THE USE OF BIVALVES TO INVESTIGATE TRACE METAL AND ORGANOCHLORINE CONTAMINATION IN THE MARINE AND ESTUARINE WATERS OF NEW SOUTH WALES, AUSTRALIA

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Declaration

All work contained in this thesis is the result of my own investigation, except where indicated.

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Abstract

The thesis describes a means of assessing the level of contamination that is occurring in an area as a result of the discharge of very low levels of potentially detrimental substances such as trace metals and organochlorine compounds. There are two main aims:

1. to develop and understand the strengths and limitations of a method of monitoring trace contaminants in estuarine and marine waters in NSW using bivalve molluscs.
2. To examine the range, scale and spatial and temporal variation of pollution by trace contaminants in a number of case studies.

The studies described use bivalve mollucs, primarily the oyster *Saccostrea commercialis* but also the cockle *Anadara trapezium* as "indicator" or "sentinel" organisms, reflecting relative levels of environmental contamination by trace metals and organochlorines in a manner amenable to short and long-term monitoring.

Preliminary studies examined the ability of the chosen organisms to demonstrate a gradient of pollution and to survive deployment in polluted areas. They showed that oysters were reliable indicators of gradients in pollution of organochlorine compounds, PAHs and trace metals. Cockles indicated gradients of trace metal concentrations but did not accumulate detectable concentrations of organochlorines. Oysters also showed great tenacity, surviving without losing condition in a wide range of conditions (including submersion at 75 m depth for 3 months) and pollution regimes. In some specific studies which involved testing of hypotheses about the effects of sediments, cockles were more useful due to lower rates of mortality.

A range of deployment techniques were tested and found to have little effect on mortality of oysters and no effect on concentrations of contaminants. Moorings using floating surface markers were preferred, but were subject to increased damage by both natural and human agents.

Studies of uptake and depuration of organochlorines and trace metals by oysters provided information to allow informed decisions regarding the duration of deployments or between samplings. Times to equilibrium for organochlorines ranged from about 1 week for heptachlor to 6 weeks for DDT; times to equilibrium for trace metals ranged from 12 to 42 weeks. This implies that unless a particular contaminants is being targetted, timing of sampling has to be a compromise.

Case studies showed that:

- diversion of Sydney's sewage effluent to deepwater outfalls reduced the amount of bioavailable chlordane in the inshore environment but did not result in an increase in concentrations of chlordane accumulated offshore. There were no detectable effects on accumulation of trace metals
- there was a persistent north/south gradient of contamination of the waters of Lake Macquarie by lead and cadmium, and a less consistent gradient of zinc contamination
- concentrations of cadmium, lead and zinc in cockles and oysters were influenced more by concentrations in water than concentrations in sediments.

I have, in this thesis, provided a means of identifying the presence of bioavailable contaminants in marine and estuarine waters. If further studies of ecological indicators show that the contaminant(s) is(are) causing ecological effects there is a case for environmental managers to intervene and attempt rectification and remediation. The methods described here are also relevant to temporal monitoring aimed at detecting whether remedial actions are reducing the potential for ecological impact.

The results here have demonstrated clearly that studies of bioaccumulation have a role to play in environmental management. They are cost-effective and provide easily interpreted data for surveillance and compliance monitoring of bioavailable contaminants. They have a less important role in assessing the disruption to ecological systems, except for investigations of some pathways by which contamination moves through food webs.

It is imperative that there is a good understanding of the reaction of the test organism to the contaminants of interest and it has been the role of this thesis to provide that understanding for *Saccostrea commercialis*.

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I took on this task at this stage of my life to fulfil my own aspirations but it would not have been possible without the unstinting love, support and forebearance given to me by my family - Karen, Elliot, Rohan and Huw.

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CHAPTER 1

Introduction

The generation and subsequent need to dispose of waste are inevitable consequences of the activities of humans. One of the most common forms of waste "management" is to put the waste somewhere else, most commonly a place where those that produced the waste do not live. Unfortunately, however, this has the potential to disrupt assemblages of other creatures and plants that naturally occur where the waste was put. The presence of waste causes contamination but it usually requires an obvious disruption to occur (i.e. there is an effect) before the disposal of waste is viewed as "pollution". Pollution is defined in the Oxford dictionary as "a destruction of the purity and sanctity; to make foul or filthy". This thesis will detail a means of assessing the level of contamination that is occurring in an area as a result of the discharge of very low levels of potentially detrimental substances such as trace metals and organochlorine compounds. It has two main aims:

1. to develop and understand the strengths and limitations of a method of monitoring trace contaminants in estuarine and marine waters in NSW using bivalve molluscs.
2. To examine the range, scale and spatial and temporal variation of pollution by trace contaminants in a number of case studies.

Pollution of Marine Systems

Oceans and waterways have long been regarded as a dumping ground for waste. They have been seen as having an almost unlimited assimilative capacity. In the 1970's this view changed and international bodies were set up to control the types and levels of waste put in the sea. The internationally recognised London Dumping Convention was established to control dumping at sea and many types of waste were banned (e.g. radioactive material, some metals and all organochlorines except at background trace levels). Plastics in the sea are becoming an important issue in the 1990's. Perhaps the most insidious form of pollution is by bioaccumulative elements and compounds. These substances are often released in minute quantities (and for this reason often ignored), but can accumulate in plant and animal tissue to concentrations where they are of concern both to the viability of the organism and, in some cases, represent a threat to human health.

The difficulty of determining the presence of trace contaminants in ambient waters and their far-reaching potential are two of the main factors that drive studies to attempt to quantify sources of trace contamination, particularly the "bioavailable" fractions of the waste (Phillips and Rainbow 1993).

Types of Impact and Determining Impact

Throughout this thesis, I will attempt to distinguish between the act of intruding on a system (a "perturbation", "insult", etc.) and the result of that act (the "impact") as they are different phenomena. The techniques that are being developed in this thesis are, however, mostly aimed at assessing the level of contamination in systems (i.e. a perturbation) as an early warning for the potential for impact.

There has been a number of attempts to define an "impact" on the environment. Underwood (1989) discussed the types of impact that might occur. He followed the terminology of Bender et al. (1984), describing the possible types of perturbation as a "pulse" (large and rapid change with the potential for rapid recovery), usually caused by a transitory and short-term event or a "press" which is a long term change from some hypothetical equilibrium state, usually caused by some long-term event. These definitions are theoretical conceptual models and are very much dependent on the time-scales at which the system is examined and the presence of some equilibrium state for the system (Underwood 1989, 1991a). They are, however, useful as a starting point for making predictions about the outcomes of a perturbation on various components of a system and for planning studies to determine whether there has been an impact.

All natural systems have inherent variability (e.g. Connell and Sousa 1983) and the challenge in assessment of environmental impact is to determine, against this background of change, whether a perturbation has caused an impact (Underwood 1989). All designs for detecting change should have similar underlying logic, that is, to produce unconfounded comparisons between control and putatively perturbed locations (Underwood 1991a). How this is achieved is very much determined by whether the perturbation is planned (allowing sampling before perturbation) or whether the study is an attempt to determine the effect of a perturbation that has occurred or is currently happening.

The development of strategies to detect an impact from a planned perturbation has been documented in Underwood (1991b). Green (1979) proposed that any design should include samples taken before and after the perturbation at control and putatively impacted sites. This became known as a BACI (Before After Control Impact) design. Stewart-Oaten et al. (1986) pointed out that Green's design was temporally confounded and suggested that multiple samples were required before and after. Underwood (1991b) argued that Stewart-Oaten et al.'s design is confounded spatially because there is only a single control and presented a design that includes spatial and temporal replication. He also recognised that perturbations rarely occur as replicates and discussed designs that include a single putatively impacted location compared to multiple control locations, which utilise partitioned analysis of variance models to determine whether an impact has occurred. These designs rely on the detection of an interaction between Time (Before vs After) and Treatment (Insult vs Control) and thus are capable of determining an impact even if the variation among control sites is great.

Determination of an impact where there are no "before" data is more difficult and relies on being able to establish that the measures of a variable at the putatively impacted location are well outside the range of values that might be expected for control locations. For many variables (e.g. abundances of individual species) this would be very difficult to establish due to the great natural variability in such variables, but for other measures (such as species richness or concentration of trace contaminants) this is a reasonable and logical proposition. Similar logic to that used by Underwood (1991b) where a single putative impact is compared to multiple controls by use of partitioned analysis of variance models can be employed to overcome spatial confounding.

The discussion so far has dwelt on univariate measures of impact as these are the main measures for which testable null hypotheses can be constructed and robust statistical tests for difference established. There is however a large literature (e.g. Grey et al. 1990) on the use of multivariate statistics to indicate large-scale patterns that might not be elucidated with univariate methods. Clarke (1993) has outlined significance tests for some procedures that will allow limited testing of the significance of the patterns indicated by methods such as multidimensional scaling.

A further consideration in planning a study to determine whether an impact to the environment has occurred is the power of the statistical comparison being used. Winer et al. (1991, p. 120) defined power as "the ability of an experiment to detect

treatment effects - the ability to demonstrate that a phenomenon exists. More generally it is the ability of an experiment to demonstrate the magnitude of treatment effects." This definition thus indicates the two essential components of power, the ability to establish the *probability of existence* of a phenomenon of a given *magnitude.* The probability that an experiment will fail to detect a phenomenon of a defined magnitude is defined to be β, the probability of Type II error. Type II error is failure to detect some event when it occurs. Power is formally defined as being equal to $(1 - \beta)$, where β is the probability of Type II statistical error (Table 1.1). The chance of Type II error is dependent on the chance of Type I error (α, the probability of errors by detecting some apparent phenomenon when, in fact, none has occurred), the number of samples being taken, the inherent variability of the data, the number of means being compared and the magnitude of difference between means that is considered important (Underwood 1981).

Thus, the power of a test can be altered by changes to α, the number of replicates, the number of means being compared, the estimated variances used in the analysis and the magnitude of difference among the means (often called the "effect size"), which has been defined to be the magnitude of the event being examined. Practically, however, the variances estimated are a function of the inherent variability in the data and the number of means is fixed by the hypothesis, so the only alternatives to change power are changes to the effect size, number of samples (n), or α. Changes to n may involve considerable increases in cost and are thus difficult. Alpha has conventionally been chosen to be 0.05, and this is somewhat of a sacred cow. Researchers are generally loathe to deviate from this established convention. The size of effect that is considered important is theoretically easy to manipulate. In practice, however, many researchers find it difficult to state and justify a particular magnitude of effect as being significant from an ecological perspective. This is not, however universally true. In studies of bioaccumulation, levels of contaminants determined in sampling can be compared to benchmarks or guidelines as a means of establishing an effect size.

The "power" of a statistical test has been promoted as an important consideration of experimental design in the scientific literature (e.g. Fairweather 1991). The commonly held view is that too much emphasis is placed on maintaining α at 0.05 with no regard for β. In most studies, β is not considered in planning, but is an outcome of the researchers choice of α and n. Power and effect size are rarely *a priori* considerations.

Considerations of relevant probabilities for α and β also entail a consideration of environmental philosophy. Authors such as Holt (1990), Peterman (1989, 1990) and Fairweather (1991) make a strong case for increasing alpha (with a concomitant decrease in β) in those cases where it is "preferable" to make an error in finding a significant result where none exists, rather than not find a significant result which does exist. It is argued that Type II errors are more costly, both in monetary and environmental terms than Type I errors. Type I error could conceivably lead to expenditure on the "red-herring" of chasing a non-existent impact, but should also be rectified quickly if further sampling shows that there was really no impact. False complacency (type II error) and inactivity while an impact is occurring could lead to severe environmental degradation or animal contamination, which is much more difficult, and often impossible, to rectify. Fairweather (1991) noted that industrial quality control theory recognised the greater public cost of Type II error, describing the two sources as "producer risk" (Type I) and "consumer risk" (Type II). This reversal of the onus of proof from "proof of impact" to "proof of no impact" has been embraced by many environmental groups and is a concept that will be relevant to environmental investigations in the future. It is also an underlying concept in the "precautionary principle" which has been adopted by a variety of regulatory bodies.

Table 1.1. Possible outcomes of statistical tests (adapted from Winer 1971)
Alpha and beta are the probabilities of Type I and Type II errors (respectively) occurring.

	True State Of Affairs in Populations	
Decision from test	No difference	Difference
There was a difference	Type I error, with probability alpha (α)	No error
There was no difference	No error	Type II error, with probability beta (β)

Measures of Impact

The question of what to measure when attempting to determine whether an environmental impact has occurred is a vexed one that has received much attention in the literature (see, for example, the results of an international GEEP workshop in Marine Ecology Progress Series, 1988).

Measures of impact can be divided into two primary categories - physical (including direct chemical measures) and biological. The biological category can be sub-divided according to levels of biological organisation into sub-organismic (includes cellular and sub-cellular measures), organismic (genetic, physiological and bioassay), population and community measures.

Underwood and Peterson (1988) have summarised some of the philosophies and implicit assumptions underlying attempts to determine impact at the GEEP workshop. They suggested that, if faced with a range of methods for determining impact, the methods should be assessed according to their performance in the following areas:

1. Explanation. Are the processes that result in a significant impact understood? This is necessary to be able to be sure that the patterns seen are actually a result of pollution and not a correlation with some other undetected environmental variable.
2. Evaluation. Comparison of techniques to determine whether aspects of the technique compromise or alter the ability to detect impacts of pollution (e.g. problems with the use of experimental studies in nepotisms).
3. Interpretation. Deciding what are the consequences of the pollution. This involves much discussion about what is the appropriate measure of pollution for each circumstance. The authors believe that impacts on populations of organisms should be the primary end-point of an investigation of pollution and discussed at length the abilities of various methods to be related to population structure.
4. Prediction. Can a measure be used to predict further change in a system. The authors felt that measures made at the level of individuals or lower (sub-organismal) were unlikely to relate to changes at an ecological level. These measures have a place, however, because they can often provide early warning of potential for wider scale impact.

They concluded that a strategy for monitoring pollution that includes measures of pollution at different levels of biological organisation is sensible because different measures serve different purposes.

In the following paragraphs, I will attempt to provide some examples from literature relevant to studies of bivalves (partly summarised from Bayne, 1989)) which illustrate the application of the various measures listed above and, in summary, an assessment of the uses of each type of measure for monitoring of pollution.

Sub-organismal measures

Biochemical responses

Increases in production of metal-binding proteins in mussels occur as exposure to metals increases and can be measured in comparison to the levels in animals from control areas (George 1980, Moore 1985, Viarengo 1985). The ability to increase synthesis of proteins is, however, limited and further influx of metals can eventually lead to damage to the lysosomal system and damaging consequences for the general function of cells (George and Viarengo, 1985).

Hydrocarbons are metabolised by cytochrome p-450 or mixed function oxidase (MFO) system (Moore 1985). The MFO system increases production when exposure to contaminants increases and the increased concentrations of enzymes can be measured. Toxic effects, with subsequent damage to DNA, can result if the two phases of the assimilation reactions are unbalanced by continued exposure (Livingstone 1985, Bayne 1989).

Similar responses have been observed in more mobile animals at higher levels of organisation. For example, MFO enzymes and metal-binding proteins were at greater concentrations in flounder caught in the proximity of a petrochemical complex than in those caught at a remote site (Sulaiman et al. 1991).

Cellular responses

The lysosomal system is involved in both the above biochemical processes and has been the object of much research (Moore et al. 1987a,b) The lysosomal membrane acts as a barrier between enzymes within the lysosomes and their potential substrates. This membrane is vulnerable to damage by excess free metallic ions and certain organic contaminants. Transfer of hydrolytic enzymes into the cell cytosol and cytosolic material back across the lysosomal membrane cause damage to the structure and function of cells. Moore and Viarengo (1987) showed that the stability of the lysosomal membrane was reduced when contaminants reached certain critical limits within the cell. There was a direct equivalence between stability of the membrane and degradation of cellular proteins. This indicates a possibility of equating biochemical measures such as failure of metal homoeostasis or enhanced MFO system activity with damage to the lysosomal system.

Lowe et al. (1981) and Lowe and Pipe (1985) examined cellular structure and function in mussels exposed to hydrocarbons which had previously been shown to have damaged lysosomal function. There was a reduction in substance and volume of cells and an increased lysosomal volume at elevated but sub-lethal concentrations of hydrocarbons. The amount of reproductive connective tissue (which supplies nutrients to the developing gametes) was greatly reduced and there was a consequent reduction in the numbers of ripe gametes within reproductive follicles.

Bayne (1989) claimed that the linked sequence of cause and effect through sub-cellular disturbances to pathological damage in important tissues indicates these measures of biological effect can provide confidence in the assertion that elevated contaminant levels can lead to significant toxic effects. This claim can, however, only be verified if there is an independent measure of the presence of elevated levels of contaminants. This is to prevent a circular argument of the form "if there are elevated contaminants then pathological damage will occur, so if we find pathological damage then there are elevated contaminant levels".

Organismal Measures

Physiological Responses

The major physiological measure of pollution is energy balance (Widdows 1985). Bayne et al. (1979) discussed how energy balance can be used to identify stress. Energy balance (also called "scope for growth"; Martin 1985) is the relationship between the amount of energy gained from food and the energy lost through processes of maintenance of the body. A surplus of energy allows growth and the production of gametes, but a deficit means that the animal must use stored reserves. If this is not redressed, the animal must eventually die.

Protein synthesis is a major drain on energy resources. Hawkins et al. (1986) estimated that up to 30% of the normal energy demand in mussels is due to turnover of proteins. The processes described for cellular and sub-cellular responses to excess contaminants both involve the animal increasing the rate of synthesis of proteins. This must therefore increase demand for energy and concomitantly reduce scope for growth. Martin (1985) and Widdows et al. (1988) have both shown a negative relationship between scope for growth and tissue concentration of hydrocarbons or trace metals. Whitelaw and Andrews (1988) showed a correlation of reduced scope for growth (Widdows 1985) along the path of dispersion of sewage sludge.

Condition

Indices of "condition", primarily shell thickness and tissue weight:cavity volume ratios, have also been used with the oyster *Crassostrea virginica*, in conjunction with trace metal concentrations, to identify when stresses are present (Lawrence and Scott 1982, Marcus et al. 1989). Changes in the condition index have also been correlated with changes in concentrations of coliform bacteria (Scott and Lawrence 1982).

A wider range of indices of condition, including ratios of body weight, shell weight, cavity volume and glycogen and protein levels have been correlated with a pollution gradient for *Crassostrea gigas* in New Zealand (Pridmore et al. 1990, Roper et al. 1991). The gradient of pollution was indicated by faecal coliforms and trace contaminants.

Individual bioassay

Numerous studies have demonstrated gradients of tissue concentration of trace contaminants that correlate with distance from known sources of contaminants. These studies will be examined in detail in a later section of this Chapter.

Population Measures

Population measures allow a direct test of whether pollution has resulted in changes to abundances of organisms (Underwood 1991a), but require either numerous analyses to cover all of the components of a community under question, or the selection of a few species or attributes for analysis. Deciding on which variable to analyse can be difficult, as can the analysis of rare species and for this reason many studies have relied on community analysis (see below). If these problems can be overcome, however, studies of populations are a very powerful tool in assessment of impacts. It is through analyses of populations that many of the underlying processes structuring marine communities have been elucidated (Underwood 1993, Underwood and Jernakoff 1981, Underwood and Kennelly 1990).

Community Measures

Community measures are often used to determine impacts resulting from large-scale or accidental perturbances. The techniques available (multivariate statistics) are rarely able to provide rigorous contrasts among locations, but can provide some qualitative

spatial comparisons. Examples of the use of community measures to demonstrate impact can be found in the following papers: differences in benthic fauna around offshore oil rigs (Grey et al. 1990); impact of sewage effluent on infauna of kelp holdfasts (Smith and Simpson 1992); improvements in benthic fauna after reduced pollution loadings (Shillabeer and Tapp 1989), effects of sewage sludge disposal (Whitelaw and Andrews 1988).

One criticism levelled at community-based measures is the time-consuming and labour-intensive sorting and identification of samples. Warwick (1988a,b, 1993) has suggested that this can be alleviated in part by limiting taxonomic resolution to higher taxonomic levels. This does not result in a reduction of the ability to detect impact, but does save much time and money.

Assessment of Uses of Various Measures

Direct comparisons of the range of measures discussed above are probably not really valid, because they each address different hypotheses and thus fulfil different purposes in a continuum of needs when assessing the various outcomes and impacts of a pollution event. As Underwood and Peterson (1988) pointed out, there is usually a decoupling between adult standing stock (or reproductive output) and recruitment. This is due to widespread dispersal of larvae prior to settlement. It would thus take a significant reduction in reproductive output over the entire range of dispersal to affect recruitment. Thus, if, as Underwood and Peterson (1988) suggested, the outcome of a pollution study is to determine changes in biotic communities, then it is not sensible to study anything below this level of organisation. If, however, the point of the study is to determine whether a pollution event is causing some sub-lethal detriment to organisms, that may not have yet manifested itself in more wide scale type of impact, or if the aim of the study is to establish a system for early warning of impact of pollutants then studies at organismal or sub-organismal levels are probably the most effective means.

As stated above, the aim of this thesis is to develop a system of identifying the presence of trace pollutants. Ideally, this should be achieved as quickly, cheaply and easily as possible. Studies involving individual assays and physiological measures at the organismal levels seem to be the most efficient means of achieving this aim. This has been recognised by many authors (Bayne 1989, Boyden and Phillips 1981, Goldberg 1975, Martin 1985, Martin and Richardson 1991, Phillips 1979, Phillips and

Yim 1981), but it has become apparent that techniques have to be developed for each species and situation involved. This is will be the outcome of research in this thesis.

"Bivalve Watch"

Background

In investigations of pollution in marine environments, the most common application of organismal levels of biological organisation has been to monitor trace contaminant levels by determining the concentrations of contaminants accumulated in the tissues of animals maintained near sources of pollution. The most commonly used animals are bivalve molluscs and the technique, known by the generic term "mussel watch" is widely used in Europe, Asia and the United States, where mussels are abundant (O'Connor 1992).

The "mussel watch" concept is based on the knowledge that a range of marine organisms, such as molluscs, crustaceans and fish, accumulate certain environmental contaminants in their tissues to concentrations above ambient levels in the environment. This allows such species to be used as "indicator" or "sentinel" organisms, reflecting relative levels of environmental contamination in a manner amenable to short and long-term monitoring. Phillips (1977,1978) has reviewed the use of biological indicators for quantifying trace contaminant pollution. He concluded that, whilst biological monitoring is better than chemical analysis of waters, the technique is subject to confounding by biological and environmental variables and studies must be designed and interpreted with care.

This bioaccumulation technique has been used in the United States (with mussels) to establish a ranking of sites contaminated by polynuclear aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), organochlorine compounds (OCs), and a range of trace metals (TMs) (Anon 1987). Oysters, mussels and barnacles have been used in Hong Kong to determine the comparative levels of contaminants in waters around the islands (Phillips and Yim 1981, Phillips and Rainbow 1988). Oysters have been used to monitor levels of radioactive silver near a nuclear power station (Rose et al. 1989) and to determine relative levels of trace metals in a number of other studies (Ikuta 1987, Martinic et al. 1987, Talbot and Chang 1987, Peerzada and Dickinson 1988). Amiard-Triquet (1986) used mussels and oysters to determine the relative metal concentrations in the Bay of Bourgneuf and reported little difference in bioaccumulation between the two species. Batley (1987) compared patterns in

contamination of cockles and levels of trace metals in estuarine waters.

The philosophy behind "mussel watch" is widely used to assess the status and trends in contamination of coastal seas, using a range of indicator species (algae, seagrass, plankton, worms, crustaceans, molluscs and fish). Bivalve molluscs (e.g. oysters, cockles, mussels) are particularly suitable for the study of pollution because of their sedentary habits, filter-feeding behaviour, convenient size and capacity for accumulating a variety of toxic compounds. Accordingly, bivalve molluscs have been recommended by many as the most suitable bioindicator of contaminants from marine and estuarine waters. They are sessile, easily sampled and filter large volumes of water, resulting in them concentrating chemicals which are available in such small amounts in water that they cannot be readily measured.

There is a perception that mussel watch studies are solely involved with measuring gradients of tissue concentration of trace contaminants, but, as Bayne (1989) and Martin (1985) pointed out, these studies can provide more information if variables other than the concentrations of trace contaminants are assessed. Bayne (1989) stressed the difference between the "contamination" of bivalves which is a physio-chemical measure resulting from discharge of contaminants to the environment in excess of natural concentrations and what he termed "pollution" which implies a biological effect upon the bivalve, probably resulting from chemical contamination. It is measurement of the biological effect and its correlation with the chemical concentration that makes mussel watch a powerful tool in environmental assessment. "Scope for growth" (an energy budget - see above) has been found to be negatively correlated with concentrations of petroleums and PCBs in the tissues of *Mytilus californianus* and a significant decline in physiological condition of *Mytilus edulis* along a pollution gradient has also been described (Martin 1985).

The literature on bioaccumulation in bivalves is enormous. I will use a selection of papers to indicate some of the range of topics and ideas covered.

Large-scale Monitoring

The concept of using bivalves to monitor trace contaminants was originally developed by Butler and his colleagues in the 1960s (Martin and Richardson 1991) and was soon implemented as routine monitoring programmes in a number of countries.

A review of the use of bivalve molluscs as concentrators of marine pollution by Scripps Institute of Oceanography (Goldberg 1975, 1986) recommended that an assessment of organochlorines using bivalves is particularly needed in the tropics and the southern hemisphere. These views were echoed by Martin and Richardson (1991) who felt that mussel watch programmes have achieved their potential in the northern hemisphere, but are lacking in southern hemisphere countries such as Australia.

Despite the widespread use of mussel watch techniques and their undisputed importance, Segar et al. (1985) and Phillips and Segar (1986) concluded that many mussel watch studies were poorly designed and resulted in the provision of little useful information to managers. Segar et al. (1985) stressed that a properly designed study must have clearly defined objectives stated as null hypotheses and must test these hypotheses in a statistically robust manner. Replication should be sufficient to ensure that variation at all levels of interest was quantifiable.

Phillips (1988) reviewed the Californian Mussel Watch programme and, while he stressed its value, he also identified a number of deficiencies. Among these was a temporal inconsistency in sampling sites and too often "hot spots" were targeted with the concomitant sacrifice (for financial reasons) of control areas. There was also an inequitable distribution of sampling sites with complex areas being under-sampled and less complex areas over-sampled. There were inappropriate temporal scales, particularly for compounds with a short half-life; some inappropriate selection of contaminants and outdated analytical protocols; inadequate financial commitment; political interference in direction and priorities and inadequate opportunity for related research and publication of results. Martin and Richardson (1991) identified many similar deficiencies in the limited mussel watch operated by the Victorian EPA (Australia).

Designing a "Bivalve Watch" Project

Phillips and Rainbow (1993) have devoted an entire chapter to this subject, some main points of which are summarised here for later reference.

Objectives of the Programme

At the beginning of any study it is imperative to have clearly stated objectives for that study. These objectives should set out the scope and intentions of the study, particularly with regard to the spatial and temporal scales of interest. These factors, along with the underlying rationale for conducting the study (e.g. comparison to

regulatory guidelines, identification of new sources of contaminants, effects of environmental management actions), will profoundly influence the design of the study.

Selection of a Sentinel Species

A sentinel, or biomonitoring, species should be selected for sound and well-established reasons. All too often, a species is chosen because it is locally abundant (with no regard to its usefulness as an indicator) or because it has been used elsewhere (with no regard for its local suitability). The pertinent characteristics of the sentinel species will be very much dependent on the hypotheses to be addressed and what is expected of the species (Underwood and Petersen 1988).

If the study is being done to test whether pollution is having an effect on the ecology of an assemblage, it is common to select one or two species to be representative of that assemblage. Ignoring the question of whether any species can actually represent others in the assemblage, the species chosen in this scenario would have to be one which is sensitive to any change in its environment and one which is going to show changes in abundance or population structure. The basis for selection will have to include detailed knowledge of a range of species in the assemblage. Common or widespread species, so often chosen, may well be totally inadequate because it could be their adaptability or resistance (or ability to overcome) impact that has allowed them to become so common.

On the other hand, if the study is aimed at examining body burdens of contaminants to determine trends in inputs of pollutants then "sensitive" species, which die or are physiologically affected in the presence of pollutants, are useless. What is needed are robust species, able to survive and grow under a wide range of conditions.

The next question to be addressed is whether biomonitors will be collected from wild populations or translocated to areas of interest. Techniques for translocation are well developed (see Green et al. 1986, Henry and Scanes 1992, Scanes 1992, Young et al. 1976) and the use of commercially grown animals can reduce the variability associated with age and genetic isolation in wild populations. Translocation also allows the precise definition of time of exposure in a particular area (Phillips and Rainbow 1993) often facilitating better interpretation of results. Translocation also allows the use of a well known biomonitor in areas where it does not occur, or the reintroduction into areas where it may have been previously eliminated.

A related question is the geographical scope of the study. The biomonitor used, whether wild or translocated, must be able to survive over the full geographical range that is of interest. If this is not possible, then either a rethink of the hypothesis or the use of a variety of species is necessary (e.g. NOAA Mussel Watch; O'Connor 1992). If more than one species is used, then they may have to be "calibrated" against each other in areas where they co-exist, allowing adjustments of data for comparative purposes.

In summary, if a species is being selected for use as a bioaccumulator it should also fulfil a number of basic requirements, here summarised from Phillips and Rainbow (1993).

- the organism should be able to accumulate the contaminants of interest and body burdens should show a simple correlation with its environment;
- contaminants should be accumulated without lethal impacts;
- the organism should be sedentary, so as to be representative of the area from which it was collected;
- the organism should be abundant in (or able to be translocated to) the area(s) of interest:
- the organism should be easy to sample and be hardy enough to permit manipulation and laboratory studies

Timing and Frequency of Monitoring

Once the hypothesis has been stated and a suitable species has been selected, the next step is to determine how often to sample. This must be part of the hypothesis, but a number of factors have to be taken into account. These include:

- temporal variation of inputs (if known);
- uptake and depuration kinetics of contaminants of interest;
- reason for doing study and therefore, potentially, time period of interest;

It is generally accepted that, unless there are other over-riding interests, the most appropriate time between sampling is long enough to allow the contaminants of interest to reach equilibrium (assuming a constant input). The kinetics of different contaminant vary, so what is appropriate for, say, chlordane, will not be appropriate for zinc. This means that timing with either be a compromise or samples will have to be collected at a range of different time intervals.

Timing of sampling should also take into account possible variations introduced into the data by the biology of the organisms (e.g. gametogenesis and spawning).

Selection of Sites

Again, the rationale for selection of sites should be defined in the hypothesis. The basic aims of the study will determine whether known "hot spots" or point sources are targeted, or whether a more general allocation of sites is required. The selection of sites should take into account the need for statistical analysis of data and ensure that there is appropriate spatial replication to ensure that generalisations about specific locations or comparisons among locations are not confounded (Hurlbert 1984, Underwood 1981). Care should be taken to ensure that there is spatial and temporal independence of data.

Bioaccumulation in Oysters

Oysters form part of the US mussel watch in those areas where mussels do not naturally occur (Goldberg et al. 1983, Farrington et al. 1983, O'Connor 1992). They have been used in a variety of other studies of bioaccumulation. Phillips (1979) considered the oyster *Saccostrea glomerata* to be a reliable indicator of concentrations of trace metals in Hong Kong waters. Phillips and Yim (1981) showed that concentrations of copper and zinc in *S. glomerata* reflected that of sediments in Hong Kong waters, but that the mussel *Septifer bilocularis* appeared to regulate Zn and Cu and hence would be an unreliable indicator.

A survey of metals in *Crassostrea commercialis* (= *Saccostrea commercialis*) by Mackay et al. (1975) showed that the concentration of trace metals in oyster tissue correlated with an environmental gradient of metal pollution, but there was some evidence that metal concentration decreased with increasing age and wet weight of oysters. The latter conclusion was not supported in a follow-up study by Brown and McPherson (1992). Peerzada and Dickinson (1989) and Pendoley (1992) used the closely related *S. cucullata* to investigate levels of trace metals in Darwin and polycyclic aromatic hydrocarbons (PAH) in Western Australia, respectively.

Kopfler and Mayer (1973) reported a poor correlation between concentrations of trace metals in oysters and waters. They did, however, show that oysters had tissue concentration 4 to 5 orders of magnitude greater than concentrations in the surrounding water . Water samples were filtered prior to analysis and the authors

suggested that the lack of correlation in concentrations of trace metals could be due to the oysters accumulating metals from particulate matter rather than dissolved metals.

Seasonality and/or gametogenesis are reported to be important variables that can affect the absolute concentrations of trace metals in oyster tissues (Boyden and Phillips 1981, Phelps et al. 1985, Talbot 1985,1986). This can cause temporal variability in concentrations of trace metal irrespective of ambient environmental concentrations, but it should not interfere with spatial comparisons at one time.

Studies of metal dynamics in oysters have shown that the periods of uptake and depuration for metals vary between species. The time taken for depuration of most metals are in the order of 60-180 days (Okazaki and Panietz 1981). Frazier and George (1983) showed the rate of uptake of cadmium in *Crassostrea gigas* was twice that of *Ostrea edulis*. Ward (1982) showed that uptake of cadmium was linear at moderate ambient concentrations and that cadmium was stored in different parts of the body of *Saccostrea commercialis* at different concentrations; little was stored in gonads.

Huggett et al. (1973) suggested that ratios of concentrations of trace metals can be a more sensitive indicator of pollution than comparisons of absolute concentrations, as this eliminates confounding by sources of variation such as salinity and season. For example, the ratio of copper to zinc and cadmium to zinc was found to be similar at places not near sources of pollution but was different in oysters taken from samples near sources of pollution.

Taxonomy of Oysters

The taxonomy of oysters was the subject of a great deal of discussion in the 1970's. Stenzel (1971) proposed that the existing classification of all oysters into 3 genera was inappropriate and, amongst a number of other changes, suggested that the oysters in the genus *Crassostrea* were actually from two separate genera. He separated them according to the presence of denticles in the hinges of the valves, non-denticulated oysters remaining as *Crassostrea*. Denticulated oysters were placed into the genus *Saccostrea.* Ahmed (1975) considered a number of other factors such as micro-habitat and possibility of hybridisation, but agreed with Stenzel's classification.

The Sydney Rock oyster (the primary bivalve discussed in this thesis) was originally named as a species of *Saxostrea* by Iredale and Roughley in 1933 but Thompson

(1954) reclassified it as *Crassostrea commercialis*. Stenzel (1971) considered the Sydney Rock Oyster to be a sub-species of the more ubiquitous tropical and sub-tropical *Saccostrea cucullata*. Ahmed (1975) reported this conclusion of Stenzel (1971) but did not take a particular stance. He did, however, make a case for doubting the validity of the name *cucullata* (a position originally taken by Thompson 1954) and favoured the use of *tuberculata*.

There seems to be no definite resolution to these deliberations in the literature, but common usage seems to have settled on the following conclusions:
1. Denticulated oysters are in the genus *Saccostrea*.
2. The common tropical oyster is known as *Saccostrea cucullata* (Phillips 1979, Phillips and Yim 1981, Talbot 1985, 1986).
3. The Sydney rock oyster is known as *Saccostrea commercialis* (Ward 1982, Malcolm 1987).

This nomenclature will be followed in this thesis.

Scope and Objectives of this study

As has been stated above, this thesis aims to provide a basis for determining and being able to interpret a system for monitoring pollution which will provide an easy, relatively cheap and quick assessment of the concentration or rates of change of trace pollutants in a marine or estuarine environment.

The assessment will have three main components:
1. Dynamics of contaminants - sample design;
2. Practical deployment techniques - sample design;
3. Case studies of various trace contaminants, via tissue concentrations, under different contaminant regimes;

The third component has some value as a broad indication of the potential for impact, but as Underwood (1993) has pointed out, a more rigorous appraisal of whether there has been an impact and what has caused that impact is an essential part of the procedure. This thesis will also provide some examples of how bivalves may be used to determine the cause of impacts. A major part of the investigation will be the application of monitoring bivalves to the assessment of the impact of sewage disposal off Sydney's coast. Each subsequent Chapter will include a more detailed analysis of its particular background, literature, objectives and hypotheses.

CHAPTER 2 Analytical Methods, QA/QC, Statistical Methods, Biology of Oysters

Introduction

Preparation and analysis of samples for organochlorine compounds and trace metals is a procedure common to most Chapters in this thesis. The methods for these procedures will be set out in full here to avoid duplication. These procedures will be restated in brief in each Chapter and will have been followed as stated here unless otherwise indicated.

An important part of a study which involves extensive use of analytical results is a laboratory quality assurance/quality control programme. Details of the programme established for this study and the results of that study are provided.

Contaminants Assessed

The contaminants which are being investigated are in two main categories, trace metals and organochlorine compounds, and are listed in Table 2.1 and 2.2 with the laboratories' Practical Quantification Limits (PQL).

Table 2.1 Trace metal compounds analysed (mg/kg wet weight basis)

Trace Metals	Practical Quantification Limits
Arsenic	0.01
Cadmium	0.01
Chromium	0.01
Cobalt	0.001
Copper	0.01
Lead	0.01
Mercury	0.01
Nickel	0.01
Selenium	0.01
Silver	0.001
Zinc	0.1

Table 2.2 Organochlorine compounds investigated in oysters (mg/kg wet weight basis)

Organochlorine Compounds	Practical Quantification Limit
Aldrin	0.01
alpha-BHC	0.01
beta-BHC	0.01
gamma- BHC (Lindane)	0.01
Chlordane	0.05, 0.01*
Dieldrin	0.01
DDD	0.01
DDE	0.01
DDT	0.01
Endosulfan	0.01
Endrin	0.01
Heptachlor (HPT)	0.01
Heptachlor epoxide (HPTE)	0.01
Hexachlorobenzene(HCB)	0.01
Methoxychlor	0.01
Oxychlordane	0.01
PCBs	0.05
* since June 1992	

Protocols for Handling and Preparation of Samples

Samples of bivalves were deployed in the field in open mesh polyethylene bags. When a bag is retrieved it is vigorously shaken in ambient water to remove any residual sediments or accumulated particulates. Bivalves are then removed from the mesh bag and placed in a plastic bag with an identifying tag. The bag is then sealed and placed in a cool location. As soon as possible, the samples in plastic bags were frozen to -18°C.

Frozen bivalves are allowed to partially thaw at room temperature prior to opening. Thawed bivalves are then opened using stainless steel instruments which are rinsed in hexane between samples to avoid cross contamination. The oyster is removed from the shell using stainless steel forceps, placed in a suitably prepared container and the wet weight determined. Fluid in the shell is discarded. Bottled samples are immediately refrozen prior to delivery to analytical laboratories.

Containers for organochlorine analysis are 250 ml Schott Duran glass bottles. The bottles are washed in detergent, rinsed in distilled water and double rinsed in nano-grade hexane. Plastic lids are shielded with hexane rinsed aluminium foil.

Containers for trace metal analysis are disposable polystyrene bacteriological sample bottles. The bottles are washed in detergent, rinsed in distilled water and then soaked in 5% nitric acid.

Chemical Analyses

Organochlorine Compounds

Organochlorine concentration and fat content were determined by the Australian Analytical Laboratories (AAL), Hornsby. Samples were extracted by homogenisation with acetone/acetonitrile (1:1) from a mixture of approximately 5 g of sample and 10 g of anhydrous sodium sulphate. The extract was exchanged into hexane and cleaned according to the procedures outlined in the florisil clean-up section, 983.21E of the *Association of Official Analytical Chemists* (AOAC) Official Methods of Analysis, 15th Edition, 1990.

The extract was then analysed in accordance with USEPA Method 8080, modified by the use of capillary gas chromatography (GC) using electron capture detection. Two different polarity GC columns were used to identify the organochlorines, with gas chromatography-mass spectrometry (GC-MS) used to confirm the organochlorines detected.

The chromatograph used was a Hewlett Packard 5890, with a 7673/A auto-injector. The primary column was 15m 0.32 mm id x 0.25 mm DB1701 (J and W Scientific) with hydrogen at 6 psi as the carrier gas. The confirmation column was a DB225 with helium at 10 psi as the carrier. Both columns were operated at an initial temperature of 130^{o}C and raised to 220^{o}C at 15^{o}C/min.

Lipid Content

Lipid content, expressed as a percentage of sample weight, was determined by petroleum/ether extraction, as described in acid hydrolysis method 948.15 of AOAC Official Methods of Analysis,15th Edition, 1990.

Trace Metals

Concentrations of trace metals were determined by the CSIRO, Centre for Analytical Chemistry, Lucas Heights. Samples were digested with high purity concentrated nitric acid in a microwave oven. The digests were then spiked with indium as an internal standard and analysed for a range of trace metals using inductively coupled plasma mass spectrometry (ICPMS) (modified USEPA Method 200.8, Revision 4.4, April 1991).

Note

Analyses of lipid were discontinued early in sampling because it was usually not possible to get enough tissue from an individual oyster for both lipid and organochlorine analysis and, more importantly, initial data showed no correlation between lipid and levels of organochlorine contamination.

Quality Assurance/Quality Control

Introduction

The accuracy (or bias) and repeatibility (or precision) of trace contaminant analysis is monitored by a QA/QC programme.

Methods

At the time that each batch of samples was analysed, the laboratory also analysed either a commercially available standard (trace metals) or prepared spiked samples (organochlorines). These will be referred to as Laboratory Standards. Later, reference samples with trace contaminant concentrations unknown to the laboratory were distributed with samples for analysis. These will be referred to as Reference Standards. The Laboratory Standards are used to determine whether the results being reported are close to "correct" concentrations (i.e. estimating accuracy). The Reference Standards indicate temporal variablity in concentrations being reported by the laboratories (among batch repeatibility) and repeatibility within a batch.

CSIRO laboratories (trace metals) analysed either of two Laboratory Standards at the end of each batch of samples. The standards are known as MAA2 (which is a freeze dried fish flesh) and MAM1 (which is freeze dried oyster tissue). These standards are supplied with the certified trace metal concentration expected in the samples.

Australian Analytical Laboratory's organochlorines Laboratory Standard consisted of blended oyster tissue which had been spiked with organochlorine compounds at 0.1 ppm (parts per million, equivalent to mg/kg) and 20 ppb (parts per billion; equivalent to 0.02 ppm).

To prepare the Reference Sample, a large number of oysters from an area believed to be contaminated by organochlorines and trace metals were blended together. Numerous samples of five to seven grams of this material were placed in sample containers and frozen. Samples of this reference standard were provided to the laboratories with each batch sent for analysis during 1993. These samples could not be anonymous to the laboratories because they were different in appearance. They were usually analysed at the end of the batch.

QA/QC data from studies of oysters that are not reported in this thesis have been included to provide the strongest basis for comparison. There has thus been no attempt made to identify QA/QC data with individual projects in the summaries below. The implications of specific instances and general trends will, however, be discussed in conjunction with projects where applicable in later Chapters. Throughout this section the term "Batch" is used to signify a batch of samples that went to the laboratory together and were analysed as a group.

To examine the accuracy of the laboratories' analyses, *t*-tests were used to compare the overall means for each contaminant in Laboratory Standards with the actual value which should have been obtained. Repeatibility among batches was examined by calculating the co-efficient of variation (CV; standard deviation* 100/mean, also known as %RSD) for the analyses of the Laboratory Standards and the Reference Standard. In addition, data from the reference standards were compared over time using a one factor analysis of variance. Within-batch variability was examined by calculating the precision (SE/mean) and CV for replicates within a batch at each time for the Reference Standard.

Results

Comparisons to Laboratory Standards.

Analyses of Laboratory Reference Standards available for trace metals showed that for fish tissue (MAA2), long term means of cobalt, nickel and lead were significantly less than certified concentrations and chromium was significantly greater than the certified concentration (Table 2.3). Analyses of oyster tissue (MAM1) showed that means reported for cobalt, nickel, zinc and silver were significantly less than expected and arsenic and selenium were significantly greater than expected (Table 2.3). Variation among batches was estimated by the co-efficient of variation (CV). The literature does not give clear guidance on acceptable values for CV. Gibbs and Miskiewicz (1995) believed that a CV of less than 10 % was acceptable and Tetra Tech (1986) suggest a CV of 20 % is acceptable. In this study, CVs for trace metals were variable, within and between Laboratory Standards. Variation among batches was evident in both standards; for the fish standard copper, zinc, arsenic and selenium had a CV less than 20 %, chromium, nickel, mercury and lead were less than 30 % and for the oyster tissue standard, chromium, cobalt, copper, zinc and arsenic were less than 20% and selenium and lead were less than 30 % (Table 2.3).

Analyses of spiked oyster samples for organochlorines showed that there was some variability from the expected results (i.e. the nominal spike concentration), but this variability was, with the exception of DDT, within a few percent of the expected value (Table 2.4), DDT varied up to 10 %. The overall means were statistically significantly different from expected concentrations for many contaminants (Table 2.4), but this seems to be primarily a consequence of the extremely small within-batch variability and among-batch variablity (CV, Table 2.4) leading to an ability to detect very small (and biologically insignificant) changes as statistically significant.

Co-efficients of variation for organochlorines were small, most below 10% and all below 12% indicating very small variability among batches (Table 2.4).

Table 2.3 Comparisons of long term mean results from CSIRO laboratory with MAA2 Standard and MAM1 Standard. Underlined type indicates significant difference between means (*t* test, $P < 0.05$). Proportional difference is mean/expected mean, a value of 1 indicates they are the same, greater than 1 indicates overestimation of concentrations by laboratory, less than 1 indicates underestimation.

MAA2 - Fish Tissue Standard

TRACE METAL	EXPECTED MEAN	ACTUAL MEAN	STD. ERROR	CV (%)	PROPORTIONAL DIFFERENCE
Cr	1.30	1.99	0.124	24	1.5
Co	0.08	0.05	0.004	35	0.68
Ni	1.10	0.76	0.044	23	0.69
Cu	4.00	3.73	0.233	8	0.94
Zn	33.00	30.65	1.740	11	0.93
As	2.60	2.67	0.070	9	1.02
Se	1.70	1.66	0.082	15	0.98
Ag	0.10	0.09	0.008	37	0.89
Cd	0.07	0.060	0.004	31	0.92
Hg	0.47	0.43	0.019	22	0.92
Pb	0.58	0.39	0.040	26	0.66

MAM1 - Oyster Tissue Standard

TRACE METAL	EXPECTED MEAN	ACTUAL MEAN	STD. ERROR	CV (%)	PROPORTIONAL DIFFERENCE
Cr	1.20	1.30	0.081	17	1.08
Co	0.43	0.37	0.007	5	0.87
Ni	3.70	1.44	0.406	74	0.39
Cu	317.00	315.00	4.477	4	0.99
Zn	2820.00	2664.43	46.010	5	0.94
As	10.50	12.77	0.412	9	1.22
Se	2.32	3.53	0.359	27	1.51
Ag	5.80	4.30	0.562	35	0.74
Cd	2.30	1.87	0.281	40	0.81
Hg	0.20	0.18	0.021	30	0.91

Table 2.4 Comparison of results of analyses of Laboratory Standards with expected results (i.e. 100 percent recovery). Data are mean percent recovery of spike. Underlined type indicates significant difference between means (*t* test, $P < 0.05$). Proportional difference is mean/expected mean, a value of 1 indicates they are the same, greater than 1 indicates overestimation of concentrations by laboratory, less than 1 indicates underestimation.

Organochlorines spiked at 0.1ppm (PCBs also spiked at 1.0ppm)

ORGANOCHLORINE	EXPECTED MEAN	ACTUAL MEAN	STD. ERROR	CV (%)	PROPORTIONAL DIFFERENCE
P.C.B.	100	101.00	1.26	8	1.01
P.C.B. (1.0ppm)	100	102.00	2.20	6	1.02
OXYCHLORDANE	100	99.68	1.38	8	0.99
LINDANE	100	97.92	1.86	12	0.98
HEPTACHLOR	100	101.45	1.80	11	1.10
HEPTACHLOR EPOXIDE	100	96.36	1.61	10	0.96
H.C.B.	100	99.42	1.97	12	0.99
ENDRIN	100	103.92	1.56	9	1.04
DIELDRIN	100	101.68	1.37	8	1.01
DDT	100	107.00	1.24	7	1.01
DDE	100	104.08	1.37	8	1.04
DDD	100	101.54	1.36	8	1.02
CHLORDANE	100	104.31	1.14	9	1.04
ALDRIN (0.1ppm)	100	102.08	1.64	10	1.02

Organochlorines spiked at 20ppb - chlordane at 100 ppb

ORGANOCHLORINE	EXPECTED MEAN	ACTUAL MEAN	STD. ERROR	CV (%)	PROPORTIONAL DIFFERENCE
OXYCHLORDANE	100	103.50	1.33	7	1.04
LINDANE	100	100.90	1.55	10	1.01
HEPTACHLOR	100	102.35	1.99	8	1.02
HEPTACHLOR EPOXIDE	100	102.65	1.60	7	1.02
H.C.B.	100	97.05	1.77	9	0.97
ENDRIN	100	105.60	1.52	8	1.05
DIELDRIN	100	107.05	2.38	8	1.07
DDT	100	111.42	3.60	11	1.11
DDE	100	104.85	1.60	8	1.04
DDD	100	102.90	1.18	8	1.03
ALDRIN	100	101.25	1.73	9	1.01
CHLORDANE (100 ppb)	100	101.20	1.52	7	1.01

Comparisons to Reference Standard

Co-efficients of variation among batches for trace metals were generally greater for the Reference Standard than for the Laboratory Standards, only copper, zinc and nickel were below 30%. The analyses of variance comparing times indicated that copper, zinc and mercury were the only metals that did not show significant differences among batches (Appendix 1, Table 2.5). Within batches, precision was high for all metals (except silver) and co-efficients of variation were small - arsenic, cadmium, cobalt, copper, lead, mercury, and zinc all less than 10%, and only silver greater than 21% (Table 2.6). Both these measures indicate that within-batch variability was small.

Co-efficients of variation among batches for organochlorines were also greater for Reference Standards than for Laboratory Standards, nevertheless CVs for chlordane DDE, DDD and PCB were still within acceptable limits (<32%; Table 2.5). Analyses of variance showed that concentrations of chlordane and PCB were not significantly different among batches, but DDT, DDD and dieldrin did differ (Table 2.5). Within batches, co-efficients of variation for organochlorines were all small (less than 16 %) and precision was high. Both these measures indicate that within-batch variability was small.

Discussion

Trace metal analyses showed quite small within-batch variability, but, in some cases, showed large deviations from expected concentrations and large differences among batches. This has some implications for how the data from subsequent studies can be used. In general, data could potentially be used in two ways, either for comparison to some legislative guideline or to demonstrate differences in concentrations between places or times.

In the first case, absolute concentrations of lead, chromium, cobalt, nickel, zinc, arsenic, selenium and silver should be carefully confirmed before comparison to regulatory guidelines. For most of these metals (except arsenic and selenium) the laboratory underestimated the concentrations which means that potential problems are likely to found less often than they should be.

Table 2.5 Co-efficient of variation and conclusions from analyses of variance comparing differences among batches from analyses of Reference Standard. * indicates significant differences among batches ($P < 0.05$) in ANOVA

trace metals n = 7	CV (%)	ANOVA	organochlorines n = 3	CV (%)	ANOVA
Arsenic	49	*	Chlordane	26	
Cadmium	54	*	DDE	32	*
Chromium	45	*	Dieldrin	132	*
Cobalt	35	*	DDD	21	*
Copper	1.4		PCB	18	
Lead	93	*			
Mercury	56				
Nickel	24	*			
Selenium	77	*			
Silver	206	*			
Zinc	1.2				

Table 2.6 Mean of CV and mean of precision for each batch (measures of within batch variation) from analyses of Reference Standard

trace metals n = 7	CV (%)	Precision	organochlorines n = 3	%RSD	Precision
Arsenic	7.9	0.02	Chlordane	13	0.04
Cadmium	5.6	0.03	DDE	12	0.04
Chromium	21	0.12	Dieldrin	0.01	0.06
Cobalt	5.9	0.03	DDD	16	0.05
Copper	1.9	0.01	PCB	9	0.02
Lead	6.6	0.03			
Mercury	0	0.00			
Nickel	16	0.09			
Selenium	15.4	0.08			
Silver	47	0.23			
Zinc	1.8	0.01			

The small within-batch variability (except silver) means that relative comparisons between samples from within the same batch should be valid. In the majority of studies described in this thesis, all samples from a particular study were analysed in a single batch. The exception is the study of Sydney's sewage outfalls reported in Chapter 5. In that case, however, there were samples from outfall and reference locations analysed in each batch and the statistical analyses compared the relative concentrations from the two treatments at each time. This comparison was not compromised by the results from the QA/QC programme. Straight temporal comparisons would be inappropriate in this study.

The comparisons with Laboratory Standards indicated that results of analyses of many organochlorines were significantly different from expected concentrations. The means were, however, within a few percent of expected concentrations. There was also some variability among batches in with both Laboratory and Reference standards. It must be remembered that these analyses are of a nominally homogeneous substance (and the very small within batch variablity emphasises this fact) and what is being measured is the variability of the method of analysis. The small differences being detected as significant in these analyses are actually smaller than the differences among individual samples from the same area in later studies. This implies that, even though there is some variability within and among batches in the chemical analyses, it is probably insignificant compared to differences between treatments and among replicates in field studies.

Conclusions

The results of organochlorine analyses can be used with confidence to compare to legislative guidelines and to demonstrate trends within and between batches, as long as the differences between batches are greater than about 10 %. The results of trace metal analyses are suitable for comparisons within batches, but care should be exercised in comparisons to legislative guidelines and in comparisons among batches unless appropriate controls are used (as in Chapter 5). The data on silver are probably of little use at present as they show large levels of inter- and intra-batch variability.

Biology of Oysters

The Sydney rock oyster (*Saccostrea commercialis*) occurs naturally in the estuaries and nearby rocky headlands of NSW. It has been under intensive commercial

cultivation since about 1870. Commercial culture still mostly relies on unregulated natural recruitment to cultivation structures.

The oysters spawn directly into the water during summer and two to three weeks later the larvae settle on to suitable hard substrata (Malcolm 1971). Oysters are protandrous hemaphrodites, spawning first as males and changing to females later in life (Roughley 1933). Spawning seems related to water temperature and the oysters may spawn twice if the water temperatures rise quickly in early summer (Roughley 1933).

Sydney oysters are filter feeders, pumping about 4 $l.hr^{-1}$ through their gills (J Nell pers comm) and extracting planktonic particles as food.

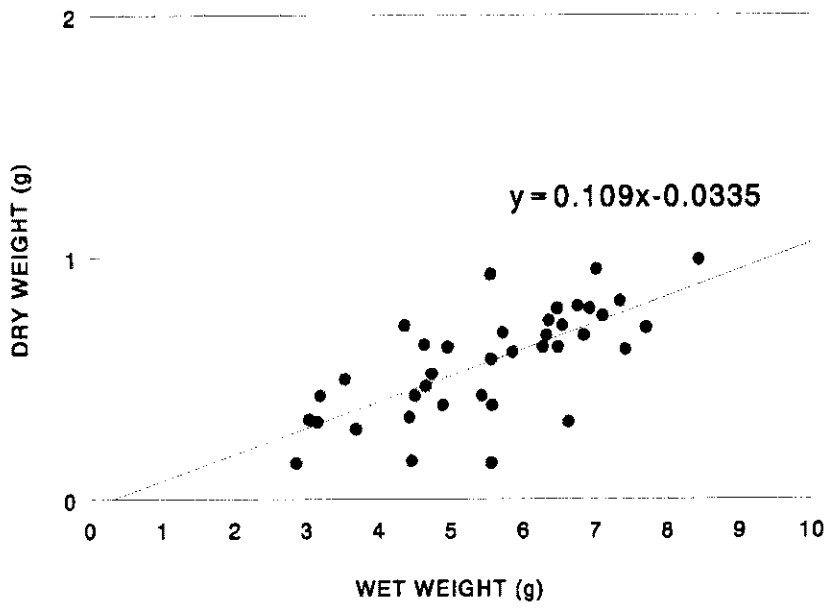
Wet versus dry weights

In this thesis, most data on contaminant concentrations are expressed on a wet weight basis. This was primarily a consequence of the difficulty in determining organochlorine concentrations on a dry weight basis. In order to allow comparisons with other data sets, I determined the relationship between the wet and dry weights of 38 *S. commercialis*. Oysters were opened and the soft parts removed and excess fluid allowed to drip off. The tissues were placed on pre-weighed foils and the wet weight determined. The oysters were then dried to constant weight at 60°C and the dry weight determined. The data were analysed by simple linear regression (Fig 2.1). The regression was significant ($P < 0.001$), had an r^2 value of 0.49.

Oysters used in experiments

All oysters were from commercial growers and were around two years in age. Their wet weights were in the range of 3 - 8 g (see Fig. 2.1).

Figure 2.1 Regression of wet and dry weights of Sydney rock oysters.



"Not detected" Data

There is no consistent procedure evident in the published literature for dealing with "not detected" values with respect to statistical analyses. The most often cited procedures are to construct the truncated part of the distribution of data using the detected values. Two common methods of estimating the value of "not-detected" data are log-probit analysis (Travis and Land 1990) and maximum likelihood estimation (Helsel 1990). Both these authors point out that substitution of zero or half the detection limit biases estimates of the mean and standard deviation of the sample. Any method of reconstructing a truncated tail, however, implies that the frequency distribution of the data is known. This may be the case when there are large data sets over constrained spatial and temporal scales that have few "not-detected" values, but in many of the situations in marine trace contaminant studies there are not enough data points at a site to provide any confidence in extrapolation. The use of data sets pooled over a number of times or locations to provide the necessary frequency distribution is also likely to lead to erroneous results, since at any given time and place it is impossible to know whether a "not detected" result actually represents zero or a low value. Therefore the distribution of data chosen to predict the probable value will strongly influence the data and this may bias the distribution's parameters much more than simple substitution.

Therefore, in these bioaccumulation studies, if the result for a contaminant was reported by the laboratory as "not detected" it was assigned a zero value. If the result for a contaminant was reported as a "trace" it was assigned a value equal to half the detection limit for that contaminant. This procedure could lead to an underestimation of total loads of organochlorines and will bias some estimates of variance but was considered to be the most conservative and parsimonious.

Assumptions of Analysis of Variance

Most data were analysed by analysis of variance (ANOVA) techniques (Underwood 1981, 1991b). The use of ANOVA involves some basic assumptions about the data. It is necessary to test to be sure that the most important assumptions are not violated. All data were tested for homogeneity of variance using Cochran's test. If data were heteroscedastic, transformations were applied to stabilise variances. The transformations used were ln (x+1), square root (x+1) and cube root (x+1).
If transformations were not successful, untransformed data were analysed and the analyses were interpreted using a protocol following the rationale in Underwood (1981) and Winer (1971). This rationale states that when n is the same, heterogeneous variances are more likely to lead to Type I error, i.e. detection of a significant difference where none exists. The protocol used was as follows:

- If differences were not significant, the interpretation of the analysis was considered valid.
- If differences were significant in the range $0.001<P< 0.05$, the results of the analysis were interpreted with caution and trends were inferred from graphs of the data.
- If differences were significant in the range $P<0.001$, then interpretations for the analysis were considered to be valid, although trends were also compared with graphs of the data.

CHAPTER 3 SPECIES AND DEPLOYMENT

Introduction

Many papers have described the use of sessile animals for monitoring trace contaminants (e.g. Amiard-Triquet et al. 1988, Cain and Luoma 1990, Hanna and Muir 1990, Klumpp and Burdon-Jones 1982, Lobel et al. 1982, Mackay et al. 1975, Martin 1985, Phillips 1976a,b, Phillips and Yim 1981, Powell and White 1990, Talbot 1985; and reviews by Phillips 1977,78, Phillips and Rainbow 1993). The choice of bioindicator usually reflects either local abundance or a desire to compare with (or emulate) studies done at another time or place.

Phillips (1977) has compiled a list of criteria that a bioindicator should fulfil. Klumpp and Burdon-Jones (1982) considered that, of these criteria, the most important were a simple correlation between body burden and concentrations in the environment and amenability to relocation. It is evident that no single organism can fulfil the role of bioindicator in a wide range of habitats and circumstances. The organism selected for a particular study should be chosen carefully, to fulfil the objectives of that study. Many of the criteria referred to by Phillips (1977) relate to the collection of wild animals and are not so relevant to studies using translocated organisms.

There have been many comparisons of different species in the same environment (e.g. Klumpp and Burdon-Jones 1982, Lobel et al. 1982, Phillips and Rainbow 1988, Phillips and Yim 1981, Powell and White 1990) to determine which were "better" biomonitors, or just to compare the species. Similarly there have been attempts to compare body burdens with environmental gradients in order to determine a suitable bioindicator (e.g. Klumpp and Burdon-Jones 1982, Martin 1985, Phillips 1976 b, 1979). These types of studies have attempted to establish that some species are useful as indicators of pollution for a variety of reasons. The reasons almost invariably rely on a correlation between body burden and an environmental gradient of pollution, either measured in the water or sediments, or implied by proximity to a point source of pollution. Species with a body burden of a particular pollutant that did not reflect the implied gradient were usually rejected as biomonitors of that pollutant.

One of the objectives of this thesis is to "develop and understand the strengths and limitations of a method of trace contaminant monitoring for estuarine and marine waters in NSW using bivalve molluscs". All aspects of this objective can not be

achieved using only one species of bivalve. Three species of bivalves are assessed in this Chapter: Sydney rock oyster *Saccostrea commercialis,* Sydney cockle *Anadara trapezium,* blue mussel *Mytilus edulis.* Oysters and cockles are primarily estuarine organisms, but occupy different substrata. Cockles live buried in estuarine sediments with only the tips of the shells exposed to allow feeding. Oysters are found attached to hard substrata in intertidal areas and smothering in sediment can cause death (Malcolm 1971). Oysters are also extensively cultivated for human consumption in NSW estuaries. Oysters and cockles are common along the entire coast of NSW. Blue mussels are primarily a cold water species (Sydney is about the northern extent of their range) and live attached to hard substrata in coastal embayments.

The objectives of this Chapter are to determine:

1. Which contaminants are accumulated by the various species and can the animals be used to discriminate between contaminated and uncontaminated sites?
2. That the different species can be deployed and retrieved in a simple and efficient manner and whether the means of deployment affects mortality and level of contamination.

Once these objectives have been met, then it should be possible to recommend the "best" species for use as a biomonitor in a given set of circumstances.
Two separate groups of experiments are described in this Chapter. The first tests hypotheses relevant to Objective 1 and the second tests hypotheses relevant to Objective 2. Overall conclusions and recommendations are provided.

