State Pollution Control Commission

Bioaccumulation in Nearshore Marine Organisms II

Organochlorine Compounds in the Red Morwong, *Cheilodactylus fuscus*, Around Sydney's Three Major Sewage Ocean Outfalls

This report was prepared by M. P. Lincoln Smith of the Ecology Lab Pty Ltd, 216 Crown Street, Darlinghurst 2010 and R. A. Mann of the State Pollution Control Commission.

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SUMMARY

1. This study examined bioaccumulation of organochlorines in marine animals near shoreline sewage ocean outfalls. Based on research conducted in 1987, a single fish species, red morwong (Cheilodactylus fuscus) was used as an indicator of organochlorine contamination in the Sydney region. Red morwong was selected as an indicator of organochlorine contamination in a particular area, due to its territorial nature.

The aims of this study were to determine:-
(1) the distribution and bioaccumulation of organochlorines in muscle tissue of red morwong at selected locations along Sydneys coastline
(2) to undertake an interlaboratory study of the analysis of organochlorines in muscle tissue of red morwong
(3) to determine the biological characteristics of red morwong from selected sites - specifically, fish length and weight, fat content of muscle, liver to body weight ratio; fish age; gut contents; and gonad state.

2. An interlaboratory comparison of organochlorines in red morwong muscle tissue showed that the chemical laboratory contracted for organochlorines determined in 1987 had used appropriate procedures and was a suitable laboratory to use, as 1988 results compared well with other Sydney laboratories. The interlaboratory comparison did indicate, however, some laboratories may be subject to error from many factors including the method employed and the subjectivity of the analyst. Accurate determination of organochlorines in fish muscle tissue is very difficult and requires further development.

3. Red morwong collected from 24 sample locations along the Sydney coast varied in length, weight, relative age and fat content; and in liver size relative to body weight. These attributes, particularly fat content, probably contributed to sources of variation in the accumulation of organochlorines, although they did not alter the overall conclusions of the study.

4. The wide range of organochlorines detected in the muscle tissue of red morwong was closely related to proximity to the shoreline ocean outfalls, particularly Malabar. In addition, there may be some input from Sydney Harbour and Botany Bay and minor outfalls, such as Diamond Bay/Vaucluse. The organochlorine found in greatest concentrations was chlordane. This was found in average concentrations exceeding the National Health and Medical Research Council (NHMRC) maximum residue limit (MRL) in the edible muscle tissue of fish at 18 of the 24 locations sampled, representing a significant degree of contamination of the nearshore marine environment. The highest concentrations (found

near Malabar) were on average more than 12 times higher than the NHMRC MRL. Another organochlorine , hexachlorobenzene (HCB) was also found in concentrations above its MRL at sites up to 3.5 km south and 1.5 km north of Malabar. Mean concentrations of HCB in red morwong occurred 0.5 km either side of Malabar, and were about 3 times the NHMRC MRL.

5. Other organochlorines detected included heptachlor epoxide (HPTE), DDT, DDE and DDD, dieldrin, oxychlordane and polychlorinated biphenyls (PCBs). HPTE and PCBs were nearly always found in association with the North Head, Bondi and Malabar outfalls; dieldrin, DDT, DDE and DDD were generally associated with the outfalls, but also displayed considerable variation along the coastline. In addition, several other chlorinated compounds, not identified as the organochlorine compounds of interest were recorded in high concentrations in fish living in close proximity to the outfalls. The anthropogenic origin of these compounds, their effects on nearshore marine communities and the possible human health effects are not known.

6. A comparison of physical characteristics of red morwong sampled in 1987 with those from 1988 at Malabar showed that in both years the fish had similar length, weight and fat contents. Total organochlorines were significantly higher in 1987, as was relative liver size. There was no difference in the concentration of dieldrin or DDTs between years. Other organochlorines , however, did show marked differences: higher concentrations of HPTE and benzene hexachloride (BHC, also known as hexachlorocyclohexane, expressed as alpha and beta isomers) were found in 1987, while HPTE levels were lower and BHC absent in 1988. Chlordane and HCB were not detected in 1987, but were present in 1988.

7. One possible explanation for the different occurrence and concentrations of organochlorines found is as follows. Several studies have shown that different organochlorines persist in marine organisms in periods ranging from several days (eg BHC) to months (eg DDT may persist for over 6 months). Thus chlordane and HCB may have resulted from a substantial input prior to the time of collections. Uptake and clearance of organochlorines should be further investigated for local species in view of the possible variations of organochlorines in the nearshore environment.

8. The results of the bioaccumulation studies indicate that a wide range of organochlorines may be present in muscle tissue of red morwong collected along the Sydney coastline. However, the persistence of organochlorines in aquatic organisms residing near variable inputs from a variety of sources must be taken into account in any monitoring programme of the nearshore or offshore environments.

1.1 BACKGROUND TO THE STUDY

The presence of organochlorines (referred to here as OCs) in aquatic organisms collected near highly urbanised communities is well documented (Robinson, 1973; Gerlach, 1981; Schmitt et al., 1985). OCs accumulate in aquatic organisms as a result of their low water solubility and high solubility in fats. They are persistent in the environment and in high concentrations may be toxic to flora and fauna and pose a significant health threat to humans.

A study conducted by the (then) Department of State Fisheries from 1977 to 1979 investigated the OCs present in rocky reef fishes near Sydney and Newcastle shoreline ocean outfalls (Scribner et al., 1987). The highest concentrations of pesticides were detected in the muscle tissue of red morwong (Cheilodactylus fuscus) and blue groper (Achoerodus viridis) collected near the Sydney ocean outfalls. The pesticides DDT (and its metabolites) and dieldrin were recorded at mean concentrations of 0.11 and 0.10 mg/kg (wet weight basis) in red morwong collected near the Sydney ocean outfalls. Other compounds detected included PCB's (polychlorinated biphenyls), HCB (hexachlorobenzene) and HPTE (heptachlor epoxide) although these were well below the NHMRC recommended maximum residue limits (MRL's) for human consumption.

During 1987, the SPCC and The Ecology Lab Pty Ltd. conducted a study, funded by the Water Board, into the bioaccumulation of organochlorine compounds and trace metals in rocky reef aquatic organisms residing near the sewage outfall at Malabar (Lincoln Smith and Mann 1987, in press). Eight replicates of 3 fish species (red morwong, blue groper and rock cale, Crinodus lophodon) and 3 invertebrate species (red bait crab, Plagusia chabrus, black lip abalone, Haliotis ruber, and sea tulip, Pyura pachydermatina) were collected and various tissues analysed. The results obtained were compared to 8 replicates of each of the 6 species collected from a control site near Pt Hacking and one near Terrigal. All collections were made by SCUBA divers, between 26 May and 19 June, 1987.

The trace metals showed a complex pattern of bioaccumulation throughout the species and sites studied (Lincoln Smith and Mann 1987, in press). All results were well below the NHMRC MRL's except for muscle tissue of the blue groper from Malabar where the mean mercury concentration was just above the MRL.

Several OCs were detected in this study, including beta-hexachlorocyclohexane (BHC), heptachlor epoxide (HPTE), dieldrin, DDE and DDD. At Malabar, BHC was detected in red morwong, blue groper, rock cale and the red bait crab; DDE was detected in red morwong; and dieldrin was detected in all fish species as well as the red bait crab. In the edible muscle tissue of the red morwong

the mean concentration of BHC was 122 times and HPTE 52 times NHMRC recommended MRL. In the blue groper muscle tissue, BHC was 20 times and HPTE 4.8 times the MRL's. All other OCs were below the NHMRC MRLs.

At Pt Hacking, BHC and HPTE were 1.3 times the NHMRC recommendations in red morwong while only trace or zero concentrations of other pesticides were detected in all other species. No OCs were detected in any species at Terrigal.

The Malabar ocean outfall and urban runoff were implicated as probable sources of the OCs. Further, the study also concluded that the red morwong is a suitable OC bioindicator on rocky reefs in the nearshore environment. Red morwong was selected as an indicator of OC contamination in a particular area due to its territorial nature.

Discussions between the Water Board, NSW Agriculture and Fisheries and the SPCC raised a number of alternatives to account for observed differences in OCs reported between the Malabar outfall and the control sites during 1987 (Lincoln Smith and Mann 1987, in press). Five possible alternatives were:

(1) That the OCs were derived from recent sewage discharge from the Malabar outfall.

(2) That the OCs were derived from past discharges.

(3) That the OCs may have been derived substantially from urban runoff, via estuaries or stormwater drains entering coastal waters, rather than via the sewage outfall at Malabar.

(4) That biological characteristics of the sampled animals may have varied among sites, contributing to variation in OC uptake.

(5) That the laboratory determinations of OCs were inaccurate.

The experimental design developed for the present study examined possible causes of variation in OCs among sites, using red morwong as an indicator of OC bioaccumulation at a particular site. The study had the following aims:-

(1) To determine the distribution and bioaccumulation of OCs in muscle tissue of red morwong at selected distances along Sydney's coastline.

(2) To undertake an interlaboratory study of the analysis of OCs in muscle tissue of red morwong.

(3) To determine biological characteristics of red morwong from selected sites - specifically, fish length and weight, fat content of muscle, liver to body weight ratio; fish age; gut contents; and gonad state.

This study, funded by the Water Board, was conducted jointly by the NSW State Pollution Control Commission (SPCC) and The Ecology Lab Pty. Ltd.

1.2 TERMINOLOGY OF ORGANOCHLORINES, OUTFALLS, BIOACCUMULATION, MRLs

Organochlorines are hydrocarbons containing several chlorine atoms. The strength of the chlorine carbon bonding makes these compounds exceptionally stable. In the past they have been used extensively in the pest control industry (eg DDT, dieldrin, heptachlor etc) and are persistant compounds accumulating in fish tissues especially fat.

This study was concerned with bioaccumulation around Sydney's existing shoreline ocean outfalls, which discharge into the nearshore environment. Throughout this report, the term outfalls refers to shoreline sewage outfalls as distinct from the proposed deepwater submarine ocean outfalls, which will discharge sewage several kilometres away from the shoreline.

We define bioaccumulation as the movement of chemicals from aqueous to biotic components of an aquatic ecosystem. The two main pathways/mechanisms (described in Figure 1.1) are bioconcentration (uptake via the gills and respiratory system) and biomagnification (uptake via the food chain) (Connell, 1988).

A maximum residue limit (MRL) is the maximum limit allowable for a substance in regulations under the Pure Act. Limits are expressed in milligrams (mg) of the substance per kilogram (kg) of the food (ie parts per million). Levels differ in various substances in the same food and, sometimes, for the same substances in different foods. The National Health and Medical Research Council (NHMRC) sets the limits and they are incorporated into State food law and therefore have legal status (Dr. D. Fox, NSW Health Dept, pers comm.).

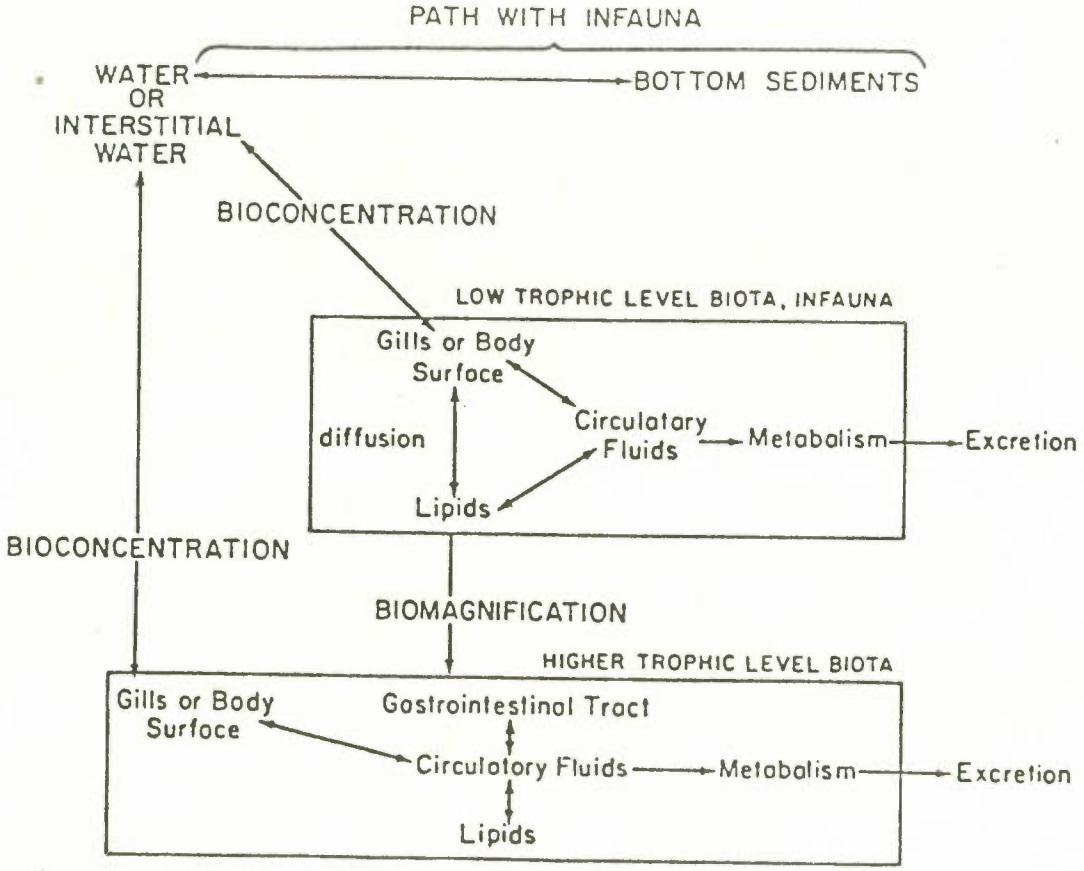


Figure 1.1: Transfer patterns of organochlorine compounds during bioconcentration and biomagnification (After Connell 1988)

CHAPTER 2. METHODS

2.1 SAMPLE LOCATIONS

Red morwong were collected from 24 sites along the Sydney coastline, between Curl Curl and Cape Banks. These sites were selected at 0.5, 1.5, 2.5 and 3.5 km either side of the outfalls at North Head, Bondi and Malabar (Figure 2.1). The distances from each outfall were determined from a scaled map; they are straight-line distances from each outfall. They do not take into account for any indentations along the coastline, current movements or variations in fish density, feeding habits, etc.

2.2 SELECTION OF INDICATOR SPECIES

During the 1987 study 3 fish and 3 invertebrate species were selected to examine patterns of contaminations, following criteria listed by Hellawell (1986) and summarised as follows :-

(1) The organisms should be readily identified, as taxonomic uncertainties can confuse data interpretation.

(2) They should be relatively easy to sample.

(3) They should have a cosmopolitan distribution, at least at the scale of investigations persued.

(4) They are associated with abundant autecological data, which can assist in analysing survey results and devising pollution, or biotic indices.

(5) They should have economic importance as a resource or nuisance or pest, as they will have intrinsic interest.

(6) They should readily accumulate pollutants, especially so as reflect environmental levels since this facilitates understanding of the distribution in relation to pollutant levels.

(7) They are easily cultured or maintained in the laboratory, which assists in relating experimental studies of their responses to pollutants and field observations.

(8) They should have low variabilty, both genetic and in their role (niche) in the biological community.

From the species selected in 1987, the red morwong (Cheilodactylus fuscus: CHEILODACTYLIDAE) was regarded as an appropriate indicator for the present study. The red morwong is not a significant commercial species (B. Richardson, NSW

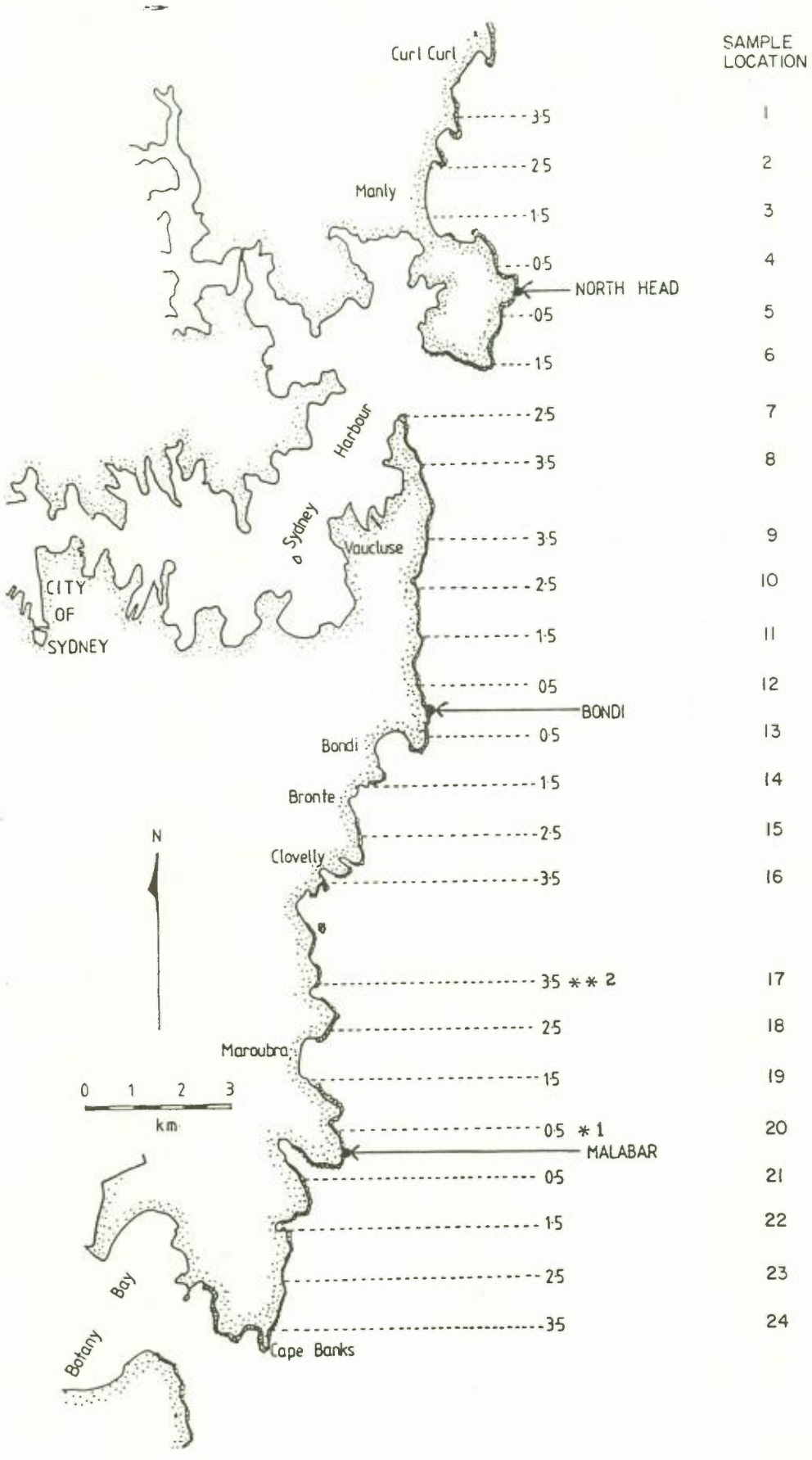


Figure 2.1: Location of sampling sites for *C. fuscus* showing their distances (km) from the three major outfalls off Sydney (* and ** are sites used for inter-laboratory study)

Agriculture and Fisheries, pers. comm.), but it is commonly taken by recreational fishermen, especially spearfishermen, on shallow rocky reefs (Lincoln Smith et al. in press).

The highest concentrations of OCs detected in 1987 occurred in red morwong (Lincoln Smith and Mann 1987, in press). Available information indicates that the species is a carnivore, feeding on benthic invertebrates, such as crabs, polychaetes and amphipods, taken from rocky reefs (Bell, 1979). It is also thought to be relatively sedentary (although males may occur in deeper water) and long lived, in the order of a decade or more. Red morwong are also abundant on shallow rocky reefs and relatively easy to collect.

2.3 SAMPLE COLLECTION AND PREPARATION

2.3.1 Bioaccumulation study

Eight red morwong were collected from each of the 24 sites between July 13 and August 22 1988. While these fish constituted the 8 replicates used for chemical and statistical analysis, some additional small fish were also speared for age determination. The fish were speared by divers using a rubber-powered speargun whilst on SCUBA. Wherever possible adult red morwong were collected, in order to provide samples large enough for proper chemical determinations.

Samples were selected randomly from among the larger size range. Fish were usually speared near the head, away from liver and the portion of muscle selected for OC analysis. All dives were undertaken from an SPCC boat.

Following each dive, when about 6-8 fish were speared, the fish were returned to the boat and placed individually into plastic bags, labelled and stored on ice until delivery to the SPCC Chemical Laboratory for dissection on the day of collection.

In the SPCC Laboratory each fish was weighed, then tail fork and standard length measurements were made. Fish were then disected on polythene boards using stainless steel scalpels. All surfaces were cleaned with detergent and washed with distilled water and nanograde hexane. As OC concentrations may vary over different parts of the same tissue type (C. Kimpton, Water Board, pers. comm.), all muscle tissues were obtained from the anterior part of the body, above the lateral line. The skin and scales were removed from the muscle tissue and two subsamples were collected. The first subsample was retained for OC analysis in glass containers (prewashed with nanograde hexane and aluminium foil-lined screw cap). The second subsample was frozen and sent to the Water Board for trace metal analyses in an acid-washed polystyrene containers. Finally, the remainder of the fish, including the bony parts, was also frozen separately for fish age determination.

2.3.2 Interlaboratory study

Twelve red morwong were collected by spearing, 6 from each of 2 sites (Figure 2.1). Site 1 was 0.5 km north of the Malabar shoreline sewage outfall, in an area previously found to have fish with OC contamination (Lincoln Smith and Mann 1987, in press). Site 2 was about 3.5 km north of the outfall, and was assumed to have lower levels of contamination. This assumption was later tested (Section 2.6.1).

Originally, it was intended that the 6 fish from each site used for the interlaboratory study (Chapter 3) would come from the opposite side of 6 of those 8 used for the bioaccumulation study (Chapter 4). The muscle tissue obtained from some of these fish, however, was insufficient to divide among all participating laboratories and a few extra fish were collected. In all, 9 fish were used in both studies.

A muscle fillet of 80 to 100 g was dissected from each fish on the day of sampling. Skin and scales were removed from the flesh, and each sample was homogenised in a blender for 10 minutes (Braun Type MX32). The homogenate was divided into 8 equal portions (6 - 18 g each, depending on the size of the fish), each of which was stored and frozen in prepared glass jars. Two of the jars for each fish were distributed randomly to each of the laboratories. Thus each laboratory received 24 jars, made up of 2 replicates of homogenate from 6 fish collected from each of 2 sites.

2.4 BIOLOGICAL CHARACTERISTICS OF RED MORWONG

2.4.1 Physical measurements

For this study, fish length (measured to fork length (LCF) and standard length, STD), body weight, fat content and liver weight were measured in all red morwong used for determination of OC concentrations. In addition, the relative ages of red morwong were determined by measurements of otolith bones. Two measures of fish length were obtained: fork length was measured for consistency with the 1987 study, standard length was required for comparison when conducting age determinations.

Fish length, weight and liver weight were measured on the day of collection. Liver weight is expressed throughout this report as a percentage of body weight.

2.4.2 Sex and age

Gonads of all fish collected were examined and sex determined by gross visual examination.

Ageing of fish was undertaken by Mr. D. Ferrell of the University of Sydney. His report on ageing is presented in Appendix 4.3. Essentially, age was determined as an index of otolith bone weight. This simple method was developed to avoid the time-consuming sectioning and counting of bands within the otoliths.

Ferrell found very good agreement between the number of growth bands observed in sectioned otoliths and otolith weight in a subsample of 50 fish (the relationship between the two had an r squared of 0.89) Thus the number of otolith bands was accurately predicted by the otolith weight for red morwong. It should be noted that while growth bands may represent yearly increments, this must be evaluated by comparison with fish of known age. In the present study this evaluation was not undertaken due to cost and time restrictions, so only the relative ages of fish were compared.

2.5 CHEMICAL ANALAYSES IN BIOACCUMULATION STUDY

Chemical analyses were conducted by Australian Analytical Laboratories Pty Ltd (AAL), Thornleigh, NSW. Fats are expressed as a percentage of tissue analysed and concentrations of OCs are expressed as mg/kg, wet weight basis.

2.5.1 Fat Determination

Fat content was determined by extraction of a blended portion of the sample (>3 g wet weight) with ether/petroleum ether, as described in Association of Official Analytical Chemists (AOAC), 14th edition, Sections 18.043 - 18.044 (e) (Williams, 1984).

2.5.2 Organochlorine Compounds

OC analysis of fish muscle was conducted by a NATA-registered method based on an AOAC method. At least 3 g of fish muscle tissue was extracted with acetonitrile and petroleum ether. The residues were purified by low temperature freezing. The OCs were then quantified by gas chromatography (GC) equipped with an electron capture detector. Two GC columns were used to identify the OCs of interest. The OCs were then confirmed by Gas Chromatography/Mass Spectrometry (GC/MS).

The OCs requested for analysis were: DDE, DDD, DDT, DDT(R) (sum of DDD+DDE+DDT), aldrin, dieldrin, lindane, alpha and beta BHC, HCB, heptachlor, HTPE, chlordane, oxychlordane, endosulfan, methoxychlor and PCB's (expressed as Arochlor 1254). Appendix 2 contains the nomenclature, common names and usage of OCs.

Limits of detection, and the NHMRC MRLs for fish or shellfish are shown in Table 2.1. It should be noted that the detection limit for chlordane, oxychlordane and BHC equals their MRL, which

is a limitation of the existing methods.

2.6 STATISTICAL ANALYSES AND DATA PRESENTATION

The experiments described in this report were designed for statistical analysis by analysis of variance (ANOVA). ANOVA is described in detail by Sokal and Rohlf (1969), Snedecor and Cochran (1980) and Underwood (1981). Where significant ANOVAs were found, sample means were compared using Student Newman Keuls (SNK) tests (eg. Sokal and Rohlf, 1969; Winer, 1970; Underwood, 1981). Other tests used include regression and t-tests (Chapter 4).

An important assumption of ANOVA is that the sample variances for a particular test do not statistically differ. The presence of heterogeneous variances could lead to a significant ANOVA and the conclusion that sample means are significantly different, when they are not. For this study, Cochrans C Test (Underwood, 1981) was used to compare variances prior to ANOVA. Where variances were heterogeneous according to Cochrans Test, data were transformed - usually by a log natural transformation of data as mg/kg or ug/kg - in order to make them homogeneous (ie., so that they were not significantly different using Cochrans Test).

Table 2.1. Limits of detection for organochlorine analyses from AAL and shellfish, and the Australian NHMRC maximum residue limits (MRLs) for edible muscle tissue for fish and shellfish (Australian NHMRC, 1987).

