THE INTERACTION BETWEEN LIFE HISTORY AND DIOXIN LEVELS IN FISH FROM SYDNEY HARBOUR, AUSTRALIA.

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Abstract

The contamination of Sydney Harbour with dioxin like compounds created a significant contamination gradient along its length. A recent large scale study of the concentrations of these compounds in seventeen species of fish and crustacea provided a unique data set from which we examined relationships between species life history and response to a contamination gradient. Apart from a two exceptions, the results showed that differences between trophic level and levels of dioxin were probably typical of what may be expected but that species responses along the gradient varied markedly. The levels in sea mullet (*Mugil cephalus*) and bream (*Acanthropagrus australis*) did not reflect the harbour wide exposure gradient but were similar over a wide spatial scale. The levels in prawn species were indicative of annual patterns of movement and growth, whereas other species (e.g. luderick, *Girella tricuspidate*) showed a strong relationship between environmental exposure and tissue levels.

Introduction

In addition to environmental concentrations several factors are considered to contribute to the total levels of dioxins found in individual biota. Tissue lipid concentration is a primary variable because it is the primary sorbing medium in fish for hydrophobic organic chemicals[1]. Trophic level is an important determinant because environmental exposure of an organism is largely determined by its trophic position (eg herbivores should have less trophic exposure than predators). Biotransformation, reproductive loss and growth dilution can also contribute significantly to variation in tissue levels among species[2]. Another factor, which is rarely considered, is the degree of movement over the life of an organism. The residence time of a species at a location will vary; some species are territorial and hence will remain for extended periods whilst others move continuously and this will greatly affect their environmental exposure.

Recent investigations of dioxin levels in recreational and commercial fish species in Sydney Harbour[3] has provided a very large data set of 17 species and over 400 samples spanning the length of Sydney Harbour (20 km). This provided a unique data set which allowed us to examine relationships between life history (eg trophic level and movement) and dioxin levels in detail.

Here we compare dioxin levels among fish species along a significant contamination gradient. We examine differences among species with similar trophic patterns (eg among predators) and among those with vastly different trophic patterns (eg herbivores, detritivores, omnivores and piscivores).

Materials and Methods

Seventeen species of fish were sampled along a putative dioxin gradient in Sydney Harbour. Fish were sampled using commercial fishing methods including trawling, gill or seine netting. The fish were immediately transferred to the laboratory and frozen at minus 20^{o}C until subsampling for tissue concentration. Up to five composite samples of muscle tissue were taken from 10 adult individuals of each fish or crab species or up to 200 prawns from five regions within Sydney Harbour.

Each sample was analysed for total lipid and dioxin and dioxin like compounds.

Analytical Methods

Sample preparation

Samples were analysed using either of the two following methods:

1) Accelerated solvent extraction was used where lyophilised samples were mixed with hydromatrix using an ASE 100 (Dionex, Utah, USA) with toluene as the extracting solvent at a temperature and pressure of 150^{o}C and 1500 psi, respectively. Between 1 and 10g of the extracted lipid was spiked with the respective PCDDs/PCDFs and dioxin-like PCB isotopically labeled $^{13}C_{12}$ surrogates. The extracts were then cleaned-up on the Power-PrepÔ system using standard elution programs as supplied by the manufacturer.

2) Samples were prepared and dioxin like compounds extracted in accordance with the procedures described with USEPA 1613b (Dioxins/furans) and USEPA 1668a (Dioxin-like PCBs). Weighed aliquots of the samples (approximately 40g) were blended with sodium sulfate, spiked with isotopically labelled internal standards and extracted with organic solvent (methylene chloride:hexane; 1:1) by Soxhlet extraction 18 hours. The lipid content was determined gravimetrically by evaporating the sample extract to constant weight. Isotopically labelled clean-up standards were added to the extract which was purified by acid and base modified silica gel, alumina, and carbon

column chromatography. The final extract was spiked with recovery standard and evaporated to a reduced volume for HRMS analysis.