Objective 1: Comparisons of accumulation among species and along putative gradients

The first three experiments describe the results of pilot studies for a large scale monitoring program aimed at assessing whether sewage outfalls off Sydney were significant sources of trace contaminants such as trace metals and organochlorine compounds (for further details see Chapter 5). These pilot studies were done to assist in the choice of an appropriate bivalve for the large scale monitoring by examining capability to accumulate contaminants and detect putative gradients. The fourth experiment examines similar questions in an estuarine environment, whilst the fifth experiment examines whether oysters are capable of accumulating other contaminants such as phenols, volatile organics and PAHs and indicating gradients for these compounds.

These experiments are grouped together because they address a common set of hypotheses:

- That bivalves deployed at a putatively contaminated location will accumulate more contaminant than those at control locations.
- That the concentrations of contaminants accumulated by each species of bivalve will be the same at each location.

In all experiments, oysters were obtained from commercial leases in the lower Georges River and cockles from sea-grass beds off Silver Beach, Botany Bay (Fig. 3.1). These animals were brought to the EPA laboratory and kept in polyethylene mesh bags in a recirculating salt water aquarium system for three to five days until they were deployed in the field. Mussels were obtained live from growers in Jervis Bay (southern NSW, Fig.3.1).

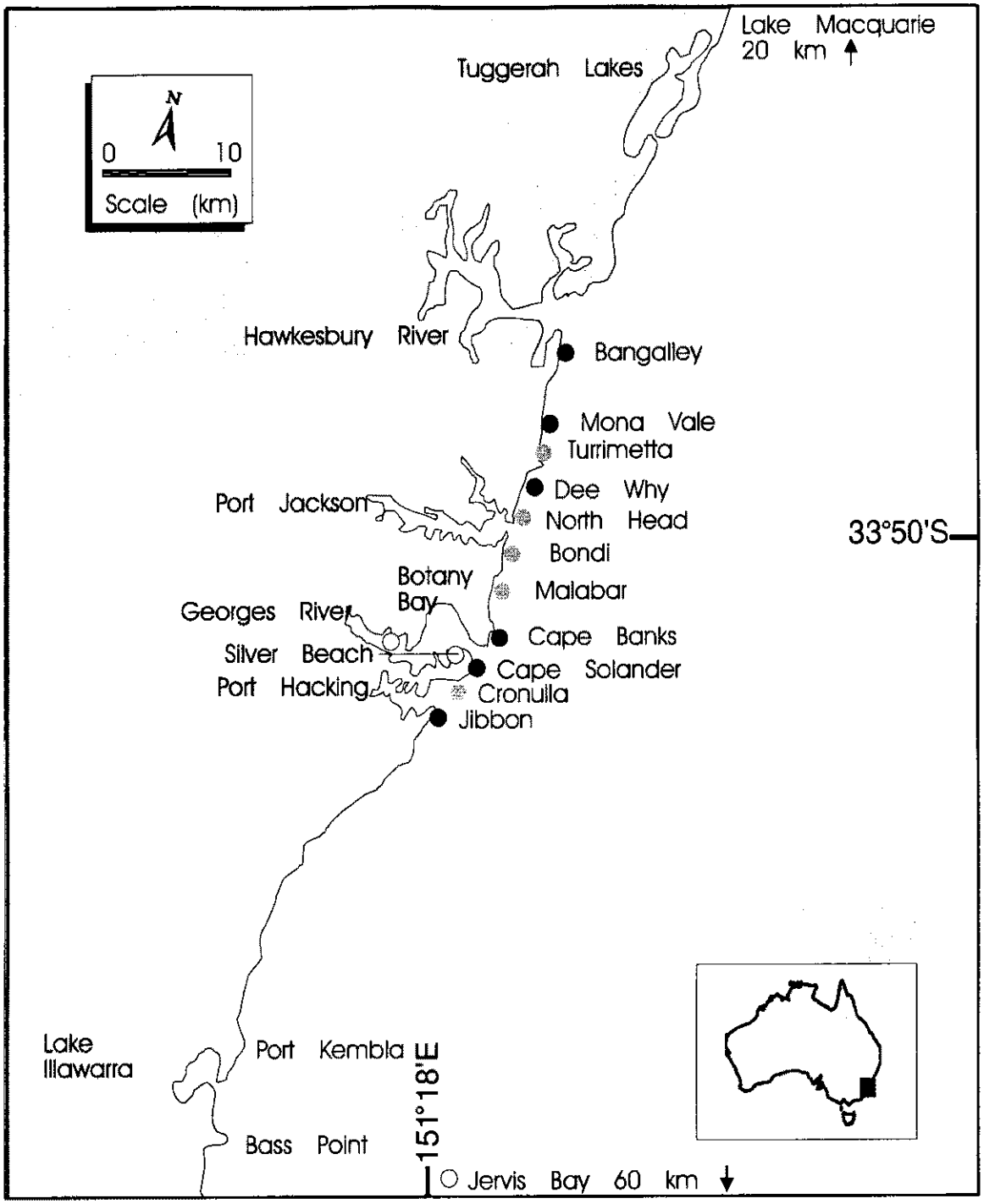


Figure 3.1 Locations where oysters and cockles were collected and deployed in Experiments 1, 2, 3, 4 and 6

- ○ **collection sites**
- ◍ **outfall sites**
- ● **control sites**

Experiment 1: Comparison between oysters and cockles at, 2 sites. (January 1990 - March 1990)

Introduction

A pilot study was set up at two sites to establish the ability of oysters and cockles to bioaccumulate trace metals and organochlorines and their survival during experimental deployments. It was hypothesised that bivalves deployed near the Malabar sewage treatment plant outfall would accumulate greater concentrations of trace metals and organochlorines than those deployed at the Cape Banks control location. Previous studies (Lincoln-Smith and Mann 1989 a,b) have indicated that Malabar outfall was a significant source of trace metals and organochlorines.

Methods

Oysters and cockles (between 20 to 50 animals) were deployed on commercial oyster growers sticks (oysters only) or in polyethylene mesh bags (oysters and cockles), inside stainless steel wire mesh cages. The cages were bolted to rock in approximately 10 m of water by divers. The locations used were a control location at Cape Banks (CB) and an outfall location approximately 500 m south of the Malabar shoreline sewage outfall (M) (Figure 3.1). These were set up on 23 December 1989 and 9 January 1990 respectively. Animals were retrieved by divers from Cape Banks on 2 March 1990 and from Malabar on 5 March 1990 and frozen prior to dissection. Oysters from sticks and bags were pooled for analyses of contaminants. Five animals of each species were retained prior to deployment for determination of background contamination.

Results

Survival

The cockles deployed at Cape Banks were not located and were presumed lost due to a weakness in the bags in which they were deployed.

Rates of survival for oysters and cockles deployed in wire mesh cages at Cape Banks and Malabar were determined for each bag of animals. Of the animals recovered from the Malabar location, the proportional survival of cockles (0.62, $\pm$ 0.1 se) was smaller than for oysters (0.70 $\pm$ 0.03 se). The proportional survival of oysters was smaller at Malabar (0.70 $\pm$ 0.03 se) than at Cape Banks (0.84 $\pm$ 0.05 se).

Contaminants

Oysters

Organochlorines were detected in most of the oysters. Oysters accumulated chlordane, dieldrin, aldrin and DDE. Some oysters accumulated traces of heptachlor and hexachlorobenzene.

Organochlorines were detected in two oysters retained for analysis of background (i.e. prior) contamination. A one factor analysis of variance was used to compare contaminant levels in the tissues of oysters retained for analysis of background contamination with contaminant levels in the tissues of the oysters from Cape Banks and Malabar. In all cases where there were significant differences in the analyses (Table 3.1) SNK tests showed that background concentrations were less than or not significantly different from concentrations at the control location (Cape Banks). In some cases, the SNK tests could not separate the Cape Banks and Malabar locations.

Data from the deployments were then analysed by two factor analyses of variance which compared concentrations in oysters among locations and among cages within locations (Table 3.2). These analyses indicated that significantly greater concentrations of chlordane, cobalt, nickel, arsenic and selenium were accumulated at Malabar than at Cape Banks and that there were significant within-location variations for mercury at Cape Banks and for lead at Malabar.

Table 3.1 Comparison of concentrations of organochlorines and trace metals in the tissues of oysters before deployment (B) with those after deployment at Cape Banks (C - uncontaminated location) and Malabar (M - contaminated location), Experiment 1. Table shows a summary of mean squares, F ratios and SNK tests from one factor analysis of variance. n = 5 oysters (per cage). * $P < 0.05$. Location df = 6 , Residual df = 28 . LNX = Data transformed to natural logs; * NIL = Data heteroscedastic, not transformed

for example, for chlordane

Source of Variation	Degrees of Freedom	Mean Square	F ratio	F divisor	*P*
Location	6	0.01	3.06	residual	< 0.05
Residual	28	0.0033			

The probability that differences among means for chlordane at each location occurred by chance is less than 5 % so it is concluded that there are significant differences among locations. The SNK test is used to determine which means are different.

The rest of the analyses are summarised below (MS - mean squares).

	MS Location	MS Residual	F	Transformation	Results of SNK test
Chlordane	0.01	0.003	3.06 *		Not significant
Aldrin	0.0003	0.00006	4.81 *	* NIL	B=C=C=C=M<M<M
Dieldrin	0.0003	0.0002	1.50		
DDE	0.73	0.70	1.04		
Chromium	0.04	0.01	4.19 *		B<C<C=C=M=M=M
Cobalt	0.006	0.0003	17.77 *	LNX	B=C=C=C<M=M=M
Nickel	0.05	0.003	17.79 *	* NIL	B<C=C=C<M=M=M
Copper	12333	11884	1.04	* NIL	
Zinc	79776	30494	2.62*	* NIL	Not significant
Arsenic	0.59	0.17	3.45 *		B<C=C=C<M=M=M
Selenium	0.29	0.10	2.81 *		Not significant
Silver	0.01	0.009	1.28	LNX	
Cadmium	0.06	0.02	3.31 *		B=M<C=M=M<C=C
Mercury	0.00009	0.00003	2.97 *		B<C=M=M=C=C<M
Lead	0.008	0.002	4.09 *	* NIL	C=B=C=M=C=M<M

Table 3.2 Comparisons of tissue concentrations of contaminants in oysters deployed at Cape Banks and near Malabar sewage outfall and at sites within these locations, Experiment 1. Table shows a summary of mean squares from two factor analysis of variance.

for example, for chlordane

Source of Variation	Degrees of Freedom	Mean Square	F ratio	F divisor	*P*
Location	1	0.054	20.9	Sites(L)	< 0.05
Sites(Location)	4	0.003	0.65	residual	> 0.05
Residual	24	0.004			

The probability that differences among means for chlordane at each location occurred by chance is less than 5 %, so it is concluded that there were significant differences among locations. The probability for differences among sites was greater than 0.05, so it was concluded that there were no differences. If this were < 0.05 (e.g. for mercury), then sites within each location would be considered to be significantly different.

The rest of the analyses are summarised below.

Transform		Chlordane		Aldrin (ln)		Dieldrin		DDE		Cr		Co (ln)		Ni (ln)		Cu (ln)	
	df	MS	F	MS	F	MS	F	MS	F	MS	F	MS	F	MS	F	MS	F
Locn	1	0.054	20.9 *	0.0007	3.0	0.0003	1.45	0.000003	0.06	0.06	7.14	2.21	219 *	1.0	615 *	0.04	0.21
Sites(L)	4	0.003	0.65	0.0002	3.4*	0.0002	0.95	0.00005	0.31	0.009	0.71	0.01	0.33	0.002	0.05	0.18	1.74
Resid	24	0.004		0.00007		0.0002		0.0002		0.01		0.03		0.03		0.10	

Transform		Zn (ln)		As		Se		Ag (ln)		Cd		Hg		Pb (ln)	
	df	MS	F	MS	F	MS	F	MS	F	MS	F	MS	F	MS	F
Locn	1	0.0015	0.01	1.0	8.0 *	1.26	12.1	0.05	0.67	0.08	1.8	0.0000005	0.01	0.73	1.97
Sites(L)	4	0.18	2.59	0.12	0.69	0.10	1.0	0.08	1.75	0.04	2.0	0.00009	3.2 *	0.37	2.56
Resid	24	0.07		0.18		0.10		0.045		0.02		0.00003		0.15	

Cockles

The loss of cockles at Cape Banks and the recovery of a small number of live cockles from Malabar precluded the level of statistical analysis presented above for oysters. The data for cockles and oysters at Malabar were compared to background values in a two factor analysis of variance with species and location (representing different exposures to contaminants) both considered fixed factors. Five animals of each species were selected at random from the total pool of animals available from Malabar and compared to the five background animals of each species. The results of this analysis are summarised in Table 3.3.

Organochlorines were not detected in any cockles so no analyses beyond those shown above were possible.

The full range of metals was detected in both species. Significant differences between species were found for all metals except Co, Ag and Cd (Fig. 3.2). Significant increases over background concentrations were found for Cr, Co, Ni, Cu, Zn and As, and significant difference between species for Cr, Ni, Cu, Zn, As, Se, Hg, Pb. In the case of Ni, Cu and Zn, oysters showed an increase over background and cockles did not (TxS significant, Table 3.3).

Table 3.3 Comparisons of trace metal concentrations in oysters and cockles deployed near Malabar sewage outfall with background (or starting) concentrations, Experiment 1. Table show a summary of mean squares from analyses of variance. * indicates $P < 0.05$.

for example, for copper

Source of Variation	Degrees of Freedom	Mean Square	F ratio	F divisor	*P*
Treatment	1	2420	10	residual	< 0.05
Species	1	27261	113	residual	< 0.05
Treat x Species	1	2205	9	residual	< 0.05
Residual	16	241			

The probability that differences among means for copper in each treatment and for each species occurred by chance are less than 5 %, so it is concluded that there are significant differences in each case. In this example, however, the relationship between species is different in each treatment (Treat x Species, P < 0.05). This means that it is not possible to generalise about relationships between species and treatments.

		Cr		Co		Ni		Cu		Zn		As	
	df	MS	F	MS	F	MS	F	MS	F	MS	F	MS	F
Treat	1	0.15	11.9	0.02	40 *	0.12	104 *	2420	10 *	94270	8.9 *	7.1	11.2 *
Species	1	0.15	12.2	0.001	2.78	0.08	74 *	27261	113 *	1971857	187 *	7.8	12.4 *
T x S	1	0.018	1.48	0.002	3.8	0.06	53 *	2205	9 *	88564	8.4 *	0.6	0.97
Resid	16	0.012		0.0005		0.001		241		10553		0.6	

		Se		Ag		Cd		Hg		Pb	
	df	MS	F	MS	F	MS	F	MS	F	MS	F
Treat	1	0.26	4.44	0.02	4.21	0.22	3.11	0.003	3.41	0.009	3.14
Species	1	3.36	58 *	0.009	1.7	0.01	0.14	0.06	77 *	0.11	37 *
T x S	1	0.01	0.25	0.001	0.19	0.00002	0.00	0.0008	1.14	0.0000008	0.00
Resid	16	0.05		0.005		0.07		0.0007		0.003	

Results of SNK tests to determine which species had greatest concentrations

Cr	cockle > oyster	As	cockle > oyster
Ni	oyster > cockle	Se	oyster > cockle
Cu	oyster > cockle	Hg	cockle > oyster
Zn	oyster > cockle	Pb	cockle > oyster

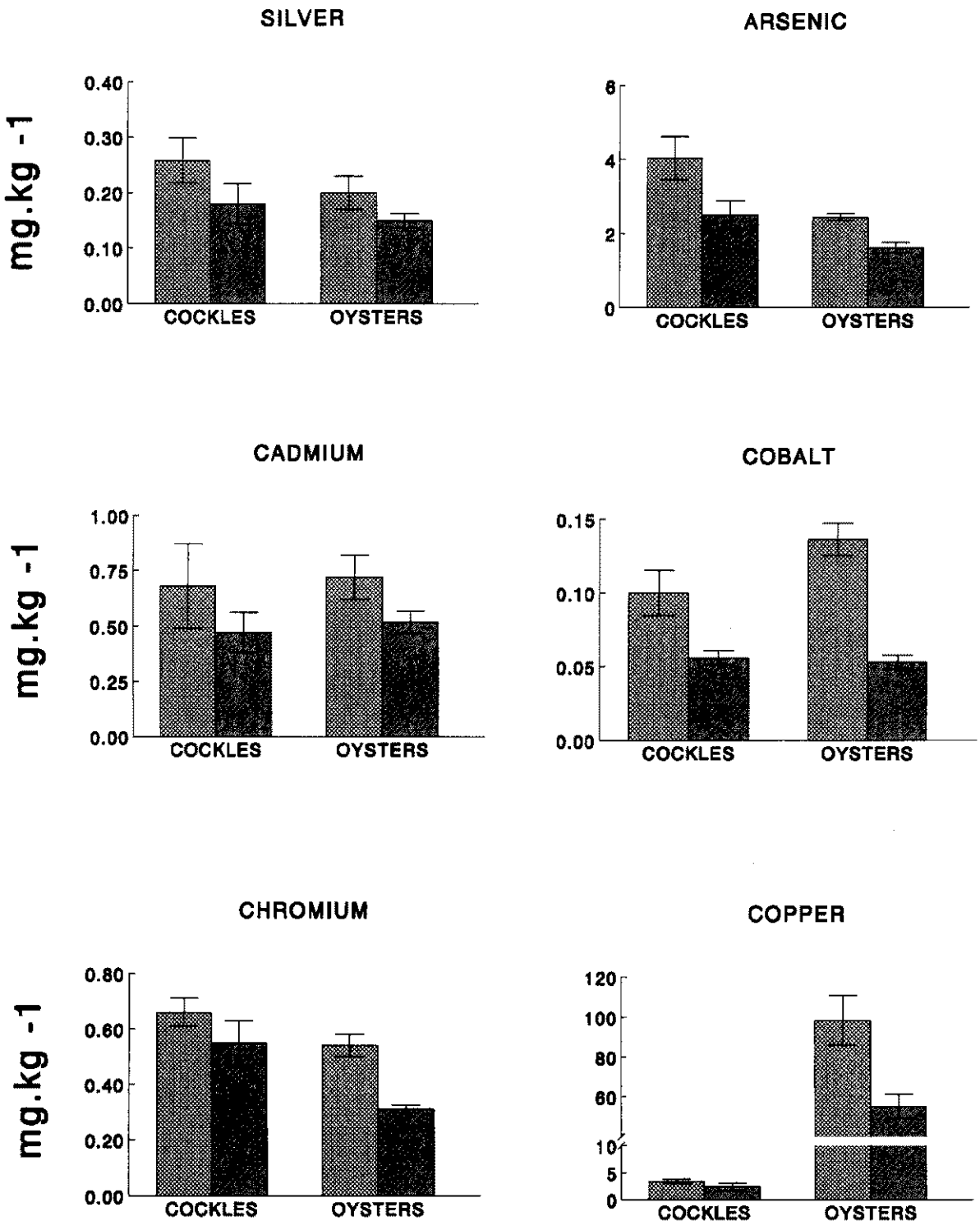


Figure 3.2 Concentrations of trace metals in cockles and oysters after (cross hatching) and prior to (diagonal hatching) deployment near the Malabar sewage outfall.

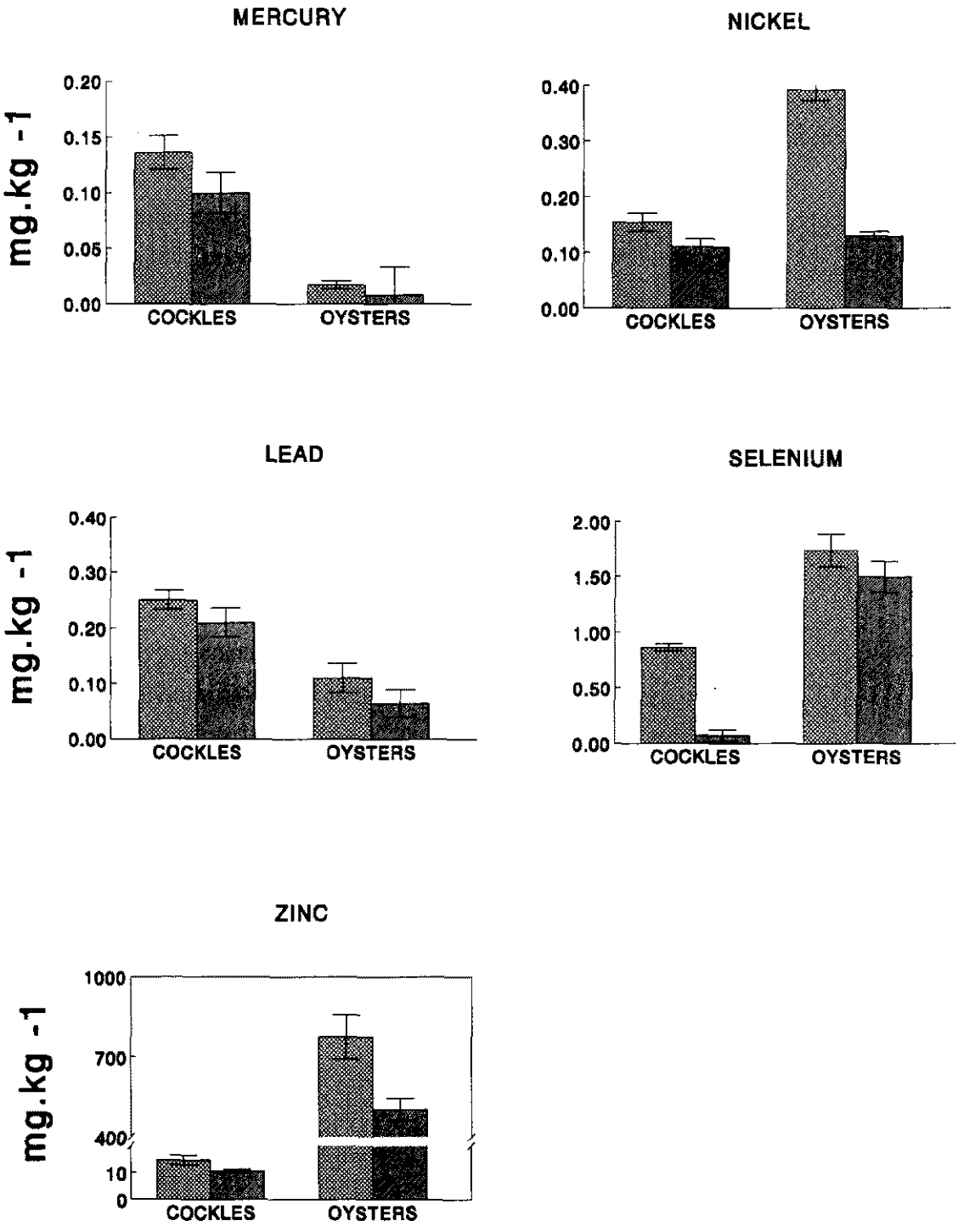


Figure 3.2 Concentrations of trace metals in cockles and oysters after (cross hatching) and prior to (diagonal hatching) deployment near the Malabar sewage outfall

Experiment 2: Comparison between oysters and cockles at 10 sites. (March - June 1990)

Introduction

Experiment 1 was limited in its spatial scope, so the comparison between oysters and cockles was repeated over a greater number of locations. In the first deployment of the main sewage outfall study (see Chapter 5), March to June 1990, cockles were included to test the consistency of the results of Experiment 1 with respect to mortality and the absence of detectable concentrations of organochlorines in cockles. The study tested the hypotheses that there will be no differences in the concentrations of trace metals and organochlorines in oysters and cockles, nor between bivalves deployed in the vicinity of sewage outfalls and at control locations.

Method

In order to reduce the time spent by divers deploying and retrieving test animals the means of deployment was modified. Two mesh bags (approximately 400 x 200 mm with 30 mm mesh), each containing either cockles or oysters, were attached by cable ties to a steel handle embedded in a concrete block. There were approximately 15 animals in each bag to allow for mortality. The blocks were then lowered to the sea bottom in rocky areas where the water depth was between 10 and 12 m.

Three locations near sewage outfalls (North Head, Bondi and Malabar) and three unimpacted reference locations (Bangalley, Dee Why and Jibbon) were used. Figure 5.1 (Chapter 5) shows the positions of these locations. At each location there were two sites, north and south, approximately 300 to 400 m apart. At outfall locations, sites were 150 to 200 m north and south of the outfalls. Three blocks were deployed at each site.

After 3 months the animals were retrieved, processed and submitted for analysis. No background samples were kept since the previous study indicated that there was no significant contamination of animals prior to deployment.

Results

Survival

Data on survival were compared using a three factor analysis of variance. In the model, species and treatment (presence of sewage) were orthogonal and sites were nested in treatment. Data were proportional mortality per bag at each site.
The loss of one set of samples from Bondi north precluded any analysis of the influence of direction from the outfall on survival. To balance the design, one unimpacted site where some bags were also lost (Bangalley north) was not considered in the analysis. There were no significant differences between species or treatments (Table 3.4), indicating that the presence of sewage effluent had no effect on the survival of either species, and that survival was not significantly different between species. The overall mean survival rate was 0.64, ±0.1 S.E. for oysters and 0.61, ±0.1 S.E. for cockles. There was a significant difference between sites within treatments due to very low survival at the Malabar south site.

Contaminants

As found in Experiment 1, cockles did not accumulate organochlorines. Oysters contained chlordane, dieldrin, DDT, DDE and DDD. There were no significant differences between outfall and control locations for any of the organochlorines (Table 3.4, Fig. 3.3).

Trace metal data were analysed by a 3 factor mixed model analysis of variance (Table 3.4) with species and treatments (outfall vs control) as orthogonal factors and sites nested within treatments. Due to losses, it was not possible to include all locations and sites, so 4 sites were randomly selected (from those that had enough samples) within each treatment. Data from three bivalves (of each species) on each of two blocks at each site were pooled.

There were significant differences between species for Cr, Co, Ni, Cu, Zn, As and Hg, but there was no consistent trend for one species to accumulate greater concentrations (Table 3.4; Fig 3.4). There were significant differences between treatments for Cr, Co, As and Pb. Lead was the only metal to have a significant interaction between species and treatment, with no difference between places for cockles, but a significant difference for oysters.

Table 3.4 Comparisons of survival and concentrations of accumulated contaminants in oysters and cockles deployed in the vicinity of sewage outfalls and control locations, Experiment 2. Table shows a summary of mean squares for analyses of variance. (ln) - data transformed to natural logarithms to stabilise variances.

	Survival				Chlordane		Dieldrin		ΣDDT	
	df	MS	F	df	MS	F	MS	F	MS	F
Treat	1	0.014	0.05	Locn	5	0.0025	1.92	0.000003	1	0.00026	1
Species	1	0.014	0.05	Sites (L)	6	0.0013	1.66	0.000003	1	0.00026	1.6
T x S	1	0.02	0.07	Resid	24	0.0008		0.000003		0.00016	
Sites (TS)	16	0.27	6.8 *								
Resid	40	0.04									

	Cr		Co		Ni		Cu (ln)		Zn (ln)		As	
	df	MS	F	MS	F	MS	F	MS	F	MS	F	MS	F
Treat	1	1.52	36 *	0.01	15.9 *	0.22	11.4 *	409	326 *	420	1706 *	7.55	30.74 *
Species	1	0.22	5.45 *	0.01	15.2 *	0.004	0.20	3.61	2.88	0.43	1.78	1.31	5.33
T x S	1	0.02	0.49	0.002	3.92	0.01	0.58	0.06	0.05	0.06	0.25	0.13	0.54
Sites (TS)	12	0.04	1.58	0.0006	2.00 *	0.02	3.31 *	1.25	1.66	0.24	1.52	0.25	2.09
Resid	80	0.026		0.0004		0.006		0.75		0.16		0.11	

	Se		Ag		Cd		Hg		Pb	
	df	MS	F	MS	F	MS	F	MS	F	MS	F
Treat	1	0.035	0.09	0.0003	0.00	0.40	1.25	0.05	15.5 *	0.01	0.78
Species	1	0.12	0.31	0.0003	0.03	0.09	0.29	0.005	1.55	0.08	6.03 *
T x S	1	0.12	0.30	0.07	0.68	0.06	0.19	0.000003	0.01	0.06	4.65
Sites (TS)	12	0.41	3.41 *	0.10	1.40	0.32	2.70 *	0.003	1.28	0.01	2.09 *
Resid	80	0.12		0.07		0.12		0.002		0.007	

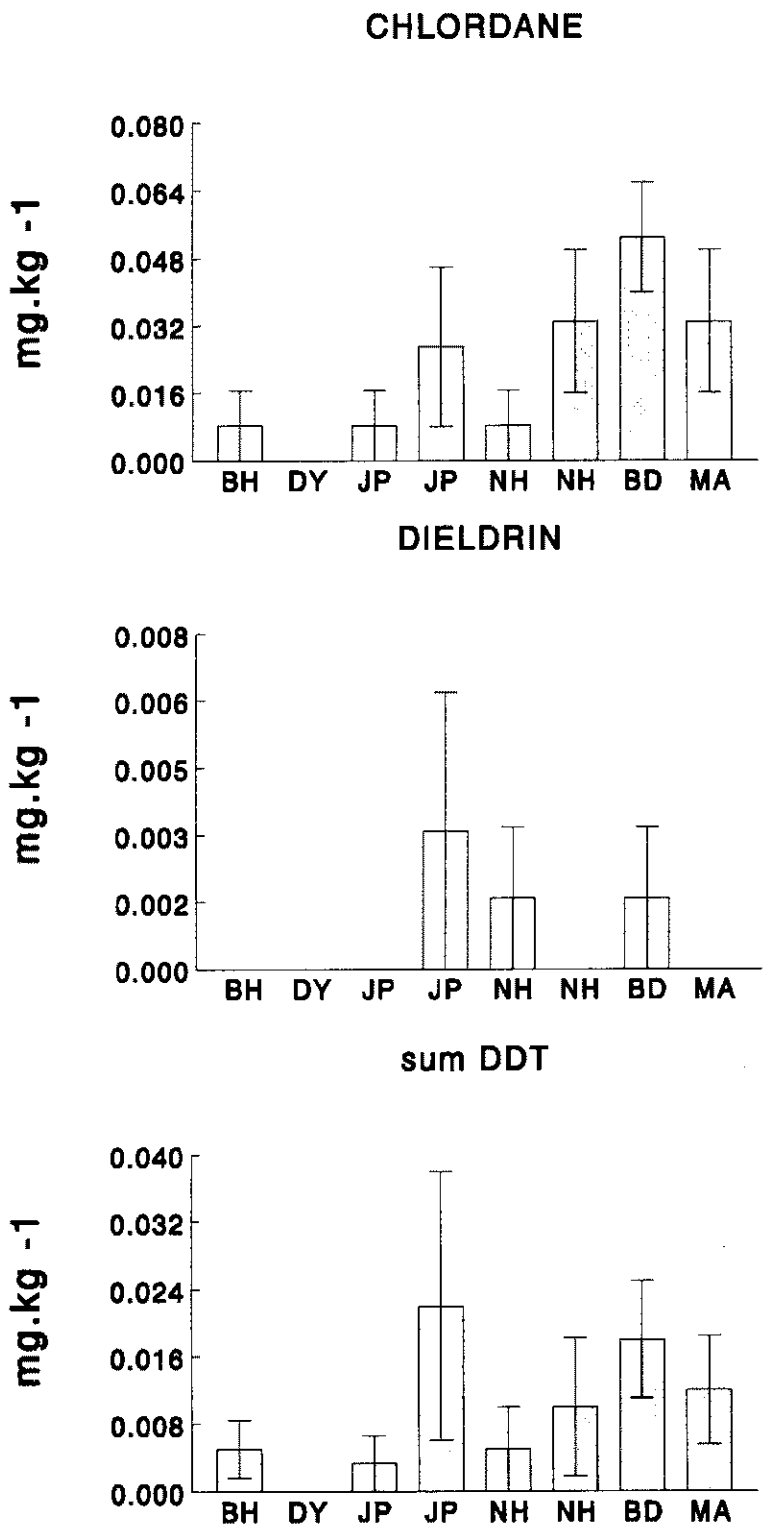


Figure 3.3 Mean concentrations of organochlorines in oysters, Experiment 2. BH - Bangalley Head, DY - DeeWhy, JP - Jibbon Point (all controls); NH - North Head, BD - Bondi, MA - Malabar (outfalls).

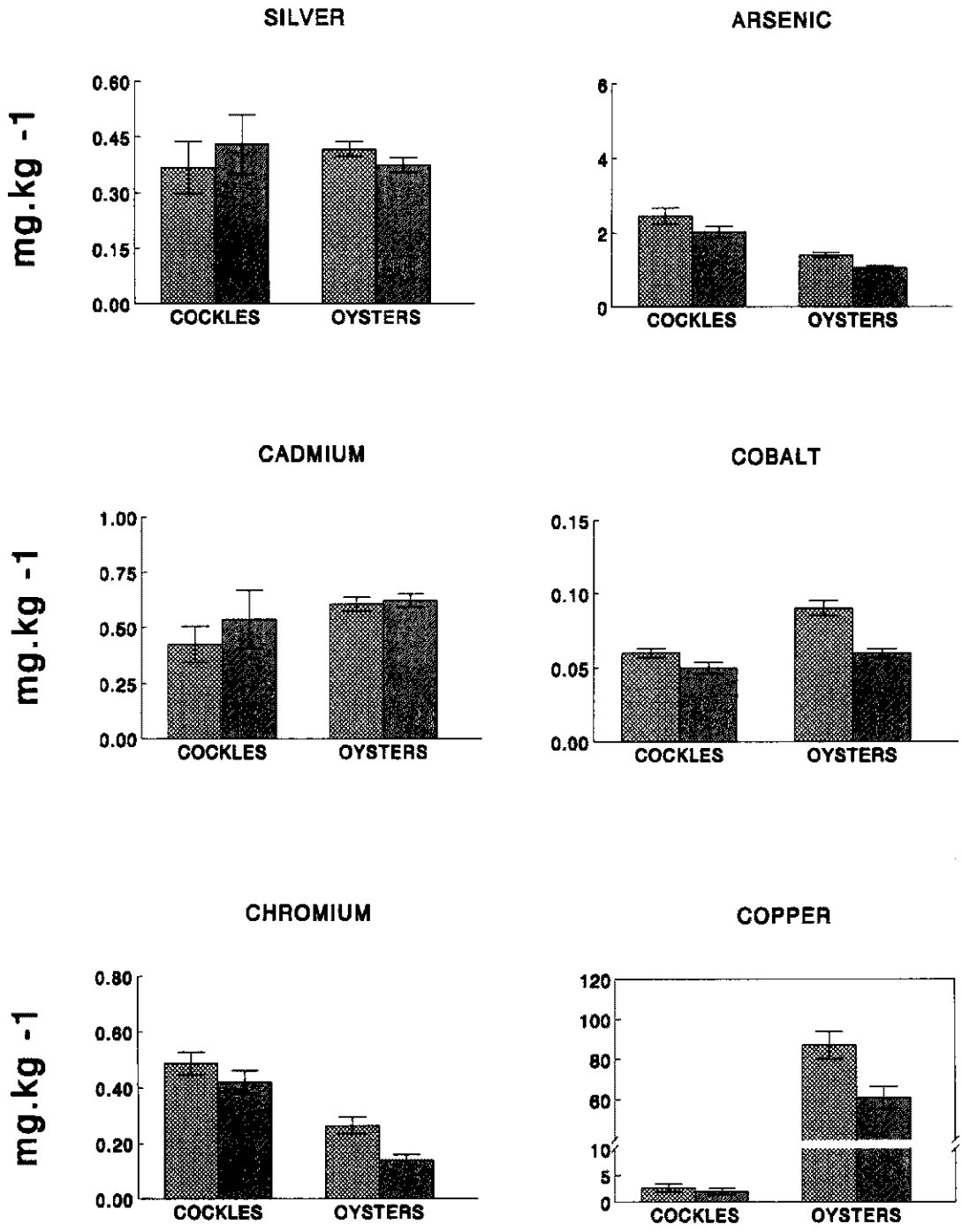


Figure 3.4 Mean concentrations of trace metals in oysters and cockles deployed near outfalls (cross hatching) and at control locations (diagonal hatching)

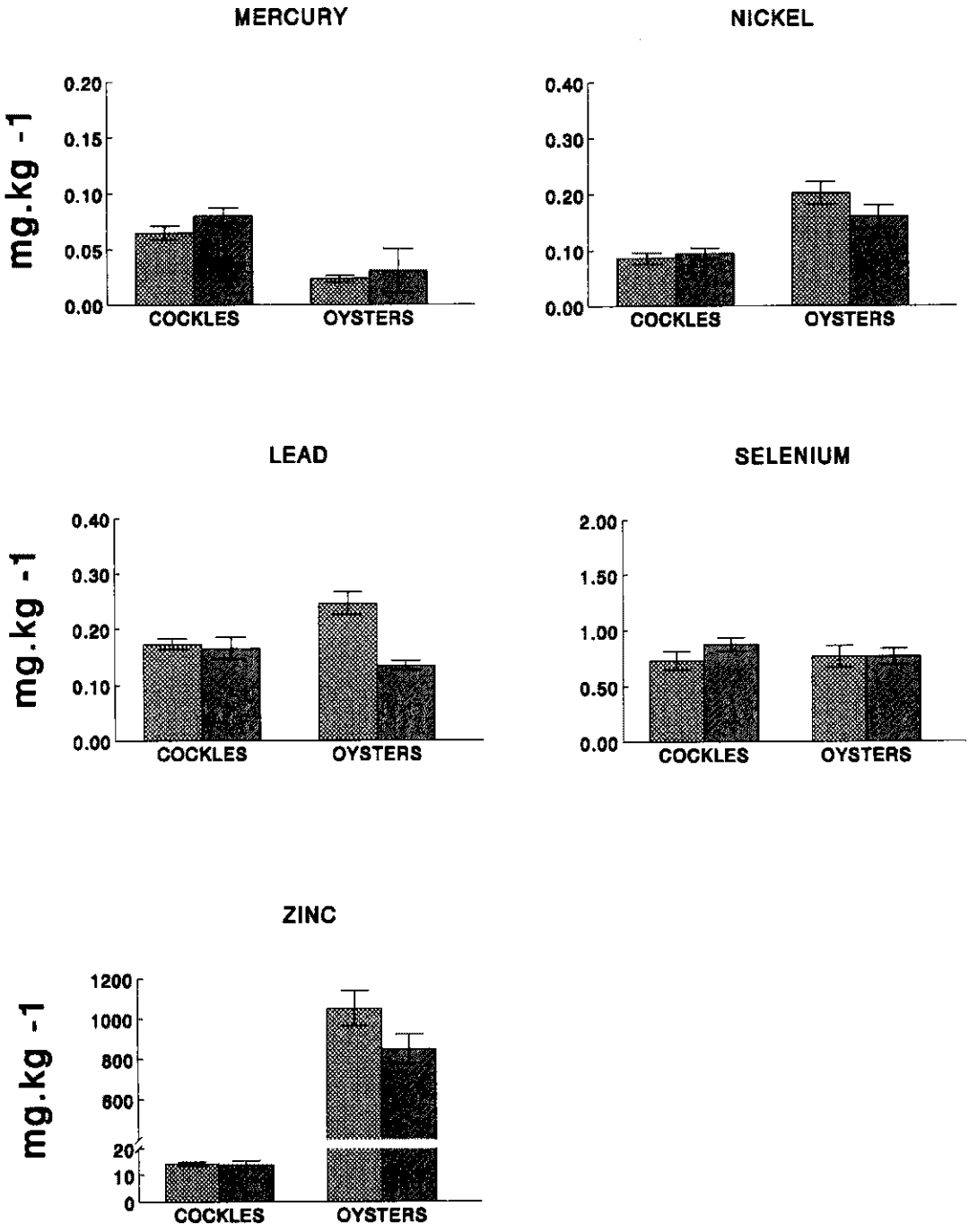


Figure 3.4 (cont)

Experiment 3: Comparisons among oysters, cockles and mussels. (May - September 1990)

Introduction

The previous two experiments concentrated on comparisons between two local bivalves, cockles and oysters. This experiment provided comparisons of the levels of trace metals and organochlorine compounds accumulated by mussels, cockles and oysters deployed near the mouth of Botany Bay.

Method

Mesh bags containing replicate animals of mussel (*Mytilus edulis*), Sydney rock oyster (*Saccostrea commercialis*) and cockles (*Anadara trapezium*) were suspended from a single mooring in 18 m water depth 300 m south of Cape Banks, Botany Bay. The animals were collected after approximately 3 months and analysed for trace metals and organochlorines. Six mussels, six cockles and four oysters were analysed.

Results

Organochlorines

Cockles did not accumulate detectable concentrations of organochlorines. One out of six mussels had 0.01 mg/kg HCB. Three out of four oysters had organochlorines. Two oysters had HCB levels of 0.02, the third had no HCB, but did have chlordane, DDE, DDD and DDT present.

Trace Metals

There were significant differences in levels of contamination of the three species for Cr, Ni, Cu, Zn, Ag, Hg and Pb. These trends are summarised in Table 3.5, and Figure 3.5. There were no consistent trends in the ranking of the three species.

Table 3.5 Comparisons of concentrations of trace metals accumulated mussels, cockles and oysters. Tables show a summary of one-factor analyses of variance.

		Cr		Co		Ni		Cu (ln)		Zn (ln)		As	
	df	MS	F	MS	F	MS	F	MS	F	MS	F	MS	F
Species	2	0.24	4.98 *	0.0003	0.4	0.12	14.7 *	23.25	273 *	21.55	109 *	0.07	0.10
Resid	9	0.05		0.0007		0.008		0.08		0.19		0.72	

		Se		Ag (ln)		Cd		Hg (ln)		Pb	
	df	MS	F	MS	F	MS	F	MS	F	MS	F
Species	2	0.16	2.71	2.52	8.02 *	0.18	1.93	2.56	25 *	0.13	5.15 *
Resid	9	0.06		0.31		0.09		0.10		0.03	

Results of SNK tests for comparisons to determine which species accumulated more of a contaminant whilst in the same location

Cr	test inconclusive
Ni	mussels = oysters < cockles
Cu	cockles = mussels < oysters
Zn	cockles < mussels < oysters
Ag	mussels < cockles = oysters
Hg	mussels = cockles < oysters
Pb	cockles = oysters < mussels

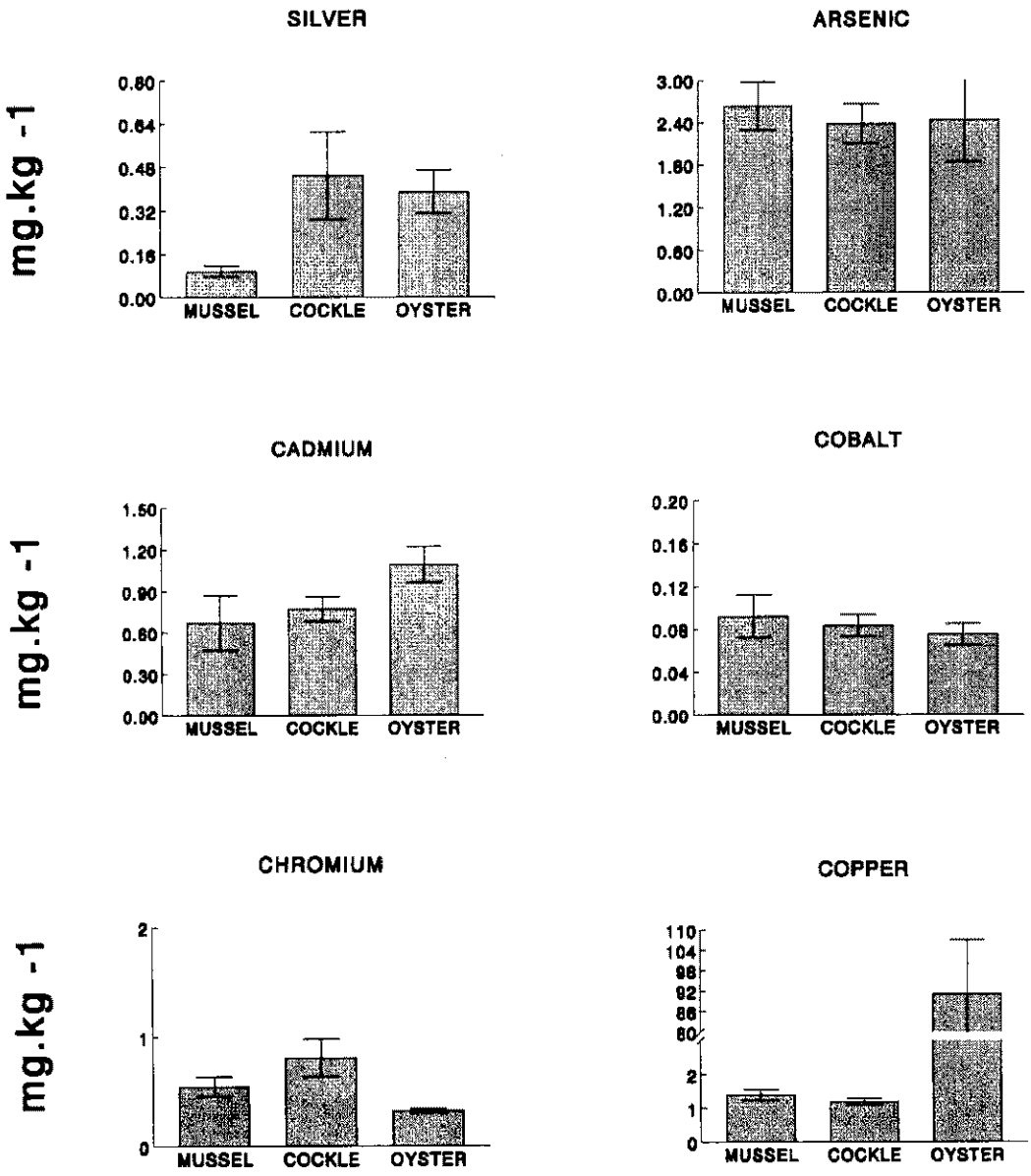


Figure 3.5 Mean concentrations of trace metals in mussels, oysters and mussels, Experiment 3

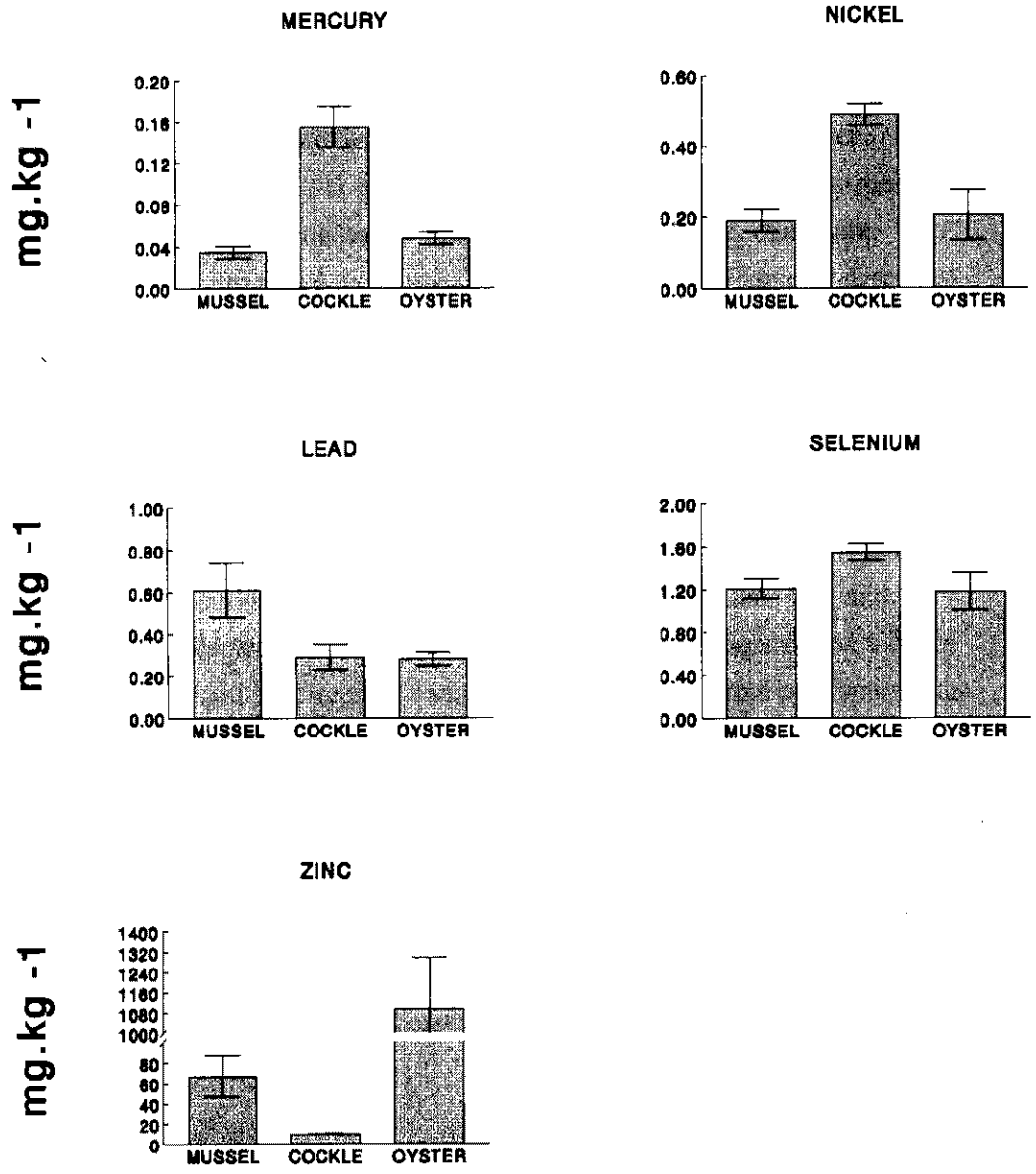


Figure 3.5 cont.

Experiment 4: Comparisons of oysters and cockles along a gradient of pollution in Lake Macquarie

Introduction

Lake Macquarie has a recognised gradient of trace metal contamination of sediments and waters from the north to the south (Batley 1989). This gradient has been used in other studies in this thesis (see Chapters 4 and 6). This experiment tests the hypotheses that oysters and cockles accumulate the same amounts of copper, cadmium, lead and zinc; and that bivalves placed in sediments and those suspended on mid-water moorings accumulate the same concentrations of contaminants. The latter two hypotheses are tested by comparison to experimental treatments described in Chapter 6, Study 2. That Study was done simultaneously with those described here.

Methods

Mesh bags containing oysters and cockles were suspended in the water column 1-1.5 m from the substratum on two replicate aluminium spar buoys for 3 months at Cockle Creek (receives contaminated effluent) and two control bays, Kilaben Bay and Crangan Bay (see Chapter 6 for details of locations). Concentrations of Cu, Cd, Pb and Zn were determined for 5 replicate animals of each species from each spar by the methods detailed in Chapter 6. Data were analysed by a three factor analysis of variance, with species and locations as fixed factors and spars nested in locations.

In addition, comparisons between the mean concentrations of metals in cockles and oysters suspended on spars and in experimental tubs of mud (see Chapter 6) in the same area, at the same time, were made by t-test. These comparisons were unbalanced (10 replicates and 45 replicates respectively for cockles, 10 and 18 for oysters) so a suitable t-test was used.

Results

Significantly more zinc and lead was accumulated at Cockle Creek by both species, with oysters accumulating more zinc than cockles and cockles more lead (Fig. 3.6). There was no interaction between species and location in either case and no significant variability among spars (Table 3.6). Oysters accumulated significantly more cadmium at Cockle Creek, but cockles did not, leading to a significant interaction between species and locations (Table 3.6). There was also significant variability between spars. The trends for copper were unclear with cockles showing

no differences and oysters showing a pattern contrary to that indicated by gradients in concentrations in the water and sediments (Batley 1989).

There were no significant differences (*t*-test, $P > 0.05$) between concentrations of trace metals in cockles or oysters embedded in the mud and suspended in the water column (Table 3.7). There was, however, a very strong correlation between concentrations of copper in oysters on spars and oysters in the sediments ($r^2 = 0.94$). This suggests that the differences among locations in concentrations of copper accumulated by oysters are not due to random variation among locations, but some unknown factor influencing copper concentrations.

Table 3.6 Comparison of concentrations of zinc, cadmium, lead and copper in oysters deployed at contaminated and uncontaminated locations in Lake Macquarie, Experiment 4. Tables show a summary of analyses of variance. * indicates $P < 0.05$

Zinc

Source	df	Mean Squares	F		df	Mean Squares	F	
Locn	2	1.06	7.66		2	1.06	9.9	*
Spar(L)	3	0.14	1.38					
Species	1	147.3	770	*	1	147.3	1364	*
L x S	2	0.21	1.1		2	0.21	1.96	
S x Sp(L)	3	0.19	1.9					
Residual	48	0.10			54	0.11		

Cadmium

Source	df	Mean Squares	F	
Locn	2	9.04	1.89	
Spar(L)	3	4.8 **	7.85	*
Species	1	54.5 *	14.1	*
L x S	2	20.44	5.28	
S x Sp(L)	3	3.9 **	6.33	*
Residual	48	0.61		

Lead

Source	df	Mean Squares	F	
Locn	2	145.3	122	*
Spar(L)	3	1.18	1.42	
Species	1	6.87*	13.31	*
L x S	2	3.17	6.14	
S x Sp(L)	3	0.51	0.62	
Residual	48	0.83		

Copper

Source	df	Mean Squares	F	
Locn	2	1997	1.32	
Spar(L)	3	1516 *	3.44	*
Species	1	10627 *	67	*
L x S	2	2124	1.33	
S x Sp(L)	3	1593 *	3.61	*
Residual	48	441		

Table 3.7 Mean concentrations (SE) of metals in cockles and oysters embedded in tubs full of sediment and suspended in the water column on spar buoys. There were no significant differences between the two methods of deployment for any metal at any location (*t*-test, *P*>0.05).

Cockles

Location	Method	Zinc	Cadmium	Lead	Copper
Cockle Ck	Tub	47.0(2.02)	4.7(0.24)	5.7(0.25)	1.4(0.04)
	Spar	48.5(2.8)	4.7(0.34)	6.1(0.52)	1.9(0.09)
Kilaben Bay	Tub	27.6(2.6)	3.9(0.11)	0.85(0.04)	1.1(0.04)
	Spar	28.3(2.7)	4.8(0.47)	0.83(0.07)	1.5(0.09)
Crangan Bay	Tub	18.83(0.52)	4.4(0.11)	0.37(0.05)	0.75(0.06)
	Spar	27.32(1.9)	5.8(0.4)	0.62(0.14)	1.3(0.17)

Oysters

Location	Method	Zinc	Cadmium	Lead	Copper
Cockle Ck	Tub	988(78)	3.02(0.14)	4.23(0.33)	46.4(4.42)
	Spar	891(69)	5.04(0.25)	4.49(0.44)	64(4.6)
Kilaben Bay	Tub	722(30)	1.67(0.10)	0.46(0.05)	55.4(4)
	Spar	772(81)	2.38(0.13)	0.52(0.06)	89(6.2)
Crangan Bay	Tub	725(45)	1.71(0.14)	0.28(0.02)	71(6)
	Spar	721(97)	2.21(0.2)	0.43(0.1)	107(17)

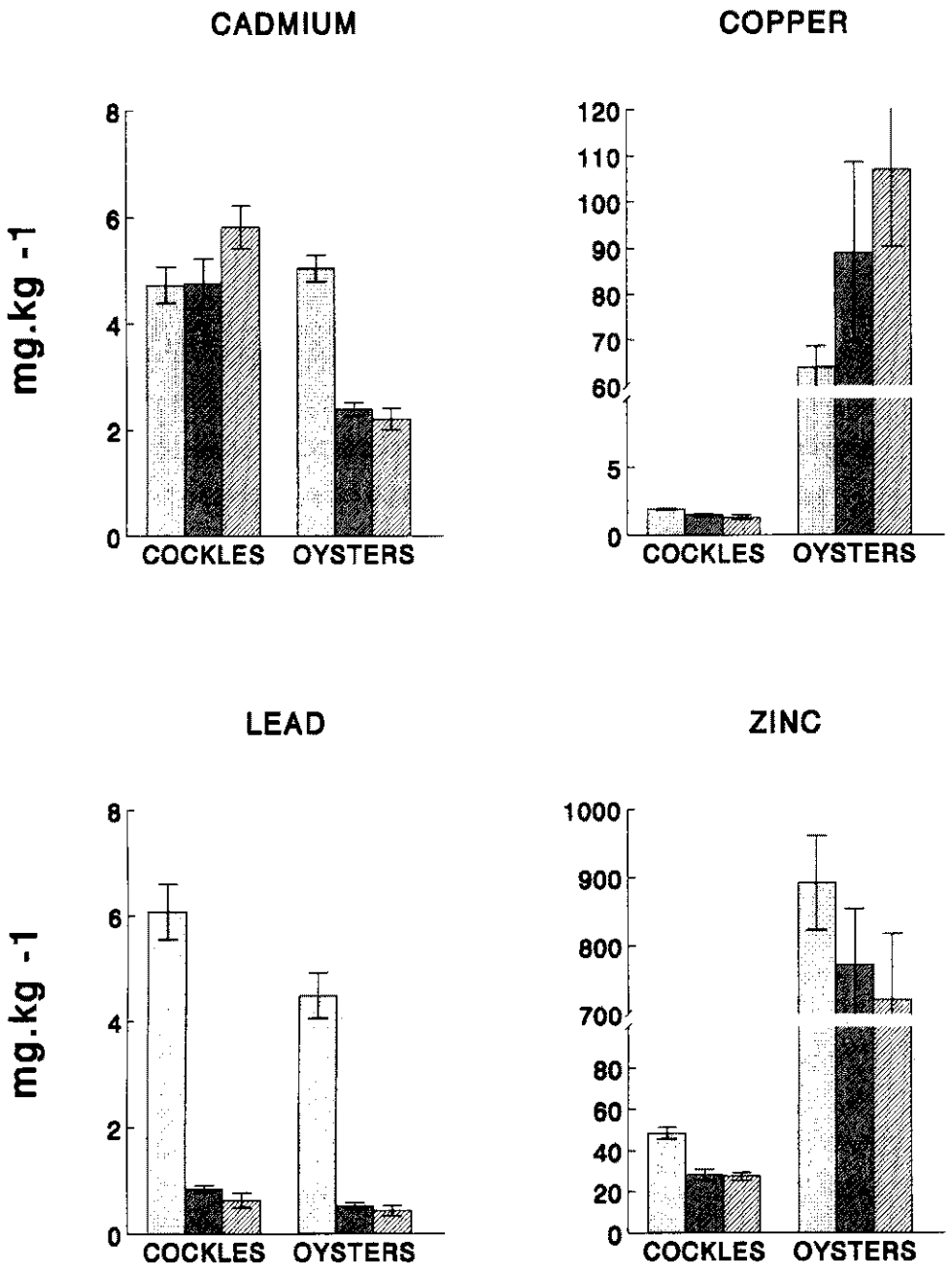


Figure 3.6 Concentrations of trace metals in cockles and oysters at a contaminated location (Cockle Ck - stippled), and two control locations (Kilaben Bay - close diagonal hatching; Crangan Bay - wide diagonal hatching).

Discussion of Trends - Experiments 1, 2, 3, and 4.

Cockles consistently accumulated more Cr, As and Hg than oysters, and oysters accumulated more Co, Ni, Cu and Zn (Table 3.8). The main anomaly is for Ni in Experiment 3 where cockles had more than oysters. This pattern of different species accumulating different concentrations of trace metals is well documented (e.g. O'Connor 1992, Phillips and Rainbow 1993).

There was very good consistency in ratios for the metals in different experiments (Table 3.8). There was also good agreement between ratios from polluted and unpolluted locations within each experiment (except cadmium). This agreement supports the concept that both species are taking up trace metals in a direct relationship to the concentrations in the environment and that the relationships between internal and external concentrations in both species remain the same despite changes in external concentrations.

The ratios for cadmium showed an interesting pattern. All oceanic deployments had a ratio of less than 1. The estuarine deployment showed a ratio of 2 for uncontaminated locations and less than 1 for the contaminated location. This could suggest that cockles regulate cadmium when under stress (e.g. exposed to large concentrations or in oceanic deployments). This speculation remains untested.

The consistency between species is reinforced by the correlations between concentrations in the two species (Table 3.9, Fig 3.7). These correlations have some limitations because, with the exception of Cu, Zn, Pb and Cd, they are based on 8 points from Experiment 2 and only 1 from Experiment 3. This restricted data set probably does not provide a great enough pollution gradient and the small data set can be influenced strongly by single outliers in the data (e.g. mercury, Fig. 3.7). Copper, zinc and arsenic showed virtually no correlation between oysters and cockles. Oysters are known to accumulate copper and zinc preferentially and the huge discrepancy in concentrations between the two species could affect the correlations. It has been shown that either species can be used to differentiate a known gradient in zinc (Experiment 4).

There are no other published data on ratios of trace metals in these bivalve species. Data from NSW Fisheries (Chvojka unpub.) provide some comparison between *S. commercialis* and *A. trapezium* collected in estuaries. There, the ratio between cockles and oysters for cadmium, zinc and copper was 2.1, 0.03 and 0.03,

respectively. The ratios for copper and zinc are the same as those found in this study. The ratio for cadmium is the same as that found for uncontaminated areas of Lake Macquarie.

O'Connor (1992) compared the oyster *Crassostrea virginica* with the mussel *Mytilus edulis* (Table 3.8) and found similar trends (i.e. which species tended to accumulate greatest concentrations) for all metals except mercury to those found here in Experiment 3.

The summary of the elucidation of a putative pollution gradient (Table 3.10), showed that oysters consistently indicated elevated levels of Cr, Co, and Zn, and also indicated elevated levels of Cu, Cd, Ni and Pb. Cockles consistently indicated elevated levels of As and also Zn, Co, Ni, and Hg at times. This analysis is, of course, dependent on whether the putative gradient actually exists. A gradient is confirmed by independent data for Experiment 4 (e.g. Batley 1989) but can not be confirmed for Experiments 1 and 2. The lack of consistency and even the absence of demonstration of a gradient could therefore be due to either the lack of a gradient, or variability in the presence of the gradient. Other data (EPA 1996) have indicated that contamination of sewage effluent is often very variable, making the second explanation likely.

The ability of oysters to show a gradient of copper seems to be variable in estuarine conditions (Experiment 4), but clearer in the other experiments. This type of experiment, which intends to demonstrate the usefulness of an organism to show a gradient, relies on the assumption that the environmental gradient of the metal is already known. The gradient can be inferred from some other independent data (e.g. sediment data) or proximity to a known source of metals (e.g. industrial or sewage outfall). There is evidence (Batley et al. 1991) that the presence of moored boats can provide a source of copper (and to some extent zinc) that is readily available for accumulation by oysters. There are moored boats in Kilaben and Crangan Bays and this could explain the results for copper in Experiment 4.

