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## Long term trends of Hg uptake in resident fish from a polluted estuary

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### 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 ( $0.5 \text{ mg kg}^{-1}$ ). 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.

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### 1. 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 and 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 that 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., 2010). 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., 2010). Assessment of the biological effects of heavy metals in the 1970s (Ratkowsky et al., 1975) showed that Hg concentrations in many species were well above recommended guidelines for human consumption ( $0.5 \text{ mg kg}^{-1}$  (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

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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 ( $<0.5 \text{ mg kg}^{-1} \text{ ww}$ ) 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., 2010), 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 (Verdouw et al., 2010; Langlois et al., 1987) are still present. Mercury concentrations in fish are influenced by length and age (Tremblay et al., 1998; Verdouw et al., 2010; Simoneau et al., 2005). Therefore, the effect of fish length on Hg concentration must be considered to assess temporal and spatial Hg variation accordingly (Goulet et al., 2008; Tremblay et al., 1998; Simoneau et al., 2005). 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 standardised fish length limits this variable's influence; however, deviation in growth rates between fish mean standardised lengths can represent fish of dissimilar age and therefore exposure time (Simoneau et al., 2005). The potential difference in Hg concentration associated with standardised 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. Methods

### 2.1. Study site

The Derwent Estuary ( $42^{\circ}54'S$ ,  $147^{\circ}18'E$ ; Fig. 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 (Margvelashvili et al., 2005; Thomson and Godfrey, 1985). The middle estuary (ME), which represents the industrialised 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 (Verdouw et al., 2010; Langlois et al., 1987).

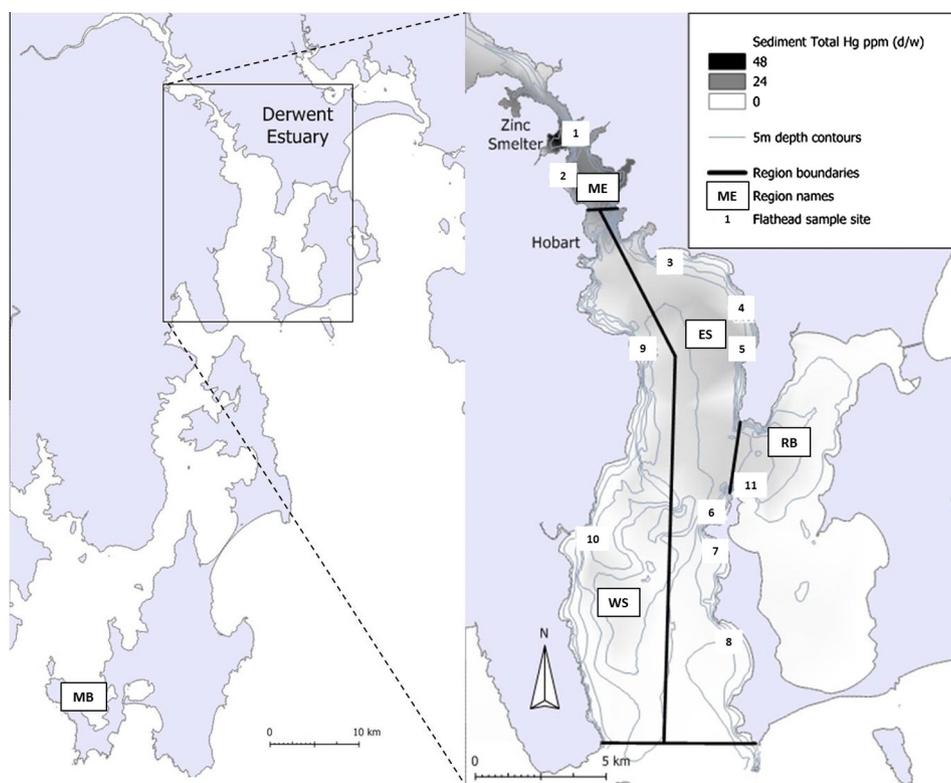
### 2.2. Sample collection

Data were collated from two sources; Langlois et al. (1987), which comprised 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. 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 was sampled wherever possible.