Gas Chromatography High-Resolution Mass Spectrometric (GC-HRMS) Analysis

1) Samples were analysed on a MAT95XL HRMS (ThermoFinnigan MAT GmbH, Bremen, Germany) coupled to an Agilent 6890 GC (Palo Alto, CA, USA) equipped with a CTC A200S autosampler. A DB-5 (J & W Scientific, Folsom, CA, USA) capillary column (60m x 0.25mm i.d., film thickness 0.25µm) was used as the primary analytical column. Resolution was maintained at 10,000 (10% valley definition) throughout the sample sequence. Multiple ion detection (MID) experiments were performed in the electron impact mode with monitoring of the exact masses of either M+ [M+2]+ or [M+4]+ ions for native and labeled compounds. Individual congeners were identified using the GC retention time and ion abundance ratios with reference to internal standards.

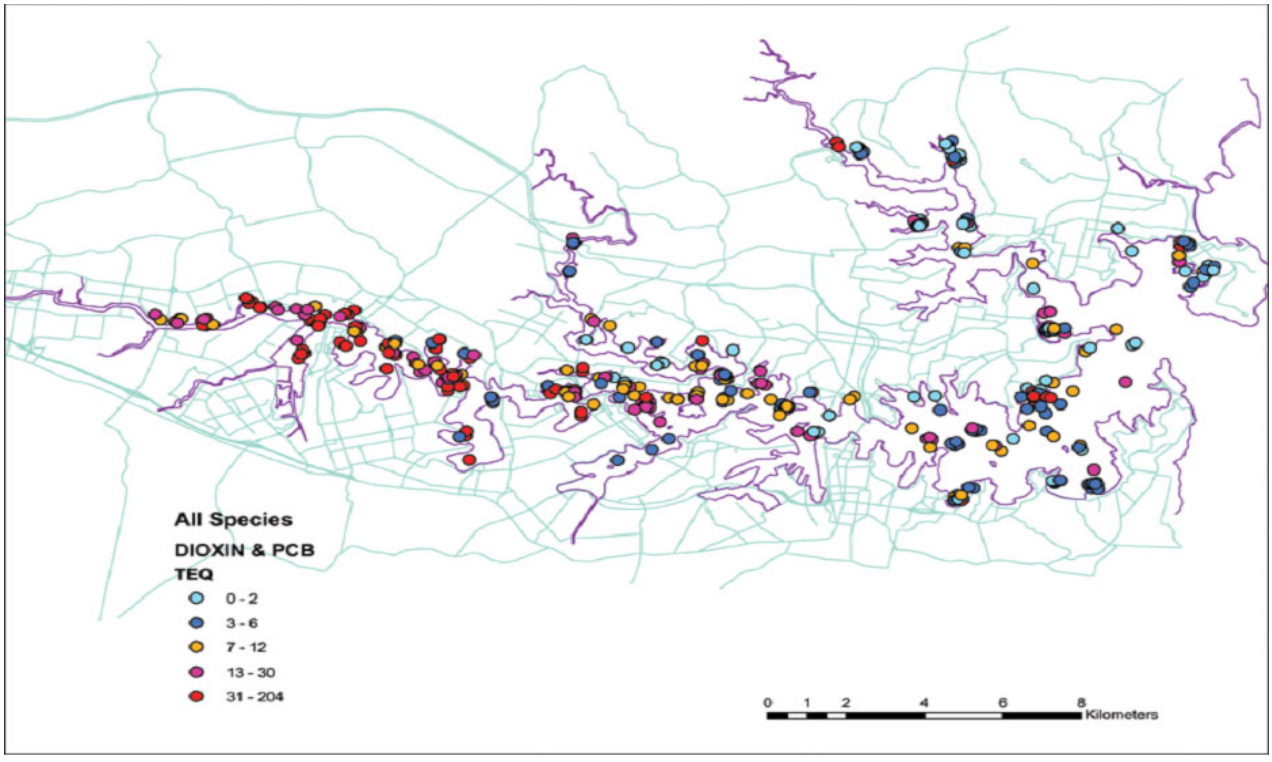
2) Samples were analysed on a Micromass Autospec Ultima HRMS (High-Resolution Mass Spectrometer) instrumentation interfaced to Agilent 6890 gas chromatographs, operating in the splitless mode and using Zebron ZB-5 capillary columns. Confirmatory analysis of the 2,3,7,8-TCDF congener was performed on a J&W Scientific DB225 capillary column. HRMS analyses were carried out in the electron impact mode. Native and labelled compounds were detected by Selected Ion Monitoring with the mass resolution being maintained at 10,000 (10% valley) throughout the analysis for mid-range masses. Chromatographic data were processed using a Waters QUANLYNX™ (V4.0) software package. Levels of target analytes were determined via the isotope dilution technique.