Table 3.8 Summary of ratios of mean concentrations of trace metals in comparison to oysters. Experiments 1, 2 and 4 compare cockles to oysters, Experiment 3 compares cockles and mussels to oysters. nd - no data.

	Experiment 1			Experiment 2			Experiment 4			Experiment 3	
	polluted	unpolluted	mean	polluted	unpolluted	mean	unpolluted	unpolluted	polluted	Cape Banks cockles	Cape Banks mussels
Cr	1.7	1.2	1.45	3	1.8	2.3	nd	nd	nd	2.6	1.7
Co	1.1	0.7	0.9	0.8	0.7	0.8	nd	nd	nd	1.1	1.2
Ni	0.8	0.4	0.6	0.6	0.4	0.5	nd	nd	nd	2.4	0.9
Cu	0.04	0.03	0.04	0.03	0.03	0.03	0.03	0.02	0.01	0.01	1.01
Zn	0.02	0.02	0.02	0.03	0.01	0.01	0.05	0.04	0.04	0.01	0.06
As	1.6	1.66	1.63	1.9	1.8	1.8	nd	nd	nd	1	1
Se	0.5	0.5	0.5	1.1	1	1	nd	nd	nd	1.3	1
Ag	1.2	1.3	1.25	1.2	0.9	1	nd	nd	nd	1.1	0.25
Cd	0.9	0.9	0.9	0.9	0.7	0.8	0.94	2.0	2.6	0.7	0.6
Hg	12.9	8	10.5	2.3	2.8	2.3	nd	nd	nd	3.2	0.7
Pb	3.3	2.3	2.8	1.2	0.7	0.9	1.4	1.6	1.5	1	2.1

Ratios of mean concentrations of contaminants in *Mytilus edulis* compared to the American oyster *Crassostrea virginica* (O'Connor 1992)

Organochlorine	Oyster	Mussel
ΣDDT	1	1.1
PCB	1	1.8
PAH	1	1.8
Chlordane	1	1.1

Metal	Oyster	Mussel
Cr	1	5.5
Ni	1	0.4
Cu	1	0.03
Zn	1	0.025
As	1	1.2
Se	1	1.6
Ag	1	0.06
Cd	1	0.5
Hg	1	1.3
Pb	1	3.3

Table 3.9 Correlations between concentrations of trace metals in oysters and cockles deployed in the same locations

Metal	r^2
Cr	0.28
Co	0.20
Ni	0.53
Cu	0.06
Zn	0.01
As	0.03
Se	0.21
Cd	0.67
Hg	0.28
Pb	0.99

Table 3.10 Summary of whether a putative environmental gradient of trace metals was reflected in the body burden of cockles and oysters (Experiments 1, 2 and 4). "O" = outfall, "C" = control, "B" = background "nd" no data.

	EXPERIMENT 1		EXPERIMENT 2		EXPERIMENT 4	
Metal	OYSTER	COCKLE	OYSTER	COCKLE	OYSTER	COCKLE
Cr	O > B		O > C		nd	nd
Co	O > B	O > B	O > C		nd	nd
Ni	O > B	O > B			nd	nd
Cu	O > B					
Zn	O > B				O > C	O > C
As	O > B	O > B	O > C	O > C	nd	nd
Se					nd	nd
Ag					nd	nd
Cd					O > C	
Hg		O > B			nd	nd
Pb			O > C		O > C	O > C

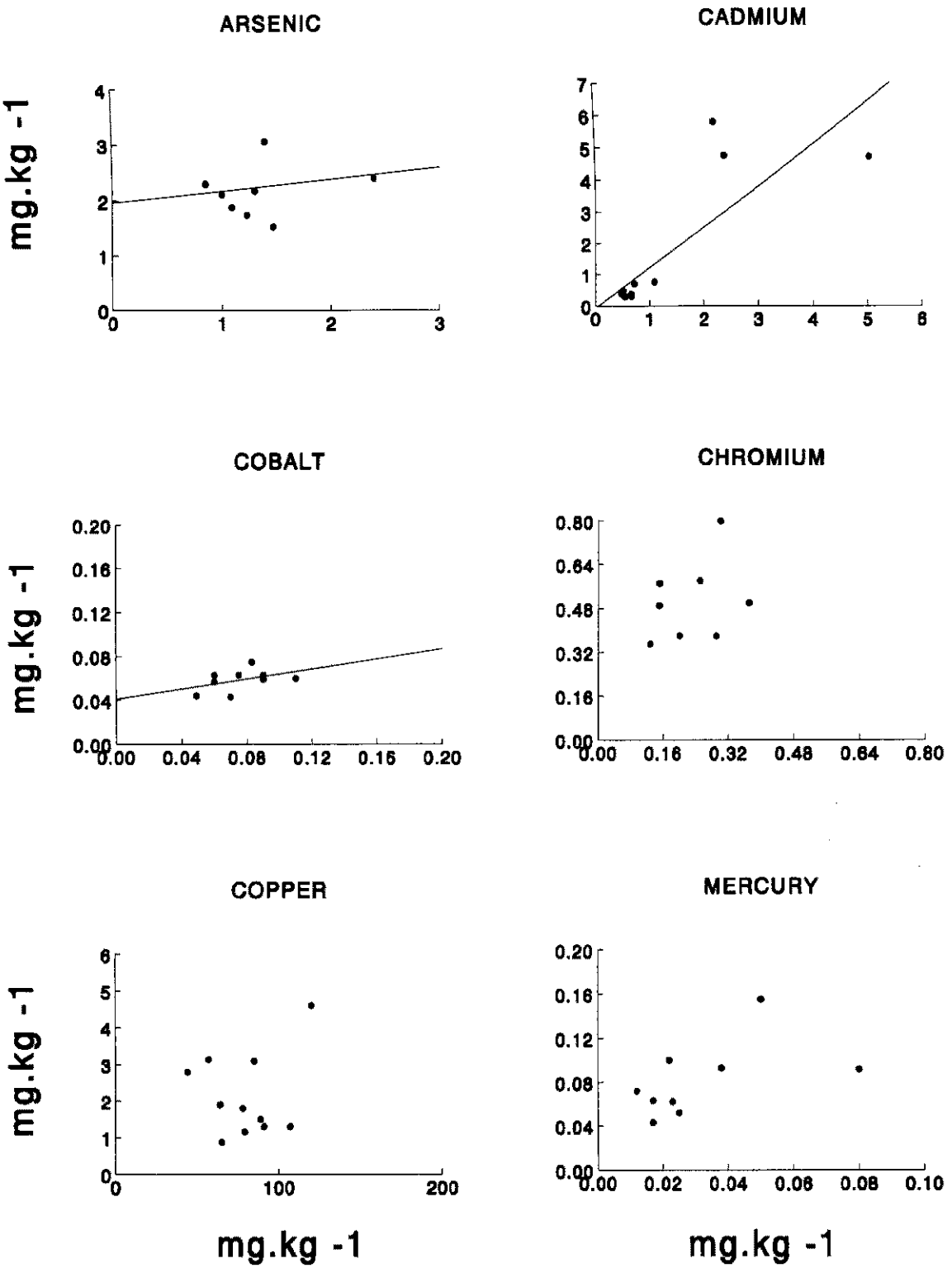


Figure 3.7 Correlations between concentrations of trace metals in cockles (y axis) and oysters (x axis).

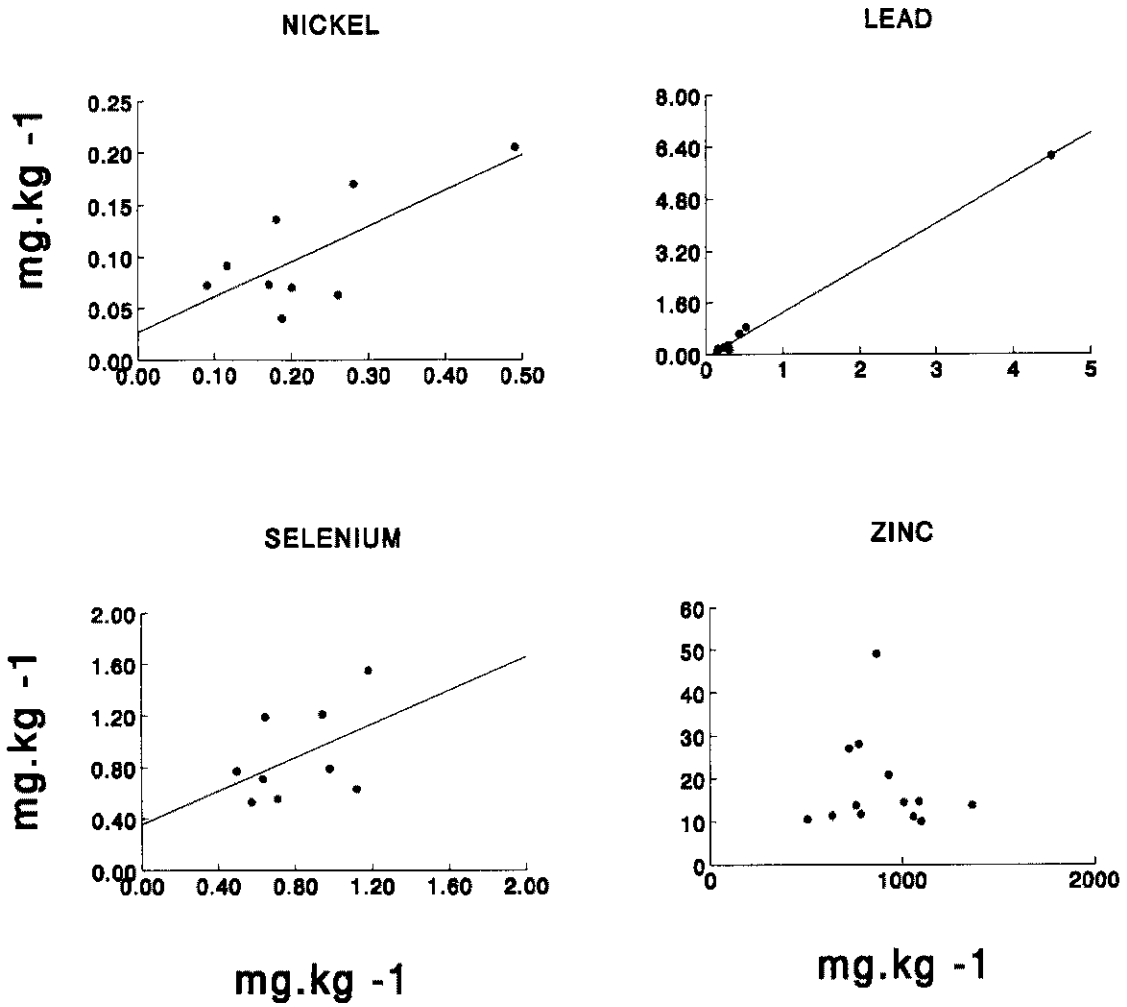


Figure 3.7 cont

Experiment 5: Detection of a Gradient in Other Contaminants (March - May 1993)

Introduction

An industrial effluent which contained a mixture of compounds including hydrocarbons and phenols is discharged to the ocean off Yena Gap (Cape Solander, Botany Bay) NSW (Figure 3.8). The discharge is via a horizontal diffuser into 8m of water. The surrounding areas in that depth of water are dominated by kelp forest habitats typical of much of the biological assemblage found at that depth along the NSW coast (Kennelly and Underwood 1993, Underwood and Kennelly 1990). This study tested the hypothesis that oysters are able to bioaccumulate trace metals, organochlorines, phenols, PAHs and volatile organics. It also tested the hypothesis that there will be smaller concentrations in oysters deployed at greater distance from the effluent's source.

Methods

Sydney rock oysters (*Saccostrea commercialis* (Iredale and Roughley)) were deployed in polyethylene mesh bags suspended about 2m off the sea floor in March 1993. Three replicate moorings were established at each location, each with enough oysters for 5 replicate analyses for each type of contaminant from each mooring. Locations were at the diffuser, approximately 250 m north of the diffuser, and two control locations at Cape Banks and Bangalley Head (Fig 3.8). Oysters deployed at a third control location, North Avoca, were included in trace metal analyses. The third control was not used for PAHs due to cost constraints.

A mooring consisted of a 80 kg (approx.) concrete weight anchoring a 2m rope suspended by a 30 cm polystyrene float. The bags of oysters were attached to the rope 200 mm below the float with stainless steel clips. Oysters were left in place for 3 months then retrieved and analysed. The contaminants analysed include polycyclic aromatic hydrocarbons (PAHs), phenols, volatile organic compounds, organochlorine compounds and trace metals (see Table 3.11 for brief description of methods and Table 3.12 for full listing of contaminants).

Table 3.11 Brief descriptions of methods for analyses of contaminants. ICP MS - inductively coupled plasma mass spectrometry, GC MS - gas chromatography mass spectrometry.

Contaminant	Method
Trace Metals	modified US EPA Method 200.8; ICP MS
Organochlorines	modified US EPA Method 8080, GC MS
PAHs	modified US EPA Method 8270, GC MS
Phenols	modified US EPA Method 8270, GC MS
Volatile organics	modified US EPA Method 8010, purge and trap.

Table 3.12 Contaminants analysed for in oysters. All results expressed as mg/kg wet weight.

Trace Metals
Arsenic, cadmium, chromium, cobalt, copper, lead, mercury, nickel, selenium, silver, zinc.
Detection Limit 0.01 except Co and Ag 0.001, Zn 0.1 mg/kg.

Organochlorines
Aldrin, alpha-BHC, beta-BHC, gamma- BHC (Lindane), chlordane, dieldrin, DDD, DDE, DDT, endosulfan, endrin, heptachlor (HPT), heptachlor epoxide (HPTE), hexachlorobenzene(HCB), methoxychlor, oxychlordane, PCBs.
Detection Limit 0.01 mg/kg.

PAHs
Naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo [a] anthracene, chrysene, benzo [b] fluoranthene, benzo [k] fluoranthene, benzo [a] pyrene, indeno [1,2,3-cd] pyrene, dibenz [ah] anthracene, benzo [ghi] perylene, total PAHs.
Detection Limit 0.05 mg/kg.

Phenols
Phenol; 2-chlorophenol; total cresols; 2-nitrophenol; 2,4-dimethylphenol; 2,4-dichlorophenol; 4-chloro-3-methylphenol; 2,4,6-trichlorophenol; 2,4-dinitrophenol; 4-nitrophenol; 4,6-dinitro-2-methylphenol; pentachlorophenol
Detection Limit 0.05 mg/kg.

Volatile Organics
Methylene chloride (0.05), benzene, toluene, ethylbenzene (all 0.1 mg/kg)

In order to assess the potential for biological impact on the oysters of deployment near Yena Gap outfall, the mortality, weight and condition index were determined for the oysters after they were retrieved. The soft tissues of the oysters were weighed to two decimal places after blotting on absorbent paper. Volume was determined by calculating the difference in displacement of water of opened and unopened oysters, this measure provides the volume of the internal cavity of the oyster in millilitres. A condition index is a measure of the size of the oyster relative to the volume of the

shell's internal cavity. It is derived by dividing the wet weight in grams by the cavity volume in millilitres (Pridmore et al. 1990, Roper et al. 1991).

Data from bioaccumulation studies were compared using analysis of variance techniques.

Results

Mortality and Condition

Mortality of oysters deployed at Yena Gap (19.9 %) was not significantly different from mortality at control locations (19.2 %) (ANOVA, $P > 0.8$); nor was wet weight (5.74g, 6.4g; $P > 0.5$) or condition index (0.61 g/ml, 0.68 g/ml; $P > 0.6$).

Bioaccumulation

Phenols and volatile organic compounds were not accumulated to detectable levels by the oysters, even though these compounds are known to be present in the effluent. PAHs were only accumulated in oysters in the vicinity of Yena Gap (Fig 3.9). The concentrations of total PAHs measured in oysters from nearer to the outfall were six times greater than those in oysters from 250m away. There were 8 different PAHs detected in oysters near to the outfall and 3 in oysters further from the outfall. In oysters from near the outfall, phenanthrene was present in greatest concentrations (Fig 3.9) followed by pyrene, napthalene and chrysene. Benzo-α-pyrene, benzo-α-anthracene, anthracene and fluorene were all present in small concentrations. Phenanthrene, pyrene and napthalene were detected in approximately the same proportions (Close 0.55:0.25:0.20; Far 0.64:0.15:0.21), but smaller concentrations, at the site further from the outfall (Fig 3.9).

The organochlorine pesticides chlordane and DDE were the only organochlorines detected in oysters. DDE was detected in very small to trace amounts in oysters from all locations and probably represents prior contamination. Chlordane was only detected in oysters from the vicinity of the Yena Gap outfall. The level of contamination of these oysters was, however, small. Eight out of fifteen oysters on the closer moorings and only five out of fifteen on the more distant moorings showed contamination. All these oysters had levels of chlordane of 0.01 mg/kg, the smallest quantifiable amount.

Copper, zinc, cobalt, nickel, selenium, lead and silver were all in greater concentrations in oysters from near Yena Gap than in oysters from control locations (Table 3.13, Fig 3.10), but, only chromium, arsenic and lead varied between the two Yena gap locations. Chromium and arsenic were in greater concentrations at the location 250 m from the outfall and lead was at greatest concentrations near the outfall. All metals except chromium and selenium varied significantly among control locations, but of these only zinc had a pattern discernible using SNK tests. Zinc was in greatest concentrations at Cape Banks, then North Avoca then Bangalley Head.

Discussion

The use of bioaccumulators as sentinel organisms in pollution studies has been criticised (Underwood and Peterson 1988) on the basis that these organisms are selected because of their tolerance to pollution and are therefore unlikely to reflect subtle changes in pollution regimes. In this study oysters showed no biological effects (e.g. mortality, tissue weight, condition) of exposure to CRLs effluent but indicated clear patterns in accumulation of anthropogenic compounds (PAHs, organochlorine compounds) which were associated with proximity to Yena Gap outfall . The data for PAHs also showed that oysters nearer the effluent accumulated more PAHs and that only those PAHs which were in greatest concentrations in the near oysters were accumulated to detectable levels in the oysters further away. Pendoley (1992) examined PAH concentrations in wild populations of the closely related (possibly a sub species of Sydney rock oyster, Stenzel (1971)) oyster *Saccostrea cucullata* around the northwest shelf (W.A., Australia) oil fields and found concentrations of 0.15 ppm, considerably lower than found in this study. Phillips et al. (1992) reported PAH concentrations of up to 3000 ppm (dry weight) with modal concentrations around 600 ppm for *Mytilus edulis* near an oil refinery in Port Phillip Bay . O'Connor (1992) reported PAH mean concentrations in the oyster *Crassostrea virginica* of 0.26 ppm (dry weight) (c.f. approx. 3.5 ppm dry wt. in oysters in this study - assuming average water content of 90% (Chapter 2)). O'Connor (1992) calculated that *Mytilus* accumulated about 1.8 times as much PAH as *Crassostrea* in the same environment. Further, the values reported for Port Phillip (Phillips et al. 1992) seem extraordinarily high as the highest concentrations reported for PAHs by the NOAA mussel watch project (NOAA 1989) are approximately 12 ppm.

Patterns in trace metal concentrations were less clearly defined with only small increases in concentrations relative to pooled controls. The exception to this was concentrations of lead which were four times greater near the outfall, and decreased to about twice control concentrations at the far Yena Gap location.

These data indicate that bioaccumulators like oysters are good indicators of the dispersion of contaminants such as organochlorines, PAHs and in this case lead, even on scales as small as 250m.

The lack of any differences in biological measures for the oysters means that the differences in contaminant levels are unlikely to be confounded by changes in the oyster's biology and that the biological measures were not good indicators of impact in this situation. In other studies (e.g. Pridmore et al. 1990, Roper et al. 1991) condition indices have been correlated with changes in tissue concentrations of some contaminants leading to the suggestion that condition indices may be a useful indicator of pollution.

Table 3.13 Comparisons of concentrations of trace metals in oysters deployed near Yena gap outfall and control locations. Tables show summaries of mean squares from analyses of variance. Where differences were indicated SNK tests were used to separate means. * indicates differences P < 0.05, ns no significant difference. "Treatment" - comparisons of all Yena samples to all control samples.

		Cr		Co		Ni		Cu		Zn		As	
	df	MS		MS		MS		MS		MS		MS	
Outfall vs Control	1	0.008		0.003	*	0.04	*	2980	*	328828	*	1.79	*
among outfall	1	0.02	*	4.1 E-5		0.001		515		5603		0.51	*
among control	2	0.004		0.002	*	0.01	*	1463	*	113361	*	1.65	*
Resid	70	0.003		0.0002		0.005		513		30652		0.21	

		Se		Ag		Cd		Hg		Pb	
	df	MS		MS		MS		MS		MS	
Outfall vs Control	1	0.28	*	2.95	*	0.01		4.0 E-5		0.33	*
among outfall	1	0.10		0.51		5.8 E-5		8.7 E-5		0.05	*
among control	2	0.05		5.65	*	0.10	*	7.7 E-4	*	0.01	*
Resid	70	0.05		0.49		0.006		4.0 E-5		0.10	

Results of SNK tests. Abbreviations: Y - Yena Gap locations, C - control locations; F - 250 m from Yena outfall, N - near diffuser; CB - Cape Banks, NA - North Avoca, BH - Bangalley Head

	Outfall vs controls	Among Outfall	Among Controls		Outfall vs controls	Among Outfall	Among Controls
Cu	Y > C		ns	As	C > Y	F > N	ns
Zn	Y > C		CB>NA>BH	Se	Y > C		ns
Cr		F > N	ns	Cd			ns
Mn			ns	Hg			ns
Co	Y > C		ns	Pb	Y > C	N > F	ns
Ni	Y > C		ns	Ag	Y > C		ns

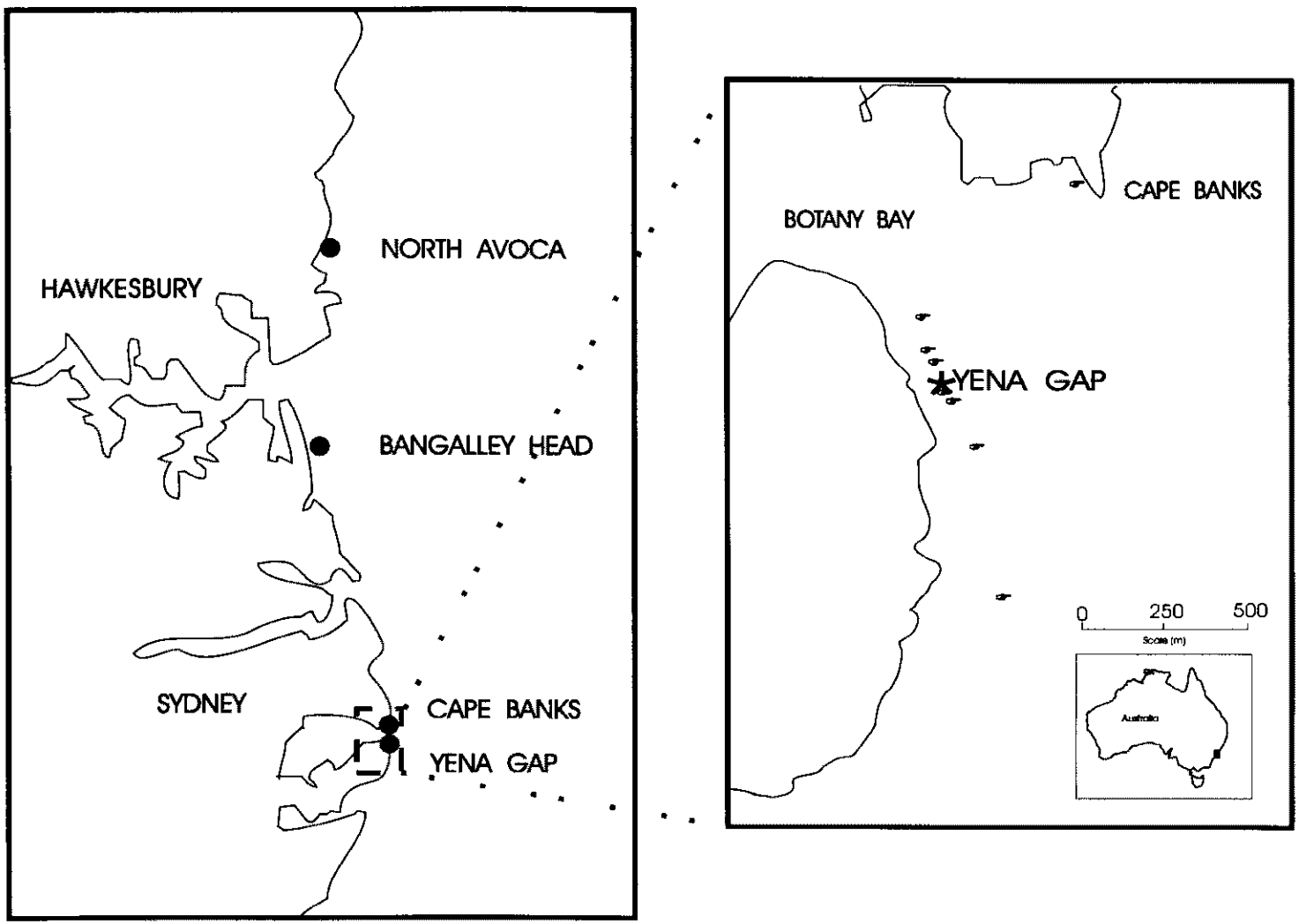
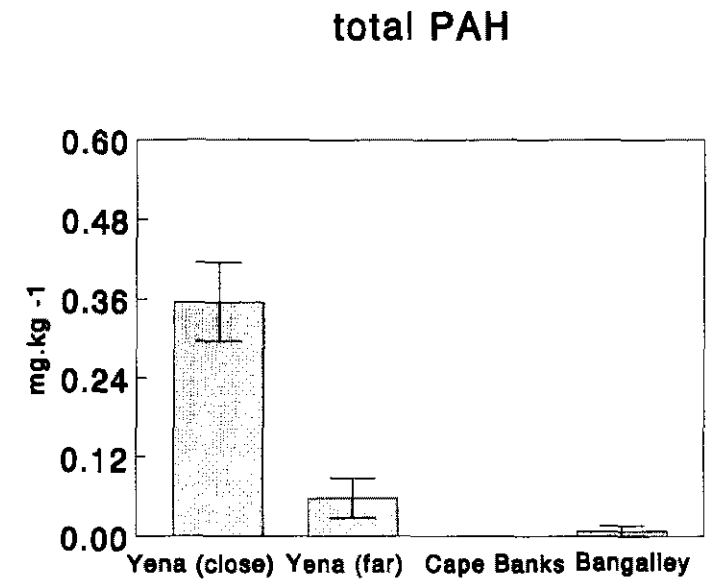


Figure 3.8 Locations of sampling in Experiment 5



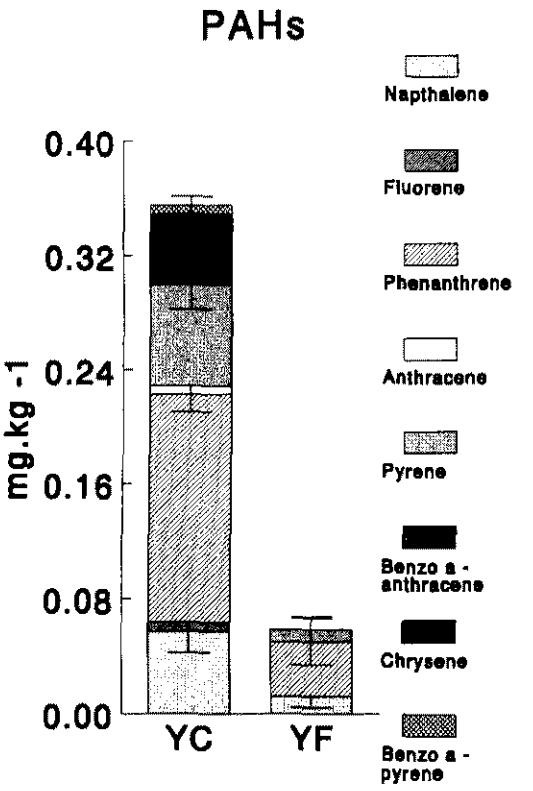


Figure 3.9 Mean concentrations of PAHs accumulated by oysters placed near Yena Gap outfall. Yena close (YC) refers to moorings within 20 m of outfall; Yena far (YF) refers to moorings 250 m from outfall.

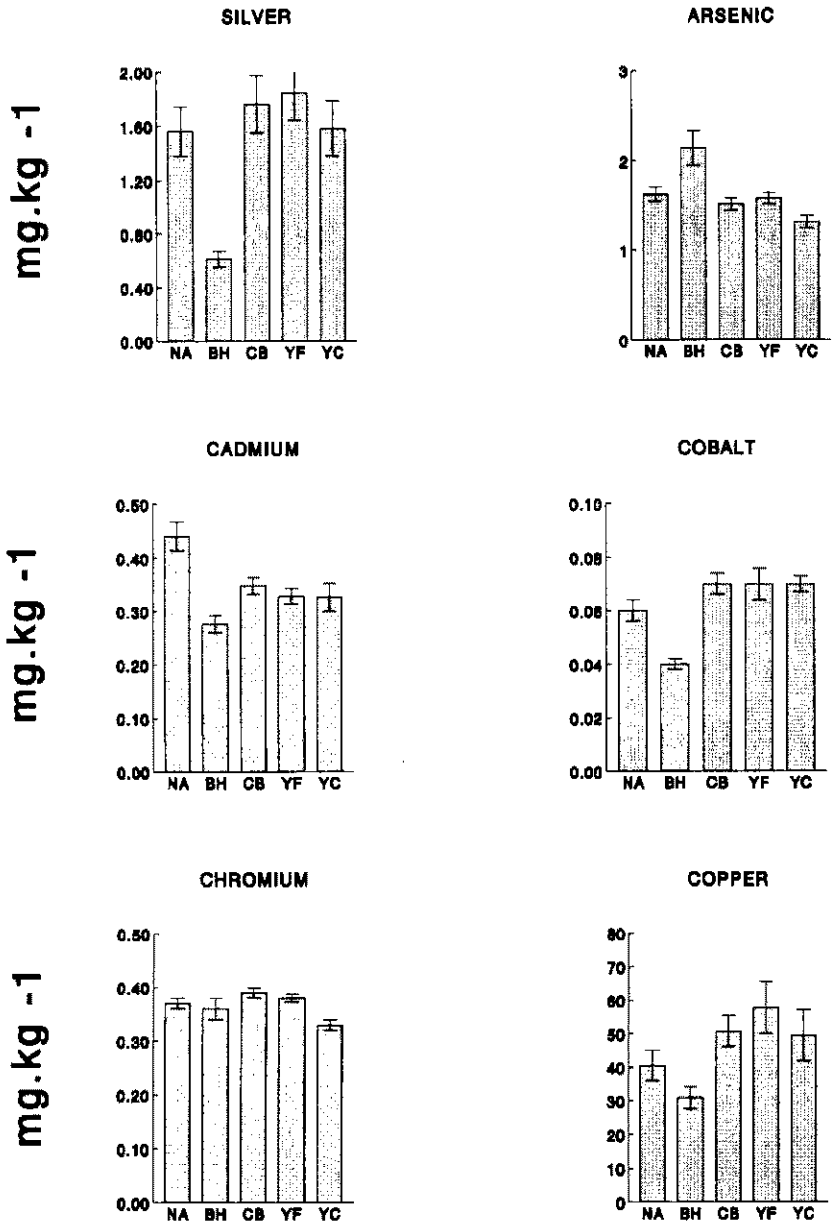


Figure 3.10 Mean concentrations of trace metals in oysters deployed near Yena Gap outfall (YC, YF) and at control locations (NA - North Avoca, BH - Bangalley Head, CB - Cape Banks).

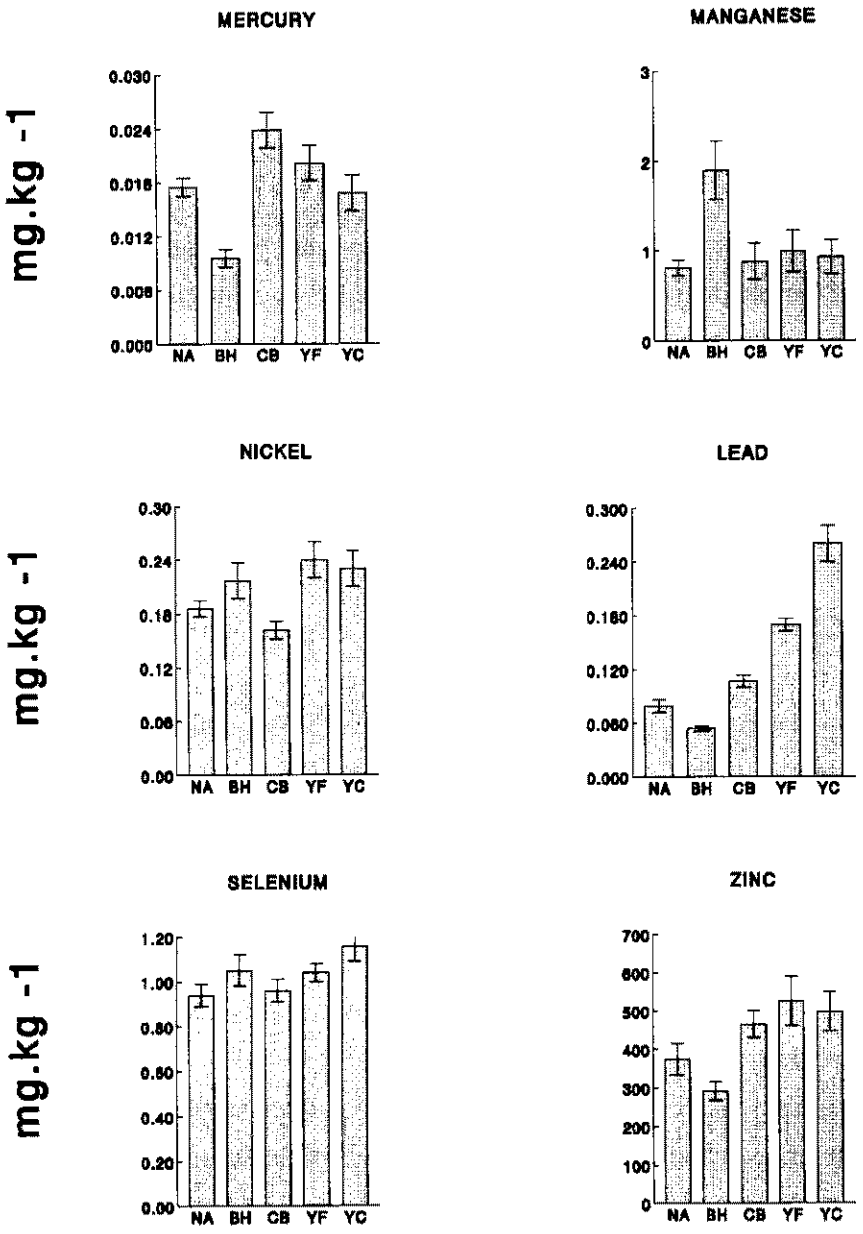


Figure 3.10 cont

Overall Conclusions - Comparisons of accumulation among species and along putative gradients

There is a reasonable level of agreement in the relative ranking of oysters and cockles in the three studies. This probably indicates that levels for these metals are different for the species, but are consistent through time and in a constant relationship with external concentrations. Differences may be due to there not being enough metal in the environment for the animals to reach saturation.

These experiments have indicated that concentrations of trace metals in oysters can, in general, demonstrate a pollution gradient for a wider range of metals than cockles. This may, in part, reflect the more widespread use of oysters. Concentrations of organochlorines in oysters were able to demonstrate gradients in aquatic concentrations whilst cockles did not. Oysters have also shown that will accumulate PAHs in a direct relationship with external concentrations and thus show gradients in the environment.

Neither bivalve detected differences for cadmium near sewage outfalls (Expts. 1,2), but oysters did when there was a very large gradient in the environment (Experiment 4). Ward (1982) showed that under laboratory conditions, the body burden of cadmium in Sydney rock oysters reflected concentration of dissolved Cd in water. That study used concentrations between 10 and 150 ug/l. Rendell and Espey (1992) measured Cd concentrations in the sewage plume from Sydney's sewage outfalls, which ranged from 0.07 to 0.6 ug/l. In general, Rendell and Espey (1992) found that metal concentrations in the plumes were about an order of magnitude greater than average background conditions (assumed to be equivalent to the concentrations at control locations). Similarly, Klumpp and Burdon-Jones (1982) used concentrations of 10 - 50 ug/l for laboratory experiments to determine whether the hairy mussel *Brachidontes hirsutus* (then called *Trichomya hirsuta*), showed a body burden that correlated with concentrations of water. Levels reported for coastal seawater in that study were between 0.2 - 3 ug/l. The use of exaggerated concentrations in laboratory studies (cf. field concentrations) may explain why field experiments do not always detect gradients when laboratory experiments have demonstrated a potential to do so.

As indicated in the introduction, the main criteria used when determining the "usefulness" of an indicator species is some correlation with concentrations in surrounding water (e.g. Klumpp and Burdon-Jones 1982, Phillips and Rainbow 1988, Powell and White 1990). This study has indicated that oysters consistently demonstrated such a correlation for Cr, Co and Zn and have, at times, indicated

elevated levels of Cd, Ni, Cu, Pb, chlordane, dieldrin and PAHs. Klumpp and Burdon-Jones (1982) reported that *Crassostrea (= Saccostrea) commercialis* may be a useful monitor of Cu and Zn. Phillips (1979) reported that the closely related *Saccostrea glomerata* was a reliable indicator of Zn, Cu, Cd and Fe. The usefulness of oysters in determining gradients of Cu should be qualified by the knowledge of the influence of moored boats on the results.

Cockles consistently indicated elevated levels of As and also Co, Ni, and Hg. Other data have demonstrated that oysters can also show gradients of As, Se, Ag, Hg, Pb and organochlorines (Scanes and Henry 1992). Cockles can also show gradients of Cu, Pb and Zn (Scanes 1993, Chapter 7).

The study has also shown that there can be considerable differences in the body burdens of bivalves maintained in the same area. Similar patterns of differences between species have been shown by Klumpp and Burdon-Jones (1982) and Lobel et al. (1982).

Phillips (1976) cautioned that the results of bioaccumulation monitoring studies could be confounded by environmental and biological vagaries. He suggested that exhaustive studies of the effects of environmental variables and aspects of the animal's biology (e.g. age, size, sex etc) should be completed before any organism can be nominated as a biomonitor. Much of Phillips' argument is based on considerations of designs which examine temporal patterns in absolute concentrations using collections of naturally occurring animals. The design of the studies described here uses deployments of animals at sites of interest and control sites. All spatial and temporal comparisons are made with reference to the controls. This approach removes confounding due to the uncontrolled effects of environmental factors in Phillips' (1976) review. Further, all oysters were obtained from commercial growers, so their age and history is similar at both the control and assayed sites. This eliminates confounding due to different biologies at the different sites.

Some of the studies reported here formed part of the pilot phase for a large scale investigation of spatial and temporal trends in contaminant distribution around sewage outfalls. It had been recommended that oysters were suitable for use in bioaccumulation studies near the outfalls and that some further information regarding the rates of uptake and depuration should be obtained. Oysters were initially considered in preference to *Mytilus* because *Mytilus* is at the northern limit of distribution at Sydney, but *Saccostrea* are common along the entire eastern Australian

coast. This work provides support for the suggestion in Phillips (1979) that oysters of the genus *Saccostrea* could become the standard biomonitor of trace contamination in tropical and sub-tropical waters where *Mytilus* is not found.

Objective 2 Determining and verifying the most effective means of deployment

Experiment 6 (December 1991 - March 1992)

Introduction

Bioaccumulation experiments need to be done in a variety of habitats and consequently there is a need for a variety of methods of deployment. The use of different methods of deployment could confound comparisons of concentrations in animals from different experiments. The following experiment examined the survival and concentrations of contaminants in oysters deployed on four different types of mooring.

Methods

Two methods of benthic deployment and two methods of mid-water deployment were trialed at each of two locations near sewage outfalls (Cronulla and Turrimetta) and two control locations (Cape Solander and Mona Vale) (Fig. 3.1). The benthic deployments consisted of a 600 x 400 x 400 mm stainless steel cage with 50 mm mesh which was bolted to a concrete slab, bags of oysters were suspended inside the cage; or a 300 x 300 x 300 mm concrete cube with an embedded steel anchor point where bags of oysters were attached. Both deployments were placed in rocky areas on the sea floor by divers. These will be referred to as cages and blocks in the following text. Mid-water deployments consisted of a car tyre filled with concrete anchoring either a 200 mm diameter polystyrene mid water float or a 1.8 m aluminium spar buoy. These will be referred to as tyres and spars in the following text. Bags of oysters were suspended 2 m from the seafloor on the mooring ropes of these two deployments.

Three replicates of each deployment were placed at each location. Oysters in mesh bags were attached to each deployment device and were collected after 3 months. Data were collected on loss or damage of deployments, mortality of oysters and level of contamination of oysters.

Results

Deployments

Each method of deployment has an associated cost, ease of construction, installation and subsequent relocation (Table 3.14).

Table 3.14 Summary of estimated costs and ease of use for each method of deployment. For ease of construction, installation and location a scoring system is used. Five is most difficult, one is easiest.

Method	Cost	Ease of building	Ease of installation	Ease of finding
Tyre and float	$20	3	3	2
Cage on slab	$30	4	3	3
Spar and mooring	$90	5	4	1
Concrete block	$20	1	1	4

After 3 months, divers searched the areas where the experiments were deployed. They recorded the presence and condition of all deployments that were found. Oysters were collected from 0 spars, 3 tyres, 5 blocks and 7 cages from a possible 12 of each type of deployment (Table 3.15).

Mortality

Mortality of those oysters that were able to be retrieved was calculated as a proportion of all shells recovered from the bags (Table 3.16). Some treatments were completely buried in sand and would have had total mortality, but since the oysters were not able to be recovered they are not included in this analysis.

An unbalanced 1 factor analysis of variance indicated that there was no significant difference ($P > 0.05$) between the mean mortality for each treatment.

Table 3.15 Fate of deployments at each site.
Key to Types: Tyre - tyre with float; Cage - stainless cage on concrete slab; Spar - spar buoy moored to tyre; Block - concrete block.

Type	Potter Pt	Cape Solander	Turrimetta	Mona Vale
Tyre 1	holes worn in oyster bags	lost	lost, probably covered by sand	good condition
Tyre 2	good condition	lost	lost, probably covered by sand	lost
Tyre 3	holes worn in oyster bags	holes worn in oyster bags	lost, probably covered by sand	lost
Cage 1	good condition	covered by sand	lost, probably covered by sand	good condition
Cage 2	good condition	covered by sand	lost, probably covered by sand	cage crushed, 1 oyster bag holed
Cage 3	good condition	cage crushed, 1 oyster bag holed	lost, probably covered by sand	lost
Spar 1	spar lost, rope and mooring found	lost	lost, probably covered by sand	spar lost, rope and mooring found
Spar 2	spar lost, rope and mooring found	lost	lost, probably covered by sand	spar lost, rope and mooring found
Spar 3	spar lost, rope and mooring found	spar lost, rope and mooring found	lost, probably covered by sand	spar lost, rope and mooring found
Block 1	oyster bags lost	good condition	lost, probably covered by sand	good condition
Block 2	good condition	lost	lost, probably covered by sand	good condition
Block 3	good condition	lost	lost, probably covered by sand	good condition

Table 3.16 Proportional mortality of oysters recovered from various types of deployment. Cape Solander and Mona Vale are control locations, Potter Point is an outfall location.

Location	Deployment Type	Mortality
Cape Solander	Cage	0.57
Cape Solander	Cage	0.20
Mona Vale	Cage	0.06
Mona Vale	Cage	0.23
Potter Point	Cage	0
Potter Point	Cage	0
Potter Point	Cage	0.06
Mean		0.16
Cape Solander	Block	0.63
Mona Vale	Block	0.03
Mona Vale	Block	0.09
Potter Point	Block	0.13
Potter Point	Block	0.10
Mean		0.20
Mona Vale	Tyre	0.16
Potter Point	Tyre	0.03
Potter Point	Tyre	0
Mean		0.06

Contamination

Organochlorines

Data for organochlorines have been analysed in two ways. First, a comparison of the levels of contamination of five replicate oysters from those moorings where oysters were retrieved from two replicates of the type of mooring at Potter Point (outfall location). These analyses indicated that the concentrations of chlordane, total DDT and PCBs (which were the only organochlorines detected) were not significantly different between moorings, nor among types of mooring (Fig 3.11, Table 3.17a).

To ensure that the ability to detect differences in concentrations of organochlorines among types of mooring was not a result of poor statistical power *post hoc* power analyses were done. The power analyses considered two alternative hypotheses, that the mean concentrations of organochlorines for one type of mooring would be 50% and 100% greater than the other two. The analyses indicated a power greater than 0.99 for both scenarios. This suggests that the experiments had adequate power and that the conclusion of no difference is unlikley to represent a Type II statistical error.

Data for organochlorines from Potter Point and Mona Vale were compared in two analyses. Initially, a model which had locations and types of mooring (two replicates

of each type) was considered. This analysis was unbalanced because only one tyre was recovered from Mona Vale. It indicated a significant difference between the two locations (Table 3.17b, Fig 3.12), but no differences among types of mooring and no interactions between the two orthogonal factors. A second balanced (but pseudoreplicated) analysis using data from only one randomly selected representative of each type of mooring at each location confirmed the significant differences between locations, but indicated for chlordane and DDT significant differences among types of mooring and a significant interaction between location and type of mooring (Table 3.17c). Given that the original analysis with replicated types of mooring did not indicate a difference among types of mooring, the significant difference found in among types of mooring in the second analysis is considered to be an artifact of the confounded design.

Trace metals

Analyses of data for trace metals compared mooring types at Potter Point outfall and compared two replicate moorings for cages and blocks at Potter Point and Mona Vale. Where there were no differences among moorings, moorings were pooled together and data reanalysed. Concentrations in three replicate oysters were replicates for each mooring.

Analyses comparing the types of mooring at Potter Point showed no differences (Table 3.18a, Fig 3.13) for all metals except As. There were greater concentrations of As in oysters from tyres. There were no significant differences among replicates of type of mooring.

Analyses of concentrations of contaminants at Mona Vale and Potter Point, with two nested replicates of cages and blocks at each location showed no differences between locations for any metal (Table 3.18b, Fig 3.14). There were no significant differences among types of mooring, between replicate moorings or for the location x mooring interaction for any metal. When moorings were pooled and the data reanalysed, arsenic concentrations were significantly greater at Mona Vale.

Conclusions

The survival of the various types of mooring suggests that, as deployed, spars were the least successful method of deployment, with tyres next and there was little difference between blocks and cages. Intuitively, low profile methods such as cages and blocks

are more susceptible to covering by sand. Blocks are by far the easiest to obtain and deploy, but are relatively difficult to relocate. Tyres were moderately successful, moderately easy to construct, more difficult to deploy, but easy to find and less susceptible to covering.

This study has also demonstrated deficiencies in the methods of deployment as they were originally done. It has also provided valuable information on how to set up these deployments more successfully in the future. Tyres and spars should be set up so that it will not be possible for the ropes to abrade on nearby rocks. This would substantially reduce the loss of oysters. Spars and tyres also were least suitable for high energy environments in the experiments described. The suitability of spars in low energy environments has been demonstrated in Lake Macquarie (a coastal lagoon, see Experiment 4 above) where nine spars have been deployed for over 12 months without loss.

Spars and tyres were considered very useful in offshore environments due to their ease of location, which is an asset when the period divers can spend on the bottom becomes limiting. Therefore, further oceanic trials with modified designs of spar and tyre moorings (Experiment 5 this chapter; Chapters 4 and 6) were done. These studies had 100 % survival of spars and tyres over 3 months, including a storm with swell greater than 5m. In addition, Ajani (1995) has successfully used spars and tyres in exposed locations off Newcastle.

There were no significant differences among mean mortalities of oysters for each type of mooring. The unbalanced nature of the analysis, however, makes it difficult to be unequivocal about these results. The mean mortality of oysters on tyres was smallest. This coincides with observations of likely sources of mortality which indicate that invertebrates such as whelks, octopus and starfish are the carnivores most commonly found in bags or seen in the vicinity. The lack of severely crushed or damaged shells in the bags suggests that large predators such as fish are not involved. Oysters suspended on floats above tyres would be least susceptible to the invertebrate predators identified. This conclusion is further verified by consideration of mortality in oysters deployed on blocks in the experiments described in Chapter 5, which is often as large as 50%, in comparison with that experienced in the depuration experiments (Chapter 4) where mortality was less than 5% on tyre moorings.

There was strong evidence from all the analyses presented for contaminants that the type of mooring does not influence the level of contamination of oysters over the

period of this experiment. This result is important because it implies that the results of experiments using different means of mooring can be directly compared, provided, of course, that there are appropriate controls.

Table 3.17 Summary of mean squares from analyses of variance comparing concentrations of organochlorines accumulated in oysters on a variety of deployment types.

a Cages, blocks and tyres at Potter Point. two replicate moorings, 5 replicate oysters per mooring.

		Chlordane		PCB		ΣDDT	
	df	MS	F	MS	F	MS	F
Type	2	0.006	0.74	0.03	0.88	0.001	0.88
Mooring(T)	3	0.008	1.73	0.04	1.34	0.001	2.10
Resid	24	0.0045		0.03		0.0007	

b Cages, blocks and tyres at Potter Point and Mona Vale. Moorings (2) not partitioned, 10 replicate oysters per mooring type, except Mona Vale tyres which had 5 replicate oysters. Both factors fixed. Unbalanced, no spatial confounding.

		Chlordane		PCB		ΣDDT	
	df	MS	F	MS	F	MS	F
Location	1	0.035	9.83 *	0.112	5.34 *	0.003	5.32 *
Type	2	0.0009	0.27	0.013	0.63	0.0004	0.85
L x T	2	0.004	1.11	0.014	0.67	0.0005	0.95
Resid	49	0.004		0.021		0.0005	

c Cages, blocks and tyres at Potter Point and Mona Vale. One mooring, 5 replicate oysters per mooring. Both factors fixed. Balanced, spatially confounded.

		Chlordane		PCB		ΣDDT	
	df	MS	F	MS	F	MS	F
Location	1	0.024	8.85 *	0.002	5.05 *	0.094	5.19 *
Type	2	0.005	1.68	0.0015	3.98 *	0.036	1.98
L x T	2	0.010	3.78 *	0.0016	4.17 *	0.042	2.33
Resid	49	0.003		0.0004		0.018	

Table 3.18 Summary of mean squares from analyses of variance comparing concentrations of trace metals accumulated in oysters on a variety of deployment types.

a Cages, blocks and tyres at Potter Point. two replicate moorings, 3 replicate oysters per mooring.

		Cr		Co (ln)		Ni		Cu (ln)		Zn		As	
	df	MS	F	MS	F	MS	F	MS	F	MS	F	MS	F
Type	2	0.002	0.29	0.013	0.07	0.0015	1.48	0.17	0.63	228776	1.96	0.69	33.4 *
Mooring(T)	3	0.007	1.82	0.187	1.51	0.0010	0.59	0.27	1.26	117007	1.14	0.02	0.11
Resid	12	0.004		0.124		0.0017		0.21		102721		0.18	

		Se		Ag (ln)		Cd (ln)		Hg (ln)		Pb	
	df	MS	F	MS	F	MS	F	MS	F	MS	F
Type	2	0.052	9.37	0.39	0.86	0.07	1.59	0.15	3.07	0.0002	0.08
Mooring(T)	3	0.006	0.07	0.45	1.99	0.05	0.89	0.05	0.2	0.0022	1.6
Resid	12	0.076		0.23		0.05		0.25		0.0014	

Table 3.18 cont

b Cages and blocks at Potter Point and Mona Vale. two replicate moorings, 3 replicate oysters per mooring.

		Cr		Co (ln)		Ni		Cu (ln)		Zn		As	
	df	MS	F	MS	F	MS	F	MS	F	MS	F	MS	F
Location	1	0.003	0.5	0.024	0.14	0.0003	0.22	0.71	1.8	362604	2.66	4.08	5.99
Type	1	0.014	2.13	0.004	0.02	0.0057	3.79	0.25	0.63	59004	0.43	1.08	1.59
L x T	1	0.002	0.36	0.058	0.34	0.0051	3.39	0.00007	0.00	16538	0.74	0.70	1.03
L x Mooring(T)	4	0.007	1.96	0.174	1.56	0.0015	0.49	0.40	1.60	136538	1.40	0.68	1.44
Resid	16	0.003		0.111		0.0031		0.25		97779		0.47	

		Se		Ag (ln)		Cd (ln)		Hg (ln)		Pb	
	df	MS	F	MS	F	MS	F	MS	F	MS	F
Location	1	0.22	6.44	0.13	0.25	0.044	1.21	0.06	0.26	0.0003	0.14
Type	1	0.22	6.44	0.002	0.00	0.044	1.21	0.14	0.66	0.0001	0.04
L x T	1	0.0003	0.01	0.76	1.46	0.020	0.56	0.08	0.38	0.0007	0.3
L x Mooring(T)	4	0.035	0.47	0.52	1.70	0.037	0.73	0.22	0.78	0.002	1.68
Resid	16	0.075		0.31		0.501		0.28		0.001	

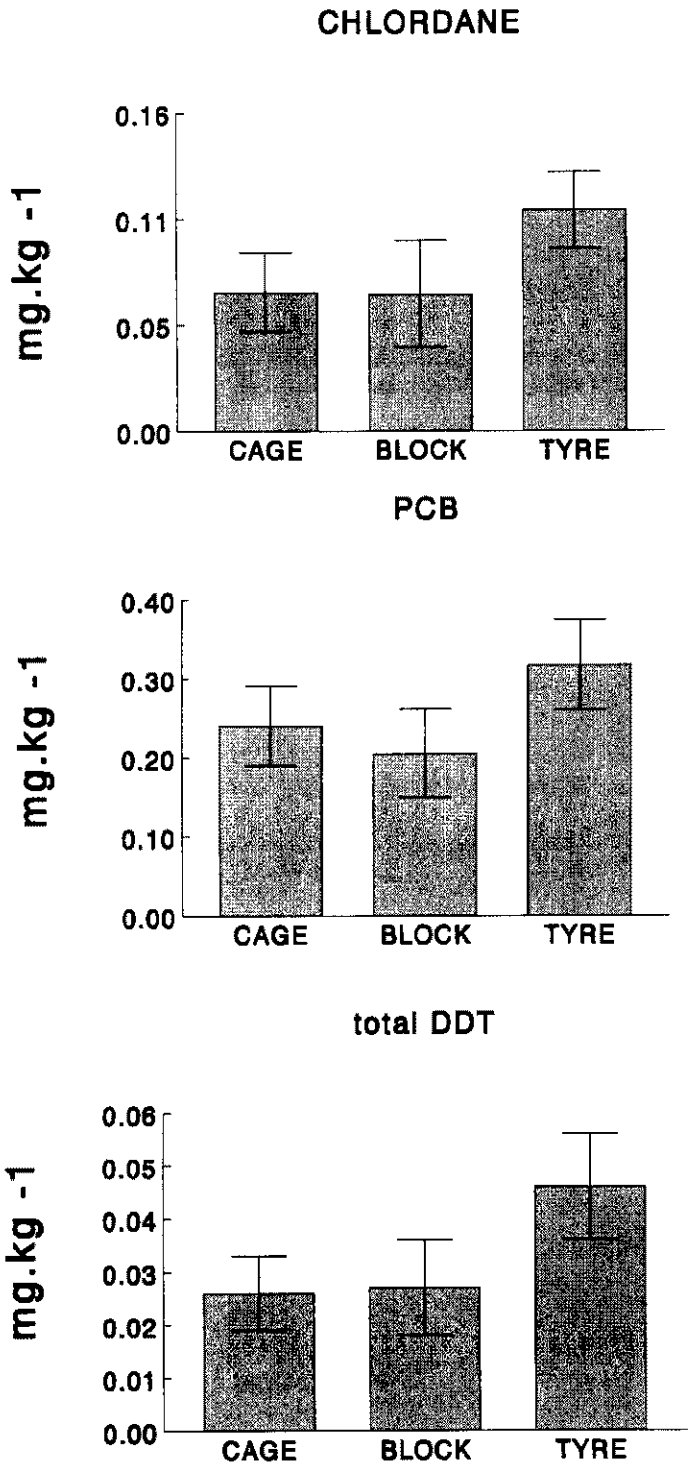


Figure 3.11 Concentrations of organochlorines in oysters on three types of moorings at the outfall location.

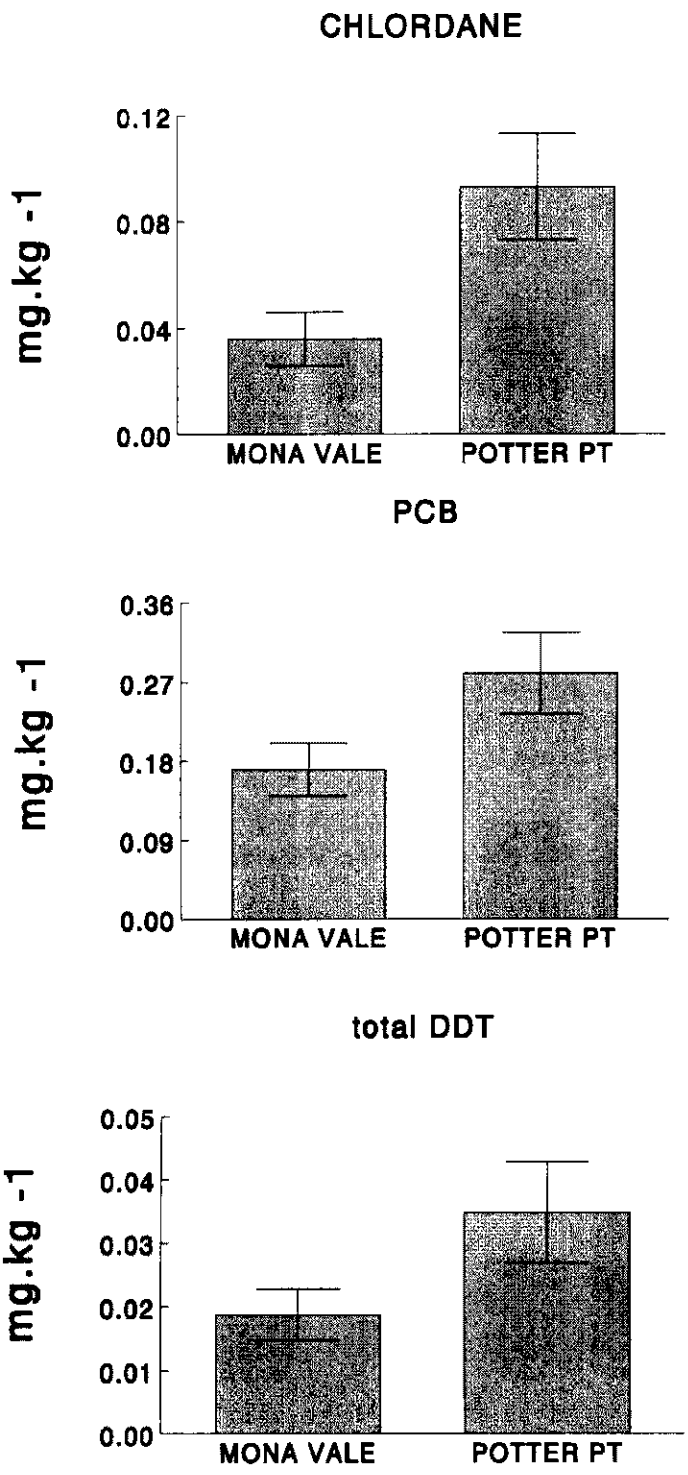


Figure 3.12 Mean concentrations of organochlorines in oysters deployed at Mona Vale (control) and Potter Point (outfall).

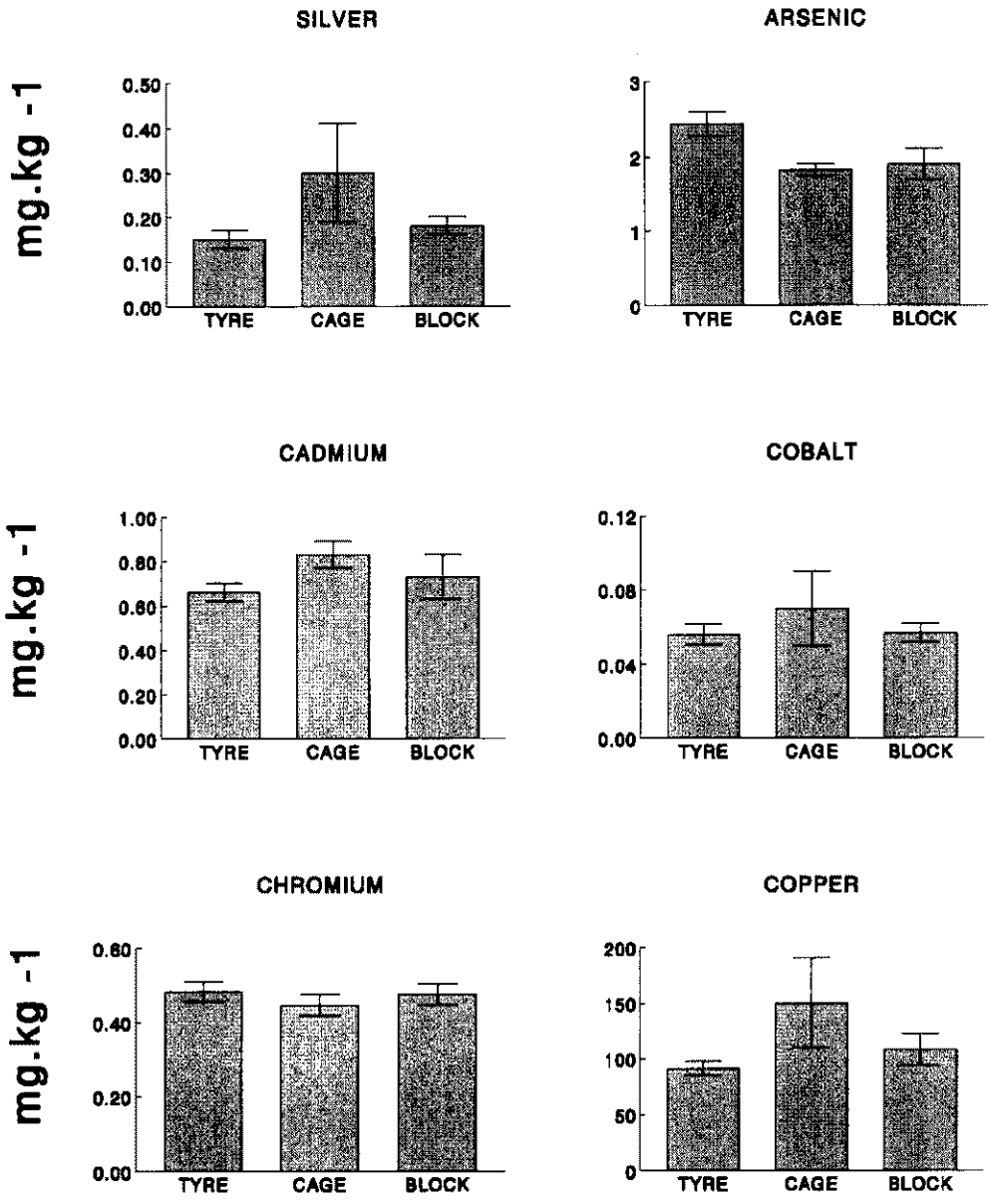


Figure 3.13 Mean concentrations of trace metals on three types of mooring at the outfall location.