Sand flathead were caught using rod and line from October to 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  $-40^{\circ}\text{C}$ . 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).

### 2.3. 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  $0.1 \text{ mg kg}^{-1}$  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. (2010). A 1 g ( $\pm 0.1$  g) subsample of homogenised, skinless muscle tissue was digested in acid ( $\text{HNO}_3$  67% v/v plus  $\text{H}_2\text{SO}_4$  33% v/v) at  $\sim 97^{\circ}\text{C}$  for 3 h. After cooling, Hg was extracted by adding potassium permanganate ( $\text{KMnO}_4$ ) and potassium persulphate ( $\text{K}_2\text{S}_2\text{O}_8$ ) and allowing the resulting solution to react for 12 h. The extraction process was then repeated until the colour stabilised, with any excess  $\text{KMnO}_4$  reacted with hydroxylamine hydrochloride ( $\text{NH}_2\text{OH}\cdot\text{HCl}$ ) 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  $0.02 \text{ mg kg}^{-1}$ , 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 ( $<\text{LoD}$ ), certified reference materials (CRMs) DOLT-4 (NRC, Canada)  $\bar{x} = 2.81 \text{ mg kg}^{-1}$  ( $\pm 0.10$ ), certified value =  $2.58 \text{ mg kg}^{-1}$  ( $\pm 0.22$ ) and blank matrix spike (25% of the theoretical value of  $1.00 \mu\text{g L}^{-1}$ ) with every 20 samples analysed. All Hg data are reported in  $\text{mg kg}^{-1}$  wet weight (ww) for skinless muscle tissue. Although the two methods vary slightly in digestion and detection procedures, recovery rates of standards and QC mea-



**Fig. 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) 48 km south. Location of sample sites for sand flathead (numbered 1–11), 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.

tures 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).

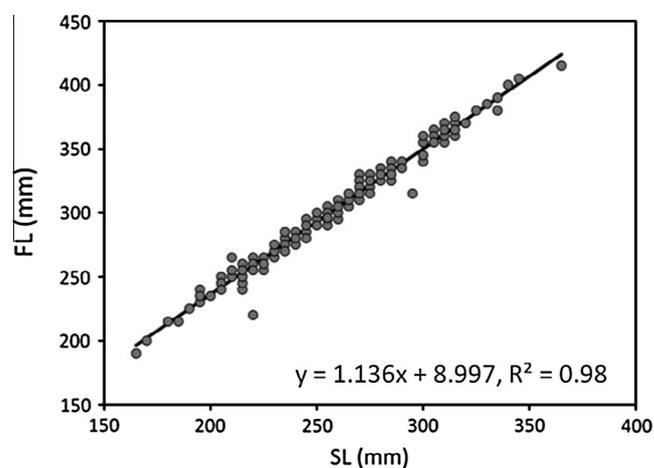
#### 2.4. Data analysis

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 to 2011

( $n = 200$ ), when both measurements were recorded (Fig. 2). The FL to Hg relationship in flathead has previously been reported as linear (Verdouw et al., 2010), however, as flathead growth curves are best examined with Von Bertalanffy curves (Jordan et al., 1998) both linear and quadratic terms were modelled.

Polynomial 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 (Goulet et al., 2008; Lavigne et al., 2010; Simoneau et al., 2005), where inadequacies in the linear regression relationships complicated the mean Hg concentration comparison (Tremblay et al.,



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

1998). To achieve normality and remove collinearity between FL and FL<sup>2</sup>, Hg concentrations in the present study were log<sub>10</sub> 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):

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

where Hg is the concentration of Hg within the fish tissue (mg kg<sup>-1</sup> ww), *L* is the centred fork length, *a*, *b*, *c*, are model coefficients and  $\varepsilon$  is an error term. Annual variation or regional comparisons can then be described by introducing binary variables (e.g. *B*<sub>1</sub>, *B*<sub>2</sub>) where each binary represents a year or region, and *d* estimates the difference in mean Hg concentration between two binaries (Tremblay et al., 1998):

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

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<sub>std</sub>) 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):

$$TL = L_{\infty}(1 - e^{-K(t-t_0)}) \quad (3)$$

where *TL* is fish length in mm, *L*<sub>∞</sub> asymptotic length, *K* the growth coefficient, *t* is the fish age and *t*<sub>0</sub> 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 three standardised lengths (FL<sub>std</sub>): 261 mm, 293.9 mm, 300 mm. The first value represents the 1st quartile of FL within the dataset, 294 mm 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*<sub>0</sub> and the end of the year is at time *t*<sub>1</sub>. The fish age between start and end of the year was then calculated as a fraction of that year.

