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Mercury and Organochlorines in Black Bream, *Acanthopagrus butcheri*, from the Gippsland Lakes, Victoria, Australia: Evidence for Temporal Increases in Mercury levels

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Total mercury and organochlorine insecticide (α-HCH, lindane, β-HCH, heptachlor, δ-HCH, aldrin, heptachlor epoxide, α- and β-endosulfan, dieldrin, 4,4′–DDE, endrin, 4,4′-DDD, 4,4′-DDT) concentrations were measured in black bream from 10 widely separated sites within the estuarine Gippsland Lakes, Victoria south-east Australia. Mercury concentrations (mean 0.22 μg g^{-1} wet weight) in the axial muscle tissues were below the maximum concentration in fish permitted for human consumption (0.5 μg g^{-1}), but they were at least 58% higher than those for black bream tested during 1978–79. The cause of the observed increase was not investigated in this study. However, while most of the mercury in the Gippsland Lakes catchment is thought to be due to the legacy of past gold mining, atmospheric emissions from coal burning power plants are likely to contribute to the existing load.

Organochlorine insecticides were detected in all fish, but the concentrations were < 10 ng g^{-1} (wet weight) in both axial muscle and liver tissues. These levels are at least one order of magnitude below the maximum residue levels permitted in fish for human consumption. The bischlorophenol (DDT) group of insecticides was the major component in most cases, and lipid weight concentrations varied between 12 and 85 ng g^{-1} (mean 39 ng g^{-1}) for axial muscle tissues and between 17 and 61 ng g^{-1} (mean 35 ng g^{-1}) for liver tissues.

Keywords: mercury; organochlorine insecticides; bioaccumulation; fish; black bream; *Acanthopagrus butcheri*.

Contamination of fish with toxic chemicals such as mercury and organochlorine insecticides is of ecological and health concern world-wide (Goldberg, 1995). These contaminants persist in the environment long after inputs have ceased and often reach water bodies where they can accumulate in fish. Anthropogenic compounds that accumulate in fish have the potential to impair reproduction or development through their neurotoxic, oestrogenic and immunogenic activities in both the fish and human consumers (Shenker *et al*., 1998).

The Gippsland Lakes, situated in a rural environment in eastern Victoria (south-east Australia), consist of a group of estuarine lagoons separated from Bass Strait by broad sandy barriers (Fig. 1). The lakes are fed by five major rivers – the Latrobe, Avon, Mitchell, Nicholson and Tambo Rivers – which drain a 20 600 km^2 catchment. Of these, the Latrobe and Mitchell Rivers together drain almost one-half of the total catchment. The Gippsland Lakes system is connected to Bass Strait by a permanent narrow opening maintained by suction dredging at Lakes Entrance. Activities within the catchment vary from heavy industrial (such as the mining and use of brown coal for Victoria's electricity supply) to agricultural (such as vegetables and ornamental plants, sheep grazing and cattle grazing), and commercial and recreational fishing. This use of the catchment's natural resources has been heavy and continuous since the 1840s when European settlement began.

There are no natural sources of mercury in the Gippsland Lakes catchment. Historical mercury contamination occurred from the treatment of gold ores with mercury during the late 1890s, waste-water discharges from industrial processes to rivers and streams and atmospheric emissions from coal-fired power plants in the Latrobe Valley (Glover *et al*., 1980). In particular, atmospheric fallout of anthropogenic mercury emissions is now thought to be responsible for the elevated levels of methylmercury that have been detected since the late 1980s in piscivorous fish from aquatic environments

