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Biochemical and cytogenetic biomarkers of pollutant exposure in marine fish (*Aldrichetta forsteri* Valenciennes and *Sillago schomburgkii* Peters) near industrial and metropolitan centres in South Australia

M. HAMANN[1], V.A. BOXALL[1], K.S. EDYVANE[2], J.W. EDWARDS[3*] AND K.L. SOOLE[1]

[1] School of Biological Sciences, and [3] Environmental Health Unit, School of Medicine, Flinders University of South Australia

[2] South Australian Research & Development Institute, West Beach, South Australia

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This project aimed to measure biochemical and cytogenetic biomarkers in marine fish (*Aldrichetta forsteri* and *Sillago schomburgkii*) associated with industrial and urban centres in South Australia. These sites were Port Pirie (affected by metal-contaminated outflows), Barker Inlet (adjacent to Metropolitan Adelaide), and Wills Creek (reference site). The biochemical biomarkers included sorbitol dehydrogenase (SDH) and alanine aminotransferase (ALAT) in serum, adenylate levels (ATP, ADP and AMP) and adenylate energy charge (AEC) in gill and liver, and sodium/potassium ATPase (Na^+,K^+-ATPase) in gill. Erythrocyte micronucleus frequency was a marker of cytogenetic effect. Serum enzyme levels were generally higher in fish from Port Pirie and Barker Inlet than in those from Wills Creek, with SDH demonstrating the clearest site-associated differences. Tissue adenylates were consistently lower at Port Pirie than elsewhere, suggesting a greater metabolic strain in fish at this site. AEC in gill and liver were consistently lower at Port Pirie than at Wills Creek, with Barker Inlet generally between these two. The reversed rank order was observed with erythrocyte micronucleus frequencies. Seasonal variations in the biomarkers may be attributed either to seasonal physiological changes in fish or changes in pollutant input levels or compositions. Na^+,K^+-ATPase did not differ between sites nor seasons in this study. This work shows that biochemical and cytogenetic differences occur in marine fish at specific locations in South Australia. It also shows that of these tests, serum SDH and erythrocyte micronuclei are potentially the most sensitive and reliable biomarkers of pollutants effects on marine fish. The results also suggest that these data may be used as a baseline against which future changes in marine water quality, and their consequent biological effects, can be compared.

Keywords: fish, serum enzymes, micronucleus, adenylates, adenylate energy charge.

Introduction

Coastal marine environments in Australia and worldwide, are susceptible to a wide variety of human activities which may result in adverse effects to local or regional ecologies (Zann 1995). Estuaries and coastal seas are important sites of marine resources such as commercial and recreational fisheries and their feeding, breeding and nursery areas, yet these are also sites of major and increasing human coastal population. As such, coastal estuaries and seas worldwide are increasingly at risk from toxic contamination from land-based discharges, coastal industries and shipping. The impact of environmental contamination is frequently apparent only after major effects on the population of marine organisms or human health have occurred. However, pollutant stress may be manifest in the impairment of physical functions such as respiration, osmoregulation and energy metabolism, or may

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appear as decreased reproductive efficiency, increased susceptibility to disease or predation, and a decreased adaptability to environmental change (Adams 1990). Biochemical and cytogenetic responses of marine organisms may act as useful biomarkers of effect when examining the role of effluents and chemical discharges in the health of populations, communities and ecosystems.

Although biomarkers of effect have been applied to many laboratory studies of the effects of stressors on fish and invertebrate species, they have not been widely applied to field studies in Australia. This is because more attention has been directed to analytical chemical techniques of assessing environmental contamination. As a consequence, the causal links between pollutants and biomarker effects have not been fully established and the relationships between specific pollutants and pollutant mixtures, biomarker responses and subsequent effects in populations or ecosystems are not understood (Mayer *et al.* 1992). A wide range of biomarkers are available and the specific utility of individual tests may depend on study site, target species and pollutant characteristics. Biomarkers of effect that have been investigated recently include DNA adducts (Karakoc *et al.* 1997), cytochrome P450 (Bucheli and Fent 1995, Gadagbui and Goksøyr 1996) and other enzyme activities (George 1994, Gadagbui and Goksøyr 1996, Karakoc *et al.* 1997).

