CONTAMINANT (PAHS, OCS, PCBS AND TRACE METALS) CONCENTRATIONS ARE DECLINING IN AXIAL TISSUE OF SAND FLATHEAD (*PLATYCEPHALUS BASSENSIS*) COLLECTED FROM AN URBANISED CATCHMENT (PORT PHILLIP BAY, AUSTRALIA)

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ABSTRACT

Concentrations of PAHs, OCs, PCBs and trace metals were determined in the white muscle of sand flathead *Platycephalus bassensis* collected at 6 locations in Port Phillip Bay during 2015. No PAHs, OCs or PCBs were detected in the white muscle of sand flathead at any of the locations, however measurable levels of As, Cu, Hg, Se and Zn were detected at all sites. Only As and Hg exhibited regional difference in white muscle concentrations, with As present only in a non-toxic organic form and Hg measured at levels that are comparable to levels reported in reference sites in other studies. All contaminants detected in the white muscle of sand flathead collected in Port Phillip Bay in 2015 were below Australian Food Standards guideline values, and by world standards, the Port Phillip Bay sand flathead population is considered minimally contaminated. Furthermore, tissue contaminant concentrations appear to be decreasing over time.

Most major cities of the world are located near sheltered embayments which facilitate transport of goods by sea, provide recreation and amenity and offer protection from open coastal weather extremes. However, embayments are susceptible to environmental pollution via industrial use of their banks and urbanisation of their catchment area. Port Phillip Bay is an example of a typical urban embayment. The catchment includes large urban areas from the Cities of Melbourne and Geelong with a population exceeding four million. The Bay is 1930 km^2 with a coastline length of 264 km (Sampson et al., 2014) and is relatively shallow with half of the 26 km^3 volume occupying areas less than 8 m deep and the narrow southern entrance, resulting in a flushing time of up to 12 months (EPA Victoria, 2011). The land catchment covers an area of 9,790 km^2 and includes industrial zones that contribute heavy metals, organic contaminants and industrial chemicals, as well as urban and rural areas which provide nutrient and biocide inputs into the Bay.

Initial studies of toxicants in the Bay, in the early 1970s, focussed on nutrients but subsequent studies in the late 1970s included measurements of metals of concern, namely cadmium, mercury, zinc and copper which were elevated in molluscs (Phillips et al., 1992). Later investigations in the 1980s included organic contaminants, and established that elevated concentrations of polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) were found in parts of the Bay (Phillips et al., 1992). Of special concern was the discharge of Kororoit Creek into the northern side of the bay, carrying significant loads of mercury (Hg), copper (Cu) and chromium (Cr) from industrial discharges (Scott and Christoff, 1988). Monitoring programs concerned with the deteriorating ecological health of the Bay were established, and contaminant levels were measured in various environmental compartments including water, sediments and fish. The first comprehensive study of Port Phillip Bay included the study of physical and ecological processes governing the Bay as well as nutrient and toxicant inputs. Harris et al. (1996) found that overall found that overall the Bay was relatively unpolluted but hot spots of contamination were identified in highly

industrialised/urbanised areas, with major inputs via direct effluent discharge from large industries or via the Yarra River flowing through Melbourne. Important measures were taken to protect Port Phillip Bay from detrimental effects of contamination: firstly, a system of licensing discharges which improved the quality of urban and industrial effluents entering the Bay was instated, and secondly the creation of management zones for specific tasks such as wildlife protection and aquaculture (Phillips et al., 1992). The water quality of the Yarra River and of Kororoit Creek has been further improved through increased sewering of catchments and diversion of industrial discharges into the sewerage system (Sampson et al., 2014; Scott and Christoff, 1988).

A key bioindicator species that has been used to monitor pollution in Port Phillip Bay is the sand flathead (*Platycephalus bassensis*), which is an iconic and recreationally important fish species. Sand flathead are long- lived carnivorous ambush predators that conceal themselves in fine sediments and are not strong swimmers and therefore believed to be representative of the area from where they are collected (Fabris et al., 1992). During the late 1970's, sand flathead collected in Port Phillip Bay had a mean mercury concentration of 0.5 mg/kg in edible white muscle but this level had declined to 0.23 mg/kg in the early 1990s, and to 0.16 mg/kg by the mid-1990s (Fabris, 1995). Whilst in the early 1990s, levels of organochlorine pesticides, PAHs and PCBs were mostly below the detection limits in the white muscle of sand flathead collected from various parts of Port Phillip Bay. Nevertheless, a study conducted in 1999 in urbanised areas of the Bay reported measurable levels of PAH metabolites in sand flathead biliary secretions, which are suggestive of recent exposure to petroleum hydrocarbons of pyrolytic origin (Gagnon and Holdway, 2002). Since Port Phillip Bay is a major seafood source for the city of Melbourne (Hirst et al., 2014) it is imperative to have ongoing monitoring of the Bay's health status.