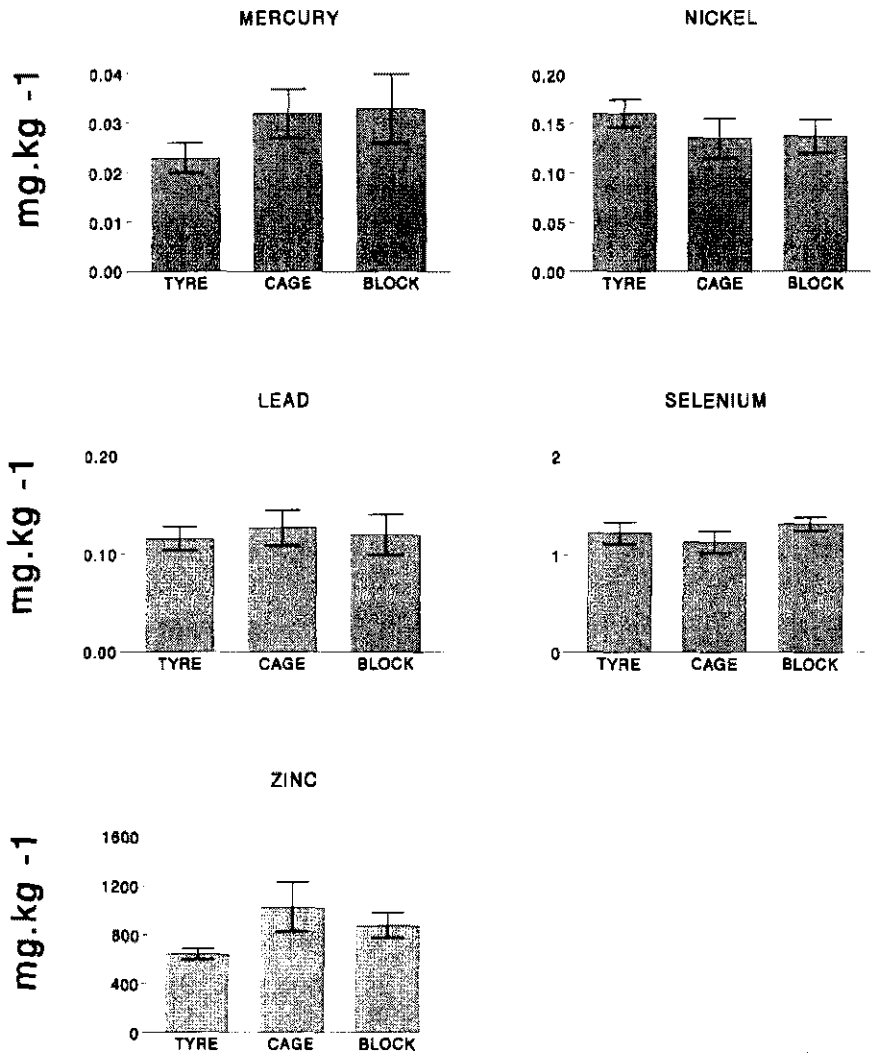


Figure 3.13 cont

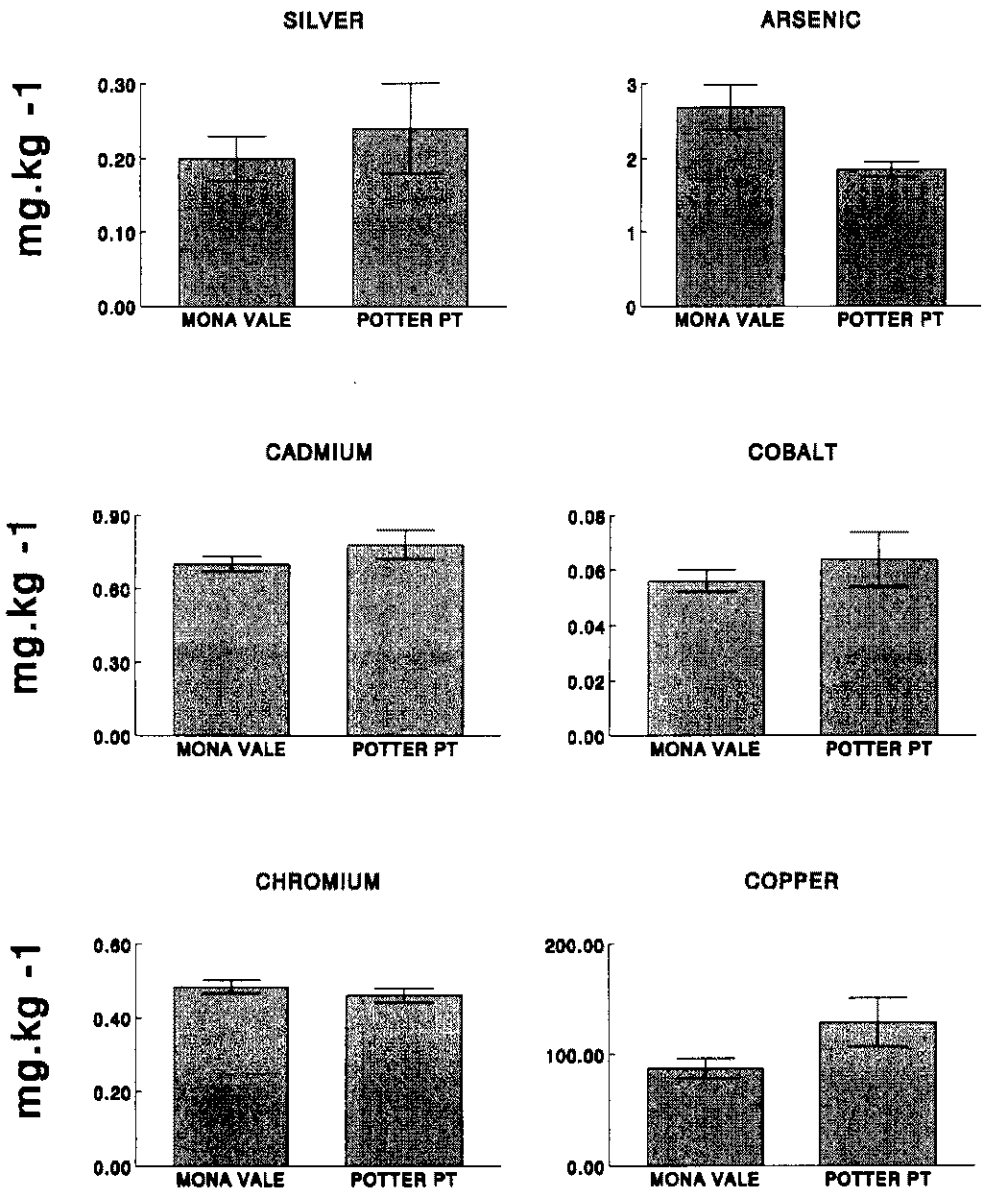


Figure 3.14 Mean concentrations of trace metals in oysters at Mona Vale (control) and Potter Point (outfall).

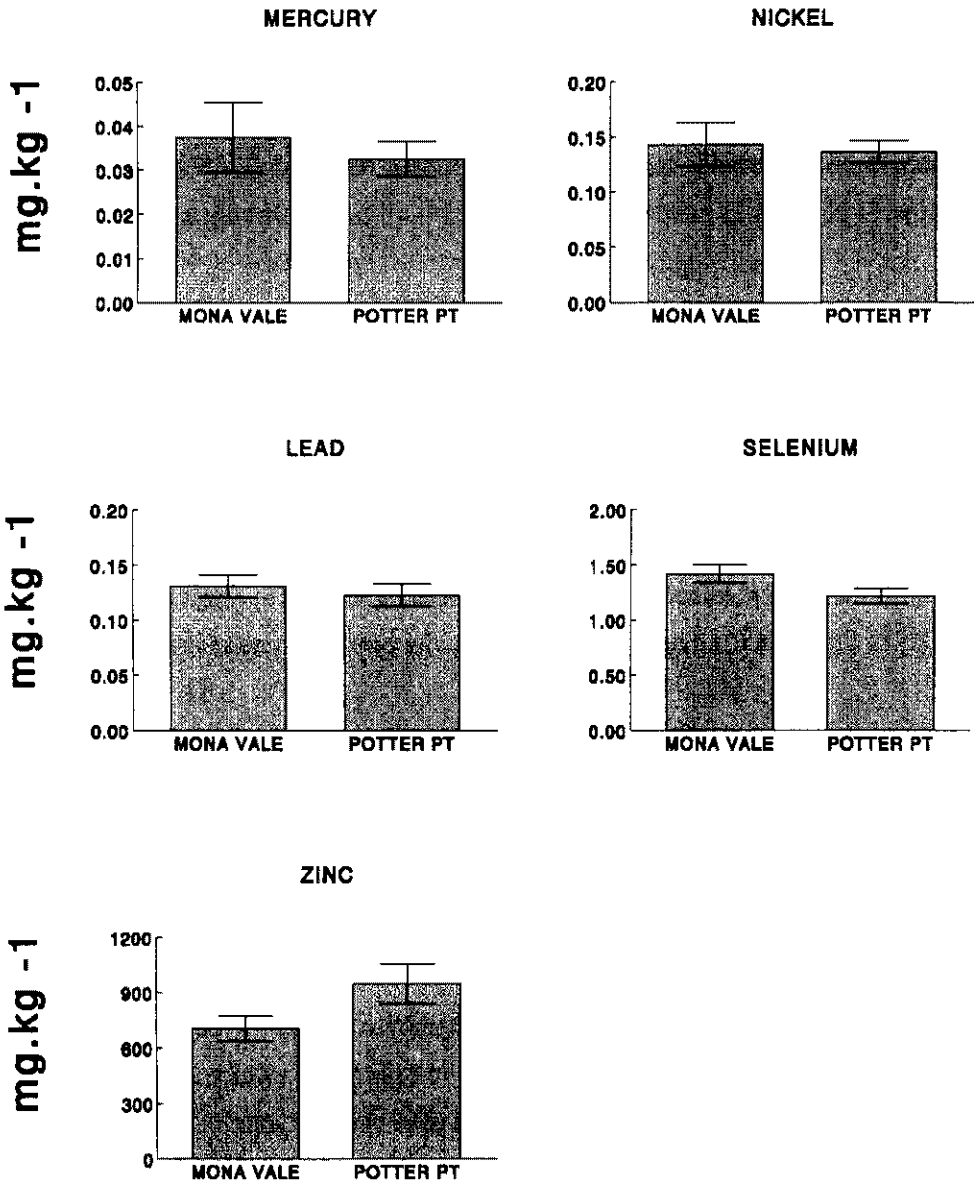


Figure 3.14 cont

Overall Conclusions

These data indicate that oysters and mussels are useful as bioaccumulators of organochlorines, that all three species are adequate as accumulators of trace metals and oysters can be used to indicate gradients in PAHs.

The data in this chapter have shown that oysters are sensitive to pollution regimes and have shown significant differences in the concentrations of trace metals, organochlorines and PAHs between outfall and control sites. There was no evidence of accumulation of organochlorines by cockles or accumulation of phenols or volatile organics by oysters.

There appear to be intrinsic relationships between the amounts of trace metals accumulated by cockles and oysters in the same environment and this relationship appears to be constant under different pollution regimes.

Given that there is probably little difference in the performance of oysters and mussels as indicator organisms, it is more logical in NSW to use a species which has statewide applicability (oysters) rather than one which is at the extreme northern limit of its distribution (mussels).

The argument that the use of mussels allows international comparisons is trivial, because oysters are commonly used in many Asian, American and European studies of bioaccumulation, and form part of the US "Mussel Watch" programme in those regions where mussels do not normally grow (O'Connor 1992).

The use of translocated animals to address specific hypotheses allows a much wider variety of studies and allows the design of studies to be unconfounded by aspects of the animal's biology. Young et al. (1976) have also advocated the use of deployed bivalves because of the flexibility of the system and the ability to place animals in locations where they do not normally occur.

The studies have shown that a bivalve which is normally found attached to intertidal hard substrata in estuaries (oysters) and one which is normally found partially buried in estuarine mud survived very well suspended in the water column in bags in marine or in estuarine conditions, with no apparent loss of condition (Chapter 6). The reverse is, however, not true and oysters suffer great mortality when buried in sediments (Chapter 6).

The choice of animal for the studies is really dependent on exactly what is required, but the poor uptake of organochlorines by cockles and the need to collect animals from wild populations (c.f purchasing oysters from commercial growers) means that oysters are the preferred animals for use in future studies. The one exception is where specific hypotheses about sediments are being tested (e.g. Chapter 6). For these studies oysters are unsuitable because their survival when buried in mud is poor compared to cockles.

The mode of deployment is also, to some extent, dependent on the specific experiment. It has been demonstrated, however, that a variety of methods of deployment had no effect on the concentrations of most contaminants. In general, the mooring which offers the best compromise between cost, ease of deployment, ease of recovery and security is a sub-surface float arrangement (e.g. "tyres" above). If security (from natural and human agents) is less of a concern, then surface floating moorings using light weight spar buoys are convenient because of their ease of recovery.

CHAPTER 4 UPTAKE AND DEPURATION

Introduction

In order to understand and interpret data on contamination and to plan studies, it is necessary to have information on the rates of uptake and depuration of contaminants in the organisms chosen as biomonitors (Phillips and Rainbow 1993). This is necessary to be able to interpret temporal patterns in data. If collections of organisms are made at time scales much greater than that required to reach equilibrium with the environment then it can not be assumed that the biomonitors are providing an integration of the entire period, they are only providing data for the period immediately prior to collection . Conversely, if the collecting period is short in relation to time to equilibrbrium of the contaminant then no equilibrium will be achieved.

Okazaki and Panietz (1981) described rates of depuration ("biological half-lives") for metals to be 23 - 60 days for *Crassostrea gigas* and 70 - 180 days for *C. virginica.* Data for organochlorines in oysters are scarce, but Mortimer and Connell (1993) have predicted rates of depuration ranging from 0.1 days for heptachlor epoxide to 60 days for compounds like DDT and PCBs.

Despite the widespread use of oysters of the genus *Saccostrea* for assessment of trace metal concentrations (Brown and McPherson 1992, Mackay et al. 1975, Peerzada and Dickinson 1988,89, Phillips 1979, Phillips and Yim 1981, Talbot 1985) there are no published studies on rates of uptake and depuration of either trace metals or organochlorines.

The experiments in this Chapter examine rates of uptake and depuration of trace metals and organochlorines by oysters (*S. commercialis)* under field conditions. Traditionally, these types of experiment are done in laboratories under artificial conditions and rarely with complex mixtures of contaminants. The aqueous concentrations of contaminants that are used are also usually far in excess of those expected in the environment (e.g. Ward 1982) because of the difficulty of measuring and maintaining very low concentrations. The results of laboratory studies may, in many situations, have questionable value when interpreting data from field studies. As a consequence, all uptake and depuration experiments described in this chapter have been done under field conditions. The locations used were chosen because they

have been shown in previous studies to be consistently contaminated by either trace metals or organochlorines.

Several experiments were used to examine hypotheses about rates of uptake and depuration of organochlorines and trace metals. Detailed descriptions of these experiments are provided below. Little was know about the kinetics of organochlorines, so an initial exploratory study (Experiment 1) was done. This provided information to suggest that studies of longer (Experiment 2) and shorter (Experiment 3) time-scales were warranted.

The experiments described are:
1) an initial examination of short-term changes (2 weeks) in both organochlorines and trace metals near the North Head (Sydney, Australia) sewage outfall;
2) long-term study of organochlorines in Long Bay near Tunks Park (hereafter referred to as Tunks Park) (Middle Harbour, Sydney).
3) very short-term study (2 days) of depuration of organochlorines in commercial oyster farmer's depuration ponds (Georges River);
4) longer-term study of trace metals (principally copper, lead, zinc and cadmium) near Cockle Creek, Lake Macquarie (see Chapter 6);

The overall aim of this chapter is to determine the time taken to reach equilibrium and the clearance rates for a range of commonly encountered contaminants. This information is crucial to planning and interpreting studies which utilise *Saccostrea* as an indicator of the presence of bio-available contaminants.

Experiment 1 Uptake and depuration of trace metals and organochlorines near North Head Sewage Treatment Plant

Introduction

During September 1991 the North Head sewage treatment plant, which was at the time discharging through deepwater outfalls 3 km from shore, was forced to divert sewage to a shore line outfall for two weeks while they undertook maintenance of the deepwater outfall. This provided an opportunity to expose oysters to sewage effluent for a short time and examine uptake and subsequent depuration of contaminants.

Aim

To examine the effects of time and distance from the outfall on concentrations of organochlorines and trace metals in *S. commercialis* placed near a major sewage outfall at North Head, Sydney. Knowledge of changes in concentrations of contaminants during fixed time periods will allow preliminary calculations of rates of uptake and depuration.

Methods

Oysters from Georges River were deployed on moorings near two control locations (Dee Why and The Gap) and near North Head shoreline sewage outfall (Fig 4.1.1) during September 1991 when untreated effluent was discharged for two weeks. Two moorings were placed at each control, and two were placed at each of two sites (20m to the north and 100m to the south) at the sewage outfall. The moorings consisted of a polystyrene float 10m above a ground weight and were established in 20 m of water at each location. Sufficient oysters were placed on each mooring to allow the retrieval of samples according to the schedule in Table 4.1.1 to be followed. Discharge began 4 days after the initial deployment. Divers in dry-suits retrieved the samples from within the barely diluted raw sewage plume (20m site).

Oysters were prepared and analysed for organochlorines and trace metals according to the methods outlined in Chapter 2.

For the period of the experiment in which concentrations of contaminants decreased, biological half lives (BHL, a measure of rates of depuration) were calculated. Half-lives for organochlorines were calculated for concentrations expressed per wet weight and were estimated as:

$$BHL = \log_e 2/k_2$$

where k_2 is the slope of the linear regression of $\log_e(C_t/C_0)$ on time and is called the rate loss constant; C_t is concentration at Time t; and C_0 is concentration at Time 0 (from Tanabe et al. 1987).

The theoretical time to equilibrium (t_{eq} ; which is actually 90% of theoretical final equilibrium) was calculated using the formula $t_{eq} = 2.3/ k_2$ (Hawker and Connell 1986; Tanabe et al. 1987; where k_2 is the rate loss constant).

Biological half-lives for trace metals were calculated using the method from Okazaki and Panietz (1981) which involves calculating the linear regression between time and the logarithm of the percentage loss from time zero. The slope of this regression (k) is substituted into the equation BHL = log 2 / k.

Table 4.1.1 Number of oysters per mooring removed from moorings, * indicates effluent being discharged.

Date	Days from start		Control OC	Control TM	Outfall OC	Outfall TM
28/8/91	0		10	10		
2/9/91	5	*	5	5	5	5
4/9/91	7	*			5	
6/9/91	9	*			5	
11/9/91	14	*	5	5	5	5
16/9/91	19	*	5	5	5	5
23/9/91	26				5	
16/10/91	49				5	

Results

Survival and Weight of Oysters

There was 100% survival of oysters on all moorings. Wet weights of oysters showed some temporal variability but no overall trends of increase or decrease (Fig 4.1.2).

Concentrations of Organochlorines

Unfortunately, the oysters were mildly contaminated with organochlorines prior to deployment. This could possibly have masked some information on uptake. Despite this, significant increases in the concentrations of chlordane, dieldrin and PCB occurred in oysters from outfall moorings between 0 and 5 days (Fig 4.1.2). These organochlorines were also all in greater concentrations in samples from moorings 20 m from the outfall than in samples 100 m from the outfall (Fig 4.1.2; Table 4.1.2 A). The difference in concentrations of chlordane at the 20 m and 100 m moorings was evident until 26 days after deployment, but concentrations of PCBs and dieldrin were no longer different after 9 and 14 days (respectively). Concentrations of chlordane, dieldrin and PCB in samples collected from outfall moorings were greater than those from control moorings after 5 days, but were not different after 14 and 19 days (Fig 4.1.2, Table 4.1.2 B). These data indicate a gradient in organochlorine concentrations

away from the outfall during the first few days of the deployment which was not evident later in the experiment.

Chlordane concentrations in oysters from near the outfalls had risen sharply by 5 days after deployment (Fig. 4.1.2) and then declined markedly over the next 4 days. Concentrations rose for the next 10 days. The concentration after 60 days was not significantly different from that at 19 days. Concentrations of chlordane in control oysters declined initially and then stabilised to a level not significantly different from that in the oysters at the outfalls after 14 days (Table 4.1.2).

Concentrations of dieldrin and PCBs followed a similar pattern to that shown by chlordane, but concentrations began to decline in oysters 100 m from the outfall earlier than in those 20 m from the outfall. Concentrations of total DDT did not show any initial increase in oysters near the outfall and decreased at the same rate as those in oysters from controls (Table 4.1.2).

The temporal patterns shown here are consistent with the model that organochlorine concentrations in the sewage effluent were not constant and tended to occur as pulses (one between 0 and 5 days, another from 10 to 19 days). Limited data from the analyses of the effluent in the plants (EPA 1996) confirms that organochlorine inputs to the plants tend to occur in pulses ontop of a low level chronic input.

Biological half-lives (a measure of rates of depuration) were calculated for dieldrin and total DDT (which did not show significant uptake at the outfalls) using data from oysters at control locations only (Table 4.1.3 a). Chlordane and PCB did show significant accumulation at the outfalls and BHLs were calculated for oysters from outfalls and controls separately (Table 4.1.3 a). There was a large discrepancy between BHLs from outfalls and controls (e.g. chlordane 4 and 38 days respectively), with faster rates of depuration occurring at the outfalls. Times to equilibrium were generally in the order of 3 to 5 weeks, except for chlordane which showed the two most extreme times, 12 and 125 days.

Table 4.1.2 (A) Results of analyses of variance comparing two outfall locations at all times.
*** $P < 0.05$, ** $P < 0.001$**

Chlordane

	d.f.	Sum-squares	Mean Square	F		Divisor
Time	6	0.106	0.018	4.38	**	resid
Near vs Far	1	0.017	0.017	4.22	*	resid
T x N	6	0.009	0.002	0.89		resid
Resid	126	0.509	0.004			

Dieldrin

	d.f.	Sum-squares	Mean Square	F		Divisor
Time	6	0.025	0.004	42	**	resid
Near vs Far	1	0.0003	0.0003	3.49		resid
T x N	6	0.003	0.0005	5.31	**	resid
Resid	126	0.012	0.0001			

PCB

	d.f.	Sum-squares	Mean Square	F		Divisor
Time	6	3.334	0.555	19.95	**	resid
Near vs Far	1	0.023	0.023	0.83		resid
T x N	6	0.944	0.157	5.65	**	resid
Resid	126	0.351	0.027			

Total DDT

	d.f.	Sum-squares	Mean Square	F		Divisor
Time	6	0.042	0.007	9.33	**	resid
Near vs Far	1	0.0009	0.0009	1.20		resid
T x N	6	0.005	0.0009	1.20		resid
Resid	126	0.094	0.0007			

Table 4.1.2 (B) Summary of analyses between outfalls (data pooled) with controls (data pooled) after 5, 14 and 19 days * $P < 0.05$, ** $P < 0.001$

Chlordane

	d.f.	Sum Squares	Mean Square	F		divisor
Time	2	0.044	0.022	4.79	*	resid
Out vs Cont	1	0.022	0.022	4.75	*	resid
T x O	2	0.017	0.008	1.84		resid
Resid	114	0.525	0.005			

Dieldrin

	d.f.	Sum Squares	Mean Square	F		divisor
Time	2	0.037	0.018	21.4	**	resid
Out vs Cont	1	0.004	0.004	4.52	*	resid
T x O	2	0.004	0.002	0.09		resid
Resid	114	0.097	0.0008			

PCB

	d.f.	Sum Squares	Mean Square	F		divisor
Time	2	3.155	1.578	73.31	**	resid
Out vs Cont	1	0.359	0.359	16.66	**	resid
T x O	2	0.107	0.053	0.08		resid
Resid	114	2.453	0.022			

Total DDT

	d.f.	Sum Squares	Mean Square	F		divisor
Time	2	0.064	0.032	8.19	**	resid
Out vs Cont	1	0.001	0.001	0.34		resid
T x O	2	0.002	0.0009	0.79		resid
Resid	114	0.443	0.004			

Table 4.1.3 a Calculated times to equilibrium and biological half lives for organochlorines

Compound	Time to equilibrium (days)	BHL (days)	r^2	k_2
Dieldrin	18	6	0.95	0.126
total DDT	34	21	0.93	0.068
Chlordane (outfall)	12	4	0.92	0.188
Chlordane (control)	125	38	0.62	0.018
PCB (outfall)	23	7	0.61	0.102
PCB (control)	37	12	0.95	0.061

Table 4.1.3 b Calculated biological half lives for trace metals

Metal	BHL (days)	Slope	r^2	significance
Lead	35	0.008	0.53	$P < 0.05$
Cobalt	33	0.009	0.65	$P < 0.05$
Selenium	76	0.004	0.73	$P < 0.05$

Trace Metals

There were no significant differences in metal concentrations among locations or moorings within locations. There were significant changes in trace metal concentrations among times for Cr, Co, Ni, Hg and Pb and significant interactions between time and location for Ag and Se (Fig. 4.1.3). With the exception of lead, cobalt and selenium, all the other changes appeared to be random variation showing no interpretable temporal patterns. Lead, cobalt and selenium showed no evidence of uptake, but showed clear patterns of depuration. Biological half lives were 35 days for lead, 33 days for cobalt and 76 days for selenium (Table 4.1.3 b).

Discussion

This experiment has shown that oysters were able to show gradients of organochlorine contamination at small (100m) and larger (5km) scales and that they respond quickly to changes in the organochlorine concentrations in the water. The rapid uptake of organochlorines early in the period of discharge and subsequent depuration are taken as indicators that concentrations of organochlorines are not constant in the discharged sewage. This conclusion is supported by data from sewage treatment plant compliance reports which also show considerable temporal variation in organochlorine contamination of raw sewage. The lack of any evidence for uptake of DDT is consistent with other studies of the sewage outfall (see Chapter 5) which also showed no elevated DDT near sewage outfalls.

There were differences in rates of depuration for outfall (recently contaminated) and control (chronically contaminated) oysters. This difference may be a result of differences in the way that recently acquired contaminants (from an intense pulse of organochlorines, as appears to have happened here) are biochemically bound in the animal compared to the situation for organochlorines which have been acquired as a result of chronic long-term exposure (control oysters prior to deployment). Vreeland (1974) suggested that first-order kinetic partitioning of organochlorines with lipids may be too simple a model to explain organochlorine kinetics. The results from this study could provide further support for this conclusion.

The biological half lives and times to equilibrium determined from this study are, however, considered extremely preliminary. They are based on regressions of 3 or 4 data points and are thus extremely susceptible to being significantly influenced by any confounding or random shifts in the data. The preliminary nature of the organochlorine data in particular is supported by consideration of the ranking of half

lives. Kinetic theory (Hawker and Connell 1986) suggests half lives and times to equilibrium should increase as a compound's affinity for lipids increases. If this were true, then the ranking should be dieldrin, chlordane, DDT, PCB. The data from this study do not rank in this order. Another factor which could be contributing to uncertainty in the data is the variable nature of the contamination, noted above.

The lack of trends for most metals is not unexpected as the period of the study was less than published equilibration times and half-lives (Okazaki and Panietz 1981) for most metals.

The good survival and unchanged condition (weight) of oysters 20 m from the outfall (which was raw sewage diluted about 1:10) emphasises the ability of this animal to be used as a biomonitor in a wide range of conditions.

Conclusions

Future uptake experiments should be done in an area where contamination is likely to be more constant. Organochlorine kinetics should be considered on a variety of time scales, ranging from 1 or 2 days to 4 to 6 months. Trace metal kinetics are slower and probably only need to be dealt with at scales of weeks to months.

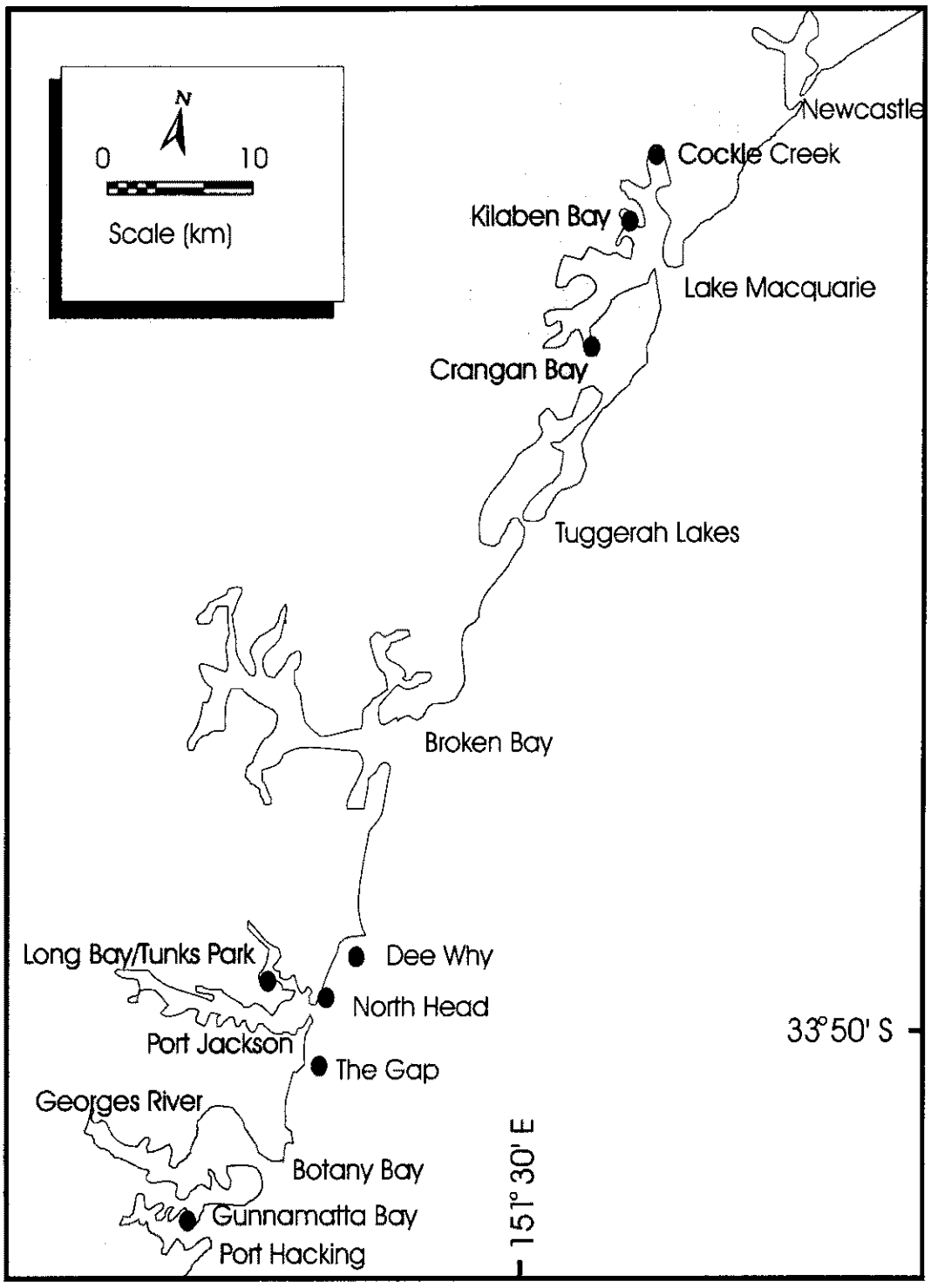


Figure 4.1.1 Locations of experiments on uptake and depuration of trace metals and organochlorines.

- **Impacted locations**
- **Control locations**

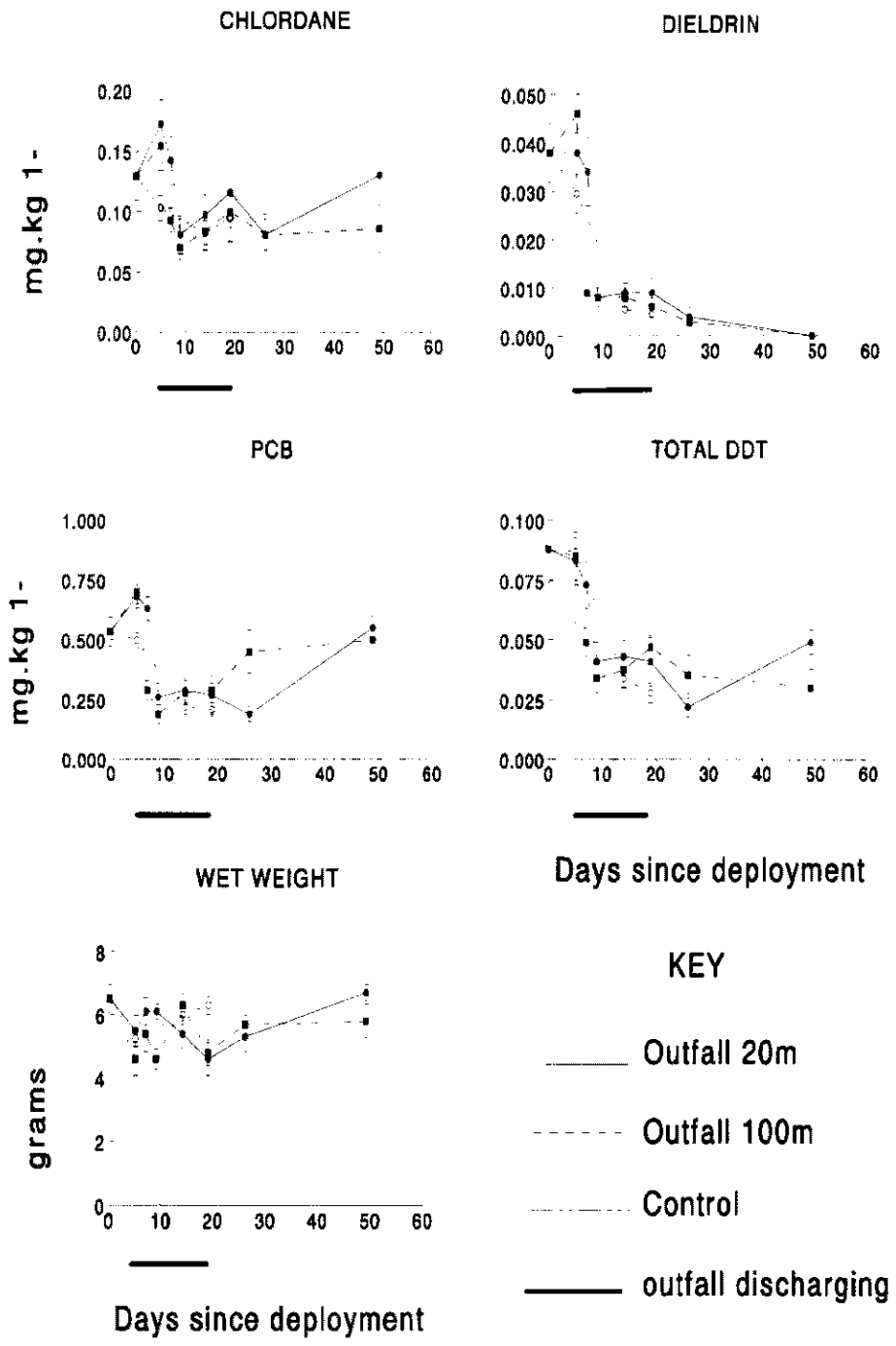


Figure 4.1.2 Mean (± 1SE) concentrations of organochlorine compounds and wet weights of oysters.

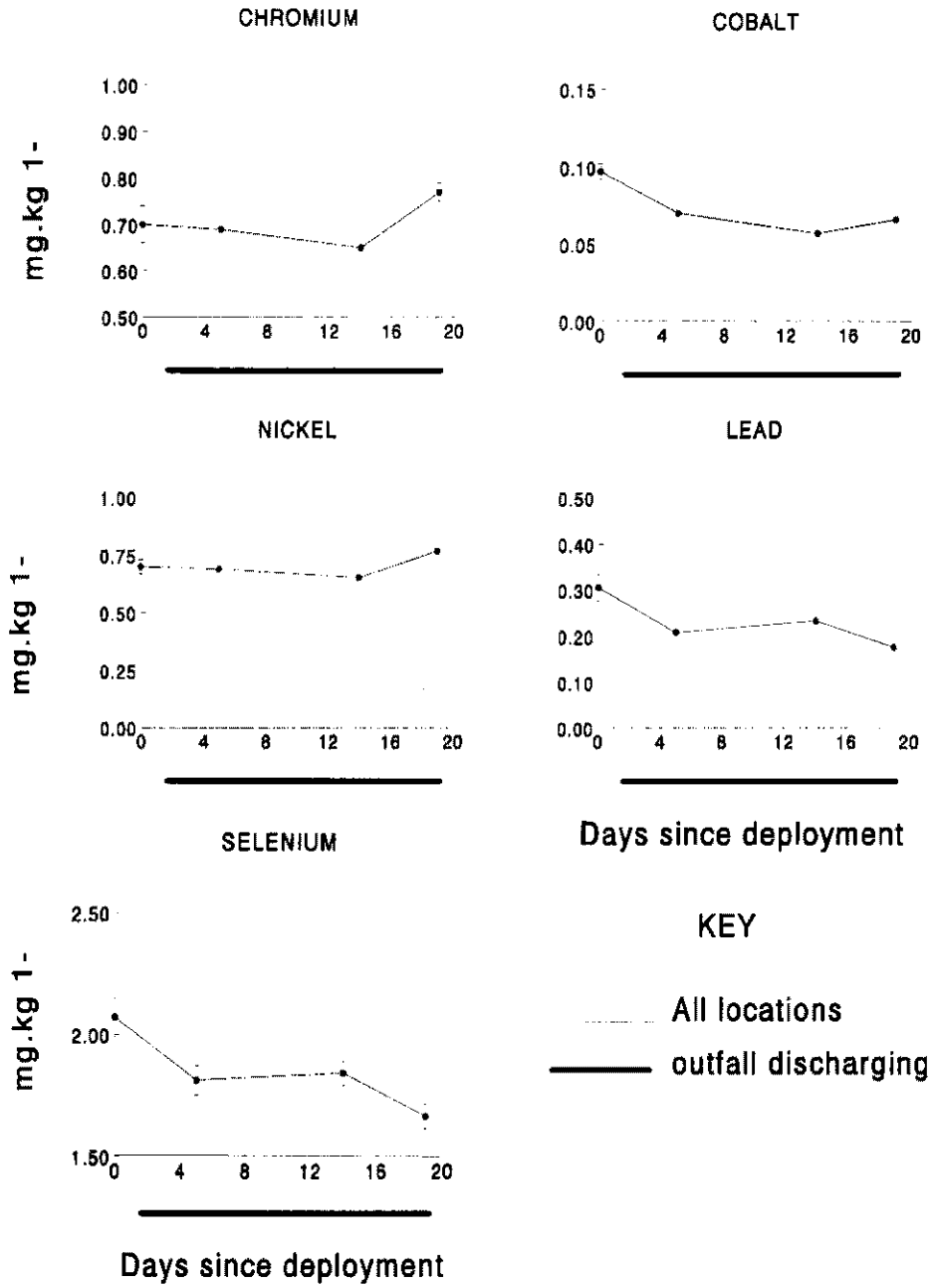


Figure 4.1.3 Mean (± 1SE) concentrations of trace metals.

Experiment 2 'Uptake and depuration of organochlorine compounds by the Sydney rock oyster (*Saccostrea commercialis*)',(Scanes 1997)

Introduction

Experiment 1 produced some data on organochlorine kinetics but, for a number of reasons, the data were only considered preliminary. It was nevertheless concluded in that short and long time-scales needed to be considered in future investigations of the kinetics of organochlorines and that the investigations should preferably be in areas with more consistent contamination.

The experiment described here takes into account the conclusions of Experiment 1 and attempts to provide better data on the medium-term kinetics of organochlorines in *Saccostrea*. It tests the hypothesis that concentrations of organochlorines in uncontaminated oysters will increase when placed contaminated waters and will subsequently decrease when those oysters are moved to uncontaminated waters. The tests of these hypotheses will provide data to allow the calculation of the biological half lives and times to equilibrium for various compounds.

The results of this experiment have been published (Scanes 1997). A copy of that paper is included as Appendix 4 and a summary is provided here.

Methods

A pool of approximately 80 uncontaminated oysters were deployed in February 1993 in open mesh polyethylene bags in Long Bay (Sydney Harbour, NSW Australia) where it is known that concentrations of organochlorines are large (chlordane in wild oysters 0.5 - 0.8 mg/kg - Scanes, Gibson and Dasey 1997). From this initial pool of oysters, five replicate individuals were removed for analysis after each of 0, 3, 12, 20, 76, 91, 125, 148 and 209 days. After 209 days, the remaining oysters were removed from the water at Long Bay and moved to Gunnamatta Bay, Port Hacking (an area not contaminated by organochlorines - Scanes, Gibson and Dasey 1997). Five replicate oysters were taken from the pool of oysters in Gunnamatta Bay after each of 1, 4, 12, 20, 40 and 56 days.

Results

Biological Variables

The mean wet weight of oysters was 9.5 g. There were significant changes in weight during the deployment but the changes did not follow any obvious pattern and were not able to be separated by SNK tests. The changes simply represent variation among the random samples taken at each time . Mean content of lipid was 2.4 % of wet weight and did not vary significantly during the experiment.

Contaminants

Prior to deployment (Day 0) the oysters had traces of DDE and one out of five had a low concentration of chlordane (0.01 $mg.kg^{-1}$). The oysters rapidly accumulated organochlorines, all showing detectable concentrations of heptachlor, heptachlor epoxide, chlordane, DDE, DDD, DDT, dieldrin and PCB after 3 days. The concentrations of heptachlor and heptachlor epoxide did not change from trace and 0.01 $mg.kg^{-1}$, respectively and so were not analysed statistically. Concentrations of DDT, DDD and DDE were also low (trace or just above practical quantifiable limits), so the concentrations of these compounds were pooled to estimate total DDT (ΣDDT) for statistical analyses and calculations of times to equilibrium and biological half lives.

Concentrations of chlordane (per unit wet tissue weight) initially rose rapidly and the rate of increase decreased dramatically between 12 and 20 days, this is considered the initial equilibrium. Dieldrin reached initial equilibrium between 3 and 12 days and PCB between 12 and 20 days. Concentrations of ΣDDT initially rose rapidly (from 0 to 20 days), then continued to rise more slowly for the rest of the uptake deployment. Connell (1988) recommended that organochlorine concentrations in monitoring studies should be expressed per unit weight of lipid. To examine whether this would lead to different conclusions than those made from data expressed per unit wet weight, the concentrations of chlordane have also been expressed as a ratio of organochlorine concentration to content of lipid. The same patterns that existed in the chlordane data based on wet tissue weight were evident in the lipid-corrected data.

When the oysters were moved to uncontaminated waters (after 209 days) the concentrations of all contaminants began to rapidly decrease. The temporal pattern of concentrations of organochlorines in this depuration phase showed the expected

exponential decay and was well modelled by linear regression of the log concentration.

Concentrations of chlordane, dieldrin and ∑DDT after initial equilibrium were strongly correlated with content of lipid, but PCBs were not as tightly correlated (Table 4.2.1).

Biological half-lives (BHLs - a measure of rates of depuration) and times to equilibrium calculated per unit wet weight and lipid weight were very similar for dieldrin, chlordane and PCB, but less similar for ∑DDT, which had the poorest fit for the two regressions (Table 4.2.2). The two methods show that chlordane and PCBs had similar biological half-lives.

Table 4.2.1 Results of regressions of percentage lipid (x) and concentrations of organochlorine compounds (y) in *Saccostrea commercialis*. Data are from oysters collected between days 21 and 148, because it was assumed that the oysters were in (or near) equilibrium with the environment during this period.

	Regression Equation	Probability	r^2
chlordane	y = 0.1144x - 0.0657	<0.001	0.77
dieldrin	y = 0.0135x - 0.0067	<0.001	0.70
ΣDDT	y = 0.0158x - 0.0114	<0.001	0.60
PCB	y = 0.0200x - 0.0970	<0.01	0.41

Table 4.2.2 Times to equilibrium and biological half lives (BHL) in days of organochlorine compounds accumulated in the Sydney rock oyster *Saccostrea commercialis*. Times were determined by three methods (see below). Theoretical times from Mortimer and Connell (1993) are provided for comparison.

Compound	time to equilibrium	BHL	k_2	r^2
(i) estimated from graphical plots of experimental data				
HPTE	less than 3			
Dieldrin	between 3 and 12			
Chlordane	between 12 and 20			
ΣDDT	72			
PCB	between 12 and 20			
(ii) calculated per unit of wet weight of tissue				
HPTE	13	4	0.170	0.77
Dieldrin	40	12	0.057	0.96
Chlordane	79	24	0.029	0.93
ΣDDT	153	46	0.015	0.79
PCB	81	25	0.028	0.93
(iii) calculated per unit lipid weight of tissue				
HPTE	14	4	0.160	0.80
Dieldrin	53	16	0.043	0.99
Chlordane	109	32	0.021	0.97
ΣDDT	124	94	0.007	0.72
PCB	115	34	0.021	0.86
Theoretical times to equilibrium and half lives.				
HPTE	0.6	0.1		
Dieldrin	4	0.7		
Chlordane	20	3		
ΣDDT	50	7.6		
PCB	63	10		

Discussion

There are few published data with which to compare the rates of uptake and depuration measured in this study and most of what is available is only for PCBs. Vreeland (1974) found that PCB in small oysters (*Crassostrea virginica*) reached equilibrium in about 28 days, which is similar to the time taken to reach the initial plateau in the present study (12 - 20 days; Table 4.2.2, Fig. 2e) but less than the times to equilibrium which were calculated (Table 4.2.2). Sericano et al. (1992) found that BHLs for non-planar PCBs (which compromise the bulk of commercial PCB mixtures) in oysters (*Crassostrea virginica*) ranged between 17 and 76 days, which is also similar to the data presented here. Tanabe et al. (1987) found that PCBs reached equilibrium in the mussel *Perna viridis* in less than 40 days and calculated a BHL (using first-order bioconcentration kinetics - Connell 1988, Hawker and Connell

1986) of 7 to 12 days. In Young et al. (1976), concentrations of PCB and DDT in *Mytilus* were very variable, but not markedly increasing after about 30 days.

Studies which measured equilibria and BHL in mussels have shown times which are less than, but of the same order as those measured for oysters in this study. This is not surprising as Hawker and Connell (1986) have shown that there is a strong consistency in organochlorine kinetics among different bivalve molluscs. Sericano et al. (1992), however, noted that the BHLs for oysters in their study were longer than those for mussels (from Tanabe et al. 1987) and suggested that it was a consequence of expressing concentrations per unit tissue weight rather than per unit lipid weight. BHLs calculated here on both tissue-based and lipid-based concentrations were similar (i.e. maximum difference was x2), but the lipid-based times were usually slightly longer. This indicates that, since BHLs calculated per unit lipid weight are not necessarily longer than those calculated per unit tissue weight, there may be a small but real difference between BHLs in oysters and mussels.

The BHLs measured for oysters are, however, between 4 and 10 times longer than predicted rates of depuration (Table 4.2.2) calculated by Mortimer and Connell (1993) using the relationships derived in Hawker and Connell (1986). Times to equilibrium estimated from graphs were similar to those predicted by Mortimer and Connell (1993) except for PCBs. The estimation of these times is based on estimates of k_2. It is obvious from Table 4.2.2 that small changes in k_2 can result in relatively large changes in times to equilibrium and BHLs. It is therefore not surprising that the estimates of Mortimer and Connell (1993) are somewhat different to those calculated from field data. PCBs are a mixture of a large number of biphenyl compounds which all have different properties and different kinetics (Tanabe et al. 1987), the differences between measured and predicted rates for PCBs is not surprising and probably a result of these predictions being made for a different set of PCB compounds than those which occurred in my experimental samples.

According to first order kinetic models, the length of BHLs and times to equilibrium should increase as the compound's affinity for lipids (measured by the logarithm of the octanol:water co-efficient) increases (Hawker and Connell 1986). This is true for BHLs and times to equilibrium measured in this study for all compounds except PCBs. Because PCBs are a mixture of many compounds and were not quantified individually, it is difficult to predict the correct location on the scale that the specific PCB mix found should occur. The fact that PCBs had such a relatively short BHL

suggests that they were mainly composed of biphenyls with lower levels of chlorination (Sericano et al. 1992, Tanabe et al. 1987).

Hawker and Connell (1986) have shown that, if the uptake of organochlorines is entirely consistent with the simple first-order kinetics model, then the time to equilibrium should be "controlled only by the magnitude of the clearance rate constant [k_2]" (p. 186). Calculations of the theoretical t_{eq} on these data (Table 4.2.2) produced times which were longer than those estimated from graphs of field data, indicating that k_2 may not be the only factor controlling uptake. Vreeland (1974) also noted that calculated t_{eq} were much longer than measured t_{eq} for oysters and suggested that mechanisms other than simple lipid/water partitioning were involved.

There is good agreement between the BHL and times to equilibria estimated from wet weight data and lipid-corrected data. This occurs despite the strong correlation between organochlorine concentration and content of lipid. This is probably a consequence of the fact that, because the variation in content of lipid is small, the variation induced in organochlorine concentrations is concomitantly small compared to the changes which result from uptake and depuration.

In this study, there was a correlation between lipid and organochlorines. In contrast, in other more wide ranging studies of bioaccumulation by bivalves, however, (e.g. Scanes 1996, O'Connor 1992) where concentrations of organochlorines were somewhat lower, a correlation did not exist. These results suggest that where ambient concentrations of organochlorines are great and there are few other external influences (e.g. in laboratory studies or special cases in the field such as that described in the experiment here), the correlation between organochlorine and lipid can be strong. In more complex situations, however, and where ambient organochlorine concentrations are small, the correlation is weaker. Further, expressing organochlorine concentrations as means based on wet weights or means of organochlorine:lipid ratios did not alter the interpretation of the data. There is, it seems, little value to be gained in this study from expressing organochlorine concentrations as a ratio of lipid content, particularly since the relationship between the two was not significant in two large sets of large-scale field contaminant data (the NOAA Mussel Watch (O'Connor 1992 and pers. comm.) and NSW EPA Oyster Watch (Scanes 1996). This assumption is only true when factors other than lipid content are more important in controlling whole animal organochlorine concentrations.

These results have shown that *Saccostrea commercialis* has strong potential as an indicator of trends in ambient concentrations of a range of organochlorine compounds. It shows a rapid uptake and depuration and, as a consequence, is providing an integration of a relatively short period (days to weeks). This has implications for the design and interpretation of studies which use oysters to assess concentrations of contaminants in aquatic systems. In particular, if a longer time integration is required then "time-bulking" procedures (Phillips and Rainbow 1993, Phillips and Segar 1986) could be employed. This study used deployment techniques to introduce oysters to polluted areas where they were not naturally found. There was very little mortality and this technique, which allows a definite starting point for accumulation in the study, would be recommended where oysters are being used to determine to pollution status of previously unstudied waters or to follow a time course of pollutant concentrations.

Saccostrea commercialis is widespread along the east coast of Australia and the closely related *Saccostrea cucullata* is found throughout tropical Australia. These species have been used to monitor trace metal and PAH concentrations in Australian waters (Brown and McPherson 1992, Mackay et al. 1975, Peerzada and Dickinson 1989, Pendoley 1992, Scanes 1993, 1995, Talbot 1986). Even allowing for probable inter-specific differences, these two species could provide an extensive biomonitoring network in temperate and tropical Australia and even further afield.

Experiment 3 Short term depuration of organochlorines

Introduction

The results of Experiment 1 (above) indicated that great changes in the concentrations of organochlorines could occur in a period of a few days. This experiment examines the depuration of organochlorines from oysters over two days. Oysters with moderate or very large starting concentrations of organochlorines were used. Counts of bacteria were also monitored to confirm that the oysters were open and active during the relatively short depuration period.

The experiment tested the hypothesis that significant changes in concentrations of organochlorine compounds accumulated in *S. commercialis* can occur over time scales of ranging from hours to 2 days.

Methods

Oysters from Georges River (moderate organochlorine contamination) and from an earlier deployment in a heavily contaminated area (Tunks Park) were placed in a commercial oyster depuration facility in November 1992. The facility is designed to reduce bacterial contamination of oysters and consists of a large tank (10 x 3 x 2 m) with recirculating water. The water (initially from the Georges River) passes through a filter and an ultraviolet light into a tank with a flow-through system which circulates the water through the filter and U.V. light. The water in the tank had a salinity of 34.1 ‰ and was kept at a constant temperature of 19 °C by a thermostat.

Oysters from each of the two sources were divided into two batches and placed into plastic tubs, which were in turn placed in random locations within the large tank. At time zero, 5 oysters from each Georges River tub were randomly selected to represent starting concentrations. Because there were few Tunks Park oysters, four were retained to represent starting condition for both Tunks Park tubs. Oysters were then removed from the tubs after 2, 12, 24, 36 or 48 hours according to the schedule in Table 4.3.1. Note again that fewer Tunks Park samples were available. The oysters were placed in plastic bags and frozen. The oysters for the final bacteria sample were placed in a hessian bag so the oysters remained closed.

Levels of bacterial contamination were measured by the Department of Health Analytical Laboratories using standard methods. This involves making a composite sample of 10 individual oysters and counting bacterial colonies on culture plates.

Table 4.3.1 Time of sampling of oysters during depuration experiment. The top line shows the number of hours since commencement and the entries show the total number of samples removed at each time.

Hours	0	2	12	24	48
Georges River	4	10	10	10	10
Tunks Park	10	10	5	5	10

Sufficient oysters were included in the experiment to allow 10 independent counts before depuration and 10 after 36 hours of depuration.

All organochlorine samples were processed and analysed according to standard protocols.

To increase power of tests, data for the two tubs from each source were pooled and analysed by one factor analysis of variance to determine differences between times.

Results

Bacterial Contamination

There was considerable bacterial contamination prior to depuration and this was significantly reduced after 36 hours depuration (faecal coliforms 1.06 to >0.3 fcu/g, $P < 0.05$; Standard plate count 561 to 79.5 counts/g, $P < 0.001$).

Organochlorine Contamination

Concentrations of chlordane in Georges River oysters did not change significantly during the experiment (ANOVA, $P > 0.35$). A *t*-test for differences between starting and final means for Georges River oysters was not significant ($P = 0.2$, Power 0.9 for H_a of 40% change).

Concentrations of chlordane in the greatly contaminated Tunks Park oysters did change significantly during the experiment (ANOVA, $P < 0.005$). Levels dropped significantly during the initial 2 hrs (SNK test, $P < 0.05$) , but did not change significantly during the following 46 hours (Fig. 4.3.1).

Oxychlordane, heptachlor and heptachlor-epoxide were significantly reduced in Georges River oysters ($P < 0.001$) during the initial two hours (Fig. 4.3.1). No other compounds showed any changes in Georges River oysters (Fig. 4.3.1).

In Tunks Park oysters, there were significant reductions in dieldrin, DDD, DDT and Σ DDT in the first two hours, non-significant decreases in the concentrations of oxychlordane, DDE and PCB were also evident in Tunks Park oysters within two hours (Fig. 4.3.1) . There was no evidence of a decrease in the concentration of heptachlor or heptachlor-epoxide in Tunks Park oysters for the duration of the experiment.

Conclusions

The rapid initial decrease in concentrations of chlordane, dieldrin and DDT isomers in Tunks Park oysters is unexpected in terms of generally accepted models of organochlorine kinetics (Connell 1988). It does suggest that, in the oysters with very large concentrations, some of the organochlorines are either not firmly biochemically bound into the animal and or its lipids. This provides some support for the ideas of Vreerland (1974), Phillips (1978) and Phillips and Rainbow (1993) who have suggested that organochlorine kinetics is a complex combination of the influences of factors such as ability to accumulate and depurate from gut, varying rates of organochlorine metabolism and binding as well as lipid content and chemical type. Shaw and Connell (1978) observed that depuration seemed to proceed in two stages and cited the explanation of Moriarty (1975) that organochlorines could be sequestered in compartments with different rates of clearance. These processes are probably not the same for different organochlorines within one species let alone for different species. This work has shown that for some organochlorines, starting concentrations may also affect fine-scale kinetics.

Thus, there was some evidence from the Tunks Park oysters that supported the rapid changes observed in chlordane in Experiment 1. The lack of any change after the first two hours suggests that after the initial, rapid changes the oysters had now

equilibrated with the organochlorines in solution within the closed system in which they were maintained.

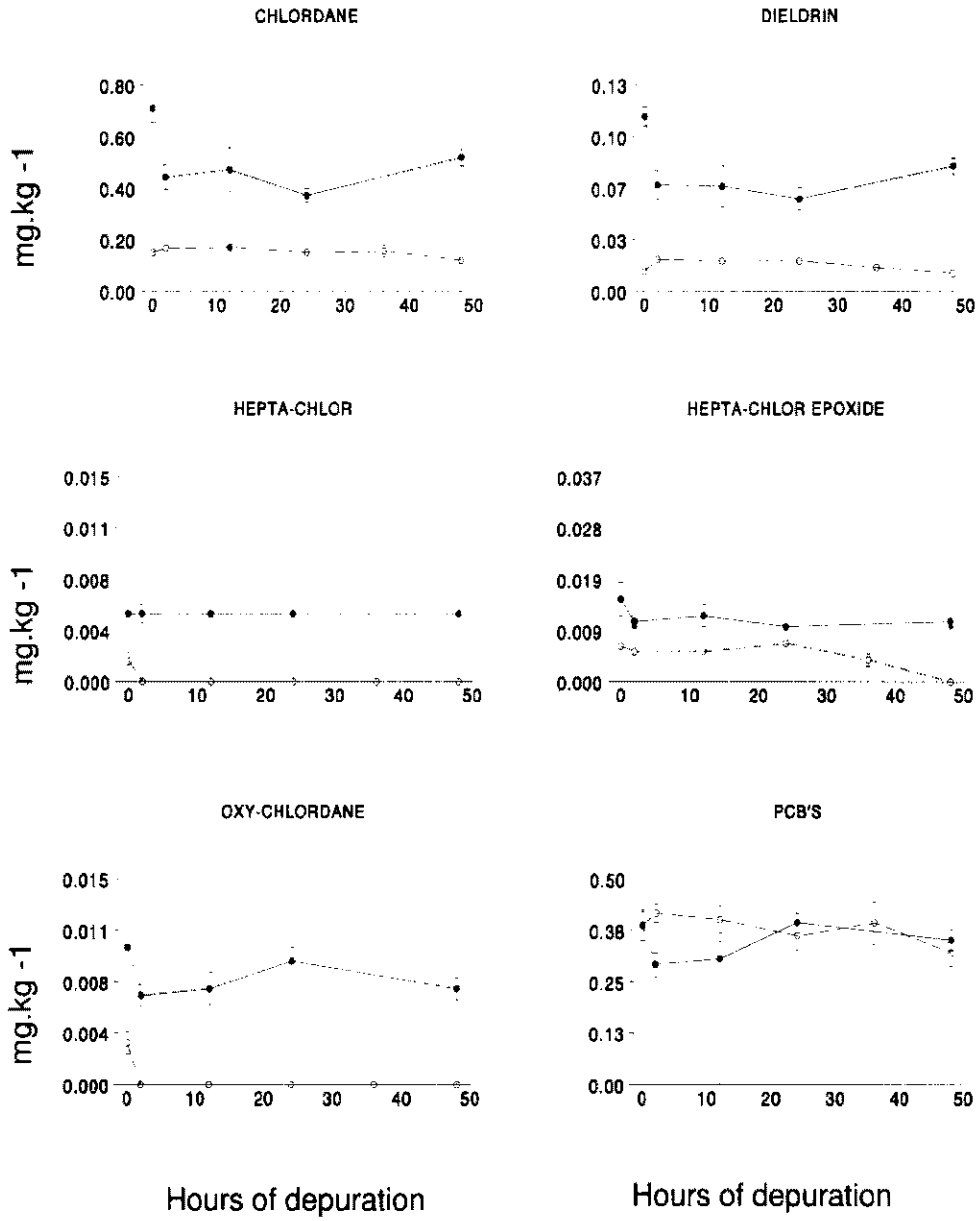


Figure 4.3.1 Mean (±SE) concentrations of organochlorines in oysters over time. "Tunks Park" oysters (closed symbols) were highly contaminated; "Georges River" (open symbols) oysters were moderately contaminated.

