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Histological alterations in fish from Sydney reefs: possible biomarkers for environmental effects?

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This study examines the utility of histopathology in the damselfish, *Parma microlepis,* as a biomarker for the effects of organochlorine pesticides and hexachlorobenzene (HCB). Linkages between organochlorine residue concentrations and contaminant-associated histological effects in fish were examined using field collections and a manipulative field experiment. The distribution of 11 frequently identified organochlorines were measured in wild fish at different spatial scales, spanning 270 km of coastline centred around the Sydney region. Histopathology was used to investigate possible effects on liver, gills and gonad tissues in these fish. Pearson correlations and correlative multivariate statistical techniques were used to explore possible associations between concentrations of organochlorines in fish collected from the field and histological alterations in tissues. Only 6 % of correlations between organochlorine residues and histological alterations in fish from field collections, were significant. A weak correlation was found between the occurrence of lamellar fusion in gills and the concentration of aldrin and dieldrin in a manipulative field experiment. A hypothetical model is suggested to explain these results.

Keywords: biomarker, histopathology, organochlorines, damselfish, Sydney.

Abbreviations: GC–ECD, gas chromatography–electron capture detector; HCB, hexachlorobenzene; MDS, multi-dimensional scaling; MMA, melano-macrophage aggregate; PAS, Periodic Acid and Schiff's reagent.

Introduction

Histological alterations in marine organisms have been identified as useful biomarkers for environmental contamination (Hinton and Laurén 1990a,b, Hinton *et al*. 1992, Munday and Nowak 1997). To date, studies of biomarkers in fish have mainly been carried out on well studied northern hemisphere species, in areas where levels of environmental contaminants are known to be relatively high (Malins *et al*. 1987, Köhler 1990, Myers *et al*. 1994). Fewer studies have been conducted in the southern hemisphere where levels of environmental contaminants may be lower and where suitable bioindicator species may not have been identified.

The territorial damselfish *Parma microlepis* (Günther) (white ear parma) is endemic to rocky reefs and is abundant along the New South Wales coast of Australia. Aspects of the ecology (Moran and Sale 1977, Holbrook *et al*. 1994), reproductive biology (Tzioumis and Kingsford 1995) and feeding biology (Moore 1994) of this fish have been documented. This fish maintains permanent territories through adult life (up to 37 years–Tzioumis and Kingsford 1999), so movement of this fish is unlikely to confound results among locations. Although little was known about the sensitivity of *P. microlepis*, biological traits including its abundance, territorial nature, diet and longevity potentially make this fish a good indicator of local levels of pollution.

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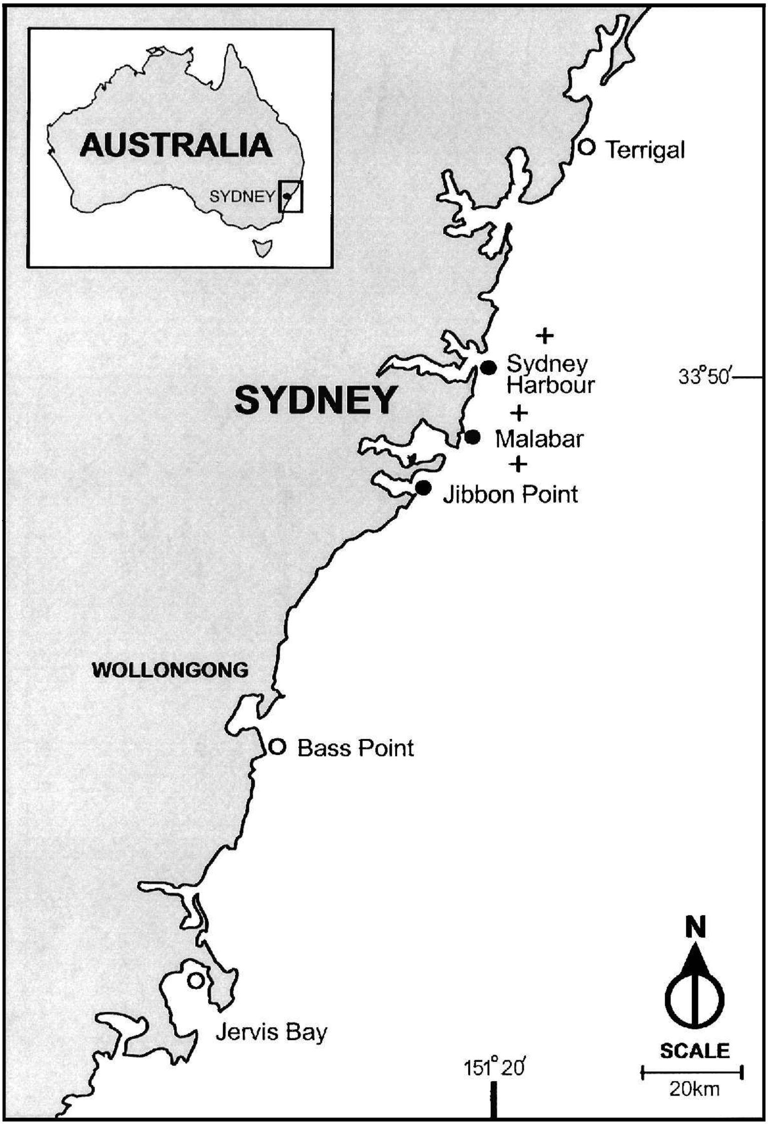

