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Accumulation of mercury in estuarine food webs: biogeochemical and ecological considerations.

By

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STATEMENTS AND DECLARATIONS

Declaration of Originality

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Date 28/10/13

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The candidate was the primary author who conceived the research idea, analysed the data and wrote the original manuscript (75 %); Catriona Macleod is the primary supervisor, providing advice on funding, framing the concept and manuscript preparation (10 %). Kerrie Swadling (10 %) provided statistical assistance and Sean Tracey (5 %) provided advice on manuscript preparation and fish biometrics. Data presented in this work was provided in part by Nyrstar Hobart, Tasmania, as part of the industry's annual monitoring program.

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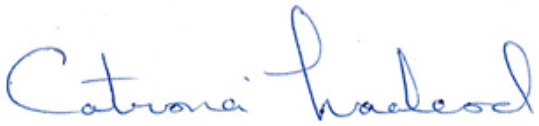

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GENERAL ABSTRACT

Estuarine systems that are exposed to industrial pollutants often retain a high loading of contaminants, including mercury (Hg), due to prevailing physical, chemical and biological conditions. Estuarine biota are principally exposed to Hg through dietary uptake, which can lead to higher order species bioaccumulating significant concentrations that can also be harmful to human health if consumed. Methylmercury (MeHg) production, bioaccumulation, and biomagnification in estuarine food webs are broadly understood but our knowledge of Hg food pathways and selenium's (Se) interaction with Hg is lacking. Current observations show poor correlation between bioaccumulation and environmental loadings, indicating that food web uptake and transfer of Hg are not straightforward. Understanding the mechanisms that underpin this variability is critical to quantifying and managing Hg exposure risks, and for developing appropriate management actions. The studies within this thesis examined the bioavailability, trophic magnification and bioaccumulation of Hg within a contaminated estuary to provide better capacity to manage the ecosystem and human health concerns.

Specifically this work focused on three areas: (1) The long-term capacity of resident fish to recover from Hg system contamination; (2) routes of Hg and Se trophic magnification within estuarine food webs; and (3) the influence of Se on Hg bioavailability and Hg toxicity. The study was based in the Derwent Estuary, Tasmania, a site of historical mercury pollution.

It was found that despite significant reduction of Hg discharges into an estuarine system, Hg concentrations in fish did not decrease, even after an extended period of time had passed (in this case, 37 years). The fact that Hg concentration in fish did not decline was only evident after application of biometric models, which suggests that monitoring of fish bioindicator species must include biological information to avoid misinterpretation of spatial and temporal trends of Hg contamination in biota.

Continuing, but spatially variable, methylation of Hg from sediments was found to be the key driver in the bioaccumulation of MeHg in resident fish. Co-contamination of Se and its close association with Hg in the sediments suggested a role of Se in reducing Hg bioavailability. Se uptake by resident fish was sufficient to maintain Se molar excess over Hg (a critical relationship in defining Hg toxicity), but an Se-based assessment of the risk of Hg toxicity to human consumers pointed to the potential for negative health effects associated with Hg in certain regions. This finding highlighted that, for human health assessments to be effective, the information on which they are based must be applied at a spatial scale appropriate to the source of Hg pollution.

To link an Hg source in the environment to fish, this research used a novel combination of Bayesian stable isotope mixing models and dietary analysis to provide refined trophic magnification models with which to evaluate Hg movement through food webs to the species of interest. The refined models reduced uncertainty in trophic magnification pathways and highlighted key benthic prey species as routes for Hg bioaccumulation.

These results provide a significant advance on the current understanding of Hg dynamics, specifically: improving our understanding of the relationship between Hg and Se; identifying issues with the way in which Hg concentrations fish are measured and reported so that the levels and risk can be more accurately understood; and identifying an improved approach for evaluating trophic interactions and bioaccumulation pathways. The findings will support estuarine management by informing existing monitoring programs and enabling better evaluation of the risks to human health in regions of Hg contamination.

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CHAPTER 1

GENERAL INTRODUCTION, OVERVIEW AND THESIS STRUCTURE

1.1 Background

Mercury (Hg) is a toxic element, which is widely distributed in the environment as a result of both natural (weathering, volcanic activity) and anthropogenic processes (mining, metal smelters, chloroalkali plants, fungicides in paper processing) (Lindberg, 2007; Amos et al., 2013). Estimates of the global pool of Hg suggest a 2.5-fold increase derived from human activities since the industrial revolution (Lindberg, 2007; Amos et al., 2013). In industrial areas, fallout from the atmosphere along with leaching from both land–surface and ground stores frequently results in elevated Hg concentrations in aquatic systems (Chen et al., 2001; Jones et al., 2003; Li et al., 2008).

Within the aquatic environment, Hg can exist in four basic forms: elemental Hg (Hg^0), ionized Hg (Hg^{2+}), mercuric sulfide (HgS), and organomercury (Compeau and Bartha, 1985). It is the methylated form of organomercury, and in particular monomethylmercury (CH_3Hg^+), that is produced by microorganisms in sediments, pore water, and the overlying water body, which becomes biologically available for uptake by aquatic organisms (Ullrich et al., 2001; Chen et al., 2008) (Fig 1.1). Methylmercury (MeHg) is initially taken up into food webs through bioconcentration at the base of food webs (Mason et al., 2000), and then biomagnified between sequential trophic levels as a result of dietary uptake and low depuration rates (Campbell et al., 2005; Chen et al.,

2009). Biomagnification of Hg in seafood and its subsequent consumption by humans is recognised as the principal source of Hg exposure in humans and, therefore, is also the main pathway for Hg-associated health issues (WHO, 1990).

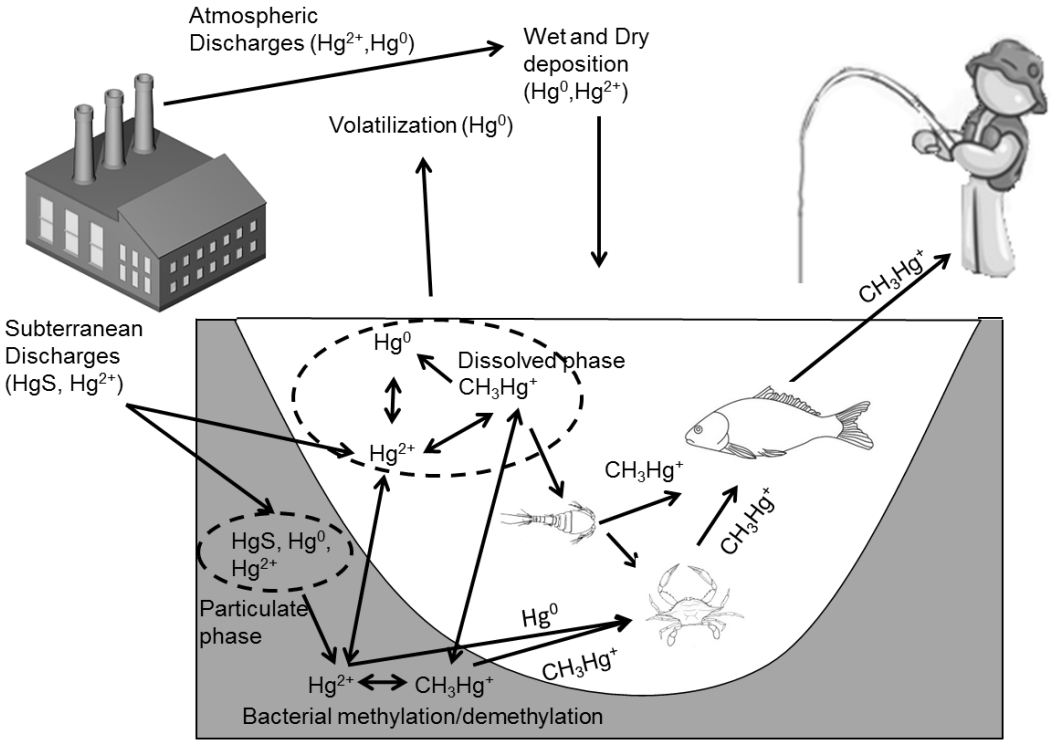

Figure 1.1. Biogeochemical cycling of Hg through sediment, water and air phases with bioaccumulation of Hg through an aquatic food web.

1.2 Study region

Estuarine systems contribute significantly to the pool of Hg in coastal areas as prevailing physical and biological processes promote regions where local conditions facilitate storage, methylation and export of Hg (Laurier et al., 2003). Estuaries can have high spatial variability in Hg contamination and there is often poor correlation between bioaccumulation in local biota and environmental loadings, indicating that food web uptake and transfer of Hg are not straightforward (Chen et al., 2009; Taylor et al., 2012). MeHg production, bioaccumulation, and biomagnification in estuarine food webs are

broadly understood; e.g. observations of higher methylation rates and bioaccumulation in environments (possibly micro-environments) that are depleted in nitrogen and carbon. But these processes are often poorly characterized due to the interrelated influences of localised Hg inputs, food web attributes and species feeding mechanisms (Davis et al., 2012; Taylor et al., 2012). Understanding the intricacies that underpin these processes is critical to quantifying and managing Hg exposure risks and to developing appropriate management actions for estuaries (Tom et al., 2010; Davis et al., 2012).

Unlike industrialised estuaries in the northern hemisphere, such as San Francisco Bay (USA), contamination of metals in the Derwent Estuary (42^{o} 54'S, 147^{o} 18'E; Fig 1.2) in southern Tasmania can be linked to a few specific point-source industrial inputs (specifically a zinc smelter and paper-pulp mill), and limited to a relatively short time frame (i.e. from early 20th century to present (Butler, 2006). Knowledge of the specific time frame and sources of Hg in this estuary makes it an excellent test site for field studies investigating the bioaccumulation and bioavailability of Hg in estuaries.

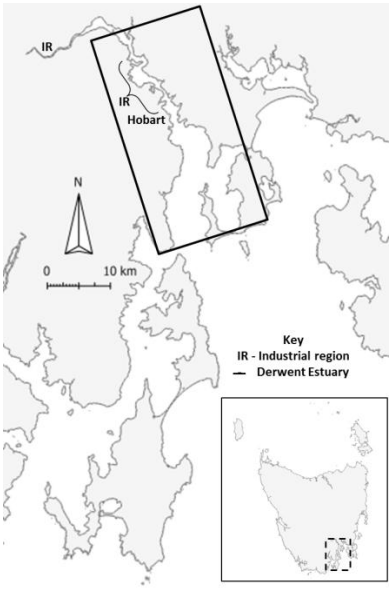

Figure 1.2. Location of the Derwent Estuary in Southern Tasmania, Australia.

The concentrations of heavy metals in the Derwent Estuary became a public concern in the early 1970's when exceptionally high concentrations of zinc were reported in harvested shellfish (Bloom and Ayling, 1977; Butler, 2006), and Hg concentrations in populations of sand flathead (*Platycephalus bassensis*) were found to be well above recommended consumption levels (Ratkowsky et al., 1975) (Table 1.1).

Region	**Study**	**Total Hg mgkg^{-1} (dw) sediments**	**Total Hg mgkg^{-1} (ww) sand flathead**
Derwent Estuary Tasmania	Bloom et al., 1974	1130	-
	Dix et al., 1975	-	1.1
	Langlois et al., 1987	-	1.6
	Jones et al., 2003	36	-
	Verdouw et al., 2011	-	1.4
Ria de Aveiro Portugal	Coelho et la., 2007	6.8	-
Minamata Bay, Japan	Kitamura 1968 (cited in Bloom et al., 1974)	2010	-0
Port Philip Bay Victoria, Australia	Fabris et al., 1992	-	0.89
Guideline values	ANZECC 2000,	1	0.5

Table 1.1. Mercury concentrations in sediments and fish from several estuaries/bays of known Hg contaminations and current Australian standards for sediment and fish concentrations.

Significant investment in abatement programs by the zinc smelter, such as ground water recovery systems and reduced atmospheric Hg emissions, have cut new Hg inputs to a fraction of former levels (Whitehead et al., 2010). However, the excessive heavy metal 'legacy' within the Derwent's sediments means that Hg concentrations continue to exceed national guidelines (Jones et al., 2003), and Hg loadings in the seafood require

health warnings advising against eating seafood from the catchment area (Simpson et al., 2005; Derwent Estuary Program, 2011) (Table 1.1).

Biological response to temporal reduction in Hg environmental concentrations is variable and dependent upon the forms of Hg within the system and the subsequent bioavailability of those species (Munthe et al., 2007); e.g. Hg compounds, such HgS, are likely to become bioavailable in a different time frame compared with organic bound Hg species (Davis et al., 2012). The pathways (pelagic vs benthic) by which Hg biomagnifies, and the feeding mechanisms of the organisms within the food webs, will further alter Hg bioaccumulation in higher-order species, such as fish. Quantification of Hg risk in an ecosystem requires a detailed understanding of the ancillary processes that might alter Hg bioavailability and bioaccumulation potential. These include: (i) the effect of selenium (Se) on Hg bioavailability and toxicity, as this element has a high affinity for Hg and has the potential to reduce Hg accumulation (Yang et al., 2008; Yang et al., 2011); (ii) change in organism growth rates, as Hg concentrations can vary as a result of differences in Hg assimilation and depuration efficiencies (Trudel and Rasmussen, 2006); (iii) and the importance of trophodynamics and food pathways on Hg biomagnification, as small changes in trophic position can result in relatively large changes in Hg concentrations in higher-order species (Campbell et al., 2005; Chen et al., 2009; Bank et al., 2007). Because of our lack of knowledge about these topics it is very difficult to design effective mitigation strategies for estuarine management or to accurately characterise human health risk. Simply monitoring the Hg concentrations in biota and the environment is not enough to explain patterns of distribution and temporal trends; the chemistry, biology and ecology that define Hg patterns in contaminated estuaries must be studied and understood.

1.3 Hg bioavailability from sediments

Sediment quality criteria (Simpson et al., 2005) form the basis for environmental management, setting acceptable limits for trigger levels based on total Hg (THg) concentration which is calculated as inorganic + organic Hg. However, the potential to evaluate ecological threat using these criteria is limited due to large uncertainties regarding the bioavailable fraction of Hg that can bioaccumulate in organisms (Mason and Lawrence, 1999; Ullrich et al., 2001; Taylor et al., 2012). Bioavailable Hg can include both inorganic Hg (InHg) and organic Hg forms, with organic MeHg being the principal form known to biomagnify and bioaccumulate in food webs (Chen et al., 2009) (Fig 1.1). Sediments are the main production site for MeHg, with variable contributions between 0.1 and 2.5% of the THg load (Ullrich et al., 2001). The bioavailability and toxicity of Hg in any given sediment is dependent on the specific make-up of the Hg complexes in that particular situation, consequently there is no simple relationship between Hg and biological availability (Ullrich et al., 2001). Therefore, it is important to know both the THg and MeHg concentrations to understand the toxicity and risk accurately.

Sulfur (S), Se, pH, organic carbon, redox, iron (Fe), nutrients and bacterial communities can all affect Hg bioavailability through their potential to influence methylation (Munthe et al., 2007), and differing combinations of these factors can result in complex and variable methylation rates (Ullrich et al., 2001). Broadly, methylation rates tend to increase in low-nitrogen (N), low-carbon (C) and low-S environments (Hortellani et al., 2005; Davis et al., 2012). Organic matter is often strongly correlated with THg, and as such may provide a reasonable predictor of THg in surface sediments (Mason and Lawrence, 1999). This is probably a result of adsorption of Hg to the organic compounds, particularly high molecular weight organic matter (Munthe et al., 2007).

However, MeHg tends to be less strongly bound to organic matter than other compounds, and, therefore, can be more easily mobilised (Mason and Lawrence, 1999). High-sulfide environments, such as those present in anaerobic sediments, severely limit Hg bioavailability for methylation by forming insoluble mercuric–sulfide (HgS) complexes (Mason and Lawrence, 1999; Ullrich et al., 2001). Hg's high affinity for organic matter and sulfur ligands results in low-solubility molecules, which are unlikely to be methylated (Shi et al., 2005) unless redox potentials and free sulfide ions allow bacterial uptake of HgS compounds (Ullrich et al., 2001). The level of oxygenation and organic enrichment status of the sediments will affect Hg bioavailability and any changes in these conditions can result in a significant change in MeHg bioavailability potential.

The presence of Se within sediments can also reduce Hg bioavailability as relatively inert mercuric–selenide (HgSe) complexes can be formed (Yang et al., 2008). Selenium exists abiotically as selenite (SeO_3^{2-}) and selenate (SeO_4^{2-}), but microorganisms and algae can metabolise these Se ions, converting them to Se^{2-} and Se^0 (Maher et al., 2010; Yang et al., 2011). Where selenite is reduced to Se^{2-} it is can sequester Hg^{2+} and form HgSe (Yang et al., 2008). This process can reduce the concentration of Hg available for bacterial methylation to MeHg (Yang et al., 2011), which will in turn suppress Hg bioaccumulation potential (Peterson et al., 2009). On the other hand, absence of Se within sediments has been linked to 'hotspots' of Hg bioaccumulation (Raymond and Ralston, 2004), and, where low level Se exists in aerobic sediments, there is increased likelihood of methylation at the water-sediment interface (Jin et al., 1997). However, although there is clear evidence of reduced Hg bioavailability in Se-rich sediments, the formation of HgSe in sediments has never been formally identified (Yang et al., 2011). Despite studies describing how sediment Hg–Se interactions regulate Hg bioavailability,

how these interactions affect Se–Hg relations in resident fish species within estuaries is still not clearly understood. It has been established that environmental Se can reduce Hg bioaccumulation in freshwater systems (Chen et al., 2001; Belzile et al., 2009), but it is not yet clear whether a similar response occurs in estuaries. It is important to understand if this occurs and the degree to which Se presence may influence Hg bioaccumulation.

1.4 Bioaccumulation of Hg in resident fish

The relationship between THg concentrations in the environment and the MeHg concentrations in resident fish is still not clearly defined, although a few studies have attempted to tackle this issue (Branfireun et al., 2005; Munthe et al., 2007). Regions with high Hg environmental loads may show low bioaccumulation if net methylation is low. Conversely, regions with low environmental Hg loads may result in high MeHg concentrations in fish tissue from high methylation efficiency (Brumbaugh, 2001). Where abiotic concentrations of THg are high, but biota concentrations are not, a threshold (saturation) point may have been achieved, such that further increases in THg loading will have no further impact on the MeHg uptake (Munthe et al., 2007). Several studies have found that resident fish and benthic invertebrates can exhibit much lower loadings in THg and MeHg than the surface sediments they inhabit (Mason and Lawrence, 1999; Southworth et al., 2000b; Coelho et al., 2008). In this situation THg concentration may no longer be limiting MeHg production, and it may be associated environmental conditions that are controlling methylation and uptake.

Contamination of sediments with Hg does lead to changes in biotic MeHg concentrations, but the magnification and timings of those changes is dependent on ecosystem-specific variables (discussed above), and species-specific biological responses

(Munthe et al., 2007). Assessing the temporal change of Hg bioaccumulation in fish is complicated by shifts in habitat, fish length, growth rates, prey preferences and the potential for seasonal movements or migration (Trudel and Rasmussen, 2006; Bank et al., 2007). As a consequence, Hg bioaccumulation in fish is highly variable among species, populations and even individuals (Andersen and Depledge, 1997; Simoneau et al., 2005). Fish are usually exposed to Hg over a number of years (Wiener et al., 2006), and the continual uptake over the course of the fish's life typically results in increases in Hg concentration with age and fish length (Tremblay et al., 1998; Simoneau et al., 2005; Verdouw et al., 2011). Therefore, the effect of fish length and age on Hg concentration must be considered in any assessment of Hg accumulation (Tremblay et al., 1998; Simoneau et al., 2005; Goulet et al., 2008).

For Hg bioaccumulation to occur within a fish, Hg intake must exceed Hg elimination and fish growth (Trudel and Rasmussen, 2006). The extent to which fish length correlates with Hg or fish age is dependent upon the relative importance of growth rate, activity costs, and Hg assimilation/depuration efficiencies (Trudel and Rasmussen, 2006). Fast-growing, short-lived species can exhibit linear correlations between fish length and Hg (Olsson, 1976; Verdouw et al., 2011), but have also shown non-linear relations (Andersen and Depledge, 1997; Magalhães et al., 2007). Fish growth is typically non-linear; therefore, models which can account for both linear and non-linear relationships are preferable when analysing bioaccumulation (Tremblay et al., 1998). Fish growth rates are often ignored in studies attempting to explain fluctuations in fish Hg concentrations, yet spatial changes in growth rates have been shown to influence Hg concentrations (Simoneau et al., 2005; Lavigne et al., 2010). Typically, lower growth rates are associated with increased muscle Hg concentrations in fish at a

given length (Lavigne et al., 2010; Cossa et al., 2012), as fast growing fish dilute Hg intake over a larger mass (Simoneau et al., 2005). However, bio-dilution explanations of Hg concentration and growth rates alone are too simplistic to provide full elucidation of Hg concentrations, as they will tend to underestimate activity costs (Trudel and Rasmussen, 2006).

Growth rates have been shown to be the dominant biological factor in accounting for differences in Hg concentration in fish populations (Simoneau et al., 2005), and suggested as a proxy for predicting Hg concentration on a regional scale (Lavigne et al., 2010). Growth rate estimates, best examined by von Bertalanffy growth models, can be used to estimate fish age at given length (Sonke and Blum, 2013), and Hg concentrations can then be correlated with these model outputs (Lavigne et al., 2010). Documented research detailing growth rates and variable Hg–fish length relationship studies are limited (Trudel and Rasmussen, 2006; Goulet et al., 2008; Lavigne et al., 2010), particularly in the estuarine environment. Without length, age and growth rate data on the fish species of interest, determination of spatiotemporal change in Hg bioaccumulation is very difficult, and, therefore, identification of management strategies for these species is severely limited.

1.5 Trophic transfer of Se and Hg

Hg biomagnification within a food web is the result of dietary Hg uptake exceeding Hg elimination (Chen et al., 2008). Both InHg and MeHg can accumulate through a food web, but only MeHg biomagnifies between successive trophic levels (Back and Watras, 1995; Estrade et al., 2011). With increasing trophic level the composition of the Hg load will alter, with the percentage contribution of MeHg increasing and percentage contribution of InHg decreasing (Andersen and Depledge, 1997; Chen et al., 2008; Kehrig

et al., 2009). For example, in zooplankton the MeHg contribution to total Hg load is generally around 10% (Al-Reasi et al., 2007), while in planktivorous fish muscle tissue MeHg contribution is typically nearer to 95% (Bloom, 1992; Evers et al., 2008). This relationship has led some researchers to only measure THg loads in fish, with the assumption that MeHg contributions essentially equal THg (Bloom, 1992; Campbell et al., 2008). In general, the overall trophic status of a species within an ecosystem can be indicated by the percentage of MeHg in its tissues (Mason et al., 2000). However, at lower trophic levels MeHg contribution to THg load varies significantly with feeding strategies (Evers et al., 2008), and life history can result in significantly different THg burdens (Coelho et al., 2008). For example, Mason and Lawrence (1999) found that deposit-feeding crustaceans had higher THg and MeHg than filter-feeding clams from the same region and Coelho et al. (2008) reported wide variation in Hg concentration in two infaunal species from the same area. As a result of varying Hg concentrations in prey, sympatric predatory fish species can have significantly different realised Hg concentrations despite similar home ranges (Bank et al., 2007).

Se has the potential to follow the same food pathways as Hg and the same trophic transfer processes (Kehrig et al., 2009). Like Hg, Se bioaccumulates with fish age and size (Cuvin-Aralar and Furness, 1991; Zhang and Wang, 2007), but as it can also be stored, recycled and eliminated when required, this relationship is not always evident (Arribére et al., 2008). It has been suggested that Se in the diet can decrease MeHg assimilation and increase Hg elimination, as HgSe complexes are less soluble in the gut (Chen et al., 2001; Yang et al., 2008; Dang and Wang, 2011). The presence of HgSe complexes in the trophic cycle may explain why Hg concentrations in fish from regions where Se is abundant are lower than in fish from regions with low Se concentrations, which may in

turn be Hg bioaccumulation hotspots (Ralston and Raymond, 2010). Whether there is indeed any benefit from Se in regulating Hg bioaccumulation remains ambiguous, with mixed results (positive, negative and lack of correlation) being reported between Se and THg (Cappon and Smith, 1981; Chen et al., 2001; Jin et al., 2006), and between Se and MeHg (Belzile et al., 2006; Kehrig et al., 2009). Belzile et al. (2006) suggested that the lack of evidence of a relationship between Hg and Se concentrations in low trophic levels may be the result of short life spans and specific variations in assimilation efficiencies. Their study found that MeHg was more closely correlated with Se concentrations than with THg, and they argued that this was because MeHg concentrations more closely reflected the actual bioassimilation process than THg. As Hg can bioaccumulate through both planktonic and benthic food webs (Chen et al., 2009; Maher et al., 2010), it follows that if Se and Hg were to bioaccumulate from separate pathways within a food web, then uncorrelated or negative bioaccumulation patterns would be possible. Equally, if Hg and Se are accumulated from the same food source, then positive correlations may exist. Under these conditions, it is important to be able to define the actual route for both Se and Hg bioaccumulation to tease out the relevant correlation patterns.

The ratios of carbon (δ^{13}C) and nitrogen (δ^{15}N) stable isotopes represent well established approaches for estimating trophic status, pathways and connections (Post, 2002). Fractionation of these two elements offers effective quantitative measures of trophic structure, providing time-integrated tracers of energy flow, dietary history and trophic position (Post, 2002). Carbon (C) isotope ratios (δ^{13}C) provide a biomarker of organic C production, identifying primary production sources (Ma et al., 2013), which show little trophic enrichment (0.8-1‰) between trophic levels (Vander Zanden and

Rasmussen, 1999). Nitrogen isotope ratios exhibit a constant rate of incremental enrichment between trophic levels, typically 3.4 ‰ (Post, 2002). This allows quantification of the trophic position of individuals in a food web, which becomes especially important when a pathway is muddled by omnivory (Vander Zanden and Rasmussen, 1999). As Hg accumulates through dietary intake δ^{15}N can provide quantitative data against which Hg biomagnification may be assessed (Cheung and Wang, 2008; Tom et al., 2010). Models based on δ^{15}N have been shown to predict changes in Hg concentrations in fish (Tom et al., 2010), provided that other parameters such as age, growth rate and location are taken into account. Studies examining the regression relationship between $\log_{10}$Hg and δ^{15}N have been applied across groups of organisms with different δ^{13}C signatures, providing ecosystem estimates of Hg biomagnification (Campbell et al., 2005; Al-Reasi et al., 2007). However, these studies do not represent direct pathways of energy/contaminant transfer to a species of concern, such as those consumed by humans, but rather an inferred trophic biomagnification process. If we wish to characterise Hg bioaccumulation in a particular species then there is a need to define specific MeHg biomagnification routes to that particular species, rather than use whole-of-ecosystem biomagnification values, as these values may not represent real MeHg biomagnification potentials or toxicity risks.

1.6 Selenium mercury interactions in fish and consequences for human health.

Currently, the most complete explanation for the protective effect of Se against Hg is where Hg or MeHg interrupt Se protein formation and sequester Se, resulting in the formation of HgSe complexes (Yang et al., 2008; Peterson et al., 2009; Ralston and Raymond, 2010). The formation of HgSe complexes significantly reduces Hg

bioavailability, lowering toxicity, but this process is also detrimental to the biological formation of the selenoenzymes, by diverting Se into HgSe complexes, and requiring supplemental Se to support continued enzyme synthesis (Raymond and Ralston, 2004). The HgSe complexes formed in this process are highly insoluble compounds with a relatively low toxicity, which then accumulate as a benign, detoxified product (Wagemann et al., 1998; Raymond and Ralston, 2004), and may later be slowly excreted (Yang et al., 2011). This suggests that over time they may offer an Hg detoxification mechanism.

For antagonistic protection from both Se and Hg toxicity, the molar concentrations of Se and Hg need to approach or exceed a 1:1 stoichiometry, as Se:Hg equal molarity suggests the formation of HgSe (Peterson et al., 2009). This is based on the assumption that Se and Hg are quantitatively bound to each other and any free Se is present in excess of the 1:1 ratio (Falnoga and Tušek-Žnidarič, 2007). Under these conditions, Hg toxicity may in fact not be dependent on the concentration of Hg present in an organism, but rather the moles of Hg relative to the moles of Se in the tissues. Se:Hg molar ratios that are > 1.0 would increasingly protect against Hg toxicity, while ratios < 1.0 offer a much lower level of protection against the adverse effects of Hg (Peterson et al., 2009).

For the majority of ocean fish, Hg molar levels do not exceed Se (Raymond and Ralston, 2004), but there are some notable exceptions among top predators: mako shark (–4.93) (Kaneko and Ralston, 2007) and lemon shark (–3.91) (Nam et al., 2011). The Se:Hg status of freshwater fish is less clear-cut (Ralston, 2008), and would appear to be system dependent, while there are currently insufficient data from temperate

estuarine systems to make an adequate assessment (Burger and Gochfeld, 2012), particularly in estuaries impacted by point source inputs.

Consumption by pregnant women of fish with high Hg concentrations has long been discouraged due to the potential for neonatal physiological damage (Harada, 1968; Grandjean et al., 1998), and there is evidence of impaired cognitive ability in children prenatally exposed to MeHg (Grandjean et al., 1998; Crump et al., 1998). The principal concern is the placental transfer of MeHg from mother to child, where it bioaccumulates on the child's side at a ratio of 7 to 1 (Mergler et al., 2007). However, the regular consumption of fish with low Hg concentrations is far more controversial as the potential health benefits associated with eating fish high in some compounds, such as omega-3 fatty acids and Se, potentially outweigh the detrimental effects of low-level MeHg accumulation (Hibbeln et al., 2007). In addition, Se uptake as part of a fish diet can offset any physiological effects of MeHg, even if some of the fish consumed contain additional MeHg (Ralston and Raymond, 2010). Advocates of fish consumption even argue that avoidance of fish during pregnancy not only fails to protect children's health, but could actually cause harm (Kaneko and Ralston, 2007), as in their view the nutritional benefits of seafood far outweigh any small adverse effect of MeHg (Flores-Arce, 2007; Hibbeln et al., 2007; Cabañero et al., 2007).

Consequently, there is ongoing debate regarding the health benefit versus health risk of consuming fish, which is further complicated by the addition of the potential effects of Se on Hg content. Current health assessments of Hg concentrations in fish are based on THg concentrations alone, both in Australia and globally (WHO, 1990; ANZECC, 2000). However, there is a growing body of research that would suggest that, in theory, consumption of fish with 1:1 Se:Hg ratio may offer protection against toxicity to the

consumer (Cabañero et al., 2007). To date, research in this field has been largely restricted to species of commercial relevance (Kaneko and Ralston, 2007; Fang et al., 2011; Burger and Gochfeld, 2012), and has ignored recreationally fished species. However, if Se health indices are employed it is important to consider spatial variation and whether there is any gradient of effect (in Se and Hg) in species living near known Hg contamination sources.

1.7 Thesis outline

The objective of this PhD is to improve our understanding of the complex interactions between Hg contamination and ecosystem functions and biological responses. In order to do this Chapter 1 introduces the background and rationale to the study, it identifies the unique situation in the Derwent and why this is such a good study site. This chapter also lays out the current knowledge and gaps in the global understanding of Hg bioavailability, bioaccumulation, trophic transfer and human health risk. The four subsequent research chapters focus specifically on one of these information gaps (Fig 1.3 chapters 2-5) and are then integrated in the general discussion and conclusions (Fig.1.3. Chapter 6) to provide an overall assessment of knowledge gained from these chapters and how they impact our understanding of the Derwent Estuary and Hg dynamics in the wider global context.

