PAPERS

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

Ralph Alquezar[1, 2, 3*] and Scott J Markich[4]

[1] Department of Environmental Sciences, University of Technology Sydney, PO Box 123, Broadway 2007, NSW, Australia.

[2] Institute for Environmental Research, Australian Nuclear Science and Technology Organisation, PMB 1, Menai 2234, NSW, Australia.

[3] Current address: Centre for Environmental Management, Central Queensland University, PO Box 1319, Gladstone 4680, Queensland, Australia.

[4] Aquatic Solutions International, Level 1, 467 Miller St, Cammeray 2062, NSW, Australia

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ABSTRACT

Metals may have direct and indirect effects on aquatic biota at different trophic levels. This study examined the metal concentrations and nutritional value (protein and lipid content) of sediment infauna consumed by the estuarine smooth toadfish (*Tetractenos glaber*) at sites with varying metal contamination in the Parramatta River (Sydney Harbour), south-eastern Australia, and the resulting influence on toadfish size and their tissue metal concentrations. Metal concentrations in sediments showed positive linear relationships (r^2 = 0.29–0.87; P < 0.001, n = 12) with metal concentrations in sediment infauna. Metal concentrations in toadfish tissues were also linearly and positively related to metal concentrations in both sediments (r^2 = 0.32–0.73; P < 0.001, n = 55) and infauna (r^2 = 0.27–0.72; P < 0.001, n = 55), indicating that sediment and infauna are an important metal exposure pathway for toadfish. Of the key toadfish prey items (sediment infauna), polychaetes (*Marphysa sanguinea*) generally had the highest metal concentrations and nutritional value followed by semaphore crabs (*Heloecius cordiformis*) and black mussels (*Xenostrobus securis*). Polychaetes from the most contaminated site generally had higher metal concentrations and higher nutritional value than those from the least contaminated site. Toadfish from the most contaminated site generally had the highest metal concentrations, and were 15% larger and 41% heavier than similarly-aged toadfish from the least contaminated site, suggesting that toadfish may be benefiting in size due to ingestion of sediment infauna of higher nutritional value.

Key words: Toadfish; infauna; sediments; metals; bioaccumulation.

INTRODUCTION

Coastal zones are under increasing pressure from urban development and rising population growth, which has led to alterations in estuarine and catchment processes (GESAMP 2001). Anthropogenic impacts represent a threat to estuarine ecosystems through deteriorating water and sediment quality, leading to the degradation and destruction of estuarine habitats. Since estuaries are depositional environments dominated by soft sediments, benthic macroinvertebrates (infauna) are a ubiquitous and important functional component (Wallace and Webster 1996). Infaunal assemblages generally integrate environmental changes in the physical, chemical and ecological characteristics of their habitat, both spatially and temporally (Hirst 2004).

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. 1999). Elevated metal concentrations in estuaries may have a direct toxic effect on macroinvertebrates and their predators (e.g. fish), 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 (Fleeger et al. 2003; Chapman 2004). Elevated environmental metal concentrations have also been linked to increased concentrations of stress proteins and decreased concentrations of lipids in benthic macroinvertebrates (Panfoli et al. 2000; Hamer et al. 2004), thus potentially affecting their nutritional value. Very few studies have reported on whether nutritional value, in the form of protein and lipid content, in infauna, influences prey selection by predators, such as fish (Yearsley 2003). Nutritional value can be an important factor affecting organism growth, health and survival (Knauer and Southgate 1996; Britz and Hecht 1997; Booth and Alquezar 2002).

Fish may be exposed to metals via different pathways, including ingestion of prey or sediments and/or uptake from the water column via the gills. Metals may affect fish directly, via acute toxicity, or indirectly, by reducing condition (e.g. size) and/or reproductive output (e.g. gonad weight), 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 a selection of genetic strains that can tolerate these high metal availabilities by decreased metal uptake, increased excretion and/or increased detoxification