In this paper we present the data as a TEQ further analysis on a concentration and lipid weight basis will be done elsewhere.

Results and Discussion

When the data are presented for all samples across Sydney Harbour as total TEQ (Figure 1) there is a clear decrease in the frequency of samples with high levels, with distance from the major dioxin source, which is located at Homebush Bay. It is this data from which many of the management decisions regarding commercial and recreational fishing closures were decided. But by far the most interesting pattern for those interested in how well we can predict effects on biota is the species by species break down of this pattern.

Figure 1 – Total TEQ of all dioxin like compounds in all fish caught in Sydney Harbour

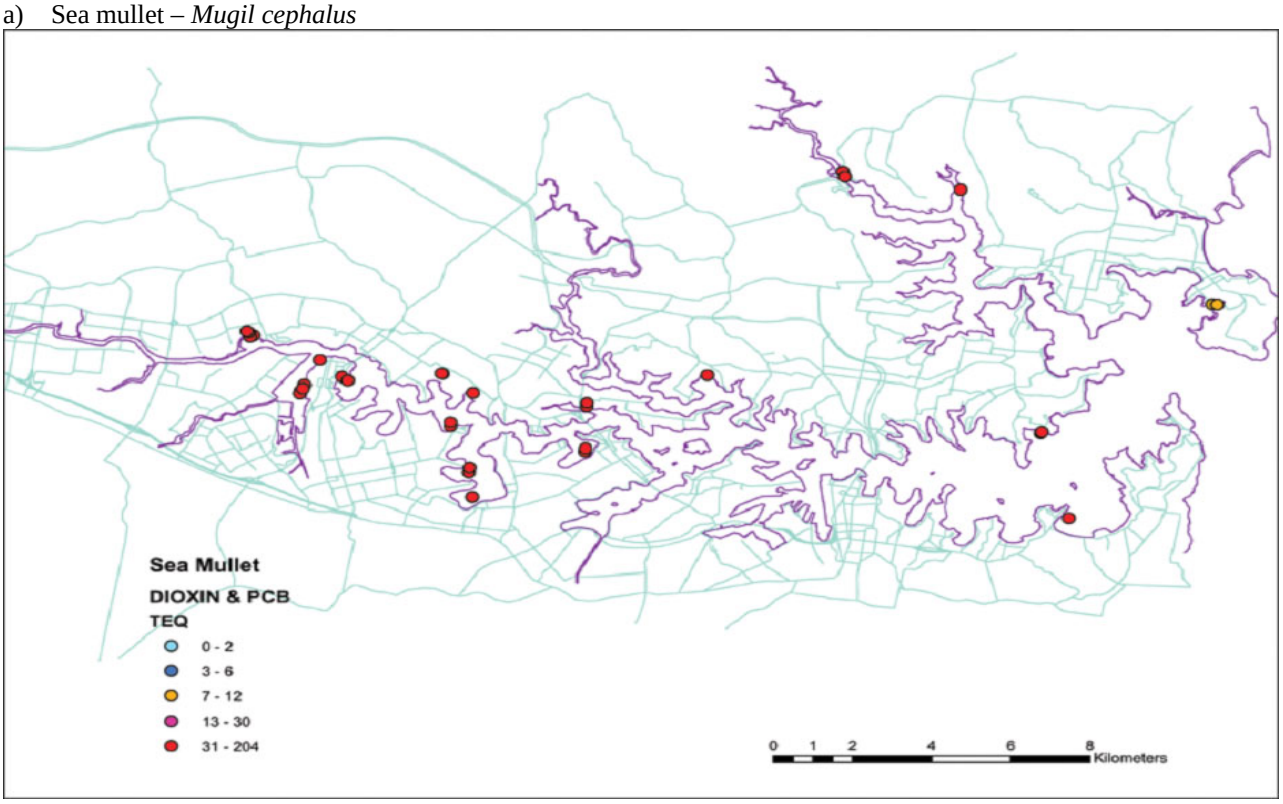


When we compare species of different trophic group and life history it can be seen that trophic level differences are evident. The levels of dioxin like compounds in the benthic detritivore, sea mullet (*Mugil cephalus*) (Figure 2a), were the highest by comparison with all other species, with a TEQ >30 (usually far higher than 30). The herbivore leatherjacket (*Monocanthis chinensis*) (Figure 2b) was the lowest with a TEQ between 0-2. This result is typical of their respective exposure pathways, sea mullet having direct exposure by consuming contaminated sediment and leatherjacket feeding predominantly on aquatic macrophytes and therefore only having water column exposure. Omnivorous and piscivorous species such as bream (*Acanthropagrus australis*) (Figure 2c) and Luderick (*Girella*

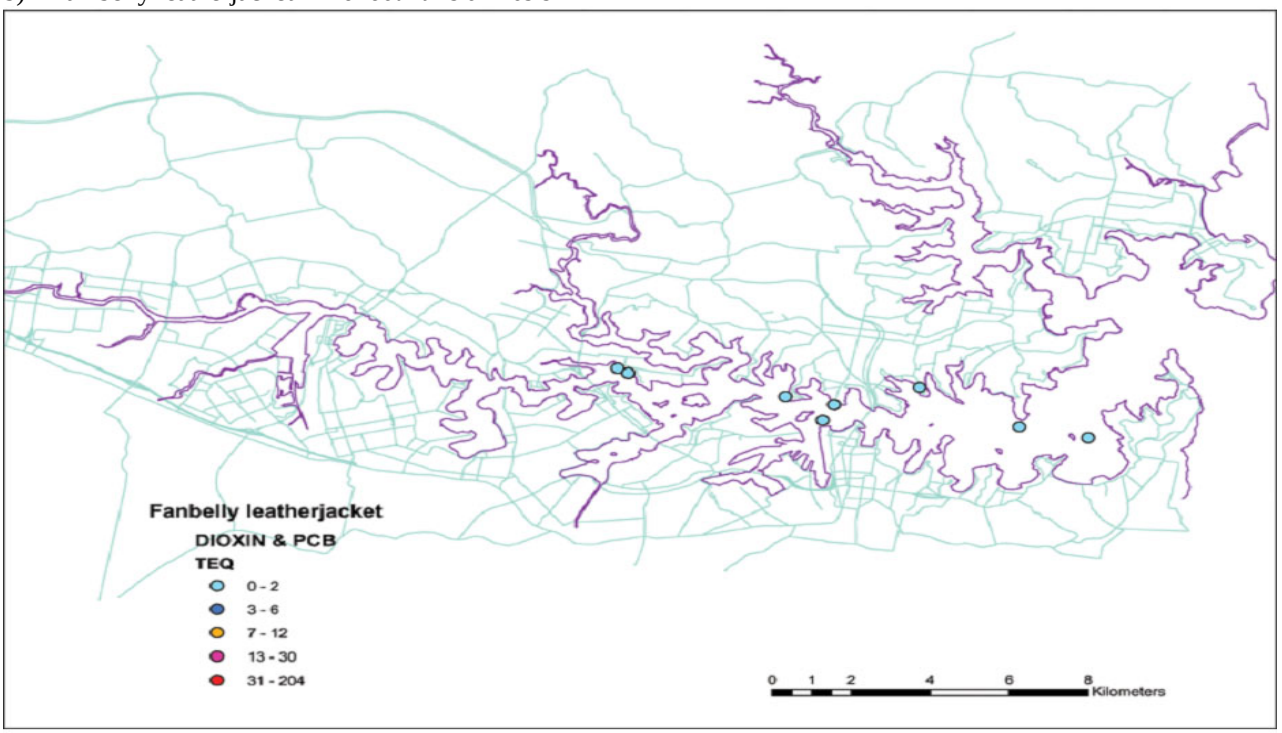
tricupidata) (Figure 2d), showed dioxin levels between the detritivores and herbivores. The concentrations in *Gerres subfasciatus* (Figure 2e), a species which was previously thought to have a largely zooplanktivorous diet, suggested a much stronger linkage to benthic food chain especially when compared to *Pseudorhombus jensyii* (Figure 2f) a known benthic feeding fish.