Sand flathead (*Platycephalus bassensis*) is currently used as a key species in the monitoring of estuarine and human health risk within the Derwent Estuary. Chapter 2 considers the relevance of the current biological monitoring approach for detecting temporal and spatial trends in the Hg concentrations of sand flathead (*Platycephalus bassensis*) in the Derwent Estuary. It is hypothesized that the current approach may miss spatiotemporal trends as it does not consider key fish biometric information. Therefore this chapter tests a set of new statistical approaches to address this and provide a clearer indication of Hg bioaccumulation in the estuary. The data presented in Chapter 2, combined with previous research (Langlois et al., 1987, Jones et al., 2003), suggest potential anomalies between Hg concentrations in sediments and biota within two Derwent Estuary regions; the point-source-impacted middle Derwent Estuary region and Ralphs Bay.

Chapter 3 (Fig 1.3) investigates the hypothesis that the observed sediment-biota Hg concentration anomaly may be the result of a biogeochemical interaction in the complex contaminant mix of the Derwent Estaury. In freshwater systems Se presence has been noted to reduce Hg bioaccumulation in resident fish (Chen et al., 2001). A similar situation may be relevant in the Derwent because there is a significant loading of Se in this system, and fish from the industrial region of the estuary may have reduced Hg concentrations as a result of these higher Se concentrations. In order to understand how the differences in concentrations of Hg and Se within a species arise it is imperative to investigate the bioaccumulation pathways of Hg and Se within the different estuary regions.

However, bioaccumulation of Hg and Se is dependent on diet (Chen et al., 2009). Chapter 4 tests whether combining stable isotope models with gut contents analysis can provide a better model of the principal bioaccumulation pathways of THg, MeHg and Se within the sand flathead than stable isotope analysis alone. The importance of understanding the accumulation of Hg and Se in sand flathead is that they are the major recreationally fished species consumed by people in Tasmania (Lyle et al., 2005). Current assessment of Hg risk to humans from seafood in Australia and globally is based on Hg concentration alone, yet research into Se based assessments of Hg can provide a more accurate projection of Hg toxicity (Ralston 2008). Research into Se based assessments of Hg toxicity is limited to commercial species on large regional scales (Ralston 2008). However, where Se and Hg concentrations vary over small spatial scales, like in the Derwent Estuary, Hg toxicity is also likely to vary. Chapter 4 tests the hypothesis that variable bioaccumulation of Se and Hg across the Derwent Estuary leads to changes in the Hg bioaccumulation and toxicity of fish from different regions.

These four research chapters combined provide fundamental information on bioaccumulation, biomagnification and toxicity of Hg, MeHg and Se within estuarine systems. The conclusions from this work will assist in governmental and industrial estuarine management plans, by providing improved global methods of assessing Hg bioavailability bioaccumulation and a more accurate assessment of potential toxicity and risk to human health. These data will provide valuable insights to inform remediation approaches and initiatives into the future.

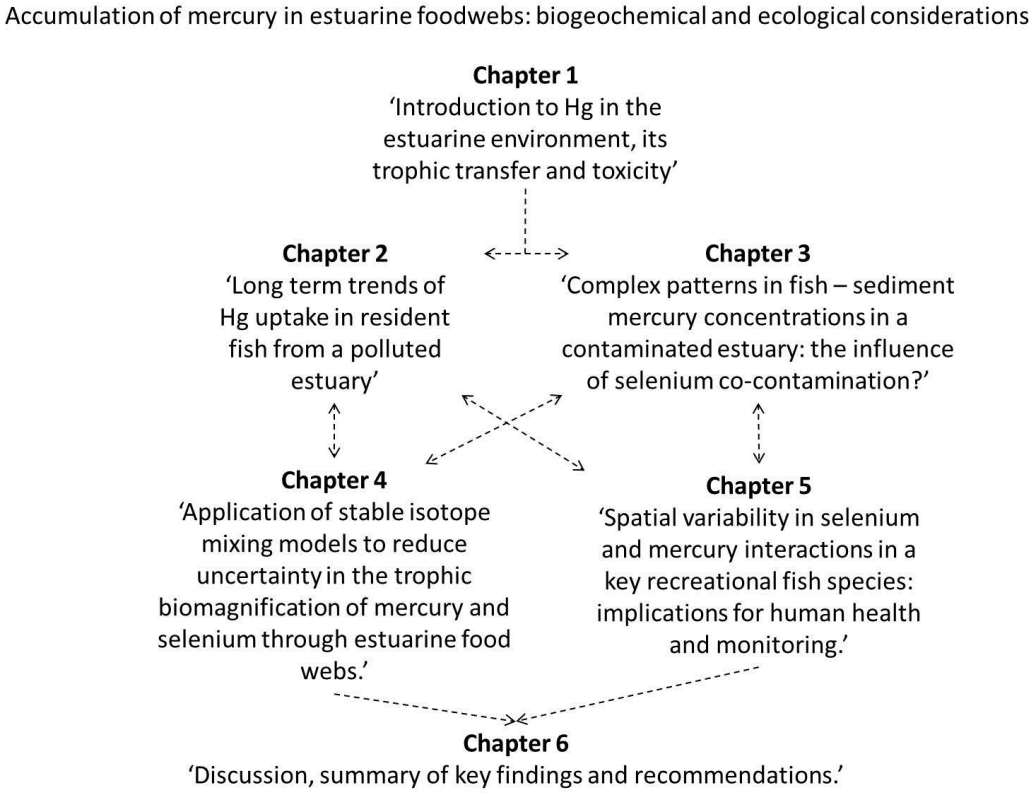

Figure 1.3. Thesis outline

CHAPTER 2

LONG TERM TRENDS OF Hg UPTAKE IN RESIDENT FISH FROM A POLLUTED ESTUARY

Preface:

One of the first objectives of this research was to evaluate existing temporal and spatial data for a resident estuarine fish species. The importance of doing this was to verify if this nominated bio-indicator was accurately portraying spatial and temporal trends. The intention was to validate this approach and if necessary propose an alternative method that may be applied to other similar temporal and spatial datasets to accurately detect long-term changes in fish Hg concentrations.

In this chapter I assess whether reductions in Hg inputs into the estuary actually resulted in observable decreases in localised fish populations and identify an assessment approach that validates the use of a resident fish species as a system bio-indicator. This has provided a statistically robust approach for the fish sampling program that will underpin the ongoing monitoring in the Derwent Estuary.

This work has been published in a refereed journal and is presented below in identical form. The citation for the original publication is:

Jones, H.J., Swadling, K.M., Tracey, S.R., Macleod, C.K., 2013. Long term trends of Hg uptake in resident fish from a polluted estuary. Marine Pollution Bulletin 73, 263-272

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2.1 Highlights

- Models examined present spatial and temporal change of mercury (Hg) concentrations in estuarine fish over a 37-year period.
- These models showed no temporal decline in the level of Hg in fish despite significant reduction of Hg inputs.
- Interannual variation in Hg concentrations accounted for by fish length variations and growth rate differences.
- The results highlight the importance of including biometric parameters for accurate interpretation of bio-indicators used for estuarine and marine monitoring programs.

2.2 Abstract

Mercury contamination of fish is dependent upon a system's ability to transform inorganic Hg into biologically available forms; however, fish biometrics also play an important role. To assess long term trends in Hg concentrations in sand flathead (*Platycephalus bassensis*) a polynomial model, corrected for fish length, was used to evaluate temporal trends and spatial variability, while growth rates were estimated using the Von Bertalanffy length-at-age model. Hg concentrations showed no decrease over time, and generally remained near recommended consumption levels (0.5 mg kg^{-1}). Previously reported spatial differences in Hg concentrations were not supported by the data once the models were corrected for fish length. Growth rate variation accounted for a large part of the previously published spatial differences. These results suggest that inclusion of fish biometrics is necessary to facilitate an accurate interpretation of spatial

and temporal trends of contaminant concentrations in long term estuarine and marine monitoring programs.

2.3 Key words

Mercury bioaccumulation, biometric modelling, Von Bertalanffy growth curves, long term monitoring, estuarine management

2.4 Introduction

The importance of long term data sets for describing environmental system change is frequently understated, but continuation of time series data should be actively encouraged as threats to ecosystems increase (Holmes, 2006). Mercury (Hg) pollution remains the least resolved of the major environmental health issue contaminants (e.g. lead, organochlorides) (Chen and Wilcox, 2008), in part due to its longevity in the environment and in part to its complex chemical behaviour. Hg-contaminated regions present a multitude of environmental challenges, with cessation of discharges often not resulting in improvements in some ecosystem sub-components for many years (Munthe et al., 2007). A review of aggressive programs to mitigate and remediate industrial Hg input into water bodies found reduced fish Hg burdens over varying time frames (Munthe et al., 2007). A major factor in the success of remediation strategies is a system's sensitivity to the translation of inorganic Hg into biologically available methylmercury (MeHg). Inorganic Hg is available to biota through bacterially-mediated methylation; the differential rates of Hg bioavailability and methylation within sediments and water are recognised as key factors contributing to spatial variation of observed Hg concentrations in fish (Ullrich et al., 2001). As individual fish bioaccumulate Hg over a number of years and depuration rates are low, changes in Hg uptake rates may not become apparent for some time after cessation of contamination inputs

(Wiener et al., 2006). Long term monitoring programs are, therefore, key to understanding temporal change in fish Hg loads, and provide valuable information in regard to human health when fish are taken as food (Rasmussen et al., 2007).

There are relatively few long term studies of point-source impact in estuaries, which have considered how biological traits of fish may affect temporal patterns of Hg bioaccumulation in a given species (Francesconi et al., 1997; Sager, 2002; Greenfield et al., 2005). Estuaries are net repositories of Hg, with only a small fraction of the Hg entering the system being exported (Chen et al., 2008). Estuarine sediments are the main production site for MeHg in the marine environment (Mason and Lawrence, 1999; Ullrich et al., 2001; Gehrke et al., 2011), and as such provide the critical interface for transfer of Hg into the food web (Taylor et al., 2012).

The Derwent Estuary in Tasmania has historical, point-sources of Hg inputs from two key riverside industries: a zinc smelter and paper-pulp mill (Verdouw *et. al.,* 2011). Despite significant reduction in Hg outputs from these sources, Hg contamination in the environment and fish remains an issue (Jones et al., 2003; Verdouw et al., 2011). Assessment of the biological effects of heavy metals in the 1970's (Ratkowsky et al., 1975) showed that Hg concentrations in many species were well above recommended guidelines for human consumption (0.5 mg kg^{-1} (FSANZ, 2004)) and resulted in sand flathead (*Platycephalus bassensis*) being selected as a bio-indicator of metal contamination by virtue of their high site fidelity, abundance and regular consumption by humans (Dix et al., 1975; Langlois et al., 1987); selection criteria still considered appropriate today (Evers et al., 2008). Hg concentrations in flathead muscle have been monitored since 1973 and an initial review covering the period 1973-1983 indicated a linear temporal decline in muscle tissue Hg concentration across the estuary with no

sign of plateau (Langlois et al., 1987). This led to the inference that the muscle Hg concentration in flathead within the Derwent Estuary could be expected to recover to a level suitable for human health (< 0.5 mg kg^{-1} w.w.) within a few years (Langlois et al., 1987). However, a more recent multi-species study of fish from the Derwent Estuary found that Hg concentrations in flathead were still significantly elevated in parts of the estuary (Verdouw et al., 2011), suggesting that the initial decline may not have continued. Considerable regional variation in Hg muscle concentrations has been detected throughout the Derwent Estuary, possibly a result of this species' high site fidelity (Tracey et al., 2011).

In this study, we test whether the temporal trend of Hg concentration decline in sand flathead (Langlois et al., 1987) and spatial variability of Hg concentration previously observed in the Derwent (Langlois et al., 1987; Verdouw et al., 2011) are still present. Mercury concentrations in fish are influenced by length and age (Tremblay et al., 1998; Simoneau et al., 2005; Verdouw et al., 2011). Therefore, the effect of fish length on Hg concentration must be considered to assess temporal and spatial Hg variation accordingly (Tremblay et al., 1998; Simoneau et al., 2005; Goulet et al., 2008). In this study, we apply a polynomial model devised for freshwater fish (Tremblay et al., 1998), to account for fish length, and thereby, more precisely evaluate temporal and spatial variability of Hg concentrations in sand flathead. Fish Hg concentration at standardized fish length limits this variable's influence; however, deviation in growth rates between fish mean standardized lengths can represent fish of dissimilar age and therefore exposure time (Simoneau et al., 2005). The potential difference in Hg concentration associated with standardized fish length, age and growth rate are considered with respect to the resultant temporal and spatial variability of Hg concentrations. The large sample size (n=3736) provides a unique opportunity to accurately describe the spatial

and temporal patterns of Hg contamination in sand flathead from the Derwent Estuary, and to enable broader inference of the implications for ecosystem health and long term recovery of estuaries degraded by Hg pollution.

2.5 Methods

Study Site

The Derwent Estuary (42^{o} 54'S, 147^{o} 18'E; Fig. 2.1) is a salt-wedge estuary that is highly stratified in its upper reaches and has well-mixed lower regions subject to an asymmetric microtidal regime (0.8 m) (Butler, 2006; Whitehead et al., 2010). The estuary has a mean depth of 15 m and reaches a maximum depth of 44 m. A large relatively shallow embayment (Ralphs Bay - RB) is located on the lower eastern shore of the estuary where depths are consistently less than 10 m (Whitehead et al., 2010). The estuarine regions referred to in this paper are used extensively in contemporary ecosystem monitoring and management programs (Whitehead et al., 2010), are consistent with previous studies on metal contamination within the region (Eustace, 1974; Langlois et al., 1987), and as well in models of hydrological flow (Thomson and Godfrey, 1985; Margvelashvili et al., 2005). The middle estuary (ME), which represents the industrialized region, is mixed predominantly by wind rather than tide (Thomson and Godfrey, 1985). The lower estuary regions – Western shore (WS), Eastern Shore (ES) and Ralphs Bay (RB) – are subject to significant refracted wave action, with freshwater flowing out along the eastern shore as a result of prevailing westerly winds (Butler, 2006). The deeper areas of the estuary are dominated by finer-grained, muddy sediments, with coarser, sandy sediments found in the shallow areas due to wave, wind and riverine influences (Green and Coughanowr, 2003). Mickey's Bay (MB), which is located approximately 48 km south of the estuary, was sampled as a marine reference

region remote from sources of contamination throughout this study (Langlois et al., 1987; Verdouw et al., 2011)

Sample collection

Data were collated from two sources; Langlois *et al.* (1987) which was comprised of 863 flathead caught between 1973 and 1983, and the estuary monitoring program where 2832 fish were sampled between 1980 and 2011. Sampling before 1991 was sporadic but from 1991 an average of 20 fish (min=18, max=43) were collected per region per annum. Three of the five regions comprised more than one sampling site (Fig. 2.1), (ME sites =1-2, ES sites =3-8, WS sites =9-10, RB =11 and MB (reference region). To achieve a representation of the size composition of fish in each region a range of sizes were sampled wherever possible.

Sand flathead were caught using rod and line from October-December each year. They were stored in resealable plastic bags and placed on ice in the field and once returned to the laboratory they were frozen at -40 ^{o}C. Fish, were measured for length and then dissected with one fillet of muscle tissue removed from each fish posterior to the pectoral fin, and refrozen prior to analysis. In 2003, 2007, 2010 and 2011, sagittal otoliths were extracted for age analysis. Determination of fish age followed the method of Jordan *et al.* (1998), where resin-mounted, sectioned sagittal otoliths were read by two independent readers, with between and within reader precision examined by index of average percent error (Beamish and Fournier, 1981).

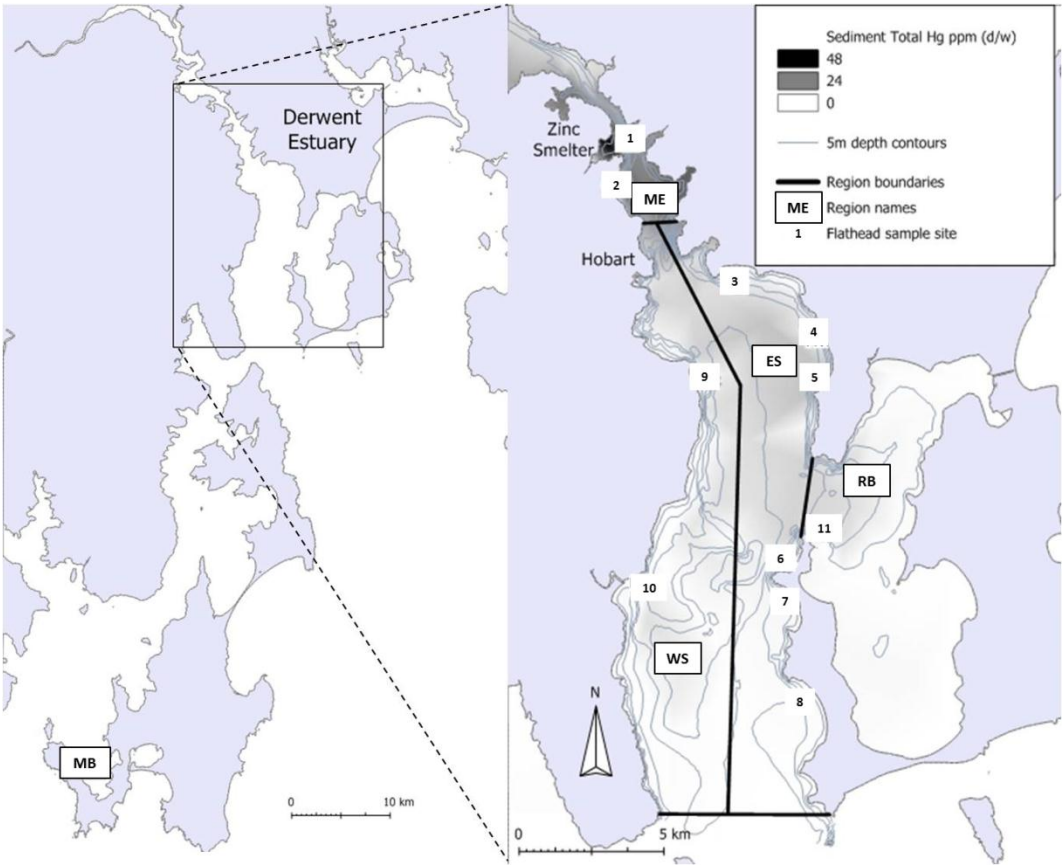

Figure 2.1. Southern Tasmania and location of the Derwent Estuary, with separation of estuary into four regions based on hydrodynamic flow and location of reference region (MB) 48km south. Location of sample sites for sand flathead (numbered 1 to 11), along with the position of Hobart city and zinc smelter, one of the historical Hg sources. 5-m bathymetric contours are also presented with previously unpublished sediment total Hg data collected in 2011, courtesy of Derwent Estuary Program .

Hg analysis

Details of the Hg analysis used by Langlois *et al.* (1987) are found within that paper. Briefly, digestion of samples (5 g) was achieved using a mixture of vanadium pentoxide (10-20 ml), nitric acid (10 ml), sulphuric acid (10 ml) and hydrogen peroxide (1:100 ml). Aliquots of the final volume (100 ml) were then analysed by cold vapour atomic absorption spectroscopy against Hg standards that were matrix-matched with samples from the reference region (MB). The detection limit of 0.1 mg kg^{-1} was never approached and inorganic Hg standards returned mean values of 100.7% recovery. All

subsequent Hg analysis followed the procedure outlined by Verdouw *et al.* (2011). A 1 g (± 0.1 g) subsample of homogenised, skinless muscle tissue was digested in acid (HNO_3 67% v/v plus H_2SO_4 33% v/v) at ~97 ^{o}C for 3 hours. After cooling, Hg was extracted by adding potassium permanganate ($KMnO_4$) and potassium persulphate ($K_2S_2O_8$) and allowing the resulting solution to react for 12 hours. The extraction process was then repeated until the colour stabilised, with any excess $KMnO_4$ reacted with hydroxylamine hydrochloride (NH_2OH HCl) to ensure a clear final solution. Hg quantification was performed using cold vapour atomic fluorescence spectrometry (CV–AFS) (PSA, UK). The limit of detection (LoD) for Hg using this approach was 0.02 mg kg^{-1}, with reported values being an average of duplicate analyses. A standard linear calibration with a correlation coefficient of 0.999 was achieved for all analyses. Quality control (QC) measures included running of blanks (<LoD), certified reference materials (CRM) DOLT-4 (NRC, Canada) $\bar{x}$ = 2.81 mg kg^{-1} (± 0.10), certified value = 2.58 mg kg^{-1} (± 0.22) and blank matrix spike (25% of the theoretical value of 1.00 µg L^{-1}) with every 20 samples analysed. All Hg data are reported in mg kg^{-1} wet weight (ww) for skinless muscle tissue. Although the two methods vary slightly in digestion and detection procedures, recovery rates of standards and QC measures suggest sufficient robustness for comparison of datasets to be acceptable. Similar comparative analysis of combined datasets using different digestion and detection procedures has been previously published within this subject area (Lavigne et al., 2010).

Data analysis

All statistical assessments were performed using the R statistical package (15.0.0; R foundation 2012). Linear regression analysis was used to examine relationships between Hg concentrations and fish biometrics. ANOVA with Tukey HSD post hoc

comparison of means and ANCOVA were used to examine spatial and temporal variations between regions. Hg concentrations were assessed via Box-Cox plots and $\log_{10}$ transformations of Hg were undertaken to conform the data to model assumptions of normality. The inclusion of 'year' as a model parameter was of specific interest in order to assess temporal change and relate any observed temporal response to those previously reported (Langlois et al., 1987). To allow incorporation of the Langlois *et al.* (1987) dataset into the overall analysis, mean annual Hg concentrations per region were calculated between 1981 and 2011. A linear regression model was applied incorporating Hg concentration as a response variable and 'region' and 'year' as co-factors. In addition, individual LOESS (locally weighted polynomial regression) curves were fitted to separate regions, and then compared against the linear regression curves with one-way ANOVA to assess goodness of fit.

A lack of structured annual data collection prior to 1991 prevented the fitting of models that included length within and between regions for this time period, so those data were eliminated from further analysis. Fish length between 1991 and 2005 was measured to standard length (SL) and thereafter as fork length (FL). SL data for all fish prior to 2005 was adjusted to FL using linear regression compiled from a sample of fish from 2010-2011 (n=200), when both measurements were recorded (Fig. 2.2). The FL to Hg relationship in flathead has previously been reported as linear (Verdouw et al., 2011), however, as flathead growth curves are best examined with Von Bertalanffy curves (Jordan et al., 1998) both linear and quadratic terms were modelled.

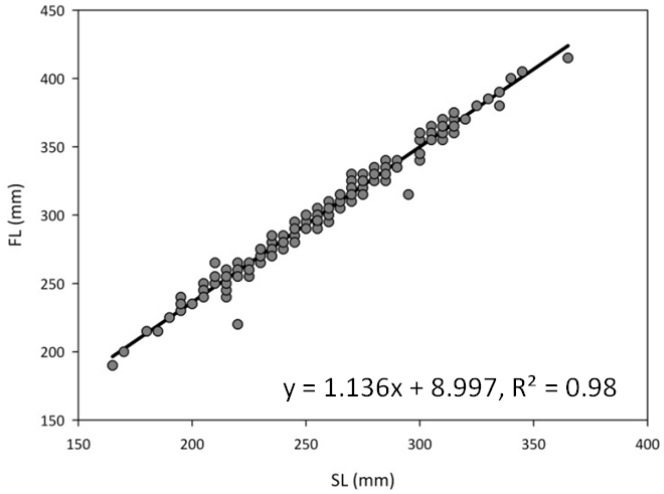

Figure 2.2. Standard length (SL) to fork length (FL) regression in sand flathead *(P. bassensis)* from the Derwent estuary, n=200, y=1.1363x +8.9974, R^2 = 0.98.

Polynominal relationships between FL and Hg allow statistical comparison between years even when the shape of the relationship between FL and Hg varies (Tremblay et al., 1998). This approach has been applied in a number of Hg monitoring studies (Simoneau et al., 2005; Goulet et al., 2008; Lavigne et al., 2010), where inadequacies in the linear regression relationships complicated the mean Hg concentration comparison (Tremblay *et al*., 1998). To achieve normality and remove collinearity between FL and FL^2 Hg concentrations in the present study were $\log_{10}$ transformed, and FL centred. A polynomial regression model was applied both temporally (1991-2011) and spatially (5 regions) using the model of (Tremblay et al., 1998):

$$Hg = a + bL + cL^2 + \mathcal{E} \quad (1)$$

Where Hg is the concentration of Hg within the fish tissue (mg kg^{-1} ww), L is the centred fork length, *a, b, c*, are model coefficients and $\mathcal{E}$ is an error term. Annual variation or regional comparisons can then be described by introducing binary variables

(e.g. B_1,B_2) where each binary represents a year or region, and *d* estimates the difference in mean Hg concentration between two binaries (Tremblay et al., 1998):

$$Hg = a + bL + cL^2 + dB + eBL + fBL^2 + \varepsilon \quad (2)$$

Step-wise elimination of non-significant coefficients (Tremblay et al., 1998), using Akaike's Information Criterion (AIC), was then applied to reduce model complexity. This model has been shown to perform well against other polynomial and linear models when describing temporal and spatial relations where fish FL varied temporally and spatially (Goulet et al., 2008). The models for each year or region were then compared based on curve shape and position as they share the same quadratic structure, and assessment of differences made through overlap between coefficient confidence intervals (P=0.05). Model prediction of mean Hg at any fish length for each year or region can then be calculated using the model equation for that year or region along with the standard error of mean response (Tremblay et al., 1996). A FL standard (FL_{std}) of 300 mm was used for comparison in this instance as this represents the minimum allowable catch size of sand flathead in Tasmanian waters, and therefore, the minimum FL of human exposure to Hg. For a subsample of fish (n=428), age data were available and a growth-rate model was fitted for each region. The relationships between FL and age were determined using the von Bertalanffy growth function (VBGF) (Chen et al., 1992):

$$TL = L_\infty(1-e^{-K(t-t0)}) \quad (3)$$

Where *TL* is fish length in mm, L_∞ asymptotic length, *K* the growth coefficient, *t* is the fish age and *t0* the hypothetical fish age at *TL*=0. Assessment of variation in the model between regions was conducted through a series of likelihood ratio analyses in accordance with the methods of Haddon (2001). Growth rate comparison between

regions was assessed using Hg concentration at 3 standardized lengths (FL_{std}): 261 mm, 293.9 mm, 300 mm. The first value represents the 1st quartile of FL within the dataset, 294mm was the mean FL of all fish caught within the dataset (n=428), and 300 mm was selected as the minimum legal FL for fishing. The upper quartile was not used due to some regions not having data within this sector. Age in this model is measured in decimal years (dy) where the start of a year is at time t_0 and the end of the year is at time t_1. The fish age between start and end of the year was then calculated as a fraction of that year.

2.6 Results

The mean FL of flathead caught between 1991 and 2011 within the Derwent Estuary ($\bar{x}$=277 mm, range = 179.6–487.4 mm), was significantly smaller ($T_{1,33}$= 151.35, P=<0.0001) than the fish caught in Mickey's Bay ($\bar{x}$=293.7 mm, range=205–482.2 mm). Within the Derwent Estuary, FL varied between regions ($F_{3, 1850}$=19.87,P= <0.0001), with RB > ES = ME >WS, and between years ($F_{18, 2279}$ = 249.2, P=<0.0001). Mean age in years of fish within the estuary was significantly lower than that of the Mickey's Bay reference region (MB) ($F_{1,426}$=33.02,P= <0.0001), (Derwent $\bar{x}$ = 4.56 (± 0.1), MB $\bar{x}$ = 5.99 (± 0.3)). A linear regression of length to age indicated a significant positive relationship but with low explanatory power ($F_{1,426}$= 148.4, P=<0.0001, R^2 = 0.26).

The mean Hg concentration of muscle tissue from fish caught in the Derwent Estuary between 1975 and 2011 was 0.49 ± 0.01 mg kg^{-1}. This was significantly higher than the concentrations reported for fish from the reference region (MB) (1981, 1991-2011, $\bar{x}$ = 0.20 ± 0.02 mg kg^{-1}) ($F_{1,2277}$= 527.5, P=<0.0001) (Table 2.1). Forty percent of all fish from the Derwent Estuary had Hg concentrations ≥ 0.5 mg kg^{-1}, rising to 56% in fish

with FL ≥ 300 mm. Only 1.6% of fish from MB had Hg concentrations ≥ 0.5 mg kg^{-1} with all of these fish greater than 300 mm FL.

Table 2.1, Mean sand flathead Hg muscle-tissue concentrations (wet weight) with standard deviation (sd) from four Derwent Estuary regions and a reference region (MB) between the years 1974–2011 (n =. n years indicates the number of years of data for each region. Superscript letter (^{a,b,c,d}), denotes significant differences (P= 0.05) assessed between regions through ANOVA with Tukey's HSD post hoc.

Region	n years	n	x̄ Hg mg kg^{-1}	sd
ME	36	796	0.49^{b}	0.13
WS	33	815	0.37^{c}	0.12
ES	35	851	0.50^{b}	0.15
RB	36	829	0.62^{a}	0.13
MB	22	445	0.18^{d}	0.06

The linear regression model applied to mean Hg concentration in fish collected between 1974 and 2011 for the four Derwent Estuary regions indicated no significant interaction between Hg concentration as a function of year and region ($F_{89, 12}$= 0.104, P = 0.85) (Table 2). There was, however, a significant difference in Hg concentration of the fish between regions ($F_{3,12}$= 26.39, P=<0.0001) (Table 2.2), with a descending order of Hg concentration RB>ME=ES>WS (Table 1). There was also significant variation of Hg concentration in fish across years ($F_{35, 12}$= 2.66, P= 0.04) (Table 2.2). Linear regression of year and Hg concentration in sand flathead provided a good fit for all four regions, with no significant difference between the linear model fit and the best fitting LOESS model for each region (Table 2.3). Despite evidence of annual variation, both linear and LOESS

model regressions by region indicated no significant temporal trend in Hg concentrations over the 37 year period for any region (Fig. 2.3).

Table 2.2, Linear model of sand flathead Hg muscle concentrations from four Derwent Estuary regions for years 1974-2011 (n= 3736), Region*Year indicates region by year interaction. Asterisks indicates significant values (*p=0.05, ***p<0.001).

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Region	3	0.135	0.045	26.39	1.436^{e-05} ***
Year	35	0.159	0.005	2.66	0.03647*
Region*Year	89	0.104	0.001	0.68	0.84849
Residuals	12	0.021	0.002		

Table 2.3. Linear and LOESS (locally weighted polynomial regression) best-fit model statistics for mean annual sand flathead Hg concentrations 1974-2011.For linear models this includes *F*-value, *P*-value, regression co-efficient (R^2), the residual Sum of Squares (^{res}SS) and degrees of freedom (df) and for the LOESS best fit model this includes the smoother index, degrees of freedom (app.df) and the residual Sum of Squares (^{res}SS). Model comparison data gives ANOVA results for contrast between LOESS best fit model and linear model with *F*-value, df and significance level (*P*-value) for the comparison.