* Author for correspondence, email: r.alquezar@cqu.edu.au

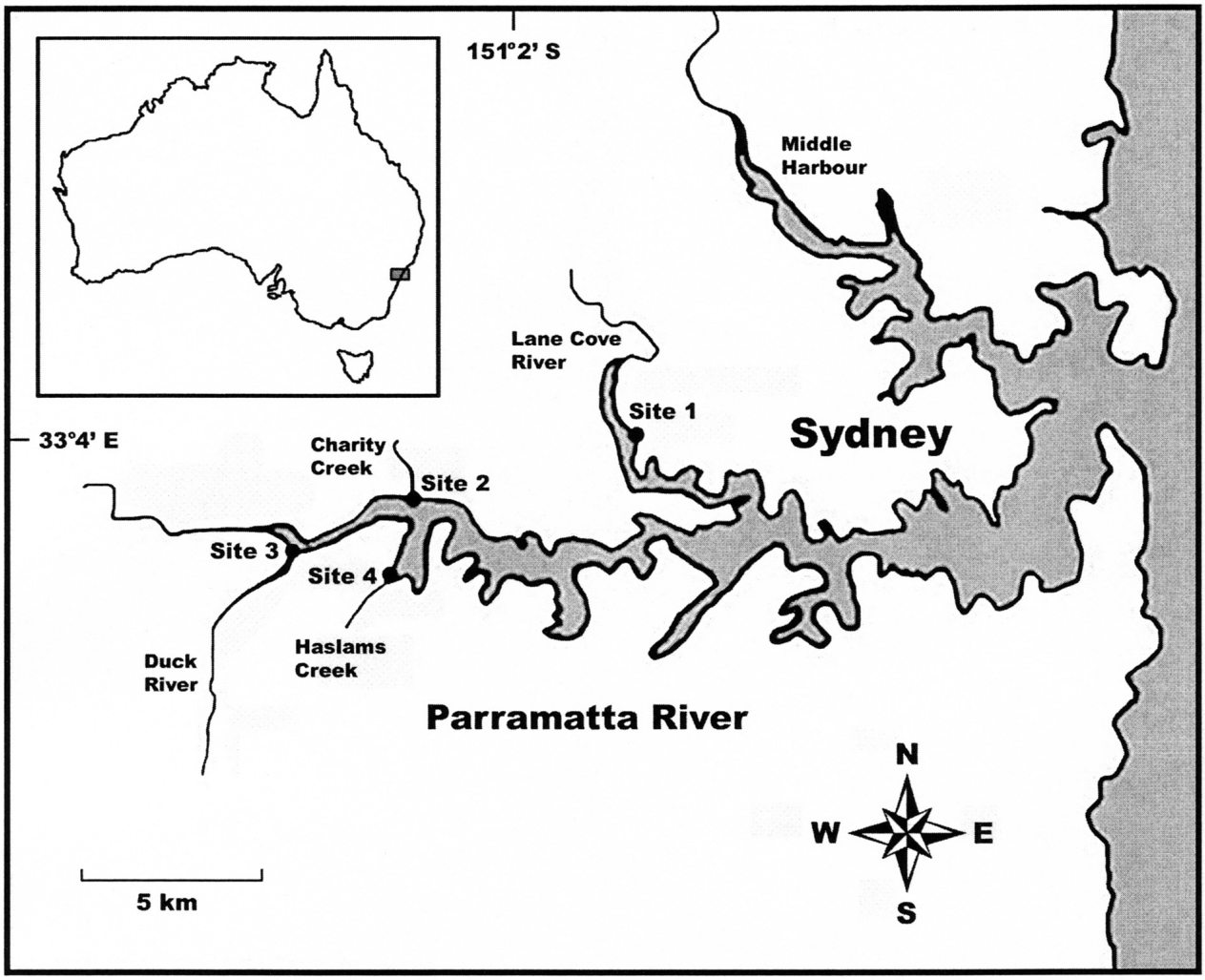

Figure 1. Map of Parramatta River/Sydney Harbour showing the four study sites (Site 1, Lovett Reserve; Site 2, mouth of Charity Creek; Site 3, mouth of Duck River; Site 4, mouth of Haslams Creek). Inset: Map of Australia showing study area.

(Klerks and Weis 1987). Such organisms may physiologically adapt to metal contaminated environments by binding metals via 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 (Klerks and Weis 1987; Belfiore and Anderson 2001).

This study examined the metal concentrations and nutritional value (lipid and protein content) of benthic macroinvertebrates (sediment infauna) consumed by the smooth toadfish (*Tetractenos glaber)* at sites with varying metal contamination in the Parramatta River (Sydney Harbour), south-eastern Australia. The specific aims of this study were to determine whether (1) toadfish consumed similar proportions of sediment infauna (dietary items) amongst sites; (2) the nutritional value of these dietary items differed between taxa and sites; (3) metal concentrations in toadfish were positively related to infauna and sediment metal concentrations; and (4) toadfish size was influenced by metal concentrations and the nutritional value of dietary items.

MATERIALS AND METHODS

Site description

Four study sites in the Parramatta River, south-eastern Australia (Figure 1), were selected *a priori* with similar surface water physico-chemistry and sediment composition (Melville et al. 2005), but with varying sediment metal concentrations (Birch 1996; Irvine and Birch 1998; Hatje et al. 2001). Site 1 was Lovett Reserve (*a priori* low metal contamination) located in the Lane Cove River, a tributary of the Parramatta River; site 2 was at the mouth of Charity Creek (*a priori* low-to-mid metal contamination), site 3 was at the mouth of Duck River (*a priori* mid-high metal contamination) and site 4 was at the mouth of Haslams Creek (*a priori* high metal contamination). Parramatta River is a drowned river valley and has a catchment area of 130 km^2 flowing into Sydney Harbour. For over two centuries, Parramatta River has been consistently exposed to metal contaminants from urban and industrial activities (Birch and Taylor 1999).

Fish species

The smooth toadfish (*Tetractenos glaber*: Tetraodontidae) is a common fish inhabiting coastal bays and estuaries of south-eastern Australia (Kuiter, 2000). It is highly territorial and feeds on resident sediment infauna, often burrowing in sediments. Toadfish are common throughout all seasons and are regarded as suitable bioindicators of their environment (Booth and Schultz 1999; Alquezar et al. 2006b).

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 sample collection devices and storage containers were washed in 5% nitric acid (AnalaR) and then washed twice with deionised water (Milli Q; 18MΩ/cm).

At each site, 12-15 sexually mature toadfish were randomly sampled 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 (n = 55), black mussels (*Xenostrobus securis*), semaphore crabs (*Heloecius cordiformis*) and polychaetes (*Marphysa sanguinea*) were selected as the main sediment infauna (prey items) consumed by toadfish. Approximately 30 individuals of each of these 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 manually sampled 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 for lipid and protein analyses (see below). At each site, five surface grab samples of sediment (0-5 cm depth) were randomly collected and stored in 500 ml polyethylene containers. 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 (sand/silt/clay) fraction only, so values could be directly compared with the national sediment quality guidelines (ANZECC and ARMCANZ 2000).

Determination of fish size, age and gender

Standard length (snout to caudal fin; mm) and wet weight (g) were determined for each toadfish. The age of individual fish was determined via the otoliths (ear stones). Otoliths were carefully extracted, polished and examined under reflected light with a compound microscope (400X magnification), following the methods described by Pease et al. (2003). The incremental otolith rings were counted three times, and rings recounted if more than 10% error occurred between counts. Fish gender was determined from a macroscopic examination of the gonads (males – testis; females – ovaries). Since the majority (95%) of toadfish across all sites was female, only females were used for this study. Toadfish were not reproductively active (i.e. oocyte development was minimal) at the time of sampling.

Toadfish gut content analysis

Gut contents of toadfish were removed to determine the number of infauna taxa present and their percentage relative biomass (wet weight) (i.e. prey selection preference) at each site.