Figure 2 Subset of the species by species breakdown of the TEQ patterns in fish throughout Sydney Harbour.

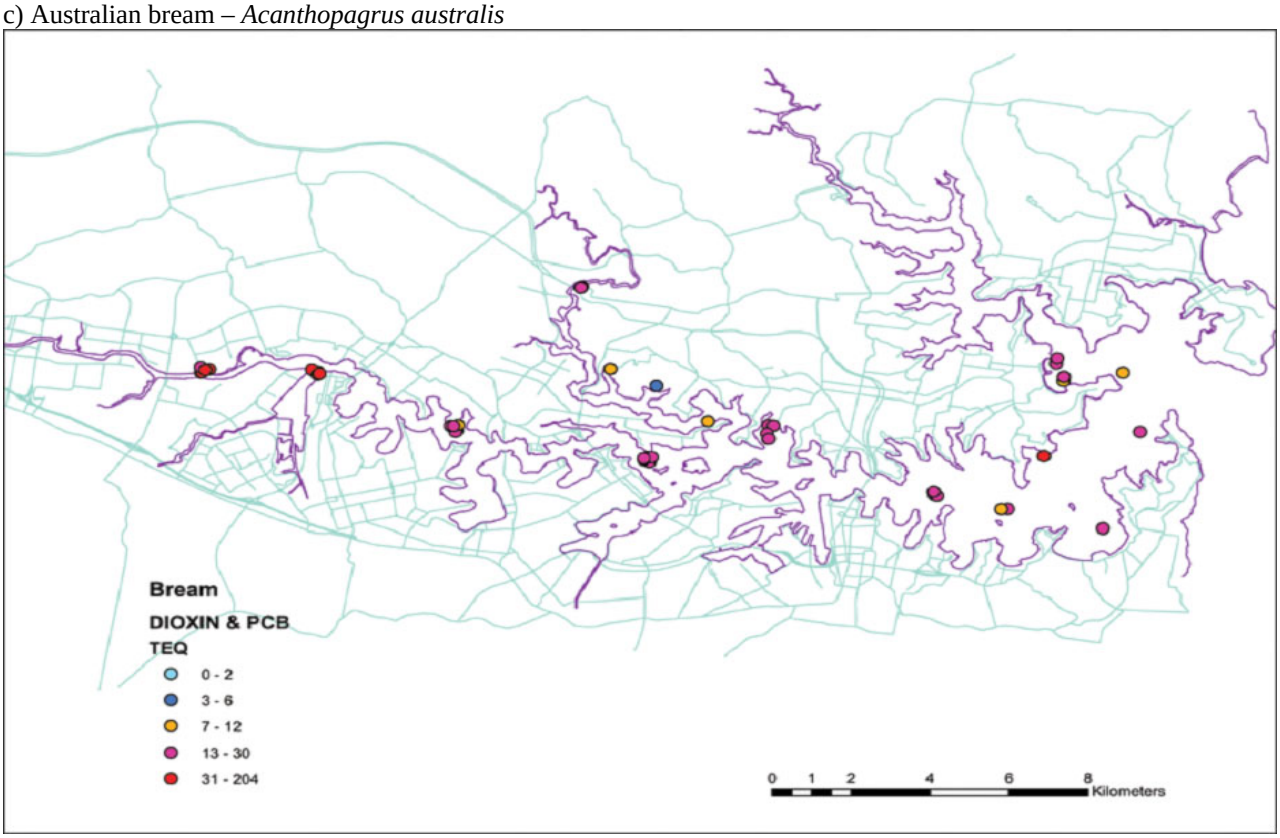