In the past decade, the continuous improvement of wastewater management by the city of Melbourne aimed at preserving water quality and ecological integrity in the Bay, however substantial population growth on the shorelines of Port Phillip Bay could intensify pollution inputs in the future. To evaluate the current health status of sand flathead in Port Philip Bay, a comprehensive study was undertaken in 2015 which included the measurement of a suite of physiological and biochemical parameters (Baker et al., in prep.) complemented by the determination of contamination levels in the white muscle of sand flathead. The suite of contaminants analysed included polycyclic aromatic hydrocarbons (PAHs), organochlorine pesticides (OCs), PCB congeners (PCBs) and ten metals commonly found as pollutants in urban or industrialised environments. It is expected that the information reported here will be useful for long term monitoring and management of the Port Phillip Bay environment.

Skinless white muscle samples were collected from 96 sand flathead originating from six separate locations within Port Phillip Bay during February 2015 (Fig 1.), as per the Department of Environment and Primary Industries permit No. RP1216. The fish were captured by hook and line and kept in aerated live tanks on board the boat, then brought back to the laboratory where they were sacrificed by iki jime (Robb et al., 2000) as per Curtin University Animal Ethics Permit # AEC-2015-05. Physiological parameters were recorded and biopsies of blood, liver, bile, brain and gonads were collected for biomarker analyses, and otoliths were retained for age determination. All tissues were collected within 10 minutes of death. Fish were immediately filleted, and a white muscle sample was placed in HPLC-grade methanol rinsed aluminium foil and stored at -20 ^{o}C prior to chemical analysis. Pooled samples of white muscle from 3 fish of similar size and sex (collected within one location) were submitted for chemical analysis.

Determination of organochlorine (OCs) pesticides and polychlorinated biphenyls (PCBs) in muscle tissue was conducted by the National Measurement Institute (NMI, North Ryde, New South Wales), according to the USEPA methods 3540C and 3620B (US EPA, 1996a, 1996b). Briefly, homogenised tissue samples were mixed with anhydrous sodium sulphate and extracted using a hexane:acetone mix. The extract was cleaned up by gel permeation chromatography (GPC) and the final extract was analysed by GC-ECD (dual column).

Polycyclic Aromatic Hydrocarbons (PAHs) were also analysed by NMI, according to the US EPA method 8270D (US EPA, 1998). Homogenised muscle samples were extracted with dichloromethane/acetone, cleaned up using GPC and silica gel, then analysed by gas chromatography with a mass selective detector (MSD) using electron impact-selective ion monitoring.

Trace metal analysis of homogenised white muscle was conducted by NMI for the following elements: total arsenic (TotAs); inorganic arsenic (InorAs); total mercury (TotHg); copper (Cu); zinc (Zn); cadmium (Cd); chromium (Cr); nickel (Ni); silver (Ag) and selenium (Se). These elements were analysed according to the USEPA methods 6010C (US EPA, 2000) and 6020A (US EPA, 1998) and to the AOAC methods 986.15 (AOAC International, 2002) and 974.14 (AOAC International, 2002, 2002a). The homogenised samples were acid-digested then analysed for trace metals using inductively coupled plasma-mass spectrometry (ICP-MS) and/or inductively coupled plasma-atomic emission spectrometry (ICP-AES).

Inorganic arsenic was measured using the hydride generation ICP-MS technique, which measures total acid extractable As^{+3} and As^{+5} from inorganic arsenic compounds plus arsenic from the partial decomposition of monomethylarsenate (MMA), as per the method of (Holak and Specchio, 1991).

For all chemical analyses, at least one blank, one duplicate, one blank spike, one sample spike and one laboratory control sample was measured for every 20 tissue samples. QA/QC also included the use of relevant certified reference materials . Limits of detection are as follows: PAHs 0.01 mg/kg; OC Pesticides 0.01 mg/kg; PCB congeners 2.0 µg/kg; total recoverable trace elements: total As, inorganic As, Cr 0.05 mg/kg; Ag 0.02 mg/kg; Cd, Cu, Pb, Hg, Ni, Se, Zn 0.01 mg/kg. The blanks were all below the limits of detection, the percent recovery of the spiked samples ranged from 95% to 103%, and sample duplication relative percent difference was between 4.8% to 15%. Concentrations are reported as either µg/kg wet weight (PCB congeners) or mg/kg wet weight (PAHs, OC pesticides, trace metals).