Biochemical indicators of effects of pollutant exposures include those relating to energy metabolism and osmoregulation. Tissue adenylates represent a short-term metabolic energy pool. Of the adenylates, adenosine triphosphate (ATP) is the primary source of metabolic energy and adenosine monophosphate (AMP) the least, with adenosine diphosphate (ADP) an intermediate source. Adenylate energy charge (AEC) is a weighted measure of the adenylate pool (Atkinson 1968, Haya and Waiwood 1983) defined by the ratio

$$AEC = \frac{ATP + 0.5\,ADP}{ATP + ADP + AMP}$$

Sodium/potassium ATPase (Na^+, K^+-ATPase) is a membrane bound protein responsible for the transport of ions across cell membranes to maintain Na^+ and K^+ gradients, and plays a central role in whole body ion regulation in marine organisms (Haya and Waiwood 1983). Both AEC and Na^+, K^+-ATPase have been found to respond to metals and other pollutants (Tucker 1979, Tucker and Matte 1980, Haya and Waiwood 1983, Haya *et al.* 1983, Dixon and Hilton 1985).

Exposure to pollutants may cause cell damage in tissues of organisms, especially sites of metabolism and excretion of xenobiotics. This damage may result in the release of organ-specific intracellular components into the circulatory system. For example, sorbitol dehydrogenase (SDH) in serum of fish is an indicator of liver damage while serum alanine aminotransferase (ALAT) indicates damage to a variety of organs including liver, kidney and heart (Dixon and Hilton 1985, Jackim 1986).

Environmental pollutants may be genotoxic, exerting an influence on the DNA of exposed organisms. This may have effects on reproduction if germ cells are affected, or on the development of neoplasia in germ and somatic cells. Cytogenetic tests such as the detection of chromosome abnormalities, sister chromatid exchanges or micronuclei have been widely used in mammalian systems and in the monitoring of human populations for exposures to genotoxic agents. The micronucleus assay of erythrocytes was developed in the early 1970s in mice

(Heddle 1973, Schmidt 1975) and provides a simple method requiring no culture of blood samples, so is attractive for field investigation of fish and other marine species. Work has been performed to measure micronuclei in molluscs (Wrisberg *et al.* 1992) and fish (Williams and Metcalfe 1992, Al-Sabti 1994, Al-Sabti *et al.* 1994), although much of this work was carried out in European waters using freshwater species. There is little known of marine species, especially those endemic to Australian waters. Some or all of these techniques may be useful non-specific biomarkers of exposure of fish to anthropogenic pollutants in South Australian coastal waters.

Methods

Site selection and description, and species selection

The three sites chosen for this study, Port Pirie, Barker Inlet and Wills Creek, support extensive stands of mangroves (*Avicennia marina*) and seagrasses (Butler *et al.* 1977) in the shallow, sheltered gulf waters of South Australia, and are important nursery areas for numerous commercial and recreational fish and crustacean species (Jones 1984, Jones *et al.* 1996). The location of these sites relative to each other are shown in figure 1. They were selected on the basis of known level and type of pollutant discharges.

Port Pirie, at the head of the Spencer Gulf, is known to be a site of high industrial point source pollution associated with the activities of one of the largest lead/zinc smelters in the world (Ward *et al.* 1986). In contrast, the Barker Inlet–Port Adelaide River estuary, adjacent to metropolitan Adelaide, is characterized by a range of point and diffuse source pollutant inputs associated with a large coastal population (i.e. 1 million residents), including industrial and urban stormwater runoff, treated sewage effluent waters, and wastes, spills and effluents associated with shipping, docking, power generation and manufacturing (Harbison 1986a,b, Thomas *et al.* 1986, Edyvane 1996, Jones *et al.* 1996). Wills Creek, at Price on eastern Yorke Peninsula, was selected as a reference site as it is relatively isolated from urban centres and known point sources of pollution, although it may be affected by agricultural run-off or localized septic tank discharges.

The commercial fish species selected in this study were yellow-eye mullet (*Aldrichetta forsteri*) and yellow-fin whiting (*Sillago schomburgkii*), since they are both estuary opportunistic species in South Australia, are potentially exposed to environmental pollutants for a significant proportion of their life history through diet, sediments, and waterborne chemicals, and are also important commercially and recreationally (Jones 1984, Jones *et al.* 1996).