a) Sea mullet – *Mugil cephalus*



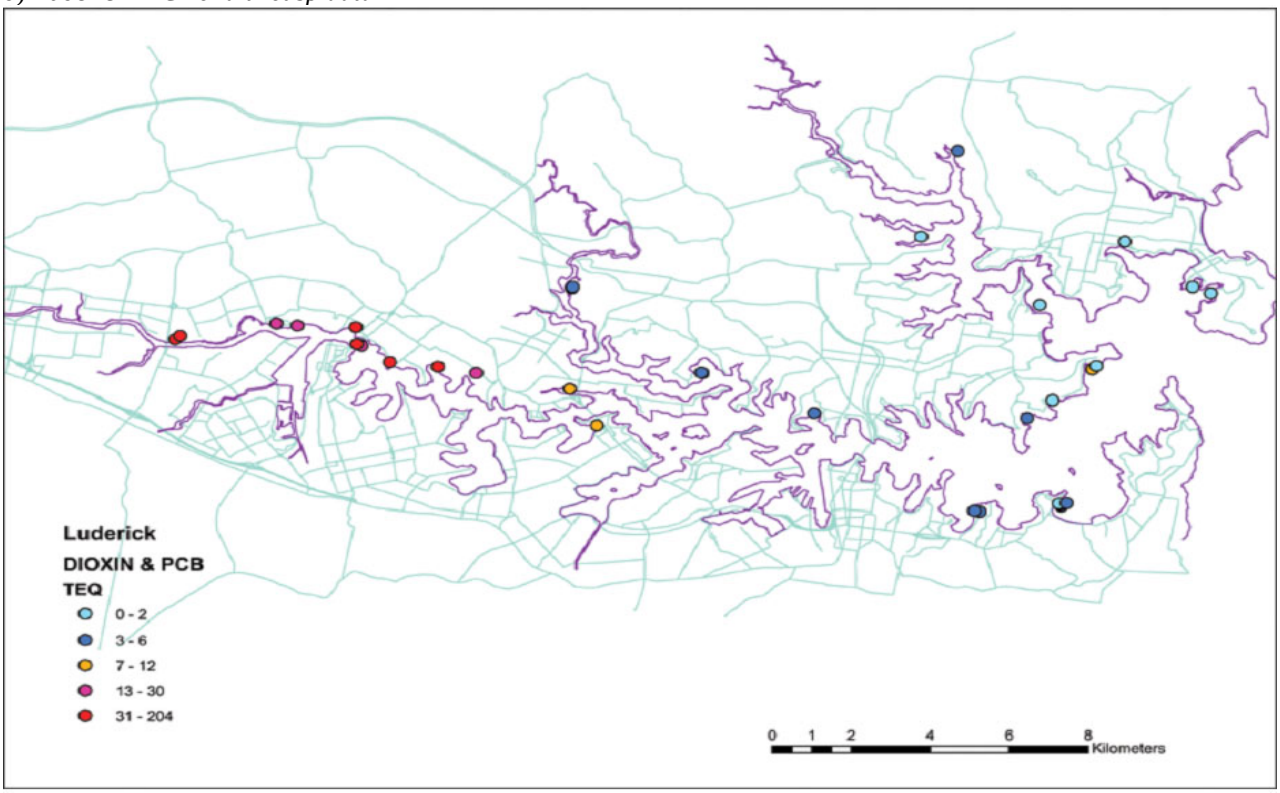
b) Fan belly leatherjacket – *Monocanthis chinesis*