To evaluate moisture content, a subsample of 2-5 g pooled white muscle from each field site was placed in a drying oven at 102°C for a minimum of 4 hours, then weighed and returned to the oven for a further 1 hour. The weighing and drying process was repeated until a constant weight was obtained. The measurements of moisture are provided in Table 1 to allow for calculations of contaminant burdens on a dry weight basis for comparisons to other results where relevant. Lipid content was similarly determined in a pooled sample of white muscle from each field collection site. Briefly, a 2 g subsample was digested in 10 mls of 10% hydrochloric acid at 80 °C for 30 minutes. The mixture was transferred to a Mojonnier tube, and the lipids extracted by successive solvent additions. Following each solvent addition, the organic layer was decanted from the Monjonnier tube into a pre-weighed glass dish. Once all extractions were completed, the organic extract was evaporated and the dish was placed in a drying oven at 102°C until a constant weight was achieved. Lipid content values are provided in Table 1 to allow for calculation of contaminant concentrations on a lipid weight basis, to enable comparisons with other studies.

Data were analysed using univariate analysis of variance (ANOVA) with Tukey B post-hoc tests to compare metal levels in muscle of sand flathead collected at different sites. Fish size (as measured by total length or weight), moisture content and percentage lipids in white muscle were also compared using ANOVA. Potential relationships between Hg tissue levels and size (length or weight) and age were evaluated using correlation analysis. All data were tested for homogenous variances (Levene) and normal distribution prior to running the statistical tests. Physiological parameters and trace metal values were $\log_{10}$-transformed prior to being statistically analysed.

No PAHs, organochlorine pesticides or PCBs were detectable in any of the samples of sand flathead white muscle collected throughout Port Phillip Bay in 2015. Similarly, inorganic As, Cd, Cr, Pb and Ag were all below the limits of detection. The only elements reported above detection limits were total (organic) As, Cu, Hg, Ni, Se and Zn (Table 2).

Arsenic levels (mg/kg) were higher in composite samples collected from St Leonards and Sorrento than those from Hobsons Bay, Corio Bay or Geelong Arm; while Mordialloc arsenic levels were statistically higher than those in Geelong Arm and Corio Bay only ($p < 0.001$, Table 2). Mercury was highest in samples from Mordialloc followed by Hobsons Bay and Corio Bay, and there was a significant difference in concentrations between Mordialloc and St. Leonards ($p = 0.011$). Concentrations of Cu, Ni, Se and Zn in white muscle tissue were not statistically different ($p > 0.05$) amongst the various Port Phillip Bay sampling locations.

Fish collected from Hobsons Bay were statistically ($p < 0.001$) larger in weight and length than fish originating from Mordialloc, Sorrento and St. Leonard but were similar to those from Geelong Arm and Corio Bay. There were no significant correlations between fish size (weight or length) or fish age and white muscle contaminant concentrations.

Moisture content in the white muscle of sand flathead did not vary amongst the sites ($p > 0.05$) and neither did the lipid content ($p > 0.05$). Moisture and lipid content were not significantly correlated to age, total length or total weight of the fish.

Early studies of contaminants in Port Philip Bay focussed on nutrient contamination and eutrophication, however, elevated concentrations of heavy metals, PAHs and PCBs have also been reported in sediments from different parts of the Bay (Harris et al., 1996). More recent studies concluded that sediment PCB concentrations were low in the Bay, and that the PCB levels measured were due to legacy contamination rather than recent PCB pollution (van Gelderen and Pettigrove, 2011). In the present survey, PAHs, organochlorine pesticides and PCBs were not detected in the edible white muscle of sand flathead collected from any of the six Port Phillip Bay locations used in this study.