Organochlorine (OC)	Limit of detection (mg/kg)	NHMRC MRL (mg/kg wet weight)
BHC (a & b)	0.01	0.01
Lindane	0.01	1.0
Aldrin	0.01	0.2 (fat of meat)£
HCB	0.01	0.1
Heptachlor	0.01	0.05
Heptachlor epoxide	0.01	0.05
Chlordane	0.05	0.05
Oxychlordane	0.05	0.05
DDE	0.01	1.0
DDD	0.01	1.0
DDT	0.01	1.0
DDT(R) *	0.01	1.0
Dieldrin	0.01	0.1
Endosulphan	0.01	0.2 (fat of meat)£
methoxychlor	0.01	3.0 (fat of meat)£
PCB's	0.05	no limit given

£ not available for fish * DDT(R) = DDT + DDD + DDE

In several cases transformed data still had heterogeneous variances. Here the acceptance criterion (alpha) was lowered from the conventional 0.05 value in accordance with the significance of the Cochrans Test result. For example, where the probability of heterogeneity was: $0.05 \geq C > 0.025$, the alpha level was changed to 0.025 for both the ANOVA and SNK tests. In some cases the C value was significant with a probability of $P \leq 0.0001$. In these cases the variances were judged intractable, and no ANOVA was done.

Analytical results and data are presented with essentially the same format for every experiment. Results of statistical tests are summarised in tables with significance of the effect tested as follows:

ns = probability, $P > 0.050$, non-significant

* = $0.050 \geq P > 0.025$

** = $0.025 \geq P > 0.010$

*** = $0.010 \geq P > 0.005$

**** = $0.005 \geq P > 0.001$

***** = $0.0001 \geq P$

Results of the SNK tests are presented by listing coded means, which increase in magnitude from left to right. For example, the coded mean "3.5" represents the mean of all data points at 3.5 km either side of all outfalls. Coded means which are underlined do not differ significantly at the alpha level used for the ANOVA. Unless otherwise shown, the alpha level is always 0.05. An example of 3 typical SNK summaries is as follows:

a) mean 1 mean 2 mean 3 mean 4

b) mean 1 mean 2 mean 3 mean 4

c) mean 1 mean 2 mean 3 mean 4

The first summary, a), is interpreted as follows: mean 1 is significantly less than all other sample means, means 2 and 3 do not differ and mean 4 is significantly greater than all others. In b), none of the sample means differ significantly. In c), means 1,2 and 3 do not differ; mean 4 is equivalent to mean 3, but statistically larger than means 1 and 2. This latter interpretation follows that of Sokal and Rohlf (1969) and Winer (1971).

Graphical presentation of data consists of bar graphs with error lines shown either as standard errors, or as the 95 % confidence limits of the sample mean. Confidence limits are used for OCs in fish muscle to show their relationship with NHMRC MRLs. Tables summarising the analyses as well as the raw data are appended.

2.6.1 Interlaboratory study

An ANOVA with a mixed design was used with the factors Labs (fixed), Sites (fixed, because they represented high and low contamination - see Fig. 2.1) and fish (nested within sites, and random). Aldrin was not detected by Lab 1 so only Labs 2, 3 and 4 were compared statistically for this OC. Lab 4 could not determine fat content, chlordane or PCBs and was excluded from analyses for these variates.

2.6.2 Bioaccumulation study

Biological characteristics and OC data were analysed by ANOVA. A 3-way design was used, the factors Distance ("Dt": 0.5, 1.5, 2.5, 3.5 km away from an outfall), Direction ("Dn": north or south of an outfall) and Outfall ("Of": North Head, Bondi, Malabar). All factors were orthogonal and fixed. Eight fish were used from each location (Figure 2.1). Biological characteristics analysed were fish length (fork and standard lengths), % fat content, liver weight to body weight ratio and relative age, as determined by otolith weights.

OCs analysed were: the number of the selected OCs detected (including trace detections), the total concentration of selected OCs detected per fish, and concentrations of chlordane, DDE, DDT(R), dieldrin, HCB and PCBs.

Recently an alternative statistical analysis was proposed by the Water Boards, consultant biometrician. However the consultant believed this alternative analysis would not alter the conclusions drawn from the analysis presented in this report.

The data were analysed twice, firstly using the OC determinations expressed as ug/kg wet weight, supplied by AAL, the contracted laboratory. Next the data were expressed on a fat corrected basis as follows:

$$\text{OC (mg/kg wet weight fat basis)} = \frac{\text{OC determination (mg/kg wet weight)}}{\text{Fat content (\%)}} \times 100$$

REGRESSIONS AND t-TESTS

Regression analysis was used to determine the relationship between OC concentrations and distance away from outfalls. The procedure outlined by Sokal and Rohlf (1969) was used, which allowed determination of deviations from linearity. Thus where deviations were found, quadratic or cubic relationships could be fitted from the data. Variances were tested for heterogeneity using Cochrans Test, but were not transformed if the C value was significant. To transform the data would probably alter the nature of the regression; instead, the alpha level was decreased where heterogeneity occurred, thus making the test more conservative.

t-tests were used to compare variates of the 8 red morwong collected from 0.5 km north of Malabar in 1987 (Lincoln Smith and Mann 1987, in press) with those collected for the present study. Variates analysed include fish length (LCF), weight, liver to body weight ratio, concentration of OCs, and concentrations of DDT(R) and dieldrin.

CHAPTER 3. INTERLABORATORY STUDY

3.1 INTRODUCTION

An interlaboratory comparison study involving four laboratories was undertaken of the analysis of OCs and fat in the muscle tissue of red morwong. Australian Analytical Laboratories (AAL, labelled Lab 1 here) was contracted for the previous study (Lincoln Smith and Mann, 1987, in press).

Each laboratory was asked to analyse fish samples for a wide range of OCs and fat content, using methods normally employed for routine analysis of OCs in fish. Gas Chromatography/ Mass spectrometry (GC/MS) confirmation was requested to confirm the OCs detected as well as the identification of any unusual peaks or interferences. In addition, a questionnaire was sent to determine differences in methodology between laboratories.

Analytical laboratory results for fish collected from 2 sites, one assumed to have a high OC concentration, the other low were compared. The OCs requested are the same as those for the bioaccumulation study (Section 2.5.2).

Following statistical analyses of the results, a detailed assessment of in-house procedures was made, to isolate potential sources of variability in analytical results.

3.2 ANALYTICAL METHODOLOGY

The method used for organochlorine analyses by each laboratory is displayed in Table 3.1. Differences in extraction, clean-up and instrumental analyses are apparent between laboratories.

3.3 RESULTS

3.3.1 Organochlorine compounds and fat content

The results of OC determinations for each laboratory are presented in Appendix 3.1. A wide range of OCs were detected in fish collected 0.5 km north of Malabar (Site 1 - Fig. 2.1) by all laboratories (Table 3.2). They all reported DDE, DDD, DDT and dieldrin, while chlordane, HCB, aldrin, heptachlor and PCBs were reported by one or more laboratories. At 3.5 km north of Malabar (Site 2), generally lower levels of OCs were detected. Typical GC chromatograms from both sites are shown in Figure 3.1. These were conducted with a similar attenuation. The figure indicates that other large peaks are present (which may be chlorinated organics) in red morwong collected at site 1 besides the OCs of interest.

Figure 3.1: Gas chromatograph analyses (mg/kg wet weight) of OCs in muscle tissue of red morwong collected from low site (3.5 km north of Malabar) and high site (0.5 km north of Malabar)

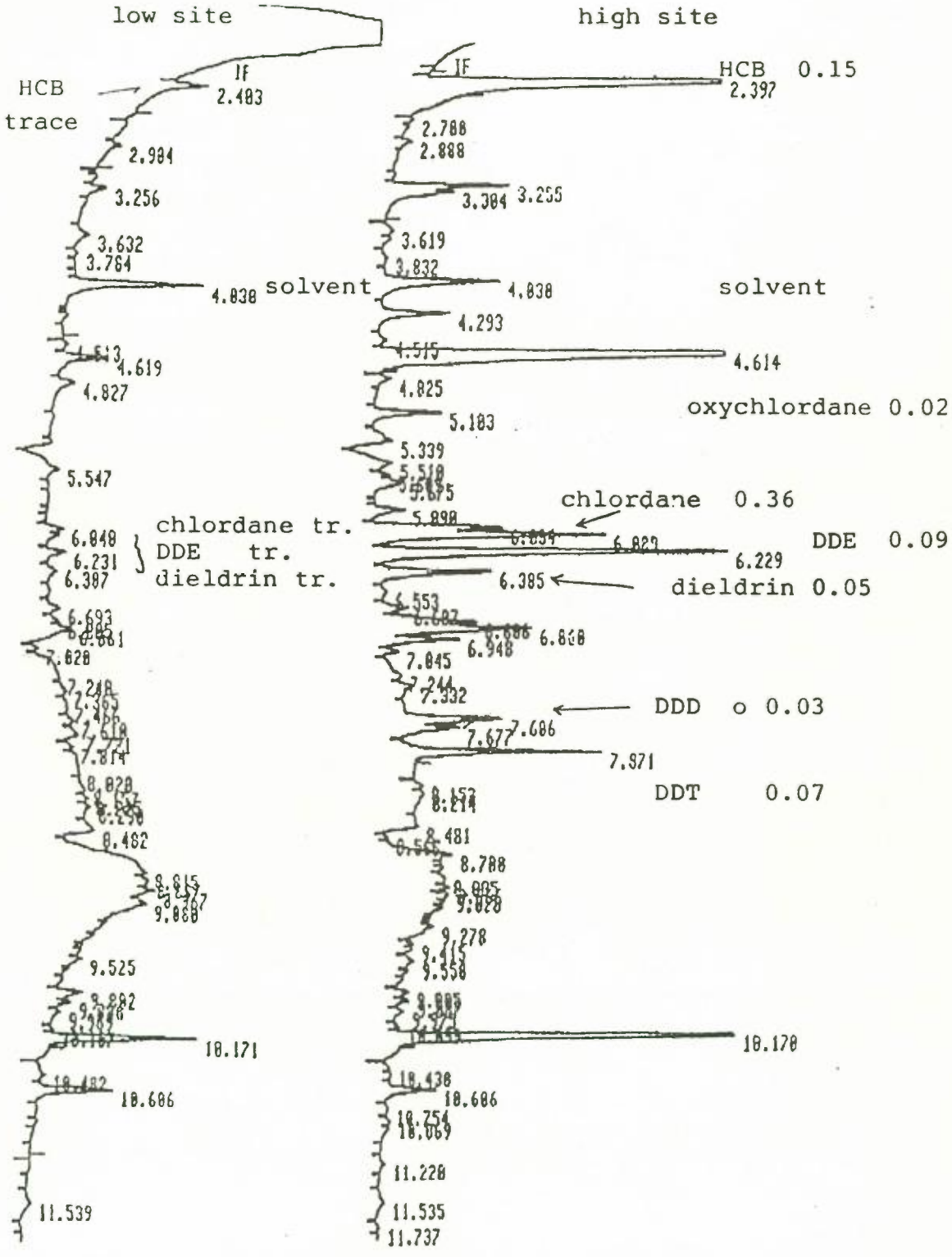


Table 3.1 Comparison of methods used by laboratories in collaborative study of organochlorine analysis of fish muscle.

Lab.	Extraction	Clean-up	GC column	Confirmation of OC's
1 (AAL)	acetonitrile pet ether		capillary	2 columns + GC/MS £
2	hexane/ acetone	gpc	capillary	1 column + GC/MS ££
3	hexane/ acetone	gpc	capillary	2 columns @
4	hexane	silicic acid	glass	1 column

Methods: Labs 1, 2 and 3: adaptations of AOAC methods
Lab 4: Ahmad and Marolt (1986)
All laboratories used GC/ECD detector
£ chlordane HCB and PCBs confirmed with GC/MS
££ chlordane and HCB confirmed with GC/MS
@ GC/MS identification of octochlorostyrene only
gpc = gel permeation chromatography
Note : the use of silicic acid column, by Lab 4, prevented fat content analyses.

Following discussions with the participating laboratories, there appear to be considerable differences in the ability of each laboratory to detect OCs, particularly at low concentrations (< 0.1 mg/kg) in fish muscle (see DLs in Table 3.1). There appeared to be many chlorinated organics present at low level in some fish, yet most laboratories reported only the common parent compounds or their metabolites. For example, Lab 3 identified one chlorinated compound from the fish collected near the Malabar outfall, which was later positively identified by GC/MS as octachlorostyrene.

Additional OCs may be present at trace concentration, or near large unidentified peaks and hence not readily identified. To check the efficiency of the extraction and analysis procedure each laboratory analyzed its own spiked fat sample. Recoveries reported (70 to 120%) compared well to other interlaboratory studies of OC analyses. Oliver and Niimi (1985) reported recoveries greater than 80 % for some OC's in spiked fish oils.

TABLE 3.2 OCs detected (D) in muscle tissue of red morwong at Site 1 by four analytical laboratories and detection limits (DL, mg/kg wet weight) assigned by each. Symbols used: * = detected in ≥ 1 fish at concentrations > DL; £ = instrument malfunction; ££ = method used cannot determine PCBs.

OC	LAB 1		LAB 2		LAB 3		LAB 4	
	D	DL	D	DL	D	DL	D	DL
Chlordane	*	0.05	*	0.01	*	0.02		0.01
Oxychlordane	*	0.05		0.01		0.02		0.01
HCB	*	0.01	*	0.01	*	0.005		0.01
DDE	*	0.01	*	0.01	*	0.01	*	0.01
DDD	*	0.01	*	0.01	*	0.02	*	0.01
DDT	*	0.01	*	0.01	*	0.02	*	0.01
DDT(R)	*	0.01	*	0.01	*	0.02	*	0.01
Dieldrin	*	0.01	*	0.01	*	0.01	*	0.01
Aldrin		0.01	*	0.01	*	0.005	*	0.01
a-BHC		0.01		0.01		0.001		0.01
b-BHC		0.01		0.01		0.001		0.01
g-BHC		0.01		0.01		0.001		0.01
Heptachlor		0.01		0.01	*	0.05		0.01
HPTE		0.01		0.01		0.05		0.01
Methoxychlor		0.01		0.01		0.05		0.01
Endosulphan		0.01		0.01		0.05		0.01
Endrin		0.01		0.01		0.05		0.01
PCBs	*	0.05		0.05	£	0.05		££

Lab 1 is AAL, used for analyses of OCs in bioaccumulation study

Of the OCs analysed, chlordane and PCBs were the most difficult to analyse. Both compounds are made up of multiple peaks which can be difficult to differentiate from other OCs present, particularly at low concentrations (eg. less than 0.05 mg/kg).

Chlordane Compounds

Lab 1 reported high concentrations of chlordane. Three main chlordane isomers in the GC analysis were identified using 2 columns. The chlordane isomers were confirmed by GC/MS. Oxychlordane, a metabolite of chlordane, was also detected by Lab 1 in most fish from Site 1 using GC.

Lab 2 reported chlordane only as 2 isomers (cis-chlordane and nonachlor). These compounds were identified on one GC column and confirmed on GC/MS. There were other compounds present which had retention times similar to oxychlordane and other isomers of chlordane, but these could not be differentiated from larger, unidentified peaks present. Thus the results from Lab 2 are lower for chlordane and further method development and additional equipment is required to positively identify other

chlordane isomers present.

Thus Lab 1 is expected to obtain higher chlordane values than Lab 2. In fact, there are many other chlordane-related isomers (including oxychlordane, trans-chlordane, cis chlordane, nonachlor-III, trans-nonachlor and cis-nonachlor - Muir et al., 1988; Norstrom et al 1988). Thus even results from Lab 1 may be conservatively low and further investigation into differentiating chlordane isomers is warranted.

Lab 3 only detected traces of chlordane (0.02 - 0.04 mg/kg) in 3 of the 12 red morwong analysed. Lab 4 could not analyse for chlordane because it used a silicic acid column clean up which would retain some of the chlordane.

Polychlorinated Biphenyls (PCBs)

In the environment, chlorinated biphenyls are complex mixtures containing many homologous and isomeric species that range from monochlorobiphenyl up to decachlorobiphenyl isomer. Commercial mixtures of these compounds are known under the trade name of Arochlors (Ryan, 1977). PCBs were only reported by Lab 1 (range 0.1 to 0.4 mg/kg at site 1). There are many chromatogram peaks associated with PCB standard Arochlor 1254 and these are often difficult to distinguish when many other OCs, their metabolites and degradation products are present.

Lab 1 detected 2 PCB isomers in red morwong muscle tissue from Site 1. These were confirmed by GC/MS. Labs 2 and 3 did not report PCBs and the method employed by Lab 4 was not suited to PCB analysis.

3.3.2 Comparison of OCs of laboratories, sites and fish

ANOVA was conducted for 6 OCs (Appendix 3.1). All showed statistical interactions between fish and laboratories, indicating that the OC concentrations reported by laboratories varied according to the fish analysed. This means that the red morwong muscle samples analysed would yield different results, depending on the laboratory used. A further complication is that variation among laboratories is not constant, but varies according to actual fish analysed.

For total OC concentration, Lab 2 and Lab 1 had the highest mean for all fish, however, there were no significant differences for fish 1,2 and 3 at Site 2, where we had assumed OC contamination was lower. At site 1, Lab 2 detected a significantly higher OC concentration on average than all other laboratories for fish 1,3 and 4, while Lab 1 detected a higher concentration than all other labs for fish 5, and a higher concentration than Labs 3 and 4 for fish 4.

For chlordane, Lab 1 detected a higher concentration than Labs 2 and 3 for fish 1,4,5 and 12, while there was no difference among laboratories in 8 fish. Lab 3 did not detect chlordane in 9 of the 12 fish. Aldrin was not detected by Lab 1 (Table 3.2). It was detected in significantly higher concentrations by Lab 2 compared with Labs 3 and 4 for fish 1,4 and 9.

DDE, DDT and DDT(R) showed similar patterns among fish and laboratories, and Lab 2 typically had a higher mean concentration than all other laboratories. At Site 1, Labs 1 and 2 recorded a higher concentration of DDE for fish 4; and for Lab 2, DDT and DDT(R) were higher than all other laboratories for fish 1 and 4. For DDE, DDT and DDT(R), fish from Site 2 and the remaining fish from Site 1 showed no significant differences among laboratories.

For % fat content, none of the three laboratories compared was clearly differentiated from the others. Lab 2, however, did obtain slightly higher (though non-significant) fat contents than Lab 1, due to different extraction procedures. Lab 2 was likely to extract proteinaceous material in addition to the fat with its method. Lab 4 did not determine fat (see Section 3.4).

3.4 DISCUSSION

3.4.1 Analytical Confidence

Site 1 was chosen because high OC concentrations were expected. The laboratories should determine high levels with greater confidence than the low sites. The actual values for site 1, however, did not reflect this expectation. Therefore the number of low OC values are confusing the issue as the confidence in these values is low. The "not detected" and "trace" results, assigned a value of zero, are influencing statistical interpretation.

Each laboratory assigned its own limit of detection for its method (Table 3.1). At the detection limit (DL) the normal error in the reported concentration is typically 100%. Thus we compared results obtained from each laboratory at a reporting limit where less than 5% error was expected. Thus results reported greater or equal to 10 x DL for each laboratory were compared to simplify interpretation (underlined data in Appendix 3.2).

Lab 3 did not produce many useful results: only three out of the ten pairs above 10 x DL duplicated (within 10 %), therefore this laboratory's results were not considered any further. Labs 1 and 2 reported many data points where chlordane, HCB, DDE, DDT, DDT(R) were at 10x DL. Lab 4 provided results which were above 10 x DL for DDE, DDD, DDT and DDT(R).

Each laboratory used pure organochlorine standards to quantitate the selected OCs. Also an international standard of

OCs in fish muscle tissue would have been useful to compare results between laboratories but was unavailable for this study. Because of the difficulties in extracting OCs from fish muscle, results reported are likely to underestimate the true concentrations. The use of an international standard would be valuable in comparing the recovery of selected OCs from fish muscle between laboratories.

3.4.2 Agreement between sample replicates

Replication in laboratory results is important when examining the precision and suitability of a particular method. Einerson and Pei (1988) found that laboratories which have poor precision in measurement of replicate samples usually have inaccurate results. Lab 1 gave reasonable agreement of duplicates for results 10 x DL for chlordane, HCB, DDE, DDT, DDT(R). Lab 2 showed reasonable agreement between duplicates for chlordane and HCB poorer duplication for DDE, DDT and DDT was found. Lab 4 gave reasonably good agreement between replicates for DDE, DDD, DDT and DDT(R).

For the simplest determination, % fat, (Appendix 3.2), Labs 1 and 2 had high agreement between duplicates but Lab 3 was extremely poor.

Some laboratories commented that the homogenised sample was not completely macerated (even after 10 mins homogenisation) and that differences between duplicates could be expected.

3.4.3 Evaluation of methods employed by participating laboratories

OC analyses are conducted in a number of stages. The first stage involves extraction of the OC from the fish matrix using an efficient and selective solvent. Interfering substances are removed from the extract in a clean-up procedure. The compounds of interest are detected by injection onto a gas chromatography (GC) column. The compounds (parent pesticides and their metabolites and some breakdown products) are estimated by comparison to standard materials. The OC's are confirmed using another column (of different chemical characteristics to the first) and if possible, confirmation by GC/MS. In general organochlorine compounds are reported if they can be easily differentiated from the background noise of the sample and if standards are available (eg some metabolites and breakdown products are sometimes not reported due to lack of proper standard material). The different stages employed by the four laboratories involved are summarised in Table 3.1.

Several aspects of these procedures are important for evaluating the methods used by each laboratory. These are discussed as follows:

Complexity of fish matrix

The analyses of fish for OCs presents some special clean-up problems due to the difficulty of removing coextracted oil. The oil causes eratic elution patterns of the residues from many chromatographic column adsorbents (Reynolds and Cooper, 1975). The efficiency of the extraction and clean-up procedures by a particular method give different recoveries and may be subject to different interferences. For example :

1) Lab 4 used a silicic acid column which prevented detection of PCBs and some organochlorines.
2) Lab 1 used solvent extraction followed by low temperature freezing as a cleanup whereas Lab 2 employs a gpc clean-up step. Thus the Lab 1 method requires less steps, with less likelihood of pesticides being adsorbed to cleanup columns.

The subjectivity of the analyst

The subjectivity of the analyst is critical in reporting low levels of OCs. The analyst must decide whether the OC is present above noise or other chromatographing species (Figure 3.1 indicates the problems in identification of trace amounts of OCs) when there is a noisy baseline. Further confirmation by one or two GC analyses is dependent on the original detection.

Interferences from other compounds present in samples

Fish collected near an ocean outfall (discharging industrial effluent) may bioaccumulate other compounds including plasticisers, benzene derivatives and other chlorinated organics which could complicate the identification of the known organochlorine pesticides. A number of unidentified peaks were observed in red morwong from Site 1 and these substances may interfere with the assignment of the OCs of interest. Lab 4 found two unknown large peaks in all samples with retention times close to alpha BHC, chlorpyrifos, or possibly aldrin metabolites. These compounds could not be identified, however, with the methods available to that laboratory.

By using a GC equipped with an electron capture detector (ECD), any molecule containing chlorine, sulphur or oxygen has some potential of giving a response to the detector (De Vries, 1968). Non-chlorinated compounds such as quinones, fumarate esters and pyridines are among some compounds which exhibit electron capturing capacity. As sewage effluent from Malabar contains a wide range of organics some of these substances, besides the OCs, may bioaccumulate.

Substances found to interfere in the GC analysis of OCs

include di-n-butyl phthalate (commonly used as a plasticiser) sulphur-containing compounds and elemental sulphur (Ruzicka, 1973). In addition, PCBs can interefere with the identification of OCs in some chromatographic systems.

3.4.4 Detections of organochlorines at low concentrations

The analysis of OCs in red morwong muscle tissue at concentrations < 0.1 mg/kg is very difficult. Better agreement between the laboratories would be expected if concentrations of OCs in the muscle tissue sent for analyses contained OCs well above 1.0 mg/kg. This strongly indicates that the OCs reported by each laboratory reflect the methods utilized (Table 3.1) and the interpretive approach of each analyst. In regard to the participating laboratories, the following evaluation is made:

Lab 1 - has previously analyzed fish from around the Malabar outfall and developed suitable methods for identification of OCs in complex matrices such as sewage sludges.

Lab 2 - have undertaken analysis of fish for OCs and sludge OCs as part of a monitoring program and are aware of the wide range of compounds present in sewage/fish near outfalls.

Lab 3 - a high throughput laboratory used existing screening method without extensive method development on the type of OCs present in fish near a sewage outfall.

Lab 4 - used a method which was designed primarily as a quick one-step extraction and clean-up procedure for the determination of DDE, DDD and DDT in fish. It was found to be poorly suited to characterising the wide range of OC's present in fish near the outfall.

We conclude that Labs 1 and 2 are most suitable for comparing analysis of OCs in fish matrix.

At least 10 laboratories would generally be needed to participate in an interlaboratory study (Einerson and Pei, 1988). With more laboratories, sufficient information would be provided on the suitability of each of the laboratories based on their performance associated with accuracy, precision, time of analysis and costing.

Past interlaboratory studies of OCs in different food matrices have documented the problems encountered in OC analyses. Twenty-nine laboratories were involved in an interlaboratory study of OCs present in meat fat (AGAL, 1987). A wide range of results were obtained and nine laboratories did not report OCs

which were known to be present; or they reported ranges well outside the known range. Moreover some laboratories reported OCs which were not present in the samples. Some results for individual OCs varied by up to 5 times between the lowest and highest recordings.

A review of an interlaboratory study on OCs in capelin fish oil involving 44 laboratories, where only 4 labs used GC/MS as confirmation concluded that the analysis was a major source of error (Hamilton, 1982). Another study used uniform calibration solutions and analytical procedures in an interlaboratory study of OCs in water, soil and sediment (Alford-Stevens et al., 1988). It found that the identification of OCs between labs was excellent, but that the internal standard peak areas were not reproducible and that improved chromatographic performances would significantly improve data quality. Additional errors were found to be due to human transcription.

In the present study, we used 4 laboratories, because of time and cost limitations. In spite of this limitation, the present interlaboratory comparison produced results which had variation typical to other studies.

3.5 CONCLUSIONS

We conclude from the interlaboratory study that the accurate determination of OCs in muscle tissue of red morwong near an ocean outfall is very difficult. More method development is required by some laboratories to identify and accurately quantify the OCs of interest, as well as their metabolites and degradation products. Thus it is important to use laboratories which have this expertise.