Fish sampling and tissue selection

Samples were collected on three occasions in 1994. In late summer, winter and spring, with approximately 20 fish collected at each site on each occasion. The proportion of each species depended upon availability at the time. Adult fish of both sexes, 22–28 cm in length, were stunned by a blow to the head and portions of gill and liver were taken from each of 10 fish for analysis of tissue adenylates and Na^+,K^+-ATPase. Gill tissues were obtained rapidly to prevent degradation of ATP and ADP. Gills were frozen *in situ* using a set of steel tongs precooled in liquid nitrogen. Snap frozen tissues were then removed, wrapped in labelled aluminium foil packages, and stored in liquid nitrogen for transport to the laboratory where they were stored overnight at –80 °C prior to analysis. Following gill removal, livers were removed as soon as possible using the snap freezing method described above and were transported and stored as for gills. Blood samples (0.5–2.0 ml) were obtained from fish by cardiac puncture using 21 gauge needles, and samples were transferred to heparinized tubes and kept on ice. About 0.1 ml whole blood was mixed with 1 ml PBS and smeared as a thin film onto a microscope slide. Smears were air-dried and fixed in methanol prior to staining. Clotted samples were centrifuged and serum transferred to fresh tubes as soon as possible upon return to the laboratory.

Tissue adenylates

Gill and liver samples were prepared for adenylate assay by the method of Haya and Waiwood (1983). For gill tissue, the filaments were removed from gill arches while frozen and prior to grinding. Frozen liver samples and gill filaments were ground to a fine powder using a mortar and pestle precooled in liquid nitrogen. One gram of pulverized tissue was homogenized at ice-bath temperature in 2 ml of 6 % perchloric acid using a Brinkman Polytron. Homogenate was allowed to stand at 25 °C for 30 min to ensure destruction of ATPases prior to analysis. Adenylates were measured in 30 000 × *g* supernatants using spectrophotometric assays as described in Jaworek *et al.* (1986), using phosphoglycerate