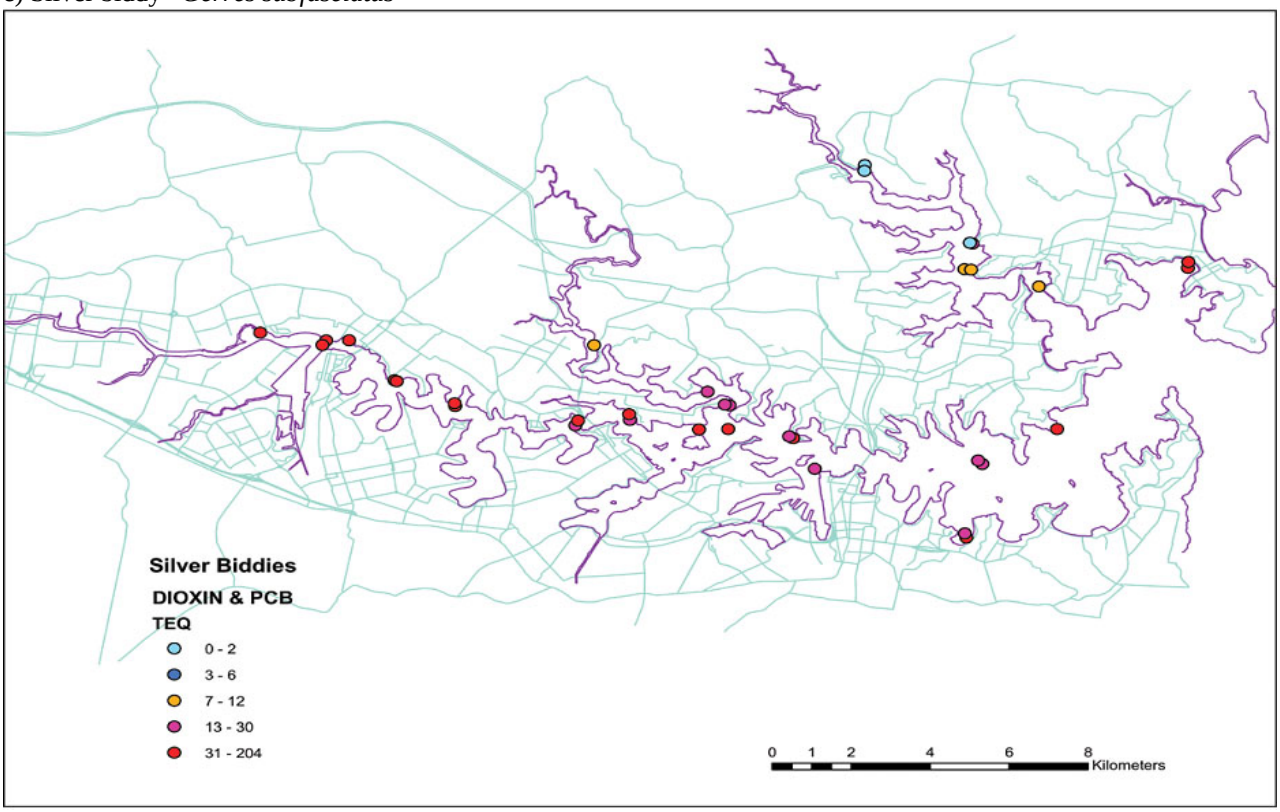
c) Australian bream – *Acanthopagrus australis*