The results indicate that although many OCs were detected in fish collected from Site 1, many of these were at concentrations well below a limit which could be considered to contain less than 5 % error, ie., 10 x DL. Thus a review of only the results which were greater than 10 x DL provided a better interlaboratory comparison. After making this comparison we found that Lab 1 and Lab 2 detected accurately a wide range of OCs. Lab 3 was found to be unsuitable for OC analyses in red morwong, as most results reported were below 10 x DL. Because of methodological constraints, Lab 4 was limited to accurate determinations of DDD, DDE and DDT. Lab 4, however, gave similar results to Lab 1 for the DDT compounds.

This study also clearly indicates that for the identification of OCs in trace concentrations (ug/kg range) three analytical procedures, such as those used by Lab 1 (ie two GC columns plus CG/MS) should be conducted in order to have a high confidence in analytical results.

Lab 1 was found to be suitable for the analysis of OCs in red morwong muscle collected near the Malabar outfall. The results obtained from Lab 1 and Lab 2 were similar (within an order of magnitude), with Lab 2 reporting consistently higher results for HCB, DDE, DDT, DDT(R) and dieldrin. However, Lab 1 had consistently higher results for chlordane than Lab 2. This was due to differences in reporting some of the isomers of chlordane: Lab 1 reported oxychlordane and 3 isomers; while Lab 2 reported no oxychlordane and only 2 isomers. Lab 2 suggested that with further method refinement and equipment they would have reported these compounds.

In conclusion, the interlaboratory study showed reasonable agreement between Labs 1 and 2 for HCB, DDE, DDT and DDT(R). The chlordane results differed typically by a factor of six to eight. This was due to clearly documented variations in methodology employed by the laboratories.

Lab 1, AAL Pty. Ltd. was found to be suitable for the determination of OCs in the muscle tissue of the red morwong.

3.6 SUGGESTED PROTOCOL FOR DETERMINATION OF ORGANOCHLORINES IN FISH MUSCLE TISSUE

The results of this study suggest that work is required in establishing a protocol for the analysis of OCs in fish tissues. The following points should be considered:

1) Further method development - use of low temperature solidifying techniques for extraction of oils.

2) Investigation of appropriate instrument analyses - for example the use of 2 GC columns, with GC MS confirmation.

3) The use of ground and sieved, freeze-dried tissue - to overcome problems with heterogeneity of wet tissue samples.

4) Identification of chlorinated compounds other than those of interest.

5) The use of international standards - to determine the extraction efficiencies of methods, and for use in interlaboratory studies (Waldichunk, 1987).

CHAPTER 4. BIOACCUMULATION STUDY

4.1 INTRODUCTION

This study addresses the variation of OCs in red morwong muscle tissue at various distances either side of three shoreline sewage outfalls, and the biological characteristics that may affect this variation. The twenty four sample locations and methods used in the study are detailed in Chapter 2.

4.2 RESULTS

4.2.1 Biological Characteristics of Red Morwong

Statistical results are summarised in Appendix 4.1 and Figures 4.1 to 4.6. The raw data are reproduced in Appendix 4.2. Fish length, measured as fork length, gave a significant interaction between distance, direction and outfall. At Malabar and Bondi, there was no significant difference in fork length across distances and directions. For North Head, however, red morwong were significantly longer 0.5 km to the south of the outfall than 1.5 to the north.

Analysis of standard length resulted in a significant interaction between outfall and direction. Fish to the north of Bondi were significantly longer (as standard length) than on any other stretch (ie 0.5 to 3.5 km north or south of an outfall) of coastline. Also, fish to the south of North Head were longer than those to the north. No difference occurred between the north and south of Malabar (Figures 4.1 and 4.2).

For fish weight, there was a significant distance effect and and interaction between direction and outfall. For distances, fish 0.5 km from the outfall were heavier than those at other distances. This may be due to a greater food supply near the outfalls. Fish were also heavier to the north of Bondi than to the north of North Head, but fish from other locations did not show significant differences (Figure 4.3).

Many of the red morwong dissected had small gonads, which did not enable determination of sex. A few fish were identified as females by their well developed ovaries, indicating that they were approaching spawning. A few fish were identified as males, but these had relatively small gonads.

The number of increments seen in otolith sections during the ageing study (Appendix 4.3) varied from 1 to 27, with very few over 20. The very low increments occurred in small fish, which were not used in OC determinations. This study did not determine whether the increments related to annual growth; if they did then the red morwong appear to be relatively long-lived, in the order

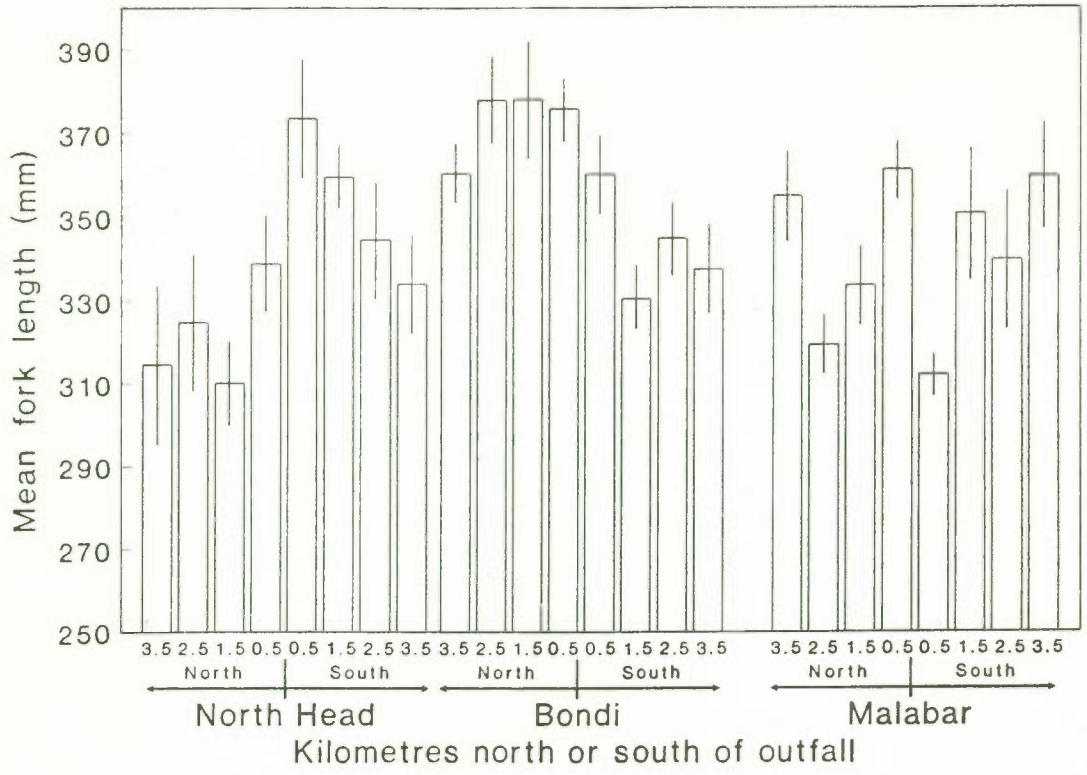


Figure 4.1: Mean fork length LCF (mm) of *C. fuscus* plus 1 standard error for all sites (n = 8)

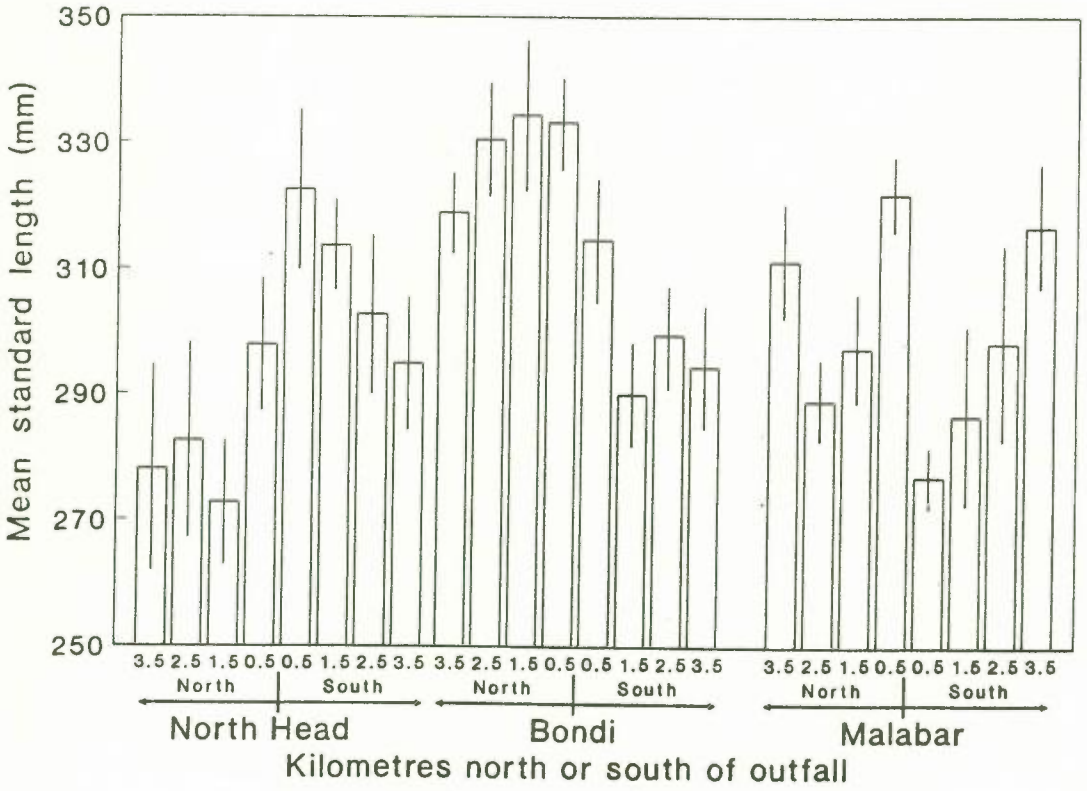


Figure 4.2: Mean standard length STD (mm) of *C. fuscus* plus 1 standard error for all sites (n = 8)

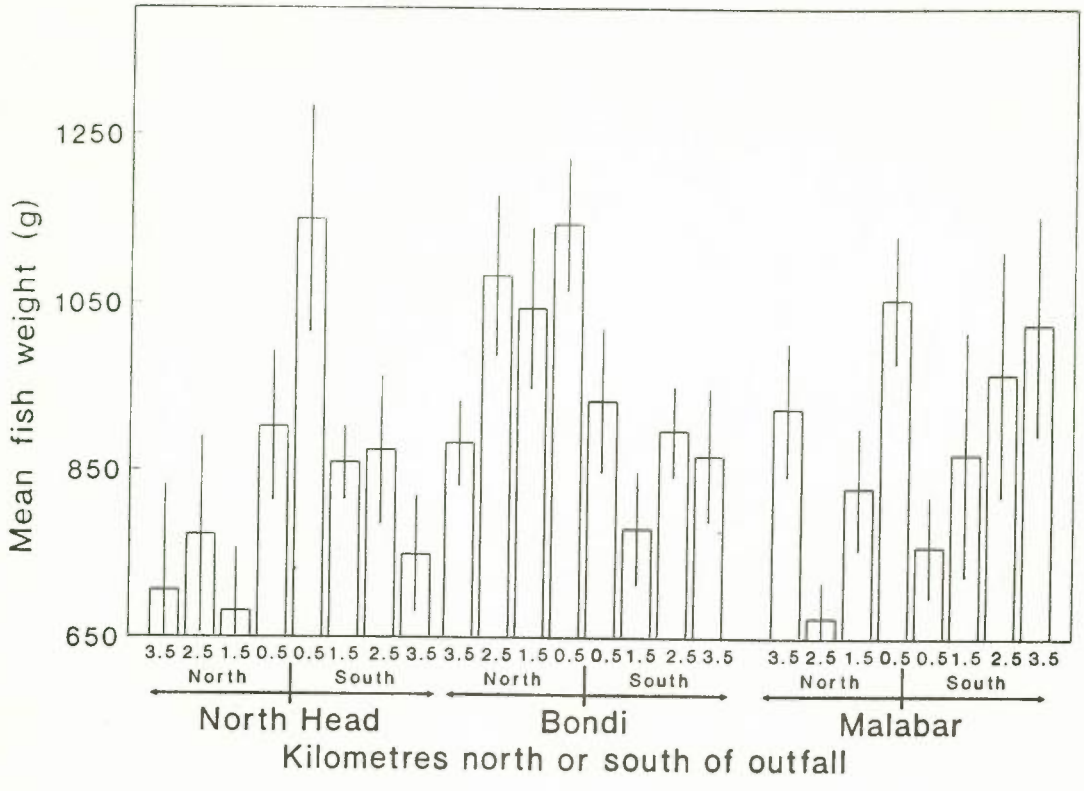


Figure 4.3: Mean fish weight (g) of *C. fuscus* plus 1 standard error for all sites (n = 8)

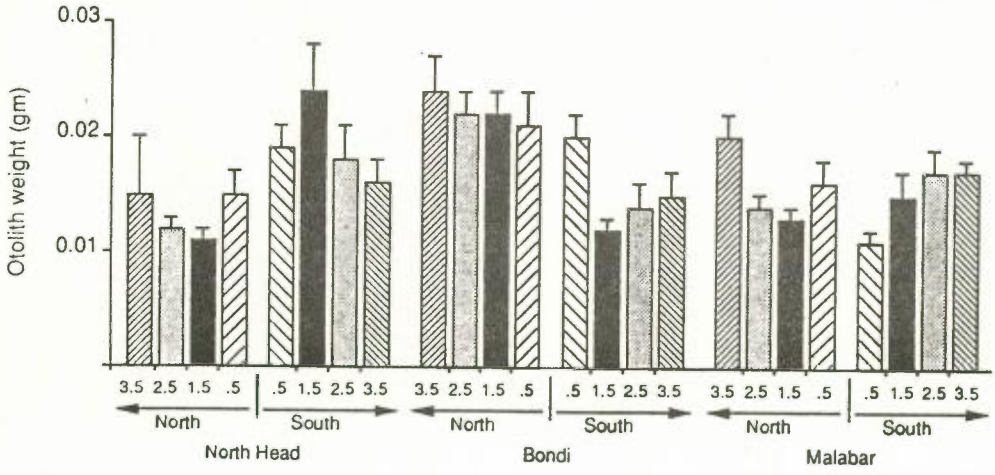


Figure 4.4: Mean otolith weight for each of the 24 sites sampled. All means from n = 8 except site 3.5 km north of North Head where only 4 available (Source: P J Ferrell, Appendix 4.3)

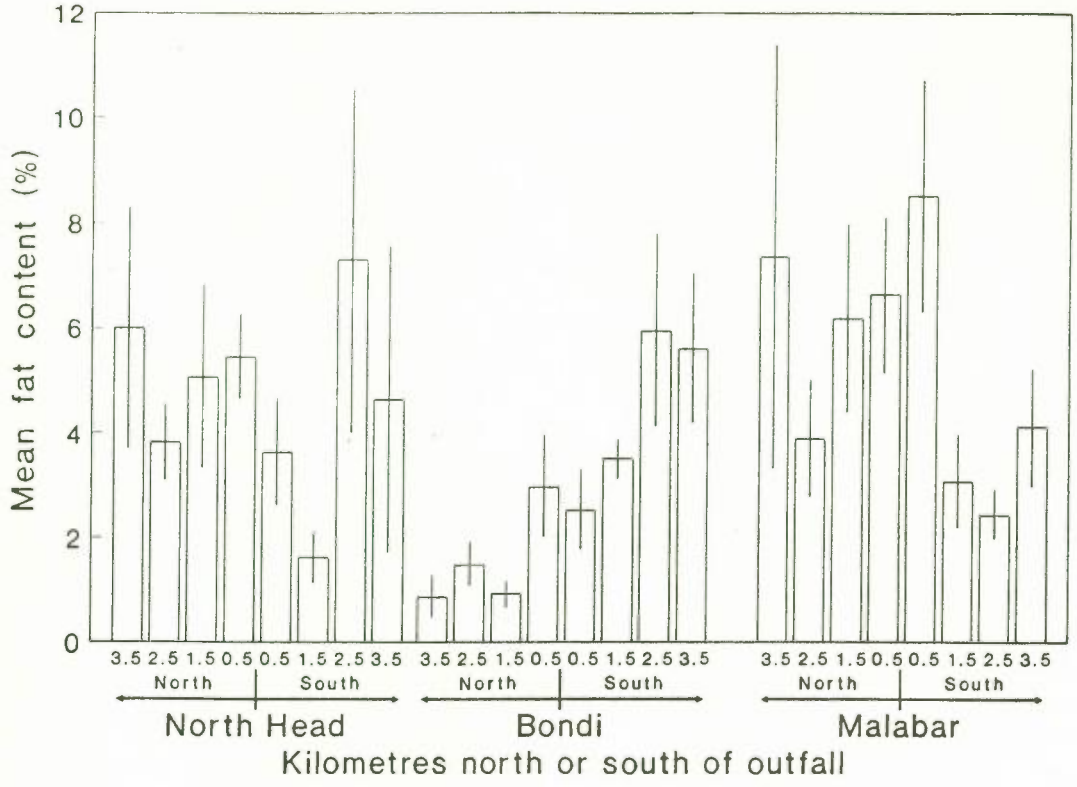


Figure 4.5: Mean fat content (%) in muscle tissue of *C. fuscus* plus 1 standard error for all sites (n = 8)

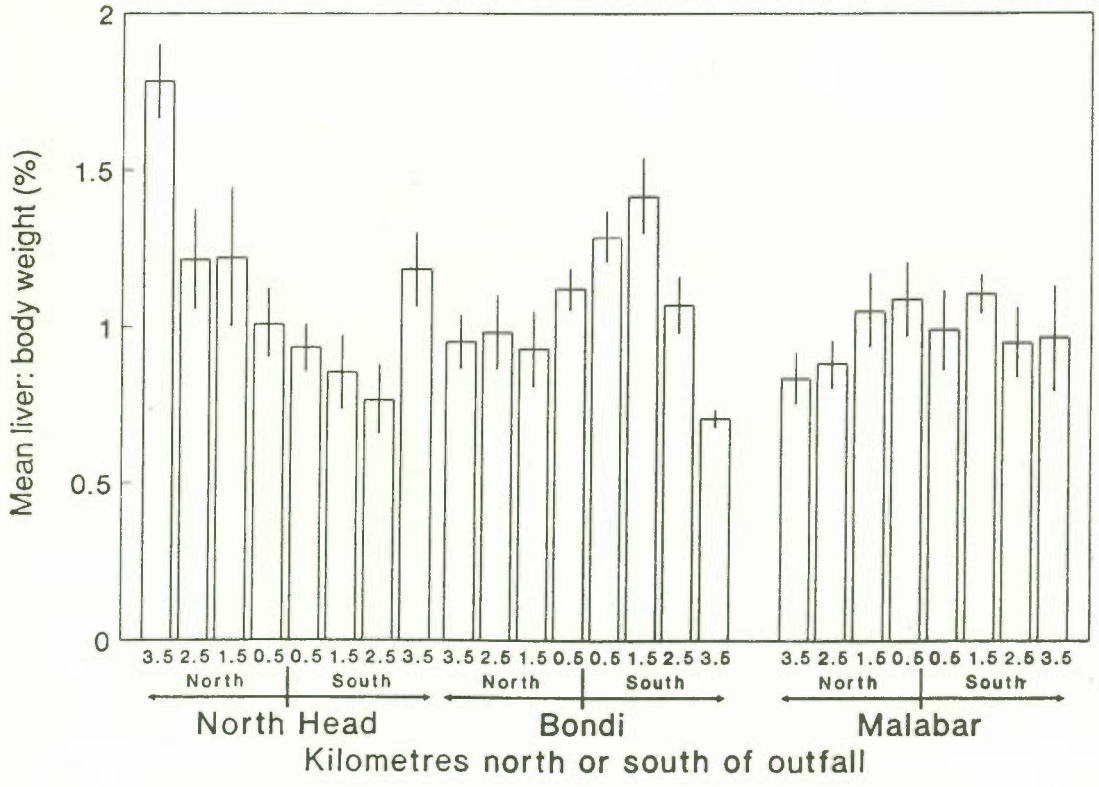


Figure 4.6: Mean liver:body weight of *C. fuscus* plus 1 standard error for all sites (n = 8)

of at least 25 years.

Relative age of red morwong, as measured by otolith weight, varied by distance, direction and outfall in a 3-way interaction. North Head-south and Bondi-north had fish with the greatest otolith weights, causing an interaction between outfall and direction, upon which was overlaid significant differences among sites at different distances, which varied among both outfalls and directions. It is likely that red morwong caught at North Head-south and Bondi-north are older than the along other stretches of coastline (Figure 4.4). Comparing sites, red morwong at 3.5km were older than 1.5km north of Malabar. All other comparisons were non-significant.

Fat content of red morwong varied as an interaction between outfall and direction. Red morwong to the north of Bondi had a lower fat content than along any other stretch of coastline, but all other stretches were statistically equivalent (Figure 4.5).

Liver weight to body weight indicated significant interactions between distance and outfall and direction and outfall. These were interpreted as follows:

1) Red morwong collected to the north of North Head had heavier livers in proportion to their bodies than any other stretch of coast, except those to the south of Bondi (Figure 4.6).

2) Red morwong from Bondi and Malabar did not differ in relative liver weight over all distances. For North Head, however, livers 3.5 km from the outfall were heavier in relation to body weight North than at 0.5, 1.5, or 2.5 km.

4.2.2 Organochlorine concentrations in red morwong

4.2.2.1 Organochlorines detected

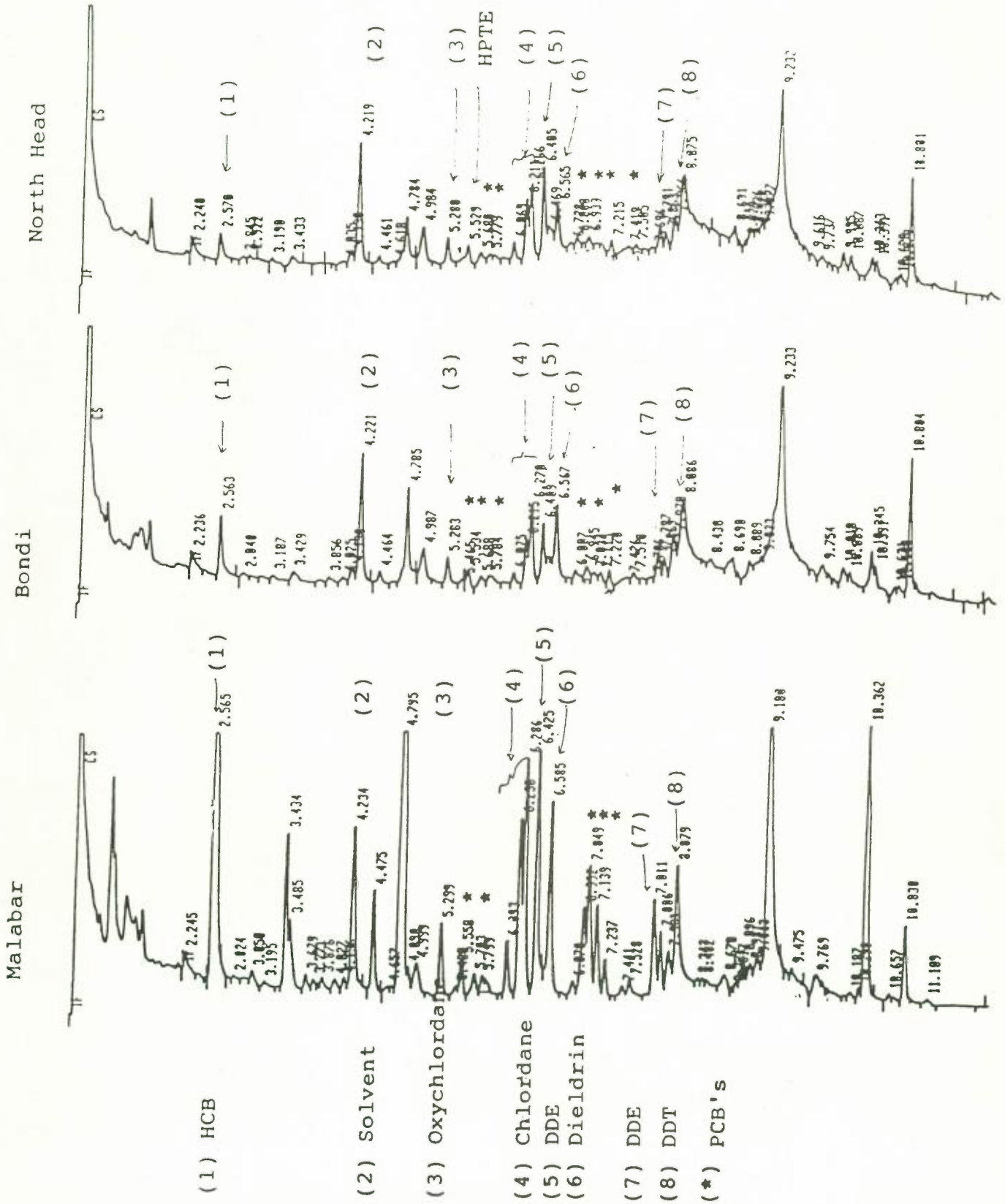
A total of nine OCs were detected in the muscle tissue of the red morwong (Table 4.1). Some of these occurred in concentrations above the NHMRC MRLs at a number of sites (Figures 4.8 to 4.16, Appendix 4.6). The graphical profiles suggest that the outfalls are principal sources of OCs. This is substantiated by statistical analyses, as reported below. It is interesting to note that the parent compounds, chlordane and DDT, were in higher concentrations than their metabolites, oxychlordane and DDE/DDT, respectively. This suggests that the red morwong sampled had been recently exposed to the parent compound.

In addition to the OCs reported, there were also a large number of unreported (and unidentified) peaks on the chromatograms (Figure 4.7). These probably represent anthropogenic compounds bioaccumulated in the red morwong muscle tissue, but we do not know their possible impacts on humans consuming this fish species, nor the impacts on the fish themselves.

TABLE 4.1 Ocs detected in muscle tissue of red morwong at 24 sites along the Sydney coast. + = recorded in concentrations above the AAL detection limit; T = trace concentration only.