Region	**Linear Model**					**Loess Model**			**Model comparison**		
	F-value	*P*-value	R^2	^{res}SS	df	Smoother index	App. df	^{res}SS	*F*-value	df	*P*-value
ME	0.91	0.35	0.03	0.42	31	5	28.00	0.20	0.07	28.00	0.79
WS	1.07	0.31	0.03	0.57	34	4.86	29.14	0.49	0.00	29.14	1
ES	0.12	0.73	0.00	0.57	33	4.82	28.18	0.45	0.07	28.18	0.79
RB	2.21	0.16	0.06	0.77	34	4.8	29.20	0.51	0.07	29.20	0.79

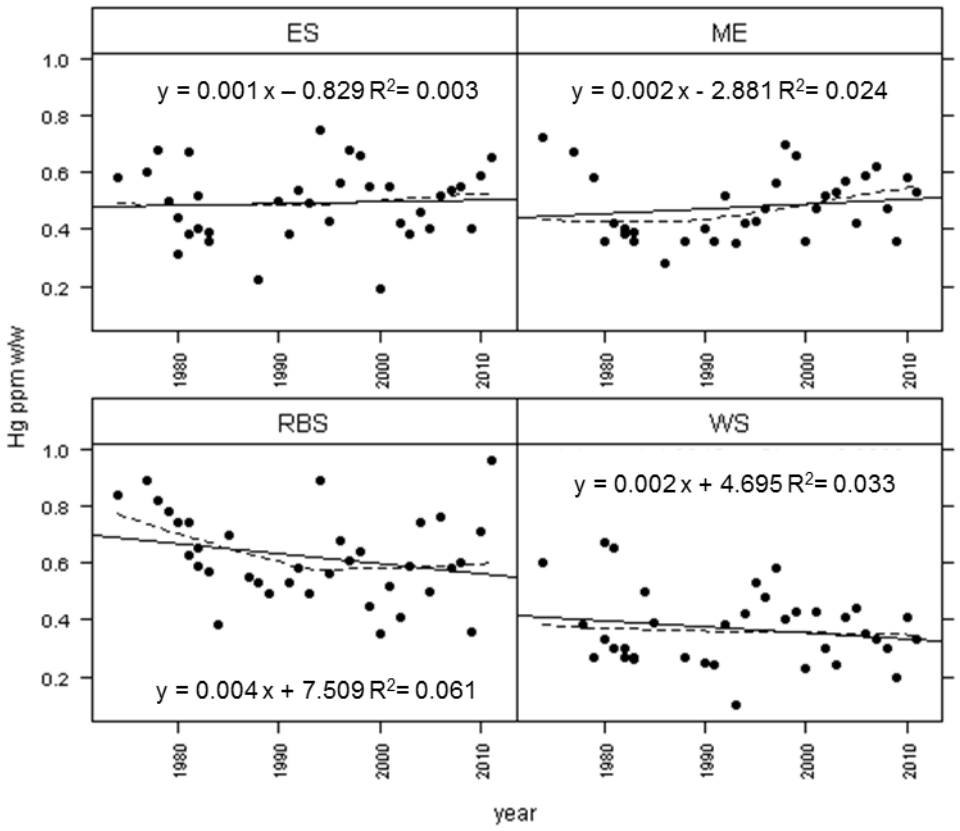

Figure 2.3. Mean Hg concentration in sand flathead muscle tissue in four Derwent regions from 1974–2011. Solid line = linear regression with intercept and slope and regression coefficient (R^2). Dotted line = LOESS (locally weighted polynomial regression) smoothed fit.

Temporal Models describing Hg bioaccumulation (1991-2011)

Step-reduced final models of temporal trends including FL returned significant quadratic terms for each region, indicating that the FL–Hg relation was not linear (Fig. 2.4). The regional models explained between 42% and 52% of the variation present within the Hg concentration of flathead muscle tissues in each region (Table 2.4). The models indicate a general increase in Hg concentration with FL, but the relation varied across years and regions (Fig. 2.4). For most years Ralphs Bay (19 of 21) and Middle Estuary (14 of 21) showed a simple, positive curvilinear relation between Hg concentration and FL (Fig. 2.4). A similar relation was also evident for approximately half

of the time (11 of 21) in both the Western Shore and Eastern Shore regions with the other years suggesting a more complicated response as a result of interaction between standardised FL and/or standardised FL^2 (Fig. 2.5). The presence of significantly different curve positions in some years, indicated by lack of overlap between confidence intervals (*P*=0.05), showed no apparent trend with time (Fig. 2.5).

Table 2.4. Evaluation of Akaike information criterion (AIC) mediated step-reduced spatial models with dummy variables for sand flathead Hg concentrations from the Derwent Estuary regions and reference region (MB) 1991-2011. Models include regression coefficient (R^2), residual standard error (se) *F*-value, *P*-value, and degrees of freedom (df).

Region	R^2	Residual se	*F*-value	df	*P*-value
WS	0.52	0.06	9.93	46, 422	2.20^{E-16}
MB	0.50	0.07	14.14	28, 396	2.20^{E-16}
ES	0.42	0.17	10.42	29, 426	2.20^{E-16}
RB	0.48	0.11	16.86	23, 422	2.20^{E-16}
ME	0.44	0.13	12.05	29, 451	2.20^{E-16}

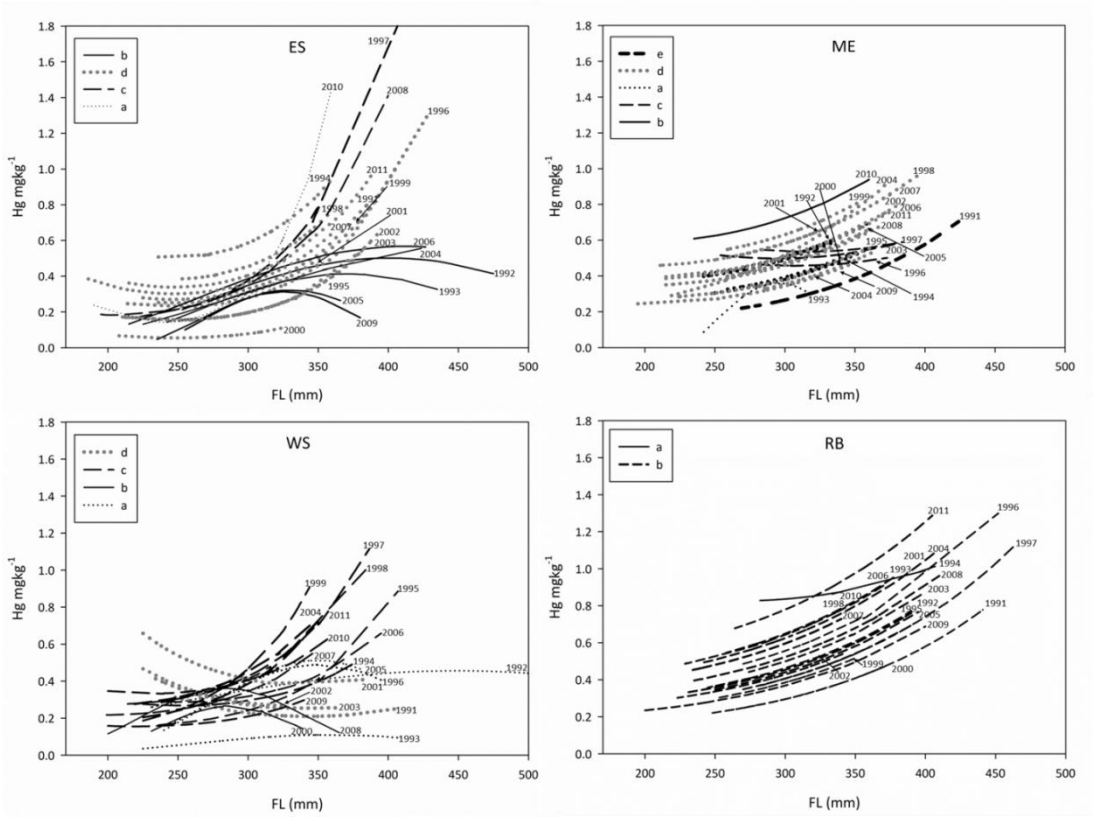

Figure 2.4. Relations between FL and Hg concentration for sand flathead in each Derwent Estuary region, data integrated over period 1991 to 2011. Each individual curve represents data from a single year, with different letters designating curves with similar shapes (i.e. where the 95% confidence interval overlaps).

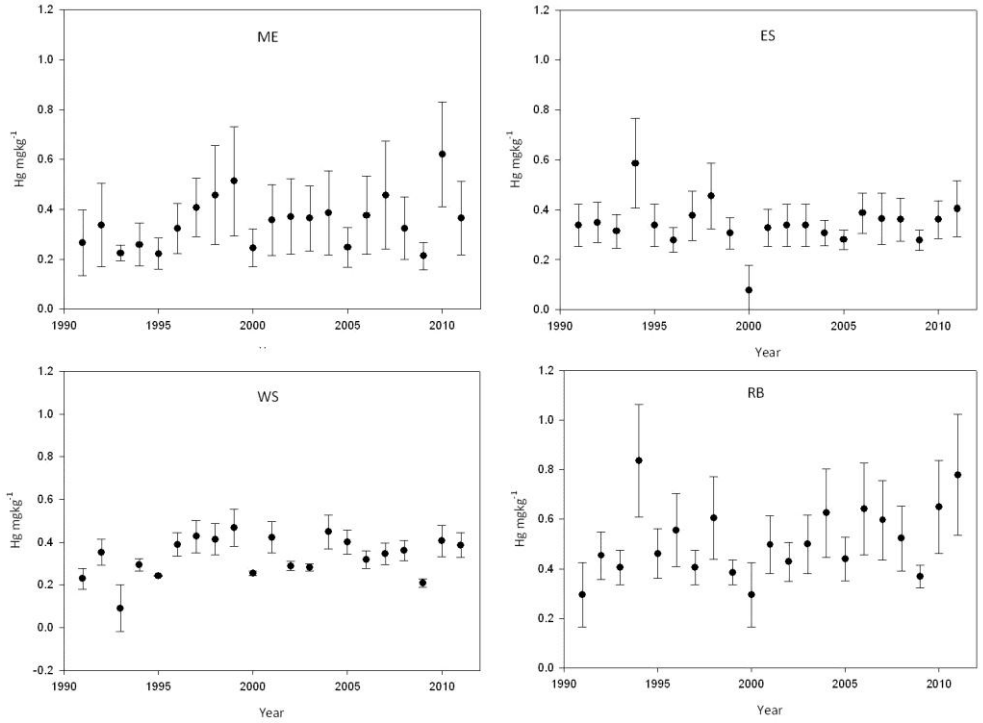

Figure 2.5. Muscle Hg concentration with 95% probability threshold for sand flathead of standardised FL (FL_{std}) (300mm) from 4 Derwent regions over 21-year sample period (1991–2011). Vertical lines represent the confidence interval (95%) around the estimated mean level. Overlap of confidence intervals suggests a lack of temporal change in Hg concentrations across this period.

Spatial Model describing Hg bioaccumulation (1991-2011)

The step-reduced model of spatial Hg concentration in sand flathead tissues fitted quadratic equations between FL and Hg (Fig. 2.6). Four out of the five regions (RB, ME, ES and MB) exhibited simple curvilinear increases in Hg with FL, while the western shore (WS) region showed an initial increase in Hg concentration with FL at lower FL but Hg concentration decreased slightly in fish with large FL (i.e. >400mm) (Fig. 2.6). Removal of year and the incorporation of a curvilinear relation between Hg concentration and FL allowed the separation of Hg concentration from these two underlying factors. This model explained 43% of the Hg variation observed across the regions (R^2=0.43, $F_{9, 2269}$=191, P= 0.0001). Standardised FL interaction in Ralphs Bay (RB) and the eastern shore (ES) regions caused significant shape variation in the FL–Hg concentration relation

between these regions and the middle estuary (ME) but the overlap in 95% confidence intervals between regions showed no significant difference in the overall position of the curves (Fig. 2.6). Lack of confidence interval overlap between the position of these three curves and that of the western shore (WS) indicate that, overall, fish from the WS region had significantly lower Hg concentrations than fish from elsewhere in the Derwent Estuary. The reference region (MB) Hg concentrations were significantly lower than all Derwent Estuary regions, suggesting that Hg concentrations in flathead from within the Derwent Estuary were elevated against background concentrations from outside of the estuary.

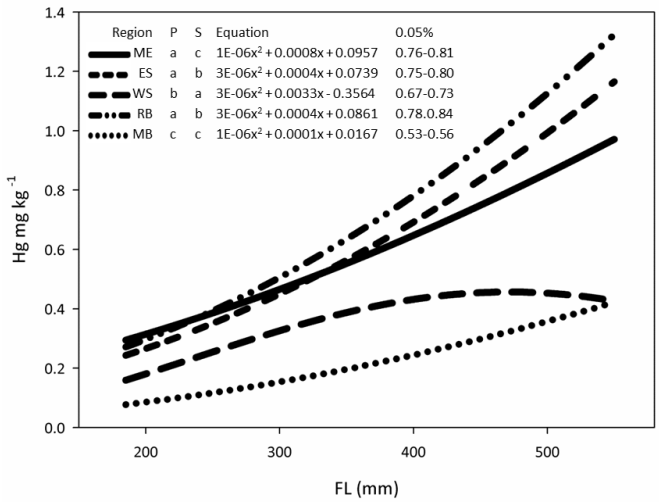

Figure 2.6. Relations between FL and muscle Hg concentrations in sand flathead for Derwent Estuary regions and reference region (MB). P= Position each letter indicates significantly different curve (p<0.05), given by lack of overlap between 95% confidence intervals between regions (0.05%). S= Curve shape, where different letters refer to significant variation in the shape of the relation between years (p<0.05), as a result of additional significant coefficients within the model. Curves labelled with decreasing complexity: a=most complex

Age data and growth rates

The relation between fish age and Hg concentration was assessed from a reduced dataset (n=428) of fish collected in four discrete years (2003, 2007, 2010, 2011) from the five regions. Fish age was positively and linearly correlated with fish muscle-tissue Hg concentration, with differences within region explaining between 19% and 40% ($\bar{x}$ = 28.4%) of the observed Hg concentration variation (Table 2.5). Von Bertalanffy growth parameters (VBGF) indicated RB fish had significantly elevated growth rates compared to all other regions examined (Fig. 2.7). In contrast, the WS region had significantly smaller fish, at any age, than other regions (including the reference region MB) (Fig. 2.7). VBGF predictions suggest that fish from this region would not reach the minimum required recreational fishing length of 300 mm, even at a maximum age of 10 years, and as a result this region was excluded from further analysis. A VBGF model was used to predict age at three standard fork lengths (FL_{std} -261 mm, 293.3 mm and 300 mm) in the remaining four regions. The average expected age for each fish length tested across the regions, reached 261 mm at 2.5 ± 0.2 decimal years (dy), 293.3 mm at 4.4 ± 0.6 dy and 300 mm at 4.5 ± 0.5 dy. The fastest growing flathead were found within the RB region, and on average reached 300 mm by age 3.3 dy while the slowest growth to 300 mm was modelled within the reference region (MB) at 5.8 dy. The ME and ES regions showed similar growth rates, taking 4.5 and 4.6 dy, respectively to reach 300 mm (Fig. 2.8). Overlap in the confidence intervals around the Hg concentrations for the three remaining Derwent Estuary regions RB, ES and ME suggested no significant difference in Hg concentration, but the higher growth rate associated with RB fish indicates that fish from this region could reasonably be expected to have higher Hg levels at a significantly younger age (Fig. 2.8).

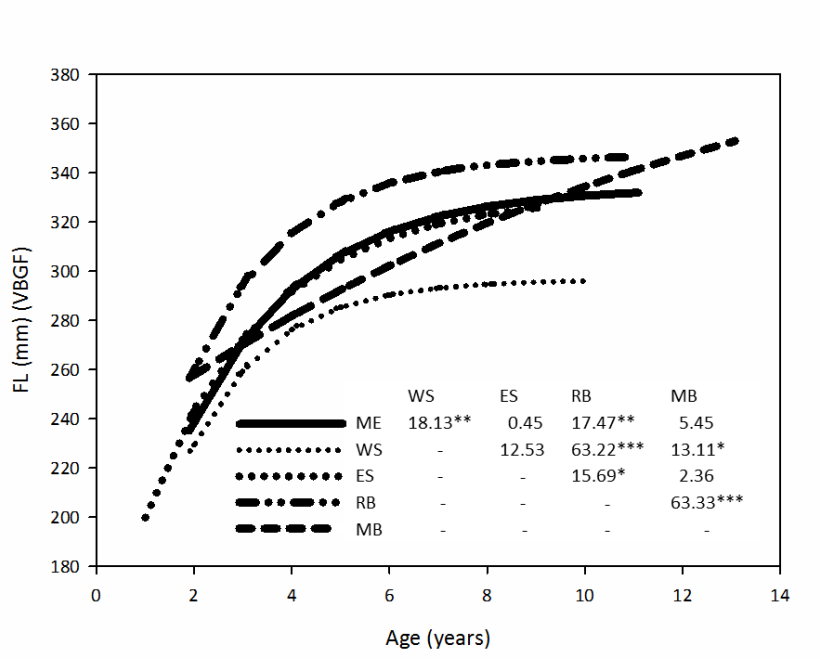

Figure 2.7. Age (years) of sand flathead for each of the Derwent regions and reference region (MB) plotted against mean fork length (FL) (mm) calculated by Von Bertalanffy growth curve function (VBGF) .Table gives chi –square test value (df=6) between regions, significant differences in curves indicated by *0.05 **0.01 ***>0.01.

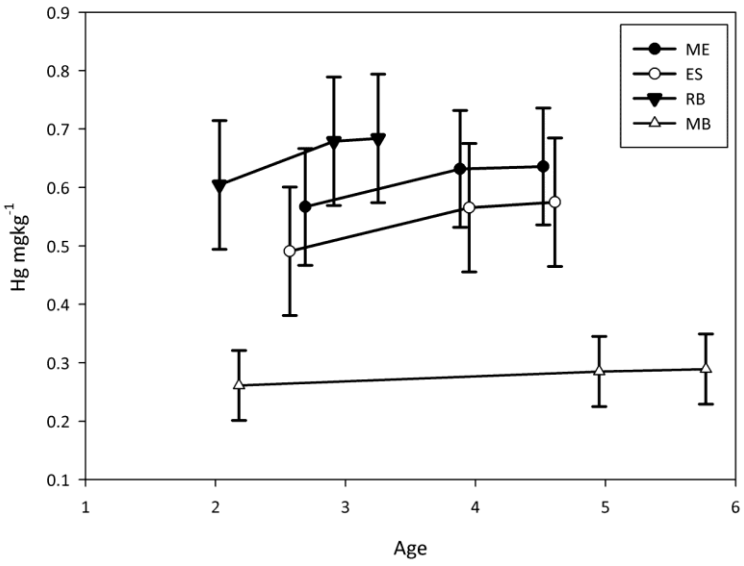

Figure 2.8. Mean Hg concentrations (± s.e.) and predicted ages of sand flathead for three FL_{std} using Von Bertalanffy growth curve predictions. FL_{std} used from left to right for each region = 261mm, 293.9mm and 300mm. Note: WS region is absent due to lack of prediction with VBGF at FL_{std} 300mm.

2.7 Discussion

The principal objectives of this study were first to determine if there had been an overall decrease in Hg concentrations in Derwent Estuary flathead over time, and second to establish the most reliable approach to measure and monitor changes in fish Hg concentrations. Both the linear model (using data from (1974-2011) and the polynomial model (using data from 1991-2011) of Hg concentrations in flathead muscle tissue, showed no decline in Hg concentrations over the full sampling period. This suggests that the reduction in Hg concentrations to the estuary over this period has not resulted in any change in Hg concentrations in muscle tissue from this species. This result does not agree with the conclusions of the earlier study in the Derwent Estuary which suggested that there had been significant reductions in Hg concentrations in flathead muscle tissues (Langlois et al., 1987). The modelling approach used in the current study was employed specifically so that we could incorporate data from a variety of sources, including previously published data (Langlois et al., 1987), and as such was able to cover a much broader period (37 years). This extended timeframe has smoothed out the periods of relatively short-term change observed in previous studies, as simply interannual variation, and therefore, suggests no temporal trend in fish Hg concentration. This trend has also been observed in another fish species, striped bass (*Morone saxatilis*) in San Francisco Bay, where a long term study showed no decrease in fish Hg concentrations despite a reduction in sediment Hg concentrations (Greenfield et al., 2005). Interannual fluctuation in Hg concentrations in striped bass were attributed to fish ecology, watershed loading, contaminated sediment exposure and variable methylation rates (Greenfield et al., 2005). Lack of reduction of Hg concentrations in fish over extended periods is atypical and other studies of estuarine benthic fish species

have recorded reductions in Hg concentration over shorter time frames, post Hg source removal (Francesconi et al., 1997; Sager, 2002).

Fish size (fork length, FL) and age are known determinants of Hg loadings in flathead (Verdouw et al., 2011). Not taking these determinants into account can lead to misinterpretation of results where mean FL varies between years (Tremblay et al., 1996). This may have contributed to the previous conclusions regarding Hg reduction within the Derwent Estuary. The polynomial model presented here returned significant correlations between Hg and FL, in part supporting previous findings (Verdouw et al., 2011) that suggested a similarly strong correlation, but we would propose that the relationship is curvilinear rather than linear. In this study, the nature of the FL–Hg concentrations relationship varied between years, explaining a maximum of 52% of the Hg concentrations fluctuation in any single year, clearly indicating that the FL-Hg concentration relationship is affected by other factors.

There was greater variation in the form of the FL–Hg concentration relationship in the western shore (WS) and eastern shore (ES) regions than in other regions. This may be the result of two independent factors influencing the sample results: firstly the number and distribution of sample sites in each region and secondly differences in fishing pressures in each region. The ES region contains six individual sample sites (see Fig. 1), as compared with either one (RB and ME) or two (WS and ME) sites for the other regions. Since the total sample set per annum from each region (typically 20 fish) is made up of fish collected from each of the within-region sites, spatial sampling of fish in the ES region will be greater than for all other regions. This approach may have inadvertently introduced within-region sampling bias to the FL–Hg concentration relationship. Another possible bias may have resulted from differences in fishing

pressure; the WS is heavily fished recreationally (Langlois et al., 1987; Verdouw et al., 2011). This fishing pressure may have resulted in changes in the population dynamics within the region, with reduced numbers of larger fish resulting in atypical FL–Hg concentration relations within the annual catch. The significantly lower mean FL recorded from the WS over the 21 year sampling period and significantly lower asymptotic length reported by the VBGF function for this region, provides some evidence that this may indeed be the case.

The length of this study far exceeds the maximum lifespan of the sand flathead caught (13 y) and therefore includes multiple generations of this species highlighting that Hg within the system continues to become biological available. Most probably this originates from the sediment. Sediment Hg sources have been specifically linked to fish Hg contamination (Gehrke et al., 2011) through bacterial methylation and bioaccumulation via benthic food webs (Sager, 2002; Lambertsson and Nilsson, 2006). Despite cessation of inputs, methylation of Hg in sediment sources can continue for significant time scales (>50 years) (Sager, 2002; Greenfield et al., 2005). Consequently changes in the geochemical status and subsequent methylation/demethylation mass balance may be the determining factor in future Hg release from sediments into the Derwent Estuary. Methylation/demethylation rates remain unexplored in this estuary, and the proportional representation of Hg species present within sediments is incompletely defined. High organic carbon content and low redox potential are known attributes of the middle estuary (Jones et al., 2003; Whitehead et al., 2010), and both can affect Hg methylation (Ullrich et al., 2001). Wind driven re-suspension of fine sediments within the middle estuary and downstream transport (Margvelashvili et al., 2005) offer a means of Hg movement from the Hg laden middle estuary sediments (Jones et al., 2003) to lower estuary regions. Concurrent contamination of the waterway

with other compounds from industrial processes, such as selenium, is also likely to have an influence on Hg bioavailability to fish (Peterson et al., 2009; Sackett et al., 2010).

The spatial variation observed in previous studies of Hg concentrations in sand flathead from the Derwent Estuary suggested that fish from Ralphs Bay had higher concentrations of Hg than fish from other Derwent Estuary regions (Langlois et al., 1987; Verdouw et al., 2011), and the mean annual data from the present study would appear to support this hypothesis. However, the spatial model incorporating the quadratic fork length terms found no significant difference between RB, ME and ES regions. The lack of significant positional differences in the FL– Hg relationship between RB, ES and ME regions, suggests that in the previous studies the observed spatial differences may have been influenced and thus compromised by between-region FL and annual catch variation. The present study found that, by examining the data for the *full* 21-year sampling period, mean FL in RB was significantly larger than the other estuary regions. If the average flathead caught in RB is larger than the other regions, it is unsurprising that previous studies have found higher Hg concentrations for this region given the positive curvilinear relationship between Hg loading and FL.

Fish length-Hg concentrations relationships in fish species are typically positive (Tremblay et al., 1998; Sager, 2002; Sackett et al., 2010), but can vary significantly as a result of differences in growth rate and Hg assimilation/depuration efficiencies (Trudel and Rasmussen, 2006). Fast-growing, short-lived species can exhibit linear trends (Olsson, 1976), but have also shown non-linear relations (Magalhães et al., 2007). Growth rates are often ignored in studies attempting to explain fluctuations in fish Hg concentrations, yet spatial changes in growth rates have been shown to influence Hg concentrations and may explain some of the observed variability (Simoneau et al., 2005;

Lavigne et al., 2010). Typically lower growth rates are associated with increased muscle Hg concentrations in fish at a given length (Lavigne et al., 2010; Cossa et al., 2012), as fast growing fish dilute Hg intake over a larger mass (Simoneau et al., 2005).

In this study, RB flathead were found to accumulate similar concentrations of muscle-tissue Hg concentrations to other regions, although in shorter timeframes. High Hg concentrations associated with fast growth rates are feasible as Hg concentration is a complex response to many factors including the fish's protective pathways against Hg (depuration and isolation), food consumption rates and fish activity costs. The exact cause of the observed growth rate variation and increased rate of Hg uptake RB is undetermined but there may be a number of possible explanations. First, intraspecific feeding differences between regions may play an important role in Hg accumulation. MeHg biomagnifies through food webs and small trophic shifts in prey between regions can result in relatively large Hg concentration changes in predators (Cabana and Rasmussen, 1994; Chen et al., 2009). Second, the conditions at RB may provide an alternate feeding environment enhancing ingestion rate and subsequently growth and arte of Hg uptake. Trudel and Rasmussen (2006) provide a detailed account of the effect of fish bioenergetics on Hg concentrations, and highlight that Hg concentrations are dependent upon the quantity of food consumed growth efficiencies in relation to size and energy allocation within the fish. Biodilution explanations of Hg concentration and growth rates alone are considered too simplistic to provide full explanations of Hg concentrations as they underestimate activity costs (Trudel and Rasmussen, 2006). Thirdly, water temperature differences may play a key role in growth rate variation. The sand flathead in this study had faster growth rates than reported from a previous study looking at continental shelf fish (Jordan, 1999), probably the result of the warmer water within the estuary. The relatively shallow depths and lower fresh water inputs allow for

higher water temperatures in RB than in the deeper main estuary channel (Whitehead et al., 2010), and this may be a contributing factor to the growth rate differences observed. Finally, the potential for higher bioavailability of Hg species from the environment within this region should also be considered. Given the high total Hg concentrations present within the ME estuary sediments (Jones et al., 2003), and the lack of difference in Hg concentrations in fish between the various regions, it is possible that a significant proportion of the Hg present in the ME is unavailable for bioaccumulation by biota. In contrast the local geochemical conditions in RB may provide a more suitable environment for methylation, and therefore enhanced bioavailability to resident biota. Further work is required in each of these areas to provide a more complete understanding of flathead Hg bioaccumulation.

2.8 Conclusions

Significant reduction of Hg discharges into an estuarine system does not necessarily result in decreased Hg concentrations in fish even after a significant time lapse (in this case 37 years), and dispersal of Hg through the system can result in elevated levels in resident fish spatially separated from the original Hg source. This study shows that it is important to ensure that temporal and spatial comparisons are of a form and at a scale consistent with the ecological dynamics of the species in question.

Of particular significance in any monitoring program seeking to evaluate seafood safety or determine risk management strategies for human health is the inclusion of fish biometrics modelling (length, age and growth rates) that realistically reflects the species dynamics. Failure to consider these variables could result in misinterpretation of spatial and temporal trends.

CHAPTER 3

COMPLEX PATTERNS IN FISH –SEDIMENT MERCURY CONCENTRATIONS IN A CONTAMINATED ESTUARY: THE INFLUENCE OF SELENIUM CO-CONTAMINATION?

Preface:

Environmental Hg loads do not always correspond to Hg concentrations in resident fish and Se presence has been reported to play a pivotal role in mitigating Hg bioaccumulation. The objective of the research presented in this chapter was to determine the interaction of Hg and Se within a contaminated estuary and establish if Se presence may be responsible for observed variability in fish Hg concentrations. Very few studies have examined both Hg and Se concentrations in the same individual animals and compared them against the localised sediment concentrations. This study differs from previous research in that it has used Se:Hg ratios, % MeHg data from sediments and biotic sediment accumulation factors (BSAF) for total Hg, MeHg and Se to determine potential hotspots of Hg methylation and bioavailability. The findings have direct relevance to management strategies seeking to characterize risk scenarios and transfer pathways for Hg and Se bioaccumulation in resident fish species.

This work is in press for publication in a refereed journal and is presented below in identical form. The citation for the original publication is:

Jones, H.J., Butler E. C.V., Macleod, C.K., in press. Complex patterns in fish – sediment mercury concentrations in a contaminated estuary: the influence of selenium co-contamination? *Estuarine and Coastal Shelf Science*

3.1 Highlights

- Low sediment Se concentrations were associated with increased MeHg bioavailability.
- Where MeHg concentration in fish was high, Se uptake also increased
- Maintaining positive Se:Hg ratios may reduce the toxicological effect of MeHg.
- Se should be a key consideration in assessments of Hg methylation/bioaccumulation.

3.2 Abstract

Environmental mercury (Hg) loads do not always correspond to Hg concentrations in resident fish and selenium's (Se) presence has been reported to play a pivotal role in mitigating Hg bioaccumulation. Total mercury (THg), methylmercury (MeHg) and Se concentrations were measured in sediments and a benthic fish species (*Platycephalus bassensis*) from a contaminated estuary (Derwent Estuary, Tasmania). We found that elevated sediment concentrations of Se did not result in increased Se concentrations in fish, but that low concentrations of Se were associated with increased MeHg bioavailability (% MeHg) from sediments to fish. Where MeHg (≈ 99% of total Hg) concentration in fish was high, Se uptake also increased, indicating that maintaining positive Se:Hg ratios may reduce the toxicity of MeHg. MeHg was detectable in sediments throughout the estuary, and a molar excess of THg over Se suggested that there was insufficient Se to prevent methylation from the sediments. Se:Hg ratios of less than 1.0 in sediments, coupled with high %MeHg fraction and high biotic sediment accumulation factors for MeHg (BSAFMeHg), indicated that the lower region of the

Derwent Estuary could be a hotspot for Hg methylation, despite having significantly lower THg concentrations. In contrast, Hg bioavailability to fish from sediments close to source may be reduced by both inorganic Hg species complexation and lower methylation rates. There was a strong association between THg and Se in estuarine sediments, suggesting that Se plays an important role in sediment Hg cycling and should be a key consideration in any future assessments of Hg methylation, bioavailability and bioaccumulation.