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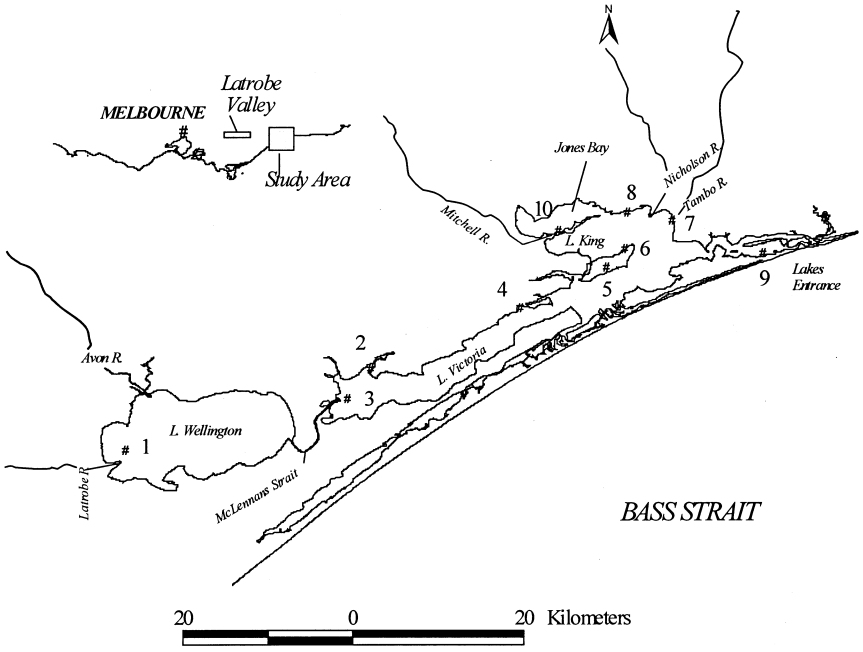

Fig. 1 Sampling sites in the Gippsland Lakes (37.95485° S, 147.66042° E), Victoria, Australia: 1. L. Wellington; 2. Blonde Bay; 3. Spoon Bay; 4. Masons Bay; 5. L. Victoria; 6. Point King; 7. Tambo; 8. Swan Bay; 9. Flanagan Is.; 10. Jones Bay.

remote from industrial sources (Fitzgerald *et al.*, 1997). With the exception of endosulfan, general use of organochlorine insecticides as agricultural chemicals has been prohibited in Victoria, Australia since 1989 (Lee, 1990). Historical usage and the environmental persistence of organochlorine insecticides such as DDT in the catchment is evident from its presence in rivers draining agricultural areas of the Gippsland Lakes (Chapman and Stranger, 1995).

The commercial black bream (*Acanthopagrus butcheri*) fishery is the most important commercial fishery for the Gippsland Lakes in terms of value and accounts for more than 89% of Victoria's catch of this species. It is an estuarine species, which can tolerate a wide range of salinities as adults and has been recorded moving into the freshwater reaches of estuaries (Gunthorpe, 1997). Black bream feed on a wide range of animal species including bivalve and gastropod molluscs, crabs and polychaete worms (Gunthorpe, 1997), but very little is known about the levels of contaminants in the black bream food chain. The concentrations of organochlorine insecticides in fish from Gippsland Lakes have not previously been monitored, whereas mercury levels were studied in detail during the late 1970s (Glover *et al.*, 1980). The present study was initiated by Fisheries Victoria as part of its fish habitat management objective to identify critical fish habitats and threatening processes.

Materials and Methods

Black bream were collected from ten sites in the Gippsland Lakes during early spring, September, 1997. The sites were chosen to provide good spatial coverage of all the water bodies within the Gippsland Lakes and its major freshwater inputs.

The fish were captured using gill-nets with a mesh size designed to capture fish of the 200–250 mm (fork length) size range. At each sampling site, the gill-nets were set during the evening and retrieved next morning. The captured black bream were sexed by inspecting the gonads, and approximately 15 male and 15 female fish from each site were selected for analysis. These samples were processed within 4 h of collection. The axial muscle tissue, without skin, and liver tissue were removed from each fish using clean stainless steel knives and wooden cutting boards. For each site, the axial muscle tissue (fillet from one side of the fish) from 15 individual fish, selected at random, were used for total mercury determinations. For organochlorine insecticide determinations, axial muscle tissues from all 30 fish were bulked into three sets of ten fish each (five males and five females) per site. The liver tissues were similarly bulked, and these were analysed for organochlorine insecticides only.

Total mercury concentrations in axial muscle tissues from individual fish were determined by the analytical procedure adopted by Fabris *et al.* (1992). An accurately weighed sub-sample (5–7 g) of homogenized fresh weight tissue was digested with 10 ml concentrated nitric acid in a 50 ml graduated borosilicate glass tube. The mixture was heated at 40°C until all the tissue had been digested, the solution was then heated at 90°C for 3 h. After cooling, the solution was diluted to 50 ml and the mercury concentration determined by cold vapour