OC	North Head								Bondi								Malabar							
	North				South				North				South				North				South			
	0.5	1.5	2.5	3.5	0.5	1.5	2.5	3.5	0.5	1.5	2.5	3.5	0.5	1.5	2.5	3.5	0.5	1.5	2.5	3.5	0.5	1.5	2.5	3.5
Chlordane	*	*	*	*	*	*	*	*	*	T	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Oxychlordane	*	*	T	T	*	T	*	T	*		T		*	T	T	T	*	*	*	*	*	*	*	*
HCB	*	*		*	*	T	*	*	*	T	*	T	*	*	*	*	*	*	*	*	*	*	*	*
DDE	*	*	*	*	*	*	*	*	*	T	*	*	*		*	*	*	*	*	*	*	*	*	*
DDD	T		*	*	*		*	*	*		*	T			T	T	*	*	*		*	*	*	*
DDT		*	T	T		*	*	*			*	*					*	*			*	*	*	*
Dieldrin	*	*	*	*	*	*	*	*	*	T	*	*	*	T	*	*	*	*	*	*	*	*	*	*
Aldrin																								
Lindane																								
b-BHC																								
g-BHC																								
Heptachlor																								
HPTE	*				*				*				*				*	*		T	*	*	*	*
Methoxychlor																								
Endosulphan																								
Endrin																								
PCBs	*		T	T	*				*		*		*			*	*	*		*	*	*	*	*

Figure 4.7: Comparison of gas chromatographs of OCs in red morwong muscle tissue collected near Malabar, Bondi and North Head outfalls



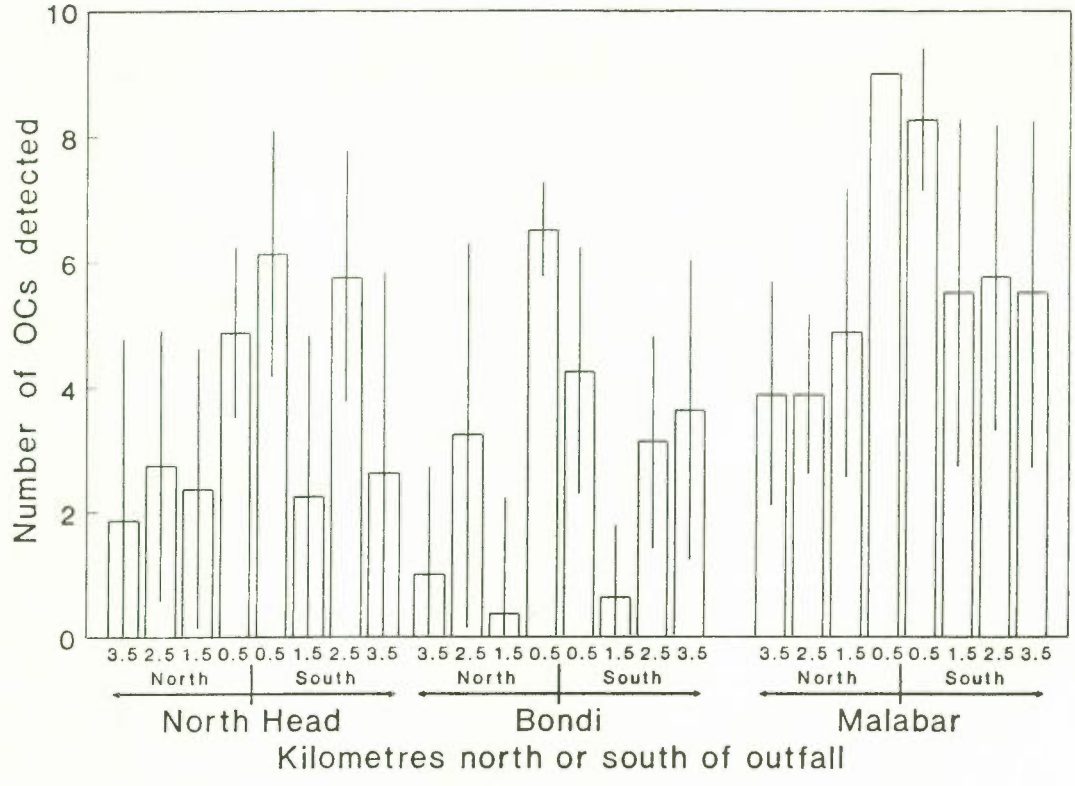


Figure 4.8: Mean number of OCs detected in muscle tissue of *C. fuscus* plus 95% confidence limits for all sites (n = 8)

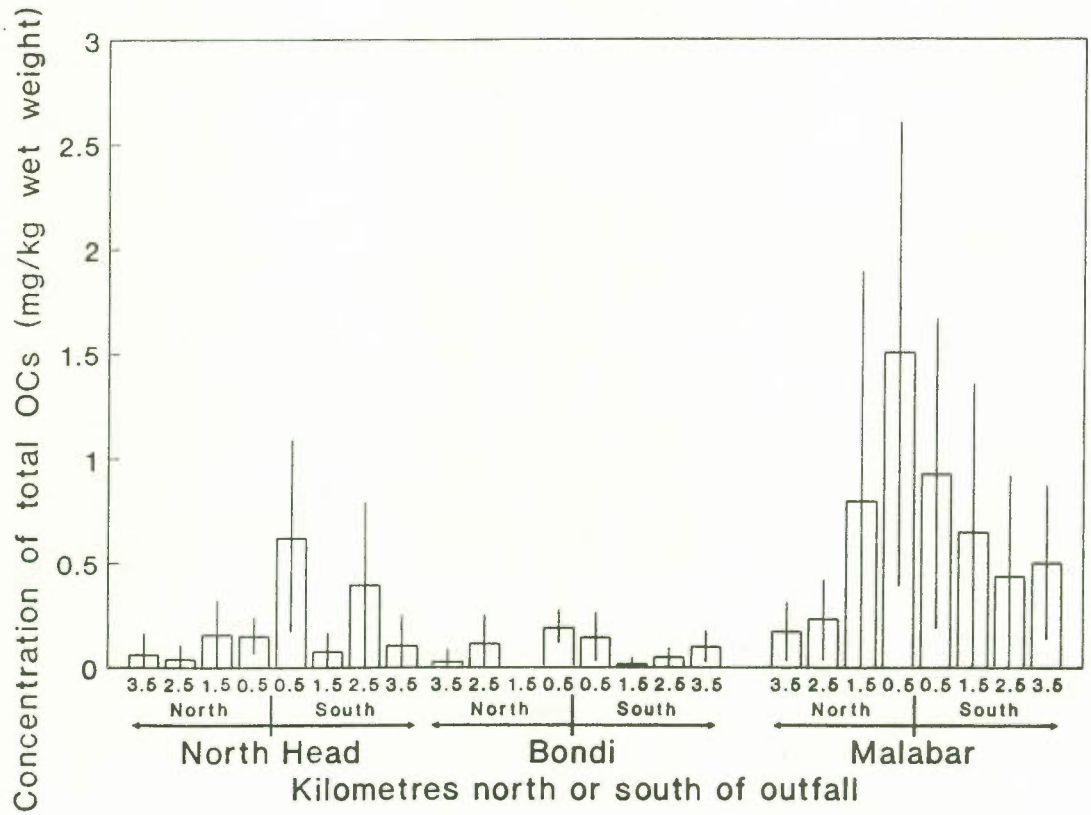


Figure 4.9: Mean concentration of total selected OCs (mg/kg wet weight) in muscle tissue of *C. fuscus* plus upper 95% confidence limits for all sites (n = 8)

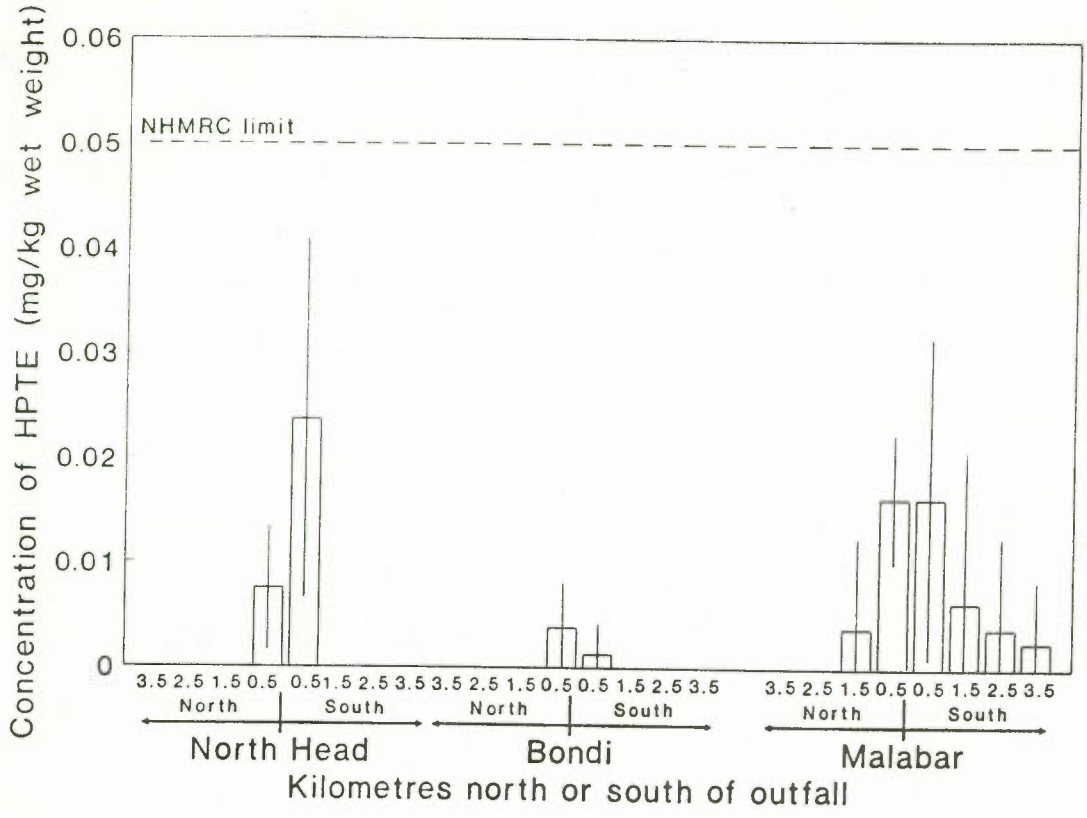


Figure 4.10: Mean concentration of heptachlor epoxide, HPTE (mg/kg wet weight) in muscle tissue of *C. fuscus* plus 95% confidence limits for all sites (n = 8)

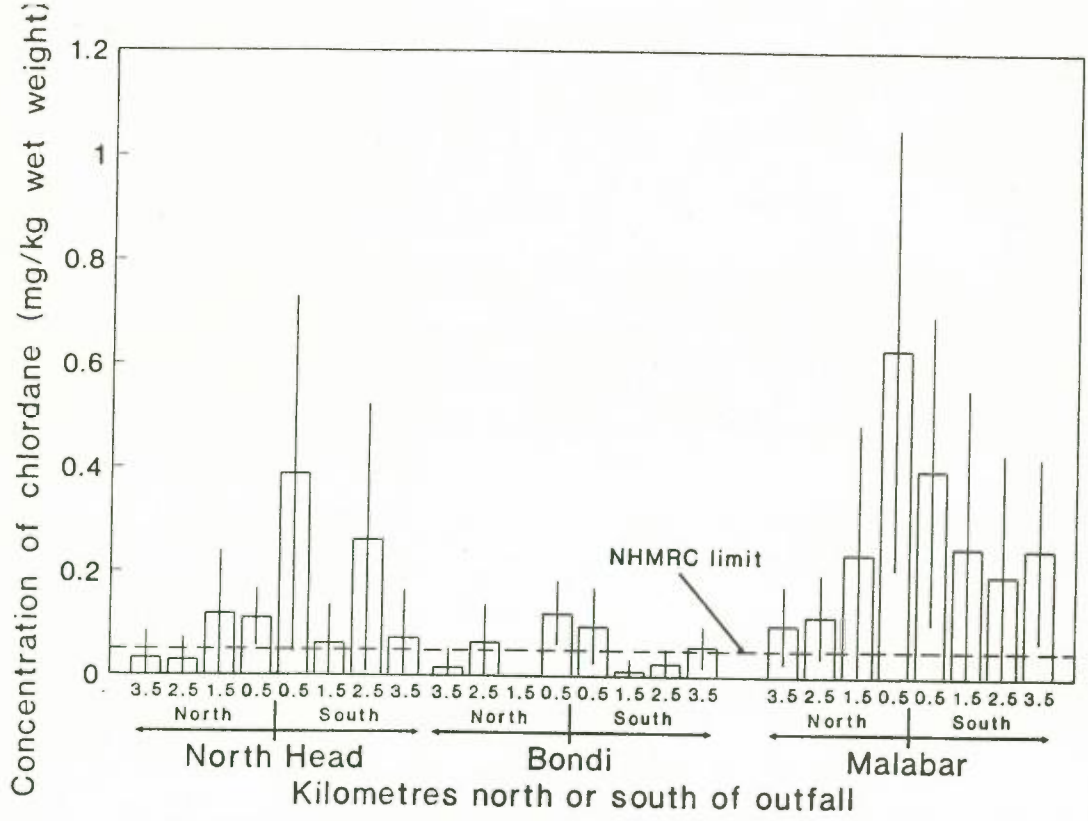


Figure 4.11: Mean concentration of chlordane (mg/kg wet weight) in muscle tissue of *C. fuscus* plus 95% confidence limits for all sites (n = 8)

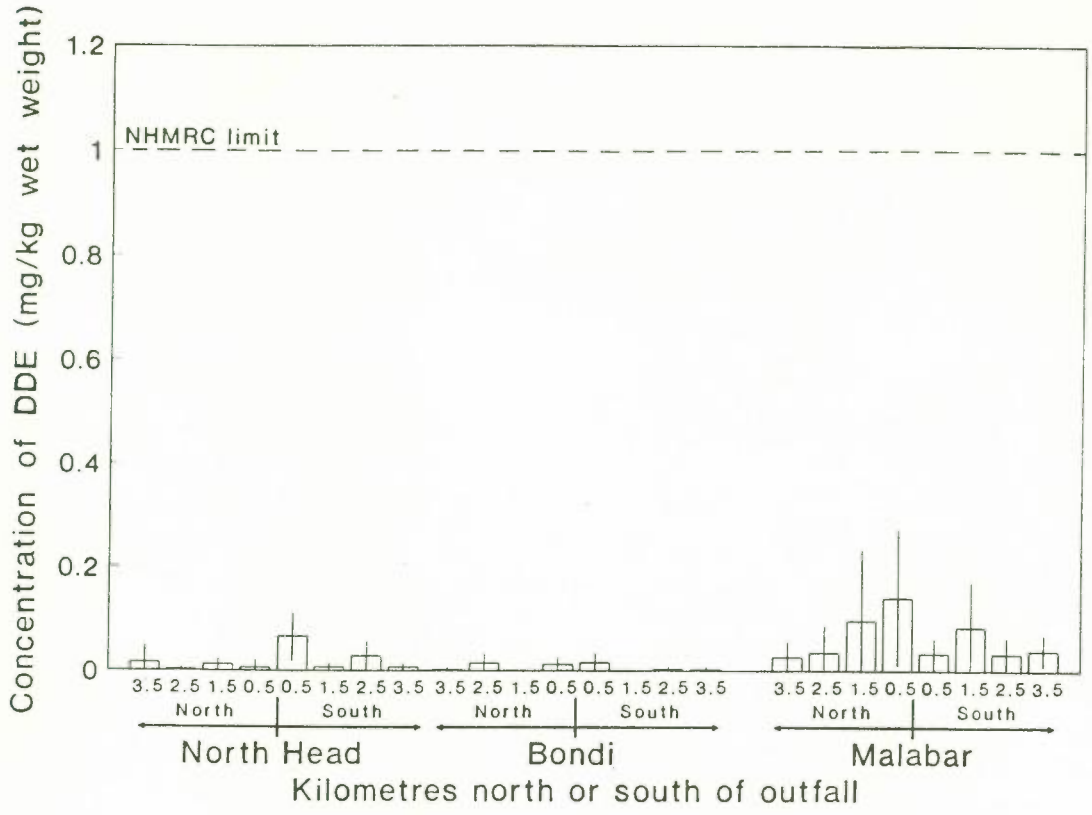


Figure 4.12: Mean concentration of DDE (mg/kg wet weight) in muscle tissue of *C. fuscus* plus 95% confidence limits for all sites (n = 8)

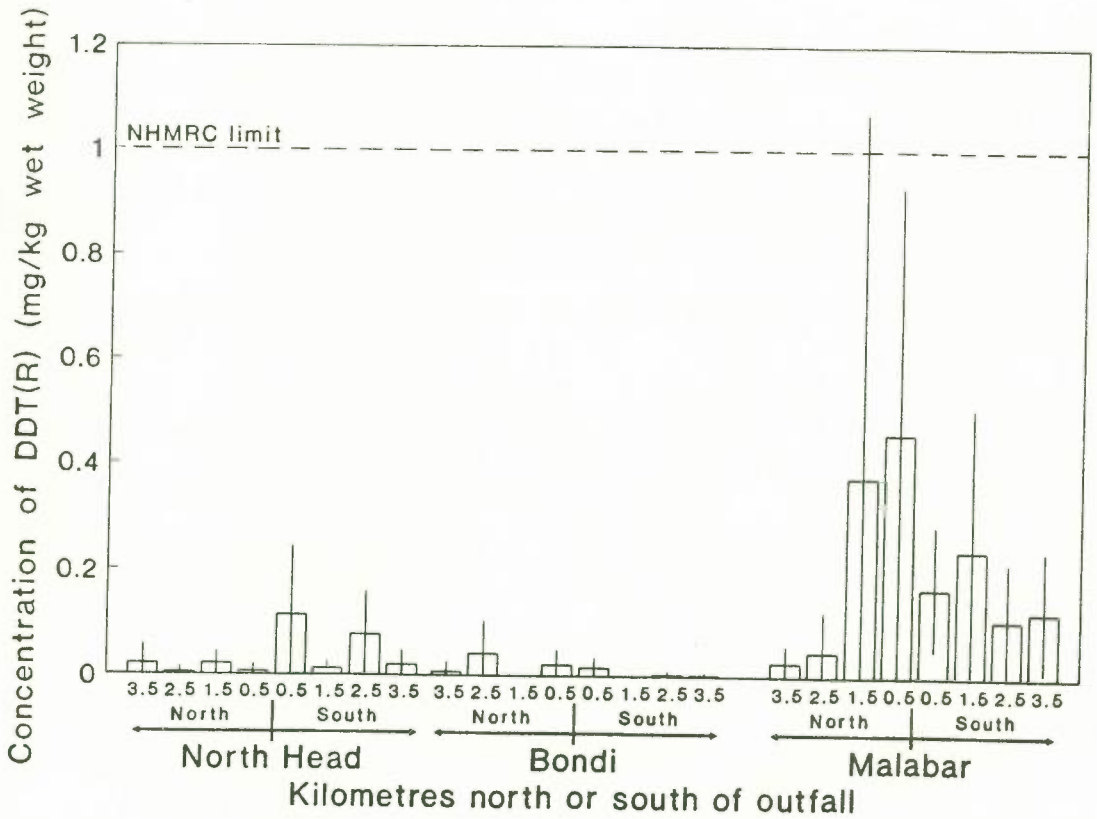


Figure 4.13: Mean concentration of DDT(R) (mg/kg wet weight) in muscle tissue of *C. fuscus* plus 95% confidence limits for all sites (n = 8)

4.2.2.2 Comparison of outfalls, direction and distance

DATA ANALYSED AS WET WEIGHT

Number of selected OCs

Significant outfall and distance effects were found for the number of OCs detected in red morwong muscle tissue (Figure 4.8, Appendix 4.4). These are interpreted as follows:

1) More OCs occurred in the samples from 0.5 km either side of all outfalls than at all other distances.

2) There was a significantly greater number of OCs detected, on average, 2.5 km away from the outfalls than 1.5 km away.

3) More Ocs were detected in red morwong collected from Malabar (over all distances) than North Head or Bondi, which in turn had statistically equivalent numbers of OCs.

A significant linear regression between distance and the number of OCs was found at Malabar (Appendix 4.1), although the regression explained only a small part of the variation, as seen from the low r-square value.

Total concentration of selected OCs and chlordane

Chlordane typically constituted over 50 % of all OCs detected per site. It occurred in mean concentrations above the NHMRC MRL at 18 of the 24 sites (Figure 4.11). The highest mean concentration was recorded 0.5 km north of Malabar, where it was nearly 13 times the MRL.

Both the concentrations of all OCs combined and that of chlordane varied in red morwong muscle tissue according to distance and outfall (Figs. 4.9, 4.11). The following interpretations were made:

1) Significantly higher concentrations occurred in red morwong collected 0.5 km from the outfalls than at any other distance.

2) Significantly higher concentrations occurred at Malabar than the other outfalls, and North Head had higher concentrations than Bondi.

There was a significant linear regression of concentrations of all OCs versus distance at Malabar, although the r-square value was low, indicating other sources of variability (Appendix 4.4). The regression equation predicts an average concentration of 1.6 mg/kg of total selected OCs in red morwong tissue at the outfall itself, during the time of sampling. A regression analysis of chlordane concentration versus distance to the north of Malabar had a probability of 0.05, but this was not accepted

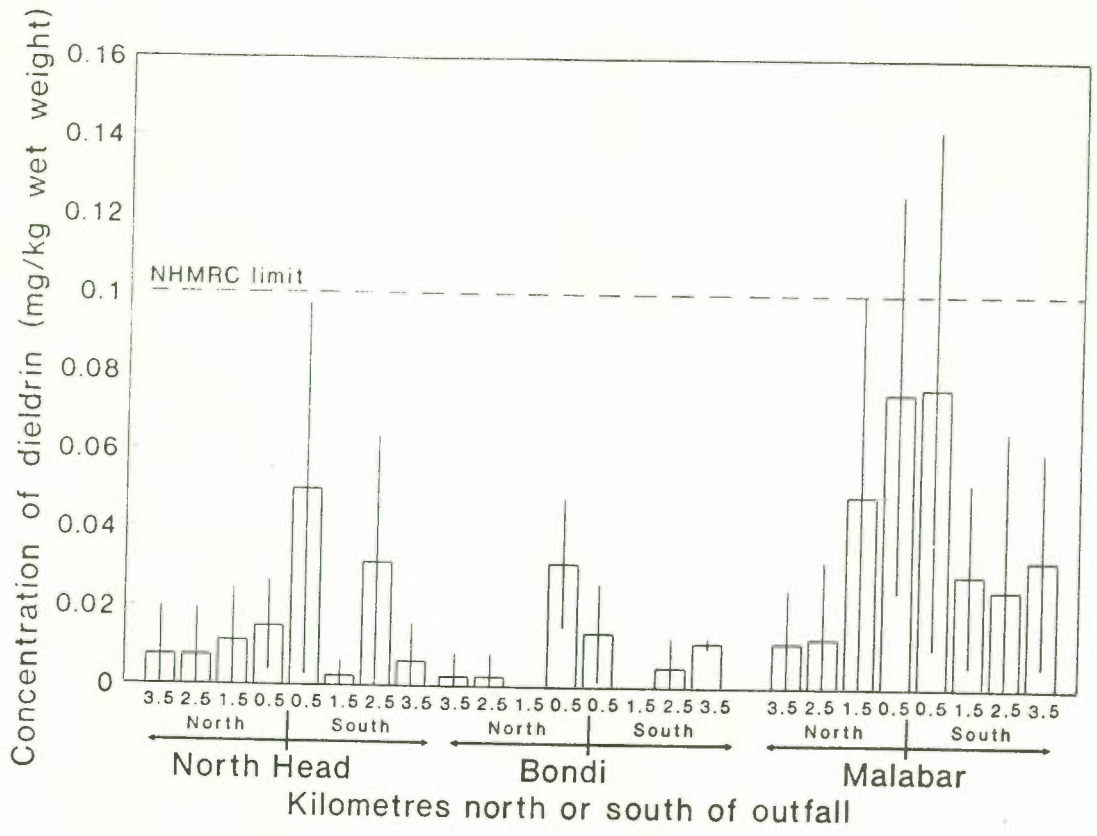


Figure 4.14: Mean concentration of dieldrin (mg/kg wet weight) in muscle tissue of *C. fuscus* plus 95% confidence limits for all sites (n = 8)

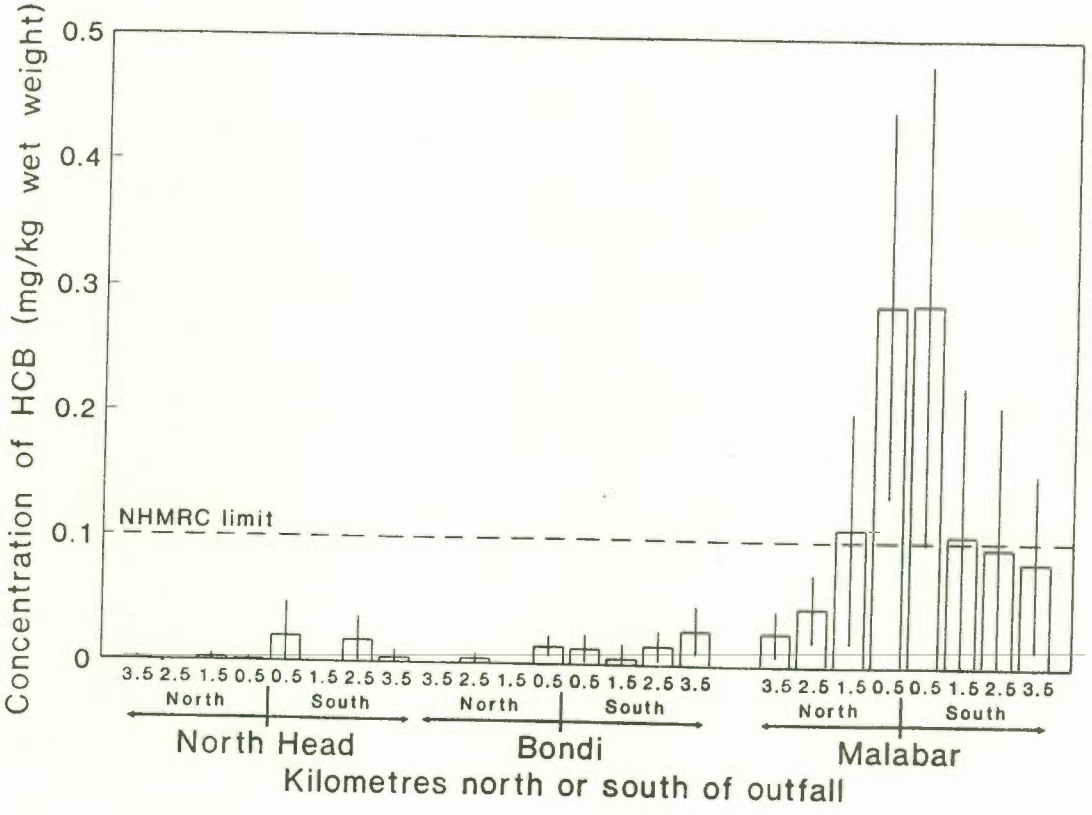


Figure 4.15: Mean concentration of HCB (mg/kg wet weight) in muscle tissue of *C. fuscus* plus 95% confidence limits for all sites (n = 8)

as significant because the Cochrans value also had a probability of heterogeneous variances of 0.05 (Section 2.6).