3.3 Key words

Derwent Estuary; biotic sediment accumulation factors; Se:Hg ratios; methylmercury; *Platycephalus bassensis*

3.4 Introduction

The spatial variation of methylmercury (MeHg) production and bioaccumulation in estuarine food webs is broadly understood but poorly characterized (Mason and Lawrence, 1999; Davis et al., 2012). Uptake and transfer of mercury (Hg) between the biotic and abiotic components is not straightforward (Chen et al., 2009), with limited data supporting the concept that elevated Hg concentration in aquatic environments leads directly to high MeHg levels in fish e.g.(Brumbaugh, 2001; Munthe et al., 2007). Regions with high Hg environmental loads may show low bioaccumulation if net methylation rates are low; conversely, low environmental Hg concentrations may result in high fish tissue loadings as a result of raised methylation efficiency (Brumbaugh, 2001). Understanding the mechanisms that underpin this variability is critical to quantifying and managing Hg exposure risks and to developing appropriate management actions (Tom et al., 2010; Davis et al., 2012).

The Derwent Estuary, in southeast Tasmania, exhibits large differences in the THg concentrations (Total Hg = inorganic Hg + organic Hg) in its sediments (Jones et al., 2003). The differences in sediment Hg values are notably not reflected in the THg concentrations of the resident benthic fish sand flathead (*Platycephalus bassensis*) (Jones et al., 2013a). The industrialized middle reaches of the estuary are located ≈20 km from the mouth, and have consistently high THg concentrations in both sediments and sand flathead as a result of historic inputs from a zinc smelter and paper mill (Bloom and Ayling, 1977; Green and Coughanowr, 2003). Conversely, a large and relatively shallow embayment on the lower eastern side of the estuary, called Ralphs Bay, has low sediment Hg levels but high Hg concentrations in fish (Jones et al., 2003; Jones et al., 2013a). Despite 40 years of Hg research in the Derwent Estuary, the reasons for this paradox remain unexplored.

Quantification of biotic exposure to MeHg is complicated by the presence of selenium (Se), a known co-contaminant from metallurgical processing (Yang et al., 2008). Formation and excretion of Se-biomolecules by sediment-dwelling organisms results in the production of the mineral selenide (Maher et al., 2010), which is capable of sequestering Hg^{2+} and forming mercuric selenide (HgSe) (Yang et al., 2008). HgSe formed in sediments is relatively inert, and may reduce the concentration of Hg available for methylation (Yang et al., 2008). This process diverts Hg away from biogeochemical cycling into methylated forms, so where Se is absent from sediments 'hotspots' of Hg bioaccumulation may occur (Ralston and Raymond, 2010). Although it is an essential trace element Se is toxic at high levels, and can bioaccumulate through food pathways similar to Hg (Cuvin-Aralar and Furness, 1991). In freshwater systems elevated

concentrations of Se in fish have been linked to reduced Hg concentrations (Chen et al., 2001; Belzile et al., 2006; Sackett et al., 2010), yet this has never been documented for estuarine systems. Se concentrations in sediments and sand flathead have never been measured in the Derwent Estuary and could explain, at least in part, the spatial disparity between Hg concentrations in sand flathead populations and the sediments.

The objective of this study was to evaluate if the co-occurrence of Se may be mitigating Hg bioavailability from sediments and reducing fish bioaccumulation, and whether this may be the reason why Hg concentrations in sand flathead are lower than might otherwise be expected. We addressed this by: (1) measuring the THg, MeHg and Se concentrations within the sediments and in the muscle tissue of resident populations of sand flathead in the Derwent Estuary; (2) examining the Se:Hg ratios in Derwent Estuary fish and sediment for evidence of spatial variation; and 3) inspecting the relationship between Se and Hg (THg and MeHg) in the sediments to assess evidence of reduced Hg bioavailability.

3.5 Methods

Site selection

The Derwent Estuary (42^{o} 54'S, 147^{o} 18'E; Fig. 1) is a micro-tidal (~1 m) drowned river valley, 52 km in length, with a maximum depth of 30 m, and is located in southern Tasmania. (Whitehead et al., 2010) The waterway has been extensively studied through monitoring and management programs and has been the focus for hydrodynamic modelling and previous studies of metal contamination (Margvelashvili et al., 2005; Jones et al., 2013a) Three regions were sampled; two of those regions (Middle Estuary

(ME) and Ralphs Bay (RB)) were within the Derwent Estuary, whilst the third region, Mickey's Bay (MB), was located 48 km south of the estuary (Fig 3.1). The Middle Estuary (ME), the industrialised region, and Ralphs Bay (RB) in the Derwent Estuary are both well-mixed (dominated by wind-driven and tidal mixing) water bodies, but vary significantly in their sediment composition (Thomson and Godfrey, 1985; Margvelashvili et al., 2005). Mickeys Bay (MB), the reference region, is an embayment similar to Ralphs Bay and was included to provide comparative data from a region that has not been contaminated with either Hg or Se (Jones et al., 2013b).

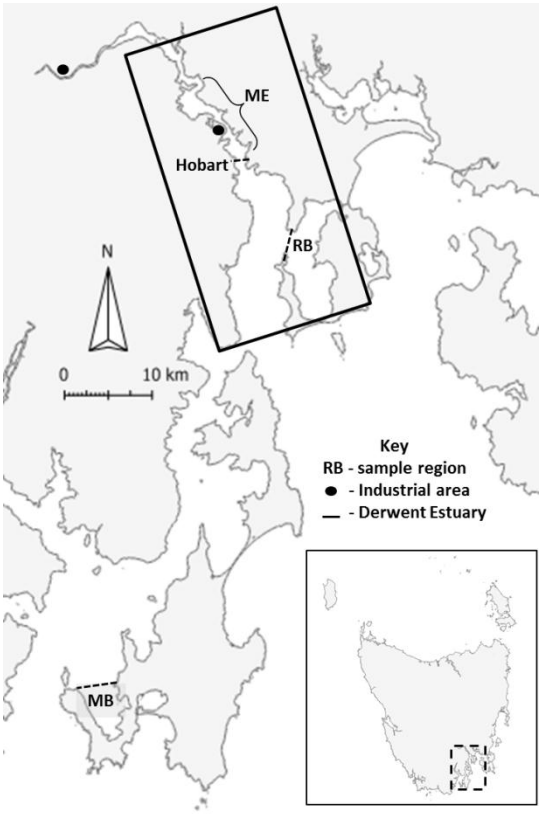

Figure 3.1. Southern Tasmania and the Derwent Estuary, with locations of the two estuary regions Middle Estuary (ME), Ralphs Bay (RB) and the reference region Mickeys Bay (MB) 48km south of the Derwent Estuary.

Fish collection

Fish (n=120) were sampled by line fishing during November and December (2010 and 2011). Each fish was sealed in a plastic bag and stored on ice until transfer to the laboratory, where they were frozen (–40 ^{o}C). Fish were measured (fork length, FL) and then dissected. One fillet of muscle tissue, posterior to the pectoral fin, was removed from each fish and refrozen in acid-cleaned polypropylene tubes. Muscle samples were lyophilized to constant mass (± 0.01 g) and homogenized, with a subsample of tissue from each region taken for separate THg, Se and MeHg analyses. Sagittal otoliths were extracted for age determination, following a validated method (Jordan et al., 1998), where resin-mounted, sectioned sagittal otoliths were read by two independent readers, with between- and within-reader precision examined by an index of average percent error (Beamish and Fournier, 1981).

Sediment collection and analyses

Sediment sites ranged in depth from 7 to 15 m. Water measurements were taken 1 m above the surface of the sediment using a multi-parameter probe (6600 v2 Sonde, YSI, Australia). Sediment cores (n=39) were collected using a purpose-built tri-corer consisting of three polycarbonate pipes (250 mm length x 45 mm internal diameter) that could be pushed into the sediment to a depth of 4 cm (± 0.5 cm). The sediment collected was transferred to glass jars and frozen (–40 ^{o}C). The three samples collected by the tri-corer were pooled, freeze-dried and sieved through 500 μm mesh to remove large shell fragments. Samples were homogenized before subsamples were taken for analysis of iron (Fe), % Sulfur (%S), % Carbon (%C), % Nitrogen (%N), acid volatile sulfide (AVS), grain size and total organic carbon (TOC), as well as THg, MeHg and Se. Quantification of %S, %C and

%N was achieved by elemental analysis (Thermo Finnigan EA 1112). AVS was determined by a rapid fluorescence method (Simpson, 2001), and grain size composition was measured by laser diffraction (ATA Scientific). Total organic carbon (TOC) was determined spectrophotometrically after oxidation with chromic acid (H_2CrO_4) and sulfuric acid (H_2SO_4) using the method outlined by Heanes (1984). Analysis of Fe content was done by inductively coupled plasma–optical emission spectrometry (ICP-OES) (Varian 730ES, Australia). Samples (1 g) were digested in 10 mL HNO_3 for 12 h at room temperature, then for 2 h at 30 °C and for 2 h at 100 °C, before being diluted to 50 mL prior to analysis.

THg and Se digestions

SEDIMENT: 1 g of sediment was cold-digested in 16 mL of HNO_3:HCl (4:1 v/v) acid mixture in lightly capped polypropylene digestion tubes before being heated to 120 ^{0}C for 4 h in an aluminium digestion block. After cooling, the tubes were filtered (Whatman GF/F) and the sample diluted to 50 mL total volume with RO water. All sediments were analysed within 60 h of digestion.

FISH: THg and Se digestions followed the method of Kaneko and Ralston (2007). In brief, dry-weight samples were digested in a digestion block in three stages with HNO_3, H_2O_2, and an acid mixture (3:1 HNO_3:HCl), before cooling and dilution to 50 mL total volume with RO. Analysis took place within 48 h of digestion.

MeHg digestion

MeHg extraction for fish and sediments was based on the method of Cai (2000).

SEDIMENT: 0.2 g (± 0.05 g) of dry sediment was weighed into glass vials, shaken with 8 mL KBr/$CuSO_4$ for 2 h, then 15 g DCM was added and the vial was shaken overnight. Samples were centrifuged for 20 min (2000 rpm), and 10 g of the bottom (DCM) layer

transferred to a clean glass vial. Sodium thiosulphate ($Na_2S_2O_3$) 0.01 M (2 mL) was added to the DCM extract, shaken for 30 min and vortexed for 30 s, after which 1.5 mL of the $Na_2S_2O_3$ was extracted into a separate glass vial. The $Na_2S_2O_3$ process was then repeated. The final extract of 3 mL $Na_2S_2O_3$ was filtered (0.45 μm) before analysis.

FISH: The full method is outlined in Jones et al. (2013b) and follows the same extraction method as the sediment, with the exception of the first digestion stage. In the first stage, 4 mL RO water was added to 0.2 g (± 0.05 g) of homogenized dry tissue within an acid-cleaned (10% HNO_3) glass vial (40 mL), along with 4 mL KOH (6 M), and shaken for 4 h. After shaking, 4 mL HCl (6 M), 8 mL $CuSO_4$/KBr/H_2SO_4 (83 g w/v, 120 g w/v, 33 mL v/v) solution and 15 g of dichloromethane (DCM) were added and the vials returned to the shaker overnight.

Hg and Se analysis

THg: Measurements were made with cold-vapour atomic fluorescence spectroscopy (CV-AFS) (10.023 Millenium Merlin, PS Analytical). A 2 % w/v tin(II) chloride reductant and argon (Ar) carrier gas was used. Calibration was achieved using traceable standards, and independent checks were undertaken using a separate stock solution.

MeHg: Aliquots were analysed by high pressure liquid chromatography–ultra-violet–atomic fluorescence spectroscopy (HPLC–UV–AFS) using an oxidant stream of acidified potassium bromide/ potassium bromate (10 % v/v HCl, 10 % v/v 0.1 M Br^-/BrO^{3-}). A mixture of 38 % methanol, 30 % (m/v) acetonitrile with ammonium pyrrolidine dithiocarbamate (APDC, 0.2464 g L^{-1}) was used for the mobile phase, with a Supelco C18 column (ODS-2) to provide species separation. An online UV photolysis/heater (PSA S570U100) and cooling module (PSA S570C100) coupled to the AFS provided oxidation

before analysis. A 2 % w/v tin(II) chloride reductant and Ar carrier gas were used for cold vapour separation prior to AFS detection.

Se: Se detection used online pre-reduction of Se with hydride-generation – atomic fluorescence analysis (HG–AFS) (Millenium Excalibur, PS Analytical). Se was reduced by mixing with pre-reductant KBr/HCl (5 % KBr, 50 % HCl) and passing through a UV heater (PSA S570U100) (150 ^{o}C) and cooling module (PSA S570C100). The sample was then mixed with the reductant (0.7 % $NaBH_4$ 0.4 %NaOH) to form selenium hydride and carried by Ar (0.3 L min^{-1}) to the detection system.

Quality assurance

All reagents used in this work were trace grade quality (Sigma-Aldrich), with all apparatus used in metal analysis and sample collection subjected to 5 d of 10% detergent bath (decon90, UK) and 5 d of 10 % HNO_3 bath, followed by a reverse osmosis (RO) water (Elga Purelab Prima) rinse. All apparatus were stored in double seal plastic bags. Linear calibration of instruments was acquired using standards diluted in the appropriate concentration range with matrix-matched reagents, and with accuracy of calibration verified by independent standards. Matrix-matched procedural blanks analysed at the beginning and end of each sample run showed no significant procedural contamination. Calibration verification (independent check and certified reference material (CRM)) was run after instrument calibration, after every 20 samples and at the end of each batch of samples. Each sample was run in duplicate, with one sample per batch spiked with 5 ng g^{-1} standard solution and recovery rates recorded. CRM DOLT-4 (NRC Canada, dogfish liver), BCR 422 (IRMM, cod muscle), ERM CA011a (European Reference Material, hard drinking

water), and IAEA 405 (International Atomic Energy Agency, Estuarine sediment) were used to verify recovery rates (Table 3.1). All results are reported as dry weight (dw).

Table 3.1. Selenium (Se), total mercury (THg) and methylmercury (MeHg) concentrations (mg kg-1) in certified reference materials (CRM) materials analysed by HG-AFS (Se), CV-AFS (THg) and HPLC-UV-AFS (MeHg) (PS Analytical, Kent. UK). Certified concentrations (c.c.) and recovery rates (% recovery) of analysis versus c.c. also displayed.

	CRM	**c.c. (± s.e.)**	**Mean (± s.e.)**	**n**	**% recovery**
Se	DOLT 4	8.3 (0.12)	7.69 (0.44)	6	92.67
	ERM CA011a	10.7 (0.7)	10.01 (0.45)	4	93.54
	IAEA 405	0.44 (0.12)	0.37 (0.09)	4	84.09
THg	DOLT 4	2.58 (0.22)	2.37 (0.13)	6	91.86
	IAEA 405	0.81 (0.04)	0.79 (0.12)	6	97.53
MeHg	DOLT 4	1.33 (0.12)	1.31 (0.09)	6	98.66
	BCR422	0.43 (0.2)	0.51 (0.03)	6	117.44
	IAEA 405	0.005 (0.0006)	0.004 (0.001)	4	80.00

Statistical analysis

Data analysis was performed using the R statistical package (version 2.15, 2012) and PRIMER with PERMANOVA package (PRIMER-E Ltd, UK). One-way analysis of variance (ANOVA) including Tukey's HSD post hoc test was used to determine if there were spatial differences between regions. Where assumptions for parametric tests could not be met, a Mann-Whitney U test was used to test for spatial differences. The reference station (MB) was compared against Derwent Estuary sediments using Kruskal-Wallis one-way analysis of variance by ranks. Principal component analysis (PCA) was employed to identify the most important gradients in the sediment data based on Euclidean distance and Pearson's correlation co-efficient. All sediment data were $\log_{10}(X+1)$-transformed prior to the analysis to normalise the data and remove scaling effects. PERMANOVA with type three sums of squares was run to examine difference between regions.

THg:Se ratios in sediments and fish were calculated by conversion of dry weight concentrations into molar mass (concentration in mg kg^{-1}/molar mass (Hg= 200.59, Se= 78.96)). As a measure of bioavailability of Hg from sediments and toxicity of fish, MeHg in each was normalised by THg: %MeHg = (MeHg/THg*100). Biota-sediment accumulation factors (BSAF = fish tissue metal concentration / sediment metal concentrations) (Tracey and Hansen, 1996) were calculated to establish associations between fish and sediment metal concentrations for THg ($BSAF_{THg}$), MeHg ($BSAF_{MeHg}$) and Se ($BSAF_{Se}$). $BSAF_{MeHg}$ for fish was determined using THg concentrations as a significantly larger sample size was available for THg (n=58); this was done on the basis that % MeHg contribution to fish muscle tissue ≈ 95% THg (Table 1) (Bloom, 1992). Hg concentration increases with age for flathead in the Derwent Estuary (Jones et al., 2013a); therefore, age was treated as a covariate in ANCOVA, where BSAF was the response variable. BSAF values were $\log_{10}$-

transformed to meet assumptions of normality (Shapiro-Wilk normality test). One-way ANCOVA was used to assess spatial variation in BSAF. Type two ANCOVA was applied initially to test for homogeneity between slopes, and, if assumptions of homogeneity were met, then type three ANCOVA was used to test for differences in intercepts.

3.6 Results

FISH: No significant difference was found in the age of flathead between regions (ANOVA $F_{2,55}$=2.04, P=0.14), although fork length FL varied significantly (ANOVA $F_{2,55}$=6.16, P=0.004) (Table 3.2). Fish from Ralphs Bay had significantly higher Se concentrations than fish from the Middle Estuary and the reference region (ANOVA $F_{2,56}$=19.38, P=<0.0001) (Table 3.2). THg concentrations in sand flathead from the Derwent Estuary were higher than the reference region, and within the estuary THg concentration in fish from Ralphs Bay exceeded that of the Middle Estuary (ANOVA $F_{2,56}$=40.84, P=<0.0001) (Table 3.2). % MeHg concentration of muscle tissue did not vary significantly between regions (ANOVA $F_{2,24}$=1.08, P=0.36), the overall mean contribution across regions being 99 % (± 4.9) (Table 3.1). There was no significant relationship between TSe and THg concentration in flathead within regions ($F_{1,53}$= 1.07, P=0.29). Se:Hg ratio in muscle tissue was consistently > 1, but the three regions varied significantly from each other ($F_{2,56}$=22.84, P=<0.0001), with Mickeys Bay>Middle Estuary>Ralphs Bay (Table 3.2). Fish age had no significant effect on the Se:Hg ratios of flathead for any region ($F_{2,52}$=2.42, P=0.13).

Table 3.2. Mean values (± s.d.) of variables measured in the benthic fish species sand flathead (*Platycephalus bassensis*) within the Derwent Estuary regions (Middle Estuary and Ralphs Bay) and in the reference region (Mickey's Bay). Variables measured were total mercury (THg), selenium (Se), methylmercury (MeHg), %MeHg (MeHg/THg*100), Se:Hg ratio ((Se mg kg^{-1}/78.96)/(THg mg kg^{-1}/200.59)), fish age and fork length (FL). $_{a,b,\ c.}$ Letters denote significant differences between regions (P <0.05).

Region	n	ME	n	RB	n	MB
THg (mgkg^{-1})	40	1.92 (1.08)$_b$	40	3.07 (1.26)$_c$	40	1.06 (0.50)$_a$
Se (mgkg^{-1})	20	0.89 (0.18)$_a$	20	1.28 (0.30)$_b$	20	0.91 (0.17)$_a$
MeHg (mgkg^{-1})	10	1.22 (0.68)$_b$	10	4.03 (1.68)$_c$	10	0.77 (0.33)$_a$
%MeHg	10	109.31 (29.96)	10	97.06 (25.58)	10	91.42 (20.63)
Se:Hg	20	2.30 (1.24)$_b$	20	1.20 (0.81)$_c$	20	3.62 (1.68)$_a$
Age (years)	40	5.2 (1.70)	38	5.2 (2.04)	40	6.3 (2.12)
FL (mm)	40	290.3 (45.06)$_a$	38	322.4 (44.38)$_c$	40	304.5 (37.22)$_b$

SEDIMENT: The three regions were significantly different from each other for all variables measured (PERMANOVA *pseudo-*$F_{2,35}$ = 28.08, P(perm) = 0.001). PCA ordination showed distinct separation of the three regions based on those variables (Fig. 3.2). Separation of the Middle Estuary and Ralphs Bay regions was strongest along PC1 and most strongly associated with concentrations of Se, THg, and Fe, along with TOC, % N and % mud concentrations. Ralphs Bay sites with low metals and organic matter concentration appeared similar to the reference region on this axis. One site in the Middle Estuary was readily distinguished from the rest of the region by its low metal concentrations and low organic load, while three sites in Ralphs Bay were distinct from the rest of the Ralphs Bay

sites by virtue of their relatively high metal levels and organic loads. Differences in MeHg concentration, AVS and %S concentration distributed the Middle Estuary sites along the second axis, whilst the reference region, Mickeys Bay, was separated from Ralphs Bay sites according to its proportionally high Se:Hg ratio (Table 3.3).

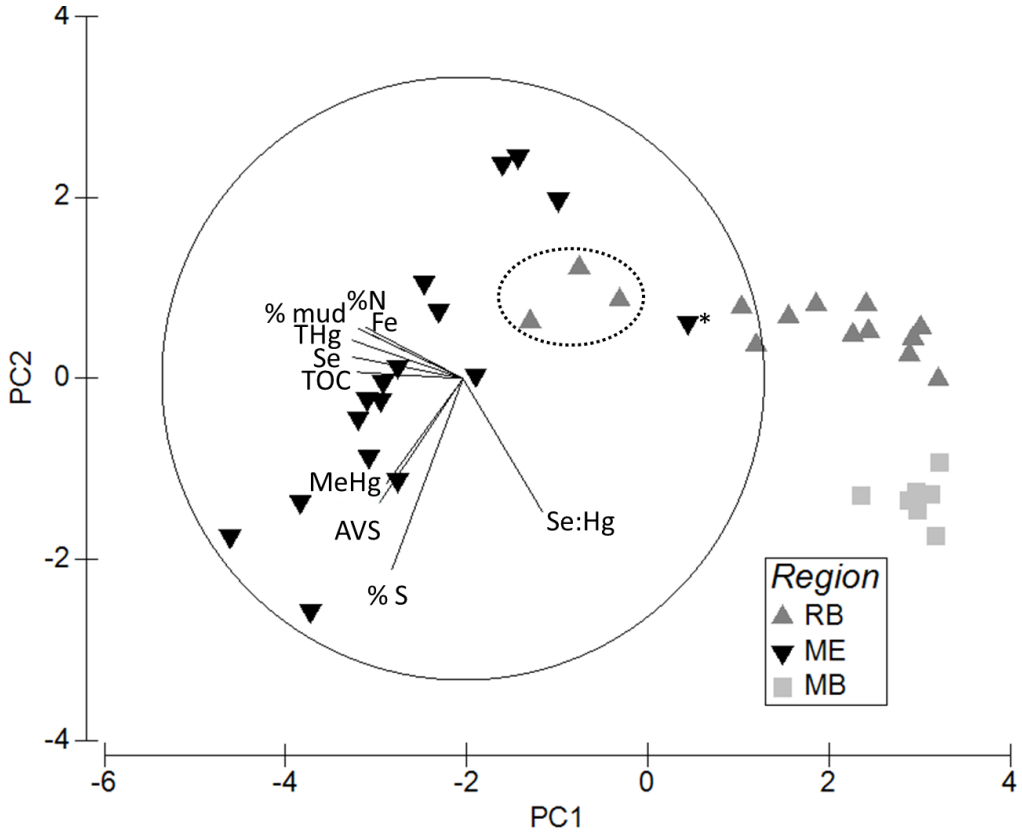

Figure 3.2. Principal component analysis (PCA) based on Euclidean distance for sediment variables from two Derwent Estuary regions: Middle Estuary (ME), and Ralphs Bay (RB). Variables examined were: % mud, Total organic content (TOC), acid volatile sulfides (AVS), sulfur (%S), Iron (Fe), total mercury (THg), methylmercury (MeHg), selenium (Se) and Hg:Se molar ratio. PC 1 explained 69.9% of the variation, while PC 2 explained a further 13.6%. Circled RB sediments had distinctly higher metal loads (THg $\bar{x}$ = 6.4, Se $\bar{x}$ = 0.67, MeHg $\bar{x}$ = 0.009) and organic content (TOC $\bar{x}$ = 3.53) compared to other sites in the region (Table 3,4). * site within the ME (THg = 2.94, Se = 0.3, MeHg = 0.009, TOC = 1) had distinctly lower concentrations than rest of that region (Table 3,4).

Table 3.3. Mean values (± s.d.) of variables measured in surface sediments (top 4 cm) and bottom water within the Derwent Estuary regions (Middle Estuary and Ralphs Bay) and in the reference region (Mickey's Bay). Sediment measurements were total mercury (THg), selenium (Se), methylmercury (MeHg), % MeHg (MeHg/THg*100), Se:Hg ratio ((Se mg kg^{-1}/78.96)/(THg mg kg^{-1}/200.59)), percentage mud (%mud), total organic carbon (TOC), acid volatile sulfides (AVS), percentage sulfur (%S), percentage iron (%Fe) and percentage nitrogen (%N). Bottom water measurements were salinity, pH and dissolved oxygen (DO). a,b,c denote significant differences between regions (P <0.05), ^ denotes concentration below detection limits.

	Region	n	ME	n	RB	n	MB
Sediment	THg (mgkg^{-1})	17	22.2_a (9.8)	15	1.8_b (2.5)	7	0.01_c (0.0)
	Se (mgkg^{-1})	17	1.1_a (0.3)	15	0.2_b (0.2)	7	0.1_c (0.0)
	MeHg (mgkg^{-1})	17	0.02_a (0.01)	15	0.01_b (0.01)	7	0.00^
	%MeHg	17	0.1_b (0.1)	15	2.7_a (1.0)	7	–
	Se:Hg	17	0.1_c (0.1)	15	0.8_b (0.8)	7	17.8_a (2.9)
	% mud	17	8.3_a (0.6)	15	4.2_b (0.5)	7	2.0_c (0.4)
	TOC	17	7.4_a (0.5)	15	1.3_b (0.3)	7	0.1_c (0.0)
	%S	17	1.3_a (0.2)	15	0.2_c (0.1)		0.8_b (0.3)
	AVS (μmol g^{-1})	17	8.8_a (2.7)	15	0.8_b (0.2)		0.7_b (0.4)
	%Fe	17	3.9_a (0.8)	15	1.2_b (0.1)	7	0.2_c (0.0)
	%N	17	0.4_a (0.1)	15	0.1_b (0.1)	7	0.0_c (0.0)
Bottom water	salinity	15	33.5 (0.1)	15	33.2 (0.0)	4	33.9 (0.0)
	pH	15	7.9 (0.0)	15	8.0 (0.0)	4	8.1 (0.0)
	DO (mg/l)	15	7.1 (0.0)	15	7.0 (0.1)	4	7.4 (0.1)

Se and THg concentrations in Derwent Estuary sediments were significantly higher than the reference region (Kruskal Wallis Se:H_2=33.77, P=<0.001, THg:H_2=31.88 P=<0.0001) (Table 3.3). Se concentrations in sediments ranged from 0.03–1.53 mg kg^{-1} within the Derwent Estuary, compared to 0.06–0.37 mg kg^{-1} at Mickey's Bay. Sediment MeHg concentrations were lower than detection limits in Mickey's Bay (<0.001 mg kg^{-1}), but ranged from 0.0025 to 0.035 mg kg^{-1} within the Derwent Estuary (Table 3.3). Middle Estuary sediments were significantly higher than Ralphs Bay sediments for MeHg concentration (Mann-Whiney U: H=37, P=<0.001) (Table 3.3). MeHg represented a small percentage of THg concentration (% MeHg) within the Derwent Estuary sediments ($\bar{x}$ =1.30% ± 0.51), however, % MeHg was elevated within the Ralphs Bay region compared to the Middle Estuary (Mann-Whitney U: H=15, P=<0.001) (Table 3.3). Se:Hg molar ratios in both Middle Estuary and Ralphs Bay regions were <1, while the reference region was >1 (Table 3.3)

Within Ralphs Bay, Se concentrations were highly correlated with THg concentration in the sediments (96.7%). In comparison, this correlation was only 46.3% in the Middle Estuary (Fig. 3.3). Se concentrations correlated less well with MeHg concentrations, accounting for only 20 % (ME) and 22.4% (RB) of the data within the Derwent Estuary. THg concentration in both Derwent Estuary regions also showed low correlation with MeHg concentration (ME = 15.7%, RB= 19.6%) (Fig. 3.3). No correlation was evident between % MeHg and Se in the Middle Estuary, although a strong relationship was evident in Ralphs Bay (Fig. 3.3).

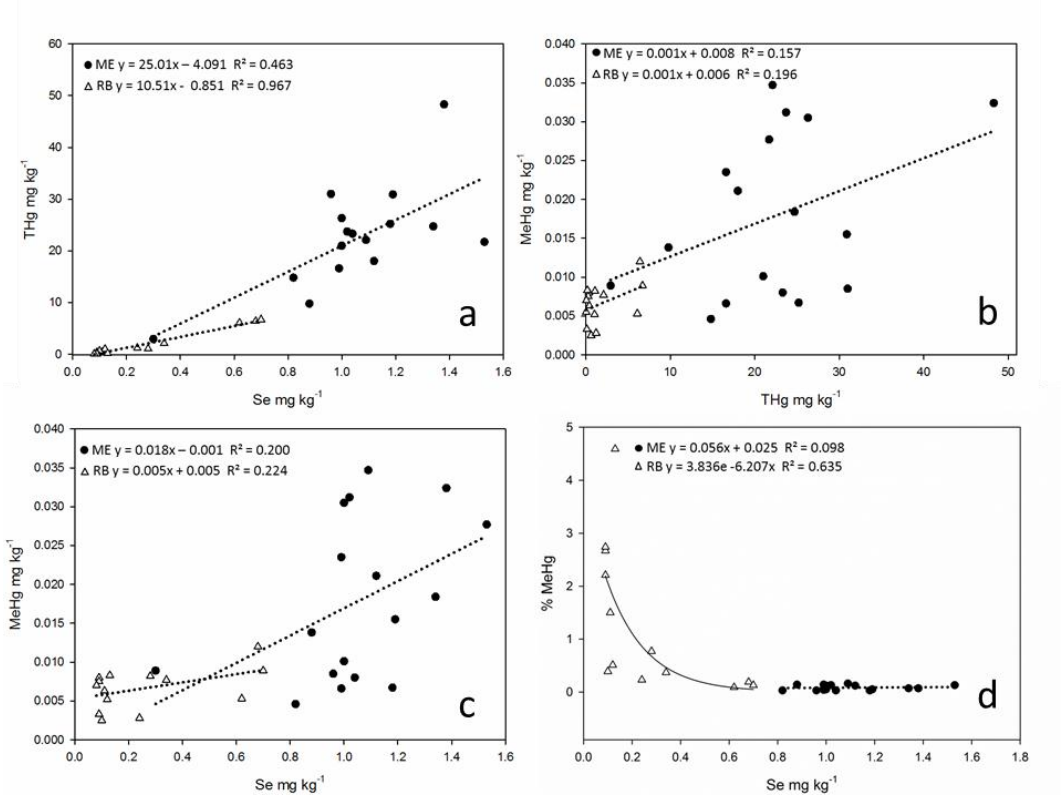

Figure 3.3. Relations between total mercury (THg) and selenium (Se) (a), THg and methylmercury (MeHg) (b), MeHg and Se (c) % MeHg and Se (d) in sediments from two Derwent Estuary regions, Ralphs Bay (RB) and Middle Estuary (ME). Dotted lines = linear regression with intercept, and regression coefficient (R^2) for each region. Solid line = exponential regression with intercept and regression coefficient

A correlation matrix (Table 3.4) was used to assess the interrelationships between sediment conditions and THg, MeHg and Se concentrations within regions. Se and THg concentrations in Ralphs Bay sediment were highly correlated with organic content (TOC and % mud), %S and Fe concentrations (R^2=<0.80). In the Middle Estuary, Fe showed the strongest correlation with sediment Se, while THg was more closely linked with Fe and AVS. MeHg showed a weak correlation with TOC, % mud and Fe in Ralphs Bay; in the Middle Estuary, MeHg was associated with %S and AVS. Sediment THg in Mickeys Bay was weakly correlated with AVS and % N, while Se concentration in this region was more strongly related to % mud and % N.