Despite the fact that urbanisation has greatly increased in recent decades around Port Phillip Bay, contamination of biota seems to have historically been, and seems to remain, at very low levels as compared to other urban embayments overseas (Sampson et al., 2014). Urban environments often contribute significant amounts of PAHs into the surrounding waters, however, in the 1990s, as in the present survey, all sand flathead samples contained very low or undetectable levels of PAHs (Nicholson et al., 1991). Fish can readily take up petroleum hydrocarbons from water, and PAHs have the potential to be detected in the muscle tissue following recent exposure (less than a week, (Gagnon and Holdway, 2000)). However, due to the rapid and extensive metabolism of PAHs by fish, standard chemical analyses of these compounds in muscle tissue are often inconclusive. In the past the Yarra River has been identified as a significant contributor of PAHs into the Bay however, but the river's water quality has improved in the past 20 years through better sewering of domestic and

industrial wastewaters (Sampson et al., 2014). In addition, the reduced usage of coal products and wood burning in the catchment are likely to contribute to declining levels of PAHs in the Bay. The present survey is the first that reports PAH concentrations in sand flathead white muscle below the limits of detection (0.1 mg/kg wet weight) using standard chemical analyses, suggesting indeed that concentrations of this group of contaminants are in decline.

In 1990, the organochlorine pesticides dieldrin and DDT were identified as widespread contaminants in sand flathead white muscle, however in 1994, a follow up study reported that all organochlorine pesticides were below the 0.1 mg/kg detection limit (Fabris, 1995). In addition, the 1990 survey indicated that PCBs were detected in fish from all sampled sites (Nicholson et al., 1991). The non-detection of a comprehensive suite of organochlorine pesticides and PCB isomers in the 2015 sampling suggests a temporal reduction of organic-type contaminant inputs in the Bay, which matches the progressive trend towards reduced contamination in biota that has been previously suggested (Sampson et al., 2014).

As in previous studies (e.g. those reported in (Harris et al., 1996) and in Table 3), Cd, Cr, Pb, Ni and Ag content in the sand flathead collected in 2015 were at very low or undetectable levels. Other metals which are metabolically regulated e.g. Cu, Se and Zn were detected in all samples and each element showed comparable tissue concentrations across all sampling sites throughout the Bay. Tissue Cu concentrations appear to be reduced in the present study relative to historical levels, with ranges of 0.23-0.66 mg/kg in 1995 (Fabris, 1995) compared with 0.10-0.16 mg/kg in 2015. The average Se levels measured in 2015 were comparable to those measured in the same species collected outside the Bay in coastal waters (0.45 mg/kg and 0.49 mg/kg, respectively (Fabris et al., 2006). While muscle Zn concentrations were comparable to levels measured 20 years earlier, which ranged from 3.8 to 6.8 mg/kg in 1995 (Fabris, 1995) compared with 5.93 to 7.16 mg/kg in 2015, the

values are also comparable to the 5.0 mg/kg baseline levels measured in sand flathead collected in marine waters outside the Bay (Fabris et al., 2006). Thus it can be concluded that the metals Cd, Cr, Pb, Ni and Ag as well as Cu, Se, and Zn are not contaminants of concern for sand flathead inhabiting Port Phillip Bay in 2015.

However, total arsenic and mercury have been detected in high levels in Port Phillip Bay waters, sediments and biota since the early 1980's (Harris et al., 1996), and our results indicated that although these two elements also follow a temporal trend towards reduced levels in white muscle, As and Hg remain the only metal contaminants showing spatial differences in sand flathead collected in Port Philip Bay in 2015. Inorganic arsenic was not detected in measurable amounts in any of the pooled samples and therefore the edible portion of sand flathead collected throughout Port Philip Bay in 2015 is well below the Australian Food Standard guideline value of 2 mg/kg for inorganic As (Food Standards Australia New Zealand, 2014). It is well established that the arsenic present in fish muscle is mainly found as the non-toxic organic forms of arsenobetaine and arsenocholine (Ng, 2005) which have little toxicological significance for human consumption. The origin of the arsenic in Port Phillip Bay is likely to be natural sediment mineralogy (Pettigrove and Hoffmann, 2003), with surface sediments leaching arsenic in the water column resulting in high (2.8 ug/L) concentrations of dissolved As (Fabris et al., 1999). Sand flathead are bottom dwellers living on sandy, shelly or muddy seafloors, and would experience higher exposure to sediment-released arsenic than other fish living in the water column, explaining why Port Phillip Bay sand flathead have a relatively high organic As muscle content relative to other reported levels, e.g. 0.17-0.48 mg/kg in Persian Gulf fishes (Raissy and Ansari, 2014), or 0.59-1.06 mg/kg in Malaysian fishes (Alina et al., 2012).