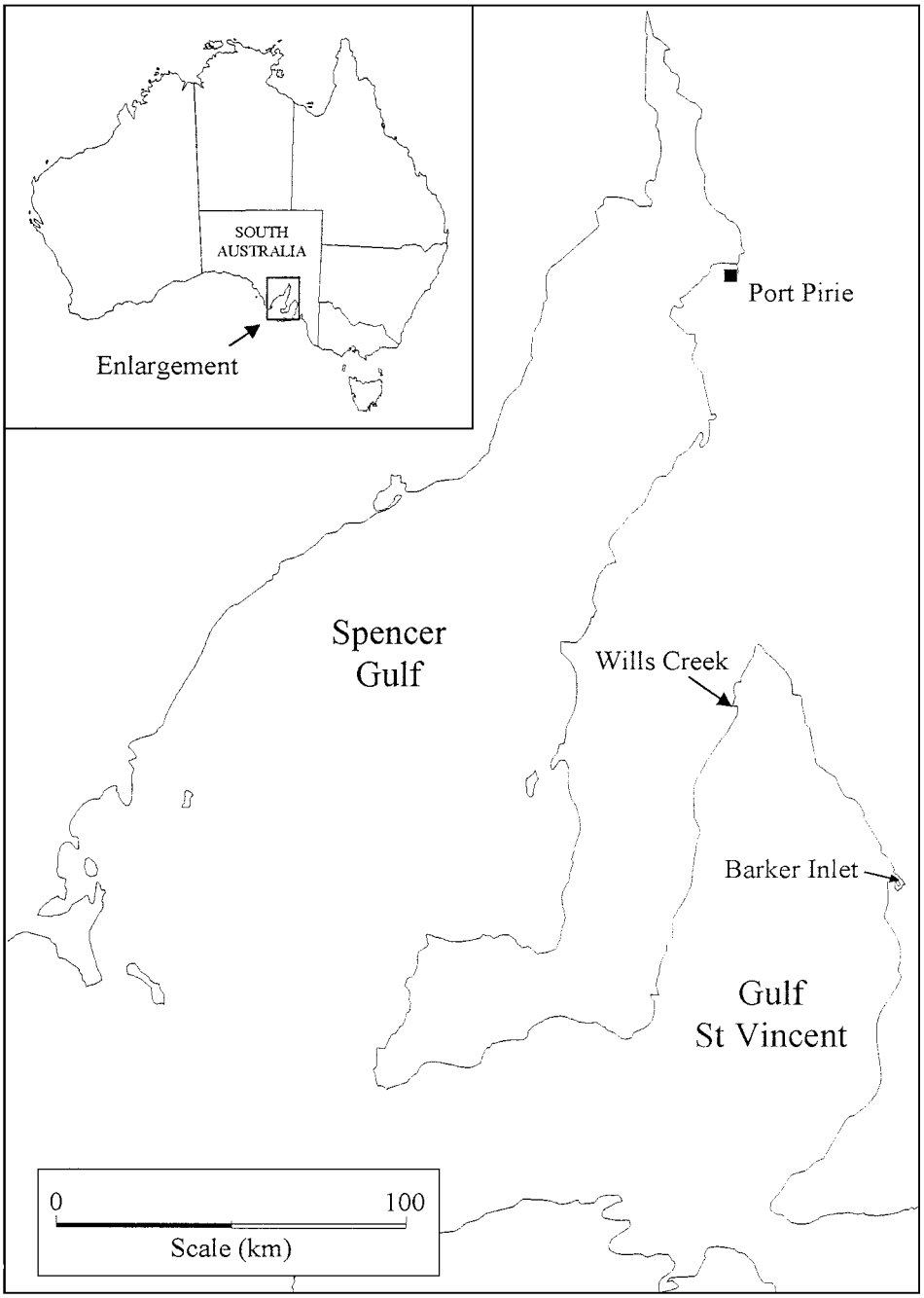

Figure 1. Location of sampling sites in South Australian coastal waters.

kinase/glyceraldehyde phosphate for ATP, lactate dehydrogenase/pyruvate kinase for ADP, and myokinase to estimate AMP.

Serum ALAT and SDH

SDH was measured by the method of Gerlach (1986) and ALAT was measured by the enzyme-linked method of Horder and Rej (1986) with modifications for fish systems from the methods of

D'Appolonia and Anderson (1980). For SDH, assay mixtures contained 0.1 M triethanolamine (pH 7.4, 2 N NaOH, 0.2 mM NADH and 0.1 ml serum (total volume 1.01 ml). This was preincubated at 25 °C for 30 min then 10mM D(–)-fructose was added and absorbance at 340 nm read at minute intervals for 6 min. For ALAT, assay mixtures contained 0.9 ml of 110 mM Tris-HCl, 6 mM alanine, 0.2 mM NADH, four units of LDH and 0.1 ml serum in a total volume of 1.015 ml. Following preincubation at 30 °C for 10 min 6 mM 2-oxoglutarate was added and absorbance at 340 nm was measured at minute intervals for 5 min. Results for both assays are expressed as nmol NADH oxidized min^{-1} ml $serum^{-1}$.

Na^+,K^+-ATPase

A method adapted from Tucker and Matte (1980) was used, in which duplicate assays for ATPase activity in homogenized gill filaments were performed in the presence of KCl (10 mM) or ouabain (1 mM), an inhibitor of Na^+,K^+-ATPase. Gill filaments were ground while frozen and 1 g was homogenized in 3 ml sucrose/EDTA (250 mM/5 mM, pH 7.4). Sample incubations contained 0.2 ml homogenate, ouabain/KCl, and 1 mM ATP with 40 mM $MgCl_2$. Inorganic phosphate (P_i) produced by ATPase was quantitated by comparison with standards containing P_i and results were expressed as µmol P_i liberated h^{-1} mg^{-1} protein. Na^+,K^+-ATPase specific activity was the difference between KCl and ouabain incubations. Protein content of tissue homogenates was estimated by a colorimetric method (Bradford 1976).

Micronuclei in erythrocytes

Fixed blood films were stained in May–Grunwald stain for 5 min, then in giemsa stain (10 % in buffered water) for 15 min. After staining, slides were rinsed in water and washed in three changes of buffered water to allow differentiation. To reduce the possibility of investigator bias, slides were coded by an independent party, labelled and scored blind for average micronucleus frequency per fish. Data were decoded only at the conclusion of the scoring process. For each slide a minimum of 2000 erythrocytes were examined for micronuclei using specific criteria (micronuclei with rounded margins within the cytoplasm and less than 5 % nuclear size, stained the same intensity as the nucleus and appearing at the same focal plane as the nucleus).

Statistical analysis

All data were analysed using Bartlett's test for homogeneity of variance. Homogeneous data were analysed by analysis of variance (ANOVA) followed by Tukey–Kramer or Dunn's multiple comparison post hoc tests. Heterogeneous data were analysed by Kruskal–Wallis ANOVA or Mann–Whitney *U*-test (Gad and Weil 1982).