Sample preparation and trace metal analysis

Toadfish were thawed and dissected into various tissues (liver, caudal-peduncle muscle, gill, gonad, kidney and gut lining) and thoroughly rinsed with deionised water (Milli Q; 18MΩ/cm) to remove any sediment or detrital material. To obtain a representative measure of metal concentrations in toadfish, the gut contents of infauna were not purged or removed, but sediment content was typically low (Alquezar 2006). Fish tissues, infauna and sediments were oven dried (40°C) to a constant measured weight. Homogenised sub-samples of each were digested using the nitric acid - hydrogen peroxide method described by Krishnamurty et al. (1976). Metal (Zn, As, Cd, Co, Cr, Ni, Pb and Se) concentrations (µg/g dry weight) were determined in digest solutions using either inductive coupled plasma mass spectrometry (Hewlett Packard 4500) or inductive coupled plasma atomic emission spectrometry (Varian Vista). Standard reference materials (Dogfish muscle - DORM-2; Dogfish Liver - DOLT-2), blank and spiked replicates were included throughout digestion and analysis protocols with minimal variation (coefficient of variation < 7%) among samples. Metal recoveries for spiked fish tissues and infauna averaged 95% (range: 85–104%), and the standard reference materials were within their 95% confidence intervals.

Lipid and protein analysis

Total lipids in black mussels, semaphore crabs and polychaetes were determined using chloroform: methanol extraction and measured gravimetrically as a percentage of the total dry body weight (Mann and Gallager 1985). Total proteins in the same infauna were determined using the Lowry method (Bovine albumin serum method) and expressed as a percentage of total dry body weight (Peterson 1977; Mann and Gallager 1985).

Data analysis

One-way analysis of variance was used to investigate differences in (a) protein and lipid content amongst infauna; (b) metal concentrations in sediments, infauna and toadfish tissues amongst sites; and (c) toadfish size, wet weight and age amongst sites. Tukey's (HSD) pair-wise multiple comparison tests were used to evaluate significant differences amongst means (Daniel 1991). 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 protein and lipid contents. All multiple comparisons were Bonferroni corrected. Data that did not meet homogeneity of variance and normality were $\log_e$ transformed.

RESULTS AND DISCUSSION

Toadfish gut content analysis

Black mussels, semaphore crabs and polychaetes were typically the most common infauna taxa present in the gut contents of toadfish sampled at several sites in Sydney Harbour, with amphipods, prawns and brown algae found in lower abundance (Alquezar 2006). Toadfish gut also contained sediment (17%), which was most likely ingested whilst toadfish sieved through sediments when consuming infauna (Alquezar 2006). There were no significant differences ($P>0.05$) in the number of taxa (~3–5) present in toadfish gut between the most contaminated and least contaminated sites (Figure 2). However, toadfish did not consume infauna in the same proportions at each site. Figure 3 shows the proportion (wet weight) of infauna found in toadfish gut at the four study sites. Semaphore crabs were the dominant prey item (43–64%) of toadfish at three sites (Lovett Reserve, Duck River and Haslams Creek). Polychaetes were the dominant prey item (31%) at Charity Creek and formed a smaller component (7–17%) of the diet at Duck River and Haslams Creek (Figure 3); however, they were absent from the toadfish diet at Lovetts Reserve due to their very low abundance at the time of sampling. Black mussels also formed a substantial component of the diet (17–31%) at all sites (Figure 3). No data are available on the prey selection of toadfish – further work is needed to ascertain whether feeding is selective or non-selective and how this may relate to the observed diet composition at each site.

Lipid and protein content in infauna

There were significant differences ($P\leq0.05$) in the percentages of proteins and lipids between infauna species, with polychaetes expressing the highest protein and lipid content (up to ten times more than black mussels), followed by semaphore crabs and black mussels (Table 1). Polychaetes from the most contaminated site (Haslams Creek) contained the highest percentage of proteins, relative to other sites, and the highest percentage of lipids compared to the least contaminated site (Lovett Reserve) (Table 1). In accord with this result, Geracitano et al. (2004) found that protein concentrations in polychaetes (*Laeonereis acuta*) from a metal-contaminated site were markedly higher than those from a control site. Conversely, there were no significant differences ($P>0.05$) in the percentages of proteins or lipids in semaphore crabs or black mussels amongst sites (Table 1). In agreement with this finding, Pederson et al. (1997) reported that protein concentrations in the gills of the shore crab, *Carcinus maenas*, were not significantly ($P>0.05$) different among sites along a metal pollution gradient. In contrast to the present results for mussels, Hamer et al. (2004) found that protein concentrations were highest in mussels (*Mytilus galloprovincialis*) from sites with elevated metal concentrations. Increased concentrations of stress proteins (such as heat shock proteins, metallothioneins and multi-xenobiotic resistance trans-membrane proteins) have been attributed to increased metal concentrations, and are used for cell damage control (Barsyte et al. 1999; Van der Oost et al. 2003; Hamer et al. 2004).

Panfoli et al. (2000) found that phospholipase activity (essential for converting phospholipids into fatty acids) was reduced in mussels (*M. galloprovincialis*) from metal-contaminated sites. Although some metals may be lipophilic, metals can inhibit lipid production and thus have a negative effect on lipid content (Panfoli et al. 2000). There was no evidence from the data obtained in the present study to suggest that elevated levels of metals in sediments inhibited lipid content in any of the three selected infauna species; in fact the converse was true for polychaetes at the most contaminated site (Table 1).

Although specific nutrient concentrations in sediments were not determined as part of this study, previous studies (Melville et al. 2004; Markich unpublished) have found that nitrogen, phosphorus and sulfur concentrations in surface sediments (0–5 cm) were very similar amongst several sites in the Parramatta River (including the selected sites in this study) with varying sediment metal concentrations. This study found that organic carbon content in sediments at the four selected sites was not significantly ($P>0.05$) different (Table 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.

Metal concentrations in sediment and infauna

Sediment composition (% gravel, sand and silt/clay) was not significantly ($P> 0.05$) different amongst sites with silt/clay (mud) being the dominant fraction (62–71%), followed by sand (22–31%) and gravel (5–8%) (Table 2). Sediment metal concentrations at Charity Creek, Duck River and Haslams Creek were always higher than those from Lovett Reserve, with Haslams Creek generally containing the highest metal concentrations (Table 2). The concentrations of Zn, As, Cd, Cr, Ni and Pb in sediments from Haslams Creek exceeded the lower Australian sediment quality guideline values (ANZECC and ARMCANZ 2000), with Zn exceeding the higher sediment quality guideline value (Table 2).

