most fish (Pauly 1989), was insufficient, or not suitable for a relatively active species such as toadfish. This species may compensate for a reduced gut length by ingesting higher rates of prey

or having a preference for prey with higher nutritive value. Further work may evaluate these hypotheses.

Cadmium-109 was predominantly located in the gut lining (60–75%) of toadfish exposed to water or food at the end of the uptake phase (Figure 6.5, Table 6.3). By the end of the loss phase, ^{109}Cd had predominantly shifted to the excretory organs - the liver (81%) in toadfish exposed to food, and the liver, gills and kidney (82%) of toadfish exposed to water (Figure 6.5, Table 6.3). These results are generally consistent with previous studies of estuarine/marine fish by Eisler (1971), Baines and Fisher (2002) and Jeffree *et al.* (2006). Eisler (1971) found that ^{115}Cd in the gut of mummichogs (*Fundulus heteroclitus*) exposed to water decreased from 36% at the end of the uptake phase to 2% at the end of the loss phase, while ^{115}Cd in the liver increased from 8% at the end of the uptake phase to 66% at the end of the loss phase. Jeffree *et al.* (2006) found that ^{109}Cd in the gut of turbot exposed to water decreased from 53% at the end of the uptake phase to 33% at the end of the loss phase, while ^{109}Cd in the liver and kidney increased from 8% at the end of the uptake phase to 14% at the end of the loss phase. This may explain why the highest amounts of Cd were present in the liver of toadfish from field study sites (Chapters 2 and 4).

Xu and Wang (2002) and Long and Wang (2005) investigated the distribution of ^{109}Cd in organs of the mangrove snapper and black bream, respectively, from both water and food. Both studies did not examine gut lining as a specific organ, but found that ^{109}Cd (80%) was predominantly located in the viscera (such as gut, liver and kidneys) following uptake from food. However, notable differences were evident between the two studies in the distribution of ^{109}Cd in organs of fish exposed to water. Xu and Wang (2002) found that ^{109}Cd was evenly distributed in the viscera (36%), muscle/bone (33%) and gills (31%), whereas Long and Wang (2005) found that ^{109}Cd was predominantly located in the viscera (60%). Neither study investigated changes in the distribution of ^{109}Cd over the loss phase. In the present study, muscle had the least ^{109}Cd, relative to other organs (Table 6.3), probably due to the fact that it represents the largest portion (by mass) of the body. The higher percentage of Cd found in the viscera of fish is most likely due to the liver and kidney being the main sequestration and detoxification organs, with metallothionein being an important chelating molecule (De Smet *et al.* 2001; Roesijadi and Robinson 1994). Long and Wang (2005) found a significant correlation ($r = 0.78$) between metallothionein and Cd concentrations in the liver of black bream. Studies to date clearly indicate that the viscera (particularly the gut), rather than gills, is the primary uptake site for Cd, since estuarine/marine fish drink considerable quantities of water to prevent dehydration by

the hyperosmotic environment (Joblings, 1995). This is in contrast to data reported for freshwater fish, which take up Cd mainly via the gills (Szebedinszky *et al.* 2001).

The distribution of ^{75}Se in toadfish organs was typically different to that observed for ^{109}Cd. For both the water and food exposures, ^{75}Se was predominantly located in the excretory organs (66–76%; gills (15–25%), kidneys and liver (41–61%)), rather than the gut lining (9–18%), at the end of the uptake phase, with little change in percentage distribution (76–83%) by the end of the loss phase (Table 6.3; Figure 6.6). In the only other study of ^{75}Se distribution in the organs of an estuarine/marine fish, Xu and Wang (2002) found that most ^{75}Se was located in the muscle/bone (58%) with smaller, but equal, amounts in the viscera (22%) and gills (20%). This is in contrast to the present study that found only 6% of ^{75}Se associated with muscle. Unlike the present study, Xu and Wang (2002) did not investigate changes in ^{75}Se distribution during the loss phase.

Differences in distributions of the two metal(loid)s amongst the selected toadfish organs may be due to differences in their physicochemistry. Selenium is a class A metalloid that reacts readily with oxygen (selenates, Se^{6+}; selenites, Se^{4+}; Hamilton, 2004). Conversely, Cd is a group B metal that reacts readily with sulphur and nitrogen, which are commonly associated with proteins (Bell et al., 2002). This may explain, in part, why the patterns of accumulation of ^{75}Se were different from ^{109}Cd in toadfish. Furthermore, cadmium (Cd^{2+}), which shares certain physicochemical similarities (e.g. ionic radii and valency) with calcium (Ca^{2+}), has the ability to cross cell membranes via Ca channels in the gut (Rainbow and Black, 2005). This may further explain why ^{109}Cd was found more predominantly in toadfish gut than ^{75}Se (Table 6.3; Figures 6.5 and 6.6).

The Trophic Transfer Factor (TTF) is useful for evaluating the magnitude of metal(loid) biotransfer in a specific food chain, as well as the contribution of dietary metals to total metal bioaccumulation. The TTF of ^{109}Cd in toadfish was 0.3, which is at the lower end of the range (TTF = 0.1–0.9) reported for estuarine/marine fishes (Suedel *et al.* 1994; Xu and Wang 2002). Biomagnification of Cd (TTF>1, or when the metal concentration in toadfish is higher than that in its prey) is rarely reported, but may occur, for predatory fish that assimilate Cd with > 20% efficiency from their prey (Wang, 2002; Xu and Wang, 2002). High Cd assimilation efficiencies may occur if a large proportion of Cd exists in the cytoplasmic fraction (most bioavailable form) of ingested food (Reinfelder and Fisher, 1994; Wallace and Luoma, 2003).

In contrast to Cd, the TTF of ^{75}Se in toadfish was higher at 0.8, but this value is also at the lower end of the range (TTF = 0.3–2.2) reported for marine fishes (Suedel et al., 1994; Wang, 2002; Xu and Wang, 2002). Biomagnification of Se

(TTF>1) is known to occur for predatory fish that assimilate Se with > 30% efficiency from their prey (Xu and Wang, 2002). The mechanism by which fish regulate metal(loid)s from their prey (food) items is poorly known and could involve concentration-dependent changes in the AE across the gut, or adaptive changes in the elimination/loss (K_e) pathways. The extent of regulation may also vary among different tissues/organs (Wang, 2002).