Figure 1. Location map of the Sydney region, showing urban and reference sampling locations and major sewage outfalls (smaller sewage outfalls not shown). ●, Urban sampling locations; ○, reference sampling locations; ✚, sewage outfalls.

Associations between organochlorine residue levels and histological alterations were examined in fish collected from a range of locations, and from a manipulative field experiment. This information is used to determine whether histological alterations in the tissues of *P. microlepis* can provide a useful biomarker for exposure to measured levels of 11 organochlorines identified in the Sydney region.

Methods

The spatial and temporal distribution of 11 organochlorines in the fish *Parma microlepis* were described over two years, at three urban locations and three reference locations along the Sydney coast (figure 1). Small scale spatial distribution patterns were also investigated at sites 100–200 m apart, at each of the six locations. Possible effects of biological factors such as gender, size and reproductive status of fish on organochlorine levels and histology, were minimized by selecting only adult females and sampling during the spring spawning season for this species. Fish were captured in hand-nets by divers and dissected immediately. Small samples of selected tissues were fixed within 2 min of dissection and processed using standard histology techniques. Remaining fish livers were frozen and used for the extraction of organochlorines. Organochlorines were identified and quantified using gas chromatography with an electron capture detector (GC–ECD). Histological alterations were identified and quantified in gill, liver and gonad tissues of 120 fish (Tricklebank 1997).

Associations between concentrations of organochlorine residues and histological alterations in the tissues of *P. microlepis* were investigated using Pearson correlation matrices. Multivariate non-metric multi-dimensional scaling (MDS) ordination procedures were also used to investigate similarities between samples based on histological data. Associations between histology data and organochlorine residue levels in each fish were then investigated using the BIOENV technique (Clarke and Ainsworth 1993). The effects of the biological factors, size and reproductive state, were minimized prior to analysis, using multiple regression.

A manipulative field experiment in which two concentrations (0.1 mg kg^{-1} and 0.5 mg kg^{-1}) of the pesticide aldrin were administered to fish using intraperitoneal injection, was carried out at the reference location, Jervis Bay. These fish were tagged and recaptured ($n = 20$) after a period of 2 weeks. Concentrations of the pesticides aldrin and dieldrin and histological alterations were identified and quantified in tissues from experimental fish. Possible associations between pesticide residues, demographic parameters and histological variables were examined using a Pearson correlation matrix.

Results

Dieldrin, HCB and DDE were the most frequent organochlorines encountered, occurring in 70–100% of samples collected. Chlordane was also reasonably common and occurred in 45% of the samples. Although each of the 11 organochlorine compounds identified had a distinct distribution pattern, multi-dimensional scaling (MDS) ordinations and analysis of similarities (ANOSIM) indicated that there were overall differences in the distribution of organochlorines between urban and reference locations. The organochlorine DDE accounted for the largest differences between urban and reference locations (Tricklebank *et al.* 2000).

Severe histological lesions such as neoplasms, usually indicative of exposure to carcinogenic chemicals, were not found in *P. microlepis*. Non-specific histological alterations in the gills and livers of fish indicated that fish may be affected by exposure to contaminants, but specific aetiologies were not identified. Significant correlations were detected between the level of glycogen in liver tissue (determined using PAS stain) and the organochlorines: HCB, dieldrin, DDE, heptachlor and alpha-chlordane. Significant correlations were also detected between the percent area of melano-macrophage aggregates (MMA) in liver and DDE and between the occurrence of epitheliocystis in the gills of *P. microlepis* and heptachlor epoxide (table 1). Multivariate MDS ordinations did not distinguish any differences between times or locations based on histological data (Tricklebank 1997). The low harmonic rank correlations (all < 0.32) between histological data matrices and organochlorine residue levels in *P. microlepis*, found using BIOENV, indicate that there was no detectable association between histological alterations and the concentrations of organochlorines (Tricklebank 1997). Superimposing the relative concentrations of the organochlorines: HCB, dieldrin and DDE on the histology data, also failed to reveal any patterns which may explain the distribution of samples based on histological variables (figure 2).

