BIOMONITORING OF HEAVY METALS IN SEAWATER

by

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STATEMENT

Except as stated herein this thesis contains no material which has been accepted for the award of any other degree or diploma in any university. To the best of my knowledge and belief, this thesis contains no copy or paraphrase of material previously published or written by another person, except where due reference is made in the text of the thesis.

Nicholas G. Elliott.

NICHOLAS G. ELLIOTT

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ABSTRACT

A strategy for the use of the mussel *Mytilus edulis planulatus* in assessing heavy metal (Cd, Cu, Pb, Zn) levels in open marine waters is suggested. Metal accumulation by mussels was linear in laboratory experiments and the rate was directly proportional to the external concentration. Depuration was minimal, and dependent on tissue concentration. Physiological and environmental factors were observed to influence accumulation rate. Smaller sized mussels accumulated metal at greater rates, while rates were higher at low water temperatures. Mussels collected from populations in areas where metal levels were high had lower rates of accumulation than mussels from more pristine areas. Rate of accumulation was shown to be a sensitive index of external metal concentration if the influence of external factors was minimized or eliminated.

When metals were presented singly, the mussel accumulated zinc and lead in direct proportion to exposure time, while this was not the case for cadmium and copper. Under combined exposure to all four metals, cadmium and lead accumulation was proportional to exposure time, while zinc and copper accumulation was not. This apparent interaction between metals during simultaneous exposure was shown to be between cadmium, copper and zinc. In general, cadmium accumulation was decreased in the presence of the other metals, while that of copper and zinc was increased. Previous exposure to either the same or another metal had no influence on the subsequent accumulation of cadmium, lead or zinc. Copper accumulation was markedly increased in mussels initially exposed for 20 days to either cadmium or zinc. The fact that these interactions occurred predominantly at high external concentrations (e.g. Cd>10 $\mu g\ \ell^{-1}$, Cu>10 $\mu g\ \ell^{-1}$, Zn>100 $\mu g\ \ell^{-1}$) suggests that they would not seriously interfere with the monitoring strategy.

In the light of experimental results, a mechanism for accumulation of each metal by *M. e. planulatus* is proposed. Cadmium and copper appear to share a similar carrier-assisted uptake mechanism, while zinc and lead appear to diffuse across membranes passively. The method of storage of lead within cells appears to be distinct from that of the other three metals.

Cadmium, lead and zinc were found to be taken up at both the gills and digestive tissue, and were generally evenly distributed throughout the mussel body, but with high concentrations in the kidney. Zinc levels were also high in the muscle and byssus/foot tissues. Copper, on the other hand, was taken up only at the gills and stored in the foot and digestive tissue (liver). The byssal threads appear to be a major excretory system for copper, and may also be important for the other three metals as well.

Field trials of the proposed monitoring techniques, involving cage suspension of organisms, revealed that the mussel provides a very sensitive and time-integrated assessment of the biologically available metals in seawater. Biomonitoring using mussels would be less costly and labour intensive than conventional techniques currently available for measuring metal levels in seawater.

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CHAPTER 1

INTRODUCTION: PRELIMINARY SELECTION OF A BIOLOGICAL MONITORING SCHEME

1.1 GENERAL INTRODUCTION

Pollution of the marine environment is defined by the United Nations Joint Group of Experts on the Scientific Aspects of Marine Pollution (GESAMP) as "such deleterious effects as harm to living resources, hazards to human health, hindrance to maritime activities or reduction of amenities" (Cole 1979b). This definition, of course, covers a wide range of pollutants. For heavy metals it is the harm to living resources and the, possibly related, hazards to human health that are of concern. Consequently, it is the biologically available metals that are important.

Heavy metals occurring naturally in aquatic systems may be described as being 'out of sight, out of mind' due to their generally low concentrations. Yet because of man's activities, in both mining and industry, increases in metal levels have and are occurring. In some instances disastrous levels for both man and marine organisms have resulted. For example (see Förstner and Wittman 1979 for review): the Minamata Disease caused by the consumption of fish and shellfish contaminated with mercury; the 'itai-itai' disease caused by the consumption of foodstuffs contaminated with cadmium; plumbism, known for over 2,000 years, is caused by lead poisoning; and copper contamination of seawater off the coast of Holland in 1965 led to innumerable deaths in fish.