Metal concentrations were generally highest in polychaetes, with similarly lower concentrations in semaphore crabs and black mussels (Table 3). Generally, infauna metal concentrations (Zn, As, Cd, Co, Cr, Ni and Pb) showed the following order:

Haslams Creek > Duck River ≈ Charity Creek > Lovett Reserve.

For all infauna, Pb concentrations were highest and Zn concentrations were lowest (Table 3). This result indicates that Zn, an essential metal, was regulated to relatively constant concentrations in the body tissues of all three invertebrates, which may be explained by the Zn uptake rates being offset by the Zn excretion (loss) rates. In contrast to the results for Zn, the relatively higher body tissue concentrations of Pb, a non-essential metal, in all invertebrates indicates that they are net accumulators of Pb, where it is stored in detoxified forms in the tissues with minimal excretion/loss. Cadmium, another non-essential metal, shows similar results (Table 3). These findings are consistent with other studies of

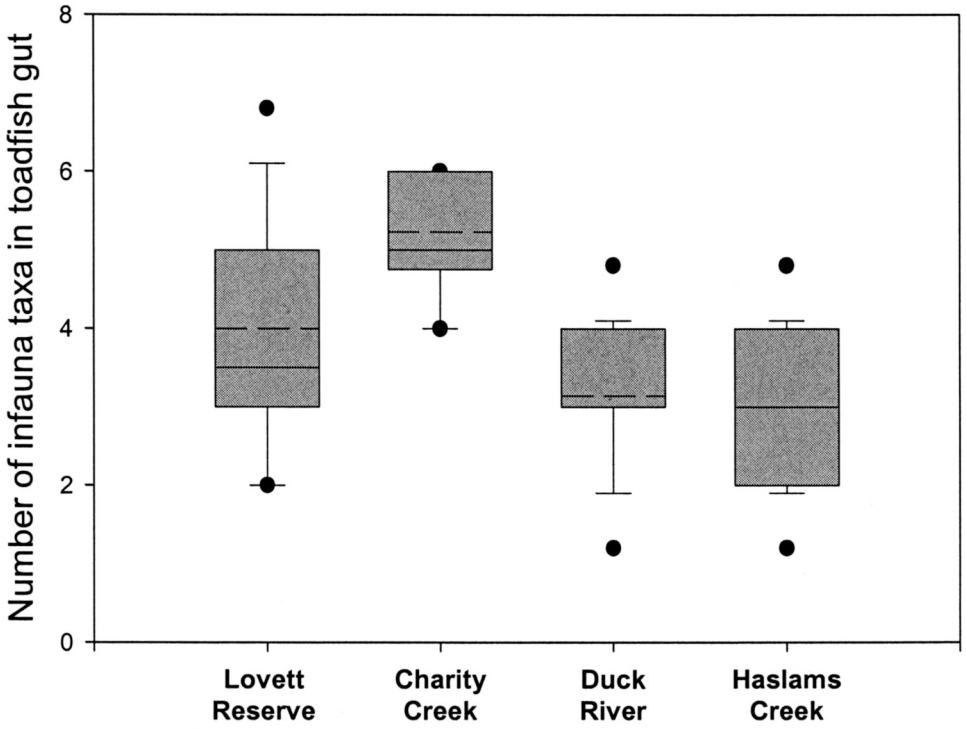

Figure 2. Box and whiskers plots showing the number of infauna taxa found within toadfish gut at each study site (n = 12). Boxes indicate interquartile range (50% of data), dashed lines indicate mean values, solid lines indicate median values, error bars represent the range (maximum and minimum) of values and solid dots indicate the 95% confidence interval.