The concentrations of aldrin and its epoxidation product dieldrin, were successfully manipulated in fish in an underwater experiment. Concentrations of the pesticides were measured in the liver, gonad and muscle tissue of each fish.

Table 1. Pearson Correlation Matrix indicating associations between each of 11 organochlorine residues (in liver tissue) and each of the histological alterations which were quantified in the gills (G) and liver (L) of *Parma microlepis* ($n = 120$).

	Dieldrin	HCB	DDE	α-Chlor.	γ-Chlor.	Hept-epox
Epi. Hyper. (G)	–0.088	–0.132	0.023	–0.052	–0.077	0.026
Lam. Fusion (G)	0.099	0.083	0.099	0.017	0.080	–0.065
Epi. Lifting (G)	–0.086	–0.031	–0.019	–0.079	–0.102	–0.028
Flukes (G)	0.035	–0.111	0.161	–0.077	0.007	0.049
Cysts (G)	–0.115	–0.021	–0.107	–0.037	–0.014	–0.054
Trichodina (G)	–0.086	–0.052	–0.072	–0.014	0.161	–0.040
Epitheliocystis (G)	–0.015	–0.075	–0.014	–0.027	–0.039	**0.260***
Necrosis (L)	0.039	0.018	0.011	–0.056	0.102	0.082
MMA (L)	–0.055	–0.047	**0.271***	0.016	–0.043	–0.057
Haemosiderosis (L)	–0.010	0.041	0.165	0.177	–0.090	–0.034
Glycogen (L)	**0.211***	**0.204***	**0.247***	**0.240***	0.078	0.023

	Lindane	DDD	Aldrin	Hept.	DDT
Epi. Hyper. (G)	0.032	0.088	–0.070	–0.113	0.045
Lam. Fusion (G)	0.158	–0.018	0.004	–0.084	–0.025
Epi. Lifting (G)	–0.103	0.083	–0.006	–0.077	–0.015
Flukes (G)	–0.114	0.179	–0.003	0.121	0.080
Cysts (G)	0.043	–0.107	–0.036	0.059	–0.065
Trichodina (G)	–0.037	–0.070	–0.049	–0.066	–0.025
Epitheliocystis (G)	–0.123	0.006	–0.012	0.112	–0.052
Necrosis (L)	0.095	–0.061	0.153	–0.015	0.091
MMA (L)	–0.092	–0.041	–0.052	0.153	–0.029
Haemosiderosis (L)	–0.062	–0.053	–0.002	0.147	–0.032
Glycogen (L)	–0.021	0.039	–0.097	**0.207***	–0.113

$r_{\text{crit } 0.05\,(2),\, df = 118} = 0.180$. * and bold typeface indicates significant values.
α-Chlor. = alpha Chlordane, γ-Chlor. = gamma chlordane, Hept-epox = heptachlor-epoxide, Hept. = heptachlor, Epi. Hyper. = epithelial hyperplasia, Lam. Fusion = lamellar fusion, Epi. Lifting = epithelial lifting, Flukes = monogenean flukes, Cysts = occurrence of all types of cysts, MMA = percent area of melano-macrophage aggregates, Glycogen = measured by the area of PAS +ve material.

Correlations indicated that only lamellar fusion in gills of experimental fish were weakly associated with concentrations of aldrin and dieldrin ($r = 0.517$, $r_{\text{crit}0.05\,(2),\, df = 17} = 0.456$) (Tricklebank in preparation). No other relationships between aldrin and dieldrin and histological alterations (using light microscope sections) were found.

Discussion

This study identified organochlorine compounds in the tissues of *Parma microlepis* collected from both urban and reference locations. Histological alterations, indicating possible exposure to contaminants, were present in fish tissues. The lack of association between the organochlorines and histological alterations suggested that *P. microlepis* are not adversely affected by present levels of the contaminants tested for.

A hypothetical model (based on Waldichuk 1979, Heath 1987) is suggested to explain the lack of an association between histological alterations and the concentrations of organochlorine compounds in fish (figure 3). The model proposes that there are two threshold concentrations of contaminants that determine the degree