HPTE

HPTE was detected in concentrations below the NHMRC MRL (Figure 4.10). At North Head and Bondi, HPTE was recorded in measureable concentrations only at 0.5 km from the outfalls. This sugests the presence of a point source of contamination from the outfalls. At Malabar, HPTE was recorded at all sites south of the outfall, and at 0.5 and 1.5 km north. No statistical comparison of sites was made.

Because of the large number of sites at which HPTE was not detected, 3-way ANOVA was not conducted. A 1-way ANOVA comparing distances to the north and south of Malabar (untransformed data) was non-significant.

HCB

This OC was detected in measurable concentrations at 19 of the 24 sites, and mean concentrations in red morwong muscle exceeded the MRL at 4 sites: 0.5 and 1.5 km north and south of Malabar. At 0.5 km, HCB was about 2.5 times its MRL (Figure 4.15).

Statistical analysis resulted in significant distance, direction and outfall effects in red morwong muscle tissue (Appendix 4.4), summarised as follows:

1) Higher concentrations of HCB were found at 0.5 km than all other distances.

2) Concentrations were higher to the south of the outfalls (over all distances) than the north.

3) Malabar had higher concentrations of HCB than North Head or Bondi, which were statistically equivalent.

Regression equations were carried out on HCB concentration versus distance to the north and south of Malabar (Appendix 4.4). These predicted that the mean HCB concentrations in red morwong muscle at the discharge point were 2.9 and 2.7 times the MRL, at the time of sampling. They also predicted that the concentration in this fish species would fall below the MRL at distances greater than 2.2 km and 2.7 km to the north and south of the shoreline outfall, respectively.

Dieldrin

Dieldrin was detected in measureable concentrations in red morwong muscle tissue at all but one of the sites (Figure 4.14). Average concentrations did not reach the MRL at any site, although the confidence limits exceeded the MRL 0.5 and 1.5 km north of Malabar.

Statistical analysis resulted in two, second order interactions, suggesting that the effect of distance could only be considered with respect to direction and outfall (Appendix 4.4). These results, which were relatively complex, are interpreted as follows:

1) Concentration of dieldrin in red morwong muscle tissue was significantly greater 0.5 km to the north or south of the outfalls than 1.5, 2.5 or 3.5 km to the north and 1.5 km to the south. Further, dieldrin was higher 0.5 km to the north of the outfalls than 2.5 or 3.5 km to the south.

2) Concentrations of dieldrin were significantly higher in red morwong 0.5 km either side of Malabar than any other distance at any outfall. Concentrations 1.5 km from Malabar and 0.5 km from Bondi were greater than 1.5 or 2.5 kim from Bondi. Concentrations 0.5 and 2.5 km from North Head, and 3.5 km from Malabar, were greater than at 1.5 km from Bondi. All other comparisons were not significantly different.

DDE and DDT(R)

The graph of DDE concentration along the coastline shows highest mean concentrations 0.5 km north of Malabar, but extreme within-site variation (Figure 4.12). While DDE was detected at 23 of the 24 sites, it was always well below the MRL. Similarly, DDT(R) had highest mean concentrations around Malabar, accompanied by very high confidence limits (Figure 4.13). All mean concentrations were below the MRL, although confidence limits exceeded the MRL 1.5 km north of Malabar.

Statistical anlaysis for DDT(R) resulted in an interaction between distance and outfalls, indicating that these factors are not acting independently of each other (Appendix 4.4). At North Head and Bondi there was no significant difference between distances, yet at Malabar the concentration was significantly higher at 0.5 km from the outfall than at all other distances. Thus, while the analysis does not indicate whether or not North Head and Bondi are sources of DDT(R), it does suggest that Malabar is.

For DDE, a third order interaction indicated that the effects of distance, direct and outfall do not affect DDE concentrations in red morwong independently (Appendix 4.4). This is interpreted as follows:

1) To the north of Malabar, and to the north and south of North Head and Bondi, no significant differences occur in DDE concentrations among distances.

2) To the north of Malabar, DDE concentrations were greater in red morwong collected 0.5 km from the outfall and 2.5 or 3.5 km away.

PCBs

PCBs were detected in red morwong muscle tissue in measureable concentrations at 13 of the locations (Figure 4.16). There is no MRL for PCBs against which the data can be compared. Department of Health, NSW, has indicated, that PCBs are not permitted in food for sale.

PCBs were only recorded in measureable concentrations at 0.5 km to the north and south of North Head, suggesting this outfall is a source. They were also recorded 0.5 km either side of Bondi (suggesting this outfall is a source) and 2.5 km to the north (near the Vaucluse/ Diamond Bay outfalls) and 3.5 km to the south. These may represent further point sources of PCBs. As with DDE, PCBs showed an interaction between distance, direction and outfall, interpreted as follows:

1) To the north of North Head no statistical difference occurred between distances, yet to the south, the concentration of PCBs was greater at 0.5 km than any other distance.

2) At Bondi, higher concentrations occurred 0.5 km to the north of the outfall than at any other distance to the north, while no significant differences occurred to the south.

3) At Malabar, higher concentrations occurred 0.5 km to the north than at any other distance to the north. To the south, higher concentrations occurred at 0.5 km than at 2.5 or 3.5 km.

DATA ANALYSED AS A WET WEIGHT FAT BASIS

Expressing OC results on a fat corrected basis did not substantially alter the conclusions obtained with data expressed as wet weight, in the previous section.

Number of OCs detected

The number of OCs showed a significant interaction between distances and outfalls, allowing comparisons to be made independently of direction. At North Head , there were significantly more OCs 0.5 km from the outfall than at 3.5 or 1.5 km (Table 5.6). There were similar numbers of OCs, on average, at 1.5 and 2.5 km, but more OCs at 2.5 km than at 3.5 km. At Bondi, there were more Ocs at 2.5 km than at any other distance, and more at 2.5km than 1.5km. At Malabar there was no significant difference in the mean number of OCs among any of the distances.

Concentrations of all selected OCs and chlordane

Both total OC concentration and chlordane gave the same results:

1) Both were in greater concentrations at 0.5 km from

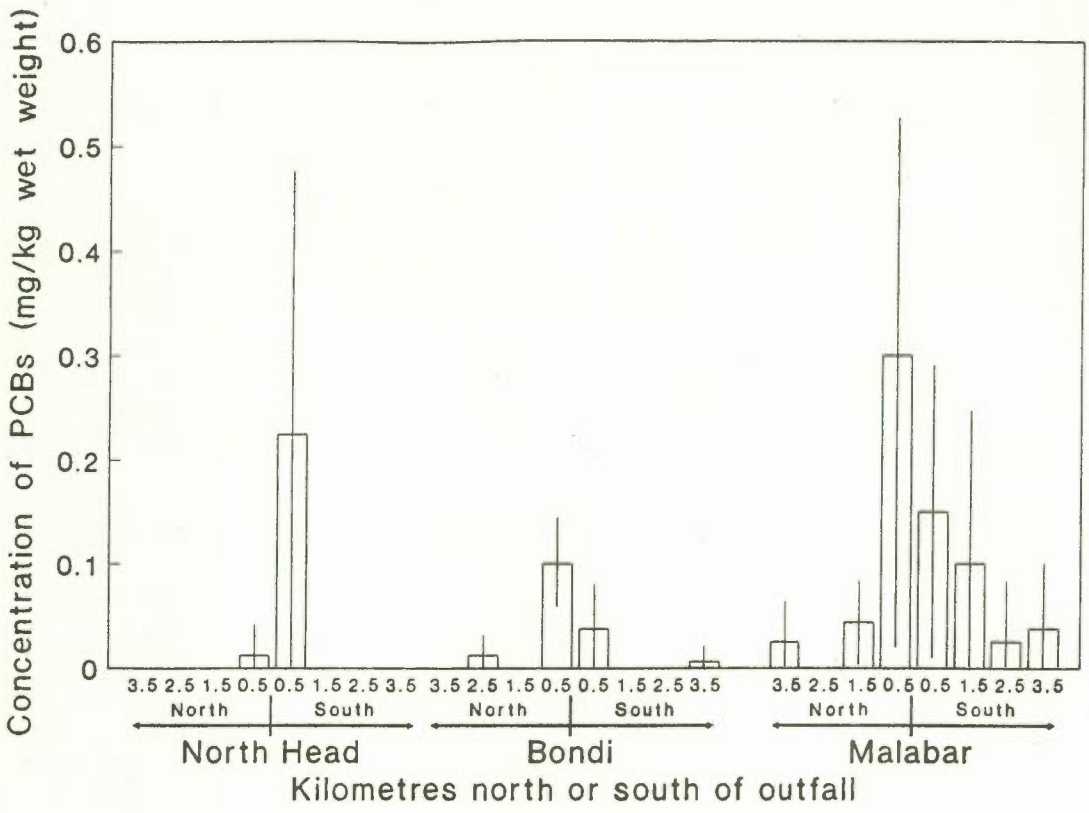


Figure 4.16: Mean concentration of PCBs (mg/kg wet weight) in muscle tissue of *C. fuscus* plus 95% confidence limits for all sites (n = 8)

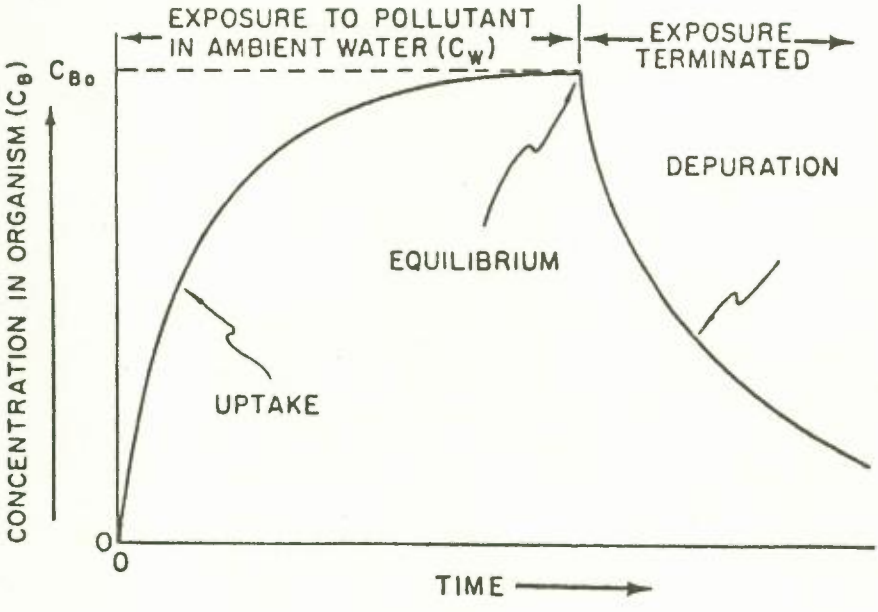


Figure 4.17: Uptake and clearance patterns of organochlorine compounds over time with an organism as represented by first order kinetics (After Connell 1988)

the outfalls than further away.

2) There was a significant direction effect, dependent upon the outfall. This second result occurred because total OC and chlordane concentrations in red morwong muscle were significantly greater to the south of North Head than to the north (Table 5.6). In addition, concentrations of total OCs were greater to the north and south of Malabar than any other stretch of coastline; and they were higher to the south of North Head than either side of Bondi (Table 5.6). For chlordane, there was no significant difference between Malabar (north or south) and North Head-south, but these were all greater than Bondi (north or south) and North Head-north.

DDE and DDT(R)

DDE and DDT(R) both showed significant interactions between outfalls, distances and directions. DDE, however, did not vary over distances for each outfall, while DDT(R) was in higher concentrations in red morwong muscle 0.5 km north of Malabar that 2.5 or 3.5 km north.

Dieldrin, HCB and PCBs (wet weight/fat basis)

Variances for each of these OCs could not be made homogeneous, when comparing all 24 sites for the full factorial design. Highest variances were associated with the North Head nearshore outfall, and no analyses were undertaken at this outfall. instead two factor analyses were used to compare distance and direction at Malabar and at Bondi. Results of these analyses are summarised in Appendix 4.5.

These OCs, calculated on a wet weight/fat basis, among distances and directions at Bondi and Malabar, indicate that higher concentrations occur nearer these outfall than further away. This suggests they are point sources of these OCs.

For dieldrin there was a significant distance effect at Bondi, but no significant effects at Malabar. There was a higher concentration of dieldrin at 0.5 km than any other distance.

For HCB there was a significant distance effect at Malabar, with higher concentrations 0.5 km from the outfall than elswhere.

For PCBs, Bondi and Malabar both showed significant distance effects (Table 5.5). At Bondi, there was a higher average concentration of PCBs 0.5 km from the outfall than at any other distance, while at Malabar there was a higher concentration at 0.5 km than 2.5 or 3.5 km, but not 1.5 km.

4.3 DISCUSSION

4.3.1 Biological characteristics of red morwong

The biological characteristics of red morwong showed considerable variation among the 24 sites. Red morwong close to the outfalls (0.5 km) were significantly heavier than further away, but they were , on average, neither longer, fatter nor relatively older nearer the outfalls. There was some variation, however, over particular stretches of coastline. Most notably , red morwong to the north of Bondi had lower fat contents than any other stretch of coastline.

Lincoln Smith and Mann 1987 (in press) noted that virtually all of the red morwong collected in 1987 were females with well developed gonads, presumably approaching spawning period. Males were rare in these samples. The samples collected for the present study were obtained later in the year (May/June in 1987 compared to July/August in 1988), and many adult fish had very small gonads, suggesting they may have spawned. The presence of fish in different conditions may have increased the within-site variations in OC concentrations.

In conclusion, the biological characteristics of the fish sampled show some differences among sample locations. Fish length, relative age and fat content varied on a site basis or within stretches of coastline. However, this variation did not explain the increased uptake of OCs in fish close to the outfall. In particular, Lincoln Smith and Mann 1987 (in press) suggested that fish age may be an important determinant of accumulation of contaminants. The results of the present study show that fish collected close to the outfalls were not older or younger, on average, than from further away. One characteristic, fish weight, did vary with distance: red morwong 0.5 km from the outfall were significantly heavier than at other distances.

The data collected for all biological characteristics would be suitable for more detailed statistical analyses (such as analysis of covariance) to assess the effects of these characteristics on concentrations of OCs in muscle tissue of red morwong. Such analyses may provide more information on the processes of bioaccumulation.

4.3.2 Organochlorines in red morwong

The results of the bioaccumulation study demonstrated:

1) Organochlorines occurred in the muscle tissue of red morwong over a large portion of the coast line of Sydney, at the time of collection. Two of these, chlordane and HCB, were on average above their NHMRC MRL at several sites.

2) The three major nearshore ocean outfalls represent the principal sources of OCs into the nearshore environment, as

indicated by the red morwong.

3) The Malabar nearshore ocean outfall represents a greater source of OCs than North Head followed by Bondi.

A further noteworthy result was that mean concentrations were often higher 2.5 km from the outfalls than 1.5 km away. In some cases the 2.5 km distance corresponds to sources of OCs into the nearshore environment : 2.5 km south of North Head was at the entrance to Sydney Harbour (a source of urban runoff), while 2.5 km north of Bondi was close to the minor outfalls of Diamond Bay and Vaucluse. In other cases, however, the location of the 1.5 km distance was behind a large headland, and possibly out of the main effluent path. This occurred to the north of North Head and Malabar (Figure 2.1).

Thus the higher concentrations of OCs at 2.5km in some cases may be caused by a combination of factors, including location of sources of OCs, and the shape of the coastline , which may affect the extent of exposure of red morwong to sewage.

The OC compounds present in aquatic organisms may be derived from the following sources : domestic, industrial, agricultural waste discharge or runoff (eg., Brown, 1984; SCCWRP, 1984, SPCC 1986, Arruda, 1987; DouAbul, 1987; Ober, 1987; Rico et al., 1987).

The general sources of OCs into the near shore marine environment include sewage outfalls and stormwater runoff. The OCs may, in turn, be derived from industrial wastes (either trade waste disposal or runoff), the pest control industry, illegal dumping and possibly tip leachates. Further work is necessary to determine the relative importance of each.

The Malabar shoreline ocean outfall disposes of about 50% of Sydneys industrial effluent, followed by North Head (30%) and Bondi (20%). The relatively high concentrations of OCs in red morwong at Malabar may be attributed to the high industrial effluent loading. Sewage sludges and effluents have been shown to contain a wide range of OCs (Van Luin and Van Starkenburg, 1984). Studies conducted in late 1988 on Malabar influents and sludges found high concentrations of HCB and chlordane, lower concentrations dieldrin and occassional traces of aldrin and heptachlor (Mann, unpubl.). Variations were found in both occurrence and concentrations of OCs. It should be noted that both chlordane and HCB occurred in high concentrations in red morwong collected in near Malabar in the present study.

4.3.3 Bioaccumulation and persitence of organochlorines

Bioaccumulation of organochlorine compounds by aquatic organisms occurrs through two processes, bioconcentration (via respiratory surfaces such as gills and transfer by circulatory fluids into fatty tissues) and biomagnification (via the food

chain where OCs are transferred from food to fatty tissues). These processes were summarised schematically in Figure 1.1. There is considerable debate in the literature as to which is the most significant process. For example, Goerke (1977), Khan (1977), Phillips (1978), Elleghausen (1980) and Connell (1988) considered bioconcentration to be the main process, whereas Connolly and Pederson (1988), Kawano et al. (1988) and Oliver and Niimi (1988) consider biomagnification as the main process.

Many factors have been documented as affecting bioaccumulation of OCs in fish (Elleghausen, 1980; Phillips, 1980: Kawano et al. (1980) and Oliver and Niimi (1988), including the following :

1) the ability of the species to metabolise or excrete OCs
2) the degree - time and concentration - of exposure
3) the reproductive condition of the species at the time of sampling
4) the size and age
5) the lipid content
6) chemical characteristics of the OCs, including:
    * water solubility
    * lipid solubility
    * partition coefficient (octanol/water)
    * stability of the parent compound
    * degradation in biological systems and sunlight
    * molecular weight
7) interaction between different OCs bioaccumulated

Numerous laboratory and field studies have been done to determine the persitence of OCs in aquatic organisms. Persistence has been expressed either as half lives (within the study organism) or as a bioconcentration factor, BCF (the ratio of OC in the study organism to OC in water at equilibrium)). High BCFs indicate that the rate of uptake is greater than the rate of clearance.

Differences can occur between field and laboratory BCFs (Oliver and Niimi, 1988). Some OCs, notably DDT and dieldrin, have higher field than laboratory BCFs. This infers that the more persistent OCs are present long enough to be bioaccumulated through the food chain. Oliver and Niimi (1988) concluded that PCBs are very persistent in the environment and therefore bioaccumulate trophically. Connolly and Pederson (1988) assert that an organism is less likely to depurate chemicals of high BCF.

Connell (1988) described persistence of OCs in aquatic organisms as a balance between uptake and clearance (Figure 4.17). He argued that clearance is a physical process and involves the OC molecules being excreted from the fish. Laboratory studies investigating the bioaccumulation and clearance of organochlorine pesticides in fish have found that pesticides can be excreted from contaminated fish if placed in a

non-contaminated environment (Metcalf, 1977; Robinson, 1973).

According to Gerlach (1977) accumulation of OCs is a complex interrelation between these substances in the water, in food and in the organism. Hellawell (1986) concludes that the mutual effect of OCs is generally either neutral or inhibitiory. Khan (1977) stated that absorption and elimination of the OCs DDT, lindane and cyclodienes may occur simultaneously in fish.

The bioaccumulation of OCs by aquatic organisms residing near the shoreline ocean outfalls is a highly dynamic process. Consequently, the OCs determined in fish at any given time reflects the balance between equilibrium concentrations in the environment and the compounds which are bioaccumulated as well as the rate of clearance of OCs from the fish. Also, if exposure to the compound stops, clearance of the compound will occurr. Therefore the sampling of red morwong at any given time will reflect the OCs present in the water column recently or deposited in sediments and sludges, or in food items consumed.

4.3.4 COMPARISON OF 1987 AND 1988 STUDIES

Given the potential for changes in pollutant inputs, organism physiology and ambient water quality, there is no particular reason to expect similar results in muscle tissue of red morwong collected near Malabar in 1987 and 1988. Differing results obtained in the limited 1987 and 1988 studies may be due to differences in the persistence of various OC compounds.

The occurrence and persistence of the most significant OCs in red morwong collected 0.5 km north of Malabar in May 1987 and July 1988 are shown in Figure 4.18. Persistence data has been adapted from information available on other fish species, besides the red morwong, but is used here as a relative guide of the persistence of different OCs for fish. Compounds with low persistence (order of days) showed significantly different concentrations between years.

BHC, detected in 1987, has been shown to quickly clear from fish (Khan, 1977; Oliver and Niimi, 1985) and was not detected in 1988. Chlordane and HCB occurred in 1988 but not 1987. These observations may be due to fluctuating concentrations of OCs in the nearshore environment. HPTE was found in high concentrations in 1987, but in low concentrations in 1988, indicating clearance of the compound. The more persistent OCs, dieldrin and DDT, were not significantly different between years (Appendix 4.7).

Most of the biological characteristics did not differ between years (Appendix 4.7). Our observations, however, suggest that there may be susbstantial differences in the reproductive state of the morwong sampled.

The ratio of liver to body weight, which has been suggesetd as an indicator of physiological stress (Bascom, 1984), was significantly greater in 1987. This may be due to significantly

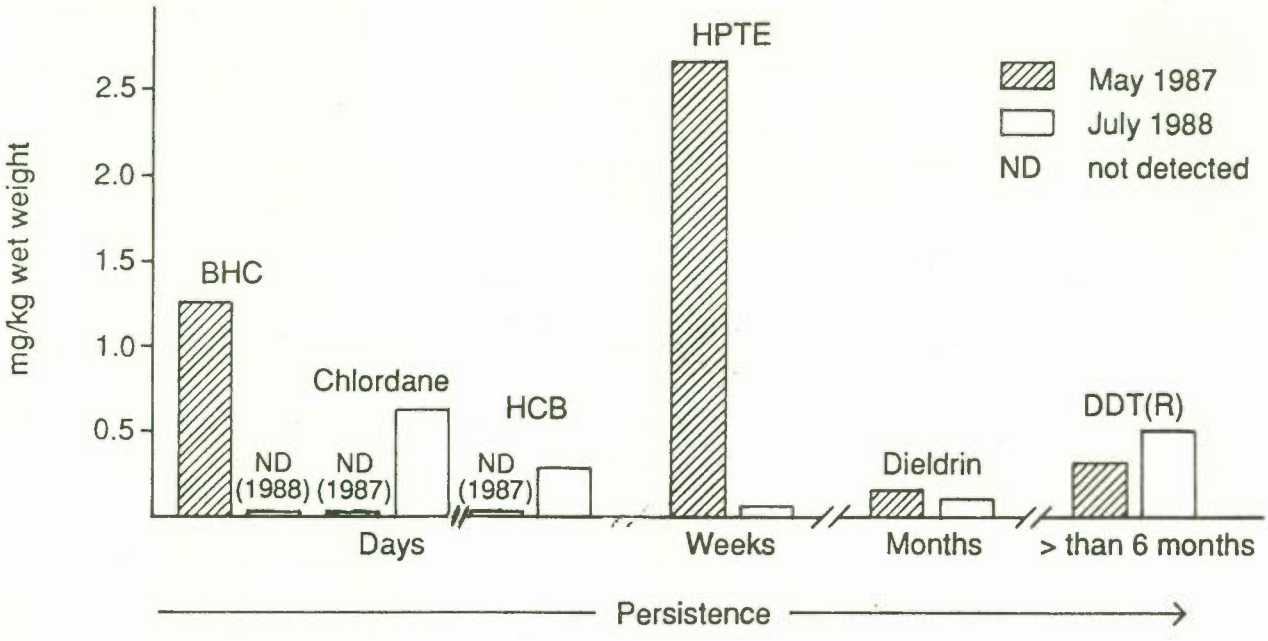


Figure 4.18: Occurrence and persistence of organochlorine compounds in *C. fuscus* (n = 8) collected 0.5 km north of Malabar in 1987 and 1988 (Time scale based on Khan [1977])

higher concentrations of total selected OCs present in the fish at that time (Appendix 4.7).

The 1987 aand 1988 results indicate that the bioaccumulation of OCs in muscle tissue of red morwong is a complex process. Samples collected infrequently near a fluctuating source of OCs may provide only a snapshot of recent exposures of the less persistent OCs.

Thus the observed differences between the 1987 and 1988 studies are not contradictory when results are reviewed with respect to the persistence of different OCs in fish.

Consequently the prediction of OC occurrence and concentration in aquatic organisms residing near a fluctuating source is difficult. This in turn raises questions as to the appropriate sampling frequency of aquatic organisms in the vicinity of a variable source.

4.3.5 Organochlorines detected in relation to NHMRC MRLs

The mean concentration of chlordane in red morwong exceeded NHMRC MRL (95% confidence limits always above MRL) at 8 of the 24 sites, and it may exceed the MRL (based on 95% confidence limits) at a further 12 sites (Table 4.3). The red morwong from all of the sites around Malabar and North Head (ie, up to 3.5 km north and south of each outfall) contained or may have contained excessive concentrations of chlordane.

HCB did exceed the MRL at 0.5 km north and south of Malabar. It may have exceeded the MRL at 1.5 N, 1.5S, 2.5S and 3.5S of Malabar. The MRL for dieldrin was found at concentrations which may have been exceeded at 0.5 km either side of Malabar, while DDT(R) may be exceeded 1.5 km north of Malabar.

Table 4.3 Locations where mean concentrations of OCs exceed, or may exceed (based on 95% confidence limits) their NHMRC MRLs at distances 0.5, 1.5, 2.5 and 3.5 km either side of each outfall. N = north, S = south (n.b. no MRL given for PCBs)

Organochlorine	Outfall	Do exceed (95% CL > MRL)	May exceed (95% CL may be > MRL
Chlordane	Malabar	0.5N, 0.5S, 3.5S, 1.5N	1.5S, 2.5S, 2.5N, 3.5N
	North Hd	0.5S, 2.5S, 0.5N	1.5N, 3.5S, 1.5S, 3.5N, 2.5N
	Bondi	0.5N	0.5S, 2.5N, 3.5S
HCB	Malabar	0.5S, 0.5N	1.5N, 1.5S, 2.5S, 3.5S
Dieldrin	Malabar		0.5S, 0.5N
DDT(R)	Malabar		1.5N

Note : CL = confidence limit

In terms of the implications on human health the muscle tissue of red morwong at 22 of the 24 sites along the Sydney coastline (Cape Banks headline to Curl Curl) was contaminated with chlordane at concentrations which did exceed or may have exceeded (based on 95% confidence limits) the NHMRC MRL. In addition HCB, dieldrin and DDT(R) concentrations indicate that the greatest extent of the OC contamination would be associated with fish caught near the Malabar shoreline ocean outfall.