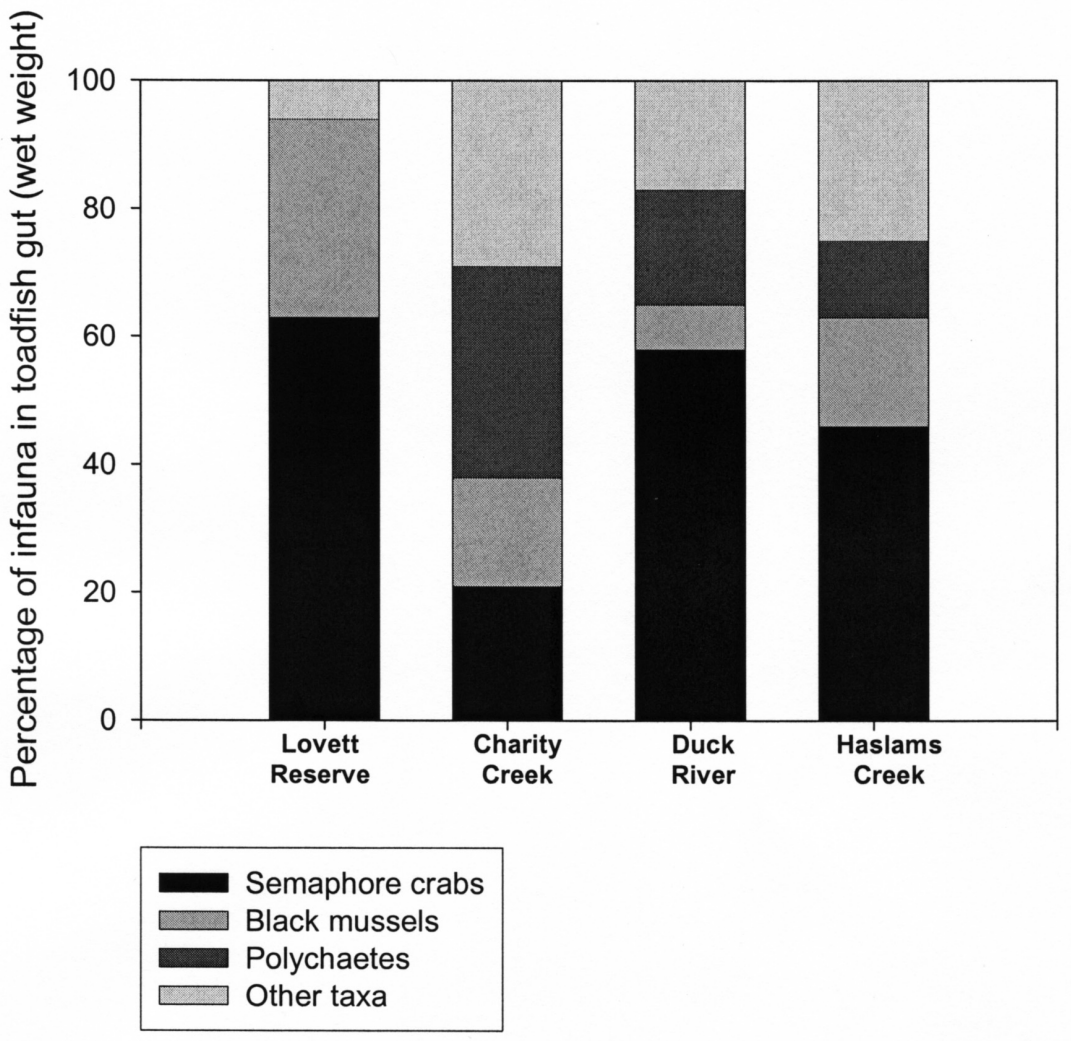

Figure 3. Percentage of key infauna (based on wet weight) present in toadfish gut at each site (n = 55).

Table 1. Percentage proteins and lipids (g^{-1} dry weight) in toadfish dietary items (infauna) at each site. Mean ± 95% confidence limits (n = 6).

Site	Semaphore crabs	Black mussels	Polychaetes
Protein			
Lovett Reserve	19 ± 1.6	3.8 ± 0.9	19 ± 1.6
Charity Creek	17 ± 1.9	5.0 ± 1.1	24 ± 5.1
Duck River	17 ± 1.7	5.5 ± 1.0	28 ± 3.6
Haslams Creek	17 ± 1.8	3.9 ± 1.3	34 ± 2.8
Lipids			
Lovett Reserve	7.4 ± 1.4	1.0 ± 0.2	8.1 ± 1.1
Charity Creek	7.0 ± 1.2	1.2 ± 0.2	12 ± 2.0
Duck River	7.5 ± 1.1	1.0 ± 0.3	10 ± 1.3
Haslams Creek	7.5 ± 1.4	1.1 ± 0.3	12 ± 1.6

the same taxa (Rainbow 2002; Wang 2003; King et al. 2004). Concentrations of Zn, As, Co, Cr and Pb were positively and linearly related ($P<0.001$, n = 60) to the percentages of lipids (r^2 = 0.26–0.73) and proteins (r^2 = 0.21–0.59) in infauna (Table 4). No significant ($P>0.05$) linear relationships were found for Cd, Ni or Se.

Metal concentrations in sediments showed positive linear relationships with those in infauna (Table 5). Concentrations of Zn, As, Cd, Co, Cr, Ni, Pb and Se in sediments were positively related to the same metals in black mussels (r^2 = 0.29(Se)–0.87(Cr)). Positive linear relationships ($P<0.001$, n = 12) were also found for polychaetes for the same metals (r^2 = 0.43(As)–0.87(Zn)), with the exception of Se. Only Pb (r^2 = 0.30) and Se (r^2 = 0.50) concentrations in semaphore crabs were also positively related to those in sediments (Table 5). These findings were consistent with those from other studies (Peters et al. 1999; Boisson et al. 2003; Selck and Forbes 2004). Peters et al. (1999) reported that Se concentrations in two benthic organisms, a bivalve (*Spisula trigonella*) and a polychaete (*Marphysa sanguinea*), were positively and linearly related to Se sediment concentrations in Lake Macquarie (south-eastern Australia).

Metal concentrations in sediment pore water were not determined in this study. Although sediment pore water may be the most bioavailable fraction to most sediment infauna, it may not be the most important exposure route (Chapman et al. 2002). The main metal exposure route for sediment infauna, is generally via direct ingestion of surficial sediment by deposit feeders and suspended sediment by suspension feeders (Forbes et al. 1998), or by sediment tube-dwelling organisms actively irrigating / bioturbating their tubes with overlying water (McCall and Tevesz 1982; Wang et al. 2001). Selck and Forbes (2004) found that sediment was the main Cd uptake pathway of the deposit feeding polychaete, *Capitella* sp. Similarly, Boisson et al. (2003) reported that ingestion of sediment was the main metal accumulation pathway in shrimp (crustaceans). 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 sulfides that are truly exposed to sediment pore waters (Warren et al. 1998; Chapman et al. 2002).

Metal concentrations in toadfish tissues and their relationship with infauna and sediment

Toadfish from Charity Creek, Duck River and Haslams Creek generally had significantly ($P\leq0.05$) higher concentrations of Cd, Co, Cr and Pb in gut lining, liver and gills (Table 5) than toadfish from Lovetts Reserve. Toadfish from Haslams Creek, the most contaminated site, generally contained the highest metal concentrations in tissues, relative to toadfish from Lovett Reserve, the least contaminated site (Table 5). There were no significant differences ($P>0.05$) in liver for Zn; gut lining for Zn, As, Cd, or Ni; muscle for As, Cd, Co, Cr, Ni or Pb; gonads for Zn, As, Cd, Cr, Ni, Pb and Se; gills for Cd, Co and Pb metal concentrations among sites. The range of metal concentrations (μg/g dry weight) among toadfish tissues were as follows: Zn, 230–8760; As, 5.2–10.1; Cd, 0.01–0.5; Co, 0.04–1.9; Cr, 0.3–3.2; Ni, 0.2–5.9; Pb, 0.7–5.7 and Se, 2.3–9.1 (Table 6). These results were comparable with those of Farkas et al. (2003), who determined the tissue concentrations (μg/g dry weight) of Cd (0.4–2.1), Pb (0.01–0.2) and Zn (10.9–82.5) in muscle, gill and liver tissues of bream (*Abramis brama*). Canli and Atli (2003) generally found higher concentrations (μg/g dry weight) of Cd (0.5–2.2), Cr (1.9–9.3), Pb (4.9–23.8) and Zn (34–85) in muscle, gill and liver tissues in six species of Mediterranean fish compared with those reported for toadfish in this study.