Results

It was intended that field samples would be collected from 10 fish of each of the investigated species at each location and at each sampling time. However, seasonal availability of fish species was variable, and some samples were over-represented by either mullet or whiting. For each sample period and location, cytogenetic and biochemical data from each species were compared (Mann–Whitney *U*-test). This showed that there were no species differences in these indicators, and the data from all fish were pooled in subsequent analyses. The availability of fish also had an impact on the data collected, with all the animals caught in winter at Wills Creek used for micronucleus analysis and none available for biochemical analyses.

Serum enzyme levels (table 1) were generally higher in the fish from Port Pirie and Barker Inlet than from Wills Creek, although the urban and industrial sites did not differ from each other. No clear effect of season was apparent in these data. Gill and liver adenylate concentrations (table 2) were consistently lower at Port Pirie than at Wills Creek and Barker Inlet, although this only reached statistical significance in a few cases. Adenylate energy charge (table 3) in gill and liver were generally lower at Port Pirie than at Wills Creek, with Barker Inlet intermediate between these two. In summer and spring, liver AEC did not differ between sites, although the lack of data from Wills Creek does not permit any conclusions to be

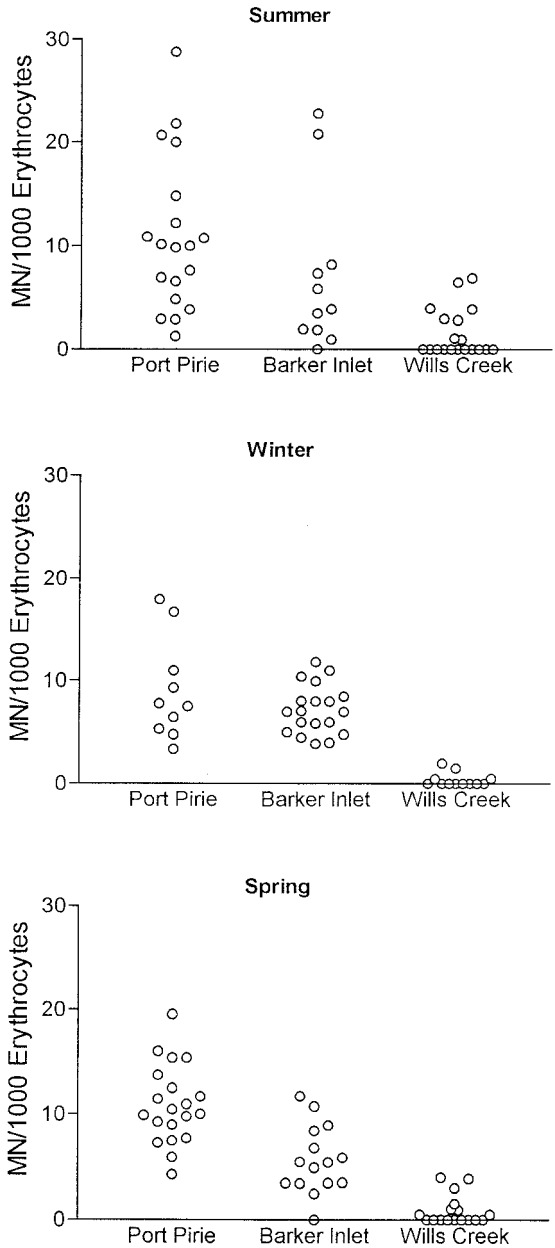

Figure 2. Erythrocyte micronucleus frequencies in *Aldrichetta forsteri* and *Sillago schomburgkii* from South Australian coastal waters.

drawn. No seasonal effects could be determined from the data. Na^+,K^+-ATPase was determined only in *A. forsteri* from each location. Median values did not differ between sites nor between seasons (table 4).