### 3. 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 ( $F_{1,426} = 33.02$ ,  $P = <0.0001$ ), (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  $0.49 \pm 0.01$  mg kg<sup>-1</sup>. This was significantly higher than the concentrations reported for fish from the reference region (MB) (1981, 1991–2011,  $\bar{x} = 0.20 \pm 0.02$  mg kg<sup>-1</sup>) ( $F_{1,2277} = 527.5$ ,  $P = <0.0001$ ) (Table 2). Forty percent of all fish from the Derwent Estuary had Hg concentrations  $\geq 0.5$  mg kg<sup>-1</sup>, rising to 56% in fish with FL  $\geq 300$  mm. Only 1.6% of fish from MB had Hg concentrations  $\geq 0.5$  mg kg<sup>-1</sup> with all of these fish greater than 300 mm FL.

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 1). There was, however, a significant difference in Hg concentration of the fish between regions ( $F_{3,12} = 26.39$ ,  $P = <0.0001$ ) (Table 1), with a descending order of Hg concentration RB > ME = ES > WS (Table 2). There was also significant variation in Hg concentration in fish across years ( $F_{35,12} = 2.66$ ,  $P = 0.04$ ) (Table 1). 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 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 years period for any region (Fig. 3).

#### 3.1. 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. 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 4). The models indicate a general increase in Hg concentration with FL, but the relation varied across years and regions (Fig. 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. 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<sup>2</sup> (Fig. 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. 5).

#### 3.2. 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. 6). Four out of the five regions (RB, ME, ES and MB) exhibited simple curvilinear increases in Hg with FL, while the Western shore

**Table 1**

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	1.436e–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**

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 (<sup>a,b,c,d</sup>), denotes significant differences ( $P = 0.05$ ) assessed between regions through ANOVA with Tukey's HSD post hoc test.

Region	$n$ Years	$n$	$\bar{x}$ Hg mg kg <sup>-1</sup>	sd
ME	36	796	0.49 <sup>b</sup>	0.13
WS	33	815	0.37 <sup>c</sup>	0.12
ES	35	851	0.50 <sup>b</sup>	0.15
RB	36	829	0.62 <sup>a</sup>	0.13
MB	22	445	0.18 <sup>d</sup>	0.06

(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. >400 mm) (Fig. 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 Ralplhs 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 but the overlap in 95% confidence intervals between regions showed no significant difference in the overall position of the curves (Fig. 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.