6.5 SUMMARY

This study demonstrated that toadfish accumulated ^{109}Cd, and to a lessor extent ^{75}Se, predominantly from water (58–85%), with a smaller contribution from food (15–42%). These results are consistent with previous studies for ^{109}Cd, but differ to those of previous studies for ^{75}Se, whereby the contribution of ^{75}Se from food in the present study was lower (about a factor of two) than expected. Further work is required to better understand the result for ^{75}Se. The distribution of metal(loids) in fish organs is important for better understanding metal(loid) kinetics and their subsequent toxicity. Toadfish exposed to ^{109}Cd in both water and food showed higher activities in the gut lining during the uptake phase followed by a shift in distribution from gut lining at the end of the uptake phase to the excretory organs, such as liver, by the end of the loss phase. This observation suggests that the main uptake pathway for water exposure was via the gut and not the gills, and possibly due to fish drinking large amounts of water to maintain osmoregulation. In contrast to ^{109}Cd, there was no appreciable shift in the organ distribution of ^{75}Se from the end of the uptake phase to the end of the loss phase, being mostly associated with the excretory organs (gills, liver and kidneys). The TTF of ^{75}Se (0.8) in toadfish was nearly three times higher than that of ^{109}Cd (0.3), but there was no indication that either metal(loid) was biomagnified (TTF > 1).

7.0 GENERAL DISCUSSION

7.1 MAJOR FINDINGS

Many studies have investigated metal concentrations in sediments and water of estuaries and freshwater systems worldwide (Birch and Taylor 1999, 2002a; Irvine and Birch 1998; Peters *et al.* 1999a; Roy and Crawford 1984). However such investigations may not fully assess bioaccumulation or effects of metals on biota (Chapman *et al.* 1998). Aquatic organisms can accumulate metals from point and diffuse sources (Birch *et al.* 1996). Physical and chemical factors acting external to organisms and biological factors acting on the surface and within organisms can affect bioaccumulation. These factors include complexation/competition between metals and ions and diffusion of metals through lipid and protein membranes and calcium channels (Borgmann 2000; Bushby 1998; He *et al.* 2001; Newman 1998). Other factors that can influence bioaccumulation include contaminant exposure route and bioavailability of metals to organisms (Boisson *et al.* 2003; Peters *et al.* 1999c; Selck and Forbes 2004; Wang and Fisher 1999). The primary objectives of this study were to investigate the effects of contaminants on the physiology, and subsequent transfer of contaminants in common estuarine biota, using an ecotoxicological approach incorporating field and laboratory techniques.

7.1.1 Metals in toadfish and the environment

Physico-chemistry of surrounding waters and surface sediments are important in metal bioavailability and can influence metal uptake (Playle, 1998). Although there were similarities in water and sediment physico-chemistry among estuaries in the present study, there were significant differences in sediment and toadfish tissue metal concentrations (Chapter 2). Differences among sediment metal concentrations, and subsequent toadfish metal concentrations, may be attributed to differences in point-source and diffusive inputs from various industries surrounding the different estuaries, with highest metal loads observed in the urban impacted estuaries of Lake Macquarie and the Parramatta River.

Metal concentrations in toadfish tissues were correlated with metal concentrations in surface sediments (Chapter 2 & 4). Previous studies have shown the main metal uptake pathways of fish are via the gills and through the guts as a result of sediment and/or food ingestion (Baudin *et al.* 1994; Garnier-Laplace *et al.* 2000; Kraal *et al.* 1995). Therefore, metal uptake via diet and water was investigated to ascertain the most important accumulation pathtway in toadfish (Chapter 6). The

results of this investigation indicated that both dietary and dissolved metal uptake could influence body burden, however the main exposure route for both was through the gut and not the gills, as has been previously been suggested in other studies (Roesijadi and Robinson 1994). This suggests that exposure through the gut was a result of direct ingestion of contaminated food and to a greater extent, contaminated water, as a consequence of elevated drinking rates due to osmoregulation (Chapter 6).

Liver tissue metal concentrations were elevated in toadfish from contaminated environments (Chapter 2 and 4). The liver is the main organ used for metal homeostasis (Heath, 1995) and has the ability to reduce metal toxicity and cellular damage by binding metals to nuclear proteins, such as metallothioneins (Cherian and Nordberg 1983). Liver uptake is likely to be a result of both dietary and water exposure (Bervoets *et al.* 2001). Cadmium-109 was accumulated in the liver of toadfish following uptake via dietary and dissolved exposure (Chapter 6). Bervoets *et al.* (2001) found a similar trend in metal accumulation in liver tissue of the Three Spined Stickelback, *Gasterosteus aculeatus*, following dietary and dissolved exposure. Selenium-75 exhibited a similar trend in toadfish liver, however this metal was also elevated in kidneys and gills, which are target organs for final deposition and metal regulation (Bervoets *et al.* 2001; Cherian and Nordberg 1983; Heath 1995). Toadfish collected in the field exhibited similar elevated concentrations of Se in the excretory organs (Chapter 2 & 4). This suggests that Cd and Se (as well as other metals) are taken up by different pathways and sequestered/stored at different concentrations, explaining the variation in toadfish tissue metal concentrations among the estuaries over time (Chapter 2).

Tissue body burden also differed between toadfish gender, perhaps as a result of a combination of factors, such as dietary preferences (Parks and Curtis 1997), foraging behaviour (Peakall and Burger 2003) or physiological metabolism in relation to reproductive strategies (Heath 1995; Olsson *et al.* 1996), with the latter being the most likely. Differences in concentrations of sex hormones, and sex-linked lipids and proteins between male and female fish have been found to influence metal uptake in various fish tissues (Brooks *et al.* 1997). There were no significant differences in prey item selection between male or female toadfish (Chapter 3), further supporting the hypothesis that gender metal differences may be due to reproduction-linked variables.

7.1.2 Effects of metals on growth, health and reproduction

Toadfish tissue (muscle and liver) concentrations of some metals (Co, Cd & Pb; Chapter 3) were negatively related to lipid concentrations, particularly in the metal impacted estuaries. Lipids are essential for energy storage, metabolism, growth and reproductive development (Brooks *et al.* 1997; Weis *et al.* 2001). Metal regulation involves active and passive mechanisms, which require energy (Newman 1998; Phillips 1995). The main form of energy from fish comes from lipids (Heath 1995). This suggests that fish from metal contaminated estuaries are expending energy for metal regulation rather than growth and development. Lipids in gonads were negatively associated with elevated metal concentrations in the gonads. Essential lipids have been linked to egg quality and embryo survival (Brooks *et al.* 1997; Carrillo *et al.* 1995; Harel *et al.* 1994), suggesting that reduced quality/quantity of lipids can affect reproductive output in teleosts. Studies have shown that juvenile fish that hatched from smaller eggs had reduced survivorship (Blaxter and Hempel 1963; Booth and Alquezar 2002; Moodie *et al.* 1989; Wootton 1994). In the present study, reduced lipid concentrations and smaller egg size and density in female toadfish from metal contaminated estuaries may have lead to disadvantages in growth and survival.