The erythrocyte micronucleus frequencies (MN per 1000 erythrocytes; figure 2) were significantly higher each season at Port Pirie (medians: 10.0, summer; 7.6, winter; 10.3, spring) than at Wills Creek (medians 0.0 each season) (Kruskal–Wallis, $p<0.001$). Barker Inlet data were intermediate (medians 3.9, 7.0, 5.4) and

Table 1. Serum sorbitol dehydrogenase (SDH) and alanine aminotransferase (ALAT) levels in *Aldrichetta forsteri and Sillago schomburgkii* from South Australian coastal waters

		SDH (nmol NADH oxidized min^{-1} ml $serum^{-1}$)		ALAT (nmol NADH oxidized min^{-1} ml $serum^{-1}$)	
	n	Median	Range	Median	Range
Summer					
Port Pirie	20	6.6	0–46.4	6.0	2.0–175.0
Barker Inlet	20	7.9	0–62.6	3.4	0–11.7
Wills Creek	20	1.2	0–11.4	2.4	0–10.7
		PP > WC (p <0.01, KW)		PP > BI (p <0.001, KW) PP > WC (p <0.001,KW)	
Winter					
Port Pirie	20	2.3	0–17.7	9.0	0–16.8
Barker Inlet	20	2.9	0–14.3	9.2	0–20.1
Wills Creek	n/a	n/a	n/a	n/a	n/a
		No significant differences (KW)		No significant differences (KW)	
Spring					
Port Pirie	10	5.9	3. 394–16.8	9.2	5.3–13.7
Barker Inlet	10	10.4	0–97.9	9.1	2.1–12.9
Wills Creek	10	0.7	0–3.6	4.5	3.1–18.0
		PP > WC (p <0.01, KW) BI > WC (p <0.01, KW)		PP > WC (p <0.05, KW)	

n/a—samples unavailable.
*—0 indicates non-detectable levels (< 0.00001 standard units SDH, < 0.0001 standard units ALAT).

Table 2. Total adenylate levels (ATP + ADP + AMP) in gill and liver of *Aldrichetta forsteri* and *Sillago schomburgkii* from South Australian coastal waters (represented by NADH oxidation[a])

		Gill adenylates (nmol NADH oxidized g $tissue^{-1}$)		Liver adenylates (nmol NADH oxidized g $tissue^{-1}$)	
	n	Mean (sem)	Range	Mean (sem)	Range
Summer					
Port Pirie	20	1.26 (0.17)	0.35–2.54	1.93 (0.09)	1.38–2.87
Barker Inlet	20 gill 19 liver	2.05 (0.08)	1.33–2.52	2.16 (0.08)	1.33–2.70
Wills Creek	10	2.01 (0.10)	1.39–2.41	2.41 (0.07)	1.96–2.70
		PP < BI (p <0.001, ANOVA) PP < WC (p <0.01,ANOVA)		PP < WC (p <0.05, ANOVA)	
Winter					
Port Pirie	10	2.01 (0.09)	1.57–2.43	2.10 (0.10)	1.49–2.69
Barker Inlet	19	2.10 (0.06)	1.66–2.67	2.10 (0.04)	1.66–2.38
Wills Creek	n/a	n/a	n/a	n/a	n/a
		No significant differences (MWU)		No significant differences (MWU)	
Spring					
Port Pirie	10	1.25 (0.10)	0.79–1.95	1.75 (0.10)	1.09–2.81
Barker Inlet	10	2.01 (0.05)	1.67–2.23	2.07 (0.07)	1.66–2.51
Wills Creek	10	2.10 (0.08)	1.83–2.50	2.04 (0.05)	1.67–2.33
		PP > BI (p <0.001, ANOVA) PP < WC (p <0.001, ANOVA)		PP < BI (p <0.05, ANOVA)	

n/a—samples unavailable.
MWU—Mann–Whitney *U*-test.
[a]In assays for adenylates 1:1 stoichiometry exists between NADH oxidation and adenylate concentration.

Table 3. Adenylate energy charge (AEC) in gill and liver of *Aldrichetta forsteri and Sillago schomburgkii* from South Australian coastal waters

		Gill AEC		Liver AEC	
	n	Mean (sem)	Range	Mean (sem)	Range
Summer					
Port Pirie	20	0.71 (0.01)	0.62–0.78	0.73 (0.01)	0.66–0.77
Barker Inlet	20 gill 19 liver	0.74 (0.01)	0.66–0.79	0.71 (0.04)	0.68–0.79
Wills Creek	10	0.77 (0.01)	0.65–0.83	0.72 (0.01)	0.66–0.76
		PP < WC (p <0.01, ANOVA)		No significant differences (ANOVA)	
Winter					
Port Pirie	10	0.73 (0.01)	0.66–0.80	0.73 (0.01)	0.66–0.81
Barker Inlet	18 gill 19 liver	0.71 (0.04)	0.69–0.78	0.73 (0.04)	0.69–0.75
Wills Creek	n/a	n/a	n/a	n/a	n/a
		No significant differences (MWU)		No significant differences (MWU)	
Spring					
Port Pirie	10	0.68 (0.01)	0.63–0.76	0.71 (0.01)	0.63–0.76
Barker Inlet	10	0.71 (0.01)	0.66–0.73	0.73 (0.01)	0.67–0.78
Wills Creek	10	0.74 (0.01)	0.72–0.77	0.75 (0.01)	0.72–0.80
		PP < BI (p <0.05, ANOVA) PP < WC (p <0.001, ANOVA) BI < WC (p <0.05, ANOVA)		PP < WC (p <0.05, ANOVA)	