Table 3.4. Correlation matrix of linear regression analysis co-efficients (R^2) for total mercury (THg), methylmercury (MeHg) and selenium (Se) against other sediment components. Data derived from surface sediment samples of two Derwent Estuary regions, ME = Middle Estuary, RB = Ralphs Bay and reference region MB = Mickeys Bay. Sediment variables include total organic carbon (TOC), percentage mud (%mud), acid volitile sulfides (AVS), percentage sulphur (%S), percentage carbon (%C), percentage nitrogen (%N) and iron (Fe). MeHg data for MB have not been included as values were below detection limits. Parentheses indicate non-significant regression coefficients (*P*=0.05).

	THg			MeHg		Se		
	RB	**ME**	**MB**	**RB**	**ME**	**RB**	**ME**	**MB**
TOC	0.96	0.16	(0.05)	0.18	0.15	0.97	0.28	(0.01)
% mud	0.97	0.32	(0.01)	0.17	(0.05)	0.94	0.12	0.43
AVS	0.42	0.40	0.28	(0.03)	0.46	0.44	0.13	(0.05)
%S	0.83	(0.01)	(0.03)	(0.09)	0.44	0.86	0.11	(0.00)
%C	0.50	(0.01)	0.11	(0.06)	0.19	0.98	0.26	(0.00)
%N	0.47	(0.01)	0.21	(0.07)	(0.03)	0.47	(0.00)	0.29
Fe	0.97	0.44	(0.05)	0.19	(0.03)	0.96	0.61	(0.01)

BSAF: $BSAF_{THg}$ increased significantly with fish age ($f_{1,112}$ = 6.99 *P*=0.009), but there was no difference in slope between regions ($f_{2,112}$ =0.21, *P*=0.27) (Fig. 4). $BSAF_{THg}$ was significantly different between each of the three regions when age was accounted for ($F_{2,112}$=371.32, *P*=<0.0001), with Mickeys Bay>Ralphs Bay>Middle Estuary (Fig. 3.4). $BSAF_{MeHg}$ also increased with age ($f_{1,72}$ =6.81 *P*=0.01) (Fig. 4), and Ralphs Bay $BSAF_{MeHg}$ was significantly higher than for the Middle Estuary ($f_{1,72}$= 22.24, *P*=<0.0001) at all ages. Analysis of $BSAF_{Se}$ between regions showed a correlation between age and $BSAF_{Se}$ in Ralphs Bay ($t_{1,52}$ =2.22, *P*=0.02) that was not present in either of the other regions (MB $t_{1,53}$=-0.23, *P*=0.82, ME $t_{1,53}$=0.02, *P*=0.98) (Fig. 3.4).

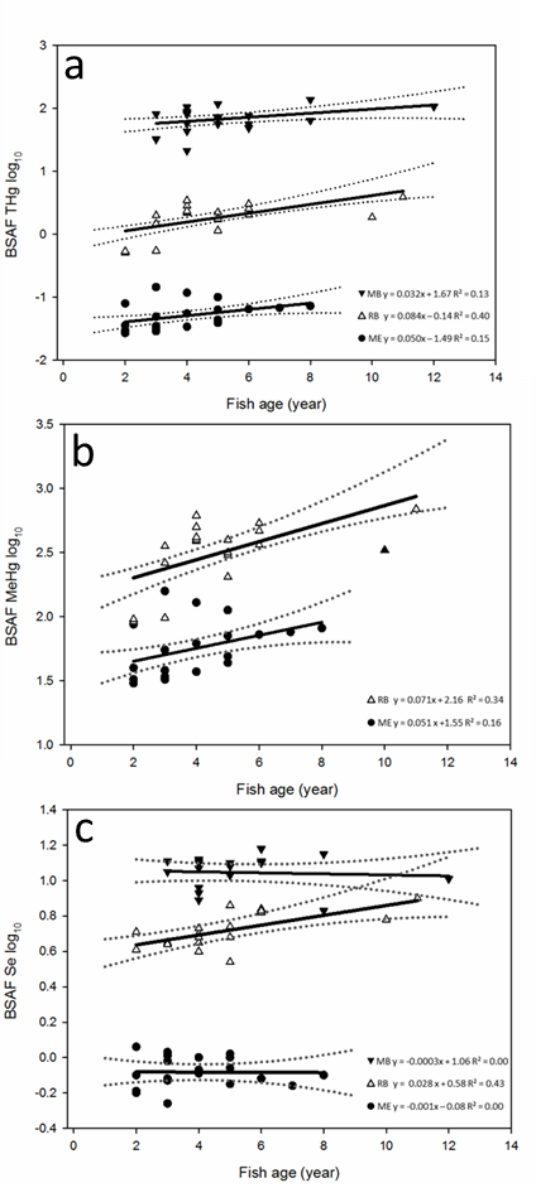

Figure 3.4. Regional linear regression analysis (R^2) with 95% confidence intervals for log_{10}-transformed biota sediment accumulation factors (BSAF) for sand flathead (*Platycephalus bassensis*) against fish age from two Derwent Estuary regions (Ralphs Bay RB and Middle Estuary ME) and reference region (Mickeys Bay MB). Graphs are shown for: a. total mercury (THg), b. methylmercury (MeHg) and c. selenium (Se). (BSAF = fish tissue metal concentration / sediment metal concentrations). The reference region MB is not included for $BSAF_{MeHg}$ as sediment MeHg were below detection limits in this region.

3.7 Discussion

Regional variations in Se concentration and %MeHg contamination in sediments, along with marked differences in the Se:Hg molar ratios in fish from the Derwent Estuary, offer strong evidence that elevated Se concentrations in sediments can have a significant effect on the bioavailability of Hg within benthic fish. Hg (both THg and MeHg) concentrations in fish caught in the Middle Estuary close to the contaminant source were significantly lower than in fish collected from Ralphs Bay, in the lower estuary. These concentrations of THg, MeHg and Se in fish ran counter to those in the surface sediments; sediment concentrations of Se and THg were significantly elevated in the Middle Estuary compared to Ralphs Bay, and concentrations in the Derwent Estuary were elevated compared to the reference region. This reduction in Hg concentrations in fish close to industrial sources compared to outlying regions has been observed in other studies, where reduced Hg loads were linked to high Se tissue concentrations, with the suggestion that the Se present mitigated the Hg concentrations in fish muscle (Chen et al., 2001; Sackett et al., 2010). However, this explanation is insufficient to explain the results of the Se concentrations in Ralphs Bay fish that were significantly higher than those of fish in the Middle Estuary, where the Se concentrations were consistent with the reference region.

Fish from all three regions had Se:Hg molar ratios greater than one, however, Se concentrations in Ralphs Bay fish were higher than in other regions, and if this had not been the case this region might have had a Se:Hg ratio less than 1.0. Organisms living in Se-poor environments have lower MeHg elimination rates than those in Se-rich environments (Belzile et al., 2006; Yang et al., 2008). The raised Se concentrations in flathead from Ralphs Bay may be the result of additional active uptake of Se, which

provides antagonistic protection against Hg toxicity (Peterson et al., 2009), and to maintain basal biochemical needs. Se typically bioaccumulates at much lower rates than Hg (Wang, 2002; Zhang and Wang, 2007), and body concentrations are regulated according to tissue requirements (Falnoga and Tušek-Žnidarič, 2007). Se is known to bioaccumulate with fish age (Cuvin-Aralar and Furness, 1991), and this was evident in Ralphs Bay but not in the reference region or Middle Estuary fish. The significantly lower Se:Hg ratios of the Ralphs Bay fish, in conjunction with the increase in THg and Se with age, suggest that the differences in Se bioaccumulation in this region may not be a temporary adaptation, but a biological requirement necessary to offset the higher Hg bioavailability.

Increased MeHg bioavailability in the Ralphs Bay region is plausible given that the sediments there were found to contain a higher fraction of THg as MeHg (%MeHg) than the sediments of other regions. Furthermore, sediment Se:Hg ratios of less than 1.0 and low Se concentrations within this region indicate that there is insufficient Se to bind all sediment Hg and would appear to suggest that Ralphs Bay may be a methylation 'hotspot' for Hg and a potential source of Hg in sand flathead. BSAF for THg, MeHg and Se clarify the linkage between flathead and sediment metal concentrations; both Hg and Se are known to bioaccumulate primarily through diet, and, therefore, have the potential to be taken up through the same food pathway (Hamilton, 2004; Zhang and Wang, 2007; Kehrig et al., 2009). The elevated $BSAF_{MeHg}$ and $BSAF_{THg}$ in Ralphs Bay, as compared with the Middle Estuary, indicate that the sediments of the Middle Estuary have less bioavailable Hg, despite having significantly higher THg concentrations.

Sand flathead, as benthic carnivores, are most likely exposed to Hg and Se through dietary intake of epibenthic fauna such as crabs (Dix et al., 1975), which in turn feed at

the sediment surface where MeHg can be produced (Ullrich et al., 2001). MeHg from sediments is readily incorporated at the base of benthic food webs and biomagnifies through successive trophic levels (Chen et al., 2008) and this pathway has been highlighted by Hg isotopes, which showed a clear link between surface-sediment MeHg and bioaccumulated MeHg in fish (Gehrke et al., 2011). Although contribution from pelagic pathways should not be discounted, benthic food pathways are likely to be significant routes for Hg bioaccumulation for this species.

Broadly, methylation rates in the Derwent Estuary followed conventional theory, with higher methylation rates (%MeHg) present in low N, C and S environments (RB) (Davis et al., 2012; Taylor et al., 2012). Within the enriched (TOC, N, S, Se) sediments of the Middle Estuary, MeHg correlated with S and AVS, which is to be expected given that its principal source is sulphate-reducing bacteria (SRB) (Compeau and Bartha, 1985). Within Ralphs Bay, no single sediment characteristic correlated well with MeHg concentration. The best (albeit relatively weak) correlations were between MeHg and Se, Fe, TOC and % mud. MeHg sediment concentration is complex and dependent upon both simultaneous methylation – demethylation pathways and flux of MeHg at the sediment-water interface (Marvin-DiPasquale and Agee, 2003; Lambertsson and Nilsson, 2006). Certain forms of MeHg are mobile in surface sediments, moving both vertically though the sediment and within the overlying water, driven by redox chemistry (Mason and Lawrence, 1999; Tomiyasu et al., 2008). MeHg eluted from surface sediments can result in a higher %MeHg concentrations in the water column than in the sediments themselves (Tomiyasu et al., 2008), and may then be available for biological uptake. Sediments in Ralphs Bay are structured by riverine flow and wind forcing, which provide intermittent transfer and deposition of suspended sediments into the region

(Margvelashvili et al., 2005). Turbulent, wind-mixed zones, like Ralphs Bay, result in frequent resuspension of particles and periodic diagenetic transformation of sediments as a result of oxidation of organic matter (Laurier et al., 2003). These conditions may lead to stimulation of microbial activity (Lambertsson and Nilsson, 2006), and enhanced methylation where anoxia-hypoxia occurs at the sediment-water interface (Sunderland et al., 2006). Unmeasured temporal change in net methylation rates may explain the poor correlation between MeHg and sediment variables and between THg and MeHg in Ralphs Bay (Marvin-DiPasquale and Agee, 2003). Hg bound to organic matter within the sediment layers of the Middle Estuary may act as a source into the lower estuary (such as Ralphs Bay) within suspended particles where Hg methylation is higher. This continued supply and turnover of Hg and its subsequent methylation in Ralphs Bay may result in significantly increased MeHg bioavailability over time in this region. It is also important to note that MeHg production could occur at depths below that sampled in this study (4 cm), with diffusion of MeHg up through the sediments to the redox line (Mason and Lawrence, 1999). Equally, efficient uptake of MeHg by biota after sediment-based production will also affect MeHg detection and correlations in the sediment.

The variability in the MeHg – THg association in this study once again stresses that THg concentrations in sediments are not reliable proxies for establishing surficial sediment Hg methylation and bioavailability (Mason and Lawrence, 1999; Lambertsson and Nilsson, 2006; Taylor et al., 2012). This study shows clear correlations between Se and THg, and Se known affinity for Hg^{2+} would suggest that some of the Hg present in the sediments may be bound as HgSe complexes (Yang et al., 2008; Yang et al., 2011). The THg association with Se, Fe and AVS in the Middle Estuary is likely the result of proximity to the industrial Hg source and the fact that inorganic Hg entering the system

may be still incorporated within compounds such as selenides, ferrites, pyrite and sulphides in which it was deposited. The positive association of Fe with Se indicates the potential of Se to sequester metals under reducing conditions, and may suggest FeSe formation (Peters et al., 1997).

Atmospheric Hg inputs from zinc refineries are typically Hg^0 and Hg^{2+} (Pirrone and Mahaffey, 2005), which could allow transformation of these Hg forms into HgS and HgSe. Hg complexes from neighbouring terrestrial sources are probably a combination of surface dusts and subsurface waters, with the make-up dependent upon the source of the original waste material (e.g. zinc ferrite waste, jarosite waste, ore feeds). Hg in jarosite waste is Hg hydroxide (Lyne et al., 1994), but the form of Hg in the other outputs remains unknown. Currently the forms present, and the transport and geochemical changes that Hg and its associated compounds undergo, in the Derwent Estuary are also unknown. However, the change in THg associations with other measured components measured in the sediments (%S, TOC, %S, %N) in this work suggest that there may be significant variation in Hg form and input between the source region and the lower estuary. Recent Hg isotope analysis has shown that the contributions of particular Hg isotopes vary markedly with distance from the Hg source (Foucher et al., 2013; Jones et al., 2013b), suggesting complex transfer and transformation dynamics. Application of these advances within the Derwent Estuary would provide important information about Hg source, movement, Hg species structure and bioavailability, thus greatly increasing our understanding of mercury cycling in estuaries.

3.8 Conclusions

The aim of this study was to evaluate if Se presence in an Hg contaminated estuary had a detectable effect on the bioavailability of Hg from the sediments and bioaccumulation of Hg in a resident fish species. The molar excess of THg over Se within the estuary's sediments suggests that there is insufficient Se to bind all the available sediment Hg. Se:Hg sediment ratios < 1.0, when coupled with high %MeHg and high $BSAF_{MeHg}$, highlighted potential methylation hotspots in the estuary's sediments. Near to the contamination source the bioavailability of Hg for fish may be reduced by a combination of inorganic Hg species complexation and lower net methylation rates, with Se presence in the sediments potentially playing a key role in this process. TOC and THg concentrations were found to be poor indicators of Hg methylation potential. This study also showed that high uptake of MeHg in fish can also be associated with increased Se uptake, potentially reducing the Hg toxicological effect.

The exact conditions that drive Hg net methylation in systems like the Derwent Estuary are unclear, but future work in developing Hg dynamic models needs to consider the role of Se in reducing Hg bioavailability. Hg isotope analysis may offer one method of determining the forms, and, therefore, potential interactions of Hg present. Analysis of the Se species, in conjunction with Hg isotope analysis of the sediments, would also aid system understanding. The strong association between THg and Se in both sediments and fish from this work suggests that Se plays an important role in balancing the impacts of Hg contamination, and should be considered in future assessments of Hg methylation and bioaccumulation from estuarine sediments.

CHAPTER 4

APPLICATION OF STABLE ISOTOPE MIXING MODELS FOR DEFINING TROPHIC BIOMAGNIFICATION PATHWAYS OF MERCURY AND SELENIUM

Preface:

The objective of this research was to determine the contributing routes of Hg exposure to resident fish species, while examining the data for evidence of spatial variation. Trophic models based on nitrogen stable isotope ratios ($\delta^{15}N$) have been shown to predict changes in Hg concentrations in fish, however they are usually applied at the ecosystem scale and rarely to specific species or food webs. Current research in this field has not considered the novel combination of gut contents and stable isotope analyses ($\delta^{15}N$ and $\delta^{13}C$) with a Bayesian isotopic mixing model to provide quantitative measures of Hg and Se biomagnification in an estuarine food web.

This chapter identifies Hg bioaccumulation pathways to key predatory species, and provides evidence to address causes of spatial discrepancies between estuarine regions.

Reducing uncertainty in food pathways to top predators significantly improves the ability to observe biomagnification potential of contaminants, and presents an additional tool for ecosystem management strategies.

This work is in review for publication in a refereed journal and is presented below in identical form. The citation for the original publication is:

Jones, H.J., Swadling K. M., Butler E. C.V., Macleod, C.K., 2013. Application of stable isotope mixing models for defining trophic biomagnification pathways of mercury and selenium. *Limnology and Oceanography*

4.1 Abstract

Trophic models based on nitrogen stable isotope ratios (δ^{15}N) have been shown to predict changes in mercury (Hg) concentrations in fish, however, they are usually applied at the ecosystem scale and are rarely directed at known trophic pathways. Here we discuss a novel approach in which we combined gut contents analysis and stable isotope analyses (δ^{15}N and δ^{13}C) with a Bayesian isotopic mixing model to provide a quantitative estimate of Hg and Se biomagnification in an estuarine food web. Estimates of the relationship between total mercury (THg) and methylmercury (MeHg) were significantly improved in mixing model-adjusted food webs, with trophic magnification factors (TMF) for THg increasing under this scenario. Spatial variation in MeHg biomagnification, when assessed in conjunction with marked differences in diet and bioavailability, offers strong evidence that food web differences can have a significant effect on the biomagnification of Hg within benthic fish species. While no evidence of Se biomagnification was found, lower Se:Hg ratios at higher trophic levels could be attributed to increasing trophic Hg concentration. Furthermore, stable isotope analysis linked Hg and Se biotransfer from benthic sources to fish. Overall, the findings highlight that isotope mixing models can be a significant aid in assessments of contaminant biomagnification, particularly when it is important to define food pathways to top predators.

4.2 Introduction

To delineate the pathways involved in the accumulation of mercury (Hg) and selenium (Se) in marine organisms, it is necessary to examine the trophic position of the species and the route of biomass acquisition (Wang, 2002; Chen et al., 2009). Total mercury (THg) and methylmercury (MeHg) concentrations typically increase with trophic level (Beneditto et al., 2012), as can Se concentrations (Besser et al., 1993; Wang, 2002; Hamilton, 2004). Despite the role of Se in mitigating Hg toxicity (Yang et al., 2008; Kehrig et al., 2009; Peterson et al., 2009), quantification of Se concentration against trophic position is almost absent from recent research (Campbell et al., 2005). Consumers' tissues are ultimately derived from the food they eat, consequently stable isotope ratios of carbon (δ^{13}C) and nitrogen (δ^{15}N) offer an effective quantitative measure of trophic structure, providing time-integrated tracers of energy flow, dietary history and trophic position (Post, 2002; Phillips and Gregg, 2003). Carbon (C) isotope ratios (δ^{13}C) provide a biomarker of organic C production, enabling identification of primary production and bioaccumulated contaminant sources (France, 1995; Chen et al., 2009; Gehrke et al., 2011). Nitrogen isotope ratios (δ^{15}N) exhibit a constant rate of incremental enrichment between trophic levels (typically 3.4 ‰), supplying a quantitative measure of trophic hierarchy (Post, 2002) against which contaminant biomagnification can be assessed (Cheung and Wang, 2008; Tom et al., 2010). Regression slopes between $\log_{10}$Hg and δ^{15}N are used as a measure of Hg biomagnification in ecosystems (Chen et al., 2009; Coelho et al., 2013). Notably, $\log_{10}$Hg – δ^{15}N regression slopes appear relatively constant (~0.2) despite changes in aquatic habitats, Hg source and food pathways (Campbell et al., 2005; Al-Reasi et al., 2007; Chen et al., 2009). However, previous studies have not represented direct pathways of contaminant transfer from prey to predator, instead

tending to infer ecosystem trophic biomagnification across a range of carbon sources and trophic levels (Campbell et al., 2005; Al-Reasi et al., 2007; Chen et al., 2009). Seldom have biomagnification studies been applied to functional food pathways where direct trophic links between prey and predator have been established by stomach contents and stable isotope analysis (Cossa et al., 2012). Bayesian stable isotope mixing models (BSIMM) have been designed specifically to allow incorporation of prior information (stomach contents), to account for multiple prey sources and to estimate the proportional contribution of prey to consumer tissues (Phillips and Gregg, 2003; Moore and Semmens, 2008). Not all prey consumed by a predator contribute significantly to predatory biomass, despite sharing similar δ^{13}C values, and BSIMM can quantify source contributions, which, in turn, allows elimination of non-significant sources (Bond and Diamond, 2011). Contaminant – δ^{15}N regressions optimized by preliminary BSIMM may offer a solution to identifying key species responsible for the transport of contaminants and may assist in refining model fit.

Intra–estuarine variation in feeding strategies and available prey has been shown to result in major changes in both stable isotope signatures and Hg concentration of estuarine fish (Adams and Paperno, 2012). In the Derwent Estuary, Tasmania, both Se and Hg contamination occur in a predatory fish species, sand flathead (*Platycephalus bassensis*) (Jones et al., 2013a), as a result of point source industry inputs (Dix et al., 1975; Bloom and Ayling, 1977). Small-scale spatial variation in contaminant concentrations for this species may be a result of dietary related biomagnification differences (Jones et al., 2013a; Jones et al., 2013b). The aims of this study were to: (1) Quantify the trophic position of sand flathead and its prey through stable isotope analysis and determine key food pathways through gut-contents and BSIMM; (2)

Compare and contrast spatial variability in the trophic magnification of THg, MeHg and Se; and (3) Evaluate the effectiveness of applying BSIMM to the patterns of trophic biomagnification through this particular food pathway.

4.3 Method

Study region

The Derwent Estuary, located in southern Tasmania (42°53'44 S, 147°22'08 E; Fig. 1), is a micro-tidal (1.2 m) estuary, 52 km in length and with a maximum depth of 30 m (Green and Coughanowr, 2003). The choice of study regions was based on previous research assessing Hg and Se concentration in sand flathead (Jones et al., 2013a; Jones et al., 2013b). Two estuary regions were selected: i) the industrialized middle estuary (ME), which has consistently high Hg concentrations in sediments and flathead, and ii) Ralphs Bay (RB), a large and relatively shallow embayment on the lower eastern side of the estuary, which exhibits relatively low Hg levels in the sediment (Bloom and Ayling, 1977; Jones et al., 2003), but high Hg concentrations in fish (Jones et al., 2013b). A reference region, Mickey's Bay (MB), located south of the estuary, was included to provide comparative concentrations from a region that has not been contaminated with heavy metals (Jones et al., 2013b) (Fig. 4.1).

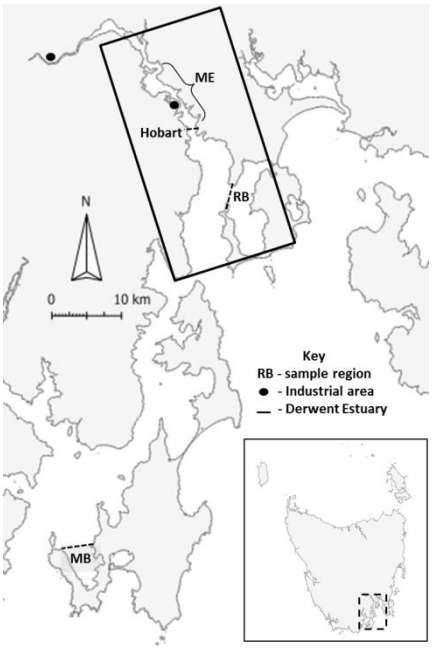

Figure 4.1 Southern Tasmania and location of the Derwent Estuary (boxed), with locations of the two estuary regions Middle Estuary (1), Ralphs Bay (2) and location of reference region (MB) (3) 48km south.

Sample collection

All containers and apparatus used in sample processing were either high density polyethelene (HDPE) or, where available, polytetrafluoroethylene (Teflon). Acid-cleaned (10-20 % HCL, 1 week bath) laboratory and non-contaminating techniques were employed throughout all sample processing and storage steps.

Fish: (n=60) were sampled in Nov–Dec 2011 by line fishing. Fish were individually sealed in plastic bags, stored on ice and frozen (–40 ^{o}C). Processing followed the procedure described by Verdouw *et. al.*(2010): morphometric measurements of each fish included fork length (FL) (±1 mm), wet weight (whole ± 0.1 g), and sex. The stomach of each fish was weighed full and the contents were then separated into lowest determinable taxonomic groups. These groups were weighed and the number of

individuals counted. Whole fish were lyophilized to constant mass (± 0.1 g) and homogenised.

Invertebrate prey: Two sampling methods were used for the collection of invertebrates: firstly, a dredge (mesh size: 2 mm sides, 12 mm base) towed behind a vessel for approximately 100 m; secondly, a venturi pump (aperture: 90 mm) operated by divers. In both cases, once the samples were retrieved the collected material was washed thoroughly in mesh bags (1 mm mesh), before being placed on ice. Samples were sorted immediately on return to the laboratory. Representatives of species that had previously been observed in the stomach contents of sand flathead were isolated from the bulk samples and left to purge overnight in aerated, filtered seawater (0.4 μm). Composite samples were prepared for each of these species, where individuals with weight or size similar to those in the gut samples were selected and pooled. These samples were then lyophilized, homogenised and sub-split for THg, MeHg, Se, δ^{13}C and δ^{15}N analyses.

Prey fish: - Undigested individual fish were extracted from the gut contents of sand flathead and thoroughly washed in reverse osmosis (RO) water (Elga Purelab Prima) to remove contaminants. Positive identification of species was generally prohibited by the initial stages of digestion, however, provided the majority of the fish was present (i.e. muscle, vertebrae, head), they were lyophilized, homogenised and sub-split for THg, MeHg, Se, δ^{13}C and δ^{15}N analyses.

Plankton: Two size fractions of plankton (63-200 μm and >200 μm) were collected from a drifting vessel on four occasions at each region between September 2010 and April 2012. Diagonal tows of 63 μm and 200 μm nets were taken from approximately 1

m above the seabed to the surface. The 63 μm samples were backwashed with filtered seawater (0.4 μm) into HDPE containers fitted with 200 μm mesh to remove the larger fraction. The containers were placed in a positive pressure glove bag where they were aerated overnight to allow the plankton time to purge. After purging, each sample was split into two equal parts, one for THg, MeHg and Se analyses and the other for stable isotope analysis. Sub-samples for metal and stable isotope analyses were captured onto 0.4 μm HTTP filters in the glove bag and scraped clean. Samples were lyophilized prior to analysis.

Trace element analysis

Digestions and analyses were performed using the method described in Jones et al., (2013a)

THg MeHg and Se digestion: THg and Se samples were digested for 2 h in HNO_3 (trace grade) in polypropylene digestion vessels at 120 ^{o}C within a deep cell digestion block. H_2O_2 was added to each sample and the vessels digested for a further 1 h, before a HNO_3:HCl mixture (3:1) was added to the vessels and heated for 1 h. Samples were diluted to 50 mL total volume with RO water and analysed within 48 h of digestion. MeHg extraction followed a serial extraction using KOH, then HCl, and finally a solution of $CuSO_4$/KBr/H2SO4. Dichloromethane (DCM) was added and the vials returned to the shaker overnight. The DCM layer was then transferred to a clean glass vial and 0.01 M sodium thiosulphate ($Na_2S_2O_3$) (2 mL) was used to extract the MeHg component. The final extract was filtered (0.45 μm) before analysis.

THg analysis: Analysis was carried out by cold vapour atomic fluorescence spectroscopy (CV-AFS) (10.023 Millenium Merlin, PS Analytical). A 2 % w/v tin(II) chloride reductant and argon (Ar) carrier gas was used.

MeHg analysis: Aliquots were analysed by high pressure liquid chromatography–ultra-violet–atomic fluorescence spectroscopy (HPLC–UV–AFS) using an oxidant stream of acidified potassium bromide/ potassium bromate (10 % v/v HCl, 10 % v/v 0.1 M Br^- /BrO^{3-}). A 38 % methanol, 30 % acetonitrile (m/v) with ammonium pyrrolidine dithiocarbamate (APDC 0.2464 g L^{-1}) was used for the mobile phase with a Supelco C18 column (ODS-2) to provide species separation. An online UV photolysis/heater (PSA S570U100) and cooling module (PSA S570C100) coupled to the AFS provided oxidation before analysis. A 2 % w/v tin(II) chloride reductant and Ar carrier gas were used for cold vapour separation prior to AFS detection.

Se: Se detection used online pre-reduction of Se with hydride-generated atomic fluorescence analysis (HG-AFS) (Millenium Excalibur, PS Analytical). Se was reduced by mixing with pre-reductant KBr/HCl (5 % KBr, 50 % HCl) and passing through a UV heater (PSA S570U100) (150 ^oC) and cooling module (PSA S570C100). The sample was then mixed with the reductant (0.7 % $NaBH_4$ 0.4 %NaOH) to form selenium hydride and carried by Ar (0.3 L/min) to the detection system.

Quality assurance

Linear calibration was acquired using standards diluted in the appropriate concentration range with matrix-matched reagents. The accuracy was verified with an independent substandard for each of the three analytical procedures. Matrix-matched procedural blanks were analysed at the beginning and after sample runs, to test for any

procedural contamination, with none observed. Calibration verification (independent check and certified reference material) was run after instrument calibration, after every 20 samples, and at the end of each the batch of samples. Each sample was run in duplicate, with one sample per batch spiked with 5 ng g^{-1} standard solution and recovery rates recorded. Certified reference materials DOLT-4 (NRC Canada, dogfish liver), mean recovery (n = 6) THg = 94.40 %, Se = 92.67 % MeHg = 98.66 %, and BCR 422 (IRMM, cod muscle), mean recovery (n = 6) MeHg = 117.44 %, were used to verify recovery rates. All results are reported as dry weight (dw).