The Department of Health, NSW, has provided the following information concerning organochlorines detected in red morwong relative to the NHMRC MRLs :-

" The MRLs are determined by toxicity studies on animals, epidemiological information such as observation of occupationally exposed workers and exposure of the general population. Information on both acute and chronic effects is evaluated.

It must be stressed that MRLs for pesticides are not, however, levels, if just exceeded, are likely to produce symtoms in humans if ingested for they include large saftey margins of at least 100 times.

HCB (hexachlorobenzene) can be taken as an example. Its MRL is 0.1 mg/ kg of fish.

A major outbreak of serious HCB poisoning occurred in Turkey between 1955 and 1959 when some 4,000 people were affected from consuming contaminated grain. The estimated daily intake per person was 50-200 mg over a prolonged period (I.A.R.C. Menographs, Vol. 20, 1979).

On the basis of fish flesh containing 0.1 mg/kg of HCB the amount of fish consumed per day would need to be 500 to 2000 kg to produce comparable effects.

The minimum doses to cause acute symptoms in humans of various ages are not known but would be much higher than the MRL.

As to long term effects such as cancer producing there is sufficient evidence that HCB is carcinogenic in mice and hamsters but the evidence that it is so in man is inadequate. It must, nevertheless, be regarded with suspicion.

Chlordane is another organochlorine pesticide which has been in use for 40 years and is more toxic than HCB. Even so, the actual lethal dose in man is between 25 and 50 mg per kg of human body weight. That is, between 1.75 to 3.5 grams in a 70 kg adult.

The MRL set for chlordane in fish is 0.05 mg/kg. At these levels one would need to consume many thousands of kilograms of fish to acquire an acute lethal dose.

The concentration of HCB and chlordane found in red morwong could not be expected to produce acute toxic effects and the effect of long term consumption is unknown.

The Counsel of common sense is that people should not consume fish of any kind caught within or near the sewage outflows. "

CHAPTER 5 : CONCLUSIONS

Biological Characteristics of Red Morwong

1 At 24 sample locations along the Sydney coastline (from Curl Curl to Cape Banks) the biological characteristics of the red morwong showed some differences. Red morwong collected varied in length, weight, relative age and fat content: and liver size relative to body weight.

2 The variation in biological characteristics did not explain the increased uptake of organochlorines in fish collected closer to the outfalls.

Interlaboratory Study of Organochlorines in Red Morwong Muscle Tissue

1 An interlaboratory study involving 4 laboratories demonstrated that significant differences can occurr in OC determinations among laboratories.

2 Australian Analytical Laboratories Pty Ltd, the laboratory contracted to analyse samples of red morwong muscle tissue in the 1987 and 1988 studies, was shown to employ suitable analytical methods for organochlorines and to be a suitable laboratory for such studies.

3 The study highlighted the need for further method development to ensure accurate identification and confirmation of selected organochlorines within fish tissue.

4 Some laboratories reported the the presence of other chlorinated compounds in the red morwong tissue which were not among the organochlorines selected for study, in samples collected near the Malabar outfall.

Bioaccumulation of Organochlorines in Red Morwong

1 The study demonstrates that there is widespread contamination of red morwong by organochlorines along the Sydney coastline (from Curl Curl to Cape Banks).

2 A wide range of organochlorines were detected in the muscle tissue of red morwong from 24 locations along the Sydney coastline. The organochlorines of greatest concentration were chlordane and HCB: other organochlorines detected included heptachlor epoxide (HPTE), DDT, DDE, DDD, dieldrin, oxychlordane and polychlorinated hydrocarbons (PCBs). Chlordane made up over 50% of the total OCs detected in most samples of muscle tissue.

3 Chlordane exceeded the National Health and Medical Research Council (NHMRC) maximum residue limit (MRL) at 18 of the 24 locations sampled. The highest concentrations of organochlorines were found within 0.5 km of the Malabar shoreline ocean outfall, where the average chlordane concentrations were more than 12

times the NHMRC MRL. HCB was also found above the MRL in an area 1.5 km north to 3.5 km south of Malabar. The highest concentrations of HCB was 0.5 km either side of Malabar, and were approximately 3 times the NHMRC MRL.

4 The study concluded that Malabar, North Head and Bondi shoreline ocean outfalls are the principal sources of organochlorines. Evidence for this is found in the significantly higher concentrations of organochlorines in the muscle tissue of red morwong collected 0.5 km from the outfalls than at distances further away.

5 There appear to be other sources of organochlorines into the nearshore environment, possibly urban stormwater runoff contained in waters flowing from Botany Bay and Sydney Harbour.

6 The Malabar shoreline ocean outfall represents a greater source of organochlorines than North Head followed by Bondi into the nearshore environment.

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APPENDICES

2 NOMENCLATURE OF ORGANOCHLORINE COMPOUNDS

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APPENDIX 2 NOMENCLATURE OF ORGANOCHLORINE COMPOUNDS

Compound	Chemical name	Principal uses and occurrences
p,p'-DDE	2,2-bis(p-chlorophenyl)-1,1-dichloroethylene	insecticide:DDT-metabolite
p,p'-DDD	2,2-bis(p-chlorophenyl)-1,1-dichloroethane	insecticide;DDT-metabolite
p,p'-DDT	2,2-bis(p-chlorophenyl)-1,1,1-trichloroethane	insecticide
Arochlor 1254 (PCB's)	polychlorinated biphenyl averaging 48% chlorine by weight	transformer fluid, hydr fluid, lubricants,inks etc.
aldrin	1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydr-1,4-endo-exo-5,8-dimethanonapthalene	insecticide
dieldrin	1,2,3,4,10,10-hexachloro-exo-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-endo-exo-5,8-dimethanonapthalene	insecticide;aldrin metb.
endrin	1,2,3,4,10,10-hexachlor-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydr-1,4-endo,endo-5,8-dimethanonapthalene	insecticide
endosulphan	6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzo[e]-dioxathiepin-3-oxide	insecticide
heptachlor (HPT)	1,4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4,7 methanoindene	insecticide; minor constituent of technical chlordane
heptachlor epoxide (HPTE)	1,4,5,6,7,8,8-heptachloro-2,3-epoxy-3a,4,7,7a-tetrahydro-4,7-methanoindane	heptachlor metabolite
cis-chlordane	1-exo-2-exo-4,5,6,7,8,8-octachloro-3a,4,7,7a-tetrahydro-4,7-methanoindane	insect. constituent of technical chlordane

Appendix 2 continued

Compound	Chemical name	Principal uses and occurrences
trans-chlordane	1-exo-2-endo-4,5,6,7,8,8-octachloro-3a,4,7,7a-tetrahydro-4,7-methanoindane	insect.chlord. const.
cis-nonachlor	1-exo-2-exo-3-exo-4,5,6,7,8,8-nonachloro-3a,4,7,7a-tetrahydro-4,7-methanoindane	insect.chlord. const.
trans-nonachlor	1-exo-2-endo-3-exo-4,5,6,7,8,8-nonachloro-3a,4,7,7a-tetrahydro-4,7-methanoindane	insect.chlord. const.
oxychlordane	1-exo-2-endo-4,5,6,7,8,8-octachloro-exo-2,3-epoxy-3a,4,7,7a-tetrahydro-4,7-methanoindane	chlordane metabolite
alpha - benzene hexachloride (BHC)	alpha isomer of 1,2,3,4,5,6-hexachlorocyclohexane	const. of insect. mixture containing various BHC isomers
beta - benzene hexachloride (BHC)	beta isomer of 1,2,3,4,5,6-hexachlorocyclohexane	as above also metab. of BHC in fish
gamma - benzene hexachloride (BHC, lindane)	gamma isomer of 1,2,3,4,5,6-hexachlorcyclohexane	insect. BHC constituent
hexachlorobenzene (HCB)	perchlorobenzene	fungicide, industrial intermediate, biproduct of chlorinated solvent manufacture
methoxychlor	2,2-bis(p-methoxyphenyl)-1,1,1-trichloroethane	insecticide

APPENDIX 3 INTERLABORATORY STUDY

APPENDIX 3.1 ANOVA and SNK tests

TABLE 1 Summary of 3-way ANOVAs comparing OCs in red morwong from different laboratories, sites and fish.

OC	Labs compared	Statistical significance of factors compared				
		Main effects			2-way interactions	
		Lab	Site	Fish	LabxSite	LabxFish
Total OC conc.	all	****	ns	****	ns	**
Chlordane	1,2,3	****	*	****	**	****
DDE	all	***	*	****	*	****
DDT	all	****	*	****	*	****
DDT(R)(+a)	all	****	**	****	**	***
Aldrin	2,3,4	****	ns	****	ns	***
% Fat(+a)	1,2,3	****	ns	****	ns	**

+: data=ln(x+1) a: alpha=0.025.

TABLE 2 Summary of SNK tests comparing labs for different fish at each site. Labs 1 to 4 are shown under SNKoutcome with means recorded above.

	FISH	ORGANOCHLORINE TESTED			
		Total Oc conc.	Chlordane	Aldrin	DDE
		SNK outcome	SNK outcome	SNK outcome	SNK outcome
MEANS x 10^{-2}		17 42 55 137	1 16 29	0 1 6	3 3 7 17
SITE 1	1	4 3 1 2	3 2 1	4 3 2	4 1 3 2
		16 20 28 39	0 6 16	1 1 2	3 3 5 6
	2	4 3 1 2	3 2 1	4 3 2	1 4 3 2
		17 46 53 103	0 9 22	1 1 4	4 5 7 14
	3	3 1 4 2	3 2 1	3 4 2	1 3 4 2
		45 66 140 224	0 18 65	1 1 6	10 14 23 36
	4	3 4 1 2	3 2 1	3 4 2	3 4 1 2
		21 60 86 133	0 9 69	1 2 3	5 12 14 18
	5	3 4 2 1	3 2 1	3 4 2	3 4 2 1
		13 33 62 79	0 6 29	0 0 2	3 6 6 12
	6	3 4 1 2	3 2 1	3 4 2	3 4 1 2
		4 11 12 16	0 1 8	0 1 3	1 1 2 2
SITE 2	7	3 1 4 2	3 2 1	3 2 4	1 4 3 2
		0 2 4 10	0 1 0	0 1 1	0 0 1 2
	8	1 3 4 2	3 2 1	3 2 4	1 4 3 2
		13 19 31 47	2 7 18	1 2 5	3 3 3 7
	9	3 4 1 2	3 2 1	3 4 2	1 3 4 2
		5 6 16 32	0 3 9	0 0 1	2 2 3 6
	10	3 4 1 2	3 2 1	4 3 2	3 4 1 2
		4 6 19 34	2 4 12	0 1 4	2 2 5 5
	11	3 4 1 2	3 2 1	4 3 2	1 4 3 2
		12 14 22 45	0 3 25	0 1 2	4 4 5 8
	12	4 3 2 1	3 2 1	4 3 2	4 2 3 1

TABLE 2 continued

FISH		ORGANOCHLORINE TESTED		%FAT
		DDT	DDT(R)	
		SNK outcome	SNK outcome	SNK outcome
	-2			-1
MEANSX10		0 2 3 25	6 8 13 45	x10 67 65 105
SITE 1	1	3 1 4 2	1 3 4 2	3 1 2
	2	2 2 8 11 1 3 4 2	5 7 14 20 1 3 4 2	23 23 29 3 2 1
	3	0 5 22 46 3 1 4 2	5 9 46 63 3 1 4 2	23 35 36 3 1 2
	4	9 18 29 94 3 1 4 2	20 46 59 139 3 1 4 2	29 106 84 3 1 2
	5	0 12 23 32 3 1 4 2	5 34 47 51 3 1 2 4	18 53 69 3 1 2
	6	2 6 13 24 3 1 4 2	5 15 28 40 3 1 4 2	14 36 57 3 1 2
SITE 2	7	0 0 3 4 3 1 2 4	1 2 5 8 1 3 2 4	6 11 25 3 1 2
	8	0 0 0 4 3 1 4 2	0 1 3 6 1 3 4 2	3 4 5 1 2 3
	9	1 1 4 10 3 1 4 2	4 4 11 18 3 1 4 2	37 74 76 3 1 2
	10	0 2 3 14 3 1 4 2	2 4 6 21 3 1 4 2	5 6 9 1 3 2
	11	0 1 3 9 3 1 4 2	3 5 5 13 1 3 4 2	27 37 43 3 1 2
	12	0 6 7 9 3 1 4 2	5 11 13 15 3 4 2 1	14 25 30 1 2 3

APPENDIX 3.2 Data used for interlaboratory study.
Concentrations ≥ to 10 times the detection limit of each lab are underlined.

LAB	Fish number	Chlordane	oxy-chlordane	HCB	DDE	DDD	DDT	DDT(R)	Dieldrin	PCBs	Aldrin	No. OCs	OC conc.	Fat %
							µg/kg wet weight							
LAB 1	1	0.20	0.01	0.10	0.00	0.02	0.01	0.03	0.03	0.1	0.00	8	0.37	6.3
Site 1	1	0.38	0.02	0.18	0.02	0.04	0.03	0.09	0.06	0.2	0.00	8	0.73	6.6
	2	0.18	0.00	0.06	0.00	0.03	0.02	0.05	0.02	0.1	0.00	8	0.31	2.8
	2	0.13	0.00	0.06	0.00	0.02	0.02	0.04	0.02	0.1	0.00	7	0.25	3.0
	3	0.25	0.01	0.08	0.00	0.04	0.05	0.09	0.02	0.1	0.00	8	0.45	3.3
	3	0.19	0.01	0.07	0.01	0.03	0.05	0.09	0.02	0.1	0.00	8	0.47	3.6
	4	0.59	0.03	0.21	0.06	0.20	0.17	0.43	0.06	0.2	0.00	8	1.32	4.2
	4	0.70	0.03	0.22	0.05	0.25	0.18	0.48	0.06	0.4	0.00	8	1.49	6.4
	5	0.64	0.03	0.20	0.04	0.15	0.12	0.31	0.06	0.2	0.00	8	1.24	5.8
	5	0.74	0.03	0.22	0.04	0.21	0.11	0.36	0.07	0.3	0.00	8	1.42	4.7
	6	0.36	0.02	0.15	0.03	0.09	0.07	0.19	0.05	0.2	0.00	8	0.77	3.8
	6	0.21	0.01	0.10	0.02	0.04	0.05	0.11	0.03	0.2	0.00	8	0.46	3.3
Site 2	7	0.11	0.00	0.01	0.00	0.01	0.00	0.01	0.01	0.05	0.00	8	0.14	1.1
	7	0.05	0.00	0.01	0.00	0.00	0.00	0.00	0.01	0.00	0.00	5	0.07	1.1
	8	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	4	0.00	0.3
	8	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	4	0.00	0.3
	9	0.16	0.01	0.05	0.00	0.02	0.02	0.04	0.02	0.05	0.00	7	0.28	7.9
	9	0.20	0.01	0.06	0.00	0.03	0.00	0.03	0.03	0.10	0.00	6	0.33	6.8
	10	0.09	0.00	0.02	0.00	0.03	0.01	0.04	0.01	0.00	0.00	7	0.16	0.6
	10	0.08	0.00	0.02	0.00	0.02	0.02	0.04	0.01	0.00	0.00	6	0.15	0.4
	11	0.10	0.00	0.03	0.00	0.01	0.00	0.01	0.01	0.00	0.00	6	0.15	4.8
	11	0.13	0.00	0.04	0.00	0.02	0.02	0.04	0.02	0.00	0.00	7	0.23	2.5
	12	0.34	0.01	0.04	0.02	0.12	0.06	0.20	0.02	0.10	0.00	8	0.61	1.5
	12	0.16	0.00	0.02	0.01	0.04	0.05	0.10	0.01	0.00	0.00	7	0.29	1.3
LAB 2	1	0.15	0.00	0.45	0.04	0.17	0.24	0.45	0.14	0.00	0.08	7	1.27	10.6
Site 1	1	0.16	0.00	0.49	0.02	0.17	0.26	0.45	0.33	0.00	0.045	7	1.475	10.4
	2	0.08	0.00	0.11	0.02	0.09	0.13	0.24	0.045	0.00	0.02	7	0.495	2.25
	2	0.03	0.00	0.065	0.00	0.055	0.09	0.15	0.025	0.00	0.01	6	0.28	2.44
	3	0.10	0.00	0.24	0.03	0.15	0.41	0.59	0.075	0.00	0.03	7	1.035	3.8
	3	0.08	0.00	0.18	0.025	0.125	0.51	0.66	0.065	0.00	0.045	7	1.03	3.3
	4	0.19	0.00	0.52	0.095	0.42	1.03	1.54	0.165	0.00	0.07	7	2.49	8.1
	4	0.165	0.00	0.425	0.095	0.30	0.84	1.235	0.12	0.00	0.045	7	1.99	8.66
	5	0.065	0.00	0.19	0.03	0.095	0.21	0.335	0.05	0.00	0.02	7	0.66	6.57
	5	0.12	0.00	0.22	0.00	0.175	0.42	0.595	0.09	0.00	0.04	6	1.065	7.25
	6	0.075	0.00	0.23	0.025	0.105	0.15	0.28	0.095	0.00	0.01	7	0.69	6.07
	6	0.05	0.00	0.22	0.03	0.14	0.32	0.49	0.11	0.00	0.02	7	0.89	5.42
Site 2	7	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	1	0.02	2.45
	7	0.02	0.00	0.04	0.00	0.04	0.05	0.09	0.14	0.00	0.01	6	0.30	2.62
	8	0.015	0.00	0.01	0.00	0.02	0.045	0.065	0.015	0.00	0.00	5	0.105	0.39
	8	0.01	0.00	0.008	0.00	0.01	0.04	0.05	0.02	0.00	0.00	5	0.088	0.34
	9	0.05	0.00	0.08	0.00	0.09	0.07	0.16	0.09	0.00	0.045	6	0.425	7.90
	9	0.08	0.00	0.135	0.02	0.055	0.12	0.195	0.06	0.00	0.05	7	0.52	7.22
	10	0.025	0.00	0.025	0.00	0.02	0.085	0.105	0.04	0.00	0.01	6	0.205	1.07
	10	0.04	0.00	0.04	0.02	0.09	0.20	0.31	0.04	0.00	0.01	7	0.44	0.78
	11	0.04	0.00	0.065	0.00	0.04	0.09	0.13	0.04	0.00	0.03	6	0.305	3.97
	11	0.04	0.00	0.09	0.00	0.055	0.08	0.135	0.04	0.00	0.06	6	0.365	4.58
	12	0.03	0.00	0.04	0.00	0.045	0.11	0.155	0.02	0.00	0.02	6	0.265	2.59
	12	0.02	0.00	0.025	0.00	0.03	0.075	0.105	0.02	0.00	0.01	6	0.18	2.36

APPENDIX 3.2 continued

LAB	Fish number	Chlordane	oxy-chlordane	HCB	DDE	DDD	DDT	DDT(R)	Dieldrin	PCBs	Aldrin	No. OCs	OC conc.	Fat %
							mg/kg wet weight							
LAB 3	1	0.02	0.00	0.12	0.03	0.00	0.00	0.03	0.03	0.00	0.00	5	0.205	4.2
Site 1	1	0.00	0.00	0.40	0.13	0.00	0.00	0.13	0.08	0.00	0.02	5	0.64	9.2
	2	0.00	0.00	0.04	0.03	0.00	0.00	0.03	0.01	0.00	0.005	4	0.085	1.5
	2	0.00	0.00	0.14	0.07	0.00	0.04	0.11	0.04	0.00	0.01	6	0.305	3.1
	3	0.00	0.00	0.09	0.05	0.00	0.00	0.05	0.02	0.00	0.01	4	0.17	2.0
	3	0.00	0.00	0.10	0.05	0.00	0.00	0.05	0.02	0.00	0.007	4	0.177	2.5
	4	0.00	0.00	0.17	0.08	0.00	0.00	0.08	0.03	0.00	0.01	4	0.29	2.4
	4	0.00	0.00	0.24	0.12	0.01	0.18	0.31	0.05	0.00	0.01	6	0.61	3.3
	5	0.00	0.00	0.11	0.05	0.00	0.00	0.05	0.02	0.00	0.006	4	0.236	1.7
	5	0.00	0.00	0.08	0.04	0.00	0.00	0.04	0.02	0.00	0.006	4	0.186	1.8
	6	0.00	0.00	0.05	0.02	0.00	0.00	0.02	0.02	0.00	0.00	3	0.09	0.9
	6	0.00	0.00	0.07	0.04	0.00	0.03	0.07	0.02	0.00	0.00	4	0.16	2.4
Site 2	7	0.00	0.00	0.02	0.01	0.00	0.00	0.01	0.01	0.00	0.00	3	0.04	0.4
	7	0.00	0.00	0.01	0.02	0.00	0.00	0.02	0.01	0.00	0.00	3	0.04	0.7
	8	0.00	0.00	0.004	0.01	0.00	0.00	0.01	0.006	0.00	0.00	3	0.02	0.2
	8	0.00	0.00	0.01	0.01	0.00	0.00	0.01	0.005	0.00	0.00	3	0.025	0.8
	9	0.03	0.00	0.08	0.03	0.00	0.02	0.05	0.01	0.00	0.00	6	0.175	0.4
	9	0.00	0.00	0.04	0.02	0.00	0.00	0.02	0.01	0.00	0.01	4	0.08	3.3
	10	0.00	0.00	0.03	0.02	0.00	0.00	0.02	0.01	0.00	0.004	4	0.064	0.8
	10	0.00	0.00	0.01	0.01	0.00	0.00	0.01	0.01	0.00	0.00	3	0.03	0.4
	11	0.04	0.00	0.11	0.08	0.00	0.00	0.08	0.03	0.00	0.01	5	0.027	2.8
	11	0.00	0.00	0.02	0.01	0.00	0.00	0.01	0.01	0.00	0.005	4	0.045	2.5
	12	0.00	0.00	0.09	0.05	0.00	0.00	0.05	0.03	0.00	0.01	4	0.18	4.5
	12	0.00	0.00	0.04	0.04	0.00	0.00	0.04	0.01	0.00	0.005	4	0.095	1.4
LAB 4	1				0.03	0.12	0.04	0.19	0.05		0.00	7	0.24	
Site 1	1				0.02	0.03	0.02	0.07	0.02		0.00	7	0.09	
	2	n	n	n	0.03	0.02	0.11	0.16	0.02	n	0.01	7	0.19	n
	2	o	o	o	0.03	0.03	0.05	0.11	0.02	o	0.00	7	0.13	o
	3	t	t	t	0.08	0.19	0.24	0.51	0.07	t	0.01	7	0.59	t
	3				0.06	0.16	0.19	0.41	0.05		0.01	7	0.47	
	4	d	d	d	0.13	0.17	0.32	0.62	0.07	d	0.01	7	0.70	d
	4	e	e	e	0.14	0.16	0.25	0.55	0.05	e	0.01	7	0.61	t
	5	t	t	t	0.09	0.10	0.13	0.32	0.04	t	0.01	7	0.37	e
	5	e	e	e	0.14	0.22	0.33	0.69	0.11	e	0.02	7	0.82	r
	6	r	r	r	0.07	0.09	0.13	0.29	0.05	r	0.00	7	0.34	m
	6	m	m	m	0.05	0.09	0.12	0.26	0.05	m	0.00	7	0.31	i
Site 2	7	i	i	i	0.00	0.05	0.04	0.07	0.02	i	0.03	7	0.13	n
	7	n	n	n	0.01	0.02	0.04	0.09	0.01	n	0.02	7	0.11	e
	8	e	e	e	0.00	0.00	0.00	0.02	0.00	e	0.00	6	0.02	d
	8	d	d	d	0.00	0.01	0.00	0.03	0.02	d	0.01	7	0.06	
	9				0.03	0.02	0.03	0.08	0.05		0.01	7	0.16	
	9				0.03	0.05	0.05	0.13	0.05		0.02	7	0.22	
	10				0.02	0.01	0.03	0.06	0.00		0.00	6	0.06	
	10				0.02	0.01	0.02	0.05	0.00		0.00	6	0.05	
	11				0.02	0.01	0.03	0.06	0.02		0.00	6	0.08	
	11				0.01	0.00	0.02	0.03	0.01		0.00	6	0.04	
	12				0.03	0.02	0.06	0.11	0.00		0.00	5	0.11	
	12				0.04	0.00	0.07	0.11	0.01		0.00	6	0.12	

APPENDIX 4 BIOACCUMULATION STUDY

APPENDIX 4.1 Biological characteristics of red morwong - ANOVA and SNK tests

TABLE 1 Summary of 3-way ANOVAs comparing physical characteristics of red morwong at fixed distances north and south of North Head, Bondi and Malabar shoreline ocean outfalls. For characteristic, (+) = data transformed ln(x+1); for Statistical significance, Dt = distance from outfall, Dn = direction (north or south), Of = outfall (North Head, Bondi and/or Malabar), ns = non-significant and asterisks indicate statistical likelihood of variation in or between factors.

Characteristic	Statistical significance of factors compared						
	Main effects			2-way interactions			3-way interaction
	Dt	Dn	Of	DtxDn	DtxOf	DnxOf	DtxDnxOf
Fish length: LCF	ns	ns	****	ns	*	*****	*
Fish length: STD	ns	ns	****	ns	ns	*****	ns
Fish weight	*	ns	*	ns	ns	****	ns
Otolith weight ('age')	ns	ns	***	ns	ns	****	**
Fat content (+)	ns	ns	****	ns	ns	****	ns
Liver wt:Body wt (+)	ns	ns	ns	ns	****	*****	ns

APPENDIX 4.1 continued

TABLE 2 Summary of SNK tests used to compare means for physical characteristics of red morwong subjected to ANOVA. For Comparison, North Head North, etc = the mean of all fish at a distance and direction (n=8); all distances = the mean of 0.5 + 1.5 + 2.5 + 3.5 (n=32); all outfalls = the mean of North Head-north + North Head-south +...+ Malabar-south (n=48); and North Head, etc = the mean of 0.5 km-north + 0.5-south at North Head, etc (n=16). For SNK Outcome, 0.5, 1.5, etc = the distance from an outfall; NHN, NHS, BON, BOS, MAN, MAS = North Head-north, North Head-south...Malabar-south. Underlinings indicate means not significantly different (alpha = 0.05), means increase in magnitude from left to right.