Protein concentrations were positively associated with elevated metal concentrations in fish tissues (Chapter 3). Elevated concentrations of stress proteins (heat stress proteins and metallothioneins), used for cellular damage/tissue repair mechanisms, have been attributed to elevated metal concentrations, (Hamer *et al.* 2004; van der Oost *et al.* 2003). Therefore, toadfish may be using proteins for metal regulation also. Toadfish may also not be investing protein in growth and development, as toadfish from the metal impacted estuaries were smaller than toadfish from the reference estuaries (Chapter 3). However, an investigation into the relationship between toadfish growth and prey item nutritional value and metal concentrations at an impacted estuary showed contrasting result (Chapter 4), suggesting localised variation in metabolic and regulatory mechanisms. Polychaetes, commonly consumed by toadfish, contained elevated metal concentrations at the metal impacted sites (Chapter 4) of the Parramatta River, as well as higher protein and lipid content. Toadfish from the same impacted sites were 15% larger and 41% heavier than similarly aged toadfish from the least contaminated sites. This suggests that the higher lipids and protein content in polychaetes may contribute to size of toadfish due to better growth efficiency stemming from lower energy expenditure/metabolism, which may be of genetic origin (Hawkins and Day 1996), having developed physiological or genetic adaptation due to long term exposure to

elevated metal concentrations in their dietary prey items. However this hypothesis was not investigated in this study and should be addressed with caution. Toadfish growth and condition may be non-related and site specific, occurring on a local population level. Results should be verified with experimental manipulations.

7.1.3 Exposure routes to macroinvertebrates and smooth toadfish

Toadfish tissue metal concentrations were positively correlated to both sediments and their dietary prey items (Chapter 4). The results were consistent with results from other studies (Peters *et al.* 1999a; Peters *et al.* 1999b). In the present study, relationships between infauna, sediments and toadfish may be attributed to direct ingestion of sediments or indirect bioaccumulation through ingestion of contaminated dietary prey items (Chapter 4). However, the level of bioaccumulation may be also influenced by the type of prey items consumed, since different species may have different metal regulation strategies (Amiard *et al.* 2006; Luoma and Rainbow 2005; Rainbow 2002; Wang and Fisher 1999). Some macroinvertebrates excrete metabolically-active metals at the same rate they are accumulated, others accumulate and sequester metals in granule-like structures and/or metallothioneins (Amiard *et al.* 2006), which may act as long term sinks and thus bioaccumulate to predators (Rainbow 2002), hence the relative importance of each pathway, as well as the bioavailability of each metal, will influence the potential transfer factor to higher order predators (Boisson *et al.* 2003; Peters *et al.* 1999c; Selck and Forbes 2004; Wang and Fisher 1999).

In Chapter 5, key benthic prey items (semaphore crabs, soldier crabs, ghost shrimp, pygmy mussels and polychaetes) of toadfish were exposed to dissolved ^{109}Cd and ^{75}Se under controlled experimental conditions to determine uptake and loss kinetics. The uptake rates, concentration factors (CF's) and biological half-lives were highest/longest in pygmy mussels, ghost shrimp and polychaetes compared to semaphore and soldier crabs for both ^{109}Cd and ^{75}Se (Chapter 5). This indicates that differences in bioaccumulation and metal regulation strategies among toadfish prey items may influence bioaccumulation in toadfish. However, metal accumulation by toadfish prey items was only considered from the dissolved phase. It is recommended that other metal uptake pathways such as diet and/or particulates in macroinvertebrate prey items be investigated in order to determine the importance of each exposure route and subsequent trophic transfer to predators.

Dietary and dissolved metal exposure pathways were investigated in the smooth toadfish (Chapter 6). Toadfish accumulated ^{109}Cd from water and food in equal amounts using ghost shrimp as prey items, however ^{75}Se was predominately

taken up (>60%; Chapter 6) via water. This result is in contrast to a number of other studies using different fish species and food types (Willis and Sunda 1984; Xu and Wang 2002) suggesting that differences in fish physiology and/or type of food being eaten may account for observed differences in results. Differences in fish physiology, metal speciation, exposure pathways and metal regulation strategies will influence the extent and distribution of metal bioaccumulation to organisms, hence a sound knowledge in metal uptake pathways and trophic transfer is essential to better understand metal transfer dynamics and subsequent toxicity to aquatic biota.

7.2 MANAGEMENT IMPLICATIONS

Coastal estuaries are important ecosystems that host a number of crucial habitats including seagrass meadows, rocky substrate, mangrove forests, mud flats, and saltmarsh habitats, providing food and shelter for a number of commercially and recreationally important animals (Hindell 2006; Laegdsgaard and Johnson 2001; Paterson and Whitfield 2000; Pusey and Arthington 2003). Rivers and estuaries are also a common ground for juvenile and adult fishes, connected to other important marine and freshwater habitats (Able 2005; Ray 2005; Secor and Rooker 2005). With increasing pressure of changing land-use, reduced freshwater flows, increased nutrient and contaminant input and rates of sedimentation, many estuaries along the Australian coastline have declined in health (Birch *et al.* 1999a; Irvine and Birch 1998; Peters *et al.* 1999b; Roy and Crawford 1984).

Water and sediment quality guidelines have been implemented as part of a national water quality management strategy (ANZECC/ARMCANZ 2000). Sediments and sediment-water interfaces are considered important biologically, since many organisms inhabit them, however, investigating contaminant concentrations in sediments alone does not constitute evaluation of biological significance (Chapman 2002b). As such, the guidelines do not relate appropriately to organisms or the extent of trophic transfer to higher order predators. This investigation has provided baseline information on sediment contamination, invertebrate bioaccumulation and subsequent transfer to toadfish, and effects on condition and reproduction as a consequence of toxicity to fish in the field. Toadfish that inhabit metal contaminated estuaries, were found to have lower condition, reduced growth and longevity and a lower reproductive output (Chapter 3).

Reproduction is directly related to individual fitness, and indirectly is important for maintaining population size and survival of the species (Jones and Reynolds 1997; McHugh and Rouse 1998). Contaminants can have direct and indirect effects on populations and communities. A reduction in fish sperm and oocyte quantity and/or quality can result in larval deformities and impaired development, reducing the chances of survival to larval fish (Brooks *et al.* 1997). Furthermore, reduced egg quality (condition), smaller egg sizes and density, as has been demonstrated in this study (Chapter 3), may lead to disadvantages in growth and survival, thus affecting sustainability of future populations. Changes to populations can have a detrimental effect on community structure and ultimately ecosystem function (Frank *et al.* 2005; Power *et al.* 1996).

7.3 FUTURE DIRECTIONS

This investigation has given rise to a number of further questions:

- *Are toadfish physiologically acclimating or genetically adapting to metal contaminated sites?*

This could be tested by collecting toadfish from metal contaminated and reference sites/estuaries and simultaneously exposing them to dietary and/or dissolved metals to evaluate differences in metal accumulation between toadfish populations. This experiment could suggest possible mechanisms. The second step would be to breed toadfish from the metal contaminated and reference sites/estuaries and use second/third generation offspring to investigate changes in genetic traits.