Stable isotope analysis

Samples were analysed for δ^{13}C and δ^{15}N by Elemental Analysis (EuroVector EA3000 or Elementar varioPYROcube) and Isotope Ratio Mass Spectrometer (GV Instruments IsoPrime 100). δ^{13}C and δ^{15}N results are presented as deviations from standards, expressed as δ^{13}C and δ^{15}N using the following formula:

$$\delta X = [R_{smpl}/R_{stnd} - 1] \times 10^3 \quad (1)$$

where X is ^{13}C or ^{15}N and R is $^{13}C/^{12}C$ or $^{15}N/^{14}N$. The reference materials used were: (i) for δ^{13}C:– an IAEA reference material, IAEA C8, with an agreed value of ^{13}CV-PDB = -18.31 ‰ and (ii) δ^{15}N:– two IAEA reference materials, IAEA N^{-2} (consensus value δ^{15}NAIR = +20.3 ‰) and IAEA N^{-3} (consensus value δ^{15}NAIR = +4.7 ‰), and a USGS reference material, USGS-34 (consensus value δ^{15}NAIR = -1.8 ‰). Precision of instrument estimates was 0.1‰ for C and 0.2‰ for N. Duplicate samples were run for all samples with further repeats run if standard deviations between duplicates exceeded 0.4‰.

Statistical analysis

All statistical analyses were performed using the R statistical package (3.0.0, R foundation 2012). Percentage frequency of occurrence (%*F*) and percentage relative weight (%*W*) of species in sand flathead stomachs were calculated using the formulae published by Hyslop (1980). Kruskal-Wallis non-parametric tests and analysis of variance (ANOVA) with unplanned post-hoc comparison of means (Tukey HSD) were used to test for differences in metal concentrations and stable isotopes between regions. THg:Se ratios were calculated by conversion of dw concentrations into molar mass in order to assess molar excess:

Se:Hg = concentration in mg kg^{-1} (dw)/molar mass (Hg= 200.59, Se= 78.96) (2)

A Bayesian isotopic mixing model R package, SIAR (Stable Isotope Analysis in R) (Parnell et al., 2010), was used to assess contribution of prey items to diet within each region. The model was fitted via Markov Chain Monte Carlo (MCMC) permutations, which produces simulations of the values of dietary proportions of sources to a mixture (predator). SIAR allows incorporation of prior information to drive the model and reduce uncertainty (Parnell et al., 2010). In this study %*W* of diet was used to guide the SIAR model for dietary contributions, with the unidentified *%W* proportion split equally between identified prey. Trophic enrichment factors (TEF), the change in δ^{13}C and δ^{15}N between trophic levels, were based on mean trophic fractionations with large standard deviations that are considered global averages (TEF δ^{13}C = 0.4 ± 1.3; TEF δ^{15}N =3.4 ± 1) (Post, 2002), as no published values were available for the species sampled.

Trophic level (TL) was established through δ^{15}N ratios:

$$TL = [(\delta^{15}N_{species} - \delta^{15}N_{base})/\Delta\ \delta^{15}N] + TL_{base} \quad (3)$$

Where $\delta^{15}N_{species}$ is the $\delta^{15}N$ value of the species in question, $\delta^{15}N_{base}$ is the $\delta^{15}N$ value of the representative baseline and TL_{base} is the trophic level of that baseline. Variation in $\delta^{15}N_{base}$ is common within systems; primary consumers are typically used due to longevity and reduced seasonality in $\delta^{15}N$ compared to primary producers (Cabana and Rasmussen, 1994). In this work the primary consumer *Paragrapsus gaimardii* was treated as the representative baseline in each region and thus TL_{base}= 2 and all species $\delta^{15}N$ are given as $\delta^{15}N_{std}$ ($\delta^{15}N_{std} = \delta^{15}N_{species} - \delta^{15}N_{base}$).

Assessment of biomagnification was undertaken by calculation of trophic magnification factors (TMF):

$$Log_{10}(THg/MeHg/Se) = a + (b \times \delta^{15}N_{std}) \quad (4)$$

Where *a* is the point of intercept and *b* is the slope of the regression

$$TMF = 10^{b} \quad (5)$$

Trophic magnification is considered to occur when TMF is >1 (i.e. slope b >0.1). Biomagnification regression models were run on the full dataset by region and then on a refined dataset resulting from the BSIMM. The BSIMM regressions included only species with a mean proportional contribution to flathead diet of >5% within that region. Variation in model fit between the full dataset and the SIAR regressions was assessed by comparison of R^2 values and variation in biomagnification was assessed by comparison of TMF. Variation in biomagnification between regions within BSIMM food web was assessed by analysis of covariance (ANCOVA), with prior testing of normality using the Shapiro-Wilk test. Variations in % MeHg and Se:Hg with $\delta^{15}N$, and between regions, was also tested using linear regressions and ANCOVA.

4.4 Results

Although the species consumed by sand flathead varied between regions, crustaceans comprised the majority of the prey species throughout all regions in this study (Table 1). Within both Derwent Estuary regions (RB and ME) the benthic crab *Paragrapsus gaimardii* contributed the highest biomass (*%W*), while in the reference region the squat lobster *Munida haswelli* was the preferred prey (Table 4.1). Fish species contributed between 1.2 – 20 %*W* of the prey found in the gut contents (Table 4.1).

Table 4.1, Stomach contents of *Platycephalus bassensis* (n=40 per region) sampled from 3 regions in southern Tasmania, ME = Middle Derwent Estuary; RB = Ralphs Bay; MB = Mickey's Bay. %F = frequency of occurrence percentage: the number of stomachs containing a given prey item divided by the total number of non-empty stomachs, multiplied by 100. %W = relative weight percentage; the total weight of a given prey item divided by the total weight of all prey items in all stomachs, multiplied by 100.

	% F			**% W**		
Species	ME	RB	MB	ME	RB	MB
Paragrapsus gaimardii	59.5	82.6	2.5	62.0	40.4	16.9
Petrolisthes elongatus	5.0	12.5	1.0	2.5	10.5	0.9
Halicarcinus ovatus	5.0	2.5	7.5	0.3	1.0	4.7
Macrophthalmus latifrons	27.5	5.0	0	11.6	3.7	0
Munida haswelli	-	1.0	25.0	-	-	24.1
Palaemon intermedius	2.5	2.5	2.5	0.3	1.0	3.7
Caprella sp.	–	2.5	–	–	0	–
Teleost spp.	3.2	5.0	7.5	1.2	10.1	20.0
unidentifiable	0	2.5	5.0	2.7	23.1	29.7

δ^{13}C values ranged between -22.8 ‰ and -14.5 ‰ (Fig 4.2.). It was possible to differentiate the benthic and pelagic species in all three regions on the basis of the δ^{13}C values, with planktonic fractions being lighter in δ^{13}C (-19.7 to -22.8 ‰) than all other prey samples (-18.6 to -14.5 ‰). However, there was one notable exception to this, namely *Paleomon intermedius* from RB, which measured -20.7 ‰ (Fig 4.2). There was no significant difference between the δ^{13}C values of sand flathead and the benthic prey species from RB (Kruskal-Wallis P_{43}= 0.18). However, δ^{13}C values in both ME and MB flathead were significantly higher than that of their benthic prey (Kruskal-Wallis $P_{40/16}$ = <0.01), but lower than the plankton (Fig. 4.2). Sand flathead from RB had higher δ^{13}C values than either of the other regions examined (Kruskal-Wallis P_{15}= <0.01) (Fig 4.2) δ^{15}N values increased from prey species (8.5 – 13.6 ‰) to sand flathead (14.3 – 16.9 ‰), but only in the ME did plankton samples have lower δ^{15}N than other prey (Table 4.2). Mean fractionation of δ^{15}N between prey species and flathead increased from the RB region (4.53 ‰) through the ME (5.4 ‰) to MB (5.6 ‰), while trophic level (TL) calculations revealed that trophic level was highest in sand flathead from the reference region (TL = 4.06), and lowest in the ME (TL = 3.45) (Table 4.2). Plankton TL ranged from 1.01-2.74, indicating highly variable δ^{15}N values. Benthic prey species TL varied between 1.7 –2.84 across the regions, suggesting they were largely primary consumers (Table 4.2).

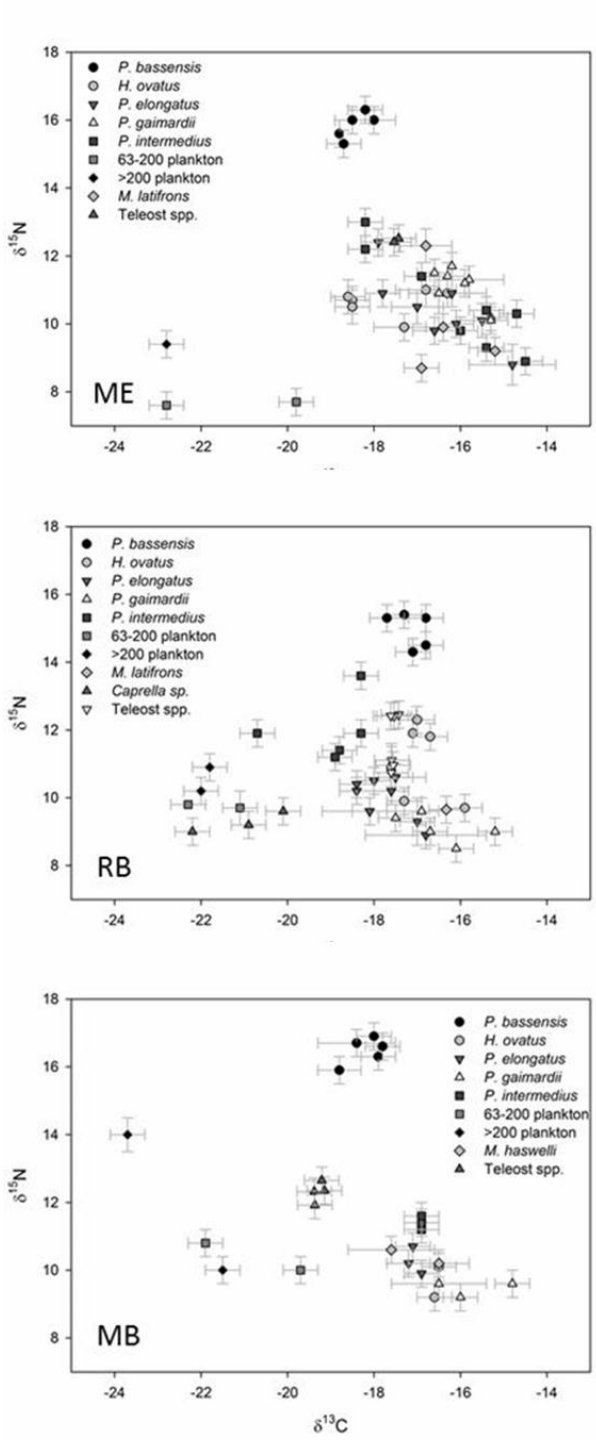

Figure 4.2. δ^{13}C and δ^{15}N values for prey species of sand flathead (*Platycephalus bassensis*) from three locations in southern Tasmania. Points represent single samples with duplicate sample standard deviations. Derwent Estuary sample regions Middle Estuary (ME), Ralphs Bay (RB), and the reference region (MB). 63-200 = 63-200 μm plankton fraction; >200 = >200 μm plankton fraction.

Table 4.2, Trophic level (TL) and mean concentrations (dry weight) of total mercury (THg), methylmercury (MeHg),selenium (Se), % methylmercury (%MeHg) and selenium:total mercury molar ratio (Se:Hg) with standard deviation from two Derwent Estuary regions (Middle estuary (ME), Ralphs Bay (RB)) and a reference region (MB) for selected species in the sand flathead (*Platycephalus bassensis*) food web . TL is calculated from TL = $[(\delta^{15}N_{species}-\delta^{15}N_{base})/\Delta\ \delta^{15}N] + TL_{base}$ where $\delta^{15}N_{species}$ is the $\delta^{15}N$ value of the species in question, $\delta^{15}N_{base}$ is the $\delta^{15}N$ value of representative baseline (*P. gaimardii*) and TL_{base} is the trophic level of that baseline. Superscript letter (^{a,b,c,}), denotes significant differences (*P*= 0.05) assessed between regions.

Species	**n**			**TL**			**THg**			**MeHg**			**%MeHg**			**Se**			**Se:Hg**		
	ME	RB	MB	ME	RB	MB	ME	RB	MB	ME	RB	MB	ME	RB	MB	ME	RB	MB	ME	RB	MB
63-200 μm plankton	4	2	2	1.01	2.23	2.28	0.08	0.3	0.1	0.02	0.02	0.00	–	11.37	9.51	0.94	1.93	1.71	6.29	18.35	60.05
							(0.05)	(0.14)	(0.05)		(0.02)			(10.28)		(0.73)	(0.28)			(3.61)	(26.64)
>200 μm plankton	6	4	5	1.52	2.47	2.74	0.13	0.11	0.09	0.02	–	0.01	3.81	–	7.08	1.53	0.60	0.55	14.7	24.38	65.59
							(0.08)	(0.05)	(0.05)	(0.01)		(0.00)	(0.95)			(0.63)			(6.01)	(7.29)	(26.95)
Paragrapsus gaimardii	40	21	9	2.00	2.00	2.00	0.64_a	0.66_a	0.19_b	0.11_a	0.19_a	0.03_b	24.97	46.36	26.32	1.12	1.12	1.52	7.87_b	32.01_a	33.82_a
Mottled shore crab							(0.46)	(0.78)	(0.24)	(0.08)	(0.26)	(0.00)	(20.72)	(26.67)	(16.57)	(0.49)	(0.32)	(0.87)	(9.74)	(55.2)	(25.47)
Petrolisthes elongates	13	12	10	1.82	2.34	2.24	0.09_a	0.09_a	0.03_b	0.04	0.08	0.06	46.10	45.06	100.0	1.57	1.36	1.18	42.36_b	42.03_b	101.01_a
New Zealand half-crab							(0.06)	(0.04)	(0.02)	(0.03)	(0.04)	(0.01)	(60.88)	(35.22)		(0.15)	(0.06)	(0.14)	(9.41)	(6.07)	(1.56)
Halicarcinus ovatus	3	6	4	1.93	2.63	2.06	0.57	0.72	0.07	0.12	0.21	0.05	14.92	29.24	71.43	3.74	0.57	–	8.23	2.89	5.45
Spider crab							(0.25)	(0.18)	(0.00)	(0.06)	(0.04)	(0.00)	(2.34)	(1.23)	(5.45)	(0.10)	(0.01)		(0.20)	(0.50)	
Palaemon intermedius	12	6	4	1.89	2.90	2.57	0.34	0.21	0.01	0.11	0.29	0.05	36.89	62.07	100.0	1.95	1.82	2.62	18.47	12.11	71.76
Caridean shrimp							(0.17)	(0.16)	(0.05)	(0.03)	(0.13)	(0.00)	(25.06)	(26.29)		(0.22)	(0.33)	(0.82)	(14.84)	(4.94)	
Munida haswelli	–	–	3	–	–	2.28	–	–	0.03	–	–	0.04	–	–	100.0	–	–	1.58	–	–	104.2
squat lobster									(0.00)			(0.00)						(0.31)			
Macrophthalmus latifrons	9	–	–	1.7	2.21	–	0.59	–	–	0.17	–	–	26.33	–	–	1.37	–	–	6.40	–	–
Southern Sentinel Crab							(0.27)			(0.13)			(19.84)			(0.27)			(1.62)		
Teleost spp.	2	4	3	2.42	2.73	2.84	0.35	0.34	0.19	0.30	0.31	0.19	85.86	92.76	100.13	1.56	1.51	1.62	11.51	11.74	22.20
Prey fish							(0.02)	(0.06)	(0.02)	(0.02)	(0.09)	(0.06)	(11.42)	(21.71)	(33.24)	(0.18)	(0.28)	(0.28)	(2.01)	(3.58)	(5.42)
Platycephalus bassensis	20	20	20	3.45	3.77	4.06	1.28_a	1.34_a	0.32_b	1.31_a	1.40_a	0.30_b	103.31	106.12	91.42	1.22	1.29	1.30	9.43_b	3.31_c	12.07_a
Sand flathead							(0.71)	(0.61)	(0.16)	(0.68)	(2.16)	(0.51)	(29.96)	(25.58)	(20.63)	(0.22)	(0.33)	(0.82)	(13.81)	(1.37)	(5.55)

The BSIMM estimated that the largest proportional contribution to diet within both Derwent Estuary regions was from *Paragrapsus gaimardii* (Fig. 4.3). *Macrophthalmus latifrons* and *Petrolithes elongatus* contributed >5 % to the mean proportion of flathead diet in both the ME and RB regions, while in RB prey fish species also contributed >5 % (Fig. 4.3). In the reference region (MB) there was a large dietary shift, with teleost prey species being the principal proportional source to diet, with other significant contributions from *Munida haswelli, Paragrapsus gaimardii*, *Halicarcinus ovatus* and *Palaemon intermedius* (Fig 4.3). In all three regions plankton contributed <5 % to diet (Fig.4.3).

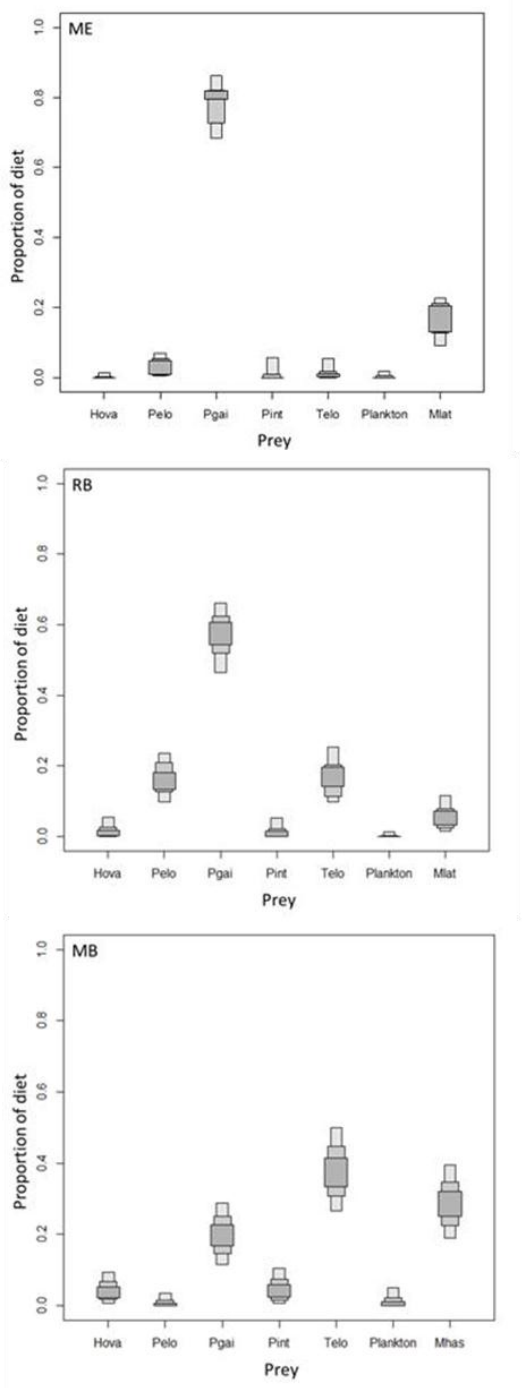

Figure 4.3. Bayesian isotope mixing model contributions to diet of sand flathead from 3 regions in southern Tasmania (ME Middle Estuary, RB Ralphs Bay, MB Mickeys Bay). Individual species proportional contributions to diet are displayed with 95%, 75% and 25% credibility intervals. Prey sources modelled are *Halicarcinus ovatus* (Hova), *Petrolisthes elongatus* (Pelo), *Paragrapsus gaimardii* (Pgai), *Palaemon intermedius* (Pint), 63-200 μm plankton (63-200), >200 μm plankton (>20 0), *Macrophthalmus latifrons* (Mlat), *Munida haswelli* (Mhas).

A summary of metal concentrations measured in each species is provided in Table 4.2. Note that low replication levels and the absence of some species from the flathead diet in certain regions prevented statistical comparison of some species between regions. THg ($F_{2,59}$ = 4.84, P=0.01) and MeHg ($F_{2,51}$ = 4.61, P=0.01) concentrations varied significantly between the Derwent Estuary and the reference region (MB) in the principal prey species *P. gaimardii*, but there was no difference in these concentrations between the regions within the Derwent Estuary (Table 4.2). Similarly, THg concentrations in *P. elongatus* did not differ between RB and ME, but concentrations were lower in the reference region (MB) than in the Derwent Estuary regions ($F_{2,20}$ = 19.21, P=<0.001). Sand flathead THg ($F_{2,59}$ = 14.54, P=0.01) and MeHg $F_{2,59}$ = 9.84, P=0.01) concentrations were significantly higher in the Derwent Estuary (RB and ME) than in the reference region (MB). However, neither Se concentration nor %MeHg differed between regions in any species examined (Table 4.2). Molar ratios of Se:Hg varied between species and regions, with the reference region having larger Se molar advantage over the Derwent Estuary regions in all but one species (*Halicarcinus ovatus*), (Table 4.2).

Biomagnification of THg, MeHg and Se was assessed by regressing $\log_{10}$ contaminant concentration against $\delta^{15}N_{std}$ within region. THg and MeHg increased with $\delta^{15}N_{std}$, with the regression strength increasing significantly with MeHg in all regions (Fig. 4.4 a–d). The regression fit of $\delta^{15}N_{std}$ – THg improved significantly in all regions within BSIMM refined trophic models (Fig. 4.4 a,b),while the regression fit of BSIMM trophic models for $\delta^{15}N_{std}$ – MeHg was improved in RB and MB regions but not in the ME against the non-BSIMM model (Fig.4. 4 c,d). Within the BSIMM refined food web there was no difference in regression slopes (biomagnification) between regions for either THg ($F_{2,60}$ =

1.21, *P*=0.30) or MeHg ($F_{2,58}$ = 1.27, *P*=0.29), but the point of intercept varied between regions THg ($F_{2,60}$ = 14.84, *P*=<0.001), MeHg ($F_{2,58}$ = 9.72, *P*=<0.001) (Fig. 4.4b,d). Se showed no correlation with $\delta^{15}N_{std}$ in any region (Fig. 4.4e), but within the BSIMM food web Se showed a weak but significant decline in ME and MB (Fig. 4.4f), although there was no difference in point of intercept between regions ($F_{2,60}$ = 1.21, *P*=0.30).

TMF_{MeHg} and TMF_{THg} (except at ME, Fig 4.4a), were ≥1, suggesting biomagnification of both Hg forms between trophic levels (Fig. 4.4a–d). TMF_{MeHg} was higher than TMF_{THg} in all regions (Fig.4.4a–d). However, there were no regional differences in TMF_{THg} for BSIMM regressions as a result of the overall variability in the full dataset (Fig. 4.4a,b). TMF_{MeHg} did not alter between BSIMM regressions and the full data set, but declined between regions with ME > MB > RB (Fig. 4.4c,d). TMF_{Se} did not exceed 1 at any location, which suggests that Se biomagnification was not occurring in any region (Fig. 4.4e,f).

In all regions %MeHg within BSIMM-refined food webs increased at a similar rate to $\delta^{15}N_{std}$ ($F_{2,55}$ = 2.57, *P*=0.09), although the regression strength varied (Fig. 4.5a). All regions exhibited equal ($F_{2,59}$ = 0.88, *P*=0.42) negative regressions between Se:Hg and $\delta^{15}N_{std}$ with a similar point of intercept ($F_{2,59}$ = 2.54, *P*=0.09) (Fig. 4.5b).

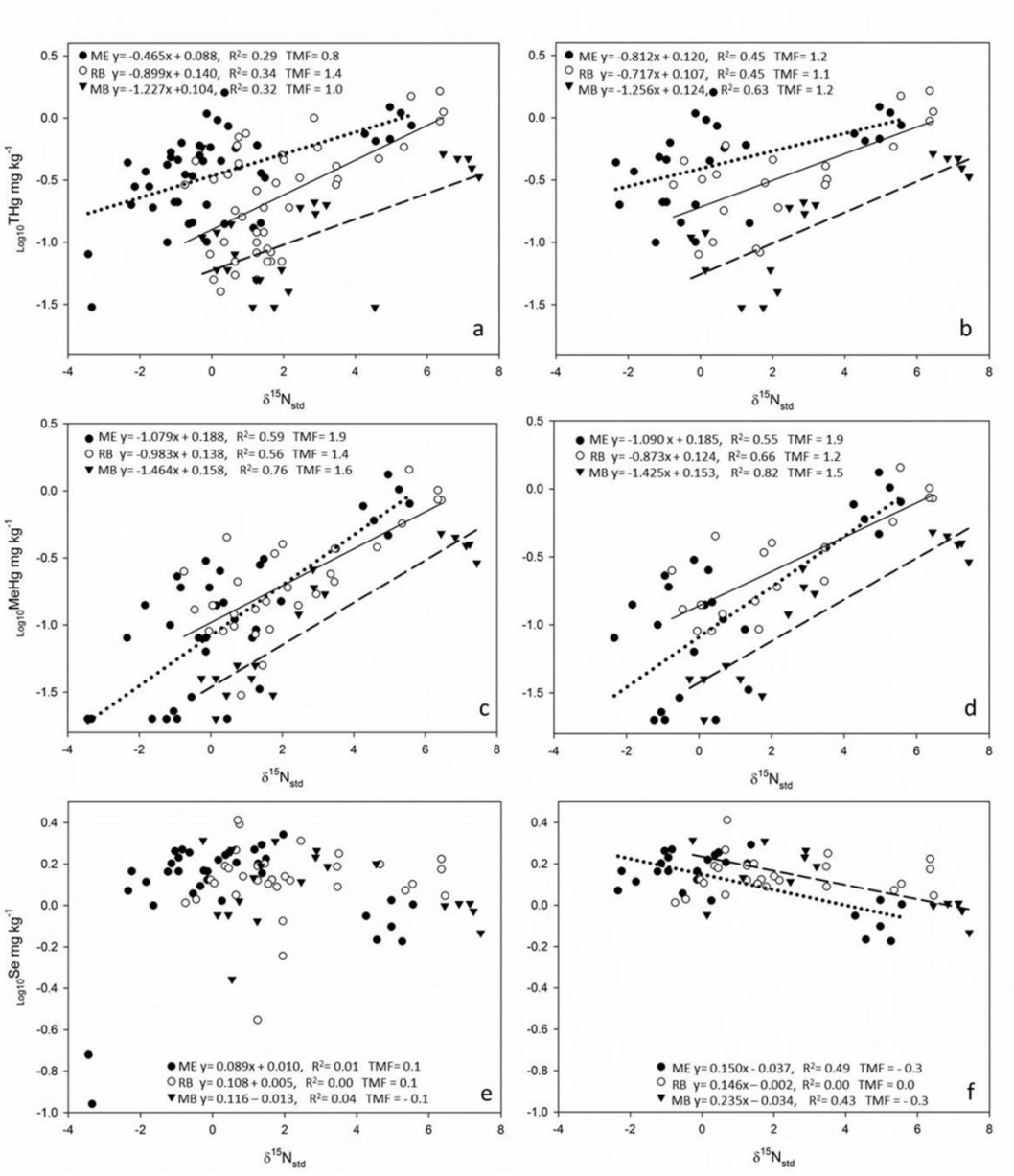

Figure 4.4. Linear regressions (R^2) between $\delta^{15}N_{std}$ and $\log_{10}$ concentrations of total mercury (THg), methylmercury (MeHg) and selenium (Se) in sand flathead food webs from two Derwent Estuary regions (Middle Estuary (ME) dotted line, and Ralphs Bay (RB), solid line) and the reference region Mickeys Bay (MB), dashed line. Left-hand figures (a,c,e) are regressions including all prey species identified in sand flathead gut contents; right-hand figures (b, d,f) are regressions of prey found to account for >5% mean proportional contribution to sand flathead diet based on stable isotope analysis in R (SIAR) mixing model.

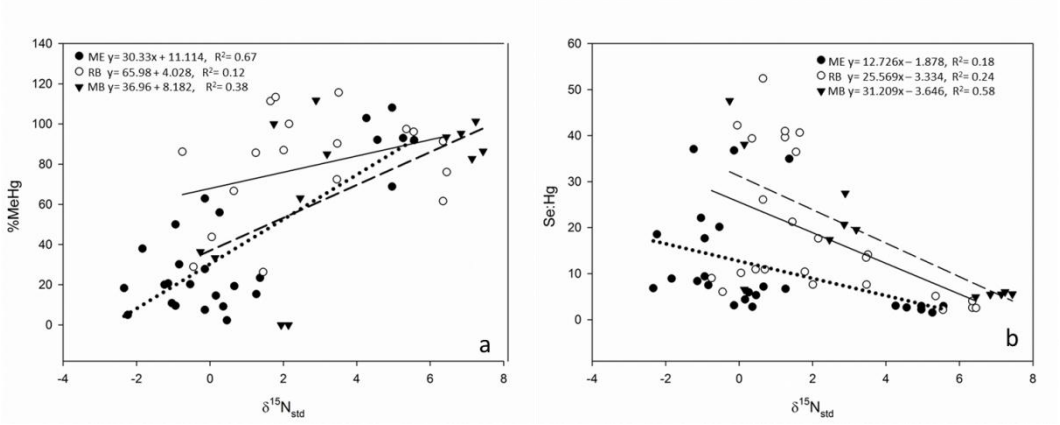

Figure 4.5. Linear regression coefficients (R^2) of select species identified by stable isotope analysis in R (SIAR) mixing model in sand flathead food webs from two Derwent Estuary regions (Middle Estuary (ME) dotted line, and Ralphs Bay (RB), solid line) and the reference region Mickeys Bay (MB), dashed line. Graphs presented are $\delta^{15}N_{std}$ versus % MeHg (MeHg/THg*100) and $\delta^{15}N_{std}$ versus Se:Hg molar ratios (MB).

4.5 Discussion

The principal objectives of this study were threefold. Firstly, to examine whether Bayesian stable isotope mixing models (BSIMM) could improve contaminant trophic models and provide a more precise guide to biomagnification pathways. Secondly, to determine if diet and biomagnification differences in a benthic fish species might be responsible for observed regional variations in Hg and Se concentrations in this species. Finally, we aimed to evaluate the effectiveness of applying BSIMM to the patterns of trophic biomagnification. To our knowledge this study is the first to use BSIMM to inform Hg trophic magnification regressions. We found that BSIMM adjusted regressions provided better model fit between Hg (THg and MeHg) concentrations and trophic level ($\delta^{15}N$) than non-adjusted regressions. The BSIMM trophic models removed inconsequential planktonic and benthic prey species, reducing variability in contaminant concentrations and uncertainty in prey trophic level. This significantly altered regression

slopes and points of intercept, and produced a more accurate evaluation of trophic magnification to sand flathead. The BSIMM model defined the trophic pathway between sand flathead and benthic prey, suggesting specific benthic dietary sources of contaminants that varied between regions in this study. The BSIMM model enables discrimination between prey species with similar isotopic values, which would not be possible with other isotope models where dietary inputs are not included (Bond and Diamond, 2011). This study suggests that, by removing bias associated with non-significant prey Hg concentrations, BSIMM provides an approach that significantly reduces uncertainty in Hg biomagnification studies where an understanding of Hg pathways to predators is required. This technique will be particularly beneficial for monitoring and toxicity risk assessments for predatory species, particularly those of high conservation value and those that are eaten by humans.

The capacity of the BSIMM model to predict dietary contribution is dependent upon assumptions regarding the trophic enrichment factors (TEF) in δ^{15}N and δ^{13}C between predator and prey (Bond and Diamond, 2011). Unfortunately, in this study TEF were not available for individual predators or prey, and therefore global means were used (Post, 2002). Although the BSIMM model is slightly weakened by the reliance on global mean data, the incorporation of the dietary information into the model strengthened the model output and separated the contributions of the various prey to the diet (Bond and Diamond, 2011). Future experiments to verify TEF for the species in this study could be used to reduce uncertainty in the BSIMM model and thereby improve the accuracy of the trophic regressions.