n/a–samples unavailable.
MWU–Mann–Whitney U-test.

Table 4. Na^+,K^+-ATPase activity in gill of *Aldrichetta forsteri* from South Australian coastal waters

		Na^+,K^+-ATPase activity (μmol P_i h^{-1} mg $protein^{-1}$)	
	n	Median	Range
Summer			
Port Pirie	10	4.20	3.12–5.01
Barker Inlet	10	4.35	3.95–4.80
Wills Creek	10	4.84	3.91–6.46
		No significant differences (KW)	
Winter			
Port Pirie	10	4.00	2.69–5.97
Barker Inlet	10	4.12	2.22–6.46
Wills Creek	n/a	n/a	n/a
		No significant differences (KW)	
Spring			
Port Pirie	10	3.26	2.06–4.69
Barker Inlet	10	3.67	2.66–4.89
Wills Creek	10	3.10	2.24–4.88
		No significant differences (KW)	

n/a—samples unavailable.
KW—Kruskal–Wallis ANOVA.
P_i—inorganic phosphate.

significantly higher than at Wills Creek (p<0.05), although these were significantly different from Port Pirie only in spring (p<0.01).

Discussion

Although several papers have described the prevalence of pollutants in South Australian waters (Olsen 1983, 1988, Jones 1989, Kemper *et al.* 1994), there is little

information on biochemical and other effects associated with them. The endpoints examined here as biomarkers of effect of environmental contamination – representing energy metabolism, clinical chemistry and cytogenetic effects – were selected as indicators of general pollutants. That is, the responses observed cannot be definitely ascribed to a single pollutant, since each of these biomarkers is influenced to different extents by metals, hydrocarbons, nutrients and other pollutants. However, these biomarkers of effect may be used to quantify the extent of pollutant impacts on individuals and populations, and in this case can be used to rank the three study areas in terms of overall pollutant stress. The effects of natural stressors, such as temperature, salinity, food availability, predation and reproductive status were not specifically examined in this study.

Port Pirie is historically a site of heavy metal contamination associated with lead smelting and refining, which commenced in 1885. Other metals, such as zinc, antimony, cadmium, copper, gold and silver are also extracted. From 1889 to 1983, over 160 000 tonnes of lead was lost to the local environment through atmospheric emissions (Ward 1983, cited by Maynard *et al.* 1993). Most concern has recently been focused on the effects of the accumulated lead sink on the residents of Port Pirie (Maynard *et al.* 1993) but it is clear that much of the metal contamination of the area (particularly lead, zinc and cadmium) has found its way into the marine environment (Harbison 1984, Ward *et al.* 1986, Edyvane 1996). Other pollutants in the region potentially arise from other urban sources, from harbour and other industrial activities, and from oil spills, such as the `Era' oil spill in 1992 (Wardrop *et al.* 1993).

Spencer Gulf is a large hypersaline marine embayment with little water exchange with the open ocean (Bullock 1975), and consequently poor flushing of pollutants. Serum enzymes, SDH and ALAT, were found in this study to be higher at Port Pirie than at the control site, suggesting significant tissue damage in fish at this location. Gill and liver adenylates and AEC were also significantly depressed at Port Pirie suggesting a disturbance in the energy budget of fish, caused either directly by marine pollutants or as a consequence of general physiological strain associated with other organ and tissue effects. Erythrocyte micronucleus frequencies clearly indicated genotoxicity expressed in animals from Port Pirie, and this may be attributed to genotoxic heavy metals or organic pollutants (Al-Sabti 1994, Al-Sabti *et al.* 1994, Belpaeme *et al.* 1996) The micronucleus assay also represents accumulated effects associated with longer term exposures to genotoxins, unlike the biochemical indicators which may respond to short term variations in chemical exposures.