Characteristic	Comparison	SNK Outcome	
Fish length: LCF	North Head:	NORTH: 1.5 3.5 2.5 0.5	SOUTH: 3.5 2.5 1.5 0.5
	Bondi:	NORTH: 3.5 0.5 2.5 1.5	SOUTH: 1.5 3.5 2.5 0.5
	Malabar:	NORTH: 2.5 1.5 3.5 0.5	SOUTH: 0.5 2.5 1.5 3.5
Fish length: STD	All distances:	NHN MAS BOS MAN NHS BON	
Fish weight	All outfalls:	1.5 3.5 2.5 0.5	
	All distances:	NHN BOS MAN MAS NHS BON	
Otolith weight	North Head:	NORTH: 1.5 2.5 0.5 3.5	SOUTH: 2.5 3.5 0.5 1.5
	Bondi:	NORTH: 2.5 0.5 1.5 3.5	SOUTH: 1.5 2.5 3.5 0.5
	Malabar:	NORTH: 1.5 2.5 0.5 3.5	SOUTH: 0.5 1.5 2.5 3.5
Fat content	All distances:	BON NHS MAS BOS NHN MAN	
Liver wt:Body wt	All distances:	NHS MAN BON MAS BOS NHN	
	North Head:	0.5 2.5 1.5 3.5	
	Bondi:	3.5 2.5 1.5 0.5	
	Malabar:	3.5 2.5 0.5 1.5	

APPENDIX 4.2 Biological characteristics data of red morwong, Cheilodactylus fuscus, collected from 24 locations (Fig. 2.1) along the Sydney coast (Data for otolith weights are provided in Appendix 4.3).

Location	Fork length mm	Standard length mm	Weight g	% Fat	Relative Liver wt. %
4	315	278	658	4.7	0.731
	374	332	1199	5.1	0.977
	318	280	756	9.5	0.888
	378	335	1243	6.7	0.970
	314	275	690	6.2	1.550
	378	330	1126	6.3	1.160
	295	258	616	3.7	0.503
	338	295	930	1.4	1.290
3	308	265	656	2.8	1.300
	305	270	562	4.1	0.915
	345	304	910	8.6	1.190
	303	261	580	0.6	1.210
	273	240	432	0.8	0.185
	340	306	882	6.8	1.520
	338	300	984	15.3	0.996
	268	237	444	1.5	2.460
2	350	305	1040	6.7	0.940
	270	226	416	2.5	1.050
	340	290	814	3.6	1.500
	403	360	1358	4.4	0.683
	274	238	432	1.2	0.787
	284	252	510	3.1	1.960
	318	276	664	2.1	1.720
	358	314	948	7.0	1.090
1	361	322	1047	5.2	1.220
	270	238	411	1.0	2.260
	311	273	677	14.8	1.670
	354	308	936	17.1	1.560
	280	247	490	6.7	2.100
	410	360	1327	1.7	1.570
	268	247	418	0.7	1.990
	262	230	346	0.8	1.880
5	382	332	1127	0.9	1.109
	378	320	1114	2.9	0.682
	382	335	1176	2.9	1.120
	395	344	1462	10.3	0.903
	355	302	922	4.4	1.160
	440	382	1832	2.8	1.020
	358	306	1032	4.2	0.911
	298	260	528	0.7	0.568

APPENDIX 4.2 continued

Location	Fork length mm	Standard length mm	Weight g	% Fat	Relative Liver wt. %
6	334	292	704	1.9	0.724
	376	324	865	0.4	0.740
	340	300	699	4.7	0.315
	360	308	847	0.5	0.697
	335	292	824	2.0	0.765
	360	315	971	1.2	1.020
	392	354	1069	0.8	1.480
	380	325	899	1.5	1.090
7	385	340	1073	5.2	0.680
	295	260	688	2.8	0.596
	395	342	1372	1.0	0.743
	375	334	980	7.6	1.420
	302	262	637	29.2	0.235
	360	315	834	2.5	0.863
	334	304	717	2.2	0.781
	310	265	696	8.0	0.805
8	350	307	793	0.7	1.230
	366	322	870	0.6	0.830
	298	262	530	1.5	1.480
	300	265	598	3.0	0.910
	324	284	637	1.6	1.570
	395	350	1140	0.8	0.760
	330	295	837	25.0	1.130
	308	274	592	4.0	1.580
12	380	345	1310	8.8	1.240
	352	310	885	3.6	1.290
	390	345	1282	2.6	1.260
	405	360	1411	0.6	1.160
	394	345	1343	5.1	1.040
	354	310	941	1.5	1.120
	350	310	848	0.5	1.180
	382	340	1126	1.0	0.710
11	355	310	933	1.6	0.351
	403	364	1188	0.6	1.304
	336	300	811	0.8	0.869
	450	395	1553	0.3	0.768
	353	314	798	0.5	1.230
	410	357	1302	0.6	0.614
	348	307	858	0.6	1.080
	370	327	899	2.4	1.234
10	376	325	970	1.5	0.711
	344	302	848	1.9	1.297
	357	314	847	1.4	1.560
	400	350	1348	1.4	0.882
	408	362	1370	4.2	1.060
	368	320	966	0.4	0.849
	345	303	820	0.3	0.476
	425	367	1486	0.7	1.040

APPENDIX 4.2 continued

Location	Fork length mm	Standard length mm	Weight g	% Fat	Relative Liver wt. %
9	366	335	935	1.2	1.040
	394	342	1090	0.1	0.591
	386	340	1095	0.1	0.879
	340	300	754	0.2	0.683
	359	318	862	0.4	1.210
	350	308	728	3.9	1.340
	352	312	784	0.7	0.897
	337	297	812	0.3	0.984
13	330	290	744	6.9	1.000
	338	280	743	5.2	1.673
	415	370	1484	1.2	1.080
	364	317	899	0.7	1.170
	365	320	948	2.3	1.230
	355	308	830	0.8	1.458
	343	304	765	0.9	1.180
	373	328	1048	2.1	1.510
14	335	290	840	2.7	1.610
	308	274	602	1.8	1.210
	306	267	582	3.7	1.870
	348	307	903	3.2	1.110
	345	304	908	3.5	0.991
	299	254	514	1.4	1.910
	340	301	827	2.3	1.340
	362	323	1067	9.4	1.310
15	325	285	726	2.9	0.600
	395	345	1160	2.2	1.250
	326	284	803	17.3	1.000
	355	300	948	4.9	1.430
	334	295	814	6.5	1.090
	367	320	1074	2.7	1.150
	330	282	884	9.6	1.220
	328	285	770	1.4	0.845
16	310	272	690	9.8	0.658
	394	342	1176	0.9	0.736
	335	290	854	9.2	0.561
	305	262	588	1.1	0.794
	305	265	633	3.3	0.742
	345	304	917	11.7	0.660
	365	320	1215	4.9	0.703
	340	300	864	3.9	0.802
20	350	320	1005	1.7	1.194
	335	295	862	8.0	1.160
	362	320	903	12.4	0.332
	338	302	841	5,0	1.430
	361	323	998	3.7	1.000
	381	341	1266	10.1	1.340
	374	330	1080	10.5	1.060
	390	344	1477	1.7	1.220

APPENDIX 4.2 continued

Location	Fork length mm	Standard length mm	Weight g	% Fat	Relative Liver wt. %
19	300	262	614	3.9	0.800
	354	317	1056	12.1	1.330
	336	296	738	3.8	1.190
	287	258	489	1.9	0.410
	333	305	837	5.3	1.020
	337	301	877	16.2	1.030
	369	324	1048	3.9	1.560
	352	316	980	2.3	1.090
18	298	265	510	1.3	0.784
	318	286	711	8.7	0.844
	305	276	648	8.8	0.460
	315	287	642	3.8	0.935
	302	277	559	4.2	1.070
	356	323	832	1.6	0.840
	345	310	866	1.9	1.040
	315	288	630	0.8	1.110
17	320	280	669	20.1	0.344
	415	358	1353	0.4	0.923
	340	300	996	30.4	0.732
	368	325	984	1.9	1.000
	320	282	655	1.0	0.834
	370	330	927	0.8	1.090
	358	310	998	2.0	0.744
	350	305	810	2.2	1.030
21	308	274	767	18.9	1.040
	301	272	763	12.5	0.655
	323	285	812	12.6	1.230
	302	265	580	2.5	0.862
	304	269	731	7.3	1.230
	343	306	1121	10.7	1.140
	311	275	728	1.9	0.357
	304	270	577	1.7	1.440
22	400	350	1513	3.8	1.060
	318	275	596	4.7	1.170
	317	282	715	1.3	1.120
	277	248	415	0.9	0.964
	405	258	1314	8.4	1.370
	345	307	855	3.2	0.820
	368	240	402	0.9	1.240
	376	333	1151	1.3	1.130
23	361	323	1126	5.0	0.533
	377	334	1186	2.1	0.930
	417	367	1445	2.0	0.830
	314	276	630	2.1	0.952
	347	303	994	1.8	0.805
	301	264	695	4.0	1.580
	267	225	954	1.9	1.260
	333	294	696	0.5	0.720

APPENDIX 4.2 continued

Location	Fork length mm	Standard length mm	Weight g	% Fat	Relative Liver wt. %
24	327	294	672	3.5	1.640
	363	323	896	2.3	1.340
	312	282	712	1.6	0.980
	352	304	872	1.2	0.630
	330	290	729	0.9	1.260
	390	343	1294	8.4	1.070
	389	338	1350	7.5	0.711
	415	360	1675	7.5	0.123

APPENDIX 4.3. Determination of relative ages of red morwong, Cheilodactylus fuscus sampled from 24 locations.

Report prepared by D.J. Ferrell, Zoology Department, University of Sydney, Camperdown, NSW, 2006

SUMMARY

To assess the potential for age determination in *Cheilodactylus fuscus* , otoliths were collected from approximately 240 fish that had been collected from different distances and directions around sewerage outfalls in the Sydney region. Sagittal otoliths showed external banding patterns but they were difficult to interpret and showed little consistency among fish. A random sample of otoliths from 50 fish were cross sectioned to enhance the view of the banding patterns. The banding seen in cross sections bore little resemblance to the patterns seen on the exterior of the otoliths but was clear and consistent with the type of annually-forming growth increments seen in other temperate reef fish. The number of bands seen in the 50 cross sectioned otoliths ranged from one to 27 and the number of bands seen in cross section could be predicted by the weight of the otolith (r^2=0.89). The ages of C. fuscus, as estimated by otolith weight, showed no trends from outfall to outfall, nor with distance from outfall.

INTRODUCTION

The object of this study was to assess the potential to determine the ages of *Cheilodactylus fuscus* using growth increments in bony structures. Age determination in fishes has historically relied upon growth increments being laid down in a predictable fashion in bony structures. Fishes' scales have been widely used for age determination but have often recently been shown to be less accurate and more difficult to interpret than otoliths (Bagenal 1974). The proximal cause of the formation of the opaque and hyaline growth increments seen in the bones of many fish has not been determined. However, changes in the proportion of protein between zones, changes in otolith growth rate (Radtke et al 1985) and metabolic changes with spawning (Samuel et al 1987) have all been hypothesised.

More recently, otoliths have been shown to differentially thicken with age, obscuring external increments(Beamish 1979, Campana 1984, Ferrell in prep.). Further, the growth in thickness of otoliths in older fish has demonstrated that accurate counts of increments are most likely to come from sectioned or broken otoliths rather than whole ones (Beamish 1979, Campana 1984, Ferrell in prep.). Otolith weight has also been suggested a good measure of otolith growth and, with it, fish age (Templeman and Squires 1956, Boehlert 1985, Ferrell in prep.)

The similarity of growth increments found in the bony structures of one species to others with proven annual periodicity is not sufficient evidence to infer annual increment formation in the study species. The timing and periodicity of increment formation must be validated (Beamish and McFarlane 1983). Acceptable validation can come from a regular sampling programme that demonstrates the changes over time in the position of the most recent growth increment relative to the edge of the otolith and the formation of the new increment (Beamish and McFarlane 1983). However, the most widely accepted form of validation of annual marks relies on marking the otoliths of captured fish, releasing them and then comparing the number of increments formed with the period at large (Beamish and McFarlane 1983).

METHODS

A pair of sagittal otoliths, one cleithra, the 3rd and 4th dorsal spines, and the first 3-4 vertebrae were dissected from each fish while semi-frozen or recently thawed. Cleithra were re-frozen for storage while all other hard structures were stored in 90% ethanol. Bony structures apart from otoliths remain in storage. The otoliths were cleaned using fine forceps and rinsed in absolute ethanol and then stored dry. The length, depth and weight of one intact otolith from each pair was measured. Measurements of external otolith dimensions were accurate to 0.001 mm and otolith weights were accurate to 0.0001 g. To

determine the variability between right and left otoliths within a pair, intact otolith sets were measured from 50 individuals.

50 otoliths were randomly selected from the pool of 240 available and were sectioned by mounting them in resin and then grinding and polishing them back to the focus of the otolith. The polished surface of the otolith was then mounted on a microscope slide and the remaining material ground away, leaving a thin (50-100 μm) section of the otolith on the slide. Thin sections were viewed using a compound microscope and reflected light at magnifications between 40 and 100X.

RESULTS

The sacular (sagittal) otoliths of *C. fuscus* were highly variable in their shape and external appearance. Sagittae from most fish were highly sculptured and had many delicate processes on the anterior end of the otolith, making dissection difficult. Whole otoliths in immersion oil under reflected light viewed with a dissecting microscope showed banding patterns similar to those often associated with annual growth increments in other fish (eg. Smith 1982). These banding patterns were not clear out to the edges of the otoliths and neither were the edges of otoliths themselves clear enough to score as opaque or hyaline (dark-clear and white bands, respectively, used for validating the periodicity of the bands). Dorsal-ventral thin sections through the focus of the otolith were did afford a view of the opaque-hyaline banding patterns that appeared to be much less ambiguous. The greatest number of growth increments seen in thin sections was more than double any of the informal counts made on intact otoliths.

The the change in size of otoliths with increasing fish size was not necessarily isometric. The relationships between both the length and the thickness of otoliths did increase linearly with fish standard length (Fig. 1). This may be contrasted with the weight of otoliths across the same range of fish sizes (Fig. 2). The relationship between fish length and otolith weight is not linear and is best approximated by an exponential curve. Otolith weight is less variable among similar-sized small fish than either otolith length or width but for larger fish, the variability is much greater. The variability in any of the measured dimensions between otoliths from a pair was independent of the size of the otoliths and was typically less than 5% of the total measurement for even the smallest otoliths.

The number of increments seen in otolith thin sections ranged from 1 to 27, with very few over 20. The weight of otoliths was an excellent predictor of the number of the rings seen in the cross sections (Fig. 3). Analysis of the residuals of this relationship showed that there was departure from linearity at the extreme values of otolith weight but not otherwise.

While the annual nature of the increments seen in cross section is only speculation without proper validation, a comparison of otolith weights among the 24 sites sampled in this study offers a preliminary way of discussing potential differences in age among the fish from different study sites. The otolith weights for five randomly selected fish from the 24 sample locations (3 outfalls, north and south of each outfall, 4 distances from each outfall) was compared using analysis of variance (Table 1). The three factors showed a significant interaction (Table 1). Mean otolith weights differed among the sampling factors, but was dependent in turn on each of the other factors (Fig 4). Student-Newman-Kuels (SNK) tests on the ranked means of otolith weight at each distance from each outfall, separated by direction, showed no consistent pattern (Table 2) (Note that the means presented in Figure 4 are not transformed and are means of eight individuals. The ANOVA and SNK tests were done using five otolith weights per cell and were transformed. Transformation can alter the ranking of means and this must kept in mind when comparing the SNK result with the means shown in Fig. 4). There is no suggestion of concordance in the ranking of the means in Table 2 (Kendall's $W=4.21$, $p > 0.25$) and the only difference detected by the SNK showed that fish from 3.5 km North of Malabar had significantly heavier otoliths than those from 1.5 km North of Malabar.

Table 1. The result of analysis of variance of otolith weight at all 24 sampling sites. N=8 for all cells except 3.5 km North of North Head where only four otolith weights were available. The mean of those four weights was used in the four empty cells and four degrees of freedom were subtracted from the residual MS. Data were transformed ($\sqrt{x}$) to stablise variances.

Source	df.	Sum Sq.	MS	F-test	Significance
Outfall (A)	2	0.00562	0.00281	5.39	
Direction (B)	1	0.000188	0.000188	0.362	
Distance (C)	3	0.00304	0.00101	1.94	
AB	2	0.018	0.00883	16.93	
AC	6	0.00443	0.000739	1.417	
BC	3	0.00145	0.000483	0.927	
ABC	6	0.00914	0.00152	2.92	$p<0.05$
Residual	164	0.088	0.000521		

Table 2. The result of SNK tests on mean otolith weights from sampling locations at different distances from outfalls (means not shown, see Fig. 4). Ranks are in ascending order (ie. 1<4) and distances that are underlined were not significantly different.

Outfall	Direction	Distance from Outfall			
	Rank:	1	2	3	4
North Head	North	1.5	2.5	0.5	3.5
North Head	South	2.5	3.5	0.5	1.5
Bondi	North	2.5	0.5	1.5	3.5
Bondi	South	1.5	2.5	3.5	0.5
Malabar	North	1.5	2.5	0.5	3.5
Malabar	South	0.5	1.5	2.5	3.5

DISCUSSION

The appearance of the growth increments seen in cross sections of *C. fuscus* otoliths appear similar to those seen in other temperate reef fish (eg. Beamish 1979, McCormick 1986, Beamish and McFarlane 1987). These accounts include reports of annual increments exceeding 50 and as high as 70 (McCormick 1986) for *C. spectabilis*, a congener from similar habitats in New Zealand. Beamish's (1979) work has been at least partially validated but validation of these proposed longevities has been difficult and remains a high priority in fisheries science (Beamish and McFarlane 1983).

The allometric growth of the otolith weight (or thickness) relative to fish length has been demonstrated a number of times (Moe 1966, Beamish 1979, Campana 1984, Ferrell in prep.). In some cases, fishes otoliths have been shown to continue to grow, even in the absence of fish somatic growth (Beamish and McFarlane 1987, Ferrell 1987). This is one explanation of the increased variance in otolith weight with increasing fish size and may be the case in *C. fuscus.*

This study supports other work that suggests both directly and indirectly that weights of otoliths may be more useful for age determination than is currently recognized. If the increments seen in cross sections of *C. fuscus* otoliths can be validated as annual, the the weight of otoliths may be a satisfactory estimator of fish age for most purposes. Templeman and Squires (1957) first pointed out that older fish had heavier otoliths than younger ones of the same size and suggested that the weight of sagittal otoliths might be useful in age determination. This proposal has been pursued more recently by Boehlert (1985) and Radtke et al. (1985) and by Ferrell (in prep.).

Provided that the growth increments seen in otolith cross sections can be demonstrated to form annually, the weights of the otoliths of *C. fuscus* will

provide a reasonable estimation of age. The predictive ability of the relationship between otolith weight and the number of increments seen in cross sections would be enhanced with more information, especially from relatively young and old fish. It may be that the best estimates do not come from a linear model, but from some other form.

The interaction among the factors of the analysis of variance in otolith weight can be explained as follows: North head-south and Bondi-north clearly had fish with the greatest otolith weights causing an outfall by direction interaction, upon which was overlaid significant differences among sites at different distances, which varied among both outfalls and directions. This interaction and the tests of the ranked means showed that there were no consistent trends in otolith weight with different distances from outfalls. The way that *C. fuscus* was sampled in this study may also have affected the outcome of this analysis. A congener of *C. fuscus* from New Zealand, *C. spectabilis*, has been show to segregate itself by sex (and therefore size) by depth and by habitat type (McCormick 1986). These variables were not accounted for in this sampling programme and may have influenced the make-up of the fish collected.

Types of Validation

As mentioned above, the periodicity of formation of the increments seen in the otoliths of *C. fuscus* can only be speculated upon. While it is reasonable to expect that those increments might be annual, Beamish and McFarlane (1983) have clearly stated why it is improper to act on that assumption without a proper validation. Irregular increment formation has been demonstrated in some species (Samuel et al. 1987) and the extreme ages found in some fish remain unconfirmed for lack of validation (Beamish and McFarlane 1987).

Validation of the timing of increment formation can be done a number of ways (Bagenal 1974) but some are considered more rigorous than others (Beamish and McFarlane 1983, Brothers 1987). The most commonly used method of validating increment timing compares the position of the outermost increment with the edge of the otolith in samples taken over a year. This is often done simply by assessing when throughout the year the edges of otoliths are either opaque or hyaline. This is called the marginal increment method and is widely accepted, however Beamish and McFarlane (1983) have pointed out some of its problems. The main problem with the marginal increment method is that it rarely works for older fish whose increments are so close together that increments at the otolith margin become very difficult to see. Consequently, the marginal increment method is generally only applied successfully to young fish. One method of validation that does not have this problem relies on physically marking fishes otoliths and returning them to the wild. When marked fish are recaptured, their otoliths are checked to see if the number of rings formed corresponds with the time at large. The antibiotic, tetracycline is most the most commonly used marking substance and makes a clear

fluorescent mark on all bones growing at the time the drug was administered (McFarlane and Beamish 1987). Other methods of validation have involved using micro-analysis to measure stable isotope ratios in different segments of otoliths (Bennett et al 1982) and using scanning electron microscopy to count 360 daily increments between the annual ones (Radtke et al 1985).

I believe the best way to proceed with an age validation for *C. fuscus* is to do both a marginal increment study and to mark a number of fish with tetracycline. Neither of these studies could be completed in less than a year, and the mark-recapture portion would benefit from a longer duration (Beamish and McFarlane 1983). The marginal increment study would involve collecting 20 fish <300mm SL every second month for one year (this is based on a need for roughly five fish in each of the three lowest age classes at each time). The 120 otoliths would then be sectioned and the number of increments counted as well as the distance from the last increment to the edge of the otolith. For each putative annual cohort, the marginal increment distance is plotted against the sample month to view the growth of the otolith over the year (eg. Ferrell in prep.). The second aspect of the age validation would involve capturing up to 100 individuals, representative of all sizes, and injecting them with tetracycline. This part of the study would need to be done where the fish were unlikely to be speared. The fish should also be marked with an external tag to facilitate recapture. A supplement to this could be to place some injected fish in the care of the Sydney Aquarium (C. Sowden, The Sydney Aquarium, pers com), or in similar facilities, collecting them after 1-2 years there.

The minimum requirement for marginal increment validation would be analysis of 5-10 fish from each of four age classes, sampled every second month over one year. The age classes would have to be estimated by size while collecting and to assure sufficient fish were collected from each of the age classes, some excess would inevitably be collected. Validation using tetracycline to mark the otoliths would require a sample of 50 to 100 fish be captured, injected, marked and released somewhere where they would be relatively safe from the depredations of spear-fishermen but where they could be recaptured again.

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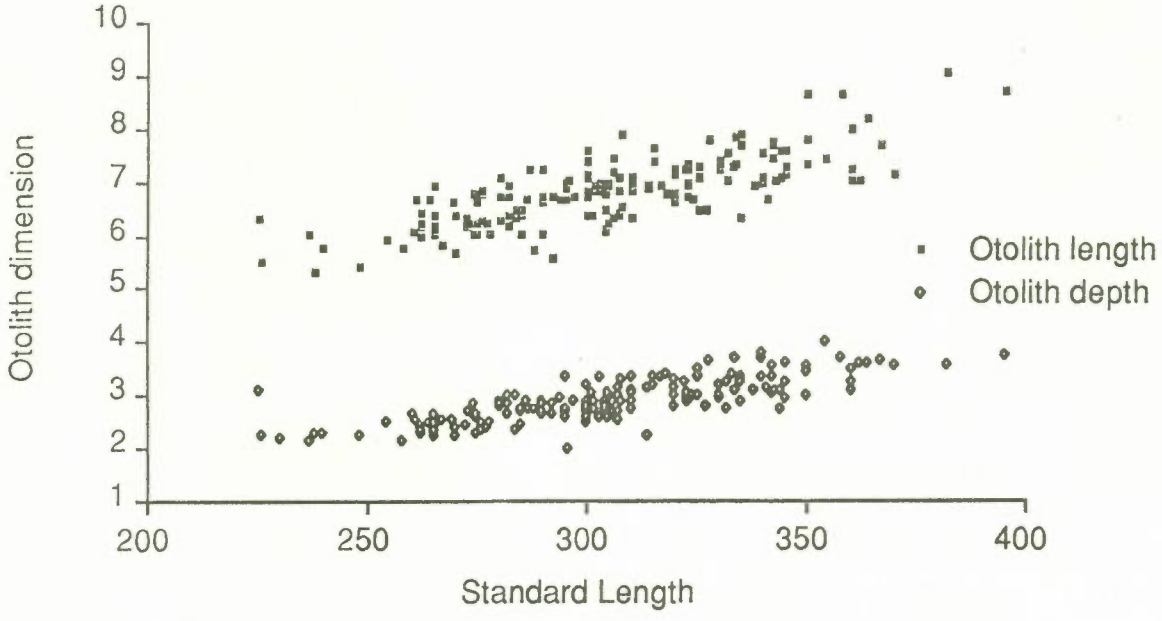

Figure 1. Otolith length and depth plotted against the standard length of the fish.

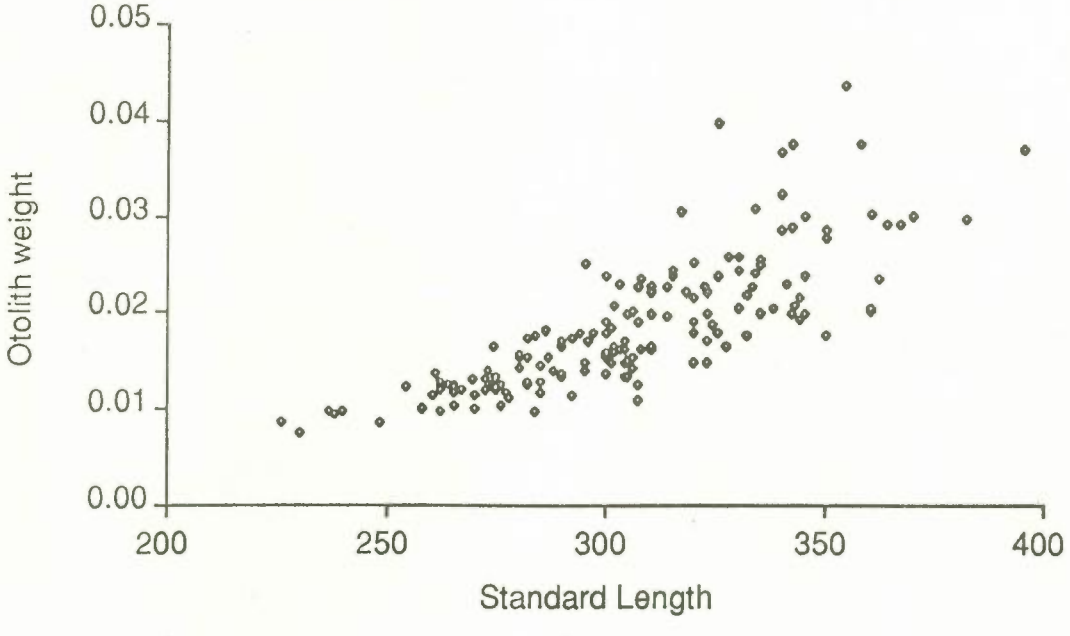

Figure 2. Otolith weight plotted against standard length of the fish. Note the slight non-linearity and greater variability at larger sizes when compared to the dimensions plotted in Fig. 1.