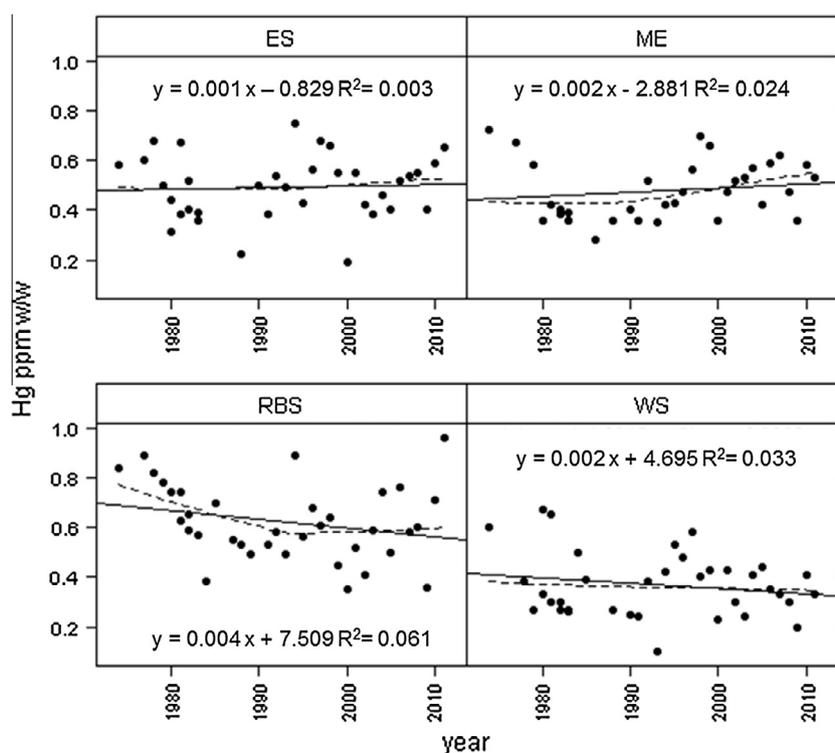
### 3.3. Age data and growth rates

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

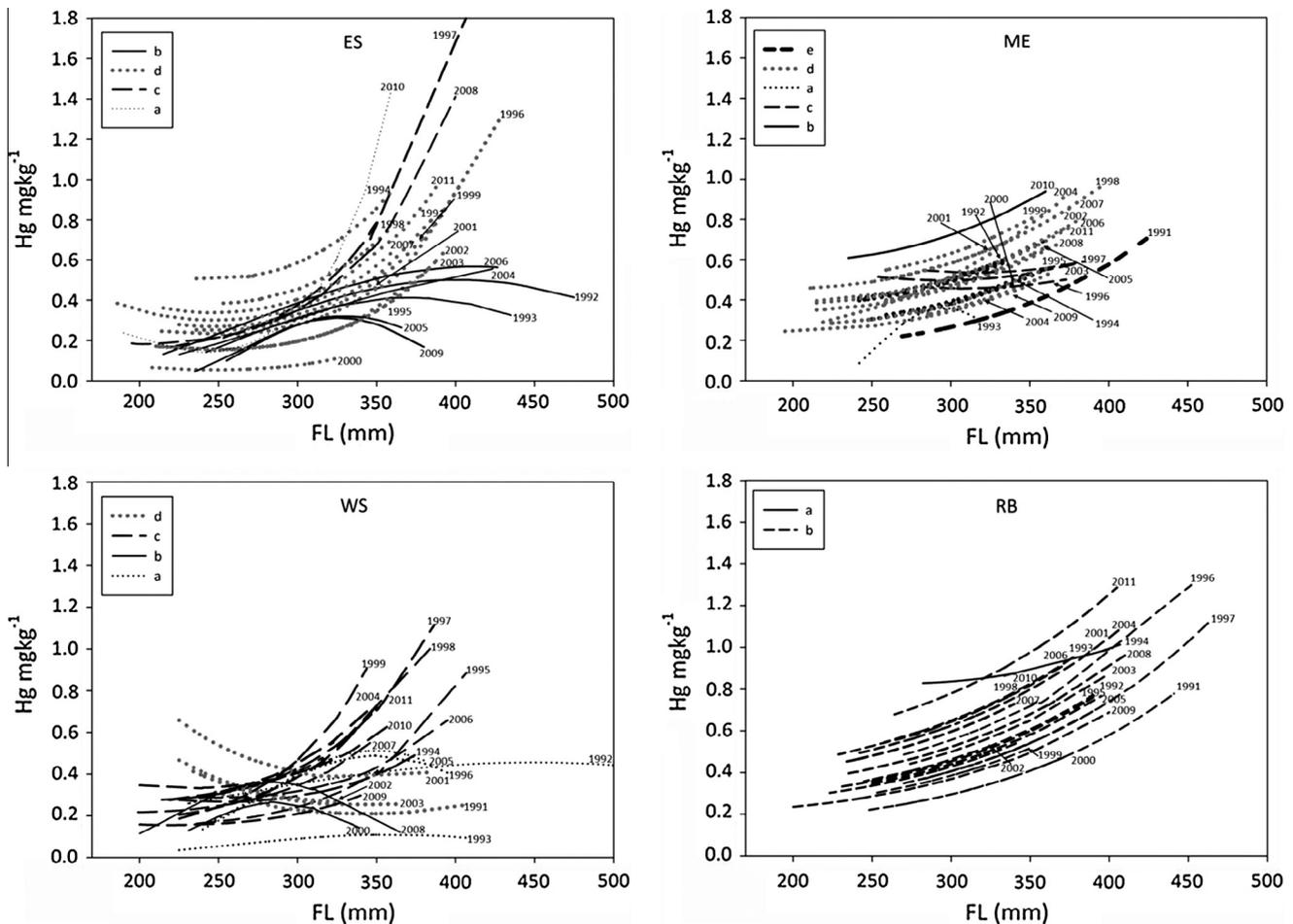
**Table 3**

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 ( $r^{res}SS$ ) 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 ( $r^{res}SS$ ). 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	$R^2$	$r^{res}SS$	$df$	Smoother index	$App. df$	$r^{res}SS$	$F$ -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



**Fig. 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.



**Fig. 4.