It has long been recognised that urbanisation of shorelines can contribute to heavy metal loads of the surrounding waters, and Port Phillip Bay is no exception. Fish collected from Mordialloc, on the

eastern side of the Bay, had the highest muscle Hg contents (average of 0.29 mg/kg) of all the sites sampled in the present study. Historical mercury sediment concentrations were found to be 'average' at this site (Fabris et al., 1999), however, regional differences in Hg sediment content have previously been observed to not be correlated with sand flathead muscle tissue Hg levels (Verdouw et al., 2011). Rather, it has been suggested that in environments contaminated by Hg, size as measured by length is the best predictor of mercury levels in ten species of fish tested (Burger and Gochfeld, 2011). A similar observation was made in sand flathead collected in southern Tasmania, with age and length being significant predictors of tissue Hg levels (Verdouw et al., 2011). In the present survey however, fish collected in the eastern side of the Bay were, on average, neither longer, heavier or older than fish from other locations, and no correlations were present between length and Hg tissue levels, so the size-Hg tissue burden relationship observed in other fish species does not apply to the present investigation. Of all pools of fish analysed for chemical content, only one pool of 3 male fish collected in Mordialloc returned Hg levels of 0.54 mg/kg which is over the Australian Food Standard of 0.50 mg/kg (Food Standards Australia New Zealand, 2014). However, fish from this pool were of average size (total length 244-252 mm, total weight: 89-99 g), further confirming that length is not a predictor of Hg muscle tissue content in Port Phillip Bay sand flathead. While size was observed to be a predictor of Hg levels in other fish species, the minimum legal size of 27 cm total length for sand flathead (Victoria State Government, 2016) does not appear to increase the likelihood of unacceptable Hg consumption by the general population.

The Hg muscle tissue contents of the fish collected at other locations in the Bay compared favourably to levels reported in reference sites in other studies. For example Verdouw et al. (2011) reported average Hg levels of 0.22 mg/kg in the muscle of sand flathead collected in a remote, unpolluted location in Tasmania while the Port Phillip Bay sand flathead muscle Hg concentrations (at locations other than Mordialloc) ranged from 0.10 to 0.20 mg/kg. Comparatively, Hg

concentrations in the muscle of the farmed Japanese tuna *Thunnus orientalis* was 0.67 mg/kg (Hisamichi et al., 2011) but was much higher in wild individuals of this species captured in Japanese waters, at 1.45 mg/kg (Hisamichi et al., 2010).

Overall, multi-decadal snapshot contaminant monitoring studies of the edible white muscle portion of sand flathead collected from Port Phillip Bay indicates a long term reduction of contaminant burden, with PAHs, PCBs, organochlorine pesticides and several metals being now under chemical detection limits, or at expected physiological levels. Similarly, As and Hg, whilst still detectable also show a lowering trend relative to the 1970s. Identifying the reasons for these declining contaminant levels in the edible portion of sand flathead is beyond the scope of the present study, however improved water quality management and a reduction in the usage of these chemicals are considered likely reasons for the declines observed.

Port Phillip Bay appears to sustain a population of recreationally important sand flathead with only minor contaminant body burdens. None of the samples contained measurable levels of organic contaminants (PAHs, organochlorine pesticides, PCBs) or some trace metals (Cd, Cr, Pb and Ag) in the edible portion of white muscle tissue, while Cu, Ni, Se and Zn were present at typical levels which do not cause concerns for consumption of fish by humans. White muscle tissue levels of As exhibited regional differences throughout the Bay and were relatively elevated, (probably due to the natural sediment mineralogy of the area) but the organic form that it was observed in is considered to be of little toxicological significance for human consumption. Hg levels in white muscle were generally low throughout the Bay with slightly higher levels observed on the eastern side compared with other areas, but nevertheless, all concentrations reported in this study are close to (0.54 mg/kg – one pooled sample from Mordialloc), or lower than Australian Food Standard guideline levels of 0.50 mg/kg. In conclusion, this study indicates that Port Phillip Bay supports a population of

relatively uncontaminated sand flathead, and that contaminant loads in this species show a declining trend through time.

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REFERENCES

Alina, M., Azrina, A., Mohd Yunus, A. S., Mohd Zakiuddin, S., Mohd Izuan Effendi, H., & Muhammad Rizal, R. (2012). Heavy metals (mercury, arsenic, cadmium, plumbum) in selected marine fish and shellfish along the Straits of Malacca. *International Food Research Journal, 19*, 135-140.

AOAC International. (2002). AOAC 974.14-1976 Mercury in fish. Alternative digestion method. Gaithersburg, MD, USA: AOAC International.

AOAC Internatonal. (2002a). Arsenic, cadmium, ead, selenium, and zinc in human and pet foods - multielement method. In: AOAC (Ed.), Official Methods of Analysis of AOAC International, Gaithersburg, 17th Edition, MD, USA.