Whilst it might be said that man causes polluted conditions, high levels of metal may occur by natural runoff where streams run through ore-bearing rocks. For example, Thomson (1979) reported that the tissues of oysters and mussels sampled from an isolated, virtually uninhabited area, where the metals derive from natural sources, had metal concentrations above the recommended health limits for human consumption. It is important, therefore, for the welfare of both the marine organisms and man, firstly that levels of the biologically available heavy metals in marine waters are constantly monitored. Secondly, it is equally

important that the levels at which these metals become hazardous is known. Only the first of these has been addressed in this thesis.

It was concluded following an Australian interlaboratory comparison of analytical methods for the analysis of cadmium, copper, lead and zinc in seawater, that without improvements in the reliability of the methods, investigations at the sublethal levels of these metals would be severely hampered (Major and Pettis 1978). Results of the ICES intercalibrations for heavy metals in seawater, however, indicated an improvement in the accuracy of analysis (Bewers *et al.* 1979). This improvement is supported by works such as that of Jørstad and Salbu (1980) using Neutron Activation to analyse seawater for heavy metals. Yet seawater analysis is not at the stage at which standards could be prepared and distributed, as are rock, sediment and biological standards (Bewers *et al.* 1978). Any degree of precision tends to be lost due to a lack of knowledge of the chemistry of the pollutants in seawater. Each element or compound may be present in a variety of physiochemical forms (Stumm and Bilinski 1973; Burton 1979). In the case of heavy metals in the marine environment, the different species of the metals, their chemical state and availability are of importance when considering biological effects (Davey *et al.* 1973). Different analytical procedures tend to estimate different 'availability' of the metals. Nygard and Hill (1979) state that there is no evidence to support any one analytical procedure as the best method to estimate the 'biologically available' metal.

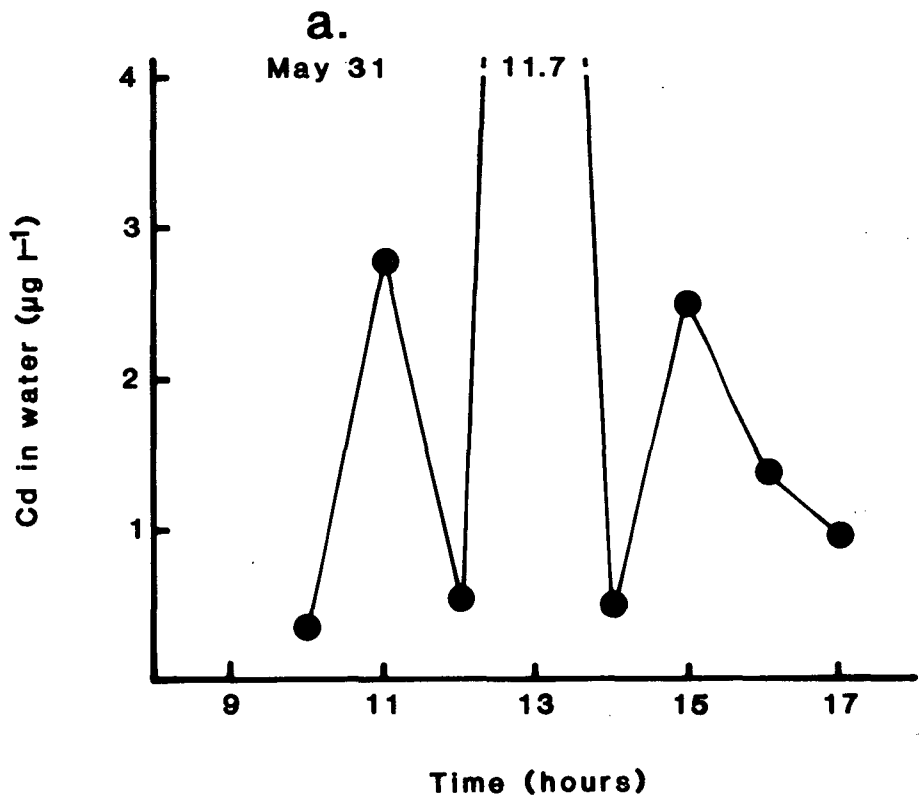
The analytical methods currently available for analysing heavy metals in seawater have other disadvantages, apart from not necessarily estimating the biologically available metal. The metal concentrations in seawater are commonly in parts per billion. Such low concentrations present problems because they often lie below the sensitivity of the

analytical instruments. Thus seawater samples require expensive and time-consuming preconcentration processes prior to analysis. Also, because of the low concentrations, the effects of any contamination from, for example, sampling equipment, reagents or glassware, are magnified. A further disadvantage is associated with the temporal variations in metal levels in aquatic systems (see Figure 1.1 and Grimshaw *et al.* 1976). Such variations may be short-term (hourly) or long-term (days/weeks), and may be dependent on tides, natural run-off, or industrial discharge. Consequently, to obtain the mean or time-integrated metal concentration at a monitoring site, would require a large number of samples, making the task very expensive and time-consuming.