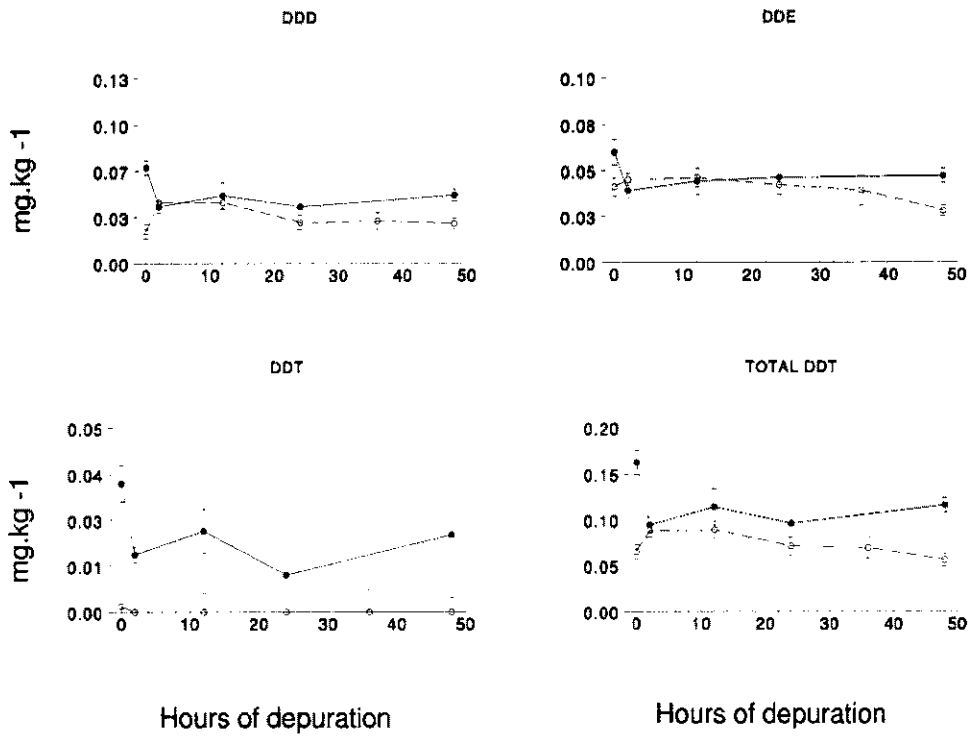


Figure 4.3.1 (cont) Mean (±SE) concentrations of organochlorines in oysters over time. "Tunks Park" oysters (closed symbols) were highly contaminated; "Georges River" oysters (open symbols)were moderately contaminated.

Experiment 4 Uptake and depuration of trace metals

Introduction

The previous experiments (2 and 3) have been extensions of investigations of the kinetics of organochlorines. This experiment examined the rate of uptake and depuration of trace metals by oysters. It was done in Lake Macquarie, where there is a known source of copper, lead, zinc, cadmium and probably chromium contamination at Cockle Creek (Fig 4.1.1, Batley 1988, Scanes 1993, this thesis Chapter 6).

Aim

To test hypotheses about the effects of distance from source and time of exposure to contaminated and uncontaminated waters on the concentrations of trace metals accumulated by *S. commercialis*.

Methods

Oysters from Georges River were deployed in September 1992 on mid-water (approx. 1 m depth) from moorings in the mouth of Cockle Creek and approx. 200m from the mouth of Cockle Creek and in Crangan and Kilaben Bays (control locations in Figure 7.1) The concentrations of zinc, cadmium, lead and copper in surface waters at these locations are shown in Table 4.4.1). There were three moorings at each location. Five replicate oysters were removed from the moorings according to the schedule in Table 4.4.2. After 12 weeks, most of the remaining oysters were moved from the Cockle Creek moorings to the Kilaben Bay moorings. The remaining oysters on each mooring at Cockle Creek were removed and then replaced as a control for disturbance. No controls for translocation (as distinct from disturbance) were included as other studies (e.g. Chapter 6) have shown this is not a significant influence on concentrations of trace metals.

Table 4.4.1 Dissolved metal concentrations in surface waters ($\mu g.l^{-1}$) from Batley (1987). * indicates concentrations from Batley's closest site

	Zn	Cd	Pb	Cu
Cockle Creek and Bay	28	2.5	2.8	1.5
Kilaben Bay*	5.6	1.5	1.7	3
Crangan Bay*	4.9	0.4	1.6	2.3

Oysters that were retrieved were prepared and analysed for trace metals according to the methods outlined in Chapter 2. Although the full suite of metals was analysed,

results will only be considered for zinc, cadmium, lead and copper which are known to be elevated in sediments in the vicinity of Cockle Creek (Batley 1987) or for metals which showed an obvious pattern of depuration (mercury and silver) or uptake (selenium) after placement in Lake Macquarie.

Table 4.4.2 Schedule for removal of oysters. "Week" is number of weeks since experiment began. R - original oysters removed, T - oysters translocated to controls, RT - translocated oysters removed, Cockle Ck - Cockle Creek moorings, Control - control moorings.

Week	0	1	2	5	9	12	13	14	17	21	24
Cockle Ck	R	R	R	R	R	R,T			R	R	R
Control	R					R	RT	RT	RT,R	RT,R	RT,R

For logistical reasons (primarily cost) it was not possible to take oysters from all sites simultaneously. The sampling scheme used (Table 4.5.2) has resulted in different numbers of oysters at each time and not all sites being sampled at each time. It is therefore not possible to combine all the data into a single analysis. The following series of analyses of variance was used, in conjunction with the figures, to determine significant trends in the data. Because I was not hypothesising about variability within sites, data from all moorings at a site were pooled. This procedure means that any variance within sites is not explicitly differentiated from variability among replicates and is included in the residual term of the analysis. It does not, however, lead to pseudoreplication as described by Hurlbert (1983) because the replicates cover the entire spatial extent of the site.

Analysis 1. Sites at Cockle Bay over time. Two factor, orthogonal, both factors fixed.
Analysis 2. Control sites over time. Two factor, orthogonal, both factors fixed.
Analysis 3. Cockle Bay compared to Controls, times 12 and 24 weeks. Three factors, Treatments, Sites within Treatments and Time. Orthogonal factors fixed.
Analysis 4. Translocated Oysters through Time. Two factor, orthogonal, both factors fixed.
Analysis 5. Controls compared to Translocated, time 12 and 24. Two factor, orthogonal, both factors fixed.

Biological half lives (BHL) were calculated by determining the linear regression between the percentage of the original mean concentration remaining at each time and

time. The slope of this regression (a) was then substituted in the equation BHL = log $2/a$ (Okazaki and Panietz 1981). Regressions have been fitted to describe the uptake of selenium and cadmium (linear) and lead (logarithmic).

Results

Weight

There were no differences between weights of oysters in the various treatments (Cockle Creek, controls, translocations), but there was temporal variability. In general, weights of oysters increased over the term of the experiment (Fig 4.4.1).

Trace Metals

In general, there was no difference between concentrations of trace metals in oysters in Cockle Creek and Cockle Bay, nor between the two control locations, so, in the following descriptions they have been considered simply as two treatments - contaminated (Cockle Creek and Bay) and controls.

Concentrations of lead in oysters at Cockle Creek rose rapidly for 2 weeks then more slowly up until 12 weeks (Fig. 4.4.1). The concentrations after 24 weeks were not different from those at 12 weeks. Concentrations at controls were not different through time but were significantly smaller than those at Cockle Creek. Concentrations in translocated oysters dropped rapidly for 5 weeks then more slowly until they were not different from controls after 24 weeks (Appendix 2).

Concentrations of cadmium at Cockle Creek increased for 12 weeks, but after 24 weeks concentrations were considerably smaller than those at 12 weeks (Fig. 4.4.1). Concentrations at controls increased slightly to 12 weeks, were not different from 12 weeks at 17 weeks and decreased slightly to 24 weeks. Concentrations in translocated oysters decreased to be only slightly greater than controls (Appendix 2).

Concentrations of zinc at Cockle Creek did not change significantly (Fig 4.4.1). Concentrations at controls decreased slowly for the first 12 weeks, then more quickly and were significantly smaller than Cockle Creek after 24 weeks but not after 12 weeks. Concentrations in translocated oysters initially increased then decreased to be not different from controls by 24 weeks (Appendix 2).

Concentrations of copper decreased at Cockle Creek for the term of the experiment (Fig. 4.4.1). Concentrations at controls dropped more slowly and remained significantly greater than those at Cockle Creek. Concentrations in translocated oysters initially increased rapidly and then followed the same time trajectory as controls to be not significantly different from controls by week 24 (Appendix 2).

Concentrations of selenium increased continually at all locations (Fig. 4.4.1). Concentrations at Cockle Creek were generally slightly less than at the controls (Appendix 2).

Concentrations of mercury and silver at Cockle Creek decreased rapidly for 2 weeks and then more slowly and seemed to equilibrate by 6 to 9 weeks (Fig. 4.4.1). At 12 weeks, concentrations of both metals had risen at the contaminated sites, and were not different from those at the controls. Thereafter, concentrations in oysters from all three treatments (contaminated, translocated and control) remained not different from each other and gradually declined to the same concentrations as those measured after about 9 weeks (Appendix 2).

Correlations

There were no really strong correlations among the different metals or between metals and tissue weight. Moderate correlations ($0.55 < r < 0.85$) were found for cadmium and chromium, zinc and copper, arsenic and selenium, arsenic and weight, cadmium and mercury, mercury and silver, mercury and weight (Table 4.4.3).

Table 4.4.3 Matrix of *r* values for correlations between metals and oyster weights. Correlations mentioned in the accompanying text are shown in bold.

	Cr	Co	Ni	Cu	Zn	As	Se	Cd	Hg	Pb	Ag
Co	0.55										
Ni	0.20	0.30									
Cu	0.38	0.18	0.01								
Zn	0.18	0.11	-0.2	**0.86**							
As	0.36	0.14	-0.2	0.40	0.51						
Se	0.08	0.02	0.05	0.40	0.48	**0.76**					
Cd	**0.58**	0.46	0.11	0.07	-0.1	-0.2	-0.5				
Hg	0.44	0.51	0.16	0.03	-0.2	-0.4	-0.5	**0.77**			
Pb	0.27	0.26	-0.1	0.06	0.01	0.26	0.01	0.36	0.11		
Ag	0.26	0.32	0.18	0.24	0.05	-0.4	-0.4	**0.70**	**0.64**	0.13	
Wt	-0.1	-0.4	-0.4	0.14	0.32	**0.55**	0.53	-0.5	**-0.6**	-0.1	-0.5

Kinetics

The only metals for which there was consistent uptake over more than 6 weeks were lead, cadmium and selenium. Linear regressions described the uptake of selenium and cadmium very well (Table 4.4.4). The slopes of the regressions indicate that cadmium is taken up 10 times faster than selenium. A logarithmic regression was a good fit for lead (Table 4.4.4) and showed that uptake was very slow after about 15 weeks, which could be considered the functional equilibrium point.

Table 4.4.4 Regressions describing uptake of Pb, Cd and Se by oysters (time in weeks).

Metal	Equation	r^2	Significance
Pb	y = 1.01 + 0.59lnx	0.70	$P < 0.005$
Cd	y = 0.68 + 0.28x	0.94	$P < 0.005$
Se	y = 0.91 + 0.02x	0.72	$P < 0.005$

Consistent depuration occurred for lead, copper, cadmium, mercury, silver and zinc and the regressions of time and log percentage loss provided a good fit for all metals (Table 4.4.5). Biological half lives ranged between 22 days for lead and 144 days for zinc.

Table 4.4.5 Calculated biological half lives (BHL) and regressions describing depuration of metals by oysters.

Metal	BHL (days)	Slope	r^2	significance
Pb	21	-0.10	0.93	$P < 0.005$
Cu	64	-0.03	0.96	$P < 0.005$
Cd	52	-0.04	0.93	$P < 0.005$
Hg	30	-0.07	0.75	$P < 0.005$
Ag	35	-0.06	0.91	$P < 0.005$
Zn	144	-0.01	0.92	$P < 0.005$

Discussion

The patterns found were not entirely expected. The oysters (from Georges River) were deployed with concentrations of metals, including copper and zinc, in excess of what they could accumulate in Cockle Creek. This led to many metals showing depuration behaviour instead of the expected uptake. Concentrations of lead and cadmium did, however, increase at Cockle Creek and then decrease after removal. Other metals such as selenium showed an general increase at all sites in Lake Macquarie.

Ward (1982) reported linear uptake of cadmium in *S. commercialis* exposed to 10 µg/l cadmium, but non-linear uptake at greater concentrations (25, 50 and 150 µg/l). Frazier and George (1983) showed linear uptake in two other oysters (*Crassostrea gigas* and *Ostrea edulis*) exposed to 100 µg/l Cd. Ward's data were expressed as dry weight concentrations, so I have converted them to wet weight (using an average moisture content of 90%, see Chapter 2). The new regression for 10 µg/l is $y = 0.82 + 0.31x$ ($r^2 = 0.95$). The intercept and slope of this regression are very close to those calculated for cadmium in this study (0.68, 0.28 respectively, Table 4.4.4) where dissolved Cd concentrations were about 2 µg/l (Batley 1987, Cockle Creek). The slightly greater slope of the regression for Ward's data probably represents the higher aqueous concentrations leading to a slightly faster rate of uptake.

Okazaki and Panietz (1981) have calculated BHLs for a range of trace metals in specific tissues (mantle, gill, digestive gland and kidney) of *Crassostrea gigas* and *C. virginica*(Table 4.4.6). With the exception of zinc, half lives for *S. commercialis* and *C. gigas* appear very similar (within an error of 1 to 2 weeks). Half lives for both species were shorter than those of *C. virginica*.

Table 4.4.6 Half lives (days) of trace metals in *S. commercialis* (Experiments 1 and 4, this study) and *Crassostrea gigas* and *C. virginica* (Okazaki and Panietz 1981). * no depuration evident in data.

	Expt 1	Expt 4	C. gigas	C. virginic
Pb	35	21	<7*	<7*
Cu		64	33	156
Cd		52	40	85
Hg		30	23	133
Ag		35	26	150
Zn		144	37	184
Se	76			
Co	33			

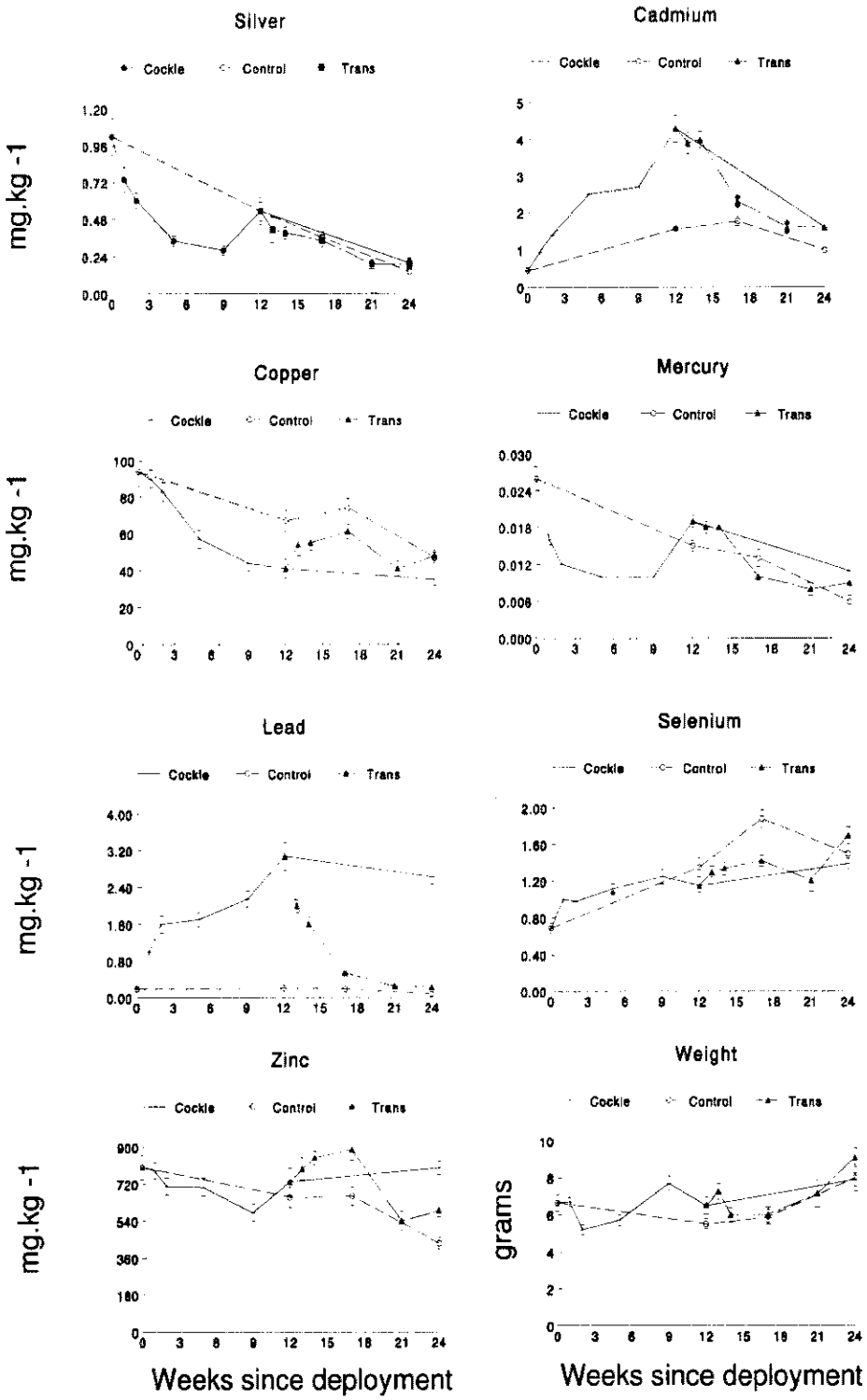


Figure 4.4.1 Mean (±SE) concentrations of trace metals in oysters. "Cockle" is a mean concentration in oysters from Cockle Creek and Bay (nominally containated); "Control" is a mean of oysters at control locations; "Trans" is a mean of concentrations in oysters translocated from Cockle to the controls.

Overall Conclusions

The experiments have shown that kinetics of organochlorines are complex. Some organochlorines have kinetic profiles with a number of components. There seems to be an initial period, which is a matter of hours long, when significant amounts of organochlorines are depurated followed by a longer period of exponentially decreasing clearance of organochlorines. The times to equilibrium of contaminants which are of concern in NSW waters range between 3 to 20 days for dieldrin, chlordane and PCBs to about 72 days for DDT. Half-lives for these contaminants range between 12 to 25 days and 50 days.

The results indicate that in some cases simple first-order kinetic models (Connell 1988) provide a good approximation of times to equilibrium and half-lives. In many cases, however, they do not, particularly with respect to prediction of times to equilibrium.

The uptake and depuration of trace metals occur over a longer period, as would be expected from the literature. There was evidence that some metals reach an equilibrium in oysters at environmental concentrations (e.g. lead) but others (e.g. cadmium and selenium) have linear uptake and did not reach equilibrium in the period of this study. The results for uptake of cadmium were very close to that published by Ward (1982) for cadmium in Sydney rock oysters. Half lives for most metals were in the range 21 to 64 days, with zinc the exception at 144 days. These are similar to results obtained for the oyster *Crassostrea gigas* by Okazaki and Paneitz (1981).

The primary aim of this Chapter was to determine rates of uptake and depuration of organochlorine compounds and trace metals in order to determine what are appropriate times of deployment, or between sampling, when utilising oysters as monitors of trace contaminants in the environment. This question is also linked to appropriate sampling strategies for biomonitoring studies. Many authors (e.g. Green 1979, Phillips and Rainbow 1993, Stewart-Oaten et al. 1986, Underwood 1989, 1991) have pointed out that the timing of sampling must be defined by the objectives of a study. In biomonitoring, timing is further constrained by the kinetics of uptake and depuration of the contaminants of interest in the biomonitor chosen (Phillips and Rainbow 1993). If the objective is to gain an integrated view of concentrations of contaminant over a particular period, then the results for those contaminants which have a half-life considerably shorter than the period chosen will not reflect an integration of the entire period, but rather only the last part of that period. The best

way to address this problem is to use a design based on nested analyses of variance, where samples are taken over a number of short time periods within the longer time span that is the primary interest.

If, however, the objective is to provide a "snap-shot" of contaminant exposure at any particular time (e.g. what are the concentrations of contaminants in wild oysters at time x) then kinetics are of less importance because the hypothesis is concerned with a particular point in time, irrespective of whether or not the animals are in equilibrium with their environment.

The data provided in this Chapter will allow the informed choice of sampling interval according to the objectives and contaminants of interest in future studies.

CHAPTER 5 MONITORING SYDNEY'S DEEPWATER SEWAGE OUTFALLS*

* The work described in this Chapter has been accepted for publication in Marine Pollution Bulletin, see Appendix 5

Introduction

Disposal of sewage and industrial waste can represent a major problem in many cities. Prior to September1990, the great majority of Sydney's sewage was discharged, after primary treatment, to the inshore marine environment through shore-line outfalls. In the period from September 1990 to June 1991, three deepwater sewage outfalls were commissioned, with the subsequent cessation of inshore sewage discharge. The deepwater outfalls were 3 to 4 km offshore in 60 to 80 m of water and were placed to take advantage of the increased opportunity for dilution and dispersion of sewage provided by the greater depth of water and the ocean currents.

Part of the approval process for the deepwater ocean outfalls was the establishment of an environmental monitoring program to determine the effects of the change in the method of sewage disposal on the coastal environment of central NSW. One known consequence of the disposal of sewage in the local inshore environment is bioaccumulation of trace contaminants (trace metals and organochlorine compounds) by fish in the vicinity of outfalls (Lincoln-Smith and Mann 1989 a,b). The studies described in this Chapter were established to examine the trends in concentrations of trace contaminants accumulated in biota in inshore and offshore areas following the change in the method of disposal of sewage. The project was designed to follow concentrations of trace contaminants in oysters placed in inshore and offshore waters before and after the change. The ability to compare inshore and offshore concentrations before and after the event allows the formulation of unconfounded conclusions about the impact of sewage on the availablity of trace contaminants in the coastal environment and the effect of the change in the method of disposal of Sydney's sewage on the availablity of those contaminants.

Aim of this Study

The aim of this study is to use oysters to determine whether environmental concentrations of organochlorine compounds and trace metals in inshore and offshore areas changed after commissioning of deepwater sewage outfalls.

The study will test the null hypothesis that there has been no change in the relative levels of contamination of oysters in inshore and offshore, control and outfall locations after sewage discharge began in offshore waters. This will be tested by analysing the spatial and temporal trends in contaminant concentrations of oysters which have been deployed in inshore and offshore areas before and after the change to offshore sewage disposal.

Methods

Background

Pilot studies (Scanes and Henry 1992) determined that oysters were a suitable organism for these studies. They had slow rates of mortality, were readily accessible and accumulated the contaminants of interest. Those studies also indicated that, inshore, the most appropriate method of deployment was in mesh bags anchored to concrete blocks and that, offshore, large counterweighted aluminium buoys (spar buoys or "spars") anchored to concrete blocks were an efficient mooring system.

The aim of the sampling was to get enough oysters to allow the determination of concentrations of organochlorines and trace metals in five replicate oysters from each inshore block or offshore depth per spar. Since it was not possible to analyse for both classes of contaminants in the same oyster, this requires at least 10 oysters per mooring.

The protocols for handling and dissection of oysters and methods of analysis for organochlorines, fats and trace metals are as set out in Chapter 2.

Sampling Designs

Inshore

Two-year-old Sydney rock oysters obtained from commercial leases in the lower Georges River (June 1990 to October 1992 inclusive) and thereafter from the Hawkesbury River were used in all studies. Ten oysters (5 for organochlorine and 5 for trace metal analyses) were immediately frozen to indicate starting concentrations.

Two mesh bags (approximately 400 x 200 mm with 30 mm mesh) containing oysters were attached, using cable ties, to a steel handle embedded in a concrete block. There

were approximately 15 animals in each bag to allow for mortality. The blocks were then placed on the sea bottom in rocky areas where the water depth was between 10 and 12 m.

Three outfall locations (North Head, Bondi and Malabar) and three control or reference locations (Bangalley, Dee Why and Jibbon) were used (Figure 5.1). At each location, there were two sites, north and south, approximately 300 to 400 m apart. At outfall locations, sites were 150 to 200 m north and south of the outfalls. Three blocks were deployed at each site.

The bags of animals attached to the blocks were retrieved and replaced with a new batch every 3 months from June 1990 to July 1993. Survival of the retrieved animals in each bag was noted. The live oysters were then frozen prior to removal from their shells. Samples for chemical analysis were frozen prior to delivery to analytical laboratories.

Offshore

Two spar buoy moorings were deployed about 200 to 400 m apart at each of the locations shown in Figure 5.1. Two bags each containing 15 oysters were attached to the mooring rope 30 m from the surface and 5 m from the bottom. The 30 m depth is the predicted level at which the sub-surface sewage effluent plume would be trapped while a thermocline is present. The 5 m depth was chosen to be as near the bottom as possible without being influenced by resuspension of sediment. The two depths are referred to in the text as "shallow" and "deep".

Since the depths were defined from the surface and the bottom, the distance between the bags of oysters on a mooring was not the same at Malabar (which is in 80 m of water) than at all the other locations (which are in 60 m of water).

After 3 months the moorings were lifted from the bottom, bags of oysters removed from the rope and replaced and the condition of the moorings inspected.
Animals for analysis were selected at random from those in each bag.

Analysis of Data

For the purposes of statistical analysis, concentrations of contaminants reported as "not detected" and "trace" were assigned values of zero and half the detection limit respectively (see Chapter 2 for justification). In some cases it was necessary to

substitute missing data with the mean of the other replicates for that date (Underwood 1981) to balance the number of replicates. The degrees of freedom of the mean square of the residual were adjusted accordingly.

Due to storms, predation and human interference it was not possible to fulfil the original designs for either inshore and offshore studies. Accordingly, a truncated design with fewer spatial scales and times of sampling has been used. In order to prevent spatial confounding, replicates for statistical analyses of differences between inshore locations or offshore regions (see below) were selected at random from all data available for that spatial scale. This means that the data came from experimental units scattered over the full area being addressed by the hypothesis and were not selected from a small sub-section of that area there-by avoiding confounding any differences between locations with any spatial differences within locations (Hurlbert 1984). Thus, variability associated with sub-sections of the main spatial scale is not explicitly examined but is included in the residual term of the ANOVA along with inter-individual variation. This residual is therefore larger than the original design would have achieved.

The North Head, Bondi and Malabar sewage outfalls have different catchments, flow-rates and effluent characteristics (Camp, Dresser & McKee 1989) and therefore may not represent true replicates for outfalls. Accordingly, data were analysed using an asymmetrical analysis of variance design (Winer 1971, Underwood 1991b, 1993). This allowed each outfall location in turn to be compared with the pooled variability associated with the three control locations.

Data were only analysed for selected contaminants. Chlordane and DDT were the only organochlorines which were detected frequently enough to provide any useful information. The metals in the graphical presentations were chosen on the basis of probable occurrence in sewage, confidence in analytical methods and suitable uptake and depuration times (see Chapters 2 and 4).

Inshore

Data from oysters retrieved in June and September 1990 (pre-commissioning), July and October 1991, January, July and October 1992 and January, April and July 1993 have been analysed by asymmetrical analysis of variance; comparing each outfall in turn with the three control locations. Data from eight randomly selected replicate oysters at each location were used in the analyses. These dates were chosen because there were enough replicates to fulfil the statistical design. In two cases (7/91, 1/92)

two and one replicates (respectively) were substituted by the mean of the other replicates for that date.

Offshore

The sampling sites were divided up into 5 regions for statistical analysis (see Fig. 5.1). The two sets of data that were analysed statistically are shown in Table 5.1.
In order to maintain a balanced design, the 10 replicates for these analyses were selected at random from the available data in those instances where more data were available.

Table 5.1 Data included in statistical analyses of data from oysters deployed offshore.

Trace Metals: 10 replicates
Regions: Far North, Near North, Outfall, Near South, Far South Dates: 5/91; 8/91; 11/91; 2/93; 5/93 (all post commissioning)
Regions: Outfall, Near South Dates: 10/90, 5/91; 8/91; 11/91; 2/93; 5/93, 8/93 (1 pre, 6 post commissioning)
Organochlorines; 10 replicates
Regions: Far North, Near North, Outfall, Near South, Far South Dates: 8/91, 11/91, 2/93, 5/93 (all post commissioning)
Regions: Outfall, Near South Dates: 10/90, 8/91; 11/91; 2/93; 5/93 (1 pre, 4 post commissioning) n.b. 5/91 not included for organochlorines because all data were below quantification limits

Results

Analyses of fat were discontinued early in sampling because it was usually not possible to get enough tissue from an individual oyster for both fat and organochlorine analysis and, more importantly, initial data (Fig. 5.2) showed no correlation between fat and levels of organochlorine contamination in field samples.

Inshore

The data obtained have been examined in two ways. First, means for all data from outfall and control locations were calculated and plotted. No statistical interpretations of these data were made because the number of samples in each mean were different. All contaminants were variable through time(examples in Fig. 5.3, remainder in Appendix 3) and there was strong concordance between outfall and control samples. Regression analyses of mean concentrations of contaminants before deployment and after retrieval indicated that, for most metals, the concentration prior to deployment was not a good predictor of the concentration after retrieval and thus did not always explain the temporal variation. There were, however, significant positive regressions for copper, zinc, cadmium and mercury (control locations only - Table 5.2). The experimental design and statistics used later should indicate if there are differences between outfall and control locations despite dissimilar starting concentrations at each time.

Temporal differences were evident for chlordane (Fig. 5.3), where concentrations were elevated near the outfalls precommissioning but were not different from control locations during the post-commissioning period. Contaminants which showed occasionally large peaks at the outfalls (, but which showed no other patterns that could be interpreted to be a consequence of a change in discharge) were arsenic, mercury, DDT, nickel, copper. Lead was consistently elevated at outfall locations throughout the study.

Table 5.2 Summary of results of regression analyses between concentrations of contaminants in oysters prior to deployment inshore and after retrieval. P indicates result of significance of test of slope of the regression, ns - not significant; * P < 0.05. Biological half lives (BHL) from Chapter 4.

	Control			Impact			
		P	r^2		*P*	r^2	BHL
Cr	y = 0.85x + 0.26	ns		y = 0.81x + 0.27	ns		
Ni	y = 0.67x + 0.11	ns		y = 1.66x + 0.06	ns		
Cu	y = 2.67x + 27.18	*	0.5	y = 3.07x + 25.23	*	0.57	64
Zn	y = 2.83x + 257	*	0.53	y = 4.10x + 180	*	0.7	144
As	y = 2.09x - 0.15	ns		y = 1.78x + 0.03	ns		
Ag	y = -0.50x + 0.66	ns		y = -0.81x + 1.11	ns		35
Cd	y = 3.81x + 0.24	*	0.68	y = 5.55x + 0.26	*	0.81	52
Hg	y = 2.36x + 0. 01	*	0.44	y = 0.60x + 0.02	ns		30
Pb	y = 0.74x + 0.15	ns		y = 0.80x + 0.17	ns		21

Secondly, a subset of data was analysed. Only the results of the analyses which showed significant differences between outfalls and controls will be discussed here, although all the results are shown in Table 5.3. Most contaminants showed significant variation among time and among the control locations and an interaction between these two factors. These significant differences are not considered to be linked to the presence of the outfalls and so will not be discussed in detail.

The concentration of chlordane at North Head outfall location compared to control areas was significantly linked with the time of sampling (Table 5.3). In the precommissioning period, concentrations at the outfalls were apparently greater than controls, but this was not the case post commissioning (Fig. 5.4). An alternative graphical presentation, which removes temporal variation by calculating the ratio of outfall and control concentrations, is presented in Figure 5.5. This shows clearly that there was more chlordane accumulated at North Head than control sites before commissioning of the deepwater outfalls, but no differences afterwards.

Table 5.3 Summaries of mean squares from asymmetrical analyses of variance of contaminants from inshore oysters. Significance is indicated as follows:* 0.05 <P>0.006; ** P>0.005. The mean squares are expressed as a negative exponential (e.g. E-3) in some cases. The general model for the analyses is also shown. Data are shown in Figure 5.4.

General Model

Source of Variation			df	MS divisor
OUTFALL VS CONTROL	fixed	T	1	L(T)
LOCATIONS WITHIN CONTROL	random	L(T)	2	RESIDUAL
DATE	random	D	9	DL(T)
T x D			9	DL(T)
D x L(T)			18	RESIDUAL
RESIDUAL			280	

NORTH HEAD vs CONTROLS

	T	L(T)	D	T x D	D x L(T)	RESIDUAL
chlordane	0.0008	9.2 E-5	0.014 **	0.008 *	0.002 *	0.0012
sum DDT	1.7 E-4	0.001 *	0.004 **	0.001	0.001 **	2.64 E-4
Cr	0.001	0.045 **	0.24 **	0.071	0.059 **	0.0079
Co	0.001	0.002 **	0.008 **	0.001	0.0008 **	3.70 E-4
Ni	0.29	0.21 **	0.21	0.03	0.09 **	0.0343
Cu	729	1715	34671 **	2133	1734 *	848.88
Zn	1524	21116	2319096**	74221	103219 *	53768.9
As	3.44	2.53 **	7.74 **	0.79	1.42 **	0.2197
Se	0.05	2.05 **	6.03 **	1.38	1.46 **	0.2363
Ag	0.03	1.36 **	8.07 **	1.09	0.93 **	0.2325
Cd	0.92	0.49 **	1.80 **	0.30	0.20 *	0.0976
Hg	5.2 E-6	0.002	0.009 **	6.8 E-4	0.002 **	6.95 E-4
Pb	0.15	0.02 *	0.08 *	0.03	0.03 **	0.00743

BONDI vs CONTROLS

	T	L(T)	D	T x D	D x L(T)	RESIDUAL
chlordane	0.0037 *	9.2 E-5	0.02 **	0.003	0.002 *	0.0013
sum DDT	1.0 E-4	0.001 *	0.006 **	0.001	0.001 **	3.07 E-4
Cr	0.03	0.065 **	0.44 **	0.06	0.06 **	0.0058
Co	2.0 E-4	0.004 **	0.008 **	0.001	7.8 E-4	4.74 E-4
Ni	2.9 E-4	0.31 *	0.60 **	0.06	0.09 ·	0.0078
Cu	188	2542	18510 **	2686	1736 **	819.8
Zn	44167	31579	1245637 **	317878*	103208**	51597
As	0.002	3.14 **	7.61 **	1.10	1.49 **	0.2036
Se	0.02	3.06 **	8.03 **	0.50	1.46 **	0.1695
Ag	1.81	1.99 **	10.57 **	0.82	0.93 **	0.2612
Cd	0.38	0.72 **	2.16 **	0.14	0.20 **	0.096
Hg	1.2 E-4	0.002 *	0.01 **	0.002	0.002 **	6.55 E-4
Pb	0.06	0.04 **	0.09 *	0.009	0.03 **	0.0054

MALABAR vs CONTROLS

	T	L(T)	D	T x D	D x L(T)	RESIDUAL
chlordane	0.003 *	9.2 E-5	0.02 **	0.003	0.002 **	0.0013
sum DDT	5.8 E-4	0.001 *	0.007 **	3.5 E-4	0.001 **	3.02 E-4
Cr	0.12	0.07 **	0.44 **	0.05	0.06 **	0.0063
Co	0.01	0.004 **	0.02 **	0.005 *	8.1 E-4 *	4.66 E-4
Ni	0.10	0.31 *	1.10 **	0.29 *	0.09	0.069
Cu	4432	2541	52916 **	5771 *	1736 *	915.2
Zn	335923	31579	2981924 **	242782	103208 *	62483
As	0.05	3.14 **	8.59 **	0.37	1.49 **	0.2414
Se	1.38	3.06 **	16.49 **	2.13	1.46 **	0.1933
Ag	0.34	2.00 **	10.37 **	0.95	0.93 **	0.2264
Cd	3.3 E-6	0.71 **	2.82 **	0.07	0.21 *	0.1030
Hg	0.005	0.002 *	0.03 **	0.003	0.002 **	7.07 E-4
Pb	0.07	0.04 **	0.16 **	0.03	0.03 **	0.0058

The concentrations of chlordane accumulated at Bondi and Malabar outfalls were significantly greater than those at controls and this was not affected by time. This implies that concentrations were not affected by commissioning of the outfalls. The differences between outfalls and controls were, however, greatest in the period prior to commissioning of the outfalls (Fig. 5.4, 5.5). Any relative differences after the deepwater outfalls began operation tended to greater concentrations at the outfalls, particularly at Bondi (Fig. 5.5). This remaining relative elevation at the outfall locations, even though it is of a much reduced magnitude, may have contributed to the statistical analyses not showing a date x treatment interaction.

Differences between concentrations of zinc accumulated at Bondi and copper and nickel at Malabar and control locations were affected by time, but there is no evidence in the graphs (Fig. 5.5) that suggests an effect due to changing the location of sewage discharge. Cobalt was significantly elevated at Malabar outfall before commissioning but not after commissioning (Figs 5.5). This provides some evidence of a reduction in inshore cobalt concentrations since offshore sewage discharge began.

Offshore

The means of all data collected between October 1990 and August 1993 were plotted and there were no obvious trends (some examples in Fig. 5.6, remainder in Appendix). If there was enhanced accumulation in offshore regions, the outfall and perhaps

near south regions should be showing consistently higher concentrations than the other regions. Such a pattern is not evident for any contaminant.

In the analyses which included all regions, the date of sampling and all interactions with date were significant (Table 5.4). There were, however, no trends in the data (Fig. 5.7) which would lead to the conclusion that there are significantly greater concentrations of metals accumulated in the outfall or "near" regions. The proportion of times that each region had the greatest concentration of a contaminant (0.29, 0.08, 0.17, 0.21, 0.25; regions north to south, all contaminants) was not significantly different from the alternative hypothesis of equal proportions (Chi squared $p = 0.54$), indicating that no particular region consistently had the highest concentrations of contaminants.

There was a significant trend to a greater accumulation of copper, cadmium and zinc in deeper waters. This trend was not, however, related to proximity to outfalls indicating that it was not caused by the presence of sewage in deeper waters (Table 5.5, Fig. 5.7).

The comparisons of outfall to near south regions confirmed the conclusion that concentrations of contaminants accumulated in the outfall region were similar to control locations (Table 5.5, Figure 5.8). This pattern did not alter after commissioning of the deepwater ocean outfalls.

Table 5.4 General model of analysis and summaries of mean squares from analyses of variance of contaminant levels in oysters from offshore oysters - all regions. Significance is indicated as follows:* 0.05 <P>0.006; ** P>0.005. Data are in Figure 5.7.

Source of Variation		df (OC)	df (TM)	MS divisor
Time - T	random	3	4	Residual
Region -R	fixed	4	4	T x R
Depth -D	fixed	1	1	T x D
T x R		12	16	Residual
T x D		3	4	Residual
R x D		4	4	T x R x D
T x R x D		12	16	Residual
Residual		360	450	

	Chlor	Cd	Cu	Hg	Pb	Zn
Time - T	**	4.11 **	68877**	0.02 **	0.30 **	4190070**
Region -R		0.14	5058	0.0006	0.08	395265
Depth -D		2.12	21158**	0.0013	0.04	842626*
T x R	**	0.60 **	3856**	0.0006 **	0.03 **	221972**
T x D	**	0.27 **	199	0.0003 **	0.01	44228
R x D		0.10	737	0.0003	0.004	46222
T x R x D	**	0.21 **	800	0.0001	0.01	37957
RESIDUAL	0.0013	0.061	1208	0.0001	0.0087	73121

Table 5.5 General model of analysis and summaries of mean squares from analyses of variance of contaminant levels in oysters from offshore oysters - outfall vs near south, time 1 represents pre-commissioning data. Significance is indicated as follows:* 0.05 <P>0.006; ** P>0.005. The mean squares are expressed as a negative exponential (e.g. E-3) in some cases. Data are in Figure 5.8.

Source of Variation		df (OC)	df (TM)	MS divisor
Time - T	random	4	6	Residual
Region -R	fixed	1	1	T x R
Depth -D	fixed	1	1	T x D
T x R		4	6	Residual
T x D		4	6	Residual
R x D		1	1	T x R x D
T x R x D		4	6	Residual
Residual		180	252	

	Chlor	Cd	Cr	Cu	Hg	Pb	Zn
Time - T	0.05 **	2.47 **	0.44 **	26086**	0.012**	0.12**	1806767**
Region -R	0.007	0.13	0.002	9189	1.9 E-5	0.07	331023
Depth -D	1.0 E-5	1.09	0.06	1818	0.002	0.004	159542
T x R	0.002	0.23**	0.26**	8515	9.1 E-4 **	0.03 *	505570**
T x D	0.003	0.30 **	0.05 **	6486	9.2 E-4 **	0.02	221103*
R x D	0.005	0.004	0.07	3289	0.001	0.002	139641
T x R x D	0.004 *	0.10	0.03 **	1625	0.001	0.009	55971
RESIDUAL	0.0012	0.045	0.0083	1470	9.73 E-5	0.0111	90605

Discussion

The use of oysters as biomonitors in NSW has received little attention in the past, despite oysters being used successfully in other countries (O'Connor 1992, Phillips 1979, Phillips and Yim 1981). The published works on Australian oysters were descriptive, indicating patterns of trace metal contamination in rivers or estuaries inferred from collections of wild or cultivated oysters (Brown and McPherson 1992, Mackay et al. 1975, Peerzada and Dickinson 1988, 1989, Peerzada and Kozlik 1992). There have been no studies describing translocation of oysters to test hypotheses and no data of any kind on concentrations of organochlorines.

The data in this study show clearly that the diversion of sewage from nearshore to offshore areas has resulted in a significant drop in the contamination of the oysters deployed inshore to assess contaminant levels. The changes in inshore concentrations of organochlorines were the most obvious. Scanes and Henry (1992) showed that during the pre-commissioning period there were significantly greater concentrations of some trace metals around the shore-line outfalls This difference was not evident in later analyses of the entire set of data. It appears that the differences in the pre-

commissioning data were mainly due to differences in September 1990. The current analyses of a longer time series shows that the same degree of difference that occurred in September 1990 was apparent at other times through the data. This suggests that the differences occurred sporadically and did not seem to be linked to the presence of sewage. The reasons for the sporadic detection of differences is unknown but could be linked to storms or urban runoff. The lack of any cyclical patterns in the data suggest that it is not a function of the biology of the oysters. In offshore areas, there were no differences detected pre-commissioning and this pattern remained through the post commissioning period although there were a number of instances when regions were different from each other. These differences were not consistently in the same region nor for a particular metal. It is again difficult to determine a cause for the differences. There was a consistent difference in concentrations of copper, zinc and cadmium between deep and shallow positions in the offshore, but again this was not confined to particular region(s) and is therefore highly unlikely to be an effect of the outfalls.

Several authors have proposed the use of power analyses to determine the probability of Type II error, i.e. failure to detect an impact when one has occurred and, post hoc, the number of replicates required to determine significant differences (Andrew & Mapstone 1987, Green 1989 and Peterman 1990). These tests are of interest primarily if tests of hypotheses do not reject the null hypothesis of no differences between samples. The calculation of power is designed to guard against making wrong managerial decisions on the basis of non-significant results. This can happen if the power of the tests that produced the results is too small to provide any surety against accepting a null hypothesis that is really false. In the pre-commissioning report for this study (Scanes and Henry 1992), consideration was given to the power of tests required to detect significant differences. Most analyses of data from inshore and offshore studies detected significant differences, so, by definition, lack of power in the tests was not likely to be the reason why there were so few significant impacts attributable to the presence of outfalls.

Modelling of the effluent fields (Roizenblit 1994) confirmed that the plumes from the three outfalls merge into a continuous and relatively homogeneous field off Sydney and that all spar moorings in the outfall region were nearly continuously subject to effluent diluted in the range 100 to 1000 times. The "Near" regions were occasionally influenced by sewage at a dilution of about 1000 to 10,000 times. The "Far" regions were beyond the area modelled, but it is a reasonable assumption that they were not ever exposed to sewage more concentrated than the "Near" regions. In all cases, the

modelling suggested that there was very little difference in the exposure to sewage of between shallow and deep oysters.

An anomaly in the results is why there was a strong correlation between lipid and concentrations of organochlorines in field uptake experiments (Chapter 4), but not in this study. In the uptake experiment, the concentrations of organochlorines reached by the oysters were an order of magnitude greater than those in this study. One effect of the great concentrations accumulated is that the analytical methods used are able to pick up the relatively small differences among individuals induced by variation in lipid (e.g. differences of 10 to 30 % are easily detectable at concentrations of 0.3 - 0.5 $mg.kg^{-1}$). In the case of this study, where concentrations of organochlorines in the oysters were much smaller (0.01 - 0.03 $mg.kg^{-1}$), the precision of the analytical method masks changes of this magnitude. Thus, it is not surprising that the correlation is poor. NOAA (1989) also found no correlation between lipid (fat) and concentrations of PCB, DDT, lindane or PAHs, and only a slight correlation for chlordane and dieldrin. It could be argued that pooling over all the locations confounded the picture, because location in addition to lipid is influencing the organochlorine concentrations, but later analyses for a longer time span at each individual location (O'Connor pers. comm.) confirmed that there was little correlation between organochlorine and lipid.

This lack of a relationship does not mean that oysters are not useful indicators. Instead, at smaller concentrations there is less need to commit resources to analyses of lipid because the results may be of little benefit in interpreting the data.

The data from the uptake experiment also indicated that oysters probably reach an equilibrium with the organochlorines in their environment within about 20 days. If the environment remains fairly constant, then so does the equilibrium. The lack of an equilibrium in the oysters deployed in inshore (post-commissioning) and offshore waters suggests that the organochlorine concentrations in these areas were very variable. This variability, combined with the relatively small variability among individuals, means that there would have been very little chance of seeing a relationship between organochlorines and lipid in such a dynamic system.

As noted above, there are few previous data with which to compare the concentrations that have been accumulated off Sydney. Existing data are either from estuaries (NSW) or from other species of oyster overseas. Post-commissioning, there was little difference among means for the Sydney outfall, control or estuarine sites (Table 5.6)

with the exception of Se, Ni, Cd and Ag which were greater in the coastal areas and organochlorines which were in smaller concentrations. The concentrations of most metals and all organochlorines were smaller in the Clyde River than in the Sydney region.

Comparisons have also been made to data from the NOAA "Mussel Watch" Project (O'Connor 1992), but these should be treated with caution because the species of oyster used (*Crassostrea virginica*) is different to the species in this study. Nevertheless, *Crassostrea* showed similar oyster: mussel bioconcentration ratios as did *Saccostrea*. O'Connor (1992) used geometric means to identify mean concentrations and what he considered to be "high" concentrations for a range of contaminants (Table 5.7). His study involved collection of wild animals from a wide range of sites remote from centres of population and from near cities. A consequence of the use of sites far from cities is that the means will tend to be reduced by the large number of small concentrations and "high" data will tend to come from near larger population centres. The data shown in O'Connor (1992) are expressed as dry weight concentrations, whereas all data in Sydney study has been expressed on a wet weight basis. Unpublished EPA studies have shown that oysters consistently have a mean water content of about 90%. Using this, we could convert wet weight concentration to dry weight by multiplying by a factor of 10. Comparisons of the converted Sydney data to those in O'Connor (1992) (Table 5.7) show that, taking into account possible small differences in bioconcentration by the two species, the concentrations of trace metals around Sydney are similar to those expected around US cities (i.e. the "high" data). Concentrations of chlordane and DDT were, however, considerably greater in the Sydney region.

CONCLUSIONS

This study has demonstrated that oysters are useful indicators of trace metal and organochlorine contamination of marine waters. Relatively large levels of contamination of inshore waters near sewage outfalls prior to deepwater sewage disposal were reduced to be not different from other parts of Sydney's coastline after the commencement of deepwater discharge. There was no concomitant increase in accumulation of contaminants in offshore waters. Further, preliminary indications are that Sydney's coastal waters are not very heavily contaminated by trace contaminants in comparison to areas of NSW with much lower levels of development; nor in comparison to data from similarly developed areas of the USA.

Table 5.6 Comparison of data from Sydney outfall studies with wild oysters from NSW estuarine areas (Clyde River - rural; Georges River, Botany Bay - urban). All data in mg/kg wet weight. Offshore outfall - data from outfall and near south regions; Inshore outfall - data from Malabar outfall; Inshore control - data from inshore controls.

	Offshore outfall	Inshore outfall	Inshore control	Clyde River*	Georges River*	Botany Bay*
As		1.81	1.84	1.48	0.63	2.24
Cd	0.87	0.79	0.79	0.27	0.41	0.26
Co		0.08	0.07	0.06	0.1	0.05
Cr	0.37	0.40	0.35	0.23	0.32	0.30
Ag		0.68	0.60	0.18	0.41	0.27
Ni		0.27	0.23	0.13	0.13	0.11
Pb	0.15	0.22	0.18	0.06	0.20	0.15
Se		1.47	1.32	0.37	0.64	0.67
Zn	622	798	724	353	1037	516
Cu	69	87	78	17	90	35
Hg	0.03	0.04	0.03	0	0.02	0.02
chlor	0.025	0.031	0.024	0	0.07	0.06
Σddt	0	0.019	0.016	0	0.05	0.02

* data from Scanes *et al.* (1996)

Table 5.7 Comparison of geometric means of data from Sydney with geometric means of data from NOAA "Mussel Watch. Data from the Sydney Outfall study have been converted to comparable units in the following ways:
Trace metal - mean wet weight (mg/kg) x 10 (mean water content = 90%) = μg/g dw
Chlordane- mean wet weight technical chlordane (mg/kg) x 0.2 x 10 x 1000 = ng/g dw (*) (factor of 0.2 has been applied to convert technical chlordane to the form of chlordane measured by NOAA)
Σddt - mean wet weight (mg/kg) x 10 x 1000 = ng/g dw(*)
Offshore outfall - data from outfall and near south regions; Inshore outfall - data from Malabar outfall; Inshore control - data from inshore controls.

	Offshore outfall mean	Inshore outfall mean	Inshore control mean	NOAA mean #	NOAA high #
As		16.2	16.8	10	17
Cd	8.05	6.9	7.0	2.7	5.7
Hg	0.19	0.26	0.21	0.094	0.24
Ni		1.71	1.75	1.7	3.3
Ag		3.0	3.3	0.17	3.7
Cu	537	614	660	150	360
Zn	5188	6170	6310	2400	5200
Pb	1.24	1.9	1.6	0.52	0.94
Cr	3.3	3.58	3.16	0.48	0.93
Se				2.5	3.5
chlor *	66	56	48	14	31
Σddt *		199	206	37	120

data from O'Connor (1992)

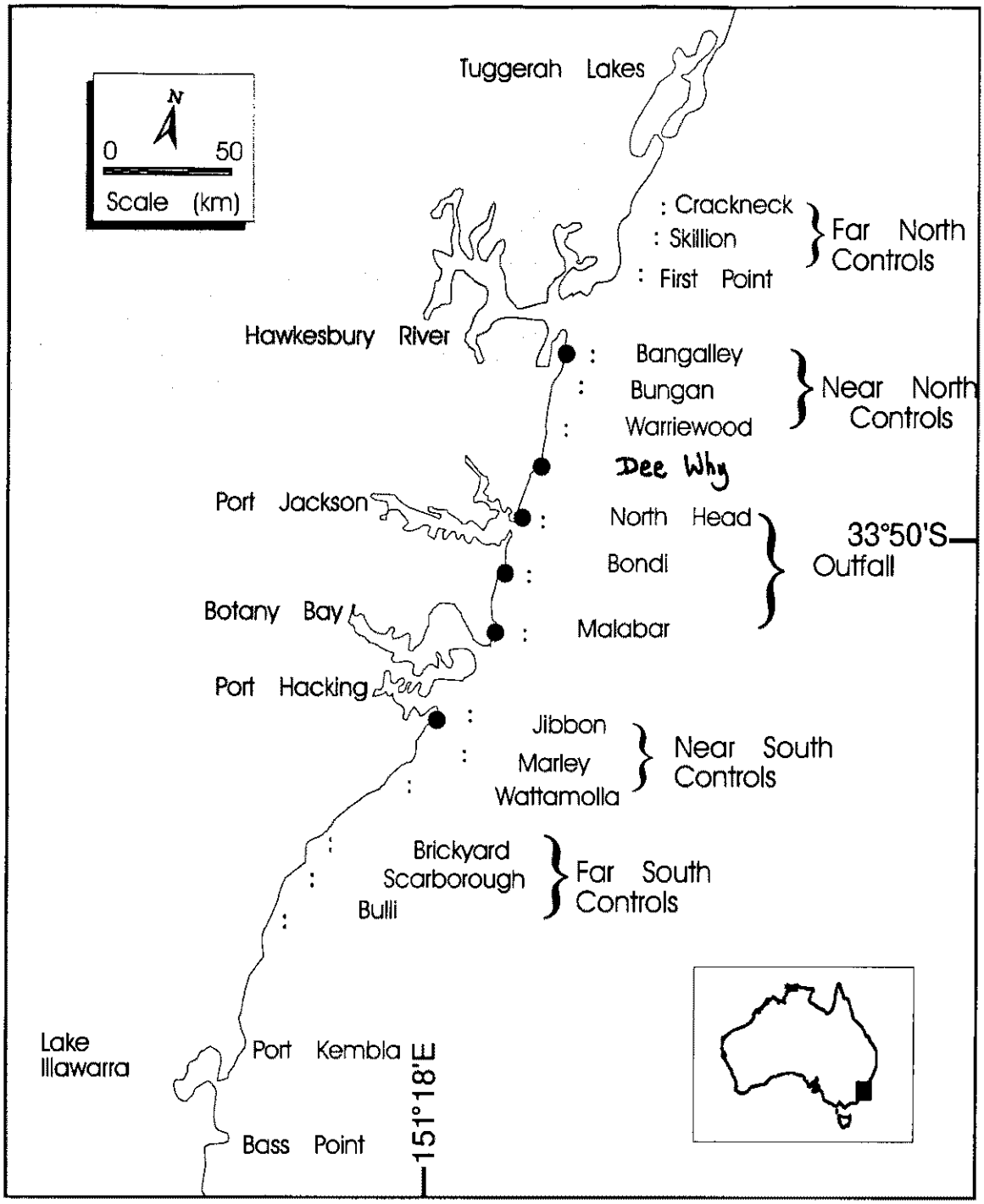


Figure 5.1 Locations of sewage outfalls and inshore and offshore deployments

● **Inshore locations**

: **Offshore locations**

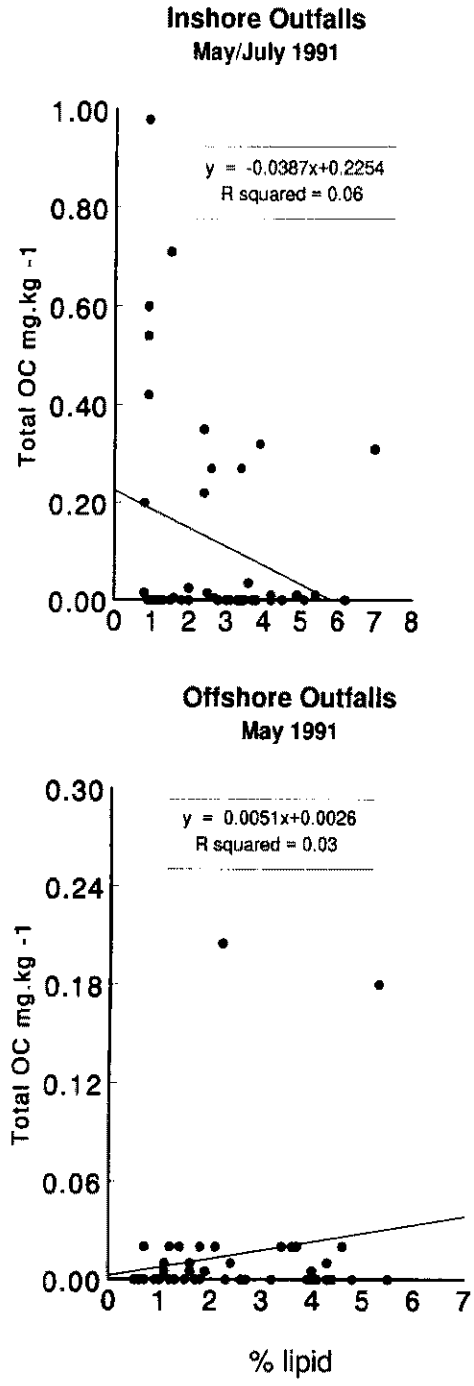


Figure 5.2. Regressions between percentage of lipid and total organochlorine concentrations in oysters from inshore and offshore outfall locations.

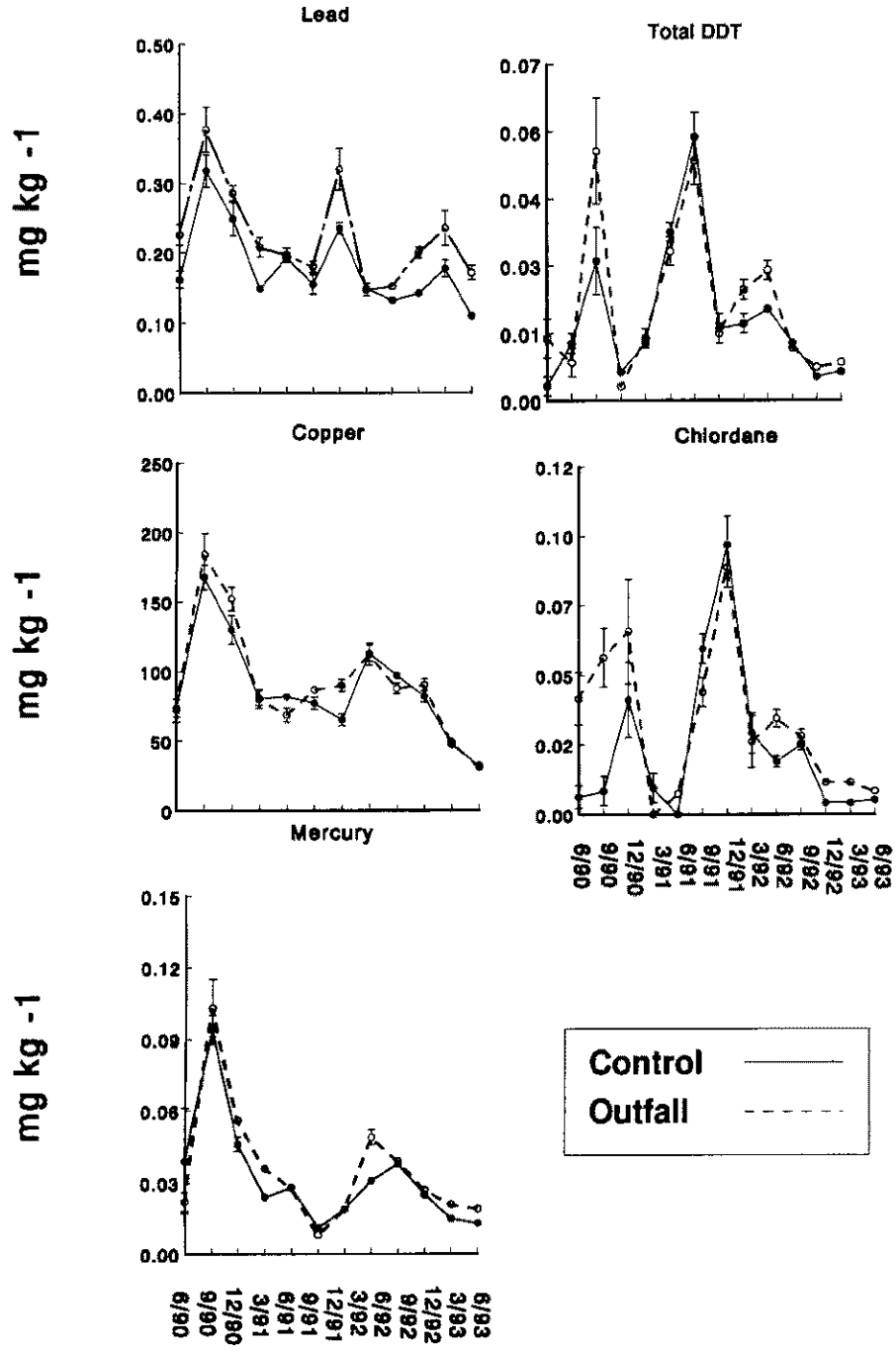


Figure 5.3. Mean (± 1S.E.) concentrations of selected contaminants in oysters deployed at inshore locations (summary of all data from outfall and control locations). Samples from 6/90 and 9/90 represent the conditions prior to commissioning the deepwater outfalls.

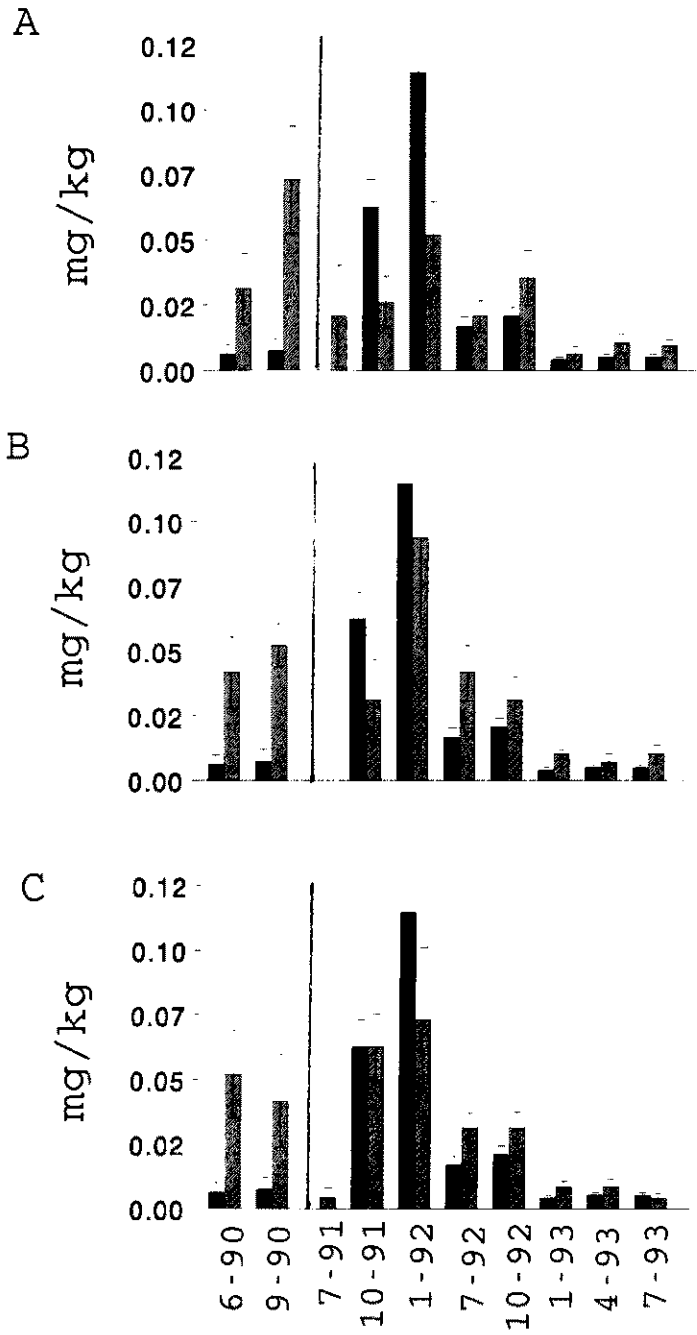


Figure 5.4 Mean concentrations of chlordane in oysters at inshore control (dark bars) and outfall locations (light bars). Vertical line shows commencement of deepwater sewage discharge.