Both THg and MeHg showed significant biomagnification in all regions, with MeHg exhibiting higher biomagnification throughout. BSIMM informed TMF_{THg} were stable spatially across all regions, while TMF_{MeHg} was spatially more variable, potentially suggesting differences in biomagnification rates between regions. However, the similarity between the regional regression slopes suggests that any difference is likely to be non-significant. The TMF_{THg} and TMF_{MeHg} across the regions were similar to TMF reported in other work (Chen et al., 2008), which supports the concept that there is considerable stability in THg and MeHg TMF across latitudes and aquatic systems (Campbell et al., 2005; Coelho et al., 2013) (Table 4.3). This is despite significant differences in Hg contamination sources between systems (Chen et al., 2008).

Table 4.3. δ^{15}N slopes and trophic magnification factors (TMF) between log-transformed mercury concentrations and δ^{15}N available in literature.

Location	**Study**	**Slope δ^{15}N THg (TMF)**	**Slope δ^{15}N MeHg (TMF)**
Ria de Aveiro, Portugal	Coelho et al., 2013	0.06 (1.15)	0.27 (1.86)
Arctic	Campbell et al., 2005	0.2 (1.59)	0.22 (1.66)
Rio de Janeiro, Brazil	Di Beneditto et al., 2012	0.25 (2.5)	–
Lake Tanganyika,Tanzania	Campbell 2008	0.13 (1.35)	–
Derwent Estuary, Australia	this study	0.12(1.16)	0.15 (1.54)

Elevated MeHg regressions and the increase of %MeHg between the successive trophic levels in this study suggest that MeHg is being preferentially biomagnified between trophic levels (Chen et al., 2008). The lower regression strength observed in

THg – $\delta^{15}N_{std}$ regressions against MeHg – $\delta^{15}N_{std}$ would appear to be the product of a number of factors. These include: (i) selective uptake of MeHg over inorganic Hg within the guts of predators, as a result of cellular partitioning (Mason et al., 1995); (ii) bioaccumulation of inorganic species of Hg through dissolved or sedimentary phases (Borgå et al., 2012; Coelho et al., 2013); (iii) the fraction of THg as MeHg in invertebrates varying widely as a result of feeding strategies and species specific habits (Evers et al., 2008; Coelho et al., 2013); and (iv) the insolubility of inorganic Hg contained within prey, such as mercuric–selenide (HgSe), making it unavailable for dietary absorption (Ralston and Raymond, 2010). All of these conditions would reduce regression strength.

The BSIMM model refined the pathway between sand flathead and benthic prey, suggesting that the primary source of contaminants was benthic, which is consistent with previous studies that have linked Hg biotransfer between sediments and benthic predatory fish species (Chen et al., 2008; Gehrke et al., 2011). The significant variation in point of intercept between regions for MeHg, THg and %MeHg suggests a differential bioavailability in Hg forms at the base of sand flathead food webs. The ME region of the Derwent Estuary has significantly higher sediment THg concentrations than RB (Jones et al., 2003), which is consistent with the higher point of intercept for THg, and suggests an increased uptake of THg at the food web base. In contrast, the higher intercept of MeHg in RB compared to ME and MB suggests that the bioavailability of MeHg in this region may be higher. The concept of RB as a potential methylation hotspot has been suggested before (Jones et al., 2013b), and would seem to infer that significant portions of that THg load in ME are biologically unavailable.

Previous work has found evidence of increased Se concentration with higher trophic levels (Barwick and Maher, 2003; Kehrig et al., 2009), but this was not observed in the present study, as no evidence of biomagnification was present (Campbell et al., 2005). Se as a micronutrient is taken up, stored and distributed as required by organisms (Yang et al., 2008). It is known to reduce Hg toxicity when at molar advantage (Peterson et al., 2009). Se maintained a molar excess over Hg in all species examined in this study, with Se concentrations never reaching those considered to be a toxic threat (Lemly, 1996). Exceptionally large Se molar advantages have been recorded through lower trophic groups (Chen et al., 2001; Belzile et al., 2006), but these molar advantages tend to decrease up the food chain to higher organisms (Yang et al., 2008; Kehrig et al., 2009; Fang et al., 2011). This relationship was also evident in the present study, with the reduced molar advantage with increasing trophic level being the result of the biomagnification of Hg across trophic levels, as Se concentrations either showed no biomagnification or weak reductions with increasing δ^{15}N. The stability of the Se concentrations across the food web may be the result of a metabolic balancing act in which Se molar advantage over Hg is offset against maintaining Se concentrations at a level that do not cause toxicity problems (Lemly, 1996). Overall, the results of this study indicate that there is no evidence for Se biomagnification or any toxic threat to organisms in the Derwent Estuary, and that there is a sufficient concentration of Se in the system to maintain basal metabolic reactions over biomagnified Hg species throughout the sand flathead food web.

Trophic models of food pathways, based on δ^{15}N, have been shown to predict changes in Hg concentrations in fish (Tom et al., 2010). The results of this study show that BSIMM can be applied prior to the running of the trophic models to refine dietary

contributions and further reduce uncertainty in Hg transfer routes. The BSIMM conducted in this work should be considered as a useful additional tool for future assessments of Hg biomagnification when there is a need to define food pathways to top predators and for species eaten by humans. The results clearly suggest that, despite the presence of significant Hg pollution within the Derwent (Jones et al., 2003) and elevated Hg concentrations in biota, the rate at which Hg is biomagnified between trophic levels is not significantly elevated against other global regions with no direct Hg input (Campbell et al., 2005; Chen et al., 2008). This work also re-establishes the theory that provided trophic status is similar throughout a food web, then it is the bioavailability of Hg at the base of the food web that is the key determinant of Hg concentration in benthic estuarine predators.

CHAPTER 5

SPATIAL VARIABILITY IN SELENIUM AND MERCURY INTERACTIONS IN A KEY RECREATIONAL FISH SPECIES; IMPLICATIONS FOR HUMAN HEALTH AND ENVIRONMENTAL MONITORING

Preface:

Current legislation on human consumption of Hg contaminated seafood typically only considers Hg concentration in isolation, despite recent research suggesting the benefit of Se based indices. The objective of this part of the PhD research was to apply Se based indices of Hg toxicity to resident fish with a view to objectively consider the effectiveness of such indices in areas contaminated with Hg. This study was also designed to assess if single values of fish toxicity are a valid method for monitoring programs seeking to determine Hg toxicity and human health risk from consumption of fish located near Hg contaminated systems.

This work has been accepted for publication in a refereed journal and is presented below in identical form. The citation for the original publication is:

Jones, H.J., Butler E. C.V., Macleod, C.K., 2013. Spatial variability in selenium and mercury interactions in a key recreational fish species: implications for human health and environmental monitoring.
Marine Pollution Bulletin 74(1) pages 231-6. doi:1016/j.marpolbul.13.06.052

SPATIAL VARIABILITY IN SELENIUM AND MERCURY INTERACTIONS IN A KEY RECREATIONAL FISH SPECIES; IMPLICATIONS FOR HUMAN HEALTH AND ENVIRONMENTAL MONITORING

5.1 Highlights

- Regional negative selenium health benefit values (Se HBV) were evident in one region, suggesting increased Hg toxicity risk.
- This study represents the first evidence that a single all-encompassing Se HBV for any given species may not be appropriate when there is strong site fidelity.
- Results highlight the importance of Se in assessments of seafood safety and spatial measurement of Se HBV for species with localised distributions.

5.2 Abstract

Selenium's (Se) protective effects against mercury (Hg) toxicity have been demonstrated; however, this is seldom considered in health assessments, where dietary exposure is still evaluated by Hg concentration alone. Se:Hg ratios and selenium health benefit values (Se HBV) offer a more comprehensive seafood safety model. Here we describe total mercury (THg), methylmercury (MeHg) and Se concentrations in fish from a Hg-polluted estuary. Spatial variation in THg, MeHg and Se was evident, though all regions maintained Se:Hg ratio values >1. Se HBV varied between regions and in one region mean negative values (-5.17) were evident. This study provides the first evidence that quoting a single all-encompassing Se HBV is not appropriate when species demonstrate strong site fidelity. It highlights the need for research into Se–Hg relationships in environments with established Hg pollution and reinforces the assertion that Se concentration be considered in assessments of human health risk to Hg exposure.

5.3 Key words

Platycephalus bassensis; Derwent Estuary; seafood safety; contaminant assessment; Se health benefit value (Se HBV).

5.4 Introduction

Human exposure to methylmercury (MeHg) can cause physiological damage (Harada, 1968; Grandjean et al., 1998), and, therefore, food sources which contain or have the potential to contain elevated concentrations of MeHg need to be carefully monitored. Consumption of seafood is the principal route of human exposure to mercury (Hg) (Clarkson, 2002), with many higher order fish containing significantly elevated concentrations of Hg (Kaneko and Ralston, 2007). Consequently, it is important to measure the Hg loadings in fish in any regions where environmental Hg concentrations are known to be elevated.

Selenium (Se) in fish has been shown to provide significant protection against MeHg toxicity (Ganther and Sunde, 2007). However, the specific mechanism for this protection remains unclear (Ralston et al., 2007; Yang et al., 2008), with the current hypothesis being that Hg or MeHg interrupts the Se protein cycle and sequesters Se, resulting in the formation of a less toxic organomercury or inorganic Hg selenide complexes (Yang et al., 2008; Peterson et al., 2009; Ralston and Raymond, 2010). Fish comprise 17 of the top 25 foods sources that are high in Se (Ralston et al., 2007). This should be considered when determining Hg toxicity and evaluating seafood safety (Kaneko and Ralston, 2007). For instance, a study on Hg concentrations in isolated island fishing communities in Japan found that despite Hg concentrations well in excess of

those that caused significant human mortalities at Minamata Bay during the middle part of the 20th century (Harada, 1968) no signs of toxicity were observed in the fishermen and their families (Hamada and Igata (1976) reviewed in Peterson *et al.,* 2009). It has been proposed that, for the Japanese fishing communities, Se in their largely ocean fish diet was offsetting the detrimental effects of MeHg bioaccumulation (Peterson et al., 2009; Ralston and Raymond, 2010).

The risks associated with eating Hg-laden fish have resulted in regulatory advisories concerning fish consumption that are designed to protect human health by minimizing MeHg exposure (Ralston and Raymond, 2010). Where Hg concentrations in fish are low the determination of risk becomes more complex, as there are recognised and significant health benefits associated with consuming fish that are high in beneficial compounds such as omega-3 fatty acids and Se, and these may potentially outweigh the detrimental effects of Hg accumulation (Hibbeln et al., 2007; Ralston, 2008). Current assessment of the potential health risk from fish consumption doesn't typically include assessment of associated Se concentrations (Kaneko and Ralston, 2007). However, a quantitative assessment of the relationship between Se and Hg within fish species may be a more relevant measure of any potential risk than the concentration of Hg or MeHg *per se* (Cabañero et al., 2005; Ralston et al., 2007), and a more accurate picture of the toxicity might be obtained by calculating the relative ratio of Se to total Hg (THg) load (Raymond and Ralston, 2004; Ralston et al., 2007).

When considering the interaction between Hg and Se from a public safety perspective, it is important that species are assessed individually: the fish which would be most favourable for human consumption will be those with the highest Se:Hg ratios

(Cabañero et al., 2005). Kaneko and Ralston (2007) proposed a selenium health benefit value (Se HBV) based on the following equation:

Se HBV = (Se:Hg molar ratio x Se (μmol kg^{-1})) — (Hg:Se molar ratio x THg (μmol kg^{-1})) (1)

(Kaneko and Ralston, 2007)

This value provides a more realistic assessment of the potential toxicity implications than the THg concentration analysis of fish muscle used to date (Peterson et al., 2009), and is now commonly applied in seafood safety research (Fang et al., 2011; Rezayi et al., 2012). Using this approach Kaneko and Ralston (2007), were able to show that shark and marine mammal flesh had negative Se HBV (-11,-80), which is consistent with our current understanding that eating such seafood is detrimental to human health. In contrast, eating ocean fish with high Se HBV (40-250) was clearly beneficial. To date, research in this field has been largely restricted to species with commercial relevance (Kaneko and Ralston, 2007; Fang et al., 2011), and has ignored recreationally fished species. Furthermore, there has been no work considering spatial variation in Se HBV, which is important for establishing whether there is a gradient of effect in species living near known Hg sources.

Current health assessment of Hg loading in Australia is based on THg concentrations (ANZECC 2000). Sand flathead (*Platycephalus bassensis*), a demersal, recreationally fished species from the Derwent Estuary Tasmania, are known to exceed the current ANZECC safe consumption levels (0.5 mg kg^{-1}) at several, but not all, locations within the estuary (Langlois et al., 1987; Verdouw et al., 2011). Sand flathead have been reported to have a high level of site fidelity (Tracey et al., 2011), and as such

might be expected to reflect Hg loadings that correlate with small scale local variability in environmental inputs/ loadings. The aims of this study were twofold: i) To determine the concentrations in THg and Se within a key recreational fishing species, sand flathead (*Platycephalus bassensis*) and, ii) to identify the potential for spatial variability in Se:Hg and Se HBV. This will provide important information for a more accurate evaluation of the potential health risks associated with consumption of this heavily fished species but also a clearer understanding of any potential limitations associated with the current seafood safety measures and important recommendations regarding the broader applicability of Se-adjusted assessments of Hg exposure risk.

5.5 Methods

Site selection

The Derwent Estuary located in southern Tasmania (42^{o}53'44 S, 147^{o}22'08 E Fig. 5.1) is a micro-tidal (1.2 m) estuary, 52 km in length with a maximum depth of 30 m which has historical Hg pollution from a zinc smelter and paper-pulp mill (Langlois et al., 1987; Green and Coughanowr, 2003). The choice of study regions was based on previous research assessing Hg concentration in sand flathead (Verdouw et al., 2011) and hydrological flow (Thomson and Godfrey, 1985). Two estuary regions were selected: the industrialized middle estuary (ME) that has consistently high Hg concentrations in sediments and flathead, and Ralphs Bay (RB), a large and relatively shallow embayment on the lower eastern side of the estuary, with relatively low sediment Hg levels (Bloom and Ayling, 1977; Jones et al., 2003), but high Hg concentrations in fish (Ratkowsky et al., 1975; Langlois et al., 1987; Verdouw et al., 2011). A reference region, Mickey's Bay (MB),

located south of the estuary, was included to provide comparative concentrations from a region that has not been contaminated with Hg or Se. (Verdouw et al., 2011) (Fig. 5.1).

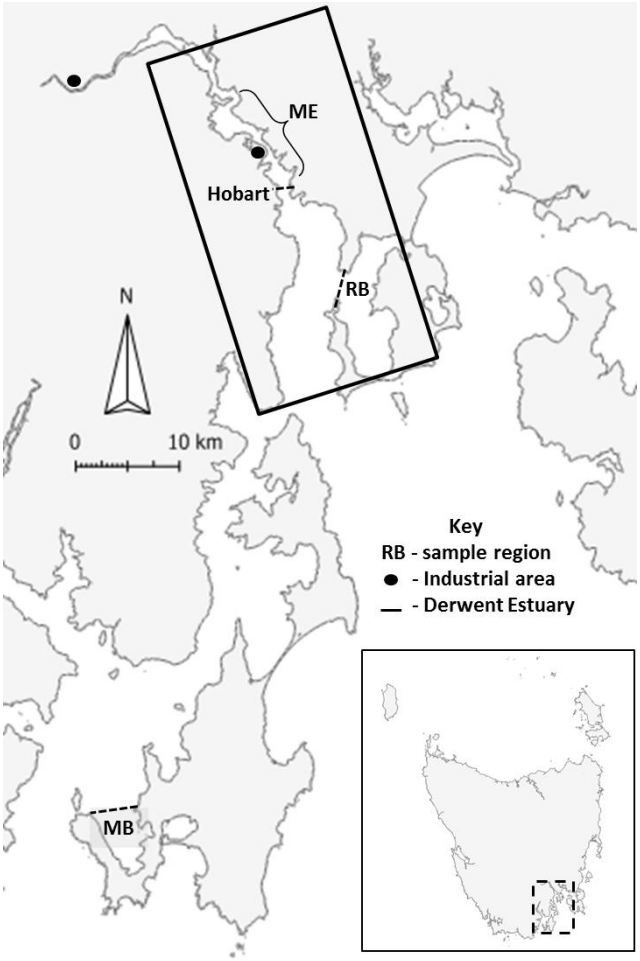

Figure 5.1. Southern Tasmania and the Derwent Estuary, with locations of the two estuary regions Middle Estuary (ME), Ralphs Bay (RB) and the reference region Mickeys Bay (MB) 48km south of the Derwent Estuary.

Flathead collection

Fish (n=60) were sampled in Nov–Dec 2011 by line fishing. Fish were individually sealed in plastic bags stored on ice and frozen (–40^{O}C). Processing followed the procedure described by Verdouw *et. al.* (2010): morphometric measurements of each

fish included fork length (FL) (±1 mm), wet weight (whole ± 0.1 g), and sex; the sagittal otoliths were extracted, cleaned and dried. Interpretation of otolith increment structure to age this species followed the method of Jordan *et. al.* (1999). Otoliths were read by two independent readers, and between and within reader precision was examined by calculating an index of average percent error (Tremblay et al., 1998). Muscle tissue was removed from each fish from an area posterior to the pectoral fin. The tissue sample was weighed (± 0.001 g) (ww), homogenized, placed in individually labelled, acid-cleaned polypropylene tubes and lyophilized to constant mass (± 0.01 g). A subsample of 30 (3 regions, n=10) fish tissue samples was split after homogenisation for MeHg analysis (± 0.01 g).

THg and Se digestions

THg and Se digestions were performed using the method of Kaneko and Ralston (2007), with minor modifications as follows: Flathead samples (20 mg d/w) were digested for 2 h in HNO_3 (trace grade) (3 mL) in lightly capped polypropylene digestion vessels at 120 ^{o}C within a deep cell digestion block, 0.5 mL. H_2O_2 was added to each sample and the vessels digested for a further 1 h. 9 mL aqua regia (3:1 HNO_3:HCl) was then added to the vessels and heated within the block for 1 h, before cooling and dilution to 50 mL total volume with reverse osmosis (RO) water (Elga Purelab Prima). Analysis took place within 48 h of digestion.

MeHg digestion

MeHg extraction was based on the method of Cai (2000). 4 ml RO water was added to 0.2 g (±0.05 g) of homogenized dry tissue within an acid-cleaned glass vial (40

mL) along with 4 mL KOH (6 M) and shaken for 4 h. After shaking 4 mL HCl (6 M), 8 mL KBr/$CuSO_4$ and 15 g of dichloromethane (DCM) was added and the vials returned to the shaker overnight. Samples were centrifuged at 2000 rpm for 20 min, and 10 g of the bottom (DCM) layer transferred to a clean glass vial. Sodium thiosulfate ($Na_2S_2O_3$) 0.01 M (2 mL) was added to the DCM extract, shaken for 30 min and agitated for 30 s with a vortex mixer, after which 1.5 mL of the $Na_2S_2O_3$ was extracted into a second clean glass vial. The $Na_2S_2O_3$ process was then repeated a second time. The final extract 3 mL $Na_2S_2O_3$ was filtered (0.45 μm) before analysis.

Trace metal analysis

THg, MeHg and Se analyses followed the procedures described by Cai (2000).

THg: Analysis was carried out by cold vapour atomic fluorescence spectroscopy (CV-AFS) (10.023 Millenium Merlin, PSA). A 2 % w/v tin(II) chloride ($SnCl_2$) reductant and argon (Ar) carrier gas was used. Calibration was achieved using traceable standards, and independent checks were undertaken using a separate stock solution.

MeHg: Aliquots were analysed by high pressure liquid chromatography-ultra violet-atomic fluorescence spectroscopy (HPLC-UV-AFS) using an oxidant stream of acidified potassium bromide/ potassium bromate (10 % v/v HCl, 10 % v/v 0.1N Br^-/BrO_3^-). A 38 % methanol, 30 % acetonitrile m/v with ammonium pyrrolidine dithiocarbamate (APDC 0.2464 g L^{-1}) was used for the mobile phase with a Supelco C18 column (ODS-2) to provide species separation. An online UV photolysis/heater (150 ^oC) (PSA S570U100) and cooling module (PSA S570C100) coupled to the CV AFS provided oxidation before

analysis. A 2 % w/v $SnCl_2$ reductant and Ar carrier gas were used for cold vapour separation prior to AFS detection (10.023 Millenium Merlin, PSA).

Se: Se detection used online pre-reduction of Se with hydride generated atomic fluorescence analysis (HG-AFS) (Millenium Excalibur, PSA). Se was reduced by mixing with pre-reductant KBr/HCl (5 % KBr, 50 % HCl) and passing through an online UV photolysis/heater (150 ^{o}C) (PSA S570U100) and cooling module (PSA S570C100). The sample was then mixed with the reductant (0.7 % $NaBH_4$, 0.4 % NaOH) to form selenium hydride with an Ar carrier (0.3 L/min) to the detection system.

Quality assurance

Linear calibration (min correlation coefficient 0.999) was acquired using standards diluted in the appropriate concentration range with matrix matched reagents. The accuracy was verified with an independent check for each of the three analytical procedures. Matrix matched procedural blanks were analysed at the beginning and end of sample runs, to test for any procedural contamination, with none observed. Calibration verification (independent check and certified reference material) was run after instrument calibration, after every 20 samples and at the end of each batch of samples. Each sample was run in duplicate, with one sample per batch spiked with 5 ng g^{-1} standard and recovery rates recorded. Certified reference materials DOLT-4 (NRC Canada, Dogfish Liver), BCR 422 (IRMM, Cod muscle), ERM CA011a and 588 (European Reference Material, hard drinking water) were used to verify recovery rates (Table 5.1). All results are reported as dry weight (dw).

Table 5.1. Selenium (Se), total mercury (THg) and methyl mercury (MeHg) concentration (mg kg^{-1} (± s.e.)) and recovery rates of certified reference materials (CRM) materials analysed by HG-AFS (Se), CV-AFS (THg) and HPLC-UV-AFS (MeHg).

Metal	CRM	c.v. (± s.e.)	Mean (± s.e.)	n	% recovery
Se	DOLT 4	8.3 (0.12)	7.69 (0.44)	6	92.67
	CA011a	10.7 (0.7)	10.01 (0.45)	4	93.54
THg	DOLT 4	2.58 (0.22)	2.37 (0.13)	6	91.86
MeHg	DOLT 4	1.33 (0.12)	1.31 (0.09)	6	98.66
	BCR422	0.43 (0.2)	0.51 (0.03)	6	117.44
	ERM588	0.075 (0.004)	0.075 (0.07)	6	99.55

Statistical analysis

Data analysis was performed using R package (version 2.15, 2012). Where necessary data were $\log_{10}$ transformed to meet assumptions of normality and homogeneity of variance. Analysis of variance (ANOVA) was used to determine whether there were differences between regions. When significant differences between mean concentrations were identified, an unplanned post-hoc comparison of means (Tukey HSD) was used to distinguish which groups were different. Analysis of covariance (ANCOVA) was used, where age was considered as a covariate. THg:Se ratios were calculated by conversion of dry weight concentrations into molar mass (concentration in mg kg^{-1}/molar mass (Hg= 200.59, Se= 78.96)) in order to assess molar excess, and potential formation of HgSe within tissues. Selenium health benefit values (Se HBV)

were calculated for individual fish, with Se and THg concentrations in mg kg^{-1} transformed to μmol kg^{-1} using equation 1 above (Kaneko and Ralston, 2007). Positive Se HBV indicate favourable Se benefits over THg uptake; Se HBV <0 are likely to be detrimental to human health (Kaneko and Ralston 2007). Regional differences in Se HBV were assessed against fish age (ANCOVA) in order to determine whether this had any influence on spatial variability. Growth rate variation in sand flathead between Derwent Estuary regions significantly affects FL–Hg correlations (Jones et al., 2013b); therefore, fish age was selected over FL as a more robust measure of fish size.

Results and Discussion

The percentage of MeHg comprising THg of muscle tissue did not vary significantly between regions (*P* =0.36), with mean contribution across regions equal to 98.8 % ± 4.9 (Table 2).This indicates that THg can be considered a proxy for MeHg in sand flathead muscle tissue, and is consistent with findings for other fish species (Bloom, 1992; Cabañero et al., 2005). Ralphs Bay (RB) had significantly higher Se (*P* =<0.0001), and THg concentrations (*P* =<0.0001) (Table 5.2) than the middle estuary (ME), with both markedly exceeding the concentrations at the reference site (MB). The lower Hg concentrations found at the industrial region (ME) compared to RB is consistent with findings of other studies (Chen et al., 2001; Sackett et al., 2010). Reduced concentrations near a known Hg source have been linked to elevated Se tissue concentrations, with the assumption that Se is mitigating a reduction in muscle Hg (Southworth et al., 2000a; Chen et al., 2001; Sackett et al., 2010). This would not appear to be the case in the current study, with Se concentrations in Ralphs Bay fish significantly higher than the other two regions (ME, MB) (Table 5.2).

Table 5.2. Mean (± s.d.) values of total mercury (THg), selenium (Se), methylmercury (MeHg), percentage methylmercury of total mercury (% MeHg), selenium mercury molar ratio (Se:THg), Selenium health benefit value (Se HBV), fish age and fork length (FL) for sand flathead (*Platycephalus bassensis*) measured within the Derwent Estuary regions: Middle Estuary (ME), Ralphs Bay (RB) and the reference region: Mickeys Bay (MB). $_{a,b,c}$ Letters denote significant differences between regions (P <0.05).

		Region			
Variable	**n**	**ME**	**RB**	**MB**	***f***
THg (mg kg^{-1})	20	1.29_b (0.70)	3.61_c (1.65)	0.72_a (0.28)	$40.84_{2.56}$
Se (mg kg^{-1})	20	0.89_a (0.18)	1.28_b (0.30)	0.91_a (0.17)	$19.38_{2,56}$
MeHg (mg kg^{-1})	10	1.22_b (0.67)	4.03_c (1.69)	0.77_a (0.33)	$24.01_{2,24}$
% MeHg	10	109.31 (10.59)	97.06 (8.09)	91.42 (6.88)	$1.08_{2,24}$
Se:Hg	20	2.30_b (1.68)	1.20_c (1.24)	3.62_a (0.81)	$22.84_{2.56}$
Se HBV	20	22.09_b (24.59)	-5.17_c (28.21)	42.25_a (21.65)	$16.26_{2,56}$
Age (years)	20	3.95 (1.69)	4.56 (2.01)	5.26 (2.09)	$2.04_{2,55}$
FL (mm)	20	283.42_a (44.47)	330.83_c (43.77)	308.95_b (36.74)	$6.16_{2,56}$

Raised Se concentration in fish from Ralphs Bay may be the result of increased Se bioaccumulation or tissue storage within the muscles, such that Se:Hg ratios >1. In the present study, Se:Hg was <1 in all regions, but there was significant variation between all three regions (P =<0.0001) with MB>ME>RB (Table 5.2). Se:Hg ratios >1 provide antagonistic protection of Se against Hg toxicity and allows selenoenzyme processes to continue unaltered (Peterson et al., 2009). If Se muscle concentrations in fish from

Ralphs Bay were similar to the other regions (mean of ME and MB = 0.90), then the mean Se:Hg ratio at Ralphs Bay would be less than 1; severely degrading Se physiological function and increasing MeHg toxicity. As a required micronutrient, Se varies in concentration according to tissue requirements and Se stored in the liver allows replenishment of Se to tissues with temporary Se depletion (Falnoga and Tušek-Žnidarič, 2007).

Variation in age can affect Se and THg concentrations (Sackett et al., 2010; Verdouw et al., 2011) but in this case there was no significant difference in age between regions (P =0.14) (Table 5.2). Se concentration showed significant interaction with age in fish from Ralphs Bay (ANCOVA $f_{5,52}$ =8.42, P =0.0006) (RB:Age $f_{5,52}$ =3.66, P =0.0006, R^2=0.53) but no relationship with age in the other regions (ME:Age $f_{5,52}$ =-0.14, P =0.89, MB:Age $f_{5,52}$=-0.11, P =0.91). The positive association between tissue Se concentration and fish age within Ralphs Bay (R^2 =0.53) suggests both a biological requirement for this element in fish muscle tissue and long term bioaccumulation. High ambient MeHg concentrations in the environment may trigger an enhanced protective accumulation of Se to bring Se:THg >1. This would come at an energy cost to the fish and may also be depend on the reservoir of bioavailable Se present. Se HBV studies may also be considered a method for assessing the health of the fish themselves, with those of higher values less metabolically stressed by sub lethal Hg toxicity. Se typically bioaccumulates at much lower rates than Hg (Besser et al., 1993; Wang, 2002; Zhang and Wang, 2007); however, due to its regulation and storage within the liver of fish (described above) this relationship is not always evident (Arribére et al., 2008). The low Se:Hg ratio in fish from Ralphs Bay coupled with the lack of variation with age (ANCOVA

f=2.42$_{2,52}$ P=0.13) and long tissue half-life for Hg (Lindberg et al., 2007) may indicate that this ratio is not a temporary adaption but a biological need in this region.

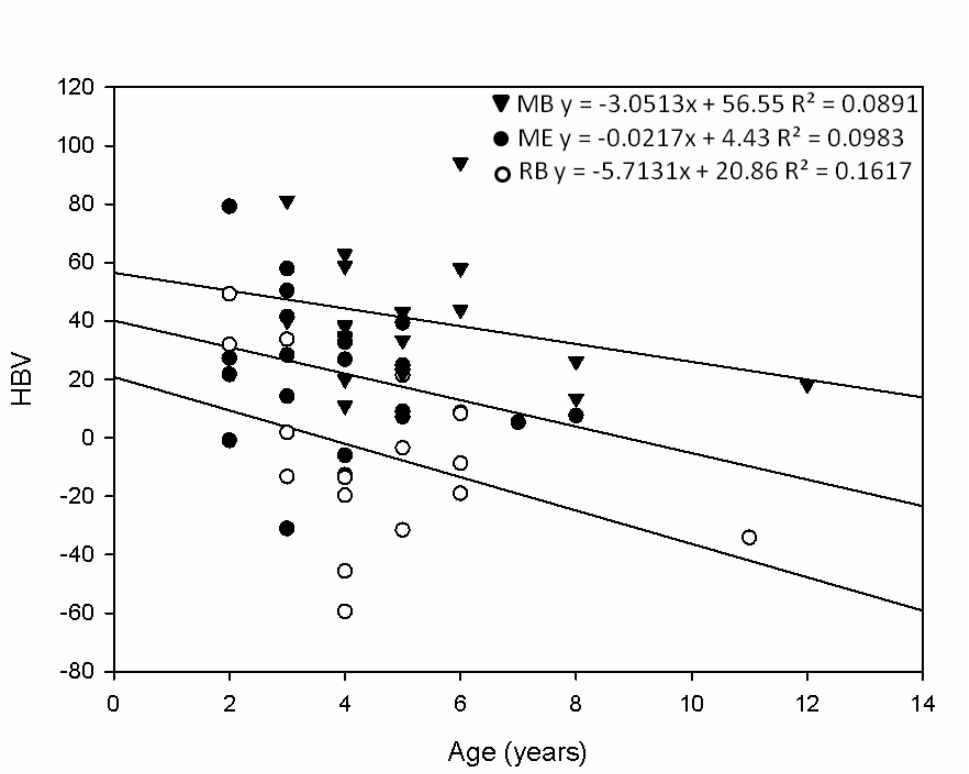

Figure 5.2. Relationship between age and selenium health benefit value (HBV) for sand flathead (*Platycephalus bassensis*) from three study regions in the Derwent Estuary (Ralphs Bay (RB) and Middle estuary (ME)) regions and reference (Mickeys Bay (MB)). Slope similarity: (ANCOVA $f_{2,56}$ =0.24, P=0.79). Intercepts differences :(ANCOVA $f_{5,54}$=14.89, P=0.0003).