Baker, J. K., Long, S., Hassell, K. L., Pettitgrove, V., & Gagnon, M. M. (in prep.). Health status of fish inhabiting an urbanised embayment, Port Phillip Bay, Victoria, as measured by biochemical markers of health in sand flathead (*Platycephalus bassensis*). *PLoS ONE*.

Burger, J., & Gochfeld, M. (2011). Mercury and selenium levels in 19 species of saltwater fish from New Jersey as a function of species, size, and season. *Science of The Total Environment, 409*, 1418-1429. doi:http://dx.doi.org/10.1016/j.scitotenv.2010.12.034

EPA Victoria. (2011). *Port Phillip Bay and Western Port Receiving Water Quality Modelling: Scenarios*. Melbourne, Victoria.

Fabris, G., Turoczy, N. J., & Stagnitti, F. (2006). Trace metal concentrations in edible tissue of snapper, flathead, lobster, and abalone from coastal waters of Victoria, Australia. *Ecotoxicology and Environmental Safety, 63*, 286-292. doi:http://dx.doi.org/10.1016/j.ecoenv.2004.11.006

Fabris, G. J. (1995). *Toxicants in aquatic biota from Port Phillip Bay: data analysis.* CSIRO Port Phillip Bay Environmental Study, Melbourne, Australia.

Fabris, G. J., Monahan, C., Nicholson, G., & Walker, T. I. (1992). Total mercury concentrations in sand flathead, *Platycephalus bassensis* Cuvier & Valenciennes, from Port Phillip Bay, Victoria *Australian Journal of Marine and Freshwater Research, 43*, 1393-1402. doi:http://dx.doi.org/10.1071/MF9921393

Fabris, G. J., Monahan, C. A., & Batley, G. E. (1999). Heavy metals in waters and sediments of Port Phillip Bay, Australia. *Marine and Freshwater Research, 50*, 503-513. doi:http://dx.doi.org/10.1071/MF98032

Food Standards Australia New Zealand. (2014). Chemicals in food - maximum residue limits. Retrieved from http://www.foodstandards.gov.au/consumer/chemicals/maxresidue/pages/default.aspx

Gagnon, M. M., & Holdway, D. A. (2000). EROD induction and biliary metabolite excretion following exposure to the water accommodated fraction of crude oil and to chemically dispersed crude oil. *Archives of Environmental Contamination and Toxicology, 38*, 70-77. doi:http://dx.doi.org/10.1007/s002449910009

Gagnon, M. M., & Holdway, D. A. (2002). EROD activity, serum SDH and PAH biliary metabolites in sand flathead (*Platycephalus bassensis*) collected in Port Phillip Bay, Australia. *Marine Pollution Bulletin, 44*, 230-237. doi:http://dx.doi.org/10.1016/S0025-326X(01)00245-4

Harris, G., Batley, G., Fox, D., Hall, D., Jernakoff, P., Molloy, R., Murray, B., Newell, B., Parslow, J., Skyring, G., & Walker, S. (1996). *Port Phillip Bay Environmental Study Final Report*. CSIRO. Canberra Australia.

Hirst, A., Rees, C., Hamer, P., Conron, S., & Kemp, J. (2014). The decline of sand flathead stockes in Port Phillip Bya: magnitude, causes and future prospects. *Recreational Fishing Grant Program Research Report, Fisheries Victoria*, 63 pages.

Hisamichi, Y., Haraguchi, K., & Endo, T. (2010). Levels of mercury and organochlorine compounds and stable isotope ratios in three tuna species taken from fifferent regions of Japan.

Environmental Science and Technology, 44, 5971–5978. doi:http://dx.doi.org/10.1021/es1008856

Hisamichi, Y., Haraguchi, K., & Endo, T. (2011). Levels of mercury and organohalogen compounds in Pacific bluefin yuna (*Thunnus orientalis*) cultured in different regions of Japan. *Archives of Environmental Contamination and Toxicology, 62*, 296-305. doi:http://dx.doi.org/10.1007/s00244-011-9696-5

Holak, W., & Specchio, J. J. (1991). Determination of total arsenic, arsenic(III) and arsenic(V), in foods by atomic absorption spectrometry. *Atomic Spectroscopy, 12*, 105-108.