A - Bondi outfall; B - North Head outfall; C - Malabar outfall

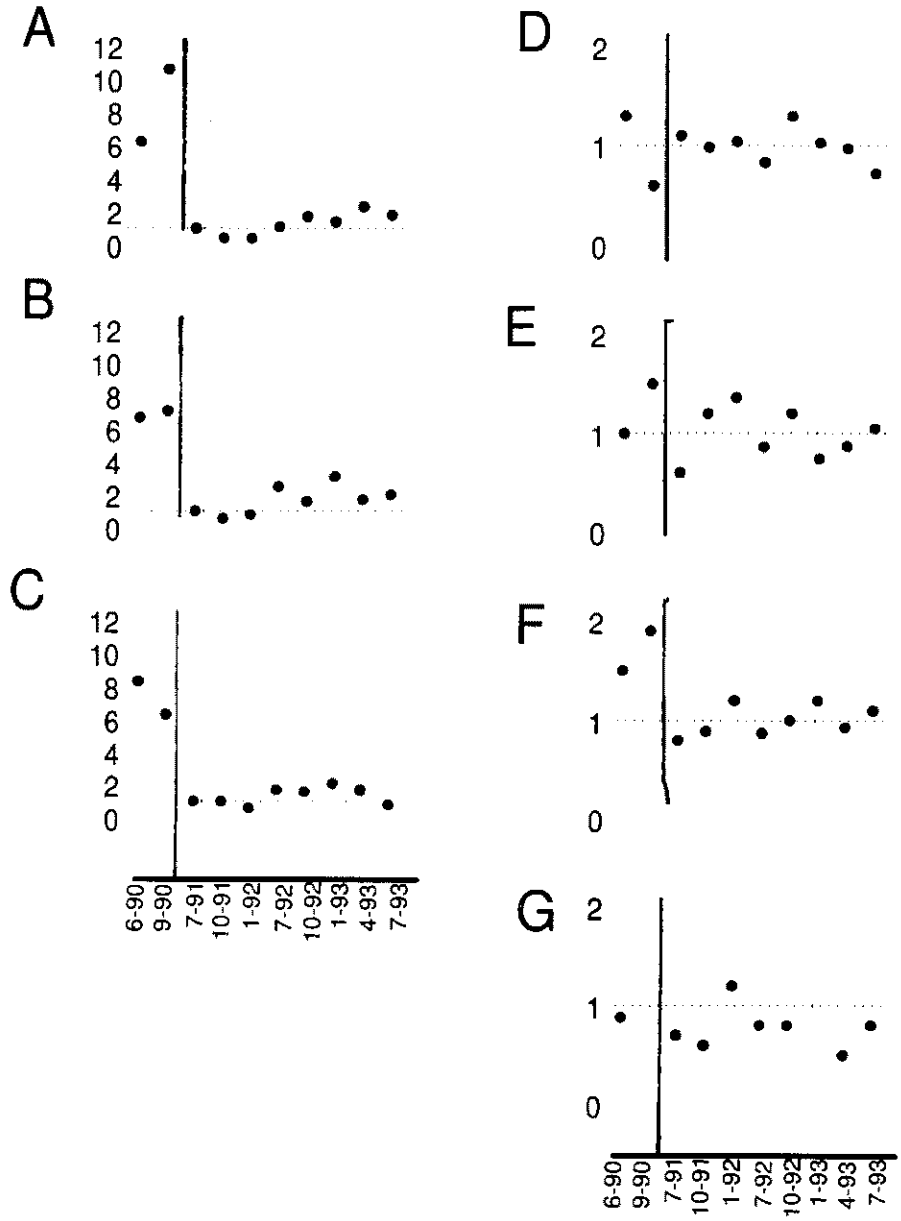


Figure 5.5. Data from analyses of variance of concentrations of contaminants in oysters deployed near inshore outfalls presented as ratios of mean concentrations from outfall and pooled control locations for each date. Samples from 6/90 and 9/90 (to left of vertical lines) represent the conditions prior to commissioning the deepwater outfalls. A - chlordane, North Head; B - chlordane, Bondi; C - chlordane, Malabar; D - zinc, Bondi; E - copper, Malabar; F - cobalt, Malabar; G - nickel, Malabar

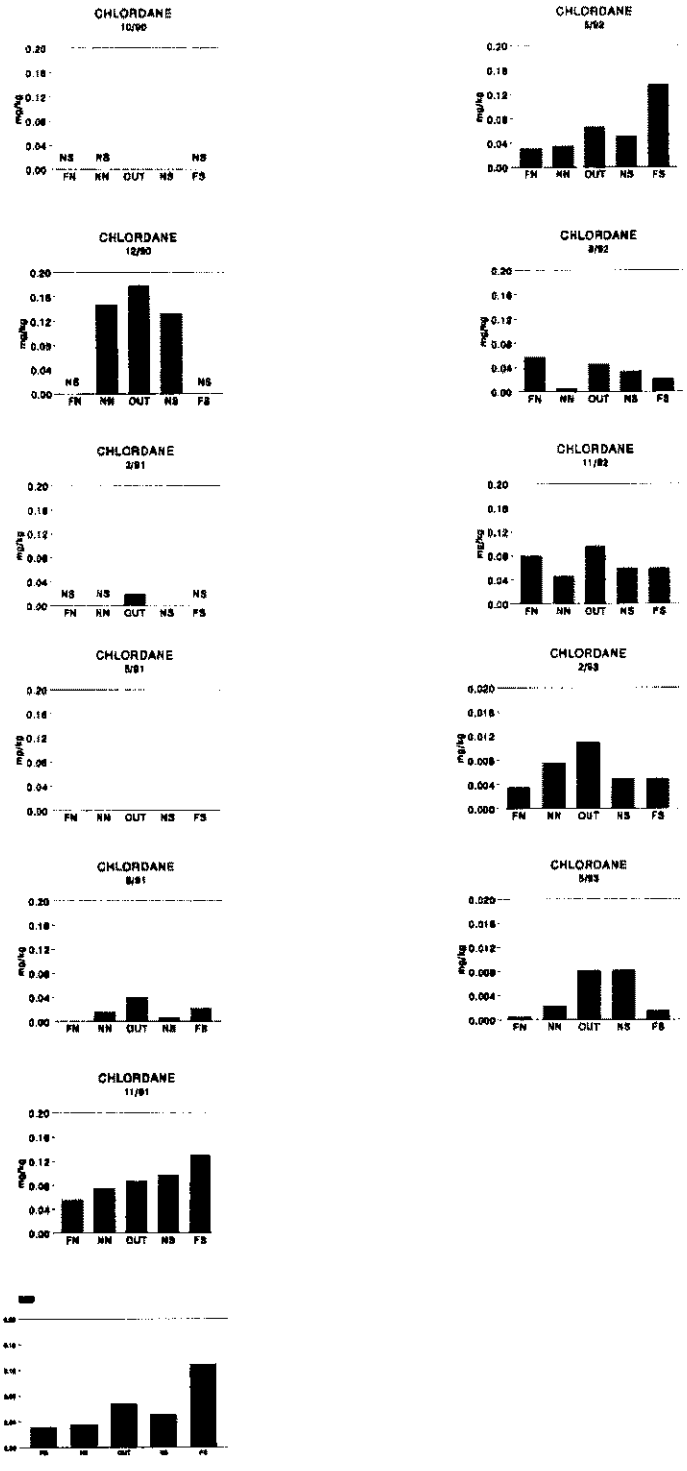


Figure 5.6 Means of all chlordane data collected for offshore locations.

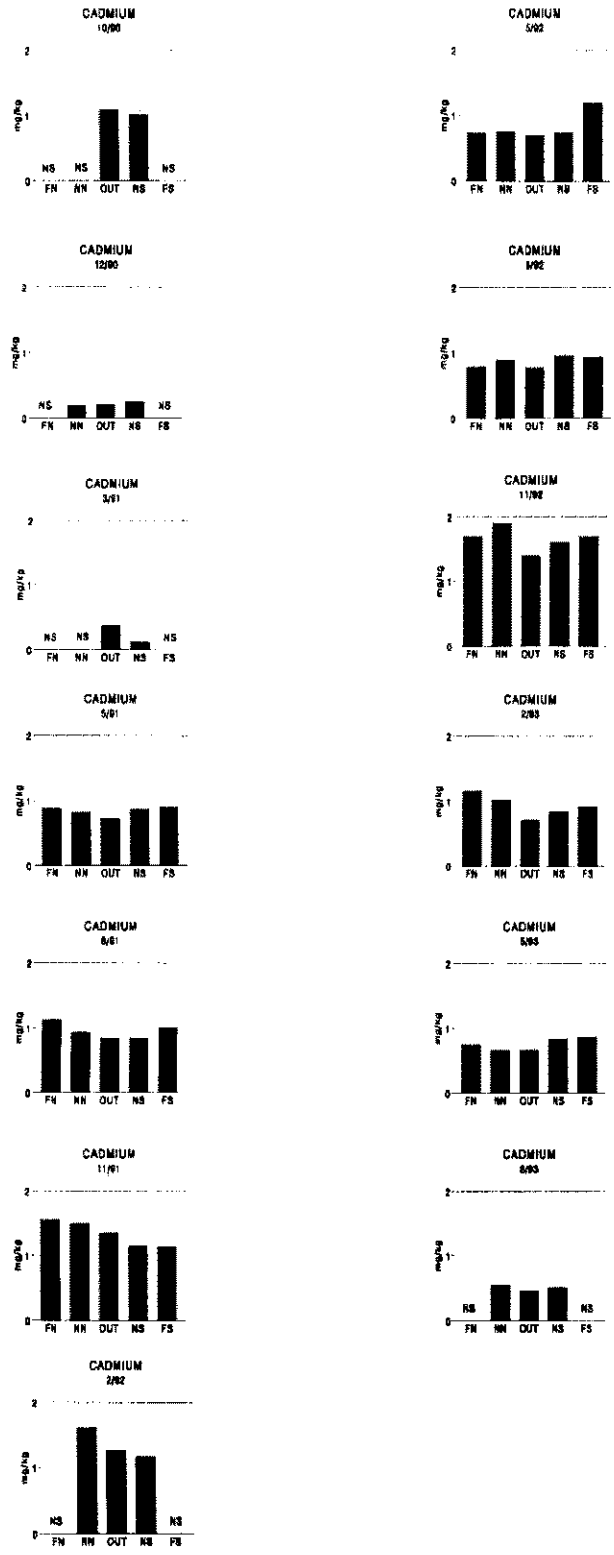


Figure 5.6 (cont) Means of all cadmium data collected for offshore locations.

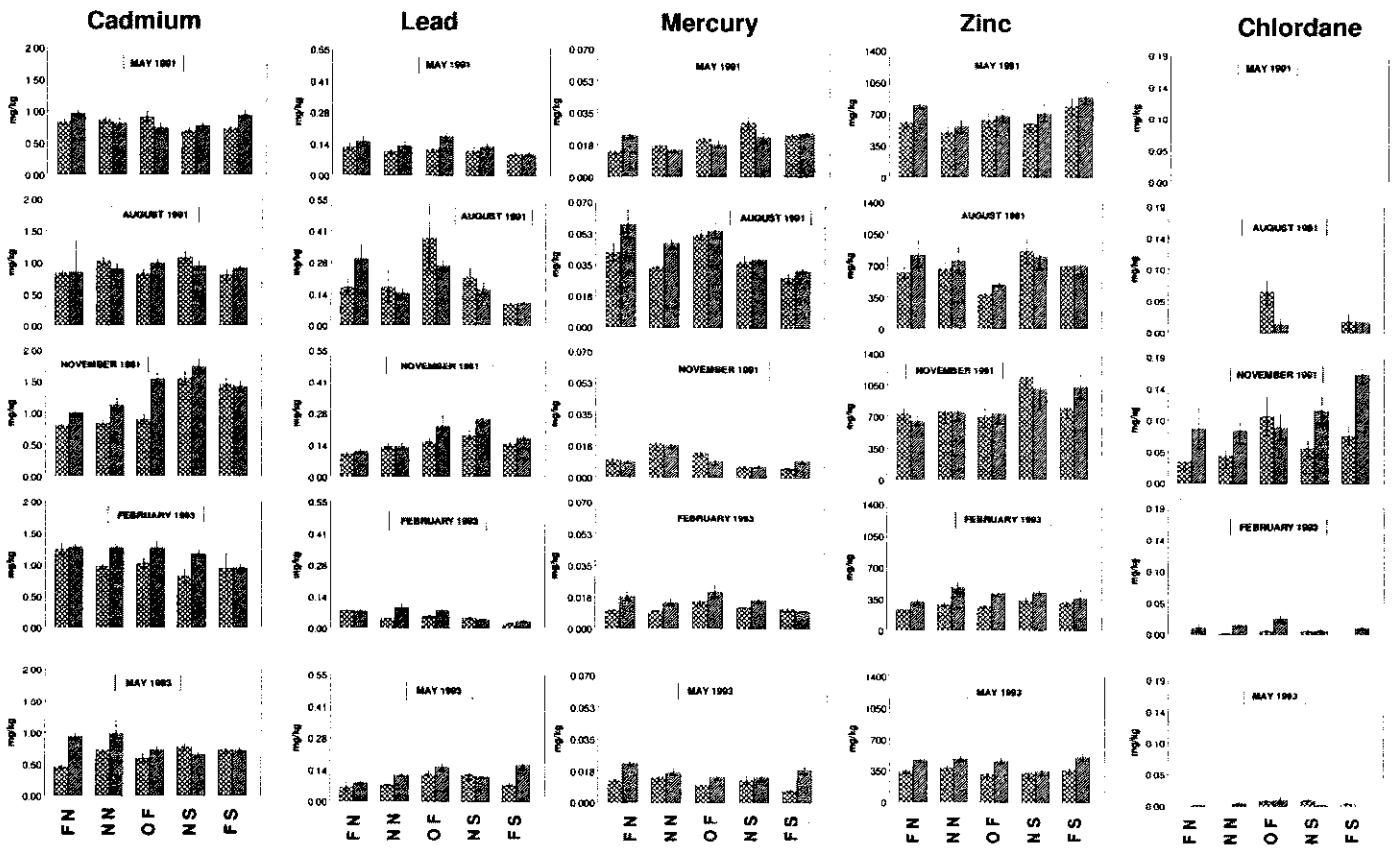


Figure 5.7. Summary of mean (± 1S.E.) concentrations at each time for selected contaminants from analyses of variance of data from oysters deployed at offshore locations. All data are from after commissioning of the deepwater outfalls. FN - far north region (see Fig 1); NN - near north; OF - outfall; NS - near south; FS - far south. Crossed bars are data from oysters suspended at 30 m from surface, hatched bars are data from oysters 5 m from sea floor.

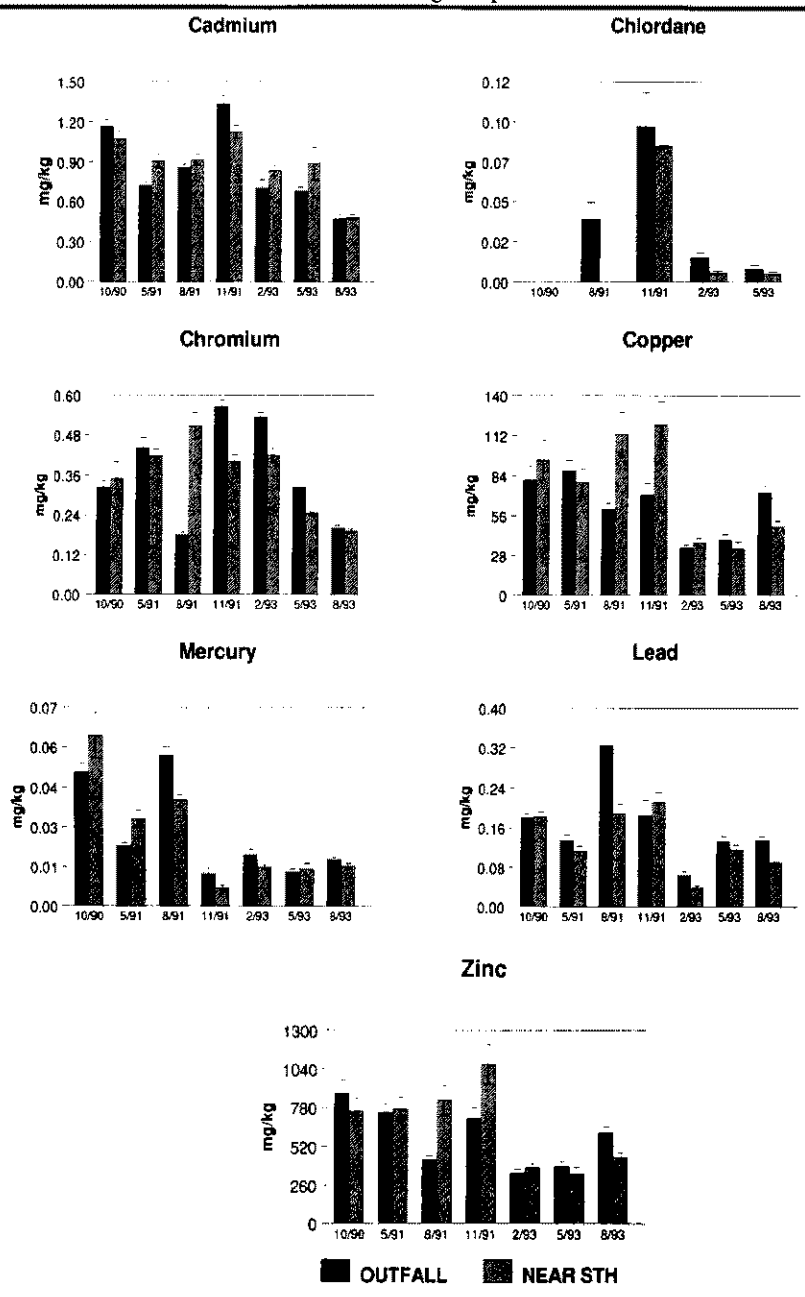


Figure 5.8. Summary of mean (± 1S.E.) concentrations at each time for selected contaminants from analyses of variance of data from oysters deployed at offshore locations. Data from 10/90 are before commissioning of the deepwater outfalls

CHAPTER 6 CASE STUDIES IN LAKE MACQUARIE, NEW SOUTH WALES

Background

Chapters 2, 3 and 4 of this thesis have considered a range of issues which were required to establish the utility of oysters and cockle as monitors of concentrations of bioavailable contaminants in marine and estuarine systems. This Chapter describes case studies where bioaccumulation techniques have been applied to address specific hypotheses in two areas:

- the pathways by which trace metals are accumulated, particularly considering whether trace metals are accumulated from sediments or water
- spatial and temporal trends in trace metal concentrations.

These case studies have been selected because they represent the most common applications of biomonitors.

The first study uses a common estuarine bivalve (cockles) which has recreational significance as a source of food. The study used experimental translocation of contaminated and uncontaminated sediments around the lake to test hypotheses about whether the cockles accumulated trace metals from contaminated sediments or contaminated waters. Full details of these experiments and their results are set out in Scanes (1993), see Appendix 6. A summary of that paper is included in this Chapter. In study 2, the experimental procedures from Scanes (1993) were extended to test the same hypotheses for oysters.

Study 3 examined spatial and temporal trends in concentrations of bioavailable trace metals. Assessment of spatial and temporal trends in contaminants is the most common application of biomonitors (e.g. this thesis Chapters 3and 5, Phillips and Yim 1981, Phillips and Rainbow 1988, O'Connor 1992). This study tested hypotheses about whether the concentrations of trace metals accumulated by oysters are consistent in space and time and whether they are well predicted by concentrations in sediments.

Study 1 "Trace metal uptake in cockles (Anadara trapezium) from Lake Macquarie, New South Wales (Scanes 1993).

Summary (The paper is in Appendix 6)

Contamination by trace metals in water, sediments and animals in northern Lake Macquarie has been identified as a matter of concern by regulatory authorities. Experimental procedures using the benthic bivalve *Anadara trapezium* (Sydney cockle) were developed to test the opposing hypotheses that the cockles accumulated trace metals from overlying water or from the sediment in which they were partially buried.

It was hypothesised that, if trace metals were accumulated from the sediment, cockles placed in clean sediment which was translocated to areas with contaminated water would not accumulate contaminants; whereas those in nearby contaminated sediments would. Conversely, if cockles were placed in contaminated sediment translocated to an area with uncontaminated water, they would become contaminated at a rate faster than those in surrounding clean sediment. Cockles in this translocated sediment would continue to become contaminated at a rate consistent with cockles left in the contaminated area.

Experiments were done in 1989 and 1991 to test these hypotheses. Pilot experiments indicated that it was possible to translocate tubs of sediments from impacted areas to unimpacted areas with no loss of trace metals and conversely with no increase in metals.

The experiments showed clearly that the presence of elevated levels of lead, copper and zinc in the water led to much greater levels in the cockles, irrespective of the concentration in surrounding sediments. There were no significant trends for cadmium.

It was concluded that the concentrations of trace metals in the sediments in which a cockle was living had little bearing on the levels of zinc, copper and lead that it accumulated, whereas the surrounding waters had a considerable effect.

Study 2 Trace metal uptake in oysters (*Saccostrea commercialis*) from Lake Macquarie.

Introduction

Study 1 provided support for the model that the concentrations of trace metals in the water column was more important than metal concentrations in sediments in determining the concentrations of trace metals in bivalves. Study 2 attempted to provide further support for this model by testing the same hypotheses as Study 1, but using oysters rather than cockles. If the tests provide support for the hypothesis, then the model would appear to be applicable to a range of bivalves.

The experiment was technically more difficult because oysters tend to live in relatively well flushed rocky areas and do not usually survive well partially embedded in mud.

Methods

Oysters were used in the same experimental design as used for cockles in Scanes (1993). Briefly, this design had three sites at each of one contaminated location (Cockle Creek) and two uncontaminated control locations (Kilaben and Crangan Bays). At each site a single replicate of the following treatments was established:

- untouched control
- disturbance control (local sediment placed in an experimental tub which was put back in the excavation hole)
- translocation 1 (sediment placed in an experimental tub and moved to another location)
- translocation 2 (sediment placed in an experimental tub and moved to another location)

The translocations involved moving sediment from each location to both of the other locations. This means that Cockle Creek had two uncontaminated translocations and that the two control locations had one contaminated and one uncontaminated translocation. The treatment identified in the following Tables and graphs as Translocation 1 for control locations always consists of contaminated sediments.

In November 1991, 20 oysters were placed by divers on plastic mesh which was buried just below the surface of the sediment in each the experimental treatments.

These oysters were collected in March 1992. The experimental procedure was intended to expose the oysters to the sediment and overlying colloidal layer as much as possible without smothering them.

After retrieval, the oysters were vigorously rinsed in ambient water to remove any attached sediment. They were then stored, processed and analysed according to the procedures in Chapter 2.

Results

As expected from the outset, survival of the oysters was poor (0 - 25%). In order to be able to analyse the data it was necessary to pool all replicates for each treatment at each location (Table 6.1).

Table 6.1 Number of live oysters retrieved from each treatment (all replicates pooled) from the possible 60 introduced at the beginning of the experiment.

	Cockle Ck.	Kilaben Bay	Crangan Bay
Control	0	7	0
Disturb. control	6	15	10
Translocation 1	8	15	7
Translocation 2	5	10	7
Total	19	40	24

Because many oysters died, it was only possible to compare control and disturbance control treatments at Kilaben Bay. There were no significant differences between the two treatments (ANOVA, $P > 0.70$) for any metals. It was assumed that this result was also true at the other two locations (as in previous studies). The differences among treatments and locations were investigated by randomly selecting data for six replicate oysters from each treatment at each location. These data were analysed by a two factor analysis of variance. The mean of the other data was substituted for the missing datum in Cockle Creek Translocation 2; the MS degrees of freedom was reduced by one.

Concentrations of lead, zinc and cadmium were greatest in oysters from Cockle Creek (SNK test, $P < 0.05$; "Location" Table 6.2; Fig 6.1), but treatments or treatment x location interactions were also significant. It can be seen, however, that the effects of treatment are minor compared to that of location and appear not to be related to the source of sediment (Fig. 6.1). If sediment was having an effect, concentrations in

oysters placed in Cockle Creek sediments (Treatment 1 at Cockle Creek and Treatment 2 at the control locations) should be greatest. Such a pattern is not obvious for lead, zinc or cadmium in Figure 6.1. Concentrations of copper showed no clear patterns with respect to either treatment or location.

Table 6.2 Summary of analyses of variance comparing effects of location and treatment on uptake of metals by oysters. * 0.05 < P < 0.001, ** P < 0.001

Lead

	df	Mean square	F ratio		divisor
Location	2	89.95	177.8	**	residual
Treatment	2	1.59	3.15		residual
L x T	9	2.33	4.61	*	residual
Residual	45	0.51			

Zinc

	df	Mean square	F ratio		divisor
Location	2	419156	9.4	**	residual
Treatment	2	177196	4.0	*	residual
L x T	9	99836	2.24		residual
Residual	45	44632			

Copper (log)

	df	Mean square	F ratio		divisor
Location	2	0.90	6.58	*	residual
Treatment	2	0.68	4.95	*	residual
L x T	9	0.07	0.53		residual
Residual	45	0.13			

Cadmium

	df	Mean square	F ratio		divisor
Location	2	10.71	46.02	**	residual
Treatment	2	1.91	8.2	**	residual
L x T	9	0.19	0.82		residual
Residual	45	0.23			

Discussion

The results of this study are very similar to those obtained for cockles (Scanes 1993). Oysters at Cockle Creek accumulated more lead, cadmium and zinc irrespective of the type of sediment in which they were embedded. Oysters at the other two locations showed no indications of elevated concentrations in those treatments with contaminated sediments. The result for copper in oysters demonstrates an unclear

picture which may be confounded by the presence of moored boats in the control bays (Batley et al. 1991) or possibly regulation of copper by oysters. Unlike cockles, oysters demonstrated a clear result for cadmium, suggesting that cockles may regulate cadmium.

The results provide further support for the model that the main factor controlling the concentrations of trace metals in bivalves is the concentration of dissolved metals in water rather than the concentrations in sediments.

Study 3 Spatial and temporal trends in trace metal concentrations in Lake Macquarie

Introduction

It has been assumed that, in most cases, the potential for bioaccumulation of trace metals can be inferred from concentrations in sediments (e.g. Batley 1987, Phillips and Yim 1981). The results of Studies 1 and 2 (this Chapter) and the trace metal uptake experiment (Chapter 4) have suggested that, in Lake Macquarie, this simple relationship may not be as reliable as is usually thought. The study described here attempts to determine whether the spatial patterns in contamination by trace metals that were found in Studies 1 and 2 are consistent in time. The hypothesis being tested is that concentrations of trace metals will always be relatively greater at Cockle Creek than at Crangan and Kilaben Bays.

Methods

Mesh bags containing oysters were attached to floating moorings in Cockle Creek, Crangan Bay and Kilaben Bay. The water depth was about 2 metres and bags were suspended in mid water. Times of deployment are shown in Table 6.3. Deployment 1 was during the repeat deployment for cockles (Scanes 1993), Deployment 2 was during the oysters in tubs experiment (Study 2 above) and Deployment 4 is the first 12 weeks of the trace metal uptake and depuration experiment (Experiment 4, Chapter 4). Five oysters from each of two moorings (n = 10) at Deployment 1 were analysed for cadmium, copper, lead and zinc by the methods in Scanes (1993); five oysters from each of three moorings (n = 15) at Deployments 2, 3 and 4 were analysed for the full range of metals by the methods in Chapter 2.

Table 6.3 Details of deployments of oysters. Dates are month/year.

	Date In	Date Out	Number of replicates	Metals analysed
Deployment 1	9/91	11/91	10	Cd, Cu, Zn, Pb
Deployment 2	11/91	3/92	15	Cr, Co, Ni, As, Se, Ag, Cd, Pb, Cu, Zn
Deployment 3	3/92	9/92	15	Cr, Co, Ni, As, Se, Ag, Cd, Pb, Cu, Zn
Deployment 4	9/92	12/92	15	Cr, Co, Ni, As, Se, Ag, Cd, Pb, Cu, Zn

Due to the limited range of metals and fewer replicates from Deployment 1, only the data on concentrations of metals from Deployments 2, 3 and 4 were analysed by a two factor analysis of variance, with deployment as a random factor and location fixed. Where available, data from all four deployments are presented for graphical comparison.

Results

The wet weights of oysters at the end of the experiment were significantly greatest from Deployments 2 and 3, lesser from Deployment 4 and least from Deployment 1, but were not different among locations, nor was there an interaction between location and time (Table 6.4, Fig 6.2).

Cadmium and lead and, to a lesser extent, zinc, consistently showed significantly greater concentrations at Cockle Creek (Fig. 6.2). There were significant interactions between time and location (Table 6.4) for all metals. In the cases of lead and cadmium, the interactions reflect non-significant small-scale changes in the relationships of Crangan and Kilaben Bays, rather than any major alterations to the overall pattern. Concentration of zinc was significantly greater at Cockle Creek in Deployments 2 and 3 and only marginally (but non-significantly) greater in Deployments 1 and 4. The pattern for copper was variable and not what would be predicted taking into account known point sources of this metal (Batley 1987). During Deployments 1 and 4, there was a trend for Cockle Creek to have the smaller concentrations, then Kilaben Bay and then Crangan Bay (this was not significant from Deployment 4 and untested from Deployment 1). There were no significant differences for concentrations of copper among locations from Deployments 2 and 3.

Variation among times for zinc and lead was most pronounced at Cockle Creek. Concentrations of zinc in oysters from Cockle Creek were smaller from Deployment 4 (and not different from Deployments 2 and 3) and concentrations of lead were

different from all three Deployments. Concentrations of zinc at Kilaben Bay were significantly larger from Deployment 3 and Deployments were not different at Crangan Bay. There were no differences among Deployments at Kilaben and Crangan Bays for lead. Concentrations of cadmium in oysters from Cockle Creek and Crangan Bay were smallest from Deployments 2 and 4, respectively, and otherwise not different.

Most other metals showed significant differences and interactions among Time and Time x Location (Table 6.4) and some examples are shown in Fig. 6.2. There was no indication of consistent patterns of contamination at any location. Cobalt, chromium and selenium were, however, significantly elevated at Cockle Creek during Deployment 2.

Table 6.4 Summary of mean squares from analyses comparing effects of time and location on uptake of metals by oysters. * indicates significance of *f* ratio (* 0.05 < *P* < 0.001, ** *P* < 0.001). "f divisor" row indicates divisor for analysis of variance calculation of *f*.

	Time (2 df)	Location (2 df)	T x L (4 df)	Residual (126 df)
f divisor	Residual	T x L	Resid	
Wet wt	219.17 **	10.49	3.96	3.55
Cr	0.29 **	0.08	0.16 **	0.004
Co	0.01 **	0.003	0.005 **	0.0006
Ni	0.97 **	0.22	0.16	0.11
As	311.1 **	108.3	41.3 **	1.7
Se	67.8 **	0.43	2.07 **	0.27
Ag	0.37 **	0.006	0.01 *	0.003
Cd	1.9	101.5 *	2.4 *	0.75
Pb	17.8 **	193.7 *	18.1 **	0.90
Cu	11612 **	1076	2510 *	953
Zn	2091579 **	2662358	491282 **	75256

Discussion

Temporal comparisons of the type done here can be difficult if significant variation due to laboratory error exists and if there are no control locations included in the design. QA/QC results (Chapter 2) indicated small variability within and between batches for copper and zinc. The temporal variations evident for these metals are, therefore, probably not an artefact. The temporal variation for zinc at Cockle Creek (but not Crangan and Kilaben Bays) is particularly interesting because Cockle Creek receives effluent from a copper, lead and zinc smelter. Further, Deployments 1 and 4, when the concentrations at Cockle Creek were not different from other locations were

during the early summer which is the onset of gametogenesis in oysters. The temporal changes at the two less contaminated locations were smaller, to the point of not being significant at Crangan Bay and concentrations from Deployment 3 being significantly greater than those from Deployments 2 and 4 at Kilaben Bay. This indicates some temporal change (possibly due to gametogenesis) on concentrations of zinc when these are large but not at more moderate concentrations. Seasonality of concentrations of trace metals in *Saccostrea* has also been investigated by Talbot (1986) and Peerzada and Kozlik (1992). The latter paper is of little value to this discussion because it only investigated the period from March to October and therefore missed the critical summer period. Talbot (1986) found that oysters collected from the wild in January had the smallest concentrations of zinc and oysters collected in October had the greatest. This pattern is very similar to that found in the present study, although Talbot (1986) found the summer low and spring high at all locations, not just the most contaminated, as found here. Talbot also found that wet weights were greatest in January and least in April (pre- and post- spawning respectively). She further surmised that, because concentrations decreased during periods of spawning, the metals were being shed in the gametes. The alternative hypothesis, that greater body weights were "diluting" the concentrations, was not considered properly. Boyden and Phillips (1991) also recorded a seasonal change in concentrations of zinc in translocated *Crassostrea gigas* which were observed over a number of years. There, again, the greatest concentrations occurred during winter/spring and least concentrations during summer/autumn. In that study, because there was a steady increase in total body load of zinc, they concluded that the changes in concentration were due to changes in body weight, probably related to gametogenesis. They then proposed that when body weight increases as gametes are formed, there is a tendency to reduce concentrations of metals in the whole animal.

In my study, where different batches of oysters were used for each period, it is not possible to follow average weights over time. Because average starting weights were known, I can, however, examine change of weight during the deployment. Oysters deployed during Deployments 1 and 4 (when concentrations of zinc in oysters at Cockle Creek were least) showed a loss of weight, but oysters from Deployments 2 and 3 showed a gain (Table 6.5). Note also that Deployments 1 and 4 were much shorter (2 and 3 months, respectively) than Deployments 2 and 3 (4.5 and 5.5 months). Deployment 4, when there was no significant difference among the locations, was during experiments on uptake and depuration of trace metals (Chapter 4). This allowed a more detailed assessment of concentrations of contaminants during the deployment. The oysters were introduced into Cockle Creek with starting

Table 6.5 Changes in mean wet weight (g) from start to end of each deployment. *n* = 10 for starting weights and Deployment 1, *n* = 15 for Deployments 2,3 and 4.

	Mean Starting Weight (SE) - all locations	Location	Mean Finishing Weight (SE)	Change in mean weights
Deployment 1	5.7 (0.21)	CC	5.08 (0.33)	-0.62
		KB	4.24 (0.33)	-1.47
(2 mo)		CB	4.15 (0.36)	-1.56
		mean	4.49	-1.22
Deployment 2	7.67 (1.20)	CC	9.42 (0.56)	1.75
		KB	9.37 (0.64)	1.70
(4.5 mo)		CB	8.17 (0.46)	0.50
		mean	8.99	1.32
Deployment 3	5.93 (0.56)	CC	9.90 (0.50)	3.96
		KB	8.71 (0.73)	2.78
(5.5 mo)		CB	9.69 (0.39)	3.76
		mean	9.43	3.50
Deployment4	6.64 (0.46)	CC	6.29 (0.56)	-0.35
		KB	4.93 (0.31)	-1.71
(3 mo)		CB	5.95 (0.33)	-0.69
		mean	5.72	-0.92

concentrations of zinc of about 800 $mg.kg^{-1}$, well below the final concentrations of 1300-1400 $mg.kg^{-1}$ in oysters from Deployments 2 and 3. During the first 9 weeks, there was a slow depuration to about 580 $mg.kg^{-1}$ and then an increase to 730 $mg.kg^{-1}$ by week 12. The average wet weight over this period also decreased and then increased, resulting in a correlation of zinc with weight that was positive and had an *r* value of 0.32. These data do not fit the "dilution" model which predicts that the organism has a static body load of metals and concentrations of metals change as the amount of tissue in which this load is distributed changes. It is more likely that the patterns being seen are not related to "seasonality" or gametogenesis but represent real variation in ambient water concentrations and differing times available for uptake.

Variation among batches in the QA/QC program was also small for cadmium and there was very little temporal variation in concentrations of cadmium at either Cockle Creek or Crangan and Kilaben Bays. There was some temporal variation in concentrations of lead at Cockle Creek and Crangan and Kilaben Bays, but this could have been confounded by the variation among batches. The concentrations of lead do, however, demonstrate the usefulness of sampling a range of sites. It is still obvious that Cockle Creek is the most affected location despite the temporal changes (or variability among batches due to variations in the laboratory). It is possible to make this conclusion confidently because there was very small variability within batches in

the QA/QC studies (Chapter 2) and because control locations were utilised in the experimental design.

There were very few consistent spatial gradients in contamination for the other metals. There were, however, many significant spatial and temporal differences in the analyses of variance. These are explained as either being due to significant variation in analytical precision among batches for these metals or, given the small variation among replicates, small changes which probably only represent random or chance variations.

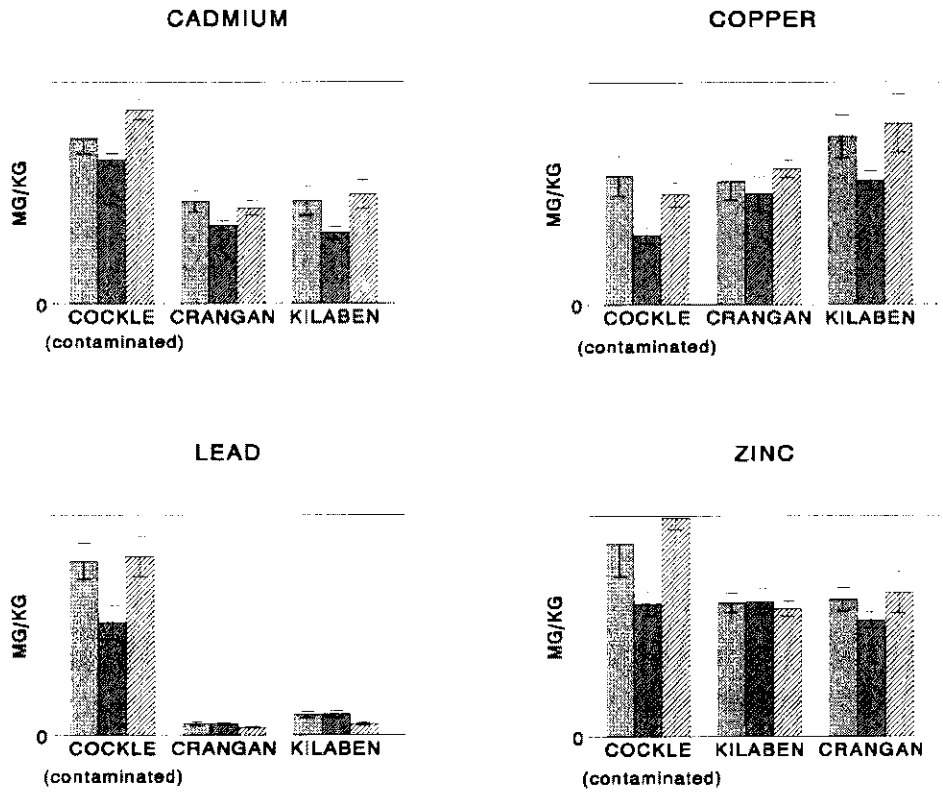


FIGURE 6.1 Mean (± S.E.) concentrations of trace metals in oysters embedded in sediments with varying metal concentrations. Labels on x axis refer to the locations of the experimental treatments. Stippled bars are for oysters in sediment from the experimental location. Hatched bars are translocated sediments: at Cockle Creek, close hatching - Crangan, wide hatching - Kilaben; at Crangan, close - Cockle Ck, wide - Kilaben; at Kilaben, close - Cockle Ck, wide - Crangan.

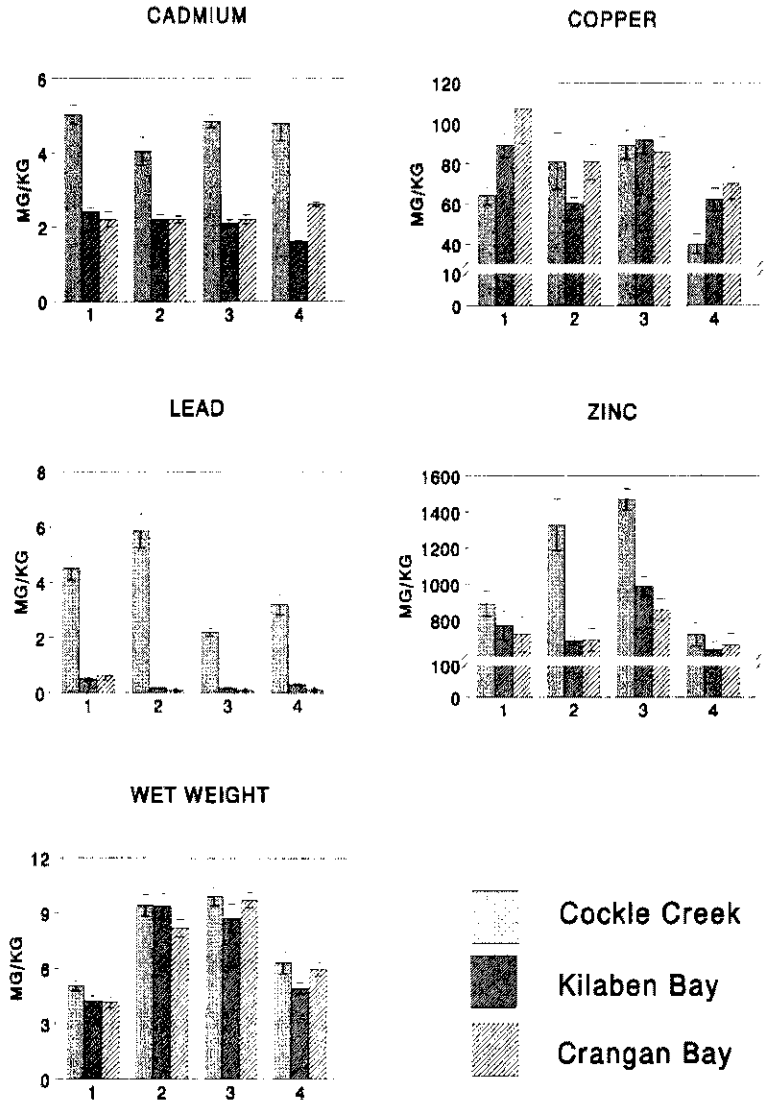


FIGURE 6.2 Mean (± S.E.) concentrations of trace metals in oysters at each location for each deployment. Numbers on x axis refer to the various deployments.

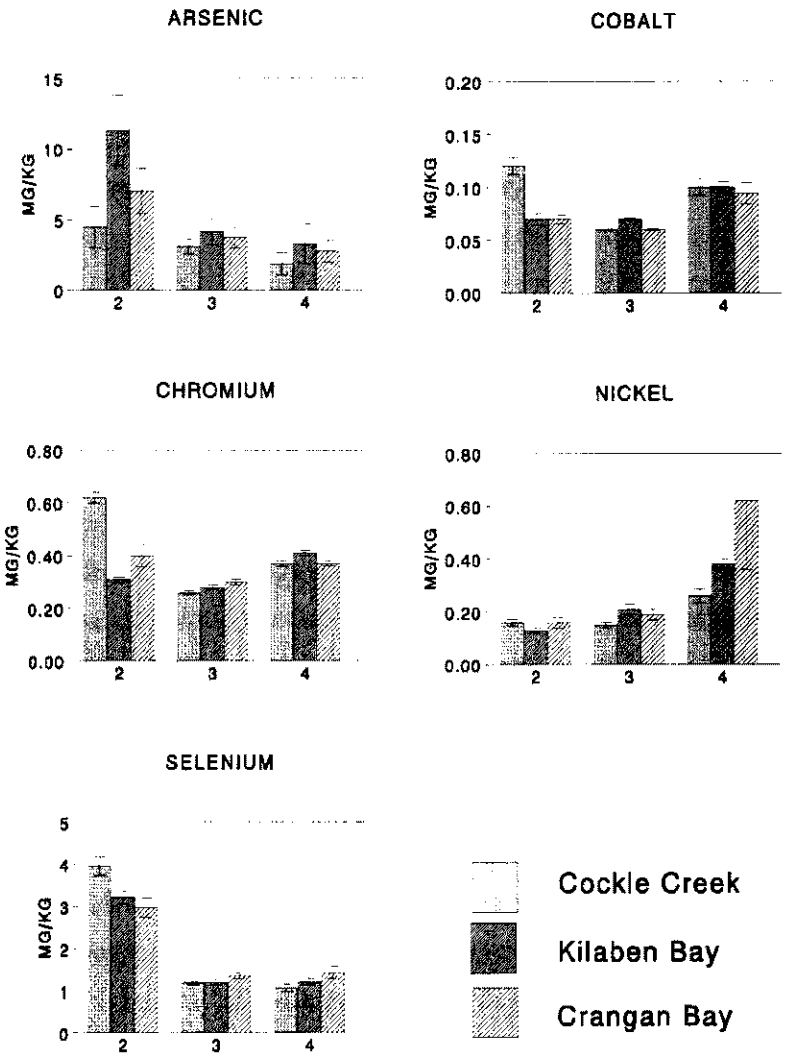


FIGURE 6.2 (cont) Mean (± S.E.) concentrations of trace metals in oysters at each location for each deployment. Numbers on x axis refer to the various deployments.

CHAPTER 7 GENERAL DISCUSSION

Bioaccumulation Programmes - Overview

The Mussel Watch concept is still forming an important component of monitoring the exposure of marine systems to chemical contaminants, particularly in the Americas and in Europe (France). There has been a recent attempt to collate all available data for oysters and mussels into a single database (Cantillo 1997) in order to assess whether there are detectable worldwide spatial and temporal trends in oceanic contamination. The database was established, but the data pre-1970 were insufficient and data were generally not spread well enough to satisfy these goals. Specific Mussel Watch programmes in the USA and France are, however, providing useful data on spatial and temporal trends in contamination of marine waters (O'Connor 1996, Beliaeff et al. 1997a, Beliaeff et al. 1997b). The recent expansion of the USA Mussel watch to Central and South America has expanded the ability to examine wider spatial patterns in detail (Farrington and Tripp 1995). These programmes are providing cost-effective descriptions of spatial and temporal trends in the range and magnitude of chemical contamination of waters. There are, however, limitations to the usefulness of the technique on very large spatial scales. These arise when there are attempts to generalise to large areas from data collected over a small scale. This is a common temptation and one that is common to many forms of ecological sampling – it is one of the main forms of confounding that Hurlbert (1984) identified in his paper on pseudoreplication. If used properly, however, data from mussel watch programmes allow investigators to focus the potentially more costly investigations of indicators of ecosystem integrity in those areas where there is the greatest potential for disturbance from chemical compounds.

Practical Application of Bioaccumulation Programmes Using Sydney Rock Oysters

As indicated in the introductory chapter, the primary aim of this thesis was to gain an understanding of how and with what limitations, bivalves (primarily the oyster *Saccostrea commercialis*) could be used in NSW marine and estuarine waters as an indicator of trace contaminant pollution. This necessitated finding out whether the animals accumulated the appropriate contaminants, whether they could be used to demonstrate gradients of pollution, whether the manner of deployment (if used) affected the bivalves' ability to accumulate contaminants, what are appropriate

sampling times, levels of replication and what are the limitations of use and interpretation of the data.

Summary of Findings

Species, Contaminants and Biological Confounding

Two main species of bivalves (oysters and cockles) were assessed in this thesis. Both demonstrated an ability to show gradients of pollution by trace metals, although cockles did not show differences for chromium, cobalt, copper and cadmium under conditions where oysters did show a difference. Mercury was the only metal for which cockles showed a gradient but oysters did not. Oysters accumulated and showed gradients of pollution for a range of organochlorines (including PCBs) and PAHs, but did not accumulate phenols. Cockles did not accumulate detectable concentrations of organochlorine compounds in any of the tests.

Based on these results and the availability of oysters through commercial growers, the majority of further studies concentrated on assessing the oyster as a biomonitor. The exception was a specific study of the effects of sediments, where cockles were more suitable due to their natural occurrence in benthic sediments.

Various authors (see Phillips and Rainbow (1993) for review) have raised the possibility that biological factors (e.g. size, sex, stage of gametogenesis, fat content) could affect concentrations of trace metals and organochlorines in bivalves. Phillips and Rainbow (1993) suggested the following strategies for dealing with such variation:

- ignore and accept different sizes, genders, etc., from different locations
- use biomonitors that do not show significant effects of biological variables
- use a normalisation procedure of some sort
- restrict the range of sizes and weights used, i.e. stratify he sampling of animals to include only certain sizes and weights

I would add the following alternative:

- if possible, randomly select animals so that effects of size and gender are randomised in each sample or stratify the sampling to include equitable sets of sizes and genders in each treatment.

The practicality (and legitimacy) of the various strategies are influenced by the circumstances and hypotheses being examined in individual studies. As a general rule, in the studies reported in this thesis, the last two strategies were employed. All studies were based on deployments of oysters of the same age obtained from commercial growers (thus restricting age and size) and were allocated randomly to various treatments and/or replicate moorings. Using this strategy, sizes were restricted and the results during the uptake and depuration experiments showed that, within the truncated size-ranges used, wet weight was not a significant determinant of equilibrial concentrations of organochlorine compounds. Weight was, however, a moderate positive determinant in the concentrations of arsenic and selenium (r^2 = 0.30 and 0.28, respectively) and was negatively correlated with concentrations of cadmium, silver and mercury (r^2 = 0.25, 0.36 and 0.25, respectively). Deploying oysters of similar age should also avoid confounding of results by different genders of oysters, because *S. commercialis* is known to be a protandrous hermaphrodite. This possibility was not investigated here. This characteristic does, however, suggest that studies which involve wild collections of different sizes are at considerable risk of confounding by gender, as are studies that contrast concentrations in oysters of different sizes. Compared to size-related differences, differences in concentrations of contaminants between genders are rare (Phillips and Rainbow 1993).

The concentration of fat in oysters was shown to influence the equilibrial concentrations of chlordane, dieldrin and DDT in experiments done in a highly contaminated estuarine area. In other studies done in more dynamic areas, with lower ambient organochlorine concentrations, there was no demonstrable correlation between organochlorine concentration and fat. Further, in the estuarine studies, the same conclusions on temporal trends in organochlorine contamination could be drawn from "raw" and "fat corrected" organochlorine data. For the type of monitoring used in this thesis, the necessity of correcting data to take account of amounts of fat is thus questioned. In cases where no relationship between lipid and concentration of organochlorines exists, corrections using weight of fat could be misleading.

Timing and Sampling Strategies

Experiments on uptake and depuration of organochlorines and trace metals were done to determine appropriate timing of sampling to allow an accurate reflection ("integration") of the sampling period for various contaminants. Estimated times to equilibrium calculated for the contaminants investigated range from < 1 week

(heptachlor expoxide) to 130 weeks for zinc (Table 7.1), indicating that selecting a suitable period for sampling is not a simple matter.

Table 7.1 Contaminants grouped according to estimated times to equilibrium.

< 1 week	1-3 weeks	3-6 weeks	6-12 weeks	12 - 42 weeks	> 42 weeks
HPTE	PCB chlordane dieldrin	DDT isomers		lead cadmium mercury silver	copper zinc

The sheer range of times to equilibrium means that unless a particular contaminant is suspected and targeted, period of deployment of bivalves has to be a compromise. Sampling intervals of 6 weeks would be suitable for most organochlorines, but much longer times of 15-20 weeks would be necessary to approach equilibrium for most metals. Other studies (in Chapters 3 and 6) demonstrated differences in ambient concentrations of metals using much shorter time periods (8 to 12 weeks). Those results probably do not reflect equilibrial conditions. Therefore ultimate concentrations could be greater. It could be argued that, for metals, collections of wild oysters might be a better option because the animals are more likely to be in equilibrium with concentrations in their local habitat. Such collections would, however, be susceptible to confounding by biological variables (as indicated above).

Design and replication of studies is very difficult to deal with in an abstract sense, except at the most general level. Here, experiments generally used between 5 and 15 replicate individual animals for each location or treatment. In most cases, power to detect moderate changes in concentration of organochlorines (50 to 100 %) was very good (0.99) with 5 to 6 replicates (Chapter 3, Experiment 6). In general, concentrations of organochlorine were less variable than concentrations of metals.

The design of any study is very much dependent on the specific hypothesis being tested. Here, experiments have concentrated on patterns at relatively large spatial scales (locations about 50 to 200 m in diameter, separated by kilometres). In all cases, animals were deployed on moorings randomly scattered about the location and the data then pooled for statistical analysis. There has been no attempt to describe smaller scales of difference because they were not relevant to the hypotheses being tested. It is, however, necessary to have replicates from all parts of the location to avoid spatial confounding or "pseudoreplication" (*sensu* Hurlbert 1984).

Habitats and Deployment

An experiment which investigated the effects of various types of structure used to deploy bivalve showed that deployments which suspended the oysters in the water column (rather than anchoring them on the sea floor) tended to have the least mortality of animals, but this difference was not significant. There were no differences in the concentrations of organochlorines or trace metals in oysters from mid-water and sea-floor moorings, with the exception of arsenic, which was in greater concentrations in oysters from mid-water moorings.

Oysters have been shown to be generally the best and most convenient biomonitor in NSW waters. The main exception to this is for studies of the effects of contaminants in sediments, where cockles were more suitable due to their natural occurrence in benthic sediments.

Relationship to other Environmental Measures

Possible "Effects" of Pollution by Trace Contaminants (the "so-what" question)

A criticism commonly levelled at bioaccumulation studies is that they do not provide information on impacts on the ecology of an area. This has been called the "so-what" question (Wolfe 1992, Chapman 1997). This thesis has not specifically addressed the "so-what" question. I have taken the position that studies of pollution occur in three distinct stages

1. Identification of a potential problem
2. Demonstration that the potential is realised in terms of biological effects
3. Intervention, rectification and remediation.

Similar models of the inter-relationships among various types of environmental indicators have proposed by a variety of authors. Wolfe (1992) suggested the following progression for monitoring marine environmental quality:

1. Distribution of toxic contaminants
2. Contaminant exposure and bioavailability
3. Distribution of "significant bioeffects
4. Concordance of bioeffects with contaminant levels (Wolfe 1992, Table 1, p 153)

He included bioaccumulation studies as an integral and necessary component of the second objective. This position is similar to the one that I have taken.

Similarly, identification of a "problem" or the potential for a problem (i.e. the presence of potential toxicants such as organochlorines, trace metals etc) has been identified by GESAMP (1995)as the first phase of any environmental study. Detection of the presence and quantification of the magnitude of contamination by bioavailable chemicals is an integral part of the approach of environmental evaluation known as the Sediment Quality Triad (Chapman et al. 1987, Chapman et al. 1991, Green and Montagna 1996).

I have, in this thesis, provided a means of identifying the presence of contaminants. If further studies of ecological indicators show that the contaminant is causing ecological effects there is a case for environmental managers to intervene and attempt rectification and remediation. The methods described here are also relevant to temporal monitoring aimed at detecting whether remedial actions are reducing the potential for ecological impact.

Various authors (e.g. Martin 1985, Bayne 1989) have suggested that sentinels used to study bioaccumulation could also be used to indicate biological effects. As I stated previously (Chapter 1), bioaccumulators and indicators of biological and ecological effects are chosen to have diametrically opposed characteristics. The former are chosen because they are hardy, adaptable and able to survive under adverse conditions. In contrast, indicators of biological effects are chosen because, as individuals or as parts of an assemblage, they react quickly to minor changes in their environment. It thus seems that, whilst it is undeniable that bivalves can be used to demonstrate biological effects (e.g. Bayne et al. 1979, Martin 1985, Bayne 1989), perhaps more subtle indicators should be chosen, indicators that are inextricably linked to the biological assemblages, which is usually the reason for being concerned about pollution (Underwood and Peterson 1988).

Bivalves commonly used for bioaccumulation studies (e.g mussels, oysters) usually do not meet the criteria for use as ecological indicators and probably require very large environmental insults before they are affected. This makes them unsuitable for use as subtle indicators. This point is well demonstrated in the *Discussion* following Bayne et al. (1979), A.V. Holden asked whether measurements of "scope for growth" might demonstrate differences over a gradient that spanned differences in body burdens of organochlorines of 3 orders of magnitude. Bayne replied that he "would guess that ....[it]... would result in measurable physiological ... effects." Further, Jorgensen

(1990) pointed out that, because scope for growth is non-linear, it is not sensitive to changes in stress until conditions become markedly sub-optimal.

The condition of bivalves (i.e. body weight: cavity volume) has been successfully used to demonstrate gradients of pollution (Pridmore et al. 1990). Condition is, however, considered a fairly crude measurement of biological effect (Phillips and Rainbow 1993). In Chapter 3, there was evidence that oysters deployed further from an industrial discharge had smaller concentrations of PAHs than those closer (oysters 200m away had 75% less PAHs than those 10m away) and the greatest concentrations achieved were about 36 times control concentrations. There were, however, no changes in the condition of oysters among outfall and control locations. Concurrent studies (Scanes 1995) demonstrated significant changes in the surrounding biological assemblages among the 10m, 200m and control locations. This demonstrates that levels of pollution which can cause large differences in body burden, can have significant effects on surrounding biological assemblages but not affect condition of the oysters. So in this case, oysters did not directly serve as indicators of some important ecological effects, but did provide information that a gradient of potential toxicants existed.

It does, however, appear that juvenile stages of some oysters may serve the purpose of indicators of biological effect as well as indicators of potential exposure to contaminants. Ringwood et al. (1996) showed reduced rates of growth and increased tissue loads of metals and PAHs in juvenile oysters deployed at contaminated sites when compared to other, control, locations. Krassoi (1995) has developed a toxicological test based on the larval stages of *Saccostrea commercialis*.

The studies described in this thesis have shown the presence of a number of potential pollutants in the waters of NSW. The primary organochlorines detected were chlordane, dieldrin, DDT, DDD and DDE. Chlordane has been linked to reductions in shell-deposition of oysters, mortality of fish and crustaceans (Parrish et al. 1976) and a reduction in the density of juvenile benthic bivalves (Pridmore et al. 1991). Aldrin (a precursor to dieldrin) has been shown to be lethal to crustaceans, but not polychaetes (Scanes et al. 1993). Trace metals and organochlorines can also cause a range of other effects to individual organisms and assemblages (see Chapter 1 and Phillips and Rainbow 1993 for review).

One effect that could confound studies of bioaccumulation is alteration of feeding-rates, which could, in turn, affect body-burdens by increasing or decreasing water flow

across surfaces of gills and changing intake of food. Phillips and Rainbow (1993) cited very few examples of this phenomenon. In all cases where it was observed, it was in laboratory experiments, where concentrations were far in excess of normal, ambient conditions. Changes in food intake would be expected to lead to changes in body weight. In the experiments described in this thesis, there was no evidence of differences in body weight of bivalves among treatments. This suggests that, whilst it is theoretically possible for contaminants to affect feeding rates, it seems that the concentrations needed to initiate such changes are very rare in field conditions in NSW. Hartwell et al.'s (1991) data, from a study which used ambient water under laboratory conditions, support this conclusion. The level of contamination of ambient waters did not influence the rates of respiration and feeding in oysters (*Crassostrea virginica*).

The link between exposure, measured by bioaccumulation and impact on organisms is receiving increased attention. Chapman (1997) presented a discussion of the use of bioaccumulation for predicting impact. His discussion was centred on an analysis of the potential for predicting the level of impact on the organism in which the levels of tissue contamination are determined. He concluded that there is potential for concentrations of contaminants in tissues to be used as a predictor of impacts. The state of knowledge about relationships between toxic concentrations and body burdens is still too poor to allow useful predictions. There is very little likelihood, given the current level of knowledge, that valid predictions could be made in the foreseeable future about the effects on higher trophic levels resulting from consumption of contaminated prey.

The clear message from these considerations is that studies of environmental disturbance will require a suite of indicators, each with their own role to play (Underwood and Peterson 1988, GESAMP 1995, Wolfe 1996, Chapman 1997). It is, however, important to understand that any component of the suite of environmental indicators will have limited or no predictive capacity outside the scope of its operational measurement (GESAMP 1995). The cumulative picture built from the individual components will provide a better understanding of the cause and effects of the disturbance than an examination of any single aspect of the study. Unfortunately, whilst there is great value in integrating the results of a variety of studies covering a broad spectrum of environmental measures (physical aspects, exposure, toxicological, sub-organismal and community impacts), this approach has not been common (Green and Montagna 1996, Kennicutt et al. 1996, Peterson et al. 1996).

Links Between Impact Assessment and Managerial Actions

GESAMP (1995) identified three general phases in measuring the condition of marine ecosystems:

1. regular, often low level, monitoring or surveillance of the system to detect changes or potential for change;
2. determination of the cause and consequences of the changes noted;
3. implementation of managerial solutions

Within each of these phases, there can be many more layers of complexity. I will focus on the role of studies of bioaccumulation in such a structure.

Projects which look at body-burdens of contaminants can be used in a number of roles within this framework:

- in Phase 1, as a "first strike" searching tool or as an indicator of potential pollution;
- followed, in Phase 2, by a more sensitive and detailed investigation of other indicators to determine if the potential problems indicated by elevated body-burdens of contaminants are translated into effects on surrounding biological assemblages. Bayne (1988) and GESAMP (1995) have, however, both noted that if the nature of chemical contamination is known or suspected (from a source other than studies of bioaccumulation) then there is little value in proceeding with studies of bioaccumulation. This position was reached because it is well known that the link between accumulated body-burdens and ecological disruption is not clear. It is therefore most cost-effective and informative to proceed directly to ecological investigations;
- to test specific hypotheses about pollution pathways (e.g. Chapter 6) in Phase 2;
- to monitor temporal patterns (surveillance monitoring, e.g. Chapter 5 and 6) in Phase 1;
- to monitor the effects of any managerial activities taken (compliance monitoring) in Phase 3 (Chapter 5);
- to provide information on acceptability for consumption or other regulatory guidelines in Phase 2.

The studies discussed in this thesis and the ongoing US Mussel Watch programme have demonstrated clearly that studies of bioaccumulation have a role to play in environmental management. They are cost-effective and provide easily interpreted

data for surveillance and compliance monitoring of bioavailable contaminants. They have a less important role in assessing the disruption to ecological systems, except for investigations of some pathways by which contamination moves through food webs.

It is, however, imperative that there is a good understanding of the reaction of the test organism to the contaminants of interest and it has been the role of this thesis to provide that understanding for *Saccostrea commercialis*.

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APPENDICES

Appendix 1 Concentrations of trace metals and organochlorines in each batch of Reference Standard analysed.