** Relations between FL and Hg concentration for sand flathead in each Derwent Estuary region, data integrated over period 1991–2011. 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).

**Table 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 ( $df$ ).

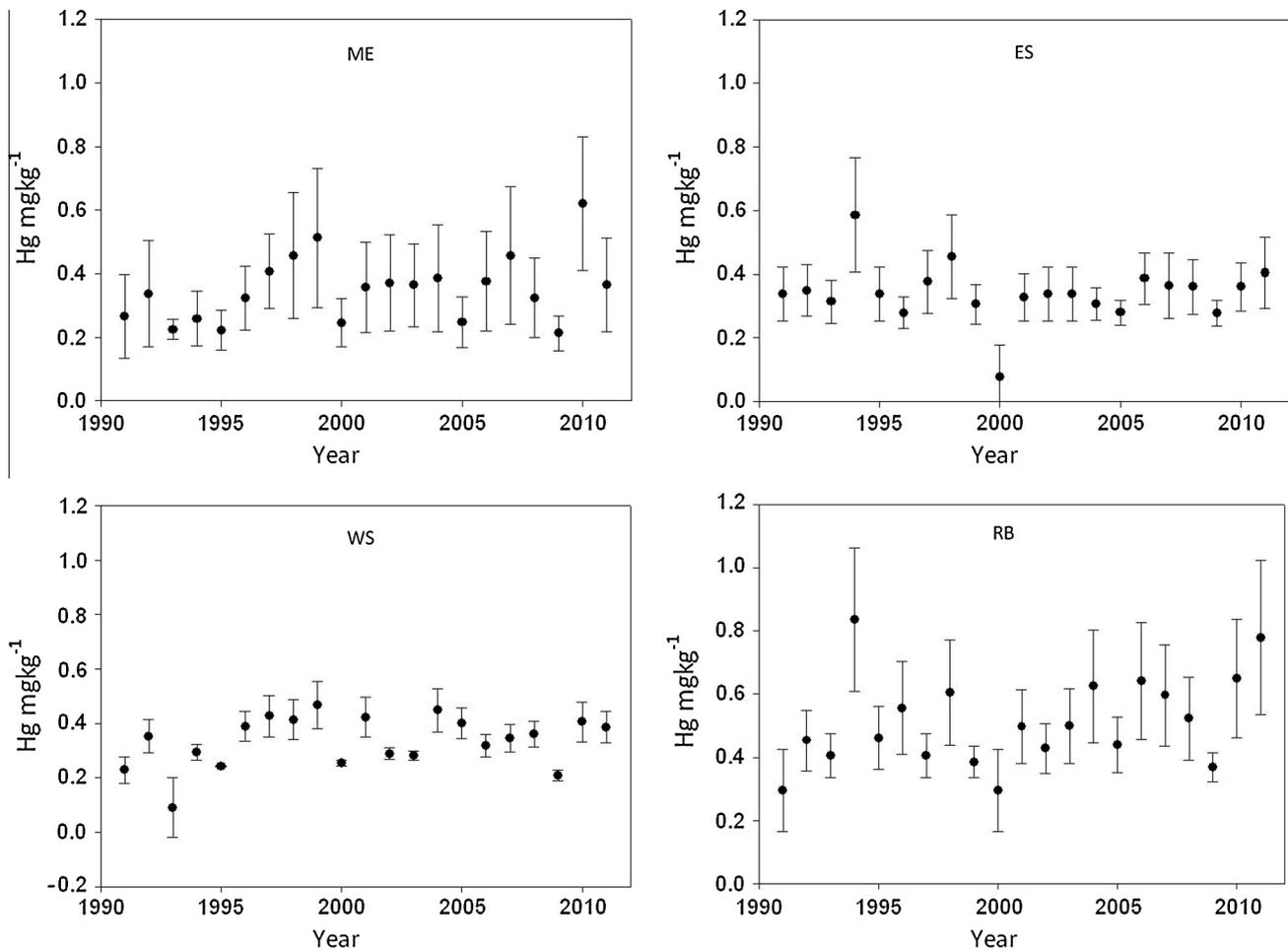
Region	$R^2$	Residual se	$F$ -value	$df$	$P$ -value
WS	0.52	0.06	9.93	46, 422	$2.20 \times 10^{-16}$
MB	0.50	0.07	14.14	28, 396	$2.20 \times 10^{-16}$
ES	0.42	0.17	10.42	29, 426	$2.20 \times 10^{-16}$
RB	0.48	0.11	16.86	23, 422	$2.20 \times 10^{-16}$
ME	0.44	0.13	12.05	29, 451	$2.20 \times 10^{-16}$

