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Relationships between body burdens of trace metals (As, Cu, Fe, Hg, Mn, Se, and Zn) and the relative body size of small tooth flounder (*Pseudorhombus jenynsii*)

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ABSTRACT

Several studies have described strong relationships between body size and the accumulation of trace metals in animal tissues. However, few of these studies have utilized aging techniques to control for age related effects. We utilized relative body size (g y^{-1}) of a model flounder species, *Pseudorhombus jenynsii*, in order to control for age related effects on growth and size measurements. We investigated links between relative body size, concentrations of trace metals in flounder muscle tissue, physico-chemical variables (temperature, salinity, pH, and turbidity), and levels of trace metals in the sediment. Flounder were sampled using an otter trawl net in the inner areas of eight estuaries that were either heavily modified or relatively unmodified by urbanization and industrial activity. Our results indicate that this commonly eaten fish is accumulating significant levels of some trace metals in their muscle tissue, both in relatively unmodified and heavily modified estuaries. Concentrations of Cu, Zn and Fe in muscle tissue, as well as temperature, showed a negative relationship to the relative body size of flounder. In contrast, Se and Hg in muscle showed a positive relationship to relative body size. Observed growth patterns indicate that these effects are not driven by age related differences in metabolic activity. Instead, our results suggest that differences in food supply or toxicological effects may be responsible for the observed relationships between relative body size and concentrations of Cu, Zn, and Se in muscle tissues. The use of otolith aging and growth measurement techniques represents a novel method for assessing the relationships between trace metal accumulation and the relative body size of fish in a field environment.

1. Introduction

The accumulation of pollutants in fish species is of significant interest to ecotoxicologists and fisheries managers. This is due both to the potential ecological impacts of those pollutants (Johnston and Roberts, 2009) and because of human health risks associated with the consumption of contaminated fish (Campbell et al., 2008). Previous field studies have shown a strong relationship between the size of fish and the accumulation of various trace metals (Al-Yousuf et al., 2000; Canli and Atli, 2003; Liang et al., 1999). These relationships have often been attributed to changes in metabolic activity as a fish ages, where it is assumed that larger (and hence older) fish are experiencing a reduction in their metabolic activity (Canli and Atli, 2003). Changes in metabolic rate as a fish ages are thought to influence the uptake or efflux of trace metals, creating a strong relationship between body size, age, and tissue concentrations of trace metals (Widianarko et al., 2000). However, most studies have not assessed these relationships by explicitly determining the age of fish and have instead linked body size and tissue concentrations without controlling for differential growth rates or age related effects (Canli and Atli, 2003; Liang et al., 1999). By controlling for the age of fish, it is possible to determine if the relationship between trace metal accumulation and body size is due to other factors unrelated to the age of the animal. Prey-mediated effects (Deb and Fukushima, 1999) or physiological impacts due to the acute toxicity of trace metals (Buckley et al., 1982; Kearns and Atchison, 1979; Waiwood and Beamish, 1978) may also play a significant role in driving the relationship between trace metal accumulation and body size.

Stressors such as trace metals can draw metabolic energy away from growth, and changes to an organism's growth rate can be indicative of a loss of fitness or health caused by physiological or environmental stress (Brown and Ahsanullah, 1971). Several studies have documented reduced growth rates in fish as a result of exposure to trace metals (Buckley et al., 1982; Kearns and Atchison, 1979; Waiwood and Beamish, 1978). However, physiological impacts can be difficult to detect or accurately measure in a field environment (Mondon et al., 2001). In many fish, age can be determined through examination of the otoliths, and enumeration of the accreted calcified layers within these bones. This data can be used to control for age related effects in body size measurements, by dividing the specimen's weight by its age. This calculates the *relative body size* of the fish, which also provides an indirect measure of the relative growth rate

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over the lifespan of an individual. Although these methods are commonly used in ecological studies, they are seldom used in toxicological studies (Francis et al., 1992). As such, the utilization of otolith aging and growth measurement techniques represents a novel method for assessing the relationships between trace metal accumulation and the relative growth of fish in a field environment.

In most estuarine environments contamination accumulates in sediments, particularly in the sheltered inner areas of estuaries where flushing from coastal currents is minimal and fine sediments are available to bind contaminants (Hesslein et al., 1980; Knott et al., 2009). Because of this, concentrations of contaminants in sediments are normally far greater than in the surrounding water column (Dafforn et al., 2012; Knott et al., 2009). Consequently, organisms which are highly associated with the benthos are thought to be exposed to greater contamination levels than their pelagic or benthopelagic counterparts (Dallinger et al., 1987). Flounders spend their entire life cycle on the benthos and frequently feed or burrow in the sediment (Gomon et al., 2008). In addition, flatfish of various species have been the subject of a variety of field eco-toxicological studies (Cossa et al., 1992; de Boer et al., 2001; Mondon et al., 2001; Plaskett and Potter, 1979), and consequently represent an ideal taxa for the evaluation of contamination impacts. *Pseudorhombus jenynsii* (small tooth flounder) is a common Paralichthyidae (sand flounder) in the estuaries of southeastern Australia. *P. jenynsii* is a fast growing species and has high fecundity (minimum population doubling time 15 months). The species is an estuarine resident, and is a secondary consumer feeding at a trophic level of 3.5 ± 0.37 (Froese and Pauly, 2010; Gomon et al., 2008). *P. jenynsii* is also a predatory generalist and most of the animals that it preys upon (e.g. sediment infauna, benthic invertebrates, and small benthic fish) also spend the majority of their life cycle in or close to the sediment (Gomon et al., 2008). Lastly, *P. jenynsii* is a highly sought after species for both commercial and recreational fishing and is consumed by humans (Froese and Pauly, 2010). For all of these reasons, *P. jenynsii* represents an excellent species for the evaluation of contamination impacts in fish (Plaskett and Potter, 1979).