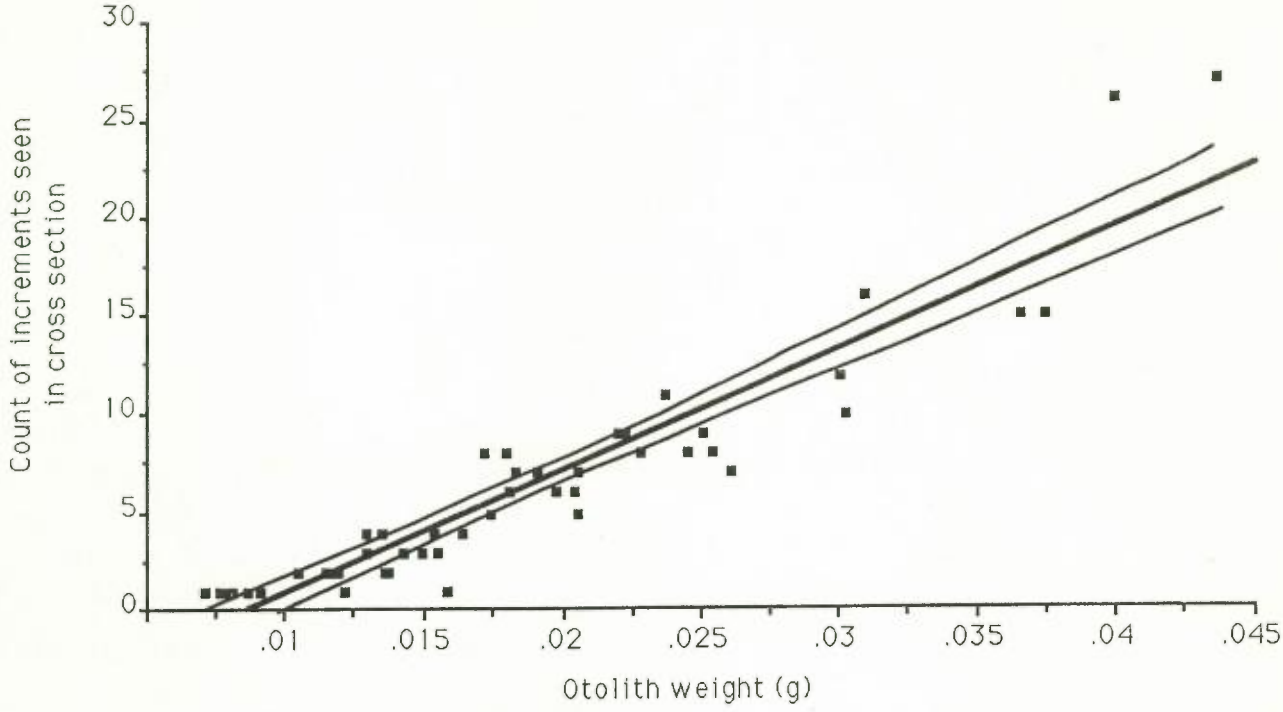

Figure 3. The number of opaque increments seen in 50 *C. fuscus* otoliths cross sectioned plotted against their wieght. R-sqared for this relationship is 0.89. Curved lines indicate 95% confidence interval for increment count.

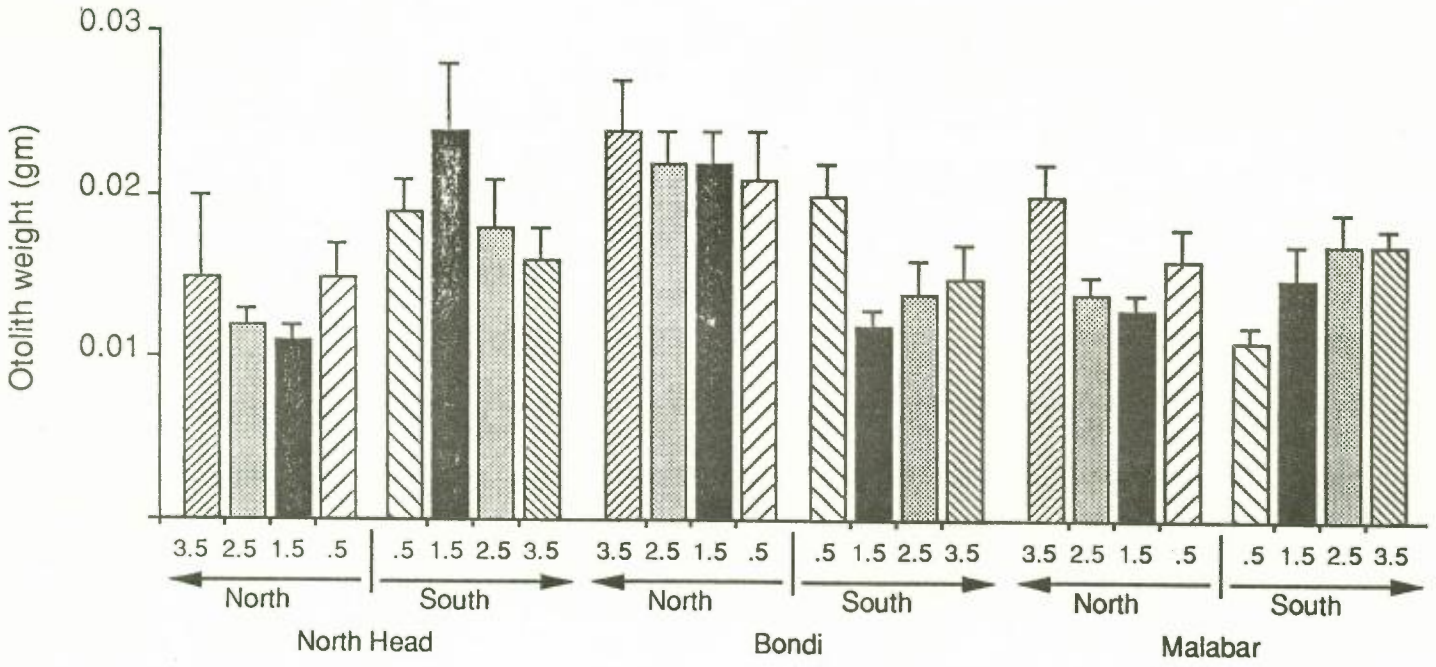

Figure 4. The mean otolith wieght for each of the 24 sites sampled in this study. All means are from N=8 except the site 3.5 km North of North Head where only four were available. Verticle lines represent standard errors.

APPENDIX 4.4 Organochlorine concentrations (wet weight) - ANOVA, SNK tests and regression

TABLE 1 Summary of 3-way ANOVAs comparing OCs in red morwong at different distances to the north and south of North Head, Bondi and Malabar shoreline outfalls.

OC	Statistical significance of factors compared						
	Main effects			2-way interactions			3-way interaction
	Dt	Dn	Of	DtxDn	DtxOf	DnxOf	DtxDnxOf
Mumber of OCs	*****	ns	****	ns	ns	ns	ns
Total OC conc +	*****	ns	****	ns	ns	ns	ns
Chlordane +	*****	ns	****	ns	ns	ns	ns
HCB (+b)	*****	***	*****	ns	ns	ns	ns
Dieldrin +	*****	ns	*****	*	*	ns	ns
DDT(R) +	****	ns	*****	ns	*	ns	ns
DDE +	***	ns	*****	ns	ns	ns	***
PCBs (+a)	*****	ns	*****	ns	ns	**	*****

+: data transformed to ln(x+1), a: alpha = 0.025, b: alpha = 0.010.

TABLE 2 Summary of SNK tests used to compare means for different OCs subjected to ANOVA. All outfalls = mean of data combined for all outfalls or all distances at an outfall; NH = both directions (north and south) of North Head outfall, etc; NHS = distances to the south of North Head outfall, BON = Bondi-north, etc. The number of data points (n) constituting each mean for each SNK comparison is shown.

OC	Comparison	SNK outcome comparing means
Number of OCs	All outfalls (n=48)	1.5 3.5 2.5 0.5
	All distances (n=64)	Bondi N Head Malabar
Total OC conc	All outfalls (n=48)	1.5 3.5 2.5 0.5
	All distances (n=64)	Bondi N Head Malabar
Chlordane	All outfalls (n=48)	1.5 3.5 2.5 0.5
	All distances (n=64)	Bondi N Head Malabar
HCB	All outfalls (n=48)	1.5 3.5 2.5 0.5
	All outfalls (n=96)	north south
	All distances (n=64)	N Head Bondi Malabar
Dieldrin	All outfalls (n=24)	N2.5 N3.5 S1.5 N1.5 S2.5 S3.5 S0.5 N0.5
	Both directions (n=16):	
		B1.5 B2.5 NH3.5 NH1.5 B3.5 M2.5 NH2.5 M3.5 NH0.5 B0.5 M1.5 M0.5
DDT(R)	North Head (n=16)	3.5 1.5 2.5 0.5
	Bondi (n=16)	1.5 3.5 2.5 0.5
	Malabar (n=16)	2.5 3.5 1.5 0.5

TABLE 2 continued

OC	Comparison	SNK outcome comparing means
DDE	N Head-north (n=8)	2.5 0.5 3.5 1.5
	N Head-south (n=8)	1.5 3.5 2.5 0.5
	Bondi-north (n=8)	1.5 3.5 0.5 2.5
	Bondi-south (n=8)	1.5 3.5 2.5 0.5
	Malabar-north (n=8)	3.5 2.5 1.5 0.5
	Malabar-south (n=8)	2.5 0.5 3.5 1.5
PCBs	N Head-north (n=8)	1.5 2.5 3.5 0.5
	N Head-south (n=8)	1.5 2.5 3.5 0.5
	Bondi-north (n=8)	1.5 3.5 2.5 0.5
	Bondi-south (n=8)	1.5 2.5 3.5 0.5
	Malabar-north (n=8)	2.5 3.5 1.5 0.5
	Malabar-north (n=8)	2.5 3.5 1.5 0.5

TABLE 3 Linear regressions of OCs against distance, with predicted distance to NHMRC MRL. Raw data used for all analyses.

Variate	Regression equation	Sig.	r^2	Distance to NHMRL MRL (km)
Number of OCs:				
MAN + MAS	y = 8.266 - 1.219x	**	0.2254	-
Total OC conc.:				
MAN	y = 1.591 - 0.457x	****	0.2461	-
Dieldrin:				
MAN	y = 0.083 - 0.023x	***	0.2572	below MRL at source
HCB:				
MAN	y = 0.288 - 0.085x	***	0.4269	2.2
MAS	y = 0.269 - 0.063x	*	0.1722	2.7

APPENDIX 4.5 Organochlorine concentrations (wet weight fat basis) - ANOVA and SNK tests

TABLE 1 Summary of results, with data calculated on a wet weight fat basis comparing OCs in red morwong at different distances to the north and south of North Head, Bondi and Malabar outfalls. Symbols and interpretation as per Appendix 4.1.

OC	Analysis used	Factors compared	Statistical significance of factors compared						
			Main effects			2-way interactions			3-way interaction
			Dt	Dn	Of	DtxDn	DtxOf	DnxOf	DtxDnxOf
Number of OCs	3-way	Dt,Dn,Of(+)	*****	ns	*****	ns	*****	ns	ns
Total OC conc.	3-way	Dt,Dn,Of(+)	*****	ns	*****	ns	ns	*	ns
Chlordane	3-way	Dt,Dn,Of(+)	*****	ns	*****	ns	ns	*	ns
DDE	3-way	Dt,Dn,Of(+1)	ns	ns	*****	ns	ns	ns	***
DDT(R)	3-way	Dt,Dn,Of(+1)	**	ns	*****	ns	ns	ns	**
Dieldrin	2-way	Bondi:Dt,Dr(+2)	***	ns	-	ns	-	-	-
	2-way	M'bar:Dt,Dr(+)	ns	ns	-	ns	-	-	-
HCB	2-way	Bondi:Dt,Dr(+4)	ns	ns	-	ns	-	-	-
	2-way	M'bar:Dt,Dr(+)	***	ns	-	ns	-	-	-
PCBs	2-way	Bondi:Dt,Dr(+1)	*****	ns	-	ns	-	-	-
	2-way	M'bar:Dt,Dr(+1)	****	ns	-	ns	-	-	-

TABLE 2 Summary of SNK tests used to compare means, with data calculated on a wet weight fat basis, of OCs subjected to ANOVA.
For **Comparison**, All outfalls = mean of data combined for all outfalls (n=48 for distance comparison; n=64 for outfall comparison).
NHS = North Head-south; MAN = Malabar-north, etc.

OC	Comparison	SNK Outcome
Number of OCs	North Head:	3.5 1.5 2.5 0.5
	Bondi:	1.5 3.5 2.5 0.5
	Malabar:	3.5 1.5 0.5 2.5
Total OC conc.	All outfalls:	3.5 1.5 2.5 0.5
	All distances:	NHN BOS BON NHS MAN MAS
Chlordane	All outfalls:	3.5 1.5 2.5 0.5
	All distances:	NHN BOS BON NHS MAN MAS
DDE	North Head:	N2.5 N0.5 N3.5 S3.5 N1.5 S1.5 S2.5 S0.5
	Bondi:	N1.5 S1.5 N3.5 S2.5 S3.5 S0.5 N0.5 N2.5
	Malabar:	N2.5 N3.5 N1.5 S3.5 S0.5 S2.5 S1.5 N0.5
DDT(R)	North Head:	N2.5 N0.5 N3.5 S3.5 N1.5 S1.5 S2.5 S0.5
	Bondi:	N1.5 S1.5 N3.5 S2.5 S3.5 S0.5 N0.5 N2.5
	Malabar:	N2.5 N3.5 N1.5 S3.5 S0.5 S2.5 S1.5 N0.5
Dieldrin	Bondi:	1.5 2.5 3.5 0.5
HCB	Malabar:	3.5 2.5 1.5 0.5
PCBs	Bondi:	1.5 3.5 2.5 0.5
	Malabar:	2.5 3.5 1.5 0.5

APPENDIX 4.6 Organochlorine concentrations in red morwong muscle tissue (mg/kg wet weight basis).

Location	No. OCs	OC conc.	HPTE	chlordane	oxy-chlordane	DDE	DDTt(R)	dieldrin	HCB	PCBs
4	5	0.10	0.00	0.10	0.00	0.00	0.00	0.00	0.00	0.00
	5	0.16	0.01	0.12	0.01	0.00	0.00	0.02	0.00	0.00
	5	0.13	0.01	0.09	0.01	0.00	0.00	0.02	0.00	0.00
	5	0.15	0.01	0.11	0.01	0.00	0.00	0.02	0.00	0.00
	4	0.07	0.00	0.07	0.00	0.00	0.00	0.00	0.00	0.00
	8	0.41	0.02	0.27	0.02	0.05	0.05	0.04	0.01	0.10
	5	0.12	0.01	0.08	0.01	0.00	0.00	0.02	0.00	0.00
	2	0.05	0.00	0.05	0.00	0.00	0.00	0.00	0.00	0.00
3	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	3	0.26	0.00	0.22	0.00	0.02	0.02	0.02	0.00	0.00
	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	4	0.11	0.00	0.09	0.00	0.02	0.02	0.00	0.00	0.00
	6	0.43	0.00	0.30	0.01	0.03	0.07	0.04	0.01	0.00
	6	0.45	0.00	0.34	0.01	0.03	0.06	0.03	0.01	0.00
	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	4	0.11	0.00	0.09	0.00	0.00	0.00	0.02	0.00	0.00
	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	8	0.22	0.00	0.14	0.00	0.02	0.035	0.04	0.00	0.00
1	8	0.16	0.00	0.10	0.00	0.02	0.04	0.02	0.00	0.00
	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	7	0.34	0.00	0.16	0.01	0.11	0.13	0.04	0.01	0.00
	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
5	1	0.06	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	6	0.36	0.01	0.29	0.02	0.04	0.04	0.00	0.00	0.10
	7	0.81	0.03	0.55	0.04	0.10	0.10	0.07	0.02	0.20
	7	1.81	0.04	1.30	0.08	0.15	0.15	0.14	0.10	0.90
	9	0.81	0.02	0.19	0.02	0.10	0.48	0.09	0.01	0.40
	7	0.48	0.00	0.34	0.03	0.10	0.10	0.00	0.01	0.10
	7	0.60	0.03	0.38	0.03	0.04	0.04	0.10	0.02	0.10
	5	0.05	0.00	0.05	0.00	0.00	0.00	0.00	0.00	0.00
6	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	6	0.23	0.00	0.18	0.00	0.03	0.05	0.00	0.00	0.00
	6	0.23	0.00	0.20	0.00	0.02	0.02	0.01	0.00	0.00
	6	0.16	0.00	0.12	0.00	0.01	0.03	0.01	0.00	0.00
	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

APPENDIX 4.6 continued

Location	No. OCs	OC conc.	HPTE	chlordane	oxy-chlordane	DDE	DDTt(R)	dieldrin	HCB	PCBs
7	5	0.08	0.00	0.06	0.00	0.02	0.02	0.00	0.00	0.00
	6	0.27	0.00	0.18	0.00	0.02	0.04	0.03	0.02	0.00
	7	1.52	0.00	1.00	0.03	0.11	0.30	0.12	0.07	0.00
	7	0.35	0.00	0.23	0.00	0.04	0.11	0.01	0.00	0.00
	7	0.35	0.00	0.21	0.01	0.02	0.07	0.04	0.02	0.00
	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	7	0.42	0.00	0.27	0.01	0.02	0.08	0.04	0.02	0.00
	7	0.17	0.00	0.15	0.00	0.00	0.00	0.01	0.01	0.00
8	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	7	0.09	0.00	0.08	0.00	0.01	0.01	0.00	0.00	0.00
	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	7	0.41	0.00	0.27	0.01	0.03	0.08	0.03	0.02	0.00
	7	0.34	0.00	0.23	0.01	0.02	0.07	0.02	0.01	0.00
12	8	0.39	0.01	0.27	0.02	0.03	0.06	0.07	0.03	0.20
	6	0.15	0.01	0.08	0.01	0.00	0.00	0.04	0.01	0.10
	7	0.28	0.00	0.19	0.02	0.02	0.02	0.03	0.02	0.10
	5	0.09	0.01	0.06	0.00	0.00	0.00	0.02	0.00	0.00
	7	0.18	0.00	0.13	0.01	0.00	0.00	0.03	0.02	0.10
	6	0.12	0.00	0.05	0.01	0.00	0.00	0.04	0.02	0.10
	6	0.08	0.00	0.05	0.00	0.00	0.00	0.02	0.01	0.10
	7	0.22	0.00	0.13	0.00	0.05	0.09	0.00	0.00	0.10
11	4	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
10	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	5	0.17	0.00	0.16	0.00	0.00	0.00	0.00	0.01	0.00
	5	0.02	0.00	0.00	0.00	0.02	0.02	0.00	0.00	0.00
	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	8	0.38	0.00	0.18	0.00	0.05	0.17	0.02	0.01	0.05
	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	8	0.34	0.00	0.18	0.00	0.05	0.15	0.00	0.01	0.05
9	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	6	0.21	0.00	0.13	0.00	0.02	0.06	0.02	0.00	0.00
	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

APPENDIX 4.6 continued

Location	No. OCs	OC conc.	HPTE	chlordane	oxy-chlordane	DDE	DDTt(R)	dieldrin	HCB	PCBs
13	7	0.23	0.00	0.15	0.00	0.03	0.03	0.02	0.03	0.10
	5	0.11	0.00	0.06	0.00	0.00	0.00	0.03	0.02	0.00
	7	0.39	0.01	0.25	0.02	0.06	0.06	0.03	0.02	0.10
	2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	2	0.06	0.00	0.06	0.00	0.00	0.00	0.00	0.00	0.00
	4	0.05	0.00	0.05	0.00	0.00	0.00	0.00	0.00	0.00
	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	6	0.30	0.00	0.19	0.01	0.04	0.04	0.03	0.03	0.10
14	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	4	0.12	0.00	0.08	0.00	0.00	0.00	0.00	0.04	0.00
15	6	0.11	0.00	0.06	0.00	0.01	0.01	0.02	0.02	0.00
	5	0.11	0.00	0.08	0.00	0.02	0.02	0.00	0.01	0.00
	2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	5	0.12	0.00	0.06	0.00	0.00	0.00	0.02	0.04	0.00
	2	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00
	3	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00
	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
16	6	0.15	0.00	0.09	0.00	0.00	0.00	0.02	0.04	0.00
	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	7	0.22	0.00	0.12	0.00	0.02	0.02	0.03	0.05	0.05
	5	0.13	0.00	0.07	0.00	0.00	0.00	0.02	0.04	0.00
	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	6	0.18	0.00	0.10	0.00	0.00	0.00	0.02	0.06	0.00
	4	0.11	0.00	0.08	0.00	0.00	0.00	0.00	0.03	0.00
20	9	1.52	0.02	0.71	0.04	0.18	0.45	0.06	0.25	0.40
	9	1.17	0.01	0.62	0.02	0.08	0.21	0.05	0.26	0.40
	9	2.05	0.02	0.95	0.06	0.14	0.39	0.15	0.48	0.50
	9	0.70	0.01	0.28	0.02	0.04	0.16	0.05	0.18	0.10
	9	0.70	0.01	0.29	0.02	0.05	0.2	0.03	0.15	0.05
	9	4.51	0.03	1.70	0.10	0.51	1.83	0.19	0.66	0.80
	9	0.83	0.02	0.32	0.01	0.07	0.27	0.03	0.18	0.10
	9	0.57	0.01	0.19	0.01	0.05	0.17	0.04	0.15	0.05
19	3	0.12	0.00	0.07	0.00	0.00	0.00	0.00	0.05	0.00
	9	3.94	0.03	0.89	0.08	0.49	2.40	0.18	0.36	0.10
	6	0.67	0.00	0.36	0.03	0.10	0.10	0.06	0.12	0.10
	3	0.11	0.00	0.05	0.00	0.00	0.00	0.02	0.04	0.00
	2	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00
	8	0.92	0.00	0.30	0.02	0.10	0.43	0.05	0.12	0.05
	6	0.57	0.00	0.24	0.03	0.08	0.08	0.08	0.14	0.10
	2	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00

APPENDIX 4.6 continued

Location	No. OCs	OC conc.	HPTE	chlordane	oxy-chlordane	DDE	DDTt(R)	dieldrin	HCB	PCBs
18	2	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00
	5	0.22	0.00	0.15	0.01	0.00	0.00	0.00	0.06	0.00
	3	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00
	5	0.33	0.00	0.16	0.02	0.04	0.04	0.04	0.07	0.00
	5	0.45	0.00	0.20	0.03	0.06	0.06	0.06	0.01	0.00
	2	0.07	0.00	0.06	0.00	0.00	0.00	0.00	0.01	0.00
	6	0.65	0.00	0.29	0.02	0.18	0.27	0.00	0.07	0.00
	3	0.10	0.00	0.08	0.00	0.00	0.00	0.00	0.02	0.00
17	3	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00
	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	5	0.16	0.00	0.08	0.01	0.00	0.00	0.02	0.04	0.10
	3	0.09	0.00	0.07	0.00	0.00	0.00	0.00	0.02	0.00
	3	0.10	0.00	0.09	0.00	0.00	0.00	0.00	0.01	0.10
	7	0.47	0.00	0.28	0.02	0.10	0.10	0.03	0.04	0.00
	6	0.36	0.00	0.17	0.02	0.06	0.06	0.04	0.07	0.00
	4	0.18	0.00	0.11	0.00	0.05	0.05	0.00	0.02	0.00
21	9	2.44	0.05	1.00	0.05	0.09	0.47	0.23	0.64	0.50
	9	1.86	0.03	0.81	0.04	0.08	0.27	0.15	0.56	0.20
	9	0.63	0.01	0.35	0.02	0.03	0.15	0.06	0.24	0.20
	9	0.38	0.00	0.15	0.01	0.00	0.09	0.02	0.11	0.00
	9	0.67	0.01	0.23	0.02	0.02	0.12	0.05	0.24	0.10
	9	1.29	0.03	0.56	0.03	0.05	0.16	0.09	0.42	0.20
	6	0.19	0.00	0.06	0.00	0.00	0.05	0.01	0.07	0.00
	6	0.12	0.00	0.05	0.00	0.00	0.03	0.00	0.04	0.00
22	7	0.42	0.00	0.18	0.02	0.05	0.05	0.06	0.11	0.00
	9	2.51	0.05	1.10	0.08	0.31	0.81	0.03	0.44	0.50
	6	0.54	0.00	0.27	0.02	0.07	0.07	0.06	0.12	0.20
	2	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00
	9	1.14	0.00	0.32	0.02	0.15	0.64	0.06	0.10	0.10
	2	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00
	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	8	0.57	0.00	0.15	0.00	0.09	0.35	0.02	0.05	0.00
23	8	1.71	0.03	0.85	0.04	0.10	0.23	0.14	0.42	0.20
	8	0.63	0.00	0.20	0.01	0.07	0.30	0.02	0.10	0.00
	6	0.17	0.00	0.08	0.00	0.02	0.06	0.00	0.03	0.00
	2	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00
	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	7	0.20	0.00	0.12	0.00	0.02	0.02	0.00	0.06	0.00
	9	0.69	0.00	0.28	0.00	0.05	0.26	0.04	0.11	0.00
	5	0.08	0.00	0.06	0.00	0.00	0.00	0.00	0.02	0.00
24	8	0.57	0.00	0.31	0.00	0.04	0.18	0.02	0.06	0.00
	8	0.89	0.00	0.41	0.03	0.11	0.34	0.03	0.08	0.20
	2	0.02	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00
	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	9	1.17	0.02	0.50	0.02	0.06	0.31	0.10	0.22	0.00
	8	0.70	0.00	0.41	0.01	0.04	0.10	0.03	0.15	0.10
	6	0.66	0.00	0.36	0.02	0.06	0.06	0.06	0.16	0.00

APPENDIX 4.7 Comparison of results (by t-Test) from 1987 with those for 1988 for Cheilodactylus fuscus at 0.5km north of Malabar. Variances for all comparisons not significantly different (F-ratio, 2 tailed) and raw data used for each comparison (n = 8).

Variate	Probability of t-test	Interpretation
Fish length (LCF)	> 0.50 ns	1987 = 1988
Weight	> 0.50 ns	1987 = 1988
Fat content (%)	0.10 > P > 0.05	1987 = 1988 £
Liver/Body weight	< 0.001 ****	1987 > 1988
Total OC's	< 0.025 **	1987 > 1988
DDT(R)	< 0.40 ns	1987 = 1988
Dieldrin	< 0.20 ns	1987 = 1988

£ possible trend towards higher fat content in 1988