Se HBV of flathead exhibited a wide range across the regions (x̄=19.84 ±4.01, max = 94.16, min = -59.43), but showed a similar (ANCOVA $f_{2,56}$ =0.24, P =0.79) negative relationship with fish age (ANCOVA $f_{5,54}$ =-2.64, P =0.01) (Fig.5.2). The three regions varied significantly from each other in respect to Se HBV (ANCOVA $f_{5,54}$=14.89, P =0.0003) (Fig. 2), MB>ME>RB (MB $f_{3,54}$=6.28 P =<0.0001: ME $f_{2,54}$=3.222, P =0.004: RB $f_{2,54}$=0.054, P =<0.0001) (Fig.5.3). The strong positive Se HBV found at the reference region (MB) suggests that there would be clear health benefits of consuming fish from

this region (Fig. 5.3). While the negative Se HBV for flathead (Fig. 5.3) from Ralphs Bay suggests likely detrimental human health effects should discourage consumption from this region. Although Se HBV have been applied to a variety of marine fish species as an approach to evaluate seafood safety (Kaneko and Ralston, 2007; Fang et al., 2011; Rezayi et al., 2012), there have been no reports of spatial variability in Se HBV within species. The results from this study (Fig. 5.2, 5.3), suggests that spatial variability should be considered where Se HBV is used to assess human exposure risk to MeHg. Variation may be significant in systems, like estuaries, where marked concentration gradients of THg are present and/or where there is a strong likelihood of small scale spatial patchiness.

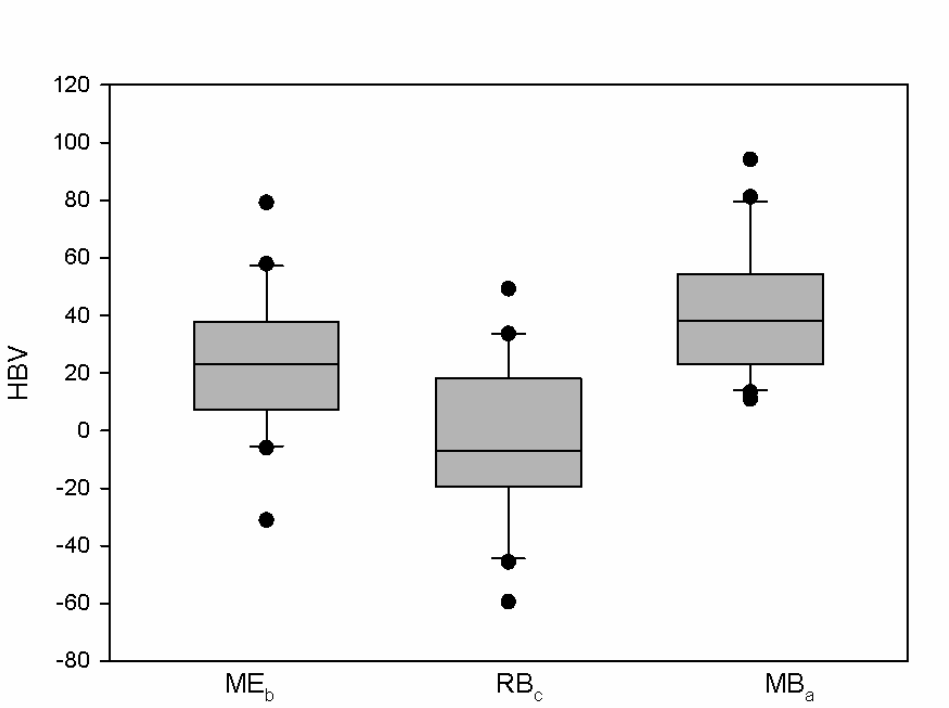

Figure 5.3. Selenium health benefit value (HBV) of sand flathead (*Platycephalus bassensis*) muscle tissue from three locations in southern Tasmania. Subscript attached to each region denotes significance of differences ANCOVA (P <0.05).

The flathead sampled in Ralphs Bay had a negative mean Se HBV (Table 5.2). The only other marine fish species so far shown to have a negative Se HBV worldwide is the top order predator, Mako shark (*Isurus oxyrinchus*), and significant health warnings are associated with consumption of these sharks (Kaneko and Ralston 2007). Unlike Mako shark, Ralphs Bay flathead had a mean positive Se:Hg ratio which may suggest that Se is in fact having an antagonistic effect on Hg toxicity; a response contrary to the low negative Se HBV. It may be that there are other marine fish species with negative Se HBV but that current research does not reflect this as it has, to date, concentrated on wide ranging pelagic species or commercial stocks (Kaneko and Ralston, 2007; Fang et

al., 2011) rather than demersal species with the potential for spatially restricted Hg exposures.

This study represents the first evidence that quoting one single Se HBV for a species may not be appropriate for species with strong site fidelities. If Se HBVs are to be considered a valid method of assessing Hg toxicity this study highlights the need for further research with respect to species fished in environments with established Hg contamination. These results reinforce the assertion that comparison of Se HBV and Se:Hg ratios provide an appropriate method for assessment of seafood safety (Kaneko and Ralston, 2007; Peterson et al., 2009) clearly establishing the realistic human health risk associated with tainted seafood and that this approach is a significant improvement on assessments based on THg values alone. The complex interaction between Se and Hg within fish can only be fully understood with in-vivo analysis of Se and Hg species (Cabañero et al., 2005; Yang et al., 2008; Dang and Wang, 2011), and until there is a reliable method for determination of the various molecular forms of Se and Hg that comprise the formation of HgSe compounds, then evaluation of Se:Hg ratios and Se HBV represent the most appropriate method for assessment of seafood safety (Kaneko and Ralston, 2007; Peterson et al., 2009).

CHAPTER 6

GENERAL DISCUSSION AND CONCLUSIONS

6.1 Key research findings

The research contained in this thesis offers a substantial body of new data and applied analytical techniques that have application to the management of metal contaminated estuaries globally and offers new insights into mercury contamination in the wider aquatic environment. Specifically this work provides a comprehensive method for the determination of spatial and temporal trends in Hg contamination in estuarine fish species. It provides clear evidence of the role of Se in Hg dynamics within an estuarine system, and highlights its importance in assessing Hg toxicity to humans resulting from the consumption of fish. Finally, it presents an improved method for the assessment of Hg biomagnification through marine foodwebs.

This thesis found that:

- Significant reduction of Hg discharges into an estuarine system may not result in decreased Hg concentrations in fish even after a significant time lapse (in this case, 37 years).
- Detection of temporal and spatial changes in fish Hg concentration can only be fully assessed when understood in the context of fish biometrics e.g. fish age, fork length and growth rate. Failure to account for these can result in misinterpretation of spatial and temporal trends of Hg contamination.

- In a highly Hg contaminated estuarine system, only MeHg was found in the muscle tissues of predatory fish species, suggesting preferential accumulation of this Hg form.
- A key driver of MeHg concentrations in resident fish is the bioavailable fraction of Hg in sediments, rather than the total concentration of Hg within the sediments.
- Se associates with Hg in the sediments and this relationship is important in reducing Hg bioavailability. Consequently, absence of Se may indicate higher Hg bioavailability.
- The role of Se in balancing the impacts of Hg contamination should be a key consideration in future assessments of Hg methylation and bioaccumulation in estuarine sediments.
- High uptake of MeHg in fish can also be associated with increased Se uptake, potentially reducing the toxicological effect of Hg.
- Se health benefit values (Se HBV) and Se:Hg molar ratios provide an improved measure of Hg toxicity over Hg concentrations alone, but must be considered at a spatial scale relevant to the contamination source to provide a truly effective measure of potential risk for consumption.
- Bayesian stable isotope models are a better means than standard trophic models for evaluation of Hg biomagnification, because they can eliminate the "noise" associated with non-significant food sources.
- Combining stomach-contents analysis and Bayesian stable-isotope modelling highlights the importance of benthic pathways of Hg biomagnification in estuarine fish.

- No evidence of Se biomagnification in estuarine food webs or fish was evident, even where Se contamination levels were significant. Se concentrations in all species examined consistently remained below those which might be considered toxic.

6.2 Synthesis of research findings

Hg contamination is a major environmental problem in the Derwent Estuary, and this study has shown that its bioavailability, trophic magnification and association with Se are complex functions of ecosystem processes and biological response. This study provides methods by which these functions can be measured and offers insight into Hg residence time, movement, and toxicity in estuarine systems, which in turn can benefit estuarine monitoring and remediation schemes.

In 2008, leading researchers in North America proposed an integrated monitoring program for estuarine systems contaminated by Hg (Evers et al., 2008). The purpose of the proposed model was to provide a comprehensive basis to detect spatio-temporal Hg trends for both human and ecological health. The authors described the need to include five categories of system indicators: abiotic (sediment/water), invertebrates, fish, marine mammals and birds, along with ancillary data that increases the interpretive power of each category; specifically Se and δ^{15}N–δ^{13}C stable isotopes. This thesis presents a synthesis of a large body of data and provides a foundation on which an integrated estuarine Hg model for the Derwent Estuary can be based. Furthermore, the improved understanding presented in each of the individual research chapters has both specific relevance to the Derwent Estuary system and broader applicability to other similarly contaminated estuaries and coastal marine systems.

Benthic fish as bioindicators

Sand flathead, the principal species studied in this work, has long been viewed as an appropriate species to test spatio-temporal change in Hg concentrations (Dix et al., 1975), indices that are consistent with recent estuarine monitoring approaches (Evers et al., 2008). Resident fish, like sand flathead, which occupy small home ranges (Tracey et al., 2011) and maintain a consistent trophic level (Chapter 4), are considered to be a good choice for bioindicators, but only when individual biometrics are accounted for (Chapter 2) (Tremblay et al., 1998; Chen et al., 2008). Monitoring that attempts to identify temporal change in Hg concentration in fish using Hg concentration alone (Langlois et al., 1987) will often fail to detect change due to the confounding effects of age, fish length and growth rates on fish Hg concentration (Tremblay et al., 1996; Goulet et al., 2008). By examining the Hg concentration of sand flathead in relation to fish length and growth rate (Chapter 2), this study was able to dismiss previous findings of temporal and spatial variation in this fish as a consequence of sampling artefacts (Langlois et al., 1987; Verdouw et al., 2011). The lack of any significant temporal change in the Hg concentration of flathead in this long-term study might be viewed as a reason for scaling back future annual monitoring work. However, the precautionary principal would advise against this, since by reducing annual monitoring the ability to detect future change may be severely compromised. The growth-rate models of Hg uptake that assisted in the explanation of spatial trends in this work were calculated from just four years data, which limits model power compared to the polynomial fish length model that used a much longer continuous dataset (21 years). Continuation of annual surveys, with the specific inclusion of fish age as a factor, would allow future data to be added to the current model increasing the interpretive capacity of the model and its ability to

detect change. In establishing sand flathead's Hg–length relationship, regional growth rate and trophic position, this research provides the benchmark against which future temporal and spatial change can be assessed. This work also provides a detailed and more accurate method of determining Hg bioaccumulation in benthic fish, which may be applied to other data sets and where fish species are routinely monitored as bioindicators.

Bioavailability of Hg in Estuaries

Continued data collection of the Hg concentration in resident fish alone is not sufficient to monitor estuarine system health, because temporal or spatial shifts in Hg bioavailability may not be observable in fish populations for a long time (Munthe et al., 2007; Evers et al., 2008). Changes in Hg system dynamics are best detected at the source level (abiotic-biotic interface), where the greatest level of bioconcentration exists and ecosystem response is rapid (Ullrich et al., 2001; Davis et al., 2012). It is very rare to have data at this source level in estuaries generally, and the Derwent is no exception; without this information there is limited evidence on which to base management actions.

A key area that is largely unexplored in the Derwent Estuary is the active portion of sediments involved in methylation – demethylation pathways. The sediment analysis in this study (Chapter 3) found evidence of MeHg within the top 4 cm of sediment, however, whether this is the site of production, storage or a transitory region is not known. It is possible that MeHg production could occur at depths below that sampled in this study, with diffusion of MeHg up through the sediments within the pore water (Mason and Lawrence, 1999; Sunderland et al., 2004). The importance of understanding

the actual depth at which methylation is taking place, and, therefore, what portion of the Hg legacy in the sediments has the potential to be active should not be understated. Derwent sediments are severely metal contaminated to a depth of ≈40 cm (Townsend and Seen, 2012), providing a considerable vertical surface area over which net methylation can occur, given the right conditions and Hg form (Sunderland et al., 2004). Currently the Hg complexes present in the Derwent Estuary sediments and water are unknown, and we know little about the transport and geochemical changes of these complexes through the estuary. Recent evidence has shown that cinnabar (HgS), widely considered to be one of the most unavailable forms of Hg (Ullrich et al., 2001), can degrade with time and become bioavailable (Davis et al., 2012). The low methylation rates observed in the Derwent Estuary suggest that much of the Hg in the ecosystem is biologically unavailable and may be in the form of HgS, other sulfide-associated forms and/or mercuric-selenide-(HgSe)-based complexes. It was postulated in Chapter 3 that the presence of Se in the environment could be viewed as an indicator of low Hg bioavailability. However, despite evidence of reduced Hg bioavailability in Se-rich sediments, there are still no specific data quantifying the formation of HgSe in sediments (Yang et al., 2011), and the degree to which highly insoluble forms of Hg, such as HgSe and HgS, are available for Hg methylation is in need of further research.

A key driver of Hg bioaccumulation potential within food webs is the presence of methylating and demethylating bacterial communities. MeHg is produced from inorganic forms of Hg by microorganisms, particularly sulfate-reducing bacteria (SRB) (Benoit et al., 1999). Consequently, inorganic Hg speciation will have an important influence on both the reduction process and bioavailability to methylating organisms (Ullrich et al., 2001). Many anaerobic micro-organisms can produce MeHg, but SRB are

the primary functional group. Hg methylation is highest where benthic methylation exceeds bacterial demethylation (Mason and Lawrence, 1999). Benthic MeHg production is based on three assumptions: (1) that the activity of Hg^{2+}-methylating bacteria is the primary driver of benthic MeHg production; (2) that inorganic Hg^{2+} is available to those bacteria; and (3) that the activity rates of MeHg-demethylating bacteria are less than those of Hg-methylating bacteria. Spatial and temporal variation in MeHg production rates are best understood by the examination of these assumptions, which are then used to calculate MeHg production potential rates (Davis et al., 2012). However, there are no empirical data available on these processes, the conditions that drive this cycle in the Derwent Estuary, or the bacterial groups present; this is an important area for future research.

The bioavailability of Hg forms in the sediments may be reduced by burial where sedimentation rates are high. In such areas the sediments will be high in organic content, and this may in turn reduce net methylation rates (Driscoll et al., 2012; Taylor et al., 2012). The present study found no correlation between MeHg and nitrogen (N) levels within the sediments (Chapter 3). This was unexpected given the well-established negative relationship between N, total organic carbon (TOC) and MeHg production found in other works (Mason and Lawrence, 1999; Lambertsson and Nilsson, 2006; Driscoll et al., 2012).

Reduction of nitrogen inputs to address eutrophication is a key management objective in many urban and industrialised estuaries, including the Derwent. But such activities could exacerbate Hg contamination problems by altering the sediment nutrient status and reducing sedimentation rates: ‘legacy’ Hg may also become more bioavailable

(Driscoll et al., 2012). In San Francisco Bay, low sedimentation rates, combined with erosional events, have increased the influence of legacy deposits of Hg in buried sediment (Davis et al., 2012). The middle estuary region of the Derwent Estuary is a net deposition zone and has high sedimentation rates (Margvelashvili et al., 2005). However, high-water-flow events (e.g. stormwater) have been linked to downstream transport of sediment-bound metals from this region to the lower estuary (Margvelashvili et al., 2005). Consequently, any changes in the sedimentation rate could alter Hg transport loads to the lower estuary and affect the Hg-bioavailable fraction in the middle estuary. Some authors argue that the theory linking methylation and eutrophication is too limited, and that reductions in organic loads do not necessarily lead to a spike in methylation (Schartup et al., 2012). However, without a detailed knowledge of how Hg is bound in the sediments throughout the estuary and the depths at which methylation is taking place, it is not possible to determine how changes in organic load may affect methylation rates. In light of this, current and future management activities targeted at improving water quality by reducing nutrient inputs should be mindful of potential impacts on Hg methylation.

Nutrient status also plays a role in how Hg is distributed within the water column, as Hg can be associated with suspended particulates (Coelho et al., 2012). Decline in suspended sediments and increased water clarity could lead to a decrease in the export of Hg from the middle estuary to the lower estuary reaches. However, the reduction of nutrients in the water column may also result in a decrease in phytoplankton productivity. Decreases in productivity have been reported to lead to increased bioaccumulation of Hg in plankton as a result of bioconcentration of Hg over a smaller

biomass (Driscoll et al., 2012). There are no regular measurements of water column MeHg concentrations in the Derwent Estuary, nor do we have any understanding of how storm events, season or tide affect MeHg production and transportation. Recent work in another impacted estuary, Ria de Aveiro, Portugal, highlighted storm events and tidal influence on MeHg transport (Coelho et al., 2012), which may have significant consequences in a changing climate with increased storm frequency. If MeHg and THg concentrations in the water column and suspended sediments can be measured in the Derwent there are detailed models of the hydrology in place for this system (Herzfeld et al., 2005), which, along with sediment transport models (Margvelashvili et al., 2005), could provide significant insight into Hg transport on a regional basis.

Selenium and mercury in estuarine food fish

It has been suggested that monitoring programs for Hg should include fish that are natural residents of the system, as well as fish that are commonly taken for human consumption; in the Derwent sand flathead satisfy both these criteria (Lyle, 2005; Evers et al., 2008). Current health advisories in Australia and worldwide suggest limiting human consumption of fish with Hg concentrations above 0.5 mg kg^{-1} (0.3 mg kg^{-1} in the US) (WHO, 1990; ANZECC, 2000). In the Derwent Estuary, this means limited consumption of sand flathead and avoidance of some other resident species (e.g. black bream *Acanthopagrus butcheri,* trout *Salmo trutta*) (Derwent Estuary Program, 2011). However, there is growing evidence to suggest that guidelines based on Hg concentration alone do not accurately portray the actual toxicity of Hg in the fish (Ralston, 2008; Peterson et al., 2009). Alternate Hg assessments that include Se should be considered, as the health benefit of fish high in compounds such as omega-3 fatty

acids and Se potentially outweigh the detrimental Hg accumulation (Hibbeln et al., 2007). The Se health benefit value (Se HBV) method and Se:Hg ratios for assessing seafood safety employed in this study (Chapter 5) support the assertion that Se-based assessments of Hg toxicity may provide an appropriate method for assessing seafood safety (Kaneko and Ralston, 2007; Peterson et al., 2009). The study also found spatial variation present in Se HBV, with respect to a species fished in an environment with Hg contamination. This suggests that previously published all–encompassing Se HBV for individual species (Ralston, 2008) may not always be valid, and that care should be taken in interpreting Se HBVs when local (spatially constrained) gradients of Hg contamination exist.

Although there is growing evidence for Se based Hg toxicity testing, and animal studies have shown that Se compounds reduce inorganic Hg toxicity, there is almost no evidence that organic forms of Se found in the human diet provide any protection from MeHg toxicity (Chen and Wilcox, 2008). Consequently, this area of Hg research requires a great deal more investigation before recommending Se indices of Hg toxicity as a reliable approach to assess human health risk (Burger and Gochfeld, 2012). That said, future monitoring of food fish from estuaries and the wider marine environment should consider the inclusion of Se analysis as a valuable reference in any “weight of evidence” approach for environmental impact assessment and an important metric in any Hg toxicity database. These data can provide a significant benchmark against which future performance and management actions can be assessed.

6.3 Future directions

Although there have been extensive efforts to reduce Hg input into the Derwent Estuary, past inputs have resulted in Hg loadings to sediments throughout the system, with clearly elevated concentrations in the sediments of the middle zone (Jones et al., 2003). This historic reservoir of Hg in the sediments is the most significant Hg source in the ecosystem, and it is this pool that, when methylated, is the predominant source of MeHg to local food webs (Chapter 3 and 4). There would appear to be sufficient Hg stored within the system to allow methylation to take place for many decades or centuries to come. Consequently, the decrease in external Hg inputs as a result of industry process improvements over the last 20–30 years has not resulted in any major reduction in MeHg in the local fish species (Chapter 2).

To manage these internal sources of MeHg we need to understand the processes that affect Hg bioavailability and toxicity. This thesis has addressed a number of these processes, although significant uncertainties still exist in key areas.

In this final section I discuss possible future research directions that will assist Derwent Estuary management specifically, but which have direct application to many coastal marine systems subject to anthropogenic Hg contamination.

Mercury stable isotopes

There is still a lack of knowledge regarding the forms of Hg, its source and methylating potential within the Derwent Estuary system, and this limits the ability to determine to what extent, and over what time scale, Hg loadings might affect concentrations in biota. Understanding the spatial and temporal patterns of Hg speciation and the factors that influence Hg uptake will improve our ability to predict

impacts associated with both natural and man-made changes in environmental conditions (i.e. changes in river flows, nutrient status (productivity), organic loads, temperature and oxygen). This understanding would in turn support more effective management strategies for this system and enable transference of them to other estuaries, nationally and globally.

Hg stable isotopes are one approach that could provide the insights into trophic bioaccumulation patterns that might resolve this issue. Research into the use of δ^{202}Hg as a tracer of Hg source has proven effective in a number of studies (Sonke and Blum, 2013), and its traceability through food webs offers a direct measure of trophic connectivity (Gehrke et al., 2011a).

Assessment of Hg source, movement and bioavailability using Hg stable isotopes would provide information that may allow adaptation of current sediment transport models to models of internal Hg transport under different flow scenarios (see Margvelashvili et al., (2005)). Hg stable isotopes have been used to track newly deposited Hg through soil systems (Branfireun et al., 2005). The stability of these isotopes in the soils has allowed researchers to assign origins (i.e. industrial contribution) to Hg sources within a mixture, based on relative isotopic abundances (Estrade et al., 2011). Examples are increasingly appearing in the literature where isotopic signatures have been used to define particular Hg sources. This approach has been applied in San Francisco Bay, where it has provided evidence of regional contributions from differing Hg sources (Gehrke et al., 2011a). In the Murray Brook mine watershed, researchers were able to show specific changes in isotope structure with distance from point of origin, and the extent of natural inputs (Foucher et al., 2013).

Researchers investigating Hg contamination in Canada used long core samples and a simple mixing model to estimate temporal and spatial change in Hg inputs from a zinc smelter (Ma et al., 2013).

As the historical industrial Hg inputs in the Derwent Estuary are well known this would be an ideal site to tease out the relative contributions to the biota using this cutting-edge approach. Application of these techniques could allow researchers to identify whether specific sources of Hg (atmospheric, subterranean stores) are more important in Hg methylation over others, and if certain areas might be more prone to net methylation. Application of stable Hg isotope work at an estuary-wide scale might unravel potential differences in MeHg bioaccumulation pathways within the estuary. For example, this study has shown that THg and MeHg are associated within the sediments with different compounds in each of the regions studied, suggesting changing Hg forms and contributions (Chapter 3). Analysis of isotopic Hg ratios (δ^{202}Hg) in water, suspended sediments and surface sediments, as well as within components of the food web reported here, could further explain the potential drivers in each of the estuary's regions. This could help optimise monitoring, provide a basis for management strategies that could reduce bioavailability, and allow spatially relevant management plans to be developed.

This study highlighted the principal route of Hg exposure to sand flathead by using stable isotopes as proxies for contaminant acquisition and energy flow (Chapter 4). The next big step in improving our understanding of Hg movement through the food webs of the Derwent is to provide direct evidence of MeHg exposure routes to predatory fish from environmental sources. Again this is possible with the use of Hg stable isotopes. A

recent study in San Francisco Bay used δ^{202}Hg isotopes to link the MeHg accumulated in fish to specific gold mines within the estuary basin (Gehrke et al., 2011b). Linking MeHg within fish to the roasted ore of the mines via the estuary's sediments, the authors were able to eliminate atmospheric Hg as the potential cause of bioaccumulation. Application of a similar Hg isotope study to the Derwent system could complement the work carried out in the studies underpinning this thesis, and further establish sediments as the main source of MeHg to sand flathead.

Sub-lethal effects: chronic/acute toxicity

Monitoring of Hg and Se exposure in this study was measured in the tissue samples of fish; providing evidence of contamination, toxicity and bioaccumulation (Chapter 3–5), but these data do not provide information on how sub-lethal exposure may affect fish health. Globally, there is little work that considers the sub-lethal toxicity of MeHg in estuarine fish, or how the antagonistic response of Se to Hg may reduce its toxicity by forming HgSe complexes. Effects on biochemical processes, and the potential for cell and tissue damage and reduced reproduction in fish, have been documented at MeHg concentrations of about 0.3–0.7 ppm in the whole body and about 0.5–1.2 ppm in axial muscle (Davis et al., 2012). These levels are consistent with Hg concentrations observed in Derwent sand flathead (Chapter 2, 5) and other resident species (Verdouw et al., 2011). It, therefore, seems plausible that there would be fish health impacts in the Derwent fish species.

One way to establish sub-lethal effects in fish and other biota is to examine biomarkers (Rodrigues et al., 2010), which should include enzyme, physiological and genetic levels of response. The initial reaction of organisms to toxic compounds will be

at the molecular and cellular levels, with target organs and tissues showing an impact (Vieira et al., 2009; Adams et al., 2010). In the case of Hg and Se, muscle, liver and gonads are generally the first to show any changes (Adams et al., 2010). Biomarkers are most effective when they can clearly link a low-level contaminant effect to a higher-level molecular response (Vieira et al., 2009), and provide evidence that the organism's exposure to the contaminant is exceeding its ability to detoxify and repair tissues. Fish exposed to Hg and Se show several sub-lethal effects, including decreased enzymatic activities, restriction on gonad development and genotoxic effects. Although there are a number of studies that have considered the effect of Hg and Se exposure independently using biomarkers, the effect of co-pollution of these two metals and whether there are any synergistic effects on organism health has never been assessed. The co-occurrence of both pollutants within the Derwent Estuary suggests that this estuary would be a suitable test area for such studies and would provide valuable information to management regarding ecosystem health. Biomarkers would improve our understanding of exposure levels that cause sub lethal effects in species and provide necessary information to evaluate subtle long term consequences to organism health.

Remediation options

Managing internal production of MeHg within estuaries is an enormous challenge, both from an economic perspective and from a scientific view point. The highly dispersed nature and scale of Hg contamination severely limits options for remediation. In other systems, particularly small reservoirs or lakes, separating the contaminated sediment from the overlying water, by sediment removal (dredging) and/or isolation (capping), is the most common option. Capping the sediments with clean material is

feasible in regions without strong mixing forces, but in regions of the Derwent Estuary, where the physical mixing zones are large (lower estuary) or where areas are subject to scouring (shallow middle estuary margins), it would be very difficult to maintain a cap. A possible alternative is identifying potential methylation 'hotspots' and focusing remediation efforts in these regions (Wiener and Shields, 2000). Targeted capping is potentially viable in areas with very high Hg loads, but has been shown to be uneconomical at an estuary wide level (Francesconi et al., 1997).

Targeted dredging is a more expensive approach, which could be employed at severely contaminated sites, such as the middle estuary industrial region of the Derwent Estuary. However, this type of dredging is likely to be of little benefit in the lower reaches of the Derwent where large volumes of sediment would have to be removed to generate any significant contaminant recovery. There are also specific concerns regarding the use of dredges, including but not limited to: (i) resuspension and transport of sediments, particularly fine organic particles which may have higher Hg loadings; (ii) exposure of 'legacy' Hg in deeper sediment layers; (iii) disposal of the removed material; (iv) impact of dredging on other metals in the sediments; (v) habitat degradation; and (vi) impact on food webs.

Chemical controls within sediments may also be a useful option in the future, but these approaches are largely developmental at present. Hydrometallurgical treatments to adsorb, oxidize or complex Hg may offer some hope currently at small scales (Wang et al., 2004). The use of activated granulated carbon to sequester Hg by encouraging diffusion into bacteria and iron amendment to perturb Hg speciation (Davis et al., 2012) are two highly novel approaches that are currently being investigated. Laboratory and

pilot-scale testing of these binding approaches have shown some promise, but the feasibility and ecological impacts of large-scale use in the natural environment have not yet been adequately assessed. Such approaches may be impractical due to costs and possible habitat damage caused if they require sediment tilling (Davis et al., 2012). One significant problem with these approaches will always be that the Hg still exists within the system, and future non-bioavailability is not guaranteed.

Phytoremediation is another novel strategy that has recently been proposed for removal of Hg in terrestrial and semi-terrestrial habitats (Moreno et al., 2004). Metals are extracted from the sediments by plants and stored either in the roots or above ground tissues, reducing sediment Hg concentrations (Moreno et al., 2004; Coelho et al., 2009). Seagrasses, for example, can sequester a large proportion of Hg into above ground tissues, and, therefore, harvesting of these plants could reduce Hg concentrations within local sediments. Distribution of Hg within the plant is of great importance with the best phytostabilizers of metal concentrations being those that maintain high levels in root systems (Weis and Weis, 2004). However, this has inherent problems in that MeHg uptake can be limited by sulphate within sediments, and mass balance calculations suggest some Hg may be volatilized back into the system (Moreno et al., 2004; Coelho et al., 2009). In addition, herbivory and detrital consumption of plant matter can also lead to export into estuarine food webs, and rapid replacement of leaves can be another potential source of export of Hg to the wider estuarine system (Coelho et al., 2009).

6.4 Conclusions

This study has shown that Hg contamination is a significant issue in the Derwent Estuary, and that bioavailability, trophic magnification and association with Se are complex functions of ecosystem processes and biological response. The large pool of Hg that is already present within the Derwent ecosystem, principally in the sediments, remains the most significant Hg source, and it is largely this pool that is converted to MeHg and accumulated in the local food webs. Unfortunately, the decrease in external Hg inputs from industrial sources over the last 30–40 years through improved industrial practices has not reduced MeHg in the local fish species. This study suggests that the current pool of Hg principally in the sediments is sufficient to allow methylation to take place for many decades or centuries to come. Effective management of this internal source of MeHg to ensure rates of methylation do not increase will require a significant knowledge base of the system processes and interactions. This study has provided baseline data for a number of these processes and interactions and as a result has improved our ability to make management decisions in relation to how fish can best be used as bioindicators and our ability to track Hg through foodwebs. The data also provide a much better understanding of the spatial bioavailability of Hg in this system and what that means for trophic transport. However, significant uncertainties still exist in number of key areas, including source of Hg methylation, transport mechanisms, and sub lethal effects to local biota. Whilst this study provided significant insights into Hg longevity, movement and toxicity in estuarine systems which will benefit estuarine monitoring and remediation schemes worldwide, further work is urgently required before we can truly characterise the risks associated with Hg in this dynamic ecosystem.

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