		Mean Conc	SE	Precision	% CV
Cu					
	Batch 1	40	0.57	0.01	2.5
	Batch 2	40.5	0.5	0.01	1.7
	Batch 3	40	0.15	0.00	1.2
	Batch 4	40.36	0.08	0.00	0.7
	Batch 5	40.2	0.37	0.01	2.1
	Batch 6	41.25	0.75	0.02	3.6
	Batch 7	40.5	0.5	0.01	1.7
	Mean			0.01	1.9
Zn					
	Batch 1	356.7	3.3	0.01	1.6
	Batch 2	360	0	0.00	0.0
	Batch 3	358	1.3	0.00	1.1
	Batch 4	357	1.5	0.00	1.4
	Batch 5	358	2	0.01	1.2
	Batch 6	367.5	6.3	0.02	3.4
	Batch 7	360	10	0.03	3.9
	Mean			0.01	1.8
Cr					
	Batch 1	0.217	0.07	0.32	55.9
	Batch 2	0.15	0.04	0.27	37.7
	Batch 3	0.15	0.005	0.03	10.5
	Batch 4	0.205	0.005	0.02	8.1
	Batch 5	0.148	0.005	0.03	7.6
	Batch 6	0.15	0.01	0.07	13.3
	Batch 7	0.29	0.03	0.10	14.6
	Mean			0.12	21.1
Co					
	Batch 1	0.053	0.006	0.11	19.6
	Batch 2	0.061	0.0005	0.01	1.2
	Batch 3	0.041	0.001	0.02	7.7
	Batch 4	0.05	0.0006	0.01	4.0
	Batch 5	0.062	0.0006	0.01	2.2
	Batch 6	0.045	0.0005	0.01	2.2
	Batch 7	0.059	0.002	0.03	4.8
	Mean			0.03	5.9

Ni					
	Batch 1	0.11	0.03	0.27	47.2
	Batch 2	0.11	0.01	0.09	12.9
	Batch 3	0.09	0.002	0.02	7.0
	Batch 4	0.08	0.001	0.01	4.1
	Batch 5	0.076	0.004	0.05	11.8
	Batch 6	0.0775	0.005	0.06	12.9
	Batch 7	0.09	0.01	0.11	15.7
	Mean			0.09	15.9
As					
	Batch 1	1.3	0.06	0.05	8.0
	Batch 2	1.1	0	0.00	0.0
	Batch 3	0.72	0.02	0.03	8.8
	Batch 4	1.27	0.12	0.09	31.3
	Batch 5	1.3	0.03	0.02	5.2
	Batch 6	0.988	0.01	0.01	2.0
	Batch 7	1.1	0	0.00	0.0
	Mean			0.03	7.9
Se					
	Batch 1	1.14	0.19	0.17	28.9
	Batch 2	0.79	0.15	0.19	26.8
	Batch 3	0.463	0.02	0.04	13.7
	Batch 4	0.68	0.02	0.03	9.8
	Batch 5	1.08	0.04	0.04	8.3
	Batch 6	0.645	0.03	0.05	9.3
	Batch 7	0.885	0.07	0.08	11.2
	Mean			0.08	15.4
Ag					
	Batch 1	0.095	0.02	0.21	36.5
	Batch 2	0.375	0.1	0.27	37.7
	Batch 3	0.106	0.03	0.28	89.5
	Batch 4	0.09	0.01	0.11	36.9
	Batch 5	0.2	0.05	0.25	55.9
	Batch 6	0.55	0.02	0.04	7.3
	Batch 7	0.126	0.06	0.48	67.3
	Mean			0.23	47.3
Cd					
	Batch 1	0.566	0.01	0.02	3.1
	Batch 2	0.46	0.07	0.15	21.5
	Batch 3	0.298	0.01	0.03	10.6
	Batch 4	0.522	0.003	0.01	1.9
	Batch 5	0.566	0.002	0.00	0.8
	Batch 6	0.413	0.003	0.01	1.5
	Batch 7	0.46	0	0.00	0.0
	Mean			0.03	5.6

Hg				
Batch 1	0.01	0	0.00	0.0
Batch 2	0.02	0	0.00	0.0
Batch 3	0.01	0	0.00	0.0
Batch 4	0.01	0	0.00	0.0
Batch 5	0.01	0	0.00	0.0
Batch 6	0.01	0	0.00	0.0
Batch 7	0.01	0	0.00	0.0
Mean			0.00	0.0
Pb				
Batch 1	0.277	0.009	0.03	5.6
Batch 2	0.28	0.02	0.07	10.1
Batch 3	0.167	0.007	0.04	13.3
Batch 4	0.12	0.002	0.02	5.5
Batch 5	0.33	0.003	0.01	2.0
Batch 6	0.213	0.003	0.01	2.8
Batch 7	0.21	0.01	0.05	6.7
Mean			0.03	6.6
Chlordane				
Batch 1	0.117	0.006	0.05	11.2
Batch 2	0.093	0.002	0.02	7.5
Batch 3	0.101	0.005	0.05	20.5
Mean			0.04	13.1
DDE				
Batch 1	0.03	0.002	0.06	15.5
Batch 2	0.03	0	0	0
Batch 3	0.04	0.002	0.05	21.6
Mean			0.04	12.4
Deildrin				
Batch 1	0.01	0.002	0.17	0.3
Batch 2	0.02	0	0	0
Batch 3	0.01	0	0	0
Mean			0.06	0.01
DDD				
Batch 1	0.04	0.002	0.06	13
Batch 2	0.03	0	0	0
Batch 3	0.03	0.002	0.08	34
Mean			0.05	16
PCB				
Batch 1	0.37	0.01	0.03	7.3
Batch 2	0.32	0.005	0.02	4.6
Batch 3	0.36	0.01	0.03	15.1
Mean			0.02	9

Appendix 2 Summaries of analyses of variance of concentrations of trace metals in oysters, Experiment 4, Chapter 4. * $P < 0.05$

Analysis 1 - Comparison of contaminated locations (Cockle Creek and Cockle Bay).

		Weight		Pb		Cd		Cu		Zn		Se		Hg		Ag	
	df	SS	F	SS	F	SS	F	SS	F	SS	F	SS	F	SS	F	SS	F
Locn	1	4.52	1.73	0.0007	0	2.11	2.94	173	0.36	2332	0.05	0.12	1.33	0.00004	5.39 *	0.05	0.46
Times	5	129.8	9.92 *	55.7	16.76 *	109.4	30.32	50814	21.0 *	299073	1.19	2.38	5.24 *	0.0009	20.8 *	4.31	8.03 *
L x T	5	47.3	3.61 *	5.28	1.59	10.01	2.77	5053	2.09	124759	0.50	0.52	1.15	0.0003	7.04 *	0.85	1.58
Resid.	84	219.8		55.8		60.6		40707		4211473		7.63		0.0007		9.03	

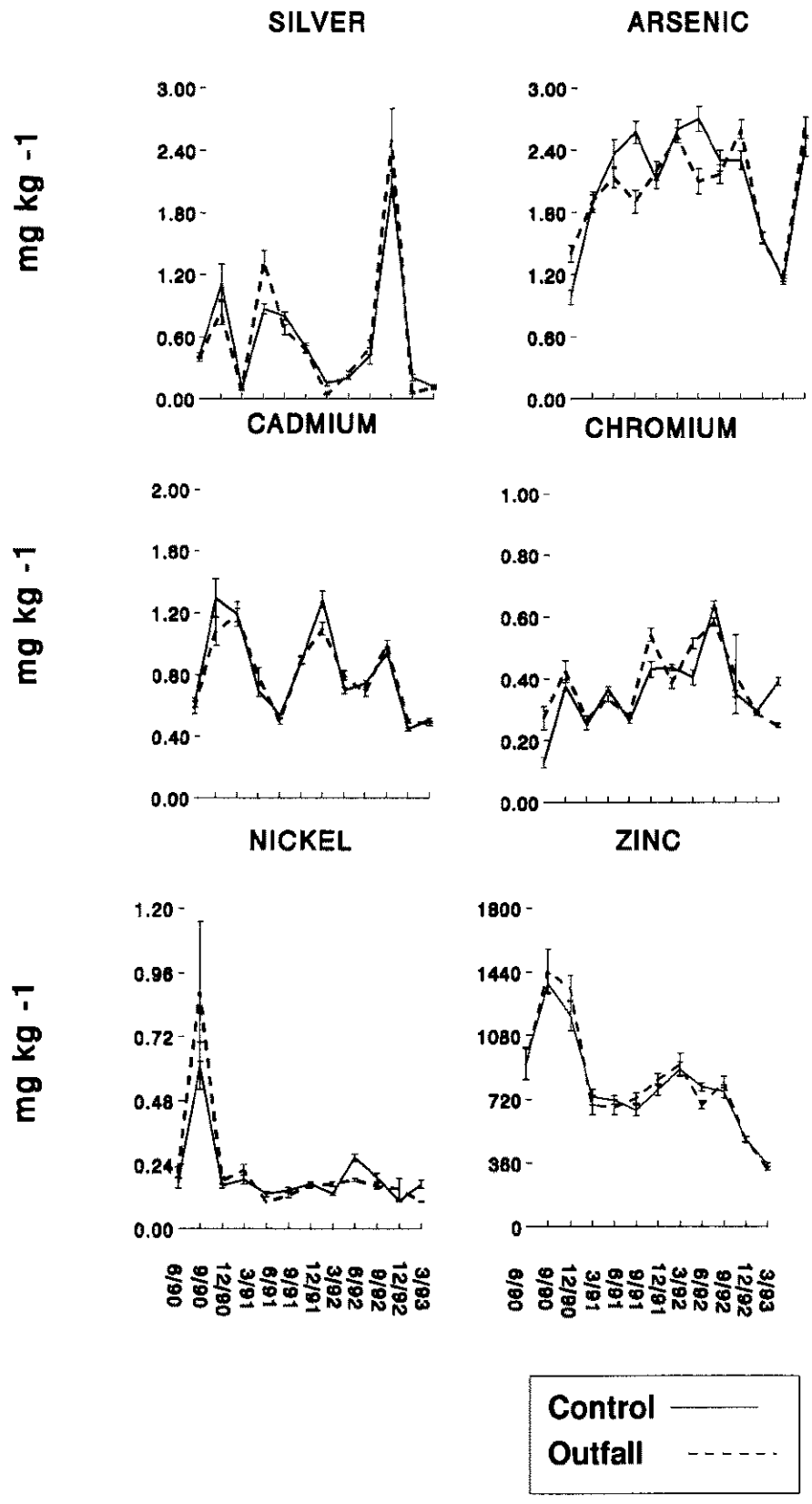
Analysis 2 - Comparison of control locations (Kilaben and Crangan Bays).

		Weight		Pb		Cd		Cu		Zn		Se		Hg		Ag	
	df	SS	F	SS	F	SS	F	SS	F	SS	F	SS	F	SS	F	SS	F
Locn	1	4.1	0.77	0.16	14.9 *	0.13	0.72	11	0.02	26557	0.51	0.16	0.78	0.0004	5.7 *	0.0006	0.35
Times	2	67.32	6.29 *	0.26	12.0 *	7.20	20.2 *	8420	5.98 *	677305	6.53	1.48	3.63 *	0.0008	16.2 *	0.0013	0.40
L x T	2	4.52	0.42	0.08	3.6 *	0.28	0.79	1462	1.04	96379	0.93	0.77	1.88	0.0001	2.95	0.0015	0.46
Resid.	42	224.8		0.46		7.51		29563		2178385		8.60		0.001		0.07	

Analysis 3 - Comparison of contaminated locations with controls, times 12 and 24 weeks.

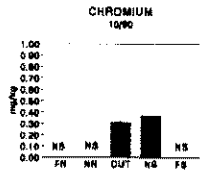
		Weight		Pb		Cd		Cu		Zn		Se		Hg		Ag	
	df	SS	F	SS	F	SS	F	SS	F	SS	F	SS	F	SS	F	SS	F
Treat	1	2.82	5.8	120	520	46.78	11.77	7521	16.12	705582	13.54 *	0.76	21.01 *	0.0003	24.0 *	0.005	0.03
S (T)	2	0.97	0.11	0.46	0.41	7.95	4.48					0.07	0.24			0.30	1.69
Times	1	87.63	7.11	2.91	2.13	40.58	22.99	3025	6.48	134479	2.58	0.11	0.28	0.0009	68.53 *	2.27	21.83 *
S x T	1	0.19	0.02	1.17	0.86	15.38	8.71	875	1.88	220910	4.24 *	0.00002	0	0.00001	0.83	0.03	0.32
T x S(T)	2	24.66	2.77	2.73	2.42	3.53	1.99					0.76	2.5			0.21	1.17
Resid.	56	249.5		31.62		49.65		27990		3127360		8.46		0.0008		4.99	

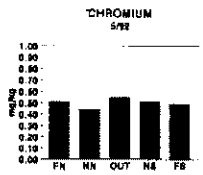
Appendix 3 Mean concentrations of trace metals and organochlorines from oyster deployments, Chapter 5

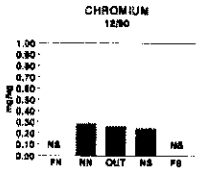


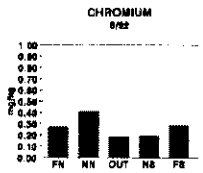
Mean of all data for inshore deployments
(see also Fig 5.3)

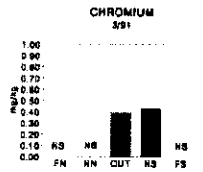
The following graphs are means of all data for offshore deployments (see also Fig 5.6)

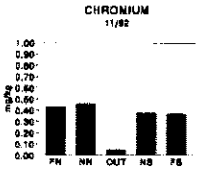


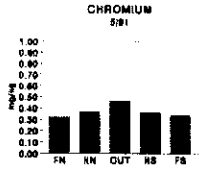


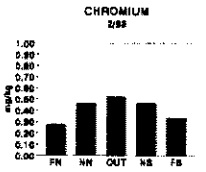


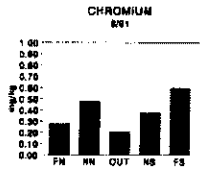


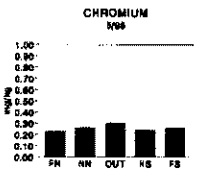


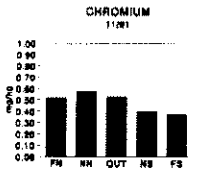


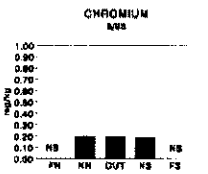


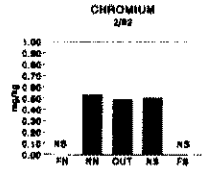


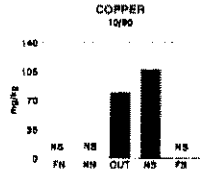








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mg/kg
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105
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35
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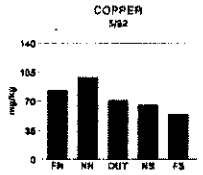
NS
FN
NN
OUT
NS
FS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mg/kg
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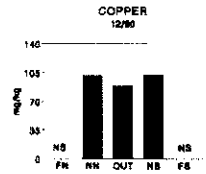
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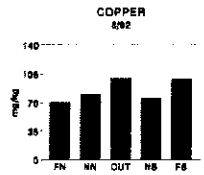
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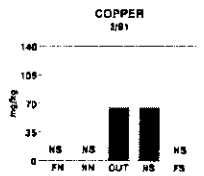
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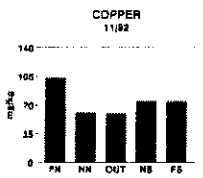
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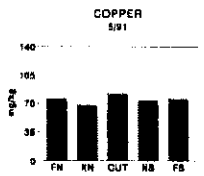
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mg/kg
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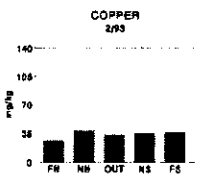
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OUT
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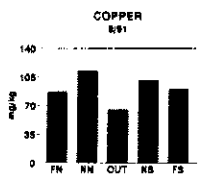
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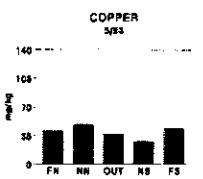
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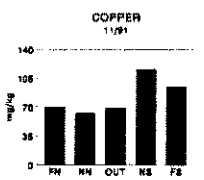
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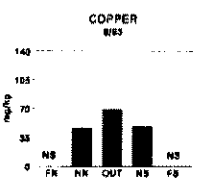
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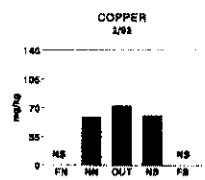
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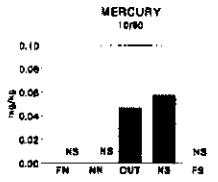
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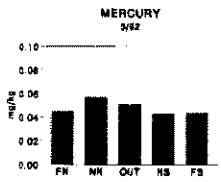
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mg/kg
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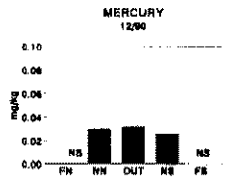
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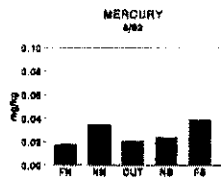
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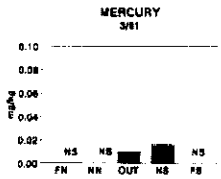
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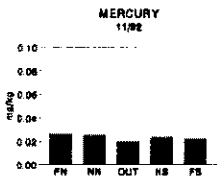
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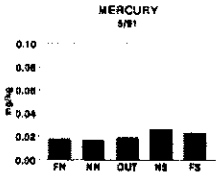
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NN
OUT
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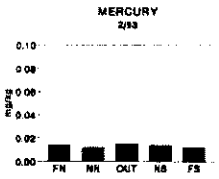
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mg/kg
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NN
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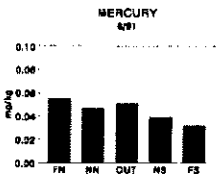
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OUT
NS
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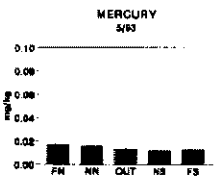
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NN
OUT
NS
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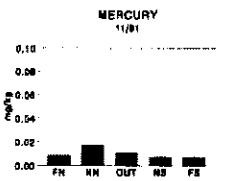
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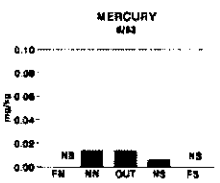
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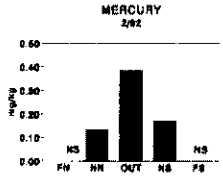
NN
OUT
NS
FS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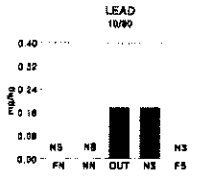
NN
OUT
NS
FS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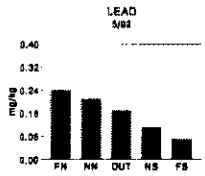
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mg/kg
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0.32
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0.08
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NS
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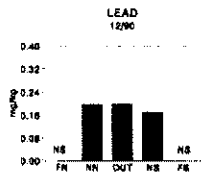
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12/90
mg/kg
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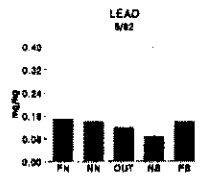
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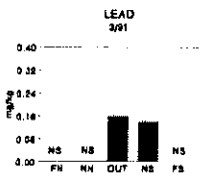
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mg/kg
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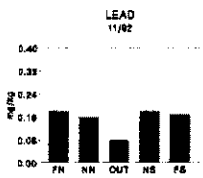
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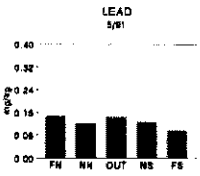
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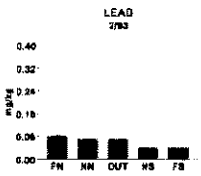
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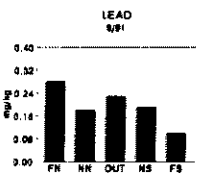
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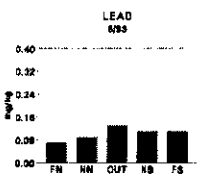
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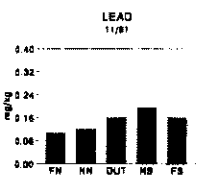
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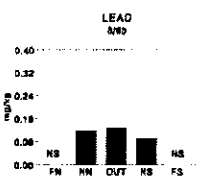
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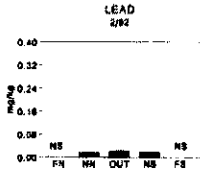
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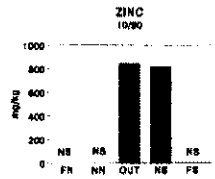
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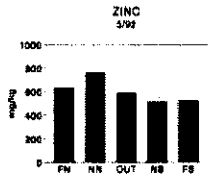
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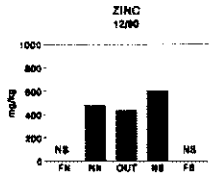
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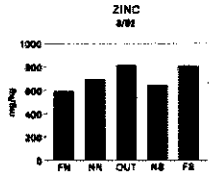
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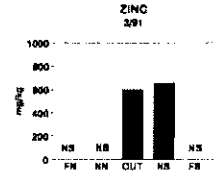
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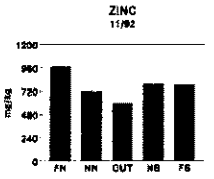
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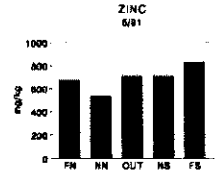
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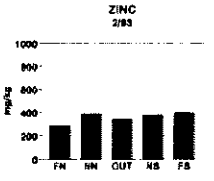
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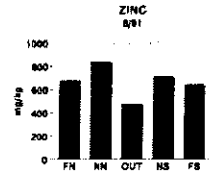
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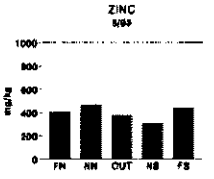
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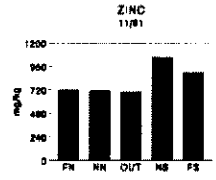
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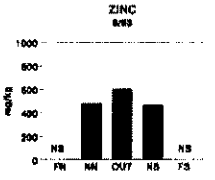
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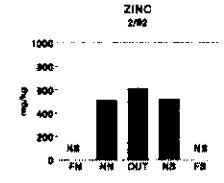
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Appendix 4 **Scanes, P. R. 1997, 'Uptake and depuration of organochlorine compounds in Sydney rock oysters (*Saccostrea commercialis*)', *Marine and Freshwater Research*, vol. 48, pp. 1-6.**

Mar. Freshwater Res., 1997, **48**, 1–6

Uptake and depuration of organochlorine compounds in Sydney rock oysters (*Saccostrea commercialis*)

Peter R. Scanes

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Abstract. Field experiments were done to determine the rates of uptake and depuration of chlordane, dieldrin, heptachlor epoxide, PCBs, DDT, DDD and DDE in Sydney rock oysters (*Saccostrea commercialis*). Oysters from a clean source were placed in a contaminated location and concentrations of organochlorines determined in samples collected at a number of times during the deployment. After 209 days (which was long enough for equilibrium to have been reached), the oysters were moved to an uncontaminated location and concentrations of the compounds were determined at various times. Mean weight of the oysters changed during the study, but not in any consistent manner. Mean content of lipids did not change significantly during the study. Detectable concentrations of all organochlorines were present after 3 days. Times to equilibrium and biological half-lives were calculated using kinetic models. Generally, rates of depuration were of the same order, but slower, than rates of uptake. There was a strong correlation between percentage lipid and organochlorine concentration, but little difference in times to equilibrium and biological half-lives derived from concentrations per wet weight and those per weight of lipid. The experiments show that times to equilibrium for each compound differ and that this should be taken into account when designing a programme to monitor these compounds.

Extra keywords: oysters, *Saccostrea commercialis*, organochlorine compounds, uptake, depuration, mussel watch, pollution monitoring.

Introduction

In investigations of pollution in marine and estuarine environments, a common approach has been to monitor ambient concentrations of trace contaminants by determination of the concentrations of contaminants accumulated in the tissues of animals maintained near sources of pollution. The animals most commonly used are bivalve molluscs, and the technique, known by the generic term 'mussel watch', is widely used in Europe, Asia and the United States, where mussels are abundant. The 'mussel watch' concept is based on the knowledge that molluscs, crustaceans and fish may accumulate certain environmental contaminants in their tissues to concentrations above ambient levels in the environment. This allows such species to be used as 'indicator' or 'sentinel' organisms, reflecting relative levels of environmental contamination in a manner amenable to short-term and long-term monitoring (Phillips and Rainbow 1993).

In order to understand and interpret data on contamination and to plan future studies, it is necessary to have information on the rates of uptake and depuration of contaminants (Phillips and Rainbow 1993). This is particularly important when interpreting temporal patterns in data. If collections are made at time-scales much greater than those required to reach equilibrium with the environment, then it cannot be assumed that the biomonitors are providing an integration of the entire period—they may be reflecting net uptake or depuration over a much shorter period. Conversely, if the collecting period is short in relation to the kinetics of the contaminant, then equilibrium will not be achieved.

Despite recommendations that bivalves be used to assess organochlorines in the tropics and the Southern Hemisphere (Goldberg 1975, 1986), and that mussel watch programmes be more fully exploited in Southern Hemisphere countries such as Australia (Martin and Richardson 1991), there are still very few studies of organochlorine contamination in the Australasian region. Oysters of the genus *Saccostrea* have been proposed as potential indicators for the assessment of contaminant concentrations in tropical areas (Phillips 1979) and have been used as biomonitors of organochlorines on the east coast of Australia (Clegg 1974; Scanes 1992), but there exist no published studies on rates of uptake and depuration of organochlorine compounds in these oysters. Traditionally, these types of experiments are done in laboratories under artificial conditions and rarely with complex mixtures of contaminants (but see Tanabe *et al.* 1987; Sericano *et al.* 1992). In most situations, however, the results of laboratory studies have questionable value in the interpretation of data from field studies. The experiment

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described in this paper examined rates of uptake and depuration of organochlorine compounds (OCs) by Sydney rock oysters (*Saccostrea commercialis* (Iredale and Roughley)) under field conditions.

Methods

Field studies

Approximately 80 4-year-old oysters were obtained from a commercial grower from the Hawkesbury River, New South Wales, Australia (Fig. 1), an area not contaminated by OCs (Scanes *et al.* 1996). These oysters were then deployed (Feb. 1993) in open-mesh polyethylene bags in Long Bay (Sydney Harbour) where organochlorine concentrations are high (chlordane in wild oysters 0·5–0·8 mg kg^{-1}; Scanes *et al.* 1996). From this initial pool of oysters, five individuals were removed for analysis after each of 0, 3, 12, 20, 76, 91, 125, 148 and 209 days. A substantial period of time was chosen in the absence of data on the time required for uptake of OCs. After 209 days, the remaining oysters were removed from the water at Long Bay and moved to Gunnamatta Bay, Port Hacking, an area minimally contaminated by OCs (chlordane in wild oysters 0·01–0·02 mg kg^{-1}; Scanes *et al.* 1996). Five oysters were taken from the pool of oysters in Gunnamatta Bay after each of 1, 4, 12, 20, 40 and 56 days.

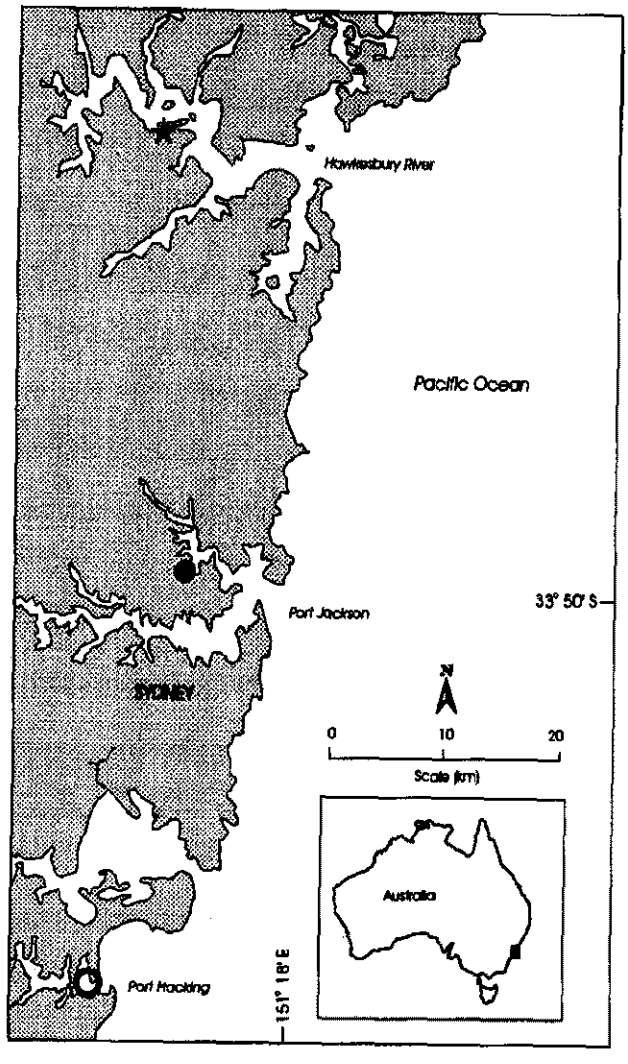

Fig. 1. Sources of oysters: (★) Hawkesbury River; (●) contaminated locality (Long Bay); (○) uncontaminated locality (Port Hacking).

All samples were stored at −18°C until analysis for the following compounds: aldrin, α-BHC, β-BHC, γ-BHC (Lindane), chlordane, dieldrin, DDD, DDE, DDT, endosulfan, endrin, heptachlor (HPT), heptachlor epoxide (HPTE), hexachlorobenzene(HCB), methoxychlor, oxychlordane and PCBs; all had a practical quantification limit of 1·01 mg kg^{-1} wet weight, except the PCBs with a limit of 0·05 mg kg^{-1}. The oysters were analysed individually (i.e. not pooled or composited), providing five independent data points for each time interval. All concentrations are expressed as mg per unit wet weight in kg of oyster tissue unless otherwise specified.

Chemical analyses

Samples (i.e. single oysters) were extracted by homogenization with acetone/acetonitrile (1:1) from a mixture of approximately 5 g of homogenized sample and 10 g of anhydrous sodium sulfate. The extract was exchanged into hexane and cleaned (Anon. 1990; florisil clean-up section, 983.21E), then analysed by USEPA Method 8080, modified by the use of capillary gas chromatography (GC) using electron-capture detection. GC columns of two different polarities were used to identify the OCs, with gas chromatography–mass spectrometry (GC–MS) used to confirm the OCs detected. The chromatograph was a Hewlett Packard 5890, with a 7673/A auto-injector. The primary column was 15m 0·32 mm id DB1701 (J and W Scientific) with hydrogen at 41·37 kPa as the carrier gas. The confirmation column was a DB225 with helium at 68·95 kPa as the carrier. Each column was operated at an initial temperature of 130°C and raised by 15°C min^{-1} to 220°C. Concentrations of PCBs were determined by comparison with standard PCB mixtures (Arochlors). Chlordane was quantified as 'technical chlordane' rather than the *cis* and *trans* isomers; the *cis* and *trans* isomers consistently make up about 20% of technical chlordane (Scanes *et al.* 1996).

Lipid content, expressed as a percentage of sample weight, was determined by petroleum–ether extraction (Anon 1990; acid hydrolysis method 948·15).

Analysis of data

Data were analysed by one-factor analysis of variance (ANOVA). Where significant differences were indicated, Student–Newman–Keuls (SNK) tests were used to separate means. To facilitate statistical analysis, data reported as not detected were assigned a concentration of zero and data reported as 'trace' were assigned a concentration of half the practical quantifiable limit.

Biological half life (BHL) for the depuration phase of OCs (after 209 days, when oysters were placed in Port Hacking), calculated for concentrations expressed per weight lipid and per wet weight, was estimated as BHL = $\log_e 2/k_2$, where k_2, the rate loss constant, is the slope of the linear regression of time and $\log_e(C_t/C_0)$, C_t is concentration at time t and C_0 is concentration at time 0 (from Tanabe *et al.* 1987).

Time to equilibrium (t_{eq}) was estimated in two ways: (*i*) from Fig. 2, the time when the rapid uptake phase was finished (i.e. the uptake curve initially became asymptotic) was determined and (*ii*) the theoretical time to equilibrium (or, practically, 90% of theoretical equilibrium) was calculated from the formula $t_{eq} = 2{\cdot}3/k_2$ (Hawker and Connell 1986; Tanabe *et al.* 1987).

Results

Biological variables

The mean wet weight of oyster tissue was 9·5 g. There were significant changes in weight during the deployment (Fig. 2*a*, ANOVA $P < 0{\cdot}01$), but these changes could not be

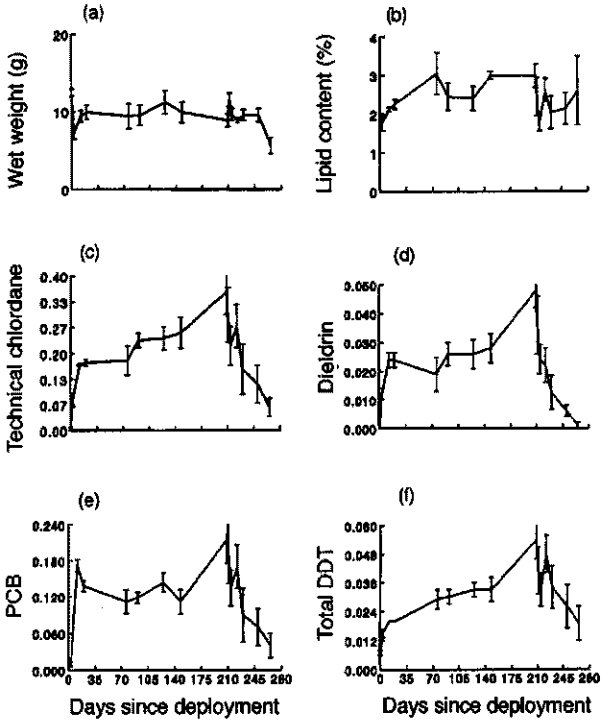

Fig. 2. (*a*) Wet weights and (*b*) lipid content of oysters and (*c–f*) wet weight concentrations (mg kg^{-1}) of organochlorine compounds during uptake and depuration phases. Values are means ± s.e. On Day 209, oysters were moved from the contaminated to the uncontaminated locality.

separated by SNK tests and simply represented variation among the random samples taken at each time. Mean content of lipid was 2·4% of wet weight and did not vary significantly during the experiment (Fig. 2*b*).

Contaminants

Before deployment (Day 0) the oysters had traces of DDE and one out of five had a low concentration of chlordane (0·01 mg kg^{-1}). The oysters rapidly accumulated OCs, all showing detectable concentrations of heptachlor, heptachlor epoxide, chlordane, DDE, DDD, DDT, dieldrin and PCB after 3 days. The concentrations of heptachlor and heptachlor epoxide did not change from trace and 0·01 mg kg^{-1}, respectively, and so were not analysed statistically. Concentrations of DDT, DDD and DDE were also low (trace or just above practical quantifiable limits), so the concentrations of these compounds were pooled to estimate total DDT (ΣDDT) for statistical analyses and calculations of times to equilibrium and biological half lives.

Concentrations of chlordane initially rose rapidly and the rate of increase fell dramatically between 12 and 20 days; this is considered the initial equilibrium (Fig. 2*c*, ANOVA $P < 0{\cdot}0001$; SNK 0<3<12=20=76=91=125=148 days). Dieldrin reached initial equilibrium between 3 and 12 days and PCB between 12 and 20 days (Figs 2*d*, 2*e*). Total DDT concentrations changed significantly over the entire exposure(Fig. 2*f*, ANOVA $P < 0{\cdot}0001$), but SNK tests did not clearly separate means. Concentrations of ΣDDT initially rose rapidly (from 0 to 20 days), then continued to rise more slowly for the rest of the uptake deployment (Fig. 2*f*). Connell (1988) recommended that OC concentrations in monitoring studies should be expressed per unit weight of lipid. In the present study, the concentrations of chlordane have also been expressed as a ratio of OC concentration to content of lipid; the patterns in the chlordane data based on wet tissue weight are also evident in the lipid-corrected data (Fig. 3).

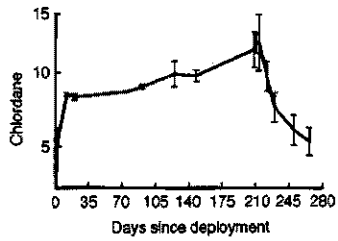

Fig. 3. Concentrations of chlordane in oysters (mean ± s.e.), expressed as mg per kg of lipid, during uptake and depuration phases.

All contaminants showed variations in concentration between 76 and 209 days (during uptake period) and this is attributed to variations in contaminant concentrations in the surrounding water. The concentrations of all main contaminants were highest after 209 days, suggesting that, even though most of the accumulation occurred quickly, the oysters continued to accumulate OCs for over 200 days. An alternative hypothesis to explain this result is that concentrations in the water continually and gradually increased during this period. This is unlikely, but without any data on concentrations in water, these two hypotheses cannot be separated.

When the oysters were moved to relatively uncontaminated waters (after 209 days) the concentrations of all contaminants rapidly decreased. The temporal pattern of concentrations of OCs in this depuration phase showed the expected exponential decay and was well modelled by linear regression of the log concentration (r^2 values, Table 1).

The experimental design did not provide for the use of control oysters at the uncontaminated locality. Unpublished data (NSW EPA) indicate that oysters deployed in Gunnamatta Bay were subject to only very small amounts of organochlorine contamination prior to and during the depuration period: early 1993 (mg kg^{-1}, n = 10) chlordane 0·017 ± 0·003, DDE 0·008 ± 0·0008, DDD 0·005

Table 1. Times to equilibrium (days) and biological half lives (BHL, days) of organochlorine compounds in the Sydney rock oyster *Saccostrea commercialis*

Times were determined by three methods. Theoretical times from Mortimer and Connell (1993) are provided for comparison

Compound	Time to equilibrium	BHL	k_2	r^2
(i) estimated from graphical plots of experimental data				
HPTE	< 3			
Dieldrin	3–12			
Chlordane	12–20			
ΣDDT	72			
PCB	12–20			
(ii) calculated per unit of wet weight of tissue				
HPTE	13	4	0·170	0·77
Dieldrin	40	12	0·057	0·96
Chlordane	79	24	0·029	0·93
ΣDDT	153	46	0·015	0·79
PCB	81	25	0·028	0·93
(iii) calculated per unit lipid weight of tissue				
HPTE	14	4	0·160	0·80
Dieldrin	53	16	0·043	0·99
Chlordane	109	32	0·021	0·97
ΣDDT	124	94	0·007	0·72
PCB	115	34	0·021	0·86
Theoretical times to equilibrium and half lives				
HPTE	0·6	0·1		
Dieldrin	4	0·7		
Chlordane	20	3		
ΣDDT	50	7·6		
PCB	63	10		

± 0·0005, PCB 0·01 ± 0·002; mid 1993 (mg kg^{-1}, n = 10) chlordane 0·028 ± 0·001, DDE 0·01 ± 0·001, DDD 0·008 ± 0·0008, PCB 0·008 ± 0·002; see also Scanes *et al.* 1996).

Concentrations of chlordane, dieldrin and ΣDDT after initial equilibrium were strongly correlated with content of lipid, but PCBs were not as well correlated (Table 2).

Biological half lives (a measure of rates of depuration) and times to equilibrium calculated per unit wet weight and lipid weight were very similar for dieldrin, chlordane and PCB, but less similar for ΣDDT, which had the poorest fit for the two regressions (Table 1). The two methods show that chlordane and PCBs had similar BHLs.

Table 2. Results of regressions of percentage lipid (x) and concentrations of organochlorine compounds (y) in *Saccostrea commercialis*

Data are from oysters collected between Days 21 and 148, when it was assumed that the oysters were in (or near) equilibrium with the environment

	Regression equation	Probability	r^2
Chlordane	$y = 0{\cdot}1144x - 0{\cdot}0657$	<0·001	0·77
Dieldrin	$y = 0{\cdot}0135x - 0{\cdot}0067$	<0·001	0·70
ΣDDT	$y = 0{\cdot}0158x - 0{\cdot}0114$	<0·001	0·60
PCB	$y = 0{\cdot}0200x - 0{\cdot}0970$	<0·01	0·41

Discussion

There are few published data with which to compare the rates of uptake and depuration measured in this study and most of what is available is for PCBs only. Vreeland (1974) found that PCBs in small oysters (*Crassostrea virginica*) reached equilibrium in about 28 days, which is similar to the time taken to reach the initial plateau in the present study (12–20 days) but less than the times to equilibrium that were calculated (Table 1). Sericano *et al.* (1992) found that BHLs for non-planar PCBs (which compromise the bulk of commercial PCB mixtures) in oysters (*Crassostrea virginica*) ranged between 17 and 76 days, i.e. they were similar to those found in the present study. Tanabe *et al.* (1987) found that PCBs reached equilibrium in the mussel *Perna viridis* in less than 40 days and calculated a BHL (using first-order bioconcentration kinetics—Hawker and Connell 1986; Connell 1988) of 7 to 12 days. Young *et al.* (1976) reported variable concentrations of PCB and DDT in *Mytilus*, but there was no marked increase after about 30 days.

Studies of equilibria and BHL in mussels have shown times that are less than, but of the same order as, those measured for oysters in this study, and Hawker and Connell (1986) have shown that there is a close similarity in OC kinetics among different bivalve molluscs. However, Sericano *et al.* (1992) noted that the BHLs for oysters in their study were longer than those for mussels (from Tanabe *et al.* 1987) and suggested that this was a consequence of expressing concentrations per unit tissue weight rather than per unit lipid weight. In the present study, BHLs calculated on both tissue-based and lipid-based concentrations were similar (the maximum difference being ×2), but the lipid-based times were usually slightly longer. This indicates that, since BHLs calculated per unit tissue weight are not necessarily longer than those calculated per unit lipid weight, there may be a small but real difference between BHLs in oysters and mussels.

The BHLs measured for oysters are, however, between 4 and 10 times longer than predicted rates of depuration (Table 1) calculated by Mortimer and Connell (1993) using the relationships derived in Hawker and Connell (1986). Times to equilibrium estimated from graphs were similar to those predicted by Mortimer and Connell (1993) except for PCBs. The estimation of these times was based on estimates of k_2. Small changes in k_2 can result in relatively large changes in times to equilibrium and BHLs (Table 1), so it is not surprising that the estimates of Mortimer and Connell (1993) are somewhat different to those calculated from field data. PCBs are a mixture of a large number of biphenyl

compounds with different properties and different kinetics (Tanabe *et al.* 1987), so the difference between measured and predicted rates for PCBs is not surprising and is probably a result of these predictions being made for a set of PCB compounds different from those measured in the present study.

According to first-order kinetic models, the length of BHL and time to equilibrium should increase as the affinity for lipids (measured by the logarithm of the octanol:water co-efficient) increases (Hawker and Connell 1986). This is true for BHLs and times to equilibrium measured in the present study for all compounds except PCBs. Because PCBs are a mixture of many compounds and were not quantified individually, it is difficult to predict the correct location on the scale applicable to the specific PCB mix used in the present study. The fact that PCBs had such a relatively short BHL suggests that they were composed mainly of biphenyls with lower levels of chlorination (Tanabe *et al.* 1987; Sericano *et al.* 1992).

If the uptake of OCs is entirely consistent with simple first-order kinetics, the time to equilibrium should be 'controlled only by the magnitude of the clearance rate constant [k_2]' (Hawker and Connell 1986, p. 186). Calculations of the theoretical t_{eq} on these data (Table 1) produced times that were longer than those estimated from field data, indicating that k_2 may not be the only factor controlling uptake. Vreeland (1974) also noted that, for oysters, calculated t_{eq} values were much longer than measured values, and suggested that mechanisms other than simple lipid/water partitioning were involved.

There is good agreement between the BHL and times to equilibria estimated from wet-weight data and lipid-corrected data, despite the strong correlation between OC concentration and content of lipid. This is probably because, with the variation in lipid content being small, the variation induced in OC concentrations is concomitantly small compared with the changes that result from uptake and depuration.

There was a correlation between lipid content and OC concentration in this study, but not in more wide-ranging studies of bioaccumulation by bivalves (e.g. O'Connor 1992; Scanes 1996) where concentrations of OCs were somewhat lower. This suggests that where ambient concentrations of OCs are high and there are few other external influences (e.g. in laboratory studies or special cases in the field such as that described in the present study), the correlation between OC concentration and lipid content can be strong. In more complex situations, however, and where ambient OC concentrations are small, the correlation is weaker. Further, expressing OC concentrations as means based on wet weights or as means of OC:lipid ratios did not alter the interpretation of the data. The present study demonstrates that little value is to be gained by expressing OC concentration as a function of lipid content, particularly since the relationship between the two has already been shown to be not significant in two sets of large-scale field contaminant data—the NOAA Mussel Watch (O'Connor 1992 and personal communication) and NSW EPA Oyster Watch (Scanes 1996). This assumption is true only when factors other than lipid content are more important in controlling whole-animal organochlorine concentrations.

The results of the present study have shown that *Saccostrea commercialis* has strong potential as an indicator of trends in ambient concentrations of a range of organochlorine compounds. The oyster shows a rapid uptake and depuration and, as a consequence, has a relatively short period of integration (days to weeks). This has implications for the design and interpretation of studies that use oysters to assess concentrations of contaminants in aquatic systems. In particular, if a longer time integration is required, 'time-bulking' procedures (Phillips and Segar 1986; Phillips and Rainbow 1993) could be employed. The present study used deployment techniques to introduce oysters to polluted areas where they were not naturally found. Mortality was low, and the technique, which uses a known starting time for accumulation of contaminants, is recommended where oysters are being used to determine the pollution status of previously unstudied waters or to follow a time course of pollutant concentrations.

Saccostrea commercialis is widespread along the east coast of Australia and the closely related *Saccostrea cucullata* is found throughout tropical Australia. These species have been used to monitor concentrations of trace metals and polycyclic aromatic hydrocarbons in Australian waters (Mackay *et al.* 1975; Talbot 1986; Peerzada and Dickinson 1989; Brown and McPherson 1992; Pendoley 1992; Scanes 1996). Despite probable inter-specific differences, these two species may be able to provide an extensive biomonitoring network in temperate and tropical waters.

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Appendix 5 **Scanes P.R. 1996, ' "Oyster Watch": Monitoring trace metal and organochlorine concentrations in Sydney's coastal waters', *Marine Pollution Bulletin*, vol. 33, pp. 182-189.**

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'Oyster Watch': Monitoring Trace Metal and Organochlorine Concentrations in Sydney's Coastal Waters

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This paper examines concentrations of trace contaminants in oysters placed in inshore and offshore waters before and after the change to offshore disposal of sewage near Sydney, NSW, Australia. The ability to compare inshore and offshore concentrations before and after the event allows the formulation of unconfounded conclusions about the impact of sewage on the availablity of trace contaminants in the coastal environment and the effect of the change in the method of disposal of Sydney's sewage on the availability of those contaminants.

The data show clearly that the diversion of sewage from nearshore to offshore areas has resulted in a significant drop in the concentrations of organochlorines in oysters deployed near sewage outfalls in the nearshore region. In offshore areas, there were no differences detected among regions prior to the commissioning of deepwater outfalls. In the post commissioning period there were a number of instances when trace metal concentrations in the regions were different from each other, but these differences were not correlated with proximity to the sewage outfalls nor was a particular metal consistently elevated, so it was concluded that the causes of difference were not related to the presence of the sewage outfalls.

This study has demonstrated that oysters were useful indicators of trace metal and organochlorine contamination of marine waters; that relatively high levels of contamination of inshore waters near sewage outfalls prior to deepwater sewage disposal were reduced, to be not different from other parts of Sydney's coastline after the commencement of deepwater discharge, and that there was no concommitant increase in accumulation of contaminants in offshore waters. Further, preliminary indications are that Sydney's coastal waters are not very heavily contaminated by trace contaminants in comparison to areas of NSW with much lower levels of development. Concentrations of organochlorines are, however, high in comparison to data from similarly developed areas of the USA. © 1997 Elsevier Science Ltd

Disposal of sewage and industrial waste represents a major problem in many cities. Prior to September 1990, the great majority of Sydney's sewage was discharged, after primary treatment, to the inshore marine environment through shoreline outfalls. In the period from September 1990 to June 1991 three deepwater sewage outfalls were commissioned, with the subsequent cessation of inshore sewage discharge. The deepwater outfalls are 3–4 km offshore in 60–80 m of water and were placed to take advantage of the increased opportunity for dilution and dispersion of sewage provided by the greater depth of water and the ocean currents.

Previous studies of Sydney's sewage outfalls indicated the bio-accumulation of trace contaminants (trace metals and organochlorine compounds) by fish in the vicinity of the inshore outfalls (Lincoln-Smith and Mann, 1989a,b). It is known that a range of marine organisms, such as molluscs, crustaceans and fish, may accumulate certain environmental contaminants in their tissues to concentrations that are above ambient levels in the environment (Phillips and Rainbow, 1993). This allows such species to be used as 'indicator' or 'sentinel' organisms, reflecting relative levels of environmental contamination in a manner amenable to short and long-term monitoring. Phillips (1977, 1978) has reviewed the use of biological indicators for quantifying trace contaminant pollution. He concluded that, whilst biological monitoring is better than chemical analysis of waters, the technique is subject to confounding by biological and environmental variables and studies must therefore be designed and interpreted with care.

The organism most commonly used for bio-accumulation studies is probably the mussel *Mytilus edulis*, however this reflects its cosmopolitan distribution as much as its utility as a test organism. The studies described in this paper use the local oyster *Saccostrea commercialis*, as it is abundant along the coast of NSW, is able to survive the test conditions, accumulates the contaminants of interest to concentrations proportional

to ambient waters, commercial growers can provide a ready supply of animals for deployment and the kinetics of trace contaminant uptake and depuration have been studied (EPA data).

Oysters are part of the US mussel watch in those areas where mussels do not naturally occur (Goldberg *et al.*, 1983; Farrington *et al.*, 1983; O'Connor, 1992). They have also been used in a variety of other studies of bio-accumulation. Phillips (1979) considered the co-generic oyster *S. glomerata* to be a reliable indicator of concentrations of trace metals in Hong Kong waters. Phillips and Yim (1981) showed that concentrations of copper and zinc in *S. glomerata* reflected those of sediments in Hong Kong waters, but that the mussel *Septifer bilocularis* appeared to regulate Zn and Cu and hence would be an unreliable indicator. A survey of metals in *Crassostrea commercialis* (= *S. commercialis*) by Mackay *et al.* (1975) showed that the concentration of trace metals in oyster tissue correlated with an environmental gradient of metal pollution, but there was some evidence that metal concentration decreased with increasing age and wet weight of oysters. The latter conclusion was not supported in follow-up studies by Brown and McPherson (1992) and Scanes *et al.* (1996).

Kopfler and Mayer (1973) reported a poor correlation between concentrations of trace metals in oysters and surrounding waters. They did, however, show that oysters had tissue concentrations that were 4 to 5 orders of magnitude greater than concentrations in the surrounding water. Water samples were filtered prior to analysis and the authors suggested that the lack of correlation in concentrations of trace metals could be due to the oysters accumulating metals from particulate matter rather than the water. Scanes (1993), however, suggested that the concentrations of trace metals accumulated by oysters were related to concentrations in ambient waters rather than surrounding sediments.

The aim of the study reported here was to use oysters to determine whether environmental concentrations of organochlorine compounds (OCs) and trace metals (TMs) in inshore and offshore areas changed after commissioning of deepwater sewage outfalls. The study tested the null hypothesis that there has been no change in the relative levels of contamination of oysters in inshore and offshore, control and outfall locations after sewage discharge began in offshore waters. This was tested by analysing the spatial and temporal trends in contaminant concentrations of oysters which have been deployed in inshore and offshore areas before and after the change to offshore sewage disposal. The ability to compare inshore and offshore concentrations before and after the event, allows the formulation of unconfounded conclusions about the impact of sewage on the availablity of trace contaminants in the coastal environment and the effect of the change in the method of disposal of Sydney's sewage on the availablity of those contaminants.

Methods

Sampling designs

Inshore. Two-year-old Sydney rock oysters obtained from commercial leases in the lower Georges River (June 1990 – October 1992 inclusive) and thereafter from the Hawkesbury River, were used in all studies. Ten oysters (5 for OC and 5 for TM analyses) were immediately frozen to indicate starting concentrations.

Two mesh bags (approximately 400×200 mm with 30 mm mesh) containing oysters were attached, using cable ties, to a steel handle embedded in a concrete block. There were approximately 15 animals in each bag to allow for mortality. The blocks were then placed on the sea bottom in rocky areas where the water depth was between 10 and 12 m.

Three outfall locations (North Head, Bondi and Malabar) and three control or reference locations (Bangalley, Dee Why and Jibbon) were used (Fig. 1). At each location there were two sites, north and south, approximately 300–400 m apart. At outfall locations, sites were 150–200 m north and south of the outfalls. Three blocks were deployed at each site. The bags of animals attached to the blocks were retrieved and replaced with a new batch every 3 months from June 1990 to July 1993. Survival of the retrieved animals in each bag was noted.

Offshore. Two moorings were established about 200–400 m apart at each of the locations shown in Fig. 1. Two bags, each containing 15 oysters, were attached to the mooring rope 30 m from the surface and 5 m from the bottom. The 30 m depth is the theoretical level at which the sub-surface sewage effluent plume would be trapped while a thermocline is present (about 70% of time, EPA data). The 5 m depth was chosen to be as near the bottom as possible without being influenced by resuspension of sediment. The two depths are referred to in the text as 'shallow' and 'deep'.

Since the depths were defined from the surface and the bottom, the distance between the bags of oysters on a mooring was not the same at Malabar (which is in 80 m of water) than at all the other locations (which are in 60 m of water).

After 3 months the moorings were lifted from the bottom, bags of oysters removed from the rope and replaced and the condition of the moorings inspected. Animals for analysis were selected at random from those in each bag.

Chemical analysis of samples

Organochlorine compounds and fat content. Organochlorine and fat content analyses were conducted on individual oysters by the Australian Analytical Laboratories, Hornsby. Samples were extracted by homogenization with acetone/acetonitrile (1:1) from a mixture of approximately 5 g of sample and 10 g of anhydrous sodium sulphate. The extract was exchanged into hexane and cleaned according to the

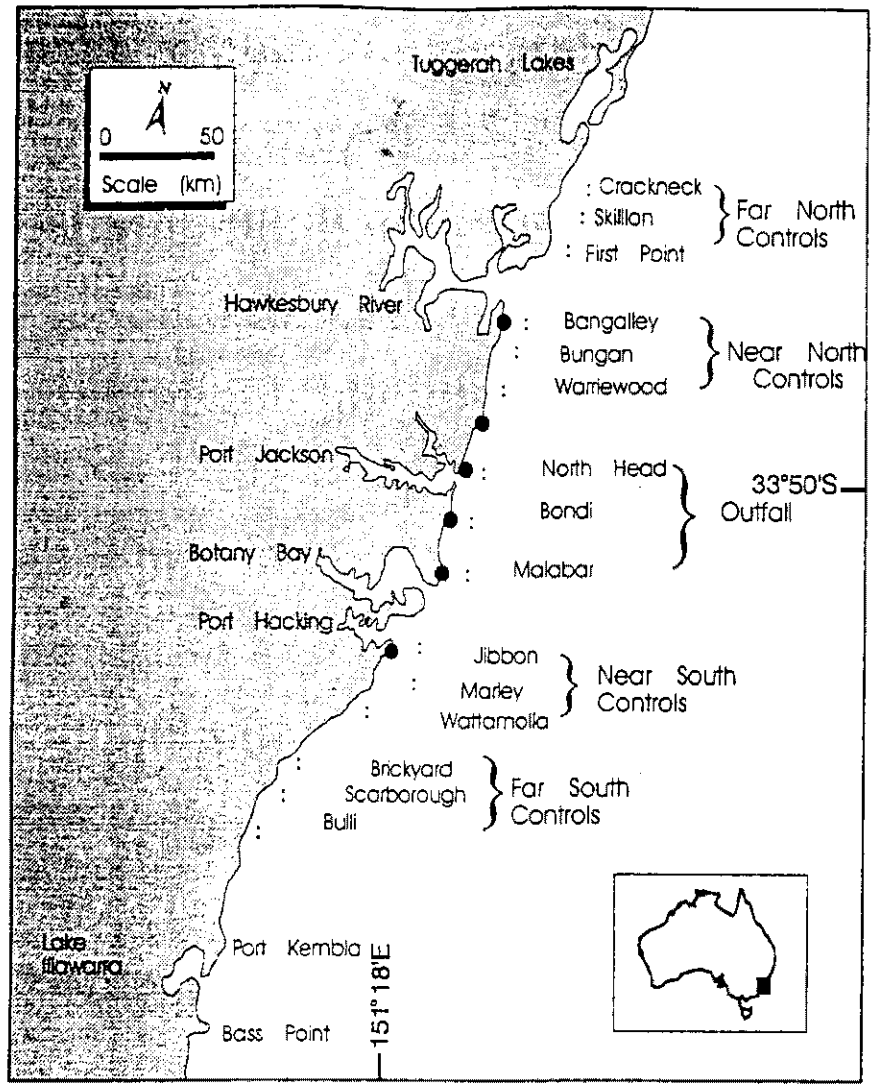

Fig. 1 Locations of inshore and offshore sampling sites.

procedures outlined in the florisil clean-up section, 983.21E of the AOAC (1990). The extract was then analysed for the organochlorines shown in Table 1 in accordance with US EPA Method 8080.

Fat content expressed as a percentage of sample weight was determined by petroleum/ether extraction, as described in acid hydrolysis method 948.15 of AOAC (1990).

Trace metals. Trace metal analyses were conducted on individual oysters by the CSIRO, Centre for Analytical Chemistry, Lucas Heights. Samples were digested with high purity concentrated nitric acid in a microwave oven. The digests were diluted with high purity water, spiked with indium as an internal standard and analysed for the trace metals shown in Table 1 using inductively coupled plasma mass spectrometry (ICPMS) (modified US EPA Method 200.8, Revision 4.4, April 1991).

Analysis of data

For the purposes of statistical analysis, concentrations of contaminants reported as 'not detected' and 'trace' were assigned values of zero and half the detection limit, respectively.

Most data were analysed by analysis of variance (ANOVA) techniques (Underwood, 1981, 1991). All data were tested for homogeneity of variance using Cochran's test. If data were heteroscedastic, transformations were applied to stabilize variances. If transformations were not successful, untransformed data were analysed and the analyses were interpreted with caution, recognizing the increased likelihood of Type I error (Underwood, 1981; Winer, 1971).

In some cases it was neccessary to substitute missing data with the mean of the other replicates for that date (Underwood, 1981) to balance the number of replicates. The degrees of freedom of the mean square of the residual were adjusted accordingly.

Due to storms, predation and human interference it was not possible to fulfil the original designs for either inshore and offshore studies. Accordingly, a truncated

TABLE 1

Summary of practical quantification limits (PQL) normally achieved for the various elements and compounds.

Trace metals	PQL mg kg^{-1} wet wt
Arsenic	0.01
Cadmium	0.01
Chromium	0.01
Cobalt	0.001
Copper	0.01
Lead	0.01
Mercury	0.01
Nickel	0.01
Selenium	0.01
Silver	0.001
Zinc	0.1
Organochlorine compounds	**PQL mg kg^{-1} wet wt**
Aldrin	0.01
α-BHC	0.01
β-BHC	0.01
γ-BHC (Lindane)	0.01
Chlordane	0.01
Dieldrin	0.01
DDD	0.01
DDE	0.01
DDT	0.01
Endosulfan	0.01
Endrin	0.01
Heptachlor (HPT)	0.01
Heptachlor epoxide (HPTE)	0.01
Hexachlorobenzene (HCB)	0.01
Methoxychlor	0.01
Oxychlordane	0.01
PCBs	0.01

design with fewer spatial scales and times of sampling has been used. In order to prevent spatial confounding, replicates for statistical analyses of differences among inshore locations or offshore regions (see below) were selected at random from all data available for that spatial scale. This means that the data came from experimental units scattered over the full area being addressed by the hypothesis and were not selected from a small sub-section of that area (therefore avoiding confounding any differences between inshore and offshore with any spatial differences within either area; Hurlbert, 1984). Thus, variability associated with sub-sections of the main spatial scale is not explicitly examined but is included in the residual term of the ANOVA along with inter-individual variation. This residual is therefore larger than the original design would have achieved.

Chlordane and DDT were the only OCs detected frequently enough to provide any useful information. The metals were chosen on the basis of probable occurrence in sewage, confidence in analytical methods (see Scanes, 1996) and suitable uptake and depuration times (Scanes, 1997). The times were chosen on the basis of enough samples being available for the statistical design.

Results

Analyses of fat were discontinued early in sampling because it was usually not possible to get enough tissue from an individual oyster for both fat and OC analysis and, more importantly, initial data (Fig. 2) showed no correlation between fat and levels of OC contamination in field samples.

Inshore

The data obtained have been examined in two ways. First, means of all data from outfall and control

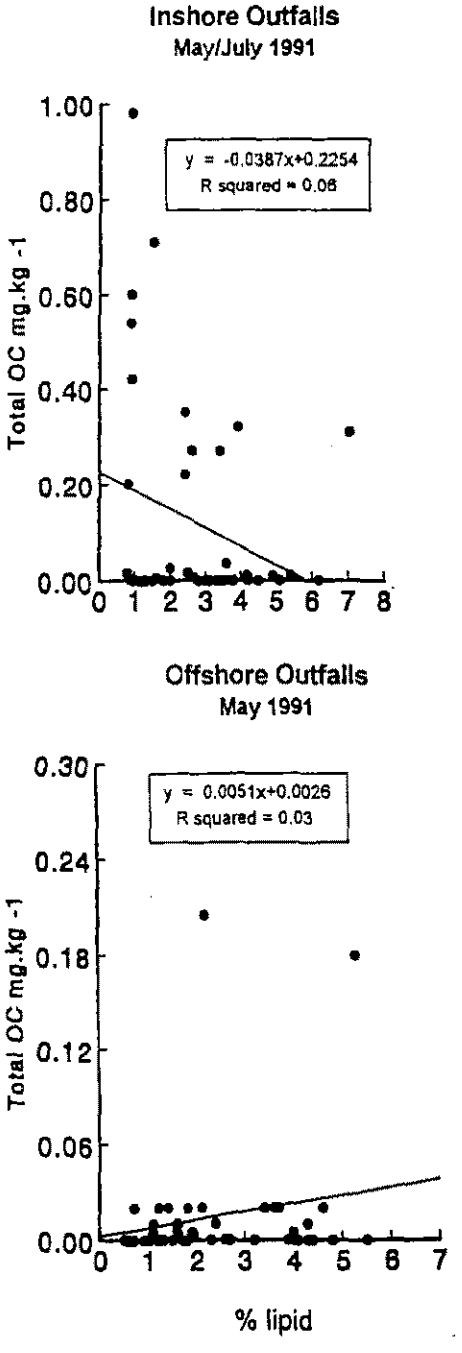

Fig. 2 Correlations between percentage of lipid and total organochlorine concentrations in oysters from inshore and offshore outfall locations.

locations were calculated and plotted. No statistical interpretations of these data were made because the number of samples in each mean were different. All contaminants were variable through time (examples in Fig. 3). Regression analyses of mean concentrations of contaminants before deployment and after retrieval indicated that, for most metals, the concentration prior to deployment was not a good predictor of the concentration after retrieval and thus did not always explain the temporal variation. There were, however, significant positive regressions for copper, zinc, cadmium and mercury (control locations only – Table 2). The experimental design and statistics used later should indicate if there are differences between outfall and control locations despite dissimilar starting concentrations at each time.