Na^+,K^+-ATPase was not found to be a reliable biomarker of effect in this study. This enzyme has been shown to be affected by pollutants including metals, pesticides and hydrocarbons (Neufeld and Pritchard 1979, Riedel and Christensen 1979, Tucker 1979, Tucker and Matte 1980, Haya *et al.* 1983, Dixon and Hilton 1985, Sola *et al.* 1994). Salinity, temperature and pH may also affect Na^+,K^+-ATPase activity and other biomarkers (Ivanovici 1980, Neufeld *et al.* 1980, Walesby and Johnston 1980, MacFarlane 1981). Although salinity and temperature dataloggers were placed at each location in this study, one was stolen and the remainder recorded data intermittently or recorded download errors. Hence the influence of these physical parameters on biomarkers could not be determined.

Barker Inlet, a shallow tidal inlet of the Port Adelaide river system, is one of South Australia's most diversely polluted estuaries (Edyvane 1996), receiving inputs from stormwater, treated sewage effluent, thermal, chemical and saline discharges

from power and industrial sites (Harbison 1986a,b, Thomas *et al.* 1986, Nias *et al.* 1993, Edyvane 1996, Jones *et al.* 1996). The complex mixture of organic and inorganic pollutants causes tissue damage in fish, represented by SDH and ALAT, and disturbances in energy balance (total adenylates and AEC) at least equivalent to that from Port Pirie. This may suggest that either the pollutant load is as great as at Port Pirie or that, unlike the single point source of Port Pirie, there may be interactions between pollution components. The genotoxic response was intermediate between those of Port Pirie and Wills Creek, suggesting a moderate exposure to genotoxic chemicals. This further suggests that the higher micronucleus frequencies at Port Pirie may be more likely associated with metal exposure.

Seasonal variations in biomarkers may be due to seasonal physiological events in fish and fish populations, or to changes in pollutant input into the marine environment. At Port Pirie, high SDH and erythrocyte MN frequencies in summer and spring, and lower SDH and MN frequencies in winter were mirrored by gill adenylate lower in summer and spring and higher in winter. Summer samples were collected in March (late summer), usually characterized by the first of the autumn and winter rains. Hence, a pulse of accumulated pollutants would possibly have been flushed from the catchments and creeks entering the Gulf resulting in higher marine concentrations. In winter, repeated rainfalls would serve to continually wash pollutants from the catchment into the sea at lower concentrations, and these concentrations would rise again toward spring as rainfall decreases. Although this may appear a plausible explanation, there was no convincing seasonal change at Port Pirie in liver adenylates or serum ALAT, nor in any marker at Barker Inlet. At Wills Creek, the lack of biochemical data in the winter confounds any examination of seasonality, although the data for MN (figure 2) suggest a similar, although less marked, pattern as for Port Pirie. Further work is needed in this area to confirm seasonal changes in these biomarkers. Seasonal measurements of various metal concentrations in sediments and fish flesh have been made at these sampling sites and their relationship with biomarkers examined (Boxall *et al.* in preparation).

Of the biomarkers examined, erythrocyte MN frequency and serum SDH appeared to most clearly demonstrate site-associated differences in pollutant levels. This may be partly because the MN frequencies and SDH levels measured at the control site were very low, with quite high values measured elsewhere. This suggests that these tests are potentially the most sensitive and reliable for medium to long term studies of this type. Changes in adenylates and AEC, although reaching statistical significance, were small relative to the control levels, and this may hamper their use in ecological studies of this type. AEC may be more applicable to studies under controlled environmental conditions such as the measurement of stress responses in aquaculture species (Edwards *et al.* in preparation). Further development and application of these assays could be achieved if they did not require the destruction of the tested individual, but that following release the same individuals could be retested to monitor changes over time (Fossi *et al.* 1994).

The data presented here demonstrate that biochemical and cytogenetic biomarkers can be applied to studies of the quality of the marine environment in South Australia using local species as indicators. Serum SDH and erythrocyte micronucleus frequencies were the most sensitive of the biomarkers tested. They show that greater biomarker responses were seen at the site expected to be most heavily contaminated (Port Pirie), and that significant effects were also seen in the

contaminated waters of metropolitan Adelaide (Barker Inlet). These data may be used as baseline data, against which future changes in marine water quality and its effects can be gauged.

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