19% and 40% ( $\bar{x} = 28.4\%$ ) of the observed Hg concentration variation (Table 5). Von Bertalanffy growth parameters (VBGF) indicated RB fish had significantly elevated growth rates compared to all other regions examined (Fig. 7). In contrast, the WS region had significantly smaller fish, at any age, than other regions (including the reference region MB) (Fig. 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<sub>std</sub> –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  $2.5 \pm 0.2$  dec-

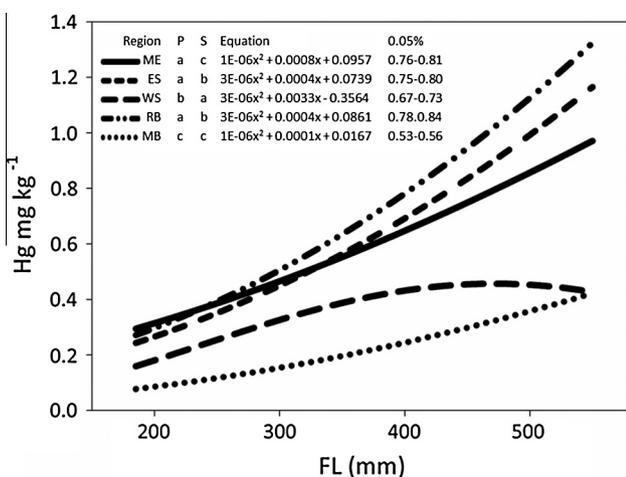
imal years (dy), 293.3 mm at  $4.4 \pm 0.6$  dy and 300 mm at  $4.5 \pm 0.5$  dy. The fastest growing flathead were found within the RB region, and on average reached 300 mm by age 3.3 dy while the slowest growth to 300 mm was modelled within the reference region (MB) at 5.8 dy. The ME and ES regions showed similar growth rates, taking 4.5 and 4.6 dy, respectively to reach 300 mm (Fig. 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. 8).

#### 4. 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 to 2011)) and the polynomial model (using data from 1991 to 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 conclu-



**Fig. 5.** Muscle Hg concentration with 95% probability threshold for sand flathead of standardised FL ( $FL_{std}$ ) (300 mm) 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.



**Fig. 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.

sions of the earlier study in the Derwent Estuary, which suggested that there had been significant reductions in Hg concentrations in

**Table 5**

Fish age and Hg concentration in sand flathead for four Derwent regions and reference region (MB), showing best-fit linear regressions. Table includes number of samples ( $n$ ),  $F$ -value,  $P$ -value, regression coefficient ( $R^2$ ), degrees of freedom ( $df$ ), model intercept and slope.