- *Does toadfish diet have an effect on metal uptake between male and female fish?*

A controlled uptake and loss kinetics experiment (as per chapter 6) using even numbers of male and female toadfish that are fed the same dietary items would be required to determine whether gender differences are associated with diet.

- *Are there differences in trophic transfer (TTF) factors among organisms with different feeding strategies?*

Other metal accumulation pathways and kinetics, such as diet/particulates, should be investigated in prey species to determine the TTF to higher order predators and

evaluate the relative importance of each pathway. Furthermore, a range of metal isotopes should be used in bioaccumulation kinetic experiments, since different metals have different chemical speciation properties (Chapter 5).

- *Do "ALL" fish accumulate metals in the same way?*

The use of other commercially and recreationally important fish species would enhance the ecological significance of contaminant exposure and sustainability of future populations.

- *Do changes in macrobenthic communities (due to indirect effects of metals) have an effect on fish health and reproduction?*

An assessment of macrobenthic invertebrate community structure, which relates to gut content (diet) in resident fish, could determine if dietary shifts in fish, in relation to indirect effects of metal contamination changing macroinvertebrate community structure, have an effect on resident fish condition and reproduction and subsequent ecosystem function.

7.4 SUMMARY OF FINDINGS

The following key outcomes were generated from the investigation:

- A detailed spatial and temporal assessment of metals in sediments and toadfish within four estuaries of the Sydney region showed differences in metal concentrations among estuaries, sampling times, toadfish gender and toadfish tissues.

- An investigation into the effects of metals on health (condition) and reproductive output in the smooth toadfish revealed that elevated metal tissue concentrations were related to reduced lipids, increased protein and reduced reproductive output.

- A detailed assessment of macroinvertebrate (commonly consumed by the smooth toadfish) nutritional value and toadfish tissue metal concentrations was related to toadfish growth along a pollution gradient in the Parramatta River.

- Differences in the uptake and loss of ^{109}Cd and ^{75}Se in selected estuarine macroinvertebrates (commonly consumed by toadfish) were influenced by aqueous chemical speciation, animal physiology, sequestration, storage and excretion mechanisms.

- Toadfish contaminant exposure pathways were investigated using isotopes of ^{109}Cd and ^{75}Se and their subsequent bioaccumulation in different tissues were observed with an increased amount being taken up via the gut, suggesting that a major exposure route is through the gut due to toadfish drinking large amounts of water to maintain osmoregulation.

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Appendix I

Table IA. Recoveries of Certified Reference Material (CRM). Reference material Dogfish Liver (DOLT-2) certified by the National Bureau of Standards, USA. n = 6.

Metal	Metal concentration ($\mu g.g^{-1}$)		% Recovery
	Certified value	Measured value	
Cd	20.8	19.8	95
Co	0.24	0.2	97
Cr	0.37	0.4	107
Cu	25.8	27.3	106
Mn	6.88	6.5	94
Ni	0.2	0.2	97
Pb	0.22	0.2	104
Zn	85.8	90.7	106

Table IB. Recoveries of Certified Reference Material (CRM). Reference material Dogfish Muscle (DORM-2) certified by the National Bureau of Standards, USA. n = 6.

Metal	Metal concentration ($\mu g.g^{-1}$)		% Recovery
	Certified value	Measured value	
Cd	0.04	0.05	109
Co	0.18	0.18	99
Cr	34.7	34.9	101
Cu	2.34	2.20	94
Mn	3.66	3.95	108
Ni	19.4	21.3	110
Pb	0.07	0.05	81
Zn	25.6	24.9	97

Appendix II

Table II. Male and female tissue metal concentrations (μg/g^{-1}) at different estuaries during the June 2002 sampling period. Values are means ± SE, n = 30.

	Zn		As		Cd		Co		Cr	
Liver	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male
Cowan Creek	130 ± 40	170 ± 60	4.4 ± 1.0	2.6 ± 0.9	0.03 ± 0.01	< 0.01	0.3 ± 0.1	0.3 ± 0.1	0.11 ± 0.03	0.06 ± 0.04
Port Hacking River	30 ± 10	70 ± 20	2.6 ± 1.3	3.2 ± 0.5	< 0.01	< 0.01	0.06 ± 0.01	< 0.01	0.02 ± 0.01	0.09 ± 0.01
Lake Macquare	110 ± 10	80 ± 10	4.4 ± 0.6	5.3 ± 0.8	1.5 ± 0.4	0.8 ± 0.2	0.2 ± 0.1	0.10 ± 0.03	0.08 ± 0.02	0.05 ± 0.01
Parramatta River	140 ± 20	100 ± 10	5.1 ± 0.8	1.7 ± 2.1	0.09 ± 0.03	< 0.01	0.6 ± 0.1	0.4 ± 0.2	0.16 ± 0.04	0.08 ± 0.06

	Cu		Ni		Pb		Se	
Liver	Female	Male	Female	Male	Female	Male	Female	Male
Cowan Creek	6.9 ± 2.7	3.4 ± 1.5	2.6 ± 1.3	1.3 ± 0.9	0.8 ± 0.2	0.4 ± 0.2	0.6 ± 0.3	0.2 ± 0.2
Port Hacking River	3.3 ± 0.2	4.3 ± 0.7	0.1 ± 0.2	< 0.01	< 0.01	0.3 ± 0.2	0.8 ± 0.4	0.1 ± 0.1
Lake Macquare	11 ± 2.0	9.3 ± 2.1	1.0 ± 0.6	2.1 ± 0.7	3.4 ± 0.8	0.6 ± 0.1	1.3 ± 0.3	0.9 ± 0.3
Parramatta River	7.0 ± 1.2	5.9 ± 0.7	1.6 ± 0.8	< 0.01	2.7 ± 0.7	0.8 ± 0.4	1.0 ± 0.2	1.6 ± 0.9

	Zn		As		Cd		Co		Cr	
Gills	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male
Cowan Creek	690 ± 60	1370 ± 250	12 ± 2.3	1.6 ± 0.6	< 0.01	< 0.01	< 0.01	< 0.01	0.6 ± 0.2	0.7 ± 0.4
Port Hacking River	1090 ± 210	1020 ± 140	8.3 ± 0.7	4.6 ± 1.7	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	0.4 ± 0.1
Lake Macquare	660 ± 80	560 ± 70	10 ± 1.3	5.31 ± 1.03	0.5 ± 0.1	0.1 ± 0.1	< 0.01	< 0.01	0.07 ± 0.03	< 0.01
Parramatta River	550 ± 40	520 ± 90	5.7 ± 0.3	2.9 ± 0.1	< 0.01	< 0.01	0.07 ± 0.03	< 0.01	2.3 ± 0.5	0.31 ± 0.05