atomic absorption spectrometry. Organochlorine insecticides in homogenized, bulked axial muscle tissues (25–30 g) and bulked liver tissues (10–20 g) were determined by gas–liquid chromatography (Varian 3400 equipped with a 30 m capillary column of 0.25 μm ID, having a 0.25 m stationary phase film of 50% phenyl and 50% methyl silicone) and electron capture detection after soxhlet extraction and silica gel/alumina column chromatography clean-up (UNEP/IOC/IAEA, 1992; Lauenstein and Cantillo, 1993). Confirmation of the compounds present at measurable levels was performed by gas–liquid chromatography and mass selective detection (HP 6890 series GC/MSD and a 30 m capillary column) using single ion monitoring mode. The quality assurance of the mercury determinations was assessed from the analysis of two National Research Council, Canada, standard reference materials prepared from fish muscle tissue (Table 1). The quality assurance of the organochlorine insecticides determinations was assessed by determination of organochlorine compounds in an International Atomic Energy Agency (IAEA) reference material prepared from shrimp homogenate (Table 2). 2,4,5,6-Tetrachloro-*m*-xylene and decachlorobiphenyl were added to samples prior to extraction as surrogate standards to monitor the efficiency of the analytical procedure. Recoveries for the two surrogate standards added to samples and blanks prior to the soxhlet extraction were 74 ± 10% and 70 ± 20% for 2,4,5,6-tetrachloro-*m*-xylene and decachlorobiphenyl, respectively.

Statistical computations of the results were performed using SAS software (SAS Institute Inc., Cary, North Carolina, USA). Cochran's test for homogeneity of variances was used to determine whether transformation of the data was required. The generalized linear models (GLM) procedure for factorial analysis of covariance with unequal replication was used as the statistical tool to determine whether or not there were significant differences in the concentrations in the muscle (mercury and insecticides) and liver (insecticides) tissues between sites (Tabachnick and Fidell, 1989). The GLM procedure was also used to compare the mercury concentrations from this study with those in black bream during 1978–79 (Glover *et al.*, 1980). In this case the least squares means, using time as the main effect and fish length as the covariate, were compared. All statistical analyses were performed on natural log transformed data. The Type III sums of squares of the GLM analyses were used to test the null hypothesis (H_0 = no difference between means). Duncan's multiple range test was used to rank any differences in mean concentrations of mercury and insecticides between sites within the Gippsland Lakes.

TABLE 1

Results (μg g^{-1}) for mercury in National Research Council, Canada, dogfish muscle standard reference material.

Material	Certified values ± 95% confidence interval	Obtained ± S.D. ($n = 8$)
DORM 1	0.798 ± 0.074	0.795 ± 0.033
DORM 2	4.64 ± 0.26	4.31 ± 0.08

TABLE 2

Results (ng g^{-1}) for organochlorine insecticides in International Atomic Energy Agency shrimp homogenate reference material (MA-A-3/OC).

Compound	IAEA ± S.D.	Obtained ± S.D. ($n = 7$)
Lindane	3.2 ± 3.5	0.8 ± 0.5
4,4′-DDE	4.7 ± 3.4	7.0 ± 2.0
4,4′-DDD	0.81 ± 0.76	0.6 ± 0.2
4,4′-DDT	3.2 ± 3.5	0.4 ± 0.2
Aldrin	0.7 ± 0.5	0.8 ± 0.4

Results and Discussion

Approximately equal numbers of male and female fish from each site were pooled and analysed for the insecticides. Male fish can have lower gonad lipid content and lower ratios of gonad weight to body weight (Hails, 1983) and although there may not be differences in organochlorine contamination between sexes for fish (Harding *et al.*, 1997; Hellou *et al.*, 1997) we wanted to ensure that consistency in the lipid content between samples was maintained since the proportion of lipophilic compounds in fish tissue is directly proportional to its lipid content (Connell and Miller, 1981).