Region	$n$	$F$ -value	$P$ -value	$R^2$	$df$	Intercept	Slope
ME	87	31.67	$2.297e^{-07}$	0.27	85	0.338	0.051
WS	89	28.94	$6.219e^{-07}$	0.25	87	0.124	0.046
ES	85	18.93	$3.838e^{-05}$	0.19	83	0.230	0.069
RB	79	31.07	$1.234e^{-07}$	0.31	76	0.305	0.092
MB	89	58.7	$2.397e^{-11}$	0.40	87	0.045	0.027

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

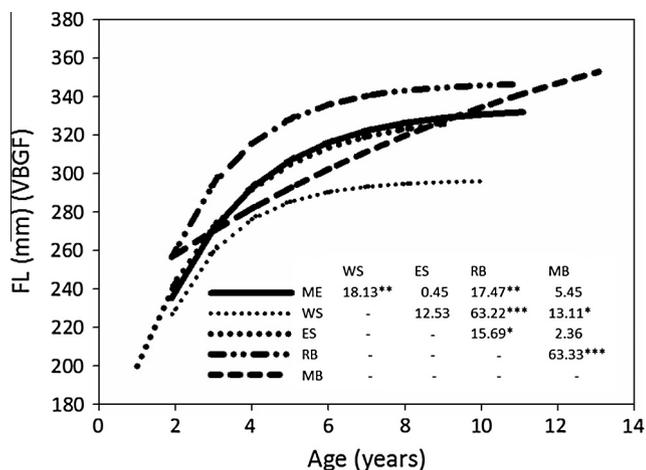


Fig. 7. Age (years) of sand flathead for each of the Derwent regions and reference region (MB) plotted against mean fork length (FL) (mm) calculated by Von Bertalanffy growth curve function (VBGF). Table gives chi-square test value ( $df = 6$ ) between regions, significant differences in curves indicated by \*  $0.05 < \chi^2 < 0.01$  \*\*\*  $> 0.01$ .

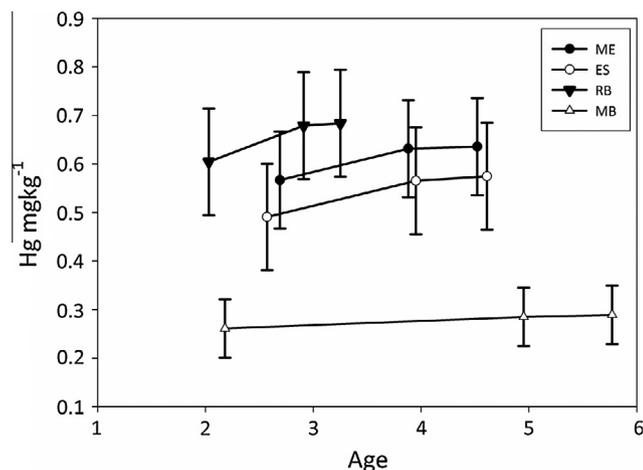


Fig. 8. Mean Hg concentrations ( $\pm$ se) and predicted ages of sand flathead for three  $FL_{std}$  using Von Bertalanffy growth curve predictions.  $FL_{std}$  used from left to right for each region = 261 mm, 293.9 mm and 300 mm. Note: WS region is absent due to lack of prediction with VBGF at  $FL_{std}$  300 mm.

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 (Sager, 2002; Francesconi et al., 1997).

Fish size (fork length, FL) and age are known determinants of Hg loadings in flathead (Verdouw et al., 2010). 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., 2010) 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 concentration 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 (Verdouw et al., 2010; Langlois et al., 1987). 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 years 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 years) and therefore includes multiple generations of this species highlighting that Hg within the system continues to become biologically 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 (Sackett et al., 2010; Peterson et al., 2009).

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 (Verdouw et al., 2010; Langlois et al., 1987), 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 (Sackett et al., 2010; Sager, 2002; Tremblay et al., 1998), 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 (Cossa et al., 2012; Lavigne et al., 2010), 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 foodwebs 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 rate 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.

## 5. 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.

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