Metal concentrations in toadfish tissues showed positive linear relationships ($P<0.001$; r^2 = 0.27–0.72) with those in infauna (Table 7). Barwick and Maher (2003) investigated the trophic transfer of a range of essential and non-essential metals in seagrass habitats in Lake Macquarie (south-eastern Australia) and devised potential trophic transfer models through different food chains (autotrophs, planktivores, herbivores, detritivores, omnivores and carnivores). They concluded that Se, and to a lesser extent Cu, Cd, Zn and Pb, were transferred and biomagnified to higher trophic levels (commercially important fishes) via diet. Concentrations of Cd, Co, Pb and Se in toadfish tissues were also positively related ($P<0.001$; r^2 = 0.32 - 0.73) to those in sediment (Table 7). In accordance with the results from this study, Peters et al. (1999) found that Se concentrations in the muscle tissues of three benthic-feeding fish species (*Mugil cephalus, Platycephalus fuscus* and *Acanthopagrus australis*) were positively related ($P\leq0:05$) with sediment metal concentrations. The presence of sediment (17%) in toadfish gut may explain why the concentrations of some metals in sediments were positively related to those in toadfish tissues (Alquezar, 2006). However, the concentrations of these metals in sediments were also positively related to those in toadfish prey, suggesting multiple exposure pathways; direct ingestion of sediments and/or ingestion of prey items that may have accumulated metals from sediments.

Table 2. Metal concentrations (µg/g dry weight) and general characteristics of sediments at each site. Mean ± 95% confidence limits (n = 4).

	Lovett Reserve	Charity Creek	Duck River	Haslams Creek	Sediment quality guidelines	
					Low[a]	High[b]
Metals						
Zn	84 ± 5.7	160 ± 24	**322 ± 42**	***434 ± 39***	200	410
As	4.0 ± 0.5	5.0 ± 0.7	**27 ± 3.4**	**23 ± 2.6**	20	70
Cd	0.12 ± 0.03	**2.3 ± 0.3**	**3.3 ± 0.5**	**2.8 ± 0.4**	1.5	10
Co	1.3 ± 0.3	7.4 ± 1.4	5.1 ± 0.6	12 ± 1.7	-	-
Cr	13 ± 1.6	37 ± 4.8	**313 ± 29**	**105 ± 13**	80	370
Ni	6.7 ± 1.0	**21 ± 3.3**	17 ± 2.2	**22 ± 2.7**	21	52
Pb	25 ± 3.5	**104 ± 16**	**120 ± 11**	**174 ± 20**	50	220
Se	0.16 ± 0.03	0.24 ± 0.06	0.52 ± 0.13	0.93 ± 0.14	-	-
Sediment characteristics[c]						
% gravel (> 2 mm)	8 ± 3	7 ± 2	6 ± 2	5 ± 2		
% sand (0.063 – 2 mm)	29 ± 5	31 ± 4	22 ± 4	26 ± 4		
% silt/clay (< 0.063 mm)	63 ± 7	62 ± 6	72 ± 8	69 ± 7		
% 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 and ARMCANZ 2000). Values shown in bold exceed this guideline value.
[b] Sediment quality guideline value above which there is high probability of biological effect (ANZECC and ARMCANZ 2000). Values shown in bold and italics exceed this guideline value.
[c] Sediment fractions and organic matter were measured as a proportion of total dry sediment weight.

Table 3. Metal concentrations (µg/g dry weight) in toadfish dietary items (infauna) at each site. Mean ± 95% confidence limits (n = 6).

	Zn	As	Cd	Co	Cr	Ni	Pb	Se
Semaphore crabs								
Lovett Reserve	0.09 ± 0.01	9.5 ± 1.0	0.18 ± 0.03	0.32 ± 0.04	1.9 ± 0.3	1.2 ± 0.2	7.4 ± 0.8	3.0 ± 0.4
Charity Creek	0.12 ± 0.01	9.0 ± 0.9	0.41 ± 0.06	0.89 ± 0.05	6.3 ± 0.7	1.7 ± 0.3	15 ± 1.7	3.3 ± 0.4
Duck River	0.11 ± 0.01	8.4 ± 0.7	0.31 ± 0.04	1.4 ± 0.15	6.3 ± 1.0	2.0 ± 0.3	13 ± 1.4	3.6 ± 0.5
Haslams Creek	0.11 ± 0.01	12 ± 1.3	0.39 ± 0.05	0.95 ± 0.12	11 ± 1.4	1.0 ± 0.2	14 ± 1.6	3.6 ± 0.4
Black mussels								
Lovett Reserve	0.02 ± 0.003	1.0 ± 0.2	0.09 ± 0.02	0.56 ± 0.07	0.36 ± 0.12	2.2 ± 0.2	2.9 ± 0.4	3.1 ± 0.4
Charity Creek	0.02 ± 0.003	1.0 ± 0.2	0.29 ± 0.04	0.51 ± 0.09	1.5 ± 0.4	2.7 ± 0.3	7.6 ± 1.1	3.2 ± 0.4
Duck River	0.02 ± 0.004	1.0 ± 0.3	0.19 ± 0.02	0.44 ± 0.05	3.0 ± 0.4	2.7 ± 0.3	6.0 ± 0.7	3.1 ± 0.4
Haslams Creek	0.03 ± 0.005	1.9 ± 0.3	0.31 ± 0.04	1.2 ± 0.2	7.0 ± 0.6	3.3 ± 0.4	16 ± 1.7	2.8 ± 0.3
Polychaetes								
Lovett Reserve	0.11 ± 0.02	19 ± 3.3	0.20 ± 0.03	1.5 ± 0.2	7.5 ± 0.8	3.2 ± 0.6	24 ± 2.8	3.1 ± 0.4
Charity Creek	0.11 ± 0.02	9.4 ± 1.5	0.10 ± 0.02	3.3 ± 0.4	24 ± 2.8	7.3 ± 2.1	36 ± 3.2	3.2 ± 0.5
Duck River	0.15 ± 0.03	23 ± 2.8	0.40 ± 0.05	3.4 ± 0.4	18 ± 1.7	4.9 ± 1.0	46 ± 4.9	2.7 ± 0.3
Haslams Creek	0.16 ± 0.04	18 ± 2.2	1.5 ± 0.2	3.7 ± 0.4	34 ± 3.7	10 ± 3.7	91 ± 8.5	3.5 ± 0.4

Table 4. Significant ($P < 0.001$, n = 60) positive linear relationships (r^2) between metal concentrations (µg/g dry weight) and nutritional value (% lipids and proteins/g dry weight) of toadfish dietary items (infauna).