Although DDT (*bis*-chlorophenol) compounds have been banned in Australia since 1989 (Lee, 1990), DDE, a by-product of DDT decomposition was the organochlorine compound most frequently detected in both the axial muscle and liver tissues. DDT and DDD, a second by-product of DDT decomposition, were detected less frequently and at lower concentrations than DDE. Total DDT (i.e. DDT + DDE + DDD) concentrations were < 10 ng g^{-1} (wet weight) for both muscle and liver tissues at all sites. Lipid weight concentrations varied between 12 and 85 ng g^{-1} (mean 39 ng g^{-1}) for muscle and between 17 and 61 ng g^{-1} (mean: 35 ng g^{-1}) for liver tissues (Table 3). Some or all of the compounds in the cyclodiene group of insecticides (aldrin, dieldrin, heptachlor, heptachlor epoxide, and endosulfan) were detected in both the muscle and liver tissues of the black bream. With the exception of samples from Tambo (site 7), the sum of concentrations of cyclodienes in axial muscle tissues and liver tissues were lower than those of the bis-chlorophenol group (Table 3). Wet weight concentrations were in all cases < 10 ng g^{-1}, while lipid weight concentrations ranged from < 10 to 94 ng g^{-1} (mean 28 ng g^{-1}) in muscle and from < 10 to 96 ng g^{-1} (mean: 25 ng g^{-1}) in liver tissues (Table 3). Lindane is primarily ($> 99\%$) composed of the gamma isomer (γ-HCH) of the hexachlorocyclodiene family of insecticides which includes the α-, β- and δ-isomers determined in this study. Wet weight concentrations were in all cases

TABLE 3

Mean insecticide concentrations (ng g^{-1}) in axial muscle and liver tissue of black bream from the Gippsland Lakes collected during 1997; ww = wet weight, lw = lipid weight.[a]

	Location (Fig. 1)	Muscle			Liver		
		ΣDDT	ΣCHC	ΣCYC	ΣDDT	ΣCHC	ΣCYC
1	L. Wellington						
	ww	< 10	< 10	< 10	< 10	< 10	< 10
	lw	49	< 10	< 10	61	< 10	< 10
2	Blonde Bay						
	ww	< 10	< 10	< 10	< 10	< 10	< 10
	lw	29	< 10	44	46	< 10	< 10
3	Spoon Bay						
	ww	< 10	< 10	< 10	< 10	< 10	< 10
	lw	27	< 10	< 10	37	< 10	< 10
4	Masons Bay						
	ww	< 10	< 10	< 10	< 10	< 10	< 10
	lw	26	< 10	< 10	17	10	< 10
5	L. Victoria						
	ww	< 10	< 10	< 10	< 10	< 10	< 10
	lw	29	< 10	< 10	33	< 10	< 10
6	Point King						
	ww	< 10	< 10	< 10	< 10	< 10	< 10
	lw	85	< 10	24	34	< 10	22
7	Tambo						
	ww	< 10	< 10	< 10	< 10	< 10	< 10
	lw	84	< 10	94	28	21	96
8	Swan Bay						
	ww	< 10	< 10	< 10	< 10	< 10	< 10
	lw	21	< 10	< 10	29	< 10	28
9	Flanagan Is.						
	ww	< 10	< 10	< 10	< 10	< 10	< 10
	lw	12	< 10	21	25	31	33
10	Jones Bay						
	ww	< 10	< 10	< 10	< 10	< 10	< 10
	lw	28	< 10	< 10	36	< 10	17

[a] ΣDDT = DDT + DDE + DDD; ΣCHC = αHCH + lindane + βHCH + δHCH; ΣCYC = aldrin + heptachlor + heptachlor epoxide + dieldrin + endrin + αendosulfan + βendosulfan.

< 10 ng g^{-1} while lipid weight concentrations ranged from < 10 to 31 ng g^{-1} (mean: < 10 ng g^{-1}) in liver tissues (Table 3). All organochlorine insecticide concentrations in the black bream were at least 10 times lower than the maximum residue limits allowed in fish for human consumption by the Australian and New Zealand Food Standards Code (ANZFA, 1998). Duncan's multiple range test ($\alpha = 0.05$, $df = 20$) did not detect any significant ($p = 0.2$) differences between sites within the Gippsland Lakes for total insecticide concentration in either the axial muscle or liver tissues.

The overall mean total mercury concentration for the individual 150 black bream samples from the 10 sites analysed in this study was 0.22 μg g^{-1} wet weight (range 0.07–0.61 μg g^{-1}; Table 4). This concentration is below the maximum permitted concentration (0.5 μg g^{-1}) allowed in fish for human consumption by the Australian and New Zealand Food Standards Code (ANZFA, 1998). Total mercury concentrations in black bream from Lake Wellington (site 1) were significantly higher than those from all other sites (Table 4), whereas fish from Spoon Bay (site 3) in Lake Victoria also had concentrations higher than those from the other eight sites (Table 4). The lowest concentrations were recorded in fish collected from Blonde Bay (site 2) and Jones Bay (site 10).