	Cu		Ni		Pb		Se	
Gills	Female	Male	Female	Male	Female	Male	Female	Male
Cowan Creek	18 ± 3.8	17 ± 6.5	0.6 ± 0.2	0.4 ± 0.3	1.4 ± 0.8	1.0 ± 0.7	< 0.01	1.0 ± 0.9
Port Hacking River	11 ± 4.1	100 ± 1.3	< 0.01	2.3 ± 1.4	1.9 ± 1.8	0.7 ± 0.4	< 0.01	< 0.01
Lake Macquare	5.1 ± 0.8	3.5 ± 0.9	1.4 ± 0.8	0.8 ± 0.7	6.5 ± 2.2	0.3 ± 0.1	0.6 ± 0.4	1.6 ± 0.7
Parramatta River	13 ± 2.0	3.1 ± 0.2	0.7 ± 0.2	< 0.01	6.0 ± 1.0	3.4 ± 2.2	0.5 ± 0.2	< 0.01

	Zn		As		Cd		Co		Cr	
Muscle	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male
Cowan Creek	30 ± 10	< 10	17 ± 2.4	15 ± 6.5	0.03 ± 0.01	< 0.01	< 0.01	19 ± 19	0.14 ± 0.05	0.3 ± 0.2
Port Hacking River	< 10	< 10	2.4 ± 0.1	11 ± 3.7	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Lake Macquare	50 ± 20	40 ± 10	13 ± 1.2	44 ± 9.2	0.4 ± 0.3	0.3 ± 0.2	< 0.01	< 0.01	< 0.01	0.2 ± 0.1
Parramatta River	40 ± 10	180 ± 160	5.2 ± 0.6	5.9 ± 3.5	< 0.01	< 0.01	1.4 ± 1.2	< 0.01	0.18 ± 0.05	0.4 ± 0.3

	Cu		Ni		Pb		Se	
Muscle	Female	Male	Female	Male	Female	Male	Female	Male
Cowan Creek	2.6 ± 1.0	4.5 ± 1.8	0.2 ± 0.1	0.2 ± 0.2	< 0.01	< 0.01	< 0.01	< 0.01
Port Hacking River	0.1 ± 0.1	2.8 ± 2.3	< 0.01	0.04 ± 0.04	< 0.01	< 0.01	< 0.01	< 0.01
Lake Macquare	3.8 ± 1.1	6.4 ± 1.7	1.5 ± 0.8	0.5 ± 0.2	2.3 ± 2.2	0.2 ± 0.2	< 0.01	< 0.01
Parramatta River	3.2 ± 0.7	1.1 ± 0.9	0.5 ± 0.1	8.5 ± 8.3	0.2 ± 0.1	0.4 ± 0.4	0.1 ± 0.1	0.7 ± 0.7

Appendix III

Table I. *Post Hoc* tests (Bonferroni) for sediment metals in June 2002 & September 2002. (P < 0.05). Codes are 1: Port Haching River; 2: Cowan Creek; 3: Lake Macquarie 4: Parramatta River.

Metal	Location (*i*)	Location (*j*)	Jun-02 Sig.	Sep-02 Sig.
As	1	2	1.000	0.655
		3	0.805	0.000
		4	1.000	0.000
	2	1	1.000	0.655
		3	1.000	0.003
		4	1.000	0.001
	3	1	0.805	0.000
		2	1.000	0.003
		4	1.000	1.000
	4	1	1.000	0.000
		2	1.000	0.001
		3	1.000	1.000
Cd	1	2	1.000	1.000
		3	0.034	0.005
		4	1.000	1.000
	2	1	1.000	1.000
		3	0.035	0.005
		4	1.000	1.000
	3	1	0.034	0.005
		2	0.035	0.005
		4	0.044	0.006
	4	1	1.000	1.000
		2	1.000	1.000
		3	0.044	0.006
Co	1	2	1.000	0.021
		3	1.000	0.000
		4	1.000	0.000
	2	1	1.000	0.021
		3	1.000	0.001
		4	1.000	0.000
	3	1	1.000	0.000
		2	1.000	0.001
		4	1.000	0.090
	4	1	1.000	0.000
		2	1.000	0.000
		3	1.000	0.090
Cr	1	2	1.000	1.000
		3	1.000	1.000
		4	0.052	0.000
	2	1	1.000	1.000
		3	1.000	1.000
		4	0.025	0.000
	3	1	1.000	1.000
		2	1.000	1.000
		4	0.085	0.000
	4	1	0.052	0.000
		2	0.025	0.000

		3	0.085	0.000
Cu	1	2	1.000	1.000
		3	0.248	0.498
		4	0.228	0.098
	2	1	1.000	1.000
		3	0.257	0.500
		4	0.236	0.099
	3	1	0.248	0.498
		2	0.257	0.500
		4	1.000	1.000
	4	1	0.228	0.098
		2	0.236	0.099
		3	1.000	1.000
Ni	1	2	1.000	0.084
		3	1.000	0.011
		4	1.000	0.000
	2	1	1.000	0.084
		3	0.808	1.000
		4	0.939	0.000
	3	1	1.000	0.011
		2	0.808	1.000
		4	1.000	0.000
	4	1	1.000	0.000
		2	0.939	0.000
		3	1.000	0.000
Pb	1	2	1.000	1.000
		3	0.111	0.001
		4	1.000	0.007
	2	1	1.000	1.000
		3	0.097	0.001
		4	1.000	0.018
	3	1	0.111	0.001
		2	0.097	0.001
		4	0.740	1.000
	4	1	1.000	0.007
		2	1.000	0.018
		3	0.740	1.000
Se	1	2	0.468	1.000
		3	0.468	0.000
		4	0.522	1.000
	2	1	0.468	1.000
		3	1.000	0.000
		4	1.000	1.000
	3	1	0.468	0.000
		2	1.000	0.000
		4	1.000	0.000
	4	1	0.522	1.000
		2	1.000	1.000
		3	1.000	0.000

Table II. *Post Hoc* tests (Bonferroni) for sediment metals in February 2003. (P < 0.05). Codes are 2: Cowan Creek; 3: Lake Macquarie 4: Parramatta River. No data available for Port Hacking River on Feb 2003.