Ng, J. C. (2005). Environmental contamination of arsenic and its toxicological impact on humans. *Environmental Chemistry, 2*, 146-160. doi:http://dx.doi.org/10.1071/EN05062

Nicholson , G. J., Theodoropoulos, T., & Fabris, G. J. (1991). Petroleum and chrolinated hydrocarbons in the axial muscle tissue and liver of sand flathed (*Platycephalus bassensis* Cuvier and Valenciennes) from Port Phillip Bay. *Internal Report No. 197*. Department of Environment and Conservation, Victoria, Australia: Marine Science Laboratories.

Pettigrove, V., & Hoffmann, A. (2003). Impact of urbanisation on heavy metal contamination in urban stream sediments: influence of catchment geology. *Australasian Journal of Ecotoxicology, 9*, 119-128.

Phillips, D. J. H., Richardson, B. J., Murray, A. P., & Fabris, G. J. (1992). Trace metals, organochlorine and hydrocarbons in Port Phillip Bay, Victoria: a historical review. *Marine Pollution Bulletin, 25*, 200-217. doi:http://dx.doi.org/10.1016/0025-326X(92)90226-V

Raissy, M., & Ansari, M. (2014). Health risk assessment of mercury and arsenic associated with consumption of fish from the Persian Gulf. *Environmental Monitoring and Assessment, 186*, 1235-1240. doi:http://dx.doi.org/10.1007/s10661-013-3452-4

Robb, D. H. F., Wotton, S. B., McKinstry, J. L., Sorensen, S. K., & Kestin, S. C. (2000). Commercial slaughter methods used on Atlantic salmon: determination of the onset of brain failure by electroencephalography. *Veterinary Record, 147*, 298-303.

Sampson, J., Easton, A., & Singh, M. (2014). Port Phillip Bay. In: *Estuaries of Australia in 2050 and beyond* (pp. 49-68). Dordrecht: Springer Netherlands.

Scott, D., & Christoff, P. (1988). State of the Environment Report 1988, Victoria's Inland Waters. Melbourne, Australia: Office of the Commission for the Environment.

US EPA. (1996a). US EPA Method 3560C. *Soxhlet extraction.* Retrieved from https://www.epa.gov/sites/production/files/2015-12/documents/3540c.pdf

US EPA. (1996b). US EPA Method 3620B *Florisil cleanup.* Retrieved from https://www3.epa.gov/wastes/hazard/testmethods/sw846/pdfs/Method%203620B,%20Revision%203%20-%201996.pdf

US EPA. (1998). Method 6020A. *Inductively coupled plasma - mass spectrometry.* Retrieved from https://www.epa.gov/sites/production/files/2015-07/documents/epa-6020a.pdf

US EPA. (2000). Method 6010C. *Inductively coupled plasma - atomic emission spectrometry.* Retrieved from https://www.epa.gov/sites/production/files/2015-07/documents/epa-6010c.pdf

van Gelderen, R., & Pettigrove, V. (2011). *Polychlorinated biphenyls (PCBs) within the Yarra/Maribyrnong rivers and Port Phillip Bay, Victoria, Australia.* Centre for Aquatic Pollution Identification and Management, Publication No. 4, Parkville, Victoria.

Verdouw, J. J., Macleod, C. K., Nowak, B. F., & Lyle, J. M. (2011). Implications of age, size and region on mercury contamination in estuarine fish species. *Water, Air, & Soil Pollution, 214*, 297-306. doi:http://dx.doi.org/10.1007/s11270-010-0424-y

Victoria State Government. (2016). Victorian Recreational Fishing Guide: The State of Victoria.

Walker, T. I. (1982). Effects of length and locality on the mercury contents of blacklip abalone (*Notohaliotis ruber* (Leach), blue mussel, *Mytilus edulis planulatus* (Lamarck), sand flathead, *Plathycephalus bassensis* Curvier & Valenciennes, and yank flathead, *Plathycephalus*

caeruleopuntatus (McCulloch), from Port Phillip Bay, Victoria. *Australian Journal of Marine and Freshwater Research, 33*, 553-560. doi:http://dx.doi.org/10.1071/MF9820553

LIST OF TABLES

Table 1. Physiological parameters (mean ± SD, with ranges in brackets) of sand flathead collected at 6 locations in Port Phillip Bay, Australia, in 2015. Values with different letters are statistically different ($p < 0.05$).