Marine organisms have been known for some time to concentrate metals within their tissues to levels well in excess of the ambient seawater levels (see Table 1.1). Concentration factors of 10^3 - 10^5 have been recorded, with metal concentrations in the tissues commonly in parts per million. The higher concentrations found in tissue samples, compared to seawater samples, can be measured with greater accuracy and would be less sensitive to contamination. Also, tissue samples do not require the expensive and time-consuming preconcentration processes required by seawater samples prior to analysis. More importantly, marine organisms would not only be accumulating the biologically available metal, but would be providing a time-integrated value of the metal concentration. Utilization of a marine organism would appear, therefore, to be an appropriate method for estimating the biologically available metal in marine waters.

Man has used other organisms to protect himself from 'unseen pollutants' since late in the 19th century, when miners used canaries to warn them of a build-up of toxic gas in coal mines. Such organisms may be classified as either indicators or monitors. The demarcation

Figure 1.1


a.
May 31
11.7
Cd in water (µg l⁻¹)
4
3
2
1
9
11
13
15
17
Time (hours)

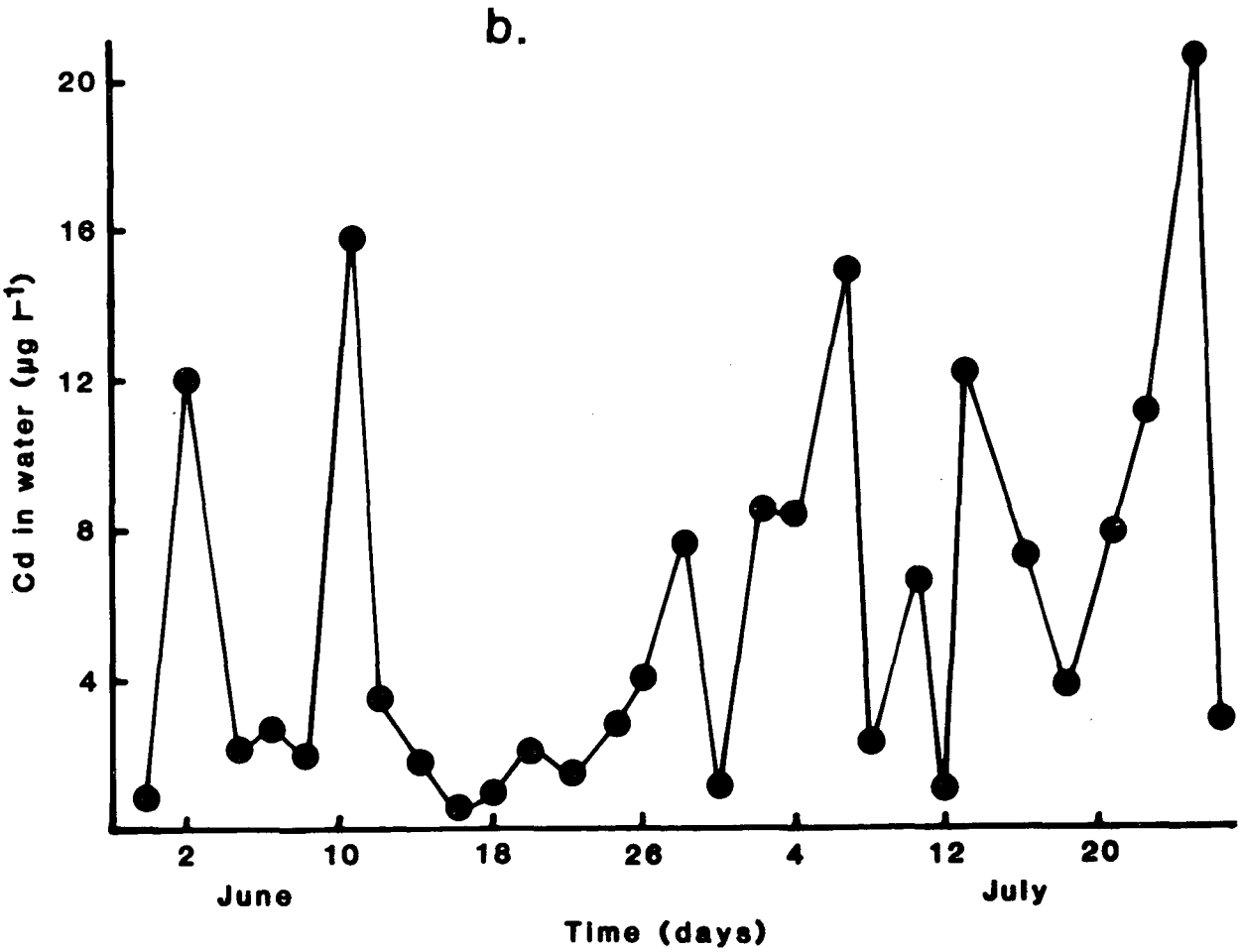

b.
Cd in water (µg l⁻¹)
20
16
12
8
4
2
10
18
26
4
12
20
June
July
Time (days)

TABLE 1.1 Selected values for comparison of metal concentrations reported in marine organisms with levels in seawater from the corresponding localities. Metal concentrations are expressed as: μg g^{-1} dry wt. for tissue, i.e. ppm; and μg ℓ^{-1} for seawater, i.e. ppb. (n.a. - not analysed; n.d. - not detected).

LOCALITY	SAMPLE	METAL CONCENTRATION				REFERENCE
		CADMIUM	COPPER	LEAD	ZINC	
Coastal waters of British Isles	Seawater	<0.01-0.62	0.18-3.75	<0.05-1.2	0.8-20.0	Preston *et al.* 1972