Glover *et al*. (1980) surveyed mercury in 99 individual black bream samples from the Gippsland Lakes during 1978–79. They collected their samples from sites located on the eastern side of the Gippsland Lakes, roughly corresponding to the regions covered by sites 4–10 in this study (Fig. 1). The length normalized least–squares mean mercury concentration for black bream from these 7 sites for the present study is higher than that obtained for the 1978–79 survey (Table 5 and Table 6.This result suggests that the mean mercury concentration in black bream from the Gippsland Lakes is now at least 58% higher than that which was recorded during 1978–79. This finding contrasts with a reported 50% decrease in mean mercury concentration in sand flathead (*Platycephalus bassensis*) from Port Phillip Bay near the city of Melbourne (Fig. 1) over the period 1975–78 to 1990 (Fabris *et al*., 1992).

The sources of mercury to the Gippsland Lakes catchment have been identified as having anthropogenic rather than natural origins (Glover *et al*., 1980). Mercury was widely used for the recovery of gold from alluvial deposits and quartz reefs, between the 1850–1930s. Glover *et al*. (1980) estimated that between 63 and 76 tonnes of mercury was released to the Gippsland Lakes catchment from this source. Because gold recovery

TABLE 4

Summary statistics for mercury concentrations in axial muscle tissues of black bream from the Gippsland Lakes.

Location (Fig. 1)	Fish length mm ± sd ($n = 15$)	Hg concentration (μg g^{-1} wet weight)		
		Mean ($n = 15$)	Min.	Max.
1 L.Wellington	236 ± 16	0.35	0.18	0.58
2 Blonde Bay	217 ± 14	0.17	0.09	0.26
3 Spoon Bay	241 ± 8	0.24	0.13	0.35
4 Masons Bay	244 ± 9	0.22	0.1	0.4
5 L. Victoria	215 ± 9	0.24	0.07	0.61
6 Point King	208 ± 9	0.18	0.08	0.29
7 Tambo	212 ± 9	0.22	0.08	0.4
8 Swan Bay	212 ± 11	0.2	0.11	0.44
9 Flanagan Is.	218 ± 9	0.18	0.08	0.35
10 Jones Bay	240 ± 6	0.16	0.08	0.27
Total mean	224 ± 14	0.22	0.07	0.61
MPC[a]		0.50		

[a] ANZFA (1998).

activities were invariably located near sources of water, most of the mercury lost in the gold recovery process would have been deposited in the rivers and streams of the catchment. Recent episodes of elevated mercury concentrations have been reported in the waters of the Latrobe, Avon, Mitchell and Tambo Rivers (Tiller, 1990). These elevated concentrations were presumed to be due to re-suspension of sediments contaminated with mercury during high flow periods. Eventually, these contaminated sediments would be deposited within the Gippsland Lakes. Some of the contaminated sediments might escape to Bass Strait, particularly during major flood events, but it is likely that most of the mercury reaching the Gippsland Lakes would be trapped within the Lakes system. Glover *et al.* (1980) also found high concentrations of mercury in the sediments, and highest concentrations were observed in the period following severe flooding of the Lakes, and it was concluded that this was due to the mobilization of contaminated riverine sediments. During 1978–79, the accumulation of mercury in sediments from Lake King was up to 58 μg g^{-1} (dry weight) and Lake Victoria up to 100 μg g^{-1}. Water quality monitoring over the past 10 years has shown that the waters from Lake Wellington have had consistently higher mercury concentrations than those found in other parts of the Gippsland Lakes (Brown *et al.*, 1998). Mercury concentrations in Lake Wellington have ranged from 2 to 40 ng l^{-1} (median: 7 ng l^{-1}) compared to a range from < 2 to 33 ng l^{-1} (median: 3 ng l^{-1}) for Lake King. The mercury concentrations in the water vary seasonally, being higher during periods of high river flows. It should be noted that the present survey was carried out during a period when south-eastern Victoria, which includes the Gippsland Lakes catchment, was experiencing a severe rainfall deficiency (Bureau of Meteorology, Australia), hence river flows were low for at least 12 months prior to this study. Additional mercury loads have also been introduced to the Latrobe River catchment in the past through wastewater discharges from a paper pulp mill incorporating a chlor-alkali plant located some 70 km west of Lake Wellington. The plant started operating during 1956 and for the period from 1956–1980, this was estimated to have contributed about 18 tonnes of mercury to the catchment (Glover *et al.*, 1980). The chlor-alkali plant stopped operating during 1986.