Metal	Location (*i*)	Location (*j*)	Feb-03 Sig.
As	2	3	0.004
		4	0.247
	3	2	0.004
		4	0.134
	4	2	0.247
		3	0.134
Cd	2	3	0.113
		4	1.000
	3	2	0.113
		4	0.230
	4	2	1.000
		3	0.230
Co	2	3	0.000
		4	0.087
	3	2	0.000
		4	0.030
	4	2	0.087
		3	0.030
Cr	2	3	0.040
		4	0.000
	3	2	0.040
		4	0.107
	4	2	0.000
		3	0.107
Cu	2	3	0.309
		4	0.374
	3	2	0.309
		4	1.000
	4	2	0.374
		3	1.000
Ni	2	3	0.000
		4	0.000
	3	2	0.000
		4	1.000
	4	2	0.000
		3	1.000
Pb	2	3	0.003
		4	0.362
	3	2	0.003
		4	0.065
	4	2	0.362
		3	0.065
Se	2	3	0.000
		4	1.000
	3	2	0.000
		4	0.000
	4	2	1.000
		3	0.000

Table III. Summary of *Post Hoc* tests (Bonferroni) for 2-way ANOVA (P < 0.05) for toadfish liver tissues among locations & sampling times.

Liver	Dependent Variable	F value	Sig.
Date	Zn	7.971	0.000
	As	4.898	0.009
	Cd	2.017	0.136
	Co	6.027	0.003
	Cr	10.122	0.000
	Cu	1.077	0.343
	Ni	3.968	0.021
	Pb	2.092	0.127
	Se	28.421	0.000
Location	Zn	0.286	0.835
	As	2.93	0.035
	Cd	29.235	0.000
	Co	17.513	0.000
	Cr	0.641	0.590
	Cu	3.004	0.032
	Ni	0.201	0.896
	Pb	6.668	0.000
	Se	4.539	0.004
Date * Location	Zn	2.68	0.016
	As	4.473	0.000
	Cd	1.755	0.111
	Co	3.407	0.003
	Cr	1.103	0.363
	Cu	0.773	0.592
	Ni	0.526	0.788
	Pb	0.694	0.655
	Se	2.667	0.017

Table IV. Summary of *Post Hoc* tests (Bonferroni) for 2-way ANOVA (P < 0.05) for toadfish liver tissues among sampling times (June 2002, September 2002 & February 2003).

Date	(I) Date	(J) Date	Sig.
Zn	1	2	0.000
		3	0.083
	2	1	0.000
		3	0.034
	3	1	0.083
		2	0.034
As	1	2	0.006
		3	0.055
	2	1	0.006
		3	1.000
	3	1	0.055
		2	1.000
Cd	1	2	0.085
		3	0.353
	2	1	0.085
		3	0.002
	3	1	0.353
		2	0.002
Co	1	2	0.013
		3	0.065
	2	1	0.013
		3	0.000
	3	1	0.065
		2	0.000
Cr	1	2	0.000
		3	0.014
	2	1	0.000
		3	0.121
	3	1	0.014
		2	0.121
Cu	1	2	0.302
		3	0.010
	2	1	0.302
		3	0.675
	3	1	0.010
		2	0.675
Ni	1	2	0.015
		3	1.000
	2	1	0.015
		3	0.005
	3	1	1.000
		2	0.005
Pb	1	2	0.753
		3	0.048
	2	1	0.753
		3	0.003
	3	1	0.048
		2	0.003
Se	1	2	0.000
		3	0.000

2	1	0.000
	3	0.000
3	1	0.000
	2	0.000

Table V. Summary of *Post Hoc* tests (Bonferroni) for 2-way ANOVA (P < 0.05) for toadfish liver tissues among sampling locations (1, Port Hacking; 2, Cowan Creek; 3, Lake Macquarie & 4, Parramatta River).

Location	(I) Location	(J) Location	Sig.
Zn	1	2	1.000
		3	1.000
		4	1.000
	2	1	1.000
		3	1.000
		4	1.000
	3	1	1.000
		2	1.000
		4	1.000
	4	1	1.000
		2	1.000
		3	1.000
As	1	2	0.000
		3	0.192
		4	0.006
	2	1	0.000
		3	0.070
		4	1.000
	3	1	0.192
		2	0.070
		4	0.878
	4	1	0.006
		2	1.000
		3	0.878
Cd	1	2	1.000
		3	0.000
		4	1.000
	2	1	1.000
		3	0.000
		4	1.000
	3	1	0.000
		2	0.000
		4	0.000
	4	1	1.000
		2	1.000
		3	0.000
Co	1	2	1.000
		3	1.000
		4	0.000
	2	1	1.000
		3	1.000
		4	0.000
	3	1	1.000
		2	1.000

		4	0.000
	4	1	0.000
		2	0.000
		3	0.000
Cr	1	2	1.000
		3	0.249
		4	1.000
	2	1	1.000
		3	1.000
		4	1.000
	3	1	0.249
		2	1.000
		4	0.455
	4	1	1.000
		2	1.000
		3	0.455
Cu	1	2	1.000
		3	0.014
		4	1.000
	2	1	1.000
		3	0.002
		4	0.911
	3	1	0.014
		2	0.002
		4	0.074
	4	1	1.000
		2	0.911
		3	0.074
Ni	1	2	1.000
		3	1.000
		4	1.000
	2	1	1.000
		3	1.000
		4	1.000
	3	1	1.000
		2	1.000
		4	1.000
	4	1	1.000
		2	1.000
		3	1.000
Pb	1	2	1.000
		3	0.028
		4	0.005
	2	1	1.000
		3	0.007
		4	0.001
	3	1	0.028
		2	0.007
		4	1.000
	4	1	0.005
		2	0.001
		3	1.000
Se	1	2	1.000
		3	1.000
		4	1.000
	2	1	1.000

		3	0.043
		4	1.000
	3	1	1.000
		2	0.043
		4	0.050
	4	1	1.000
		2	1.000
		3	0.050

Table VI. Summary of *Post Hoc* tests (Bonferroni) for 2-way ANOVA (P < 0.05) for toadfish gill tissues among locations & sampling times.

Gills	Dependent Variable	F value	Sig.
Date	Zn	9.214	0.000
	As	14.091	0.000
	Cd	0.198	0.820
	Co	0.912	0.403
	Cr	0.406	0.667
	Cu	5.011	0.008
	Ni	3.643	0.028
	Pb	0.849	0.430
	Se	40.448	0.000
Location	Zn	10.984	0.000
	As	20.124	0.000
	Cd	6.183	0.001
	Co	2.757	0.044
	Cr	11.611	0.000
	Cu	5.053	0.002
	Ni	2.148	0.096
	Pb	6.106	0.001
	Se	17.999	0.000
Date * Location	Zn	4.91	0.000
	As	7.37	0.000
	Cd	0.686	0.661
	Co	0.986	0.436
	Cr	1.626	0.142
	Cu	2.391	0.030
	Ni	3.412	0.003
	Pb	1.279	0.269
	Se	3.819	0.001

Table VII. Summary of *Post Hoc* tests (Bonferroni) for 2-way ANOVA (P < 0.05) for toadfish gill tissues among sampling times (June 2002, September 2002 & February 2003).