We explore the relationship between trace metal contamination in sediments, the occurrence of trace metals in the muscle tissues of *P. jenynsii*, and the relative body size of fish at different ages (as an indirect measure of relative growth rate over the lifespan). We assess these relationships within the context of large scale anthropogenic modification and physico-chemical variability. We hypothesize several outcomes:

1. We hypothesize that levels of trace metals in muscle tissues will be correlated with levels of trace metals in the sediment. Furthermore, we predict that flounder living in heavily modified estuaries will show higher levels of trace metals in their muscle tissues.
2. We predict that the occurrence of trace metals in muscle tissues, above concentrations which have been demonstrated to have physiological significance for fish, will show a negative relationship to the relative body size of *P. jenynsii*.
3. Lastly, we predict that the negative relationship between trace metals in muscle tissue and the relative body size of fish will be observed for all age groups.

2. Methods

2.1. Study sites

Fish were sampled in eight permanently open estuaries along the south coast of New South Wales, Australia. These included four heavily modified estuaries — Hunter River (32°55.352′S, 151°46.191′E), Port Jackson (33°44.258′S, 151°16.542′E), Botany Bay (33°59.352′S, 151°11.433′E), and Port Kembla (34°28.121′S, 150°54.410′E), as well as four relatively unmodified estuaries — Karuah River (32°38.782′S, 151°57.953′E), Broken Bay (33°32.203′S, 151°12.839′E), Port Hacking (34°04.680′S, 151°09.311′E), and the Clyde River (35°44.233′S, 150°14.272′E) (Fig. 1). The four heavily modified estuaries are all anthropogenically disturbed environments near large urban and industrial areas and are subject to intense commercial and recreational boating traffic, historic and ongoing contamination, greater recreational fishing activity, and substantial urbanization of their shoreline and catchment (Birch and Taylor, 1999; DPI, 2010; Scanes, 2010). In comparison, the relatively unmodified estuaries have fewer recreational fishers, less boating traffic (almost none of which is commercial), less urbanization of the coastline and catchment, and virtually no heavy industry (Birch and Taylor, 1999; DPI, 2010; Scanes, 2010). While these estuaries do have some degree of agricultural land use in their catchment, the majority of the catchment in all of the relatively unmodified estuaries is within conservation areas, forestry zones, or areas where anthropogenic utilization is negligible. Most significantly for this study, previous studies have indicated high levels of trace metal contamination in the four heavily modified estuaries, while the relatively unmodified estuaries have comparatively low levels of trace metal contamination (Dafforn et al., 2012).

Four sampling sites were initially selected in each estuary with three replicate trawls conducted per site to sample flounder (twelve trawls per estuary). Flounder were captured in 7–12 trawls per estuary, representing 3–4 sites. All sampling was done at a similar distance from the mouth of the estuary, within the sheltered inner areas of the estuary (Fig. 1).

2.2. Fish sampling methods

Sampling at each site was conducted with an otter trawl (6 m mouth, 12 m length, and 3.8 cm diamond mesh, with a fine 0.6 mm cod-end mesh) pulled over bare sediment at 4–12 m depth. Otter boards were rigged into a four point bridle using an approximate 3:1 warp to depth ratio. Trawls were 15 min, at a speed ~1 knot, covering a distance of ~450 m. Following trawling all fish were sorted on the boat and flounder were euthanized in a 100 mg L^{-1} benzocaine solution and frozen for transport back to the laboratory.

2.3. Tissue trace metal analysis

Fish were taken back to the laboratory and rinsed with Milli-Q water before being dissected for muscle tissue samples. All flounder were dry weighed and length measurements were taken in the lab. Acid washed tools were used for dissections and muscle tissue samples were placed in polypropylene vials and freeze-dried for 48 h prior to trace metal analysis. Freeze-dried samples were digested using microwave high pressure digestion (C-225) at 200 °C. All trace metals except Fe and Mn were measured using inductively coupled plasma mass spectrometry (ICP-MS) (C-209). Fe and Mn were measured using inductively couple plasma atomic emissions spectrometry (ICP-AES) (C-229). All samples were analyzed in batches with blanks and analytical accuracy was determined using certified reference material with every digestion batch. DORM-3 Fish Muscle and DOLT-4 Dogfish Liver reference material was obtained from the National Research Council of Canada. All recoveries were within 10% of the certified values. Limits of detection (LOD 3σ) were determined per digestion batch and are listed in the results section; where samples were below the limit of detection this has been noted (Table 1). Throughout the manuscript metal concentrations in both the flounder muscle tissue and in sediment samples are presented as μg g^{-1}.

2.4. Otolith analysis

Sagittal otoliths were extracted, cleaned, dried, and stored in plastic vials. The dorsal otolith was utilized for analysis while the ventral