Site	*N*[†]	Total Length (mm)	Total Weight (g)	White Muscle[‡] Moisture (%)	White Muscle[‡] Lipids (%)
Sorrento	18	247 ± 16.2 [a] (207-279)	91.5 ± 19.2 [a] (52-127)	75.4 ± 0.64 [a] (74.5-75.9)	0.88 ± 0.17 [a] (0.7-1.1)
Geelong Arm	12	265 ± 26.7 [ab] (225-309)	120 ± 41.1 [ab] (71-211)	76.4 ± 1.31 [a] (75.2-77.8)	0.83 ± 0.25 [a] (0.6-1.1)
St. Leonards	13	244 ± 13.9 [a] (214-261)	85.5 ± 15.9 [a] (53-108)	77.0 ± 1.65 [a] (75.5-79.3)	0.80 ± 0.16 [a] (0.6-1.0)
Corio Bay	17	264 ± 38.8 [ab] (217-368)	127 ± 63.8 [ab] (60-312)	75.1 ± 0.19 [a] (74.9-75.4)	1.08 ± 0.33 [a] (0.7-1.5)
Mordialloc	18	239 ± 22.4 [a] (198-279)	93.9 ± 26.6 [a] (54-144)	75.7 ± 1.30 [a] (74.0-77.4)	0.96 ± 0.18 [a] (0.7-1.2)
Hobsons Bay	18	285 ± 45.0 [b] (227-439)	158 ± 98.0 [b] (74-522)	75.1 ± 0.90 [a] (73.7-76.0)	0.74 ± 0.22 [a] (0.5-1.1)

[†] Weight and length averaged from all fish captured at each site

[‡] Moisture and Lipids measured on pooled samples. Ns for moisture and lipids as in Table 2.

Table 2. Trace elements (mg/kg wet weight) found in the white muscle[#] of sand flathead collected from Port Phillip Bay in Feb/March 2015. Results are presented as the mean of the composite pools for each site (± SD where possible). Values with different letters are statistically different ($p < 0.05$). Note: figures with < amounts are below the limits of detection.

Site	*N*[a]	Tot As	Cd	Cr	Cu	Pb	Hg	Ni	Se	Ag	Zn
Sorrento	4	7.30 ± 1.14 [c]	<0.01	<0.05	0.16 ± 0.05 [a]	<0.01	0.14 ± 0.04 [ab]	0.02 ± 0.00 [a]	0.45 ± 0.06 [a]	<0.02	5.93 ± 1.69 [a]
Geelong Arm	3	2.13 ± 1.36 [a]	<0.01	<0.05	0.11 ± 0.01 [a]	<0.01	0.12 ± 0.04 [ab]	0.02 ± 0.01 [a]	0.45 ± 0.06 [a]	<0.02	6.97 ± 0.25 [a]
St. Leonards	4	7.87 ± 2.23 [c]	<0.01	<0.05	0.13 ± 0.01 [a]	<0.01	0.10 ± 0.04 [a]	0.02 ± 0.00 [a]	0.44 ± 0.07 [a]	<0.02	6.70 ± 0.90 [a]
Corio Bay	5	2.38 ± 1.28 [a]	<0.01	<0.05	0.10 ± 0.01 [a]	<0.01	0.19 ± 0.06 [ab]	0.02 ± 0.01 [a]	0.47 ± 0.04 [a]	<0.02	7.16 ± 0.63 [a]
Mordialloc	5	6.40 ± 3.10 [bc]	<0.01	<0.05	0.14 ± 0.00 [a]	<0.01	0.29 ± 0.14 [c]	<0.01	0.47 ± 0.05 [a]	<0.02	6.34 ± 0.37 [a]
Hobsons Bay	5	2.89 ± 1.68 [ab]	<0.01	<0.05	0.11 ± 0.01 [a]	<0.01	0.20 ± 0.12 [ab]	<0.01	0.42 ± 0.04 [a]	<0.02	6.46 ± 0.95 [a]

[#] Each sample consisted of composite pools of 30g fillets from 3 fish of similar size and sex caught at the same site (3 x 10g from each fish)

Table 3. Historical trends in metal contaminant burdens (mg/kg wet weight) in sand flathead fillets collected from Port Phillip Bay.

Date of collection	Tot As	Cu	Hg	Ni	Se	Pb	Zn	Reference
1975-78	-	-	0.50	-	-	-	-	(Walker, 1982)
1990	-	-	0.23	-	-	-	-	(Fabris et al., 1992)
1994†	8.3	0.36	0.13	0.05	ns	<0.05	4.98	(Harris et al., 1996)
2003	13.0	0.13	0.21	-	0.49	0.05	5.0	(Fabris et al., 2006)
2015†	4.83	0.13	0.17	0.01	0.45	< 0.01	5.59	Present study

ns: not specified † *averaged from 6 locations within the Bay - : not analysed*

Figure 1. Sampling locations in Port Phillip Bay, 2015.