	Zn	As	Cd	Co	Cr	Ni	Pb	Se
Lipids	0.64	0.77	-	0.39	0.36	-	0.26	-
Proteins	0.55	0.59	-	0.37	0.35	-	0.21	-

Table 5. Significant ($P < 0.001$, $n = 12$) positive linear relationships (r^2) between metal concentrations (µg/g dry weight) in sediment and toadfish dietary items (infauna).

	Zn	As	Cd	Co	Cr	Ni	Pb	Se
Semaphore crabs	-	-	-	-	-	-	0.30	0.50
Black mussels	0.78	0.77	0.32	0.58	0.89	0.32	0.87	0.29
Polychaetes	9.87	0.43	0.52	0.67	0.75	0.58	0.74	-

Table 6. Metal concentrations (µg/g dry weight) in toadfish tissues (gut lining, liver, muscle, gonads, kidneys, gills) at each site. Mean ± 95% confidence limits ($n = 12$).

	Zn	As	Cd	Co	Cr	Ni	Pb	Se
Gut lining								
Lovett Reserve	230 ± 74	5.1 ± 3.2	0.019 ± 0.007	0.33 ± 0.035	0.7 ± 0.4	2.6 ± 2.1	3.2 ± 2.4	3.1 ± 1.3
Charity Creek	330 ± 171	6.4 ± 1.4	0.011 ± 0.002	0.62 ± 0.13	3.0 ± 2.4	1.3 ± 1.2	6.5 ± 3.8	3.8 ± 0.9
Duck River	289 ± 122	4.5 ± 1.1	0.018 ± 0.004	1.1 ± 0.4	3.4 ± 2.5	1.2 ± 1.1	3.7 ± 2.2	3.7 ± 0.6
Haslams Creek	286 ± 94	4.9 ± 1.4	0.070 ± 0.011	1.0 ± 0.5	1.8 ± 1.0	0.24 ± 0.11	3.7 ± 1.2	3.3 ± 0.4
Liver								
Lovett Reserve	184 ± 33	5.3 ± 1.5	0.18 ± 0.015	0.57 ± 0.27	0.09 ± 0.04	0.08 ± 0.02	0.4 ± 0.3	2.8 ± 1.2
Charity Creek	100 ± 16	4.6 ± 1.2	0.19 ± 0.015	0.89 ± 0.19	0.19 ± 0.07	0.12 ± 0.06	1.3 ± 0.5	2.7 ± 1.1
Duck River	148 ± 24	3.2 ± 0.8	0.63 ± 0.055	1.7 ± 0.5	0.69 ± 0.17	0.23 ± 0.08	3.9 ± 1.6	3.1 ± 1.8
Haslams Creek	171 ± 29	5.4 ± 2.0	1.1 ± 0.12	4.1 ± 1.8	0.40 ± 0.16	0.64 ± 0.17	5.4 ± 1.9	4.2 ± 1.8
Muscle								
Lovett Reserve	58 ± 19	5.8 ± 3.5	0.003 ± 0.001	0.01 ± 0.004	0.5 ± 0.3	1.0 ± 0.4	0.7 ± 0.4	2.8 ± 0.4
Charity Creek	65 ± 46	6.3 ± 1.3	0.002 ± 0.001	0.01 ± 0.004	0.2 ± 0.06	0.78 ± 0.36	0.5 ± 0.4	1.9 ± 0.3
Duck River	46 ± 23	6.6 ± 3.5	0.001 ± 0.001	0.06 ± 0.02	1.0 ± 0.6	0.79 ± 0.35	3.9 ± 2.0	2.4 ± 0.4
Haslams Creek	96 ± 54	7.6 ± 2.9	0.024 ± 0.001	0.07 ± 0.02	0.4 ± 0.2	1.1 ± 0.7	1.6 ± 1.0	2.3 ± 0.3
Gonads								
Lovett Reserve	221 ± 122	5.6 ± 2.4	--	0.18 ± 0.03	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 ± 0.001	0.52 ± 0.11	0.5 ± 0.4	0.9 ± 0.4	0.27 ± 0.17	2.9 ± 0.8
Duck River	238 ± 89	4.5 ± 2.5	--	0.87 ± 0.46	0.5 ± 0.4	1.5 ± 1.2	0.86 ± 0.36	2.3 ± 1.2
Haslams Creek	214 ± 96	7.8 ± 3.8	0.002 ± 0.001	1.8 ± 0.4	0.3 ± 0.2	0.5 ± 0.2	0.48 ± 0.13	2.2 ± 1.5
Kidneys								
Lovett Reserve	8600 ± 2500	6.4 ± 2.4	--	0.09 ± 0.02	1.4 ± 0.8	5.4 ± 2.0	1.9 ± 0.8	8.4 ± 3.3
Charity Creek	7000 ± 2400	5.0 ± 1.6	--	0.41 ± 0.09	0.9 ± 0.2	1.4 ± 1.0	0.8 ± 0.3	7.3 ± 2.7
Duck River	9300 ± 4100	3.9 ± 1.0	--	1.0 ± 0.4	1.5 ± 0.5	1.6 ± 1.0	1.7 ± 1.1	10 ± 2.4
Haslams Creek	10 020 ± 2500	5.3 ± 1.5	--	2.2 ± 0.6	1.6 ± 0.4	1.6 ± 1.0	2.5 ± 1.1	10 ± 2.6
Gills								
Lovett Reserve	519 ± 153	6.3 ± 1.6	--	--	2.0 ± 0.5	3.0 ± 1.2	4.5 ± 1.6	3.9 ± 1.1
Charity Creek	353 ± 103	5.2 ± 1.1	--	--	1.9 ± 1.2	13 ± 4.0	4.8 ± 2.8	4.6 ± 1.4
Duck River	496 ± 167	3.9 ± 1.4	--	--	4.9 ± 2.8	1.2 ± 0.6	7.3 ± 5.9	3.9 ± 1.5
Haslams Creek	545 ± 187	5.2 ± 2.0	--	--	4.0 ± 2.0	7.3 ± 2.5	6.2 ± 2.5	5.0 ± 2.1

Table 7. Significant ($P < 0.001$, $n = 55$) positive linear relationships (r^2) between metal concentrations (µg/g dry weight) in toadfish tissues, and their dietary items (infauna) and with sediment.