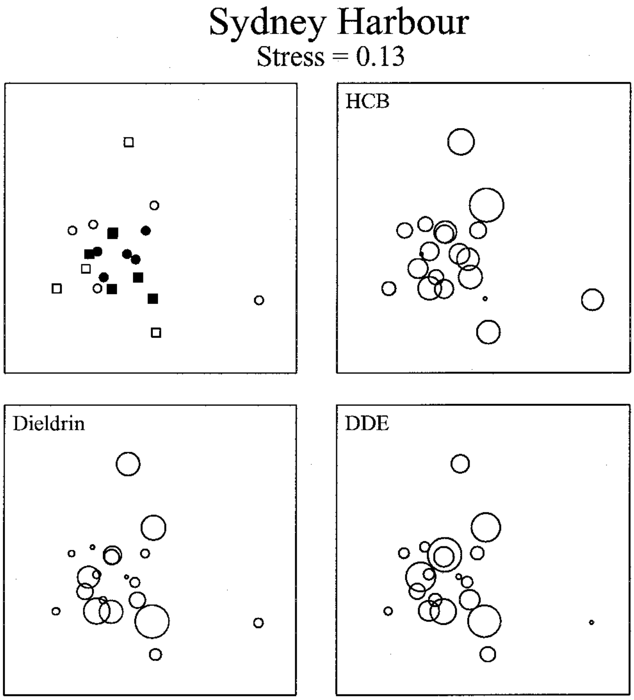

Figure 2. Two-dimensional MDS ordinations of histology variables for fish from Sydney Harbour after adjustment for the biological variables gonad somatic index (GSI) and standard length. Organochlorine residue data of the most frequently occurring compounds, HCB, dieldrin and DDE are superimposed on the ordination. The size of the circles are square root proportional in diameter to organochlorine concentrations of each sample. Fish were collected from Sydney Harbour: ●, Site A, 1993; ■, Site B, 1993; ○, Site A, 1994; □, Site B, 1994.

of biological response in organisms. Below the first critical or 'threshold' concentration of contaminants (Threshold 1) there is no response by organisms. When Threshold 1 is reached, organisms begin to respond to increasing levels of exposure by showing an initial detectable response, which may result in an induction of mixed function oxidase (MFO) enzymes. The enzymes catalyse metabolism of the xenobiotic, causing a decrease in measurable response. The net effect is one of no measurable increase in organism response. This process continues until the second 'threshold' concentration (Threshold 2) of contaminants is reached and enzyme induction in organisms can no longer regulate the level of measurable effects. Increasing concentrations of contaminants result in a corresponding positive association (dose–response curve) between contaminant levels and responses in organisms. It is suggested that the results from fish investigated in this study are between the two threshold concentrations for organochlorines and exhibit relatively low level responses with high variability between individuals.

Histological alterations do not appear to be associated with organochlorines identified in *P. microlepis*, at the concentrations measured in the current study. Threshold levels of organochlorines in tissues which induce a measurable response should be determined. Further work at higher concentrations of organochlorines is required to ascertain if the histological alterations identified in *P. microlepis* can be used as a biomarker for exposure.

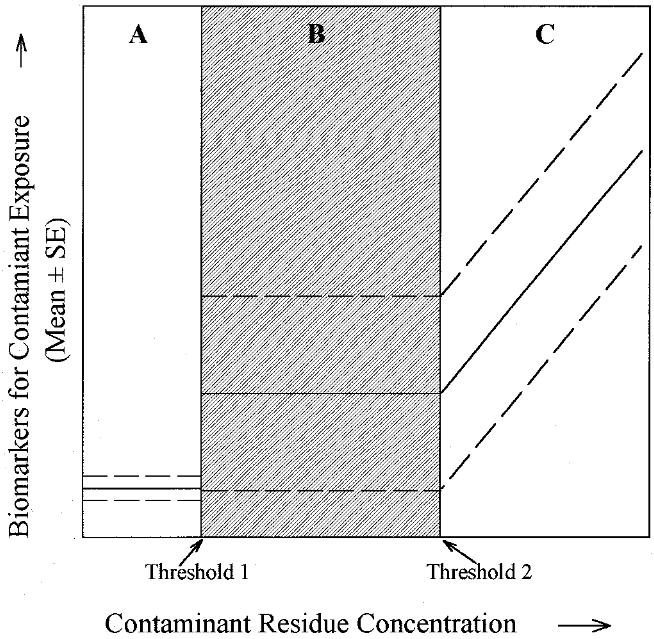

Figure 3. Hypothetical 'Threshold Model' describing three phases of response to contaminant exposure. Mean response (solid line) ± standard error (dashed line). Modified from Waldichuk (1979) and Heath (1987). (A) Low levels of contaminant exposure with no resulting effect on organisms. When a critical or 'threshold' concentration of contaminants is reached (Threshold 1) organisms begin to respond. Variability for response in different individuals is low. (B) Increasing levels of exposure resulting in highly variable response by organisms. Organisms may be regulating the effects of contaminants. This process continues until 'Threshold 2' is reached. The net effect is one of no measurable increase in organism response. (C) Increasing concentrations of contaminants result in a corresponding increased response by organisms. Individual responses are highly variable.

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