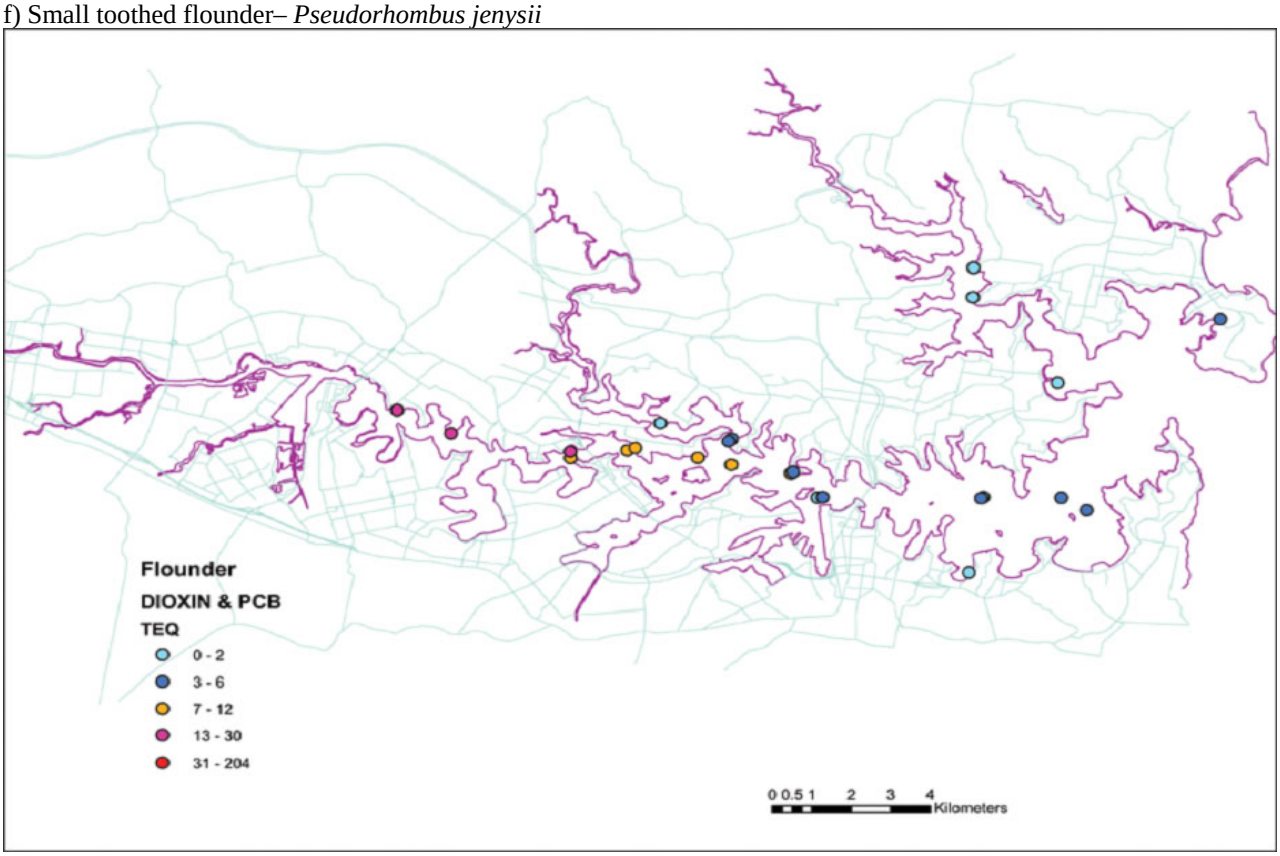
d) Luderick – *Girella tricuspidata*



e) Silver biddy– *Gerres subfasciatus*



f) Small toothed flounder– *Pseudorhombus jenysii*



The environmental levels of dioxin are largely dominated by TCDD, which presents as a concentration gradient down Sydney Harbour, with the highest concentrations located at Homebush Bay and the lower concentrations near the harbour entrance. Changes in the concentrations of the different fish species with proximity to Homebush Bay varied enormously. Concentrations in sea mullet did not vary throughout the harbour with the highest individual sample concentration obtained from fish in middle harbour the furthest sampling point from Homebush Bay. Australian bream had highest concentrations at Homebush Bay but were not significantly different from about 2 km downstream to the harbour entrance. Silver biddy showed a similar pattern to bream. Other species such as flounder

and luderick, showed a clear relationship with the TCDD gradient with concentrations decreasing with distance from Homebush Bay. Patterns in prawns (not shown here) were indicative of their life history. Their concentrations in samples taken upstream from Homebush were lower than those taken adjacent to Homebush Bay. Their concentrations then decreased downstream with distance from Homebush Bay and their body size increased. This reflects their life-history patterns of recruitment to the upper part of the estuary and movement downstream as they approach adulthood and breeding areas at the mouth of the estuary. These results clearly indicate that species such as sea mullet and bream move throughout Sydney Harbour and individuals taken far from the source of contamination can have had significant exposure to contaminants. Patterns in other species suggested that they exhibit a more constrained range of movement in the harbour and that life history in terms of movement within a system is very important. This information has significant implications for our ability to determine the magnitude of effect that contamination may have in a system.

Trophic modelling has become a much relied upon tool in the armoury of scientists to make predictions about the levels of organic contaminants in biota[2]. With knowledge of the trophic habits of fish species, some biological information and a suite of environmental data one can estimate the concentrations of organic compounds in biota for a given set of environmental exposure. These models rarely build in an understanding of the patterns of exposure a fish may experience. Our data suggest that our ability to extrapolate from these models will be improved by understanding variations contamination patterns along contaminant gradients among species.

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