Temporal differences were evident for chlordane (Fig. 3), where concentrations were elevated near the outfalls precommissioning but were not different from control locations during the post-commissioning period. Contaminants which showed occasionally large peaks at the outfalls (but showed no other patterns that could be interpreted to be a consequence of a change in sewage discharge), were arsenic, mercury, DDT, nickel and copper. Lead was consistently elevated at outfall locations throughout the study.

Secondly, a subset of data was analysed. Only the results of the analyses which showed significant differences between outfalls and controls will be discussed here, although all the results are shown in Table 3. Most contaminants showed significant variation among time and among the control locations and an interaction between these two factors. These significant differences are not considered to be linked to the presence of the outfalls and so will not be discussed in detail.

The concentration of chlordane at North Head outfall location compared to control areas was significantly linked with the time of sampling (Table 3). In the precommissioning period, concentrations at the outfalls were greater than controls, but this was not the case post-commissioning. An alternative graphical presentation, which removes temporal variation by calculating the ratio of outfall and control concentrations, is presented in Fig. 4. This shows clearly that there was more chlordane accumulated at North Head than at control sites before commissioning of the deepwater outfalls, but that there were no differences afterwards.

The concentrations of chlordane accumulated at Bondi and Malabar outfalls were significantly higher than those at controls and this was not affected by time (Table 3). This implies that concentrations were not affected by commissioning of the outfalls. The graphs (Fig. 4), however, show that the differences between outfalls and controls were greatest in the period prior to commissioning of the outfalls. Any relative differences tended to greater concentrations at the outfalls, particularly at Bondi (Fig. 4). This remaining relative elevation at the outfall locations, even though it is much smaller, may have contributed to the statistical analyses not showing a strong effect of time.

Differences between concentrations of zinc accumulated at Bondi and copper and nickel at Malabar and control locations were affected by time, but there is no evidence in the graphs (Fig. 4) that suggests an effect due to changing the location of sewage discharge. Cobalt was significantly elevated at Malabar outfall before commissioning but not after commissioning (Fig. 4). This provides some evidence of a reduction in inshore cobalt concentrations since offshore sewage discharge began.

Offshore

The means of all data collected between October 1990 and August 1993 were plotted and there were no consistent spatial trends in mean concentration. If there was enhanced accumulation in offshore regions, the outfall and perhaps near south regions (due to prevailing current structure) should be showing consistently higher concentrations than the other regions. Such a pattern is not evident for any contaminant.

In the analyses which included all regions, the date of sampling and all interactions with date were significant (Table 4). There were, however, no trends in the data (Fig. 5) which would lead to the conclusion that there are significantly higher concentrations of metals accumulated in the outfall or 'near' regions. The proportion of times that each region had the highest concentration of a contaminant (0.29, 0.08, 0.17, 0.21, 0.25; regions north to south, all contaminants) was not significantly different from the alternative hypothesis of equal proportions (Chi squared $p = 0.54$), indicating that no particular region consistently had the highest concentrations of contaminants.

There was a significant trend to a greater accumulation of copper, cadmium and zinc in deeper waters. This trend was was not, however, related to proximity to outfalls indicating that it was not caused by the presence of sewage in deeper waters (Tables 4 and 5, Fig. 5).

The comparisons of outfall to near south regions confirmed the conclusion that concentrations of contaminants accumulated in the outfall region were similar to control locations (Table 4). This pattern did not alter after commissioning of the deepwater ocean outfalls (Table 5).

Discussion

The use of oysters as biomonitors in NSW has received little attention in the past, despite oysters being used successfully in a number of other countries (Phillips and Yim, 1981; O'Connor, 1992; Phillips, 1979). The published works on Australian oysters were descriptive, indicating patterns of trace metal contamination in rivers or estuaries inferred from collections of

TABLE 2

Summary of results of regression analyses between concentrations of contaminants in oysters prior to deployment inshore and after retrieval. p indicates result of significance of test of slope of the regression, ns – not significant; $^*p < 0.05$. Biological half lives (BHL) from Scanes (unpub.). # No data.

	Control			Impact			
		p	r^2		p	r^2	BHL
Cr	$y = 0.85x + 0.26$	ns		$y = 0.81x + 0.27$	ns		#
Ni	$y = 0.67x + 0.11$	ns		$y = 1.66x + 0.06$	ns		#
Cu	$y = 2.67x + 27.18$	*	0.5	$y = 3.07x + 25.23$	*	0.57	64
Zn	$y = 2.83x + 257$	*	0.53	$y = 4.10x + 180$	*	0.7	144
As	$y = 2.09x - 0.15$	ns		$y = 1.78x + 0.03$	ns		#
Ag	$y = -0.50x + 0.66$	ns		$y = -0.81x + 1.11$	ns		35
Cd	$y = 3.81x + 0.24$	*	0.68	$y = 5.55x + 0.26$	*	0.81	52
Hg	$y = 2.36x + 0.01$	*	0.44	$y = 0.60x + 0.02$	ns		30
Pb	$y = 0.74x + 0.15$	ns		$y = 0.80x + 0.17$	ns		21

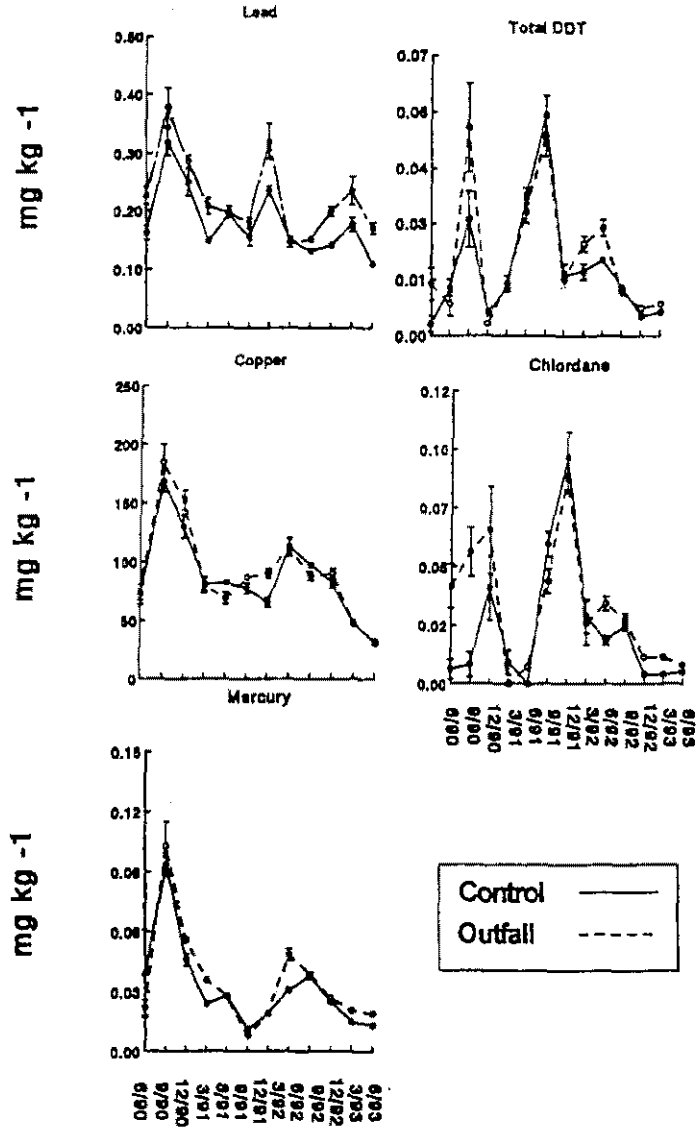

Fig. 3 Mean (±1 SE) concentrations of selected contaminants in oysters deployed at inshore locations (summary of all data from outfall and control locations). Samples from 6/90 and 9/90 represent the conditions prior to commissioning the deepwater outfalls.

TABLE 3

Summaries of F ratios from asymmetrical analyses of variance of contaminants from inshore oysters. Significance is indicated as follows: * $0.05 < p > 0.006$; ** $p > 0.005$. The mean square of the residual (MSR) is expressed as a negative exponential (e.g. E-3) in some cases. The general model for the analyses is also shown.

General model

Source of Variation			df	MS divisor
Outfall vs Control	fixed	T	1	L(T)
Locations within Control	random	L(T)	2	Residual
Date	random	D	9	L(T)
T×D			9	L(T)
D×L(T)			18	Residual
Residual			280	

North Head vs Controls

	T	L(T)	D	T×D	D×L(T)	MSR
Chlordane	9.28	0.08	6.07**	3.42*	1.84*	1.21 E-3
Sum DDT	0.13	4.94*	4.25**	1.14	3.78**	2.64 E-4
Cr	0.03	5.69**	4.09**	1.22	7.39**	7.92 E-3
Co	0.42	6.52**	9.96**	1.42	2.13**	3.70 E-4
Ni	1.37	6.20**	2.31	0.32	2.67**	0.0343
Cu	0.42	2.02	19.99**	1.23	2.04*	848.88
Zn	0.07	0.39	22.47**	0.72	1.92*	53768.9
As	1.36	11.53**	5.46**	0.56	6.45**	0.2197
Se	0.02	8.67**	4.14**	0.95	6.17**	0.2363
Ag	0.02	5.87**	8.67**	1.17	4.01**	0.2325
Cd	1.88	5.02**	8.85**	1.48	2.08*	9.76 E-2
Hg	0.00	2.50	5.51**	0.39	2.49**	6.95 E-4
Pb	5.81	3.56*	2.68*	1.09	3.99**	7.43 E-3

Bondi vs Controls

	T	L(T)	D	T×D	D×L(T)	MSR
Chlordane	40.03*	0.07	9.67**	1.37	1.71*	1.31 E-3
Sum DDT	0.08	4.26*	5.85**	1.21	3.26**	3.07 E-4
Cr	0.45	12.80**	7.48**	0.94	11.57**	5.08 E-3
Co	0.06	7.62**	10.14**	1.35	1.66	4.74 E-4
Ni	0.00	4.21*	6.48**	0.60	1.27	7.30 E-2
Cu	0.07	3.10	10.66**	1.55	2.12**	819.80
Zn	1.40	0.61	12.07**	3.08*	2.00**	51597.00
As	0.00	15.43**	5.11**	0.74	7.31**	0.2036
Se	0.01	18.06**	5.50**	0.34	8.61**	0.1695
Ag	0.91	7.65**	11.32**	0.89	3.58**	0.2612
Cd	0.53	7.49**	10.52**	0.67	2.15**	9.57 E-2
Hg	0.05	3.60*	5.73**	1.01	2.68**	6.55 E-4
Pb	1.38	7.33**	2.91*	0.32	5.45**	5.41 E-3

Malabar vs Controls

	T	L(T)	D	T×D	D×L(T)	MSR
Chlordane	36.05*	0.07	8.98**	1.54	1.78**	1.26 E-3
Sum DDT	0.44	4.33*	6.61**	0.35	3.32**	3.02 E-4
Cr	1.87	10.29**	7.56**	0.81	9.3**	6.32 E-3
Co	2.73	8.04**	26.14**	6.66**	1.74*	4.66 E-4
Ni	0.33	4.47*	11.94**	3.17*	1.34	6.91 E-2
Cu	1.74	2.78	30.47**	3.32*	1.90*	915.2
Zn	10.64	0.51	28.89**	2.35	1.65*	62483.6
As	0.02	13.01**	5.77**	0.25	6.17**	0.2414
Se	0.45	15.85**	11.29**	1.46	7.56**	0.1933
Ag	0.17	8.83**	11.10**	1.03	4.13**	0.2264
Cd	0.00	6.96**	13.74**	0.35	2.00*	0.1030
Hg	2.16	3.33*	14.55**	1.77	2.48**	7.07 E-4
Pb	1.86	6.89**	5.33**	0.91	5.12**	5.76 E-3

TABLE 4

General model of analysis and summaries of F ratios from analyses of variance of contaminant levels in oysters from offshore oysters – all regions. Significance is indicated as follows: * $0.05 < p > 0.006$; ** $p > 0.005$. MSR is the mean square of the residual.

Source of variation		df (OC)	df (TM)	MS divisor
Time – T	random	3	4	Residual
Region – R	fixed	4	4	T×R
Depth – D	fixed	1	1	T×D
T×R		12	16	Residual
T×D		3	4	Residual
R×D		4	4	T×R×D
T×R×D		12	16	Residual
Residual		360	450	

	Chlordane	Cd	Cu	Hg	Pb	Zn
Time – T	112.67**	67.05**	57.03**	254.0**	33.96**	57.3**
Region – R	2.54	0.24	1.31	0.98	2.28	1.78
Depth – D	0.85	7.93	106.09**	3.81	4.34	19.05*
T×R	2.72**	9.76**	3.19**	8.77**	3.77**	3.04**
T×D	11.39**	4.37**	0.17	5.42**	1.14	0.60
R×D	1.48	0.48	0.92	2.75	0.27	1.22
T×R×D	1.91**	3.37**	0.66	1.48	1.52	0.52
MSR	0.0013	0.061	1207.64	0.0001	0.0087	73121

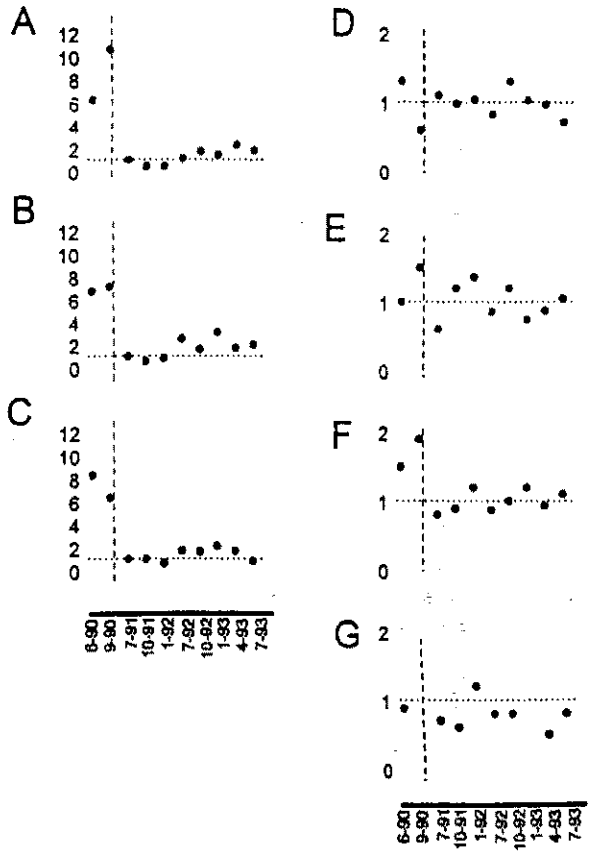

Fig. 4 Data from analyses of variance of concentrations of contaminants in oysters deployed near inshore outfalls presented as ratios of mean concentrations from outfall and pooled control locations for each date. Samples from 6/90 and 9/90 (to left of vertical lines) represent the conditions prior to commissioning the deepwater outfalls. A, chlordane, North Head; B, chlordane, Bondi; C, chlordane, Malabar; D, zinc, Bondi; E, copper, Malabar; F, cobalt, Malabar; G, nickel, Malabar.

wild or cultivated oysters (Mackay *et al.*, 1975; Peerzada and Dickinson, 1989; Brown and McPherson, 1992; Peerzada and Kozlik, 1992). There have been no studies describing translocation of oysters to test hypotheses and no data of any kind on concentrations of organochlorines exists.

The data in this study show clearly that the diversion of sewage from nearshore to offshore areas has resulted in a significant drop in the organochlorine contamination of the oysters deployed inshore to assess contaminant levels. Scanes and Henry (1992) showed that during the pre-commissioning period there were significantly greater concentrations of some trace metals around the shoreline outfalls. This difference was not evident in later analyses of the entire data set. It appears that the differences in the pre-commissioning data set were mainly due to differences in the September 1990 data. The current analysis of a longer time series shows that the same degree of difference that occurred in September 1990 was apparent at other times through the data. This suggests that the differences occurred sporadically and did not seem to be linked to the presence of sewage. The reasons for the sporadic detection of differences is unknown but could be linked to storms or urban runoff. The lack of any

Fig. 5 Summary of mean (±1 SE) concentrations at each time for selected contaminants from analyses of variance of data from oysters deployed at offshore locations. All data are from after commissioning of the deepwater outfalls. FN, far north region (see Fig. 1); NN, near north; OF, outfall; NS, near south; FS, far south. Crossed bars are data from oysters suspended at 30 m from surface, hatched bars are data from oysters 5 m from sea floor.

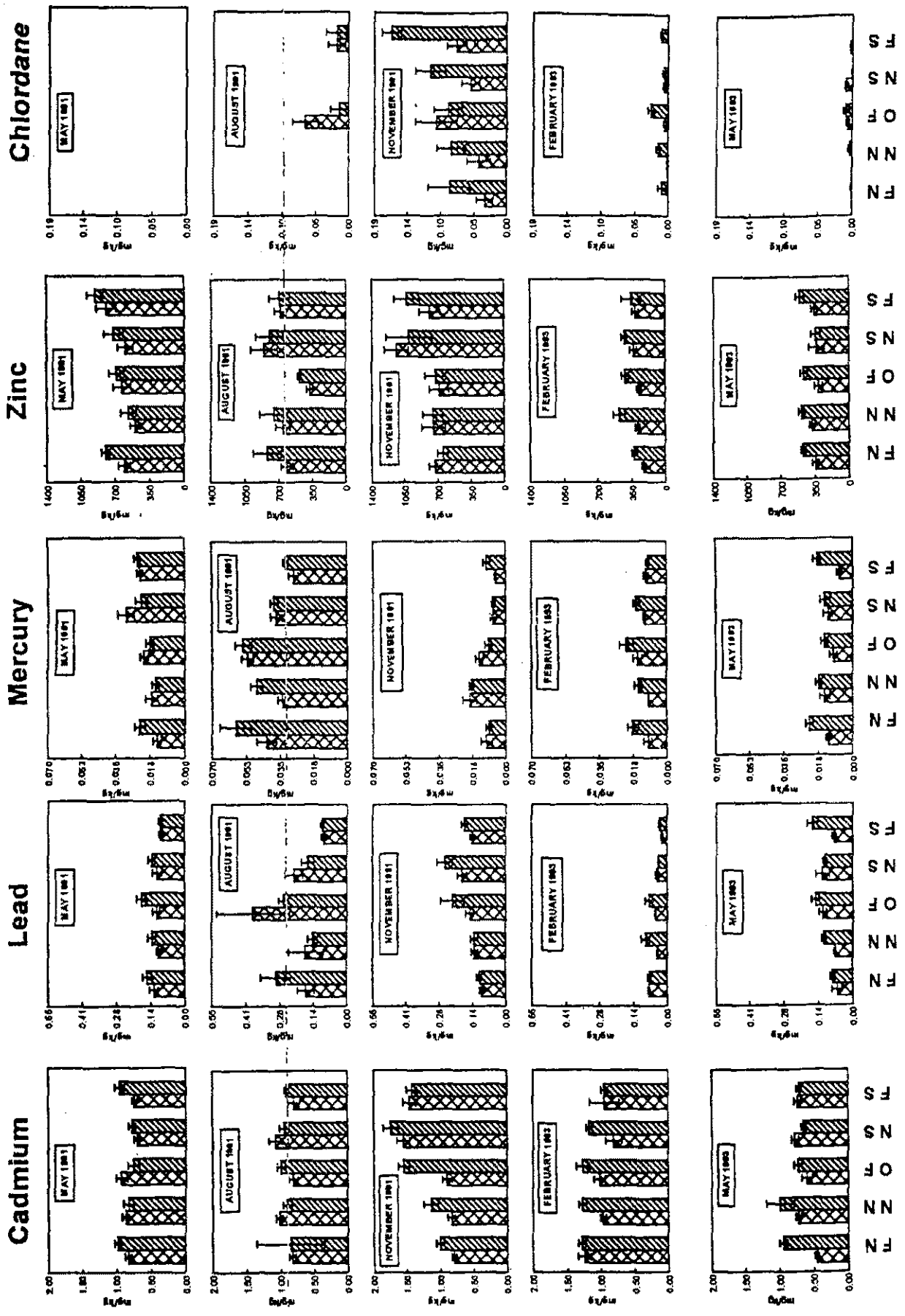
Cadmium
Lead
Mercury
Zinc
Chlordane
MAY 1981
AUGUST 1981
NOVEMBER 1981
FEBRUARY 1982
MAY 1982
FN
NN
OF
NS
FS

TABLE 5

General model of analysis and summaries of F ratios from analyses of variance of contaminant levels in oysters from offshore oysters – outfall vs near south, time 1 represents pre-commissioning data. Significance is indicated as follows: $*0.05 < p > 0.006$; $**p > 0.005$. The mean square of the residual (MSR) is expressed as a negative exponential (e.g. E–3) in some cases.

Source of variation		df (OC)	df (TM)	MS divisor
Time – T	random	4	6	Residual
Region – R	fixed	1	1	T×R
Depth – D	fixed	1	1	T×D
T×R		4	6	Residual
T×D		4	6	Residual
R×D		1	1	T×R×D
T×R×D		4	6	Residual
Residual		180	252	

	Chlordane	Cd	Cr	Cu	Hg	Pb	Zn
Time – T	42.57**	54.6**	53.3**	17.8**	124.5**	16.2**	19.9**
Region – R	3.42	0.56	0.01	1.08	0.02	2.61	0.65
Depth – D	0.00	3.60	1.09	0.28	1.89	0.17	0.72
T×R	1.83	5.03**	30.9**	5.79**	9.36**	2.38*	5.58**
T×D	2.30	6.70**	6.33**	4.41**	9.45**	2.08	2.44*
R×D	1.11	0.04	2.40	2.02	1.01	0.21	2.49
T×R×D	3.36*	2.21	3.65**	1.11	12.14**	0.78	0.62
MSR	0.0012	0.045	0.0083	1469.8	9.73 E-5	0.0111	90605

cyclical patterns in the data suggests that it is not a function of the biology of the oysters. In offshore areas, there were no differences detected pre-commissioning and this pattern remained through the post-commissioning period, although there were a number of instances when regions were different from each other. These differences were not consistently in the same region nor for a particular metal so it is again difficult to determine a cause for the differences. There was a consistent difference in concentrations of copper, zinc and cadmium between deep and shallow positions in the offshore, but again this was not confined to particular region(s) and so is highly unlikely to be an effect of the outfalls.

A number of authors have proposed the use of power analyses to determine the probability of Type II error, i.e. failure to detect an impact when one has occurred and, post hoc, the number of replicates required to determine significant differences (Andrew and Mapstone, 1987; Green, 1989; Peterman, 1990). These tests are of interest primarily if tests of hypotheses do not reject the null hypothesis of no differences between samples. The calculation of power is designed to guard against making wrong management decisions on the basis of non-significant results. This can happen if the power of the tests that produced the results is too low to provide any surety against accepting a null hypothesis that is really false. In the pre-commissioning report for this study (Scanes and Henry, 1992), consideration was given to the power of tests required to detect significant differences. Most analyses of data from inshore and offshore studies detected significant differences, so, by definition, lack of power in the tests was not likely to be the reason why there were so few significant impacts attributable to the presence of outfalls.

Modelling of the effluent fields (Roizenblit, 1994) confirmed that the plumes from the three outfalls merge into a continuous and relatively homogeneous field off Sydney and that all spar moorings in the outfall region were nearly continuously subject to effluent diluted in the range 100–1000 times. The 'Near' regions were occasionally influenced by sewage at a dilution of about 1000–10 000 times. The 'Far' regions were beyond the modelling domain but it is a reasonable assumption that they were not ever exposed to sewage more concentrated than the 'Near' regions. In all cases, the modelling suggested that there was very little difference in the exposure of shallow and deep oysters to sewage.

Other studies (e.g. Connell, 1988; Scanes, 1997) have suggested that there is, or should be, a strong link between fat content and concentration of organochlorine compounds. In a study of bio-accumulation of OCs in a contaminated estuary (Scanes, 1997) there was a strong positive regression between OCs and lipid content. In that study, however, the concentrations of OCs reached by the oysters were an order of magnitude higher than those in this study. One effect of the relatively high concentrations accumulated was that the analytical methods were able to pick up the relatively small differences among individuals induced by variation in lipid (e.g. differences of 10–30% are easily detectable at concentrations of 0.3–0.5 mg kg^{-1}). In the case of the present study, where concentrations of OCs in the oysters were much lower (0.01–0.03 mg kg^{-1}), the accuracy of the analytical method masks changes of this magnitude. Thus it is not surprising that the correlation is poor. NOAA (1989) also found no correlation between lipid (fat) and concentrations of PCB, DDT, lindane or PAHs, and only a slight correlation for chlordane and dieldrin. It could be

argued that pooling over all the locations confounded the picture, as location as well as lipid is influencing the OC concentrations, but later analyses for a longer time span at each individual location (O'Connor, pers. comm.) confirmed that there was little correlation between OC and lipid.

This lack of a relationship does not mean that oysters are not useful indicators, but rather, at lower concentrations there is less need to commit resources to lipid analyses as the results may be of little benefit in interpreting the data.

The data from the estuary study (Scanes, 1997) also indicated that oysters probably reach an equilibrium with the OCs in their environment within about 20 days, and if the environment remains fairly constant then so does the equilibrium. The lack of an equilibrium in the oysters deployed in inshore (post-commissioning) and offshore waters suggests that the OC concentrations in these areas was very variable. This variability, combined with the relatively small individual variability, means that there would have been very little chance of seeing a relationship between OCs and lipid in the outfall studies where effluent concentrations are very variable and the exposure of oysters to effluent is dependant on the ocean currents.

As noted above, there are few previous data with which to compare the concentrations that have been accumulated off Sydney, and what exists is either from estuaries (NSW) or from other species of oyster overseas. Post-commissioning, there was little difference among means for the Sydney outfall, control or estuarine sites (Table 6) with the exception of Se, Ni, Cd and Ag which were at higher concentrations in the marine areas and OCs which were in higher concentrations in estuaries. The concentrations of most metals and all OCs were lower in the Clyde River (which flows

TABLE 6

Comparison of data from Sydney outfall studies with wild oysters from NSW estuarine areas (Clyde River – rural; Georges River, Botany Bay – urban). All data in mg kg^{-1} wet wt. Offshore outfall – data from outfall and near south regions; inshore outfall – data from Malabar outfall; inshore control – data from inshore controls.

	Offshore outfall	Inshore outfall	Inshore control	Clyde River*	Georges River*	Botany Bay*
As		1.81	1.84	1.48	0.63	2.24
Cd	0.87	0.79	0.79	0.27	0.41	0.26
Co		0.08	0.07	0.06	0.1	0.05
Cr	0.37	0.40	0.35	0.23	0.32	0.30
Ag		0.68	0.60	0.18	0.41	0.27
Ni		0.27	0.23	0.13	0.13	0.11
Pb	0.15	0.22	0.18	0.06	0.20	0.15
Se		1.47	1.32	0.37	0.64	0.67
Zn	622	798	724	353	1037	516
Cu	69	87	78	17	90	35
Hg	0.03	0.04	0.03	0	0.02	0.02
chlordane	0.025	0.031	0.024	0	0.07	0.06
Σddt	0	0.019	0.016	0	0.05	0.02

* Data from Scanes *et al.* (1996).

TABLE 7

Comparison of geometric means of data from Sydney with geometric means of data from NOAA 'Mussel Watch'. Data from the Sydney Outfall study have been converted to comparable units in the following ways: trace metal – mean wet wt (mg kg^{-1})×10 (mean water content =90%) =μg g^{-1} dry wt; chlordane – mean wet wt technical chlordane (mg kg^{-1})×0.2×10×1000 =ng g^{-1} dry wt(*) (factor of 0.2 has been applied to convert technical chlordane to the form of chlordane measured by NOAA). Σ DDT – mean wet wt (mg kg^{-1})×10×1000 =ng g^{-1} dry wt(*). Offshore outfall – data from outfall and near south regions; Inshore outfall – data from Malabar outfall; Inshore control – data from inshore controls.

	Offshore outfall mean	Inshore outfall mean	Inshore control mean	NOAA mean #	NOAA high #
As		16.2	16.8	10	17
Cd	8.05	6.9	7.0	2.7	5.7
Hg	0.19	0.26	0.21	0.094	0.24
Ni		1.71	1.75	1.7	3.3
Ag		3.0	3.3	0.17	3.7
Cu	537	614	660	150	360
Zn	5188	6170	6310	2400	5200
Pb	1.24	1.9	1.6	0.52	0.94
Cr	3.3	3.58	3.16	0.48	0.93
Se				2.5	3.5
chlordane*	66	56	48	14	31
Σddt*		199	206	37	120

Data from O'Connor (1992).

through relatively undeveloped rural country on the NSW south coast) than in the Sydney region.

Comparisons have also been made to data from the NOAA 'Mussel Watch' Project (O'Connor, 1992) (Table 7), but these should be treated with caution as the species of oyster used (*C. virginica*) is different to the species in this study. Nevertheless, *Crassostrea* showed similar oyster : mussel bioconcentration ratios as *Saccostrea* (Scanes, 1996). O'Connor (1992) used geometric means to identify mean concentrations and what he considered to be 'high' concentrations for a range of contaminants (Table 7). His study involved collection of wild animals from a wide range of sites remote from centres of population as well as near cities. A consequence of the use of sites far from cities is the means that will tend to be reduced by the large number of low concentrations and 'high' data will tend to come from near larger population centres. Comparisons of the Sydney data to those in O'Connor (1992) (Table 7) show that, taking into account possible small differences in bioconcentration by the two species, the concentrations of trace metals around Sydney are similar to those expected around US cities (i.e. the 'high' data). Concentrations of chlordane and DDT were, however, considerably greater in the Sydney region. This is not surprising as the use of these chemicals was banned in the USA many years before their banning in Australia.

The use of deployed oysters in a design which included data from before and after a change in the location of sewage discharge has allowed a clear demonstration that offshore discharge of sewage has decreased the potential for accumulation of organochlorine compounds in inshore waters without significantly increasing the potential for accumulation offshore.

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Trace metal uptake in cockles *Anadara trapezium* from Lake Macquarie, New South Wales

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ABSTRACT: Trace metal contamination of waters, sediments and animals in northern Lake Macquarie has been identified as a matter of concern by regulatory authorities. Experimental procedures using the benthic bivalve *Anadara trapezium* (Sydney cockle) were developed to examine hypotheses relating to whether the cockle accumulated trace metals free in the water or from the sediment in which it is partially buried. Experiments were done in 1989 and 1991 to determine whether the concentration of trace metals in cockles was related to the concentration of metals in surrounding water or sediments. Pilot experiments indicated that it was possible to translocate tubs of sediments from impacted areas to unimpacted areas with no loss of trace metals from contaminated sediments and conversely with no increase in metals in uncontaminated sediments. Other experiments showed clearly that the presence of elevated levels of lead, copper and zinc in the water led to much greater levels in the cockles, irrespective of the concentration in surrounding sediments. There were no significant trends for cadmium. It was concluded that the sediments in which a cockle was living had little bearing on the levels of zinc, copper and lead that were accumulated in the cockle, whereas the surrounding waters had a considerable effect.

KEY WORDS: *Anadara trapezium* · Bioaccumulation · Management · Power · Sediments · Trace metals

INTRODUCTION

The detection of contamination of marine and estuarine systems by industrial pollutants such as trace metals has received considerable attention (e.g. Phillips 1977, Martin 1985). Phillips (1977) recommends that biological indicators (specifically accumulators of metals) are the best indicators of trace metal pollution as they detect that portion of the contaminant load that is available to organisms. Other methods of detecting environmental contamination include analysis of water and sediments. These have been traditionally favoured (e.g. Batley 1987) but, as Phillips (1977) pointed out, they suffer from problems in interpretation of biological effects or considerable temporal and spatial variability. Biota are perceived as being able to integrate or smooth out some of the short-term variability and reflect longer-term trends. Various attempts to determine biological relevance of analyses of sediments have been attempted, mainly by extraction of metals in different solvents (Batley 1987), some of which are said to emulate the bioavailable fractions of the total load of metals (Salomons & Forstner 1980, Batley 1987). Despite possible problems with correlating total load of metals with biological effects, contamination of sediments is often cited as an indicator of industrial contamination and potential biological impact (e.g. Phillips & Yim 1981, Gray et al. 1988).

Underwood & Peterson (1988) made the case that an important part of a study of pollution is that the methodology should elucidate the mechanism which is causing the observed biological effect. This is often overlooked in studies correlating biological effects and concentrations of pollutants in sediments (e.g. Brooks & Rumsby 1965, Cain & Luoma 1990), where it is assumed that the contamination of the sediments has led to the biological effect. This paper investigates that assumption by testing whether contaminants in the sediments or in the water column led to contamination of cockles in Lake Macquarie, New South Wales, Australia.

Lake Macquarie (Fig. 1) is a coastal lagoon which has received industrial and sewage effluent discharges

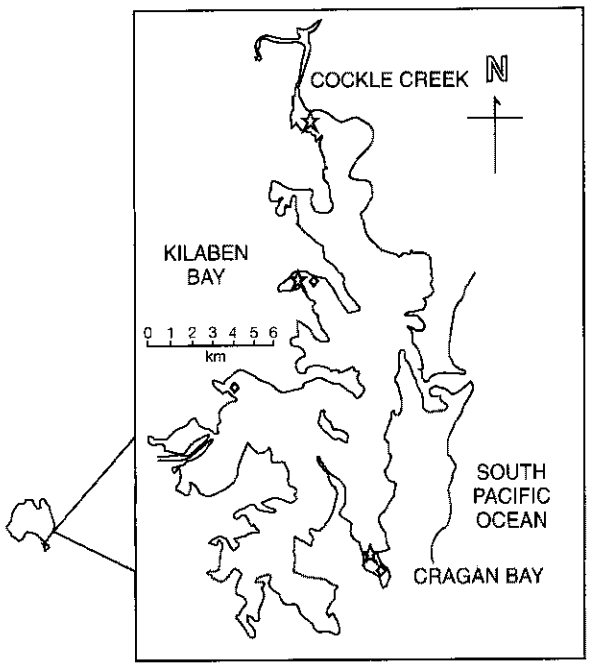

Fig. 1. Lake Macquarie (NSW, Autralia) showing position of experimental areas (☆) and areas where cockles were collected (◇)

for over 100 yr. Contamination of sediments and water by trace metals has occurred in the northern quarter of the Lake (Batley 1987). The main trace metals present above background levels in the sediments and water column were zinc, cadmium, copper and lead. The sediments were considered to have been contaminated from past and present industrial and sewage discharge into Cockle Creek, which drains into the northern end of Lake Macquarie. Batley (1987) found concentrations of total dissolved zinc, cadmium and lead in water 1 to 2 orders of magnitude greater at Cockle Creek than near to, or in areas similar to, the control sites used in the present study. Concentrations of copper appeared to be smaller at the terminal ends of bays. Concentrations of the 4 metals in sediments were an order of magnitude greater at Cockle Creek than at the control sites chosen in the present study.

A lamellibranch bivalve, the cockle *Anadara trapezium* (DeShayes, 1839), sampled from northern parts of the lake, has been shown to accumulate some trace metals to levels greater than those recommended for human consumption. The ability of bivalves to accumulate trace metals has been well documented (Phillips 1977).

Field experimentation was judged to be the most effective means of attempting to identify the source of contamination (Connell 1961, Dayton 1971, Underwood 1986, 1990). Experiments are, however, only able to identify causal factors if the design is unconfounded (Green 1979, Hurlbert 1984, Underwood 1986, 1989, 1990). The field experiments in the present study utilised control treatments for all manipulations in order to provide unconfounded conclusions.

This study addressed 3 linked null hypotheses – that there are expected to be no differences in the concentrations of trace metals in cockles exposed to water with elevated concentrations of metals compared to cockles in unaffected areas; that there are expected to be no differences in the concentrations of trace metals in cockles exposed to sediment with elevated concentrations of metals compared to cockles in unaffected areas; and that there are expected to be no differences in the concentrations of trace metals in cockles exposed to both water with elevated concentrations of metals and sediments with elevated concentrations of metals compared to those in unaffected areas. The temporal consistency of patterns indicated by the experiment was examined by repeating the whole series of experiments.

The results of the tests of these hypotheses will enable determination of whether cockles take up metals which were free in the water or bound in the sediment, and thus indicate the best future actions to reduce metal contamination of cockles in Lake Macquarie.

METHODS

In order to provide orthogonal comparisons of contaminated and uncontaminated sediments and water, reciprocal translocations of sediments between impacted areas and unimpacted areas were made. Batley (1987) showed that concentrations of trace metals in water and sediments in Lake Macquarie were elevated in the vicinity of Cockle Creek, and it was from this area that contaminated sediments were collected and treatments requiring water with elevated levels of trace metals were set up. The unimpacted areas used for the experiments, Kilaben Bay and Crangan Bay (Fig. 1), were also chosen on the basis of data from Batley (1987).

The experimental design used (Table 1) includes the use of 2 control (unimpacted) locations, and control treatments for the effects of disturbance of sediments and translocation between impact and treatment locations and between control locations. Three places were chosen in 2 m water depth within each location. One replicate of each treatment (undisturbed control, a disturbance control and 2 translocations; Table 1) was put at each place.

Undisturbed control plots were areas of 50 × 50 cm with undisturbed sediments, marked with wooden stakes at the corners. Sediments to be translocated or

Table 1. Design for trace metal study. Each treatment at each location was replicated 3 times, and there were 5 cockles analysed per treatment (n = 5). S: sediment; W: water; +, – indicate presence or absence of great concentrations of trace metals

Location	A Untouch. control	B Disturb. control	C Translocat.	D Translocat.
1. Cockle Creek	+S +W	+S +W from:	–S +W Location 2	–S +W Location 3
2. Kilaben Bay (control)	–S –W	–S –W from:	+S –W Location 1	–S –W Location 3
3. Crangan Bay (control)	–S –W	–S –W from:	+S –W Location 1	–S –W Location 2

disturbed were placed in plastic tubs (350 × 600 × 200 mm deep, volume ca 42 l) by divers, brought to the surface and placed in a boat. The tubs were then either replaced as disturbance controls or moved to another location according to the design in Table 1. Tubs were partially buried so that the top of the tubs protruded about 50 mm above the lake floor. This procedure was designed to reduce drift of sediment into or out of the tubs.

To test the efficacy of this procedure for translocating sediments, a pilot study of 6 wk duration (in 1989) compared the concentrations of metals in sediments before disturbance and 6 wk after being placed in plastic tubs and translocated. The experiment used one impact (Cockle Creek) and one control (Crangan Bay) location. Three replicate samples of sediment were taken from each tub at the beginning and end of study. The pilot study was based on the assumption that any loss of metals from the sediments would occur mainly during the disturbance and moving of the sediment.

Anadara trapezium (Sydney cockle) is a common estuarine bivalve about 40 mm long, often found in association with beds of the seagrass *Zostera capricornia* Ascherson. It is usually found embedded in sediment with about 25% of the valves protruding. It has very limited (if any) mobility. Cockles used in all tests were collected from a number of unimpacted areas in the southern and central parts of the lake (Fig. 1) and combined into a common pool. From this pool of cockles, 15 were selected at random and placed by divers into the sediment in each treatment, in their natural orientation, in September 1989 and 1991. All treatments (including untouched controls) were enclosed in plastic mesh (15 mm mesh), as experience had shown that cockles were a favoured item of benthic predators in the Lake.

The experiment was terminated in November of each year and all cockles were removed from each treatment. Cockles were immediately placed in plastic bags and frozen. Mortality and loss had reduced the number of cockles in some treatments, so 5 cockles were randomly selected for analysis from those collected for each treatment.

The tissue concentrations of zinc and cadmium were determined on undried samples by flame atomic absorption spectroscopy (flame AAS) after digestion in nitric acid and hydrogen peroxide. Copper and lead were determined in the organic phase by flame AAS after adding sodium iodide and extracting the Pb and Cu iodide complexes in *n*-butyl acetate containing 3% tri-*n*-octylamine. Concentrations were reported as $\mu g\ g^{-1}$ wet weight.

Data were analysed by analysis of variance after first checking for homogeneity of variance, and transforming data if necessary (Underwood 1981). Concentrations of metals in 5 replicate individual cockles from each treatment at each of the 3 replicate places at each location were pooled for statistical analyses (i.e. n = 15).

Post-hoc power analyses (Underwood 1981) were done on the data to determine the power of non-significant tests. The alternative hypothesis being tested was that of a 20 or 40% difference in means between experimental and pooled control 'treatments' at each time. In the absence of any data from the literature which might indicate what is an 'important' or biologically 'significant' change in concentrations of metals, I have selected differences which indicate power for changes that were much smaller than those found for the significant analyses of the effects of 'location' (where differences of 50 to 700% occurred). This rationale aims to demonstrate that both main factors had similar power to detect differences.

RESULTS

Pilot study – sediments

Concentrations of zinc, cadmium, lead and copper were all an order of magnitude larger in Cockle Creek (impacted) sediments than Crangan Bay (unimpacted) sediments (Fig. 2). There were significant differences from start to finish (Date) for all metals (Table 2) and significant Date × Place interactions (Fig. 2). The interactions resulted from significant changes in concentrations from the beginning to the end of the experiment at Cockle Creek, but not at Crangan Bay. There was also a significant Date × Treatment interaction for copper and lead. This is a result of different sediment concentrations in the 3 treatments from Cockle Creek at the start of the experiment, but similar levels at the finish.

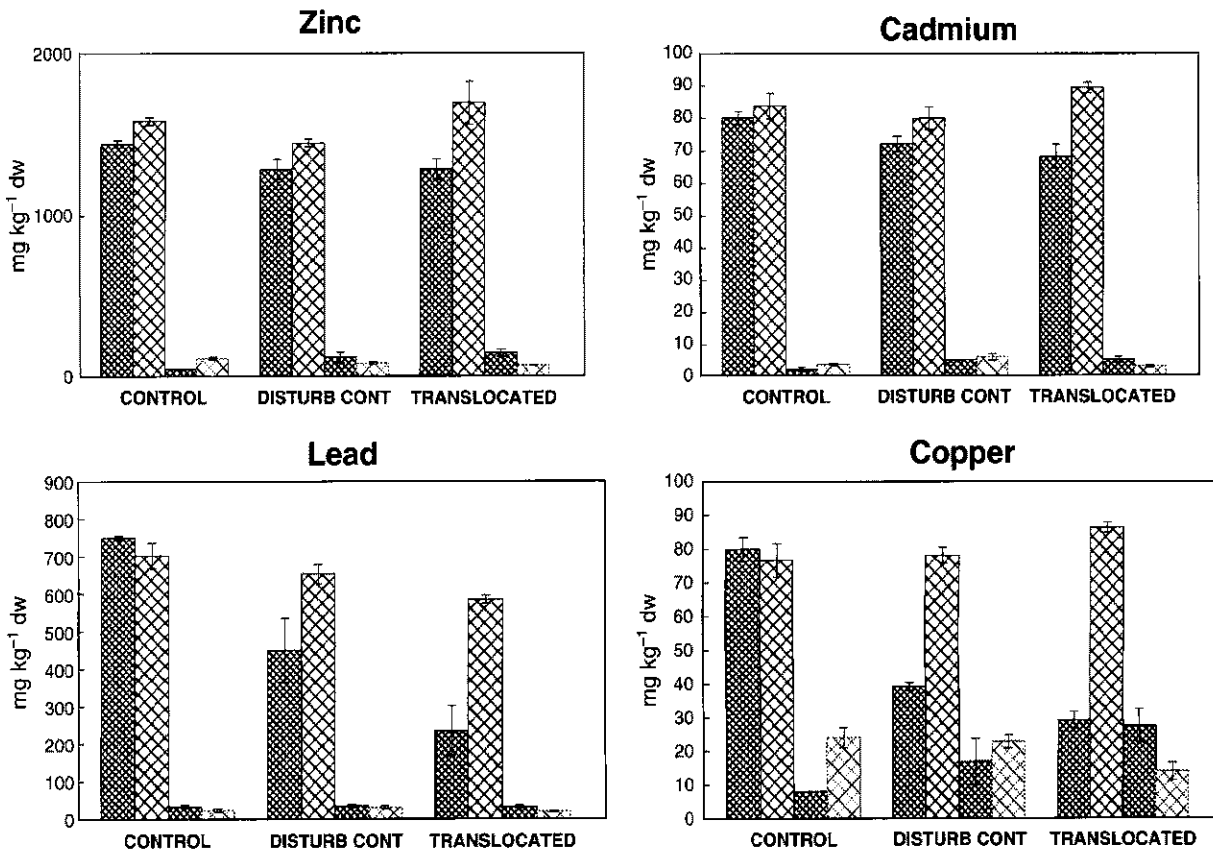

Fig. 2. Comparison of mean (± 1 SE) concentrations of trace metals in sediments before and after experimental manipulations (pilot study). Within each group of 4 bars the 2 bars on the left are data for Cockle Creek (affected) sediments, and the 2 on the right are Crangan Bay (unaffected) sediments. 'Control' treatments are untouched prior to collection of sediments, 'disturb control' treatments have been dug up, placed in a tub and put back in the same hole, 'translocated' have been moved between the 2 sites. Closely hatched bars are data from the start of the experiment, widely hatched bars are data from the end

The significant facts to note are that sediment concentrations did not fall over time, but actually seemed to increase in those instances where they changed significantly. There was also no indication that translocating sediments caused any greater changes than disturbing sediments and that the changes all seemed to be confined to the impacted Cockle Creek sediments. There was no indication that translocating contaminated sediments to an unimpacted area led to a reduction in the level of contamination of those sediments, or conversely, that moving uncontaminated sediments to an impacted area resulted in an increase in the metal concentration in the sediments.

Table 2. Summaries of results of analyses of variance comparing concentrations of trace metals in sediments after disturbance – pilot study. r: random factor with respect to the model; f: fixed factor with respect to the model.

Source		Test df	Zinc[a]	Cadmium	Lead[a]	Copper
Date	r	1,24	*	***	***	***
Location	f	1,1	*	*	ns	ns
D × L		1,24	***	***	***	***
Treatment	f	2,2	ns	ns	ns	ns
D × T		2,24	ns	ns	*	*
L × T		2,2	ns	ns	ns	ns
D × L × T		2,24	***	*	*	***

[a]Data transformed to square root $(x+1)$; all variances stabilised (i.e. Cochrans test, $p > 0.05$)

*Significant differences at $p < 0.05$

***Significant differences at $p < 0.001$

Main study – cockles

Effect of disturbance

The first comparison made was between specimens from disturbed and undisturbed controls. This comparison was required to confirm the validity of generalising the results obtained from specimens in tubs to those not in tubs. There was no significant difference

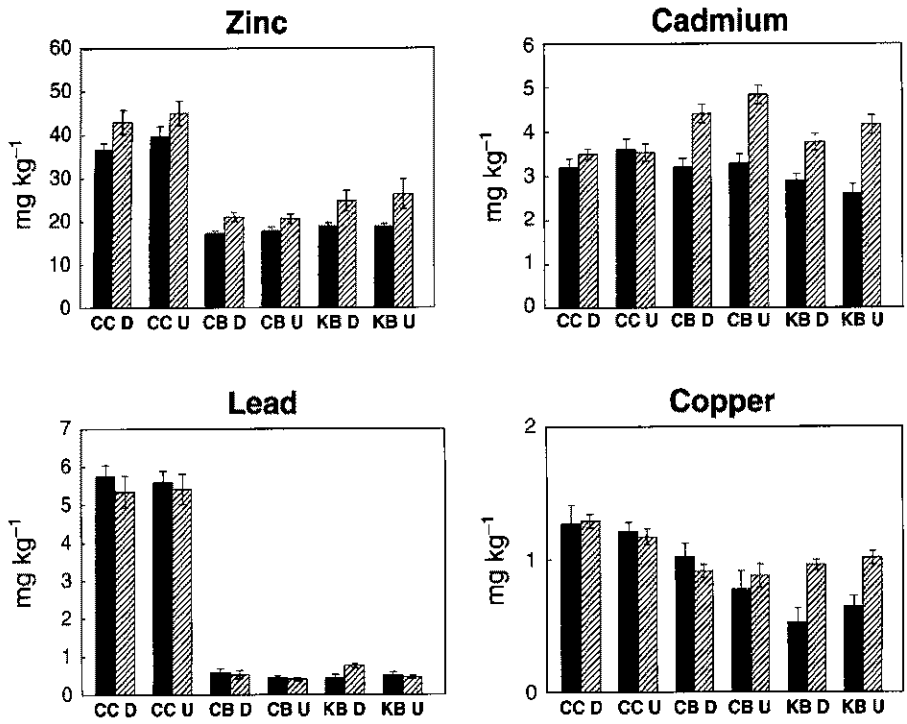

Fig. 3. *Anadara trapezium*. Comparison of mean (± 1 SE) concentrations of metals in cockles from disturbed (D) and undisturbed controls (U), main study. CC: Cockle Creek; CB: Crangan Bay; KB: Kilaben Bay. Solid bars: data from 1989; hatched bars: data from 1991

between metal concentrations in the tissue of cockles in disturbed and undisturbed controls ('Treatments'; Table 3, Fig. 3). There were significant differences between locations (i.e. concentration of metals in the water) for zinc, lead and copper, with Cockle Creek samples having significantly greater tissue loads (SNK tests, $p < 0.05$). Interactions between date and location for cadmium and copper were caused by greater concentrations at control locations in 1991. These results indicated that it was appropriate to use the disturbed controls to compare with the various translocations.

Effect of water vs sediment

An analysis of the effects of the treatments indicated that there were no significant differences attributable to the concentration of metals in the sediments ('Treatments'; Table 4). There were, however, significant differences in the tissue loads of lead, zinc and copper (Cockle Creek > Kilaben = Crangan; SNK tests, $p < 0.05$) attributable to the concentration of metals in the water column (Fig. 4, 'Location' Table 4). There was no significant difference in the tissue concentrations of cadmium between treatments and the differences among locations did not seem to be related to known patterns of concentrations of cadmium in the water (Fig. 4, Table 4).

In addition, analyses of the concentrations of zinc, lead and copper indicated significant interactions between date and location and treatment and location.

These are always complex to explain in full. Here, they are presented graphically. For example, Fig. 4A(ii) describes the date by location interaction for zinc. The asterisks above the first 2 pairs of bars indi-

Table 3. Summaries of results of analyses of variance comparing concentrations of trace metals in experimental cockles from disturbed and undisturbed controls – main study. r: random factor with respect to the model; f: fixed factor with respect to the model.

Source		Test df	Zinc[a]	Cadmium[a]	Lead[b]	Copper[b]
Date	f	1,12	ns	ns	ns	ns
Location	r	2,12	***	ns	***	***
D × L		2,12	ns	*	ns	*
Treatment	f	1,4	ns	ns	ns	ns
D × T		1,12	ns	ns	ns	ns
L × T		2,12	ns	ns	ns	ns
D × L × T		2,12	ns	ns	ns	ns

[a]Data transformed to log $(x+1)$;
[b]Data transformed to square root $(x+1)$; all variances stabilised (i.e. Cochrans test, $p > 0.05$)
*Significant differences at $p < 0.05$
***Significant differences at $p < 0.001$

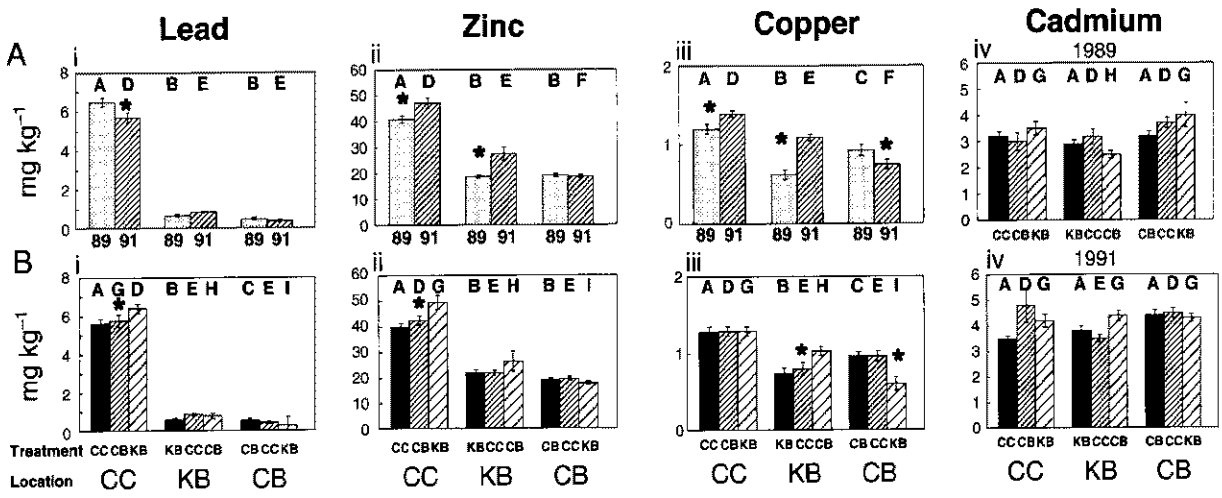

Fig. 4. *Anadara trapezium*. Comparison of mean (± 1 SE) trace metal concentrations in cockles. 'Treatment' on the *x*-axis indicates the source of the sediment in which the cockles were placed. 'Location' on the *x*-axis indicates the location at which the treatments were placed. CC: Cockle Creek (contaminated water and sediments); KB: Kilaben Bay; CB: Crangan Bay (uncontaminated water and sediments). Data in A(i–iii) are treatments pooled at a location (n = 45); B(i–iii) are times pooled for each treatment (n = 30); A(iv) and B(iv) are each treatment at each location and time (n = 15). *Significant differences among time of sampling or treatments, i.e. within a group on the graph. Significant differences among locations for a time or treatment are shown by different letters above the bars

cate significant differences between concentrations in 1989 and 1991 at Cockle Creek and Kilaben Bay. The letters above the bars with similar shading (i.e. those from the same year) indicate that in 1989 concentrations in cockles from Cockle Creek were significantly greater than in those from Kilaben and Crangan Bays. However, in 1991, cockles from Cockle Creek were significantly more contaminated than those from Kilaben Bay, which were in turn significantly different from Crangan Bay.

The data in Fig. 4A(i–iii) show that, whilst there was temporal inconsistency in the results for any particular location, concentrations of zinc, lead and copper in cockles from the Cockle Creek location were always significantly greatest. This is illustrated by the different letters above bars in the graphs. There are indications that, in 1991, significantly greater concentrations of zinc and copper were found in cockles from Kilaben Bay than in those from Crangan Bay.

There was no consistent pattern in the ranking of treatments among locations [Fig. 4B(i–iii)], indicating that sediments from each location did not consistently lead to greater concentrations of lead, zinc or copper.

The accumulation of cadmium was affected most consistently by date with most interactions involving time suggesting greater concentrations in 1991. There were no consistent patterns, leading to the conclusion that no particular treatment or location led to greater concentrations of cadmium in cockles.

This illustrates that, whilst there were small-scale alterations in the ranking of treatments and times within locations, they did not alter the conclusions. Cockles at Cockle Creek always accumulated more lead, zinc and copper than cockles at the other 2 locations and the presence of contaminated sediments did not lead to elevated levels of metals in cockles at the control locations.

Table 4. Summary of analyses of variance comparing concentrations of trace metals in experimental cockles from all treatments – main study. r: random factor with respect to the model; f: fixed factor with respect to the model

Source		Test df	Zinc[a]	Cadmium[a]	Lead[b]	Copper[b]
Date	f	1,2	ns	*	ns	ns
Location	r	2,252	***	***	***	***
D × L		2,252	*	ns	*	***
Treatment	f	1,4	ns	ns	ns	ns
D × T		1,4	ns	ns	ns	ns
L × T		4,252	*	ns	*	***
D × L × T		4,252	ns	***	ns	ns

[a]Data transformed to log (*x*+1);
[b]Data transformed to square root (*x*+1); all variances stabilised (i.e. Cochrans test, $p > 0.05$)
*Significant differences at $p < 0.05$
***Significant differences at $p < 0.001$

Post-hoc analyses of power

The most important non-significant tests were those associated with Treatments. The results of power

Table 5. Power of tests among treatments. Columns '20' and '40' show power for alternative hypotheses of 20 and 40% increase over control concentrations respectively

	20				40			
	Zn	Cd	Pb	Cu	Zn	Cd	Pb	Cu
1989	0.63	0.82	0.75	0.72	0.99	0.99	0.99	0.99
1991	0.85	0.96	0.77	0.89	0.99	0.99	0.99	0.99

analyses (Table 5) for the Treatments effect (years were treated separately) indicated that for an alternative hypothesis of 20% change, power ranges between 0.63 to 0.96 and for 40% was 0.99. This demonstrates that analyses of the effects of treatments were at least as powerful as those for the effects of locations and that the lack of an effect of treatment is not an artifact of the experimental design.

DISCUSSION

The pilot study indicated that trace metals were not lost from sediments in significant amounts even after considerable disturbance. This result was not unexpected, since laboratory elutriate tests described in Batley (1987) also indicated that the metals in Lake Macquarie sediments were not mobile. Some of the results were unexpected, particularly the differing concentrations in each treatment at the start of the experiment which did not continue to the end of the experiment. A possible explanation is that digging of the sediments disturbed the overall structure of the sediments and may have introduced lumps of less contaminated sediments to the surface of the experimental tubs, where samples were taken. Bioturbation by infauna may have homogenised the sediments during the experiment, leading to more consistent results between treatments, and results which were similar to contaminant levels in undisturbed controls at the start of the experiment.

Importantly, the experimental procedure did not lead either to a decrease in the contaminant levels in disturbed contaminated sediments, either those left at Cockle Creek or those moved to Crangan Bay; or to an increase in contaminant levels in uncontaminated Crangan Bay sediments moved to impacted areas. This allowed the unconfounded conduct of translocation experiments such as those described.

The second series of experiments showed conclusively that zinc and lead were bioaccumulated to significantly greater levels in treatments exposed to relatively elevated water concentrations. Concentrations of metals in sediments had less effect on tissue loads of these metals. It was not possible to determine the reason for the lack of any differences for cadmium, even between locations, from these experiments. Possible explanations are that cadmium accumulates over longer time periods, or cockles are able to regulate tissue loads, or that it was present (in both aqueous media and in sediments) in a form unavailable to the cockles, or that the water or sediment concentration gradient was insufficient to produce a significant result. Further work would have to be done to differentiate between these alternatives and the contribution of pore waters (Adams et al. 1992).

The consistency of the trends in the results between 2 trials 2 yr apart adds to the confidence that can be placed in both the procedure and the conclusions. Unpublished data indicate that the same results were obtained for oysters *Saccostrea commercialis* subjected to the same experimental treatments as described in this paper.

Fairweather (1991) and Peterman (1990) have stressed the potential costs in making environmental decisions on the basis of non-significant results with no consideration of the probability of Type II statistical error. They advocate strongly that the power of non-significant tests must be presented when conclusions are drawn from non-significant results (where Power = 1 – Probability of Type II error). Power calculations must, however, be based on an alternative hypothesis which states what is an environmentally/ecologically significant change in the variable being measured. This is often a difficult thing to determine and there are no satisfactory general procedures. Examples of appropriate ways of determining a suitable alternative hypothesis include comparison with legislative standards, or percentage or proportional differences found to be significant in similar studies at another time or place. In this study 2 alternative hypotheses were considered: increases in concentrations from experimental treatments over control concentrations of 20 and 40%. Given that the differences among locations were usually 50 to 700%, the hypothesised alternatives are probably conservative. The power of the tests for treatments with an alternative of 40% change was nearly 1. This allows much confidence to be placed in the conclusion that the effects of contaminated sediment on accumulation of metals by cockles are very small.

Correlations between concentration of metals in the water column and in animal tissue is cited by Phillips (1977) as one of the most important attributes of a good indicator, and has been demonstrated for a number of bivalve species (e.g. Klumpp & Burdon-Jones 1982). Mussels have been shown to be able to accumulate mercury from both dissolved and particulate phases (King & Davies 1987). McConachie & Lawrence (1991) found that bivalves accumulated cadmium to much greater levels when they were exposed to contami-

nated particulates. This study indicates that metals free in the water are responsible for contamination of cockles, but the same result would have been erroneously obtained by correlation with the existing distribution of contaminated sediments. These results underline the importance of manipulative experiments in the explanation of observed patterns. Elucidation of the cause of observed patterns is extremely important in situations such as the one described for Lake Macquarie where potentially costly management decisions may be based on the outcome of the study.

Adams et al. (1992) note that the testing of bulk sediment for trace metals has been shown to be an unreliable measure of bioavailability. The sediments used in the present study were tested for 'bioavailability' using EDTA and other chemical extraction procedures by Batley (1987). He concluded that the majority of zinc and lead in the sediments was present in a form that should be biologically available. This is somewhat in conflict with the studies described in the present paper. Further studies, along the lines of the methods suggested in Adams et al. (1992), would be required to fully assess the toxicology (including bioavailability of metals) of the sediments at Cockle Creek and the role that sediments play in introducing metals into biological systems.

There are management implications arising from this study regarding the contaminant load in the water column, sediments and biota. The study suggests that the immediate source of trace metal in cockles is the water column, not the sediments. Further work may, however, be necessary to establish the contribution the sediments are making to concentrations in the water column. Should the sediments be contributing to the contamination of the water column, then removal of the sediments may be justified to reduce water concentrations.

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Appendix 6 **Scanes, P. R. 1993, 'Trace metal uptake in cockles *Anadara trapezium* from Lake Macquarie, New South Wales', *Marine Ecology Progress Series*, vol. 102, pp. 135-42.**