	Zn	As	Cd	Co	Cr	Ni	Pb	Se
Semaphore crabs	-	-	-	-	0.27	-	-	0.47
Black mussels	-	-	-	0.50	-	-	-	-
Polychaetes	-	-	0.72	-	-	-	-	-
Sediment	-	-	0.73	0.49	-	-	0.32	0.33

Surface water is another potentially important metal exposure pathway for many sediment infauna (e.g. suspension feeders) 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 (P>0.05) 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 relative importance of sediment as a metal uptake pathway in toadfish and their prey items.

Toadfish size and age

There were no significant (P>0.05) differences in toadfish age amongst sites (Figure 4c). Toadfish from the more contaminated sites (Haslams Creek and Duck River) were 15% larger (~97 mm; Figure 4a) and 41% heavier (33–36 g; Figure 4b) than toadfish from the least contaminated sites (Lovett Reserve & Charity Creek; ~86 mm, 25–30 g, respectively; Figure 4). Thus, toadfish from the most contaminated sites (Haslams Creek and Duck River) had higher growth rates compared to toadfish from the least contaminated sites (Lovett Reserve and Charity Creek). This result is in contrast to previous studies (Canli and Atli 2003; Farkas et al. 2003) that have reported reduced size and growth rates of individual fish at metal contaminated sites. The higher percentages of lipids and proteins in polychaetes at Haslams Creek (Table 1) may contribute to the larger toadfish size at this site. Alternatively, toadfish from Haslams Creek may be larger than those from Lovett Reserve 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 and/or physiologically (non-genetic acclimation) adapted to long-term exposure of elevated metal concentrations in sediments and associated prey items. Although toadfish were larger at the most metal-contaminated site, chronic metal toxicity may lead to detrimental changes in toadfish population dynamics, via a reduction in the number of eggs and/or quality or longevity of offspring (Chapman 2002). 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 reduced longevity, compared to white suckerfish from adjacent non-contaminated lakes. Alquezar et al. (2006a) found that toadfish from contaminated estuaries in south-eastern Australia 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.

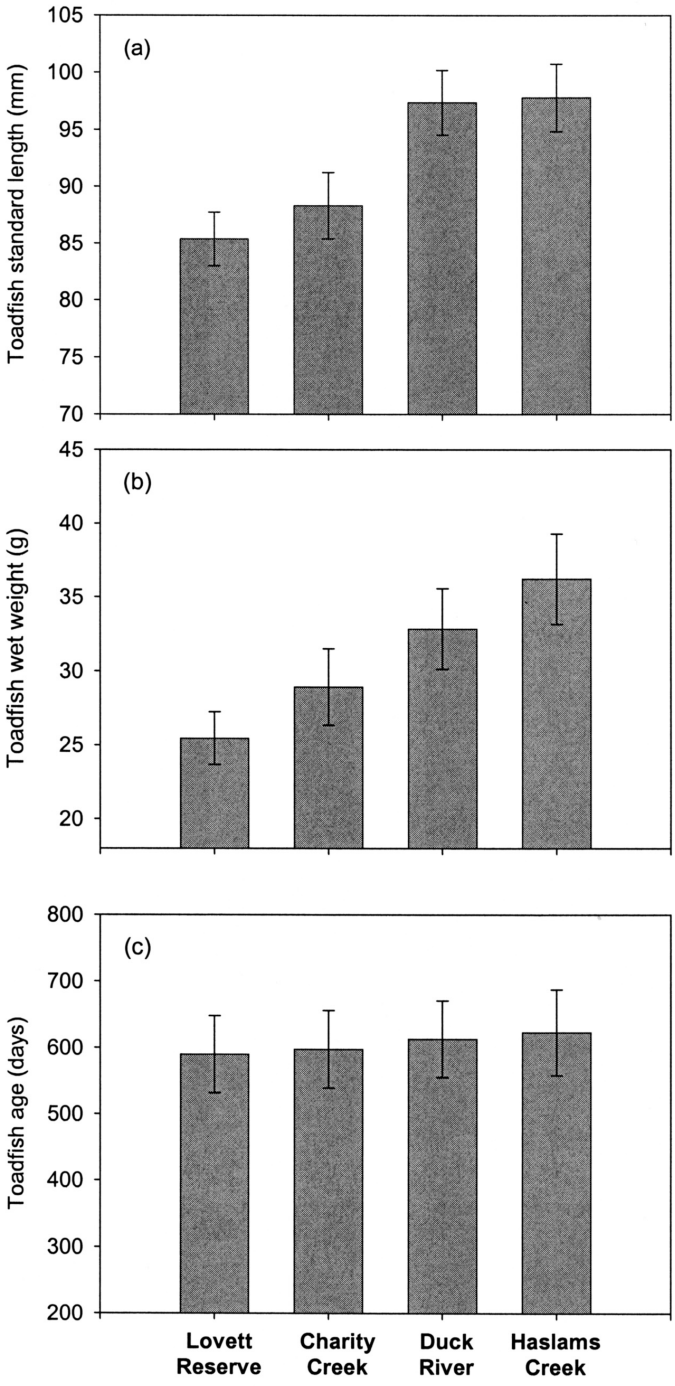

Figure 4. Growth characteristics of sexually mature toadfish at each site: (a) Standard length (mm), (b) wet weight (g), and (c) age (days). Mean ± 95% confidence limits (n = 55).

CONCLUSIONS

Metal concentrations in toadfish tissues were linearly and positively related to metal concentrations in both sediments and infauna, indicating that sediment and infauna are an important metal exposure pathway. Toadfish from the most contaminated site generally had the highest metal concentrations and were larger and heavier than similarly-aged toadfish from the least contaminated site, suggesting that toadfish may be benefiting in size due to ingestion of polychaetes with higher nutritional value. This may stem from physiological acclimation or acquired genetic resistance of sediment infauna through generations of continuous metal exposure – a working hypothesis that requires further investigation.

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