Date	(I) Date	(J) Date	Sig.
Zn	1	2	0.000
		3	0.826
	2	1	0.000
		3	0.000
	3	1	0.826
		2	0.000
As	1	2	0.041
		3	0.000
	2	1	0.041
		3	0.036
	3	1	0.000
		2	0.036
Cd	1	2	1.000
		3	1.000
	2	1	1.000
		3	1.000
	3	1	1.000
		2	1.000
Co	1	2	0.300
		3	1.000
	2	1	0.300
		3	0.119
	3	1	1.000
		2	0.119
Cr	1	2	1.000
		3	1.000
	2	1	1.000
		3	1.000
	3	1	1.000
		2	1.000
Cu	1	2	0.085
		3	0.071
	2	1	0.085
		3	1.000
	3	1	0.071
		2	1.000
Ni	1	2	1.000
		3	1.000
	2	1	1.000
		3	1.000
	3	1	1.000
		2	1.000
Pb	1	2	0.117
		3	1.000
	2	1	0.117
		3	0.128
	3	1	1.000
		2	0.128
Se	1	2	0.000
		3	0.000

	2	1	0.000
		3	0.005
	3	1	0.000
		2	0.005

Table VIII. Summary of *Post Hoc* tests (Bonferroni) for 2-way ANOVA (P < 0.05) for toadfish gill tissues among sampling locations (1, Port Hacking; 2, Cowan Creek; 3, Lake Macquarie & 4, Parramatta River).

Location	(I) Location	(J) Location	Sig.
Zn	1	2	1.000
		3	0.052
		4	0.003
	2	1	1.000
		3	0.000
		4	0.000
	3	1	0.052
		2	0.000
		4	1.000
	4	1	0.003
		2	0.000
		3	1.000
As	1	2	0.000
		3	0.003
		4	0.000
	2	1	0.000
		3	0.002
		4	0.833
	3	1	0.003
		2	0.002
		4	0.000
	4	1	0.000
		2	0.833
		3	0.000
Cd	1	2	0.448
		3	0.627
		4	0.376
	2	1	0.448
		3	0.000
		4	1.000
	3	1	0.627
		2	0.000
		4	0.000
	4	1	0.376
		2	1.000
		3	0.000
Co	1	2	1.000
		3	1.000
		4	0.575
	2	1	1.000
		3	1.000
		4	0.055
	3	1	1.000
		2	1.000
		4	0.181

	4	1	0.575
		2	0.055
		3	0.181
Cr	1	2	1.000
		3	1.000
		4	0.001
	2	1	1.000
		3	1.000
		4	0.000
	3	1	1.000
		2	1.000
		4	0.000
	4	1	0.001
		2	0.000
		3	0.000
Cu	1	2	0.010
		3	1.000
		4	0.110
	2	1	0.010
		3	0.001
		4	1.000
	3	1	1.000
		2	0.001
		4	0.014
	4	1	0.110
		2	1.000
		3	0.014
Ni	1	2	0.585
		3	1.000
		4	0.257
	2	1	0.585
		3	1.000
		4	1.000
	3	1	1.000
		2	1.000
		4	1.000
	4	1	0.257
		2	1.000
		3	1.000
Pb	1	2	1.000
		3	0.599
		4	0.435
	2	1	1.000
		3	0.004
		4	0.001
	3	1	0.599
		2	0.004
		4	1.000
	4	1	0.435
		2	0.001
		3	1.000
Se	1	2	1.000
		3	0.001
		4	1.000
	2	1	1.000
		3	0.011

	4	0.465
3	1	0.001
	2	0.011
	4	0.000
4	1	1.000
	2	0.465
	3	0.000

Table IX. Summary of *Post Hoc* tests (Bonferroni) for 2-way ANOVA (P < 0.05) for toadfish gill tissues among locations & sampling times.

Muscle	Dependent Variable	F value	Sig.
Date	Zn	7.691	0.001
	As	0.319	0.727
	Cd	2.059	0.131
	Co	1.301	0.275
	Cr	17.168	0.000
	Cu	10.376	0.000
	Mn	0.18	0.836
	Ni	4.159	0.017
	Pb	0.398	0.672
	Se	179.473	0.000
Location	Zn	1.984	0.118
	As	12.663	0.000
	Cd	1.15	0.331
	Co	0.85	0.468
	Cr	4.938	0.003
	Cu	3.443	0.018
	Mn	0.827	0.481
	Ni	4.289	0.006
	Pb	0.597	0.618
	Se	52.998	0.000
Date * Location	Zn	0.262	0.954
	As	3.486	0.003
	Cd	1.147	0.337
	Co	0.899	0.497
	Cr	2.971	0.009
	Cu	1.433	0.205
	Mn	0.829	0.549
	Ni	1.543	0.167
	Pb	0.823	0.554
	Se	24.165	0.000

Table X. Summary of *Post Hoc* tests (Bonferroni) for 2-way ANOVA (P < 0.05) for toadfish muscle tissues among sampling times (June 2002, September 2002 & February 2003).

Date	(I) Date	(J) Date	Sig.
Zn	1	2	0.074
		3	0.001
	2	1	0.074
		3	0.515
	3	1	0.001
		2	0.515
As	1	2	0.275
		3	0.825
	2	1	0.275
		3	1.000
	3	1	0.825
		2	1.000
Cd	1	2	1.000
		3	0.386
	2	1	1.000
		3	0.108
	3	1	0.386
		2	0.108
Co	1	2	0.581
		3	0.570
	2	1	0.581
		3	1.000
	3	1	0.570
		2	1.000
Cr	1	2	0.000
		3	0.000
	2	1	0.000
		3	0.056
	3	1	0.000
		2	0.056
Cu	1	2	1.000
		3	0.001
	2	1	1.000
		3	0.003
	3	1	0.001
		2	0.003
Ni	1	2	0.025
		3	0.236
	2	1	0.025
		3	1.000
	3	1	0.236
		2	1.000
Pb	1	2	1.000
		3	1.000
	2	1	1.000
		3	0.806
	3	1	1.000
		2	0.806
Se	1	2	0.000

		3	0.000
	2	1	0.000
		3	0.000
	3	1	0.000
		2	0.000

Table XI. Summary of *Post Hoc* tests (Bonferroni) for 2-way ANOVA (P < 0.05) for toadfish muscle tissues among sampling locations (1, Port Hacking; 2, Cowan Creek; 3, Lake Macquarie & 4, Parramatta River).