Atmospheric deposition of mercury escaping from stack emissions of coal-fired power stations in the Latrobe Valley, 70 km to the east of Lake Wellington (Fig. 1) is another source of mercury to the Gippsland Lakes catchment. Estimates of the quantity of mercury that is deposited from atmospheric emissions are subject to

TABLE 5

Comparison of total mercury concentrations in axial muscle tissues of black bream from eastern Gippsland Lakes during 1978–79 (Glover et al., 1980) and 1997 (present study).

Date	Sample size	Least-squares mean mercury concentration (μg g^{-1} wet weight)[a]
1978–79	99	0.11
1997	105	0.18

[a] GLM model: ln(mercury concentration) = date ln(fork length). $p < 0.0001$; $F = 53.29$ (Power = 0.8, $\alpha = 0.05$).

TABLE 6

Between site comparisons of total mercury concentrations in axial muscle tissues of black bream from the Gippsland Lakes during 1997.

	Location (Fig. 1)	Duncan grouping[a]
1	Lake Wellington	A
3	Spoon Bay	B
4	Masons Bay	BC
7	Tambo	BC
5	Lake Victoria	BC
8	Swan Bay	BC
9	Flanagan Island	BC
6	Point King	BC
2	Blonde Bay	C
10	Jones Bay	C

[a] Duncan's multiple range test ($\alpha = 0.05$ $df = 140$, $p < 0.001$). Means with the same letter are not significantly different.

large uncertainties and tend to vary widely (US EPA, 1997) since atmospheric transport of mercury depends on the chemical and physical species involved (Schroeder and Menthe, 1998) and these are not readily quantified. The brown coal mined in the Latrobe Valley contains 62% moisture and has a mean mercury concentration of 0.18 μg g^{-1} dry weight; (Bone and Schaap, 1981). Power generation in the Latrobe Valley using brown coal started early this century, and from 1917 to 1995 a total of 6.4×10^{11} tonnes of brown coal was used for power generation (Jackson, 1996). Assuming a 40% control efficiency (Pirrone *et al*., 1998), then up to 27 tonnes of mercury may have been discharged into the atmosphere in the Latrobe Valley over this 78-year period. It is likely, as has been reported in other areas (US EPA, 1997), that some of this mercury would have been deposited within the 20 600 km^2 Gippsland Lakes catchment.

The 58% increase in mercury levels in black bream compared with 20 years earlier may be due to a complex interaction of several factors including changes in ambient mercury levels, immediate past history of the fish and the age of fish used in the study. Increases in ambient mercury levels (i.e. in water) can result from current inputs or from remobilization mercury in contaminated sediments. The fact that highest mercury concentrations were recorded in fish from Lake Wellington is consistent with data from water monitoring. A confounding factor might be due to differences in the age of the fish. Older, and generally longer, fish are expected to have higher mercury concentrations in their tissues than young fish through bioaccumulation. The length-age relationship for black bream from the Gippsland Lakes is complex and while the maximum age recorded for black bream in the Gippsland Lakes is 29 years, the majority of fish in the lakes, including those used in the present study (200–240 mm length), are 5–10 years of age (Morison *et al*., 1998). To some extent, the effects due to differences in size (age) in fish between the two studies were compensated by standardizing mercury concentrations using least–squares means with fish length as a covariate (Table 5). The migratory habits of black bream have not been categorized. However, anecdotal evidence indicates that mature black bream move into the rivers draining into the Gippsland Lakes, or into the deep channels within the lakes during winter and spring, whereas during summer and autumn they are commonly found dispersed throughout the shallower areas of the lakes. The fish analysed in this study were captured at the beginning of spring, after they had probably spent winter in the various rivers and streams draining into the Gippsland Lakes, whereas the fish analysed by Glover *et al*. (1980) were captured at various times over an extended period from December 1977 to January 1979. It is possible the rivers and streams may have higher ambient mercury concentrations than the Gippsland Lakes. Given the complex nature of anthropogenic sources and bioaccumulation processes, the cause for both the differences in mercury concentration in fish from different sites and the increase in mercury concentration in black bream over the past 20 years requires investigation, particularly as there is potential for mercury levels in black bream to increase further if atmospheric deposition from coal burning is the major current source of mercury to the Gippsland Lakes catchment.

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