Location	(I) Location	(J) Location	Sig.
Zn	1	2	1.000
		3	1.000
		4	0.920
	2	1	1.000
		3	1.000
		4	1.000
	3	1	1.000
		2	1.000
		4	1.000
	4	1	0.920
		2	1.000
		3	1.000
As	1	2	0.085
		3	1.000
		4	0.000
	2	1	0.085
		3	0.000
		4	0.041
	3	1	1.000
		2	0.000
		4	0.000
	4	1	0.000
		2	0.041
		3	0.000
Cd	1	2	1.000
		3	1.000
		4	1.000
	2	1	1.000
		3	0.526
		4	1.000
	3	1	1.000
		2	0.526
		4	1.000
	4	1	1.000
		2	1.000
		3	1.000
Co	1	2	1.000
		3	1.000
		4	1.000
	2	1	1.000
		3	0.956
		4	1.000
	3	1	1.000
		2	0.956

		4	1.000
	4	1	1.000
		2	1.000
		3	1.000
Cr	1	2	1.000
		3	1.000
		4	0.161
	2	1	1.000
		3	1.000
		4	1.000
	3	1	1.000
		2	1.000
		4	0.585
	4	1	0.161
		2	1.000
		3	0.585
Cu	1	2	0.493
		3	0.256
		4	1.000
	2	1	0.493
		3	1.000
		4	0.896
	3	1	0.256
		2	1.000
		4	0.403
	4	1	1.000
		2	0.896
		3	0.403
Ni	1	2	1.000
		3	0.200
		4	1.000
	2	1	1.000
		3	0.760
		4	1.000
	3	1	0.200
		2	0.760
		4	0.688
	4	1	1.000
		2	1.000
		3	0.688
Pb	1	2	1.000
		3	1.000
		4	1.000
	2	1	1.000
		3	1.000
		4	1.000
	3	1	1.000
		2	1.000
		4	1.000
	4	1	1.000
		2	1.000
		3	1.000
Se	1	2	1.000
		3	0.000
		4	0.249
	2	1	1.000

		3	0.000
		4	0.237
	3	1	0.000
		2	0.000
		4	0.000
	4	1	0.249
		2	0.237
		3	0.000

Table XII. Summary of *Post Hoc* tests (Bonferroni) for 2-way ANOVA (P < 0.05) for toadfish gonad tissues among locations & sampling times.

Muscle	Dependent Variable	F value	Sig.
Date	Zn	0.532	0.588
	As	1.78	0.172
	Cd	0.394	0.675
	Co	1.205	0.302
	Cr	1.052	0.351
	Cu	5.219	0.006
	Mn	6.335	0.002
	Ni	3.535	0.031
	Pb	3.481	0.033
	Se	1.871	0.157
Location	Zn	0.862	0.462
	As	5.172	0.002
	Cd	0.655	0.581
	Co	15.579	0.000
	Cr	1.777	0.153
	Cu	2.079	0.105
	Mn	1.861	0.138
	Ni	0.419	0.740
	Pb	0.475	0.700
	Se	0.944	0.421
Date * Location	Zn	0.659	0.683
	As	3.193	0.005
	Cd	1.07	0.382
	Co	0.83	0.548
	Cr	0.632	0.704
	Cu	1.421	0.209
	Mn	2.223	0.043
	Ni	1.197	0.310
	Pb	0.599	0.731
	Se	2.028	0.064

Table XII. Summary of *Post Hoc* tests (Bonferroni) for 2-way ANOVA (P < 0.05) for toadfish gonad tissues among sampling times (June 2002, September 2002 & February 2003).

Date	(I) Date	(J) Date	Sig.
Zn	1	2	0.783
		3	0.807
	2	1	0.783
		3	1.000
	3	1	0.807
		2	1.000
As	1	2	0.125
		3	0.650
	2	1	0.125
		3	1.000
	3	1	0.650
		2	1.000
Cd	1	2	1.000
		3	0.746
	2	1	1.000
		3	1.000
	3	1	0.746
		2	1.000
Co	1	2	0.788
		3	0.241
	2	1	0.788
		3	0.026
	3	1	0.241
		2	0.026
Cr	1	2	0.296
		3	1.000
	2	1	0.296
		3	0.420
	3	1	1.000
		2	0.420
Cu	1	2	1.000
		3	0.112
	2	1	1.000
		3	0.044
	3	1	0.112
		2	0.044
Ni	1	2	1.000
		3	0.045
	2	1	1.000
		3	0.032
	3	1	0.045
		2	0.032
Pb	1	2	1.000
		3	0.020
	2	1	1.000
		3	0.053
	3	1	0.020
		2	0.053
Se	1	2	0.705
		3	0.279

	2	1	0.705
		3	1.000
	3	1	0.279
		2	1.000

Table XIII. Summary of *Post Hoc* tests (Bonferroni) for 2-way ANOVA (P < 0.05) for toadfish gonad tissues among sampling locations (1, Port Hacking; 2, Cowan Creek; 3, Lake Macquarie & 4, Parramatta River).

Location	(I) Location	(J) Location	Sig.
Zn	1	2	1.000
		3	1.000
		4	1.000
	2	1	1.000
		3	1.000
		4	1.000
	3	1	1.000
		2	1.000
		4	1.000
	4	1	1.000
		2	1.000
		3	1.000
As	1	2	0.090
		3	1.000
		4	0.000
	2	1	0.090
		3	0.063
		4	0.381
	3	1	1.000
		2	0.063
		4	0.000
	4	1	0.000
		2	0.381
		3	0.000
Cd	1	2	1.000
		3	1.000
		4	1.000
	2	1	1.000
		3	1.000
		4	1.000
	3	1	1.000
		2	1.000
		4	1.000
	4	1	1.000
		2	1.000
		3	1.000
Co	1	2	1.000
		3	1.000
		4	0.000
	2	1	1.000
		3	1.000
		4	0.000
	3	1	1.000
		2	1.000
		4	0.000

	4	1	0.000
		2	0.000
		3	0.000
Cr	1	2	0.333
		3	0.043
		4	0.157
	2	1	0.333
		3	1.000
		4	1.000
	3	1	0.043
		2	1.000
		4	1.000
	4	1	0.157
		2	1.000
		3	1.000
Cu	1	2	1.000
		3	1.000
		4	1.000
	2	1	1.000
		3	1.000
		4	1.000
	3	1	1.000
		2	1.000
		4	1.000
	4	1	1.000
		2	1.000
		3	1.000
Ni	1	2	1.000
		3	1.000
		4	1.000
	2	1	1.000
		3	1.000
		4	1.000
	3	1	1.000
		2	1.000
		4	1.000
	4	1	1.000
		2	1.000
		3	1.000
Pb	1	2	1.000
		3	1.000
		4	1.000
	2	1	1.000
		3	1.000
		4	1.000
	3	1	1.000
		2	1.000
		4	1.000
	4	1	1.000
		2	1.000
		3	1.000
Se	1	2	1.000
		3	1.000
		4	1.000
	2	1	1.000
		3	1.000

	4	1.000
3	1	1.000
	2	1.000
	4	1.000
4	1	1.000
	2	1.000
	3	1.000