	Macroalgae	0.4-20.8	1.7-28.4	0.5-9.0	42-962	"
Cardigan Bay, Wales	Seawater	n.a.	n.a.	n.a.	40-88	Ireland 1973
	Macroalgae	n.a.	n.a.	n.a.	216-507	"
	Barnacles	n.a.	n.a.	n.a.	4500-23100	"
	Bivalve molluscs	n.a.	n.a.	n.a.	253-779	"
Monterey Bay, California U.S.A.	Seawater	0.02-4.7	0.5-4.5	0.2-2	0.7-35	Knauer & Martin 1973
	Phytoplankton	1-6	2-45	0.5-35	10-700	"
Fjords, Norway	Seawater	<0.01-85	3.75-77	1.6-27.4	13.8-3560	Stenner & Nickless 1974
	Macroalgae	0.7-13	4-118	0.7-1200	35-3550	"
	Bivalve molluscs	1.9-140	3-130	2-3100	105-2370	"
Atlantic Coast of Spain & Portugal	Seawater	0.08-6.0	1.9-107	2.3-38	17-525	Stenner & Nickless 1975
	Macroalgae	0.5-7.4	5.5-31	4-22	59-345	"
	Barnacles	n.a.	6.3-600	n.a.	n.a.	"
	Bivalve molluscs	1.7-3.6	6.5-435	2-15	190-920	"
Derwent Estuary, Australia	Seawater	<0.5-15	n.a.	6-28	6-1500	Bloom & Ayling 1977
	Barnacles	n.a.	n.a.	n.a.	1360-10200	"
	Bivalve molluscs	4.3-174	n.a.	1.4-532	171-38700	"
Fjords, Norway	Seawater	0.5-3.1	0.3-9.4	0.2-1.7	7-610	Eide *et al.* 1979
	Phytoplankton*	<150-2350	200-2700	<150-1400	300-56800	"
Carnon Estuary, U.K.	Seawater	n.d.-7.4	2.1-395.7	n.a.	12.9-22000	Klumpp & Peterson 1979
	Macroalgae	0.1-3.8	60.1-228.3	n.a.	103.7-1321	"
	Bivalve molluscs	0.8-3.2	14.6-2220	n.a.	160.2-7207	"
Corio Bay, Australia	Seawater	0.11-5.6	1.5-25	<0.4-11	3.9-67	Smith *et al.*1981
	Bivalve molluscs	9.8-53	4.8-21	1.2-9.7	105-410	"

* were converted to dry matter using 2×10^{-5} μg $cell^{-1}$ for cell mass (Eide *et al.* 1979)

between the two is not always sharp. In general, an indicator organism is used primarily to identify a pollution problem. Monitoring organisms, by contrast, are used primarily to quantify pollution levels (Butler *et al.* 1971). A variety of marine organisms has been suggested as potentially useful monitoring or indicator organisms of contaminants of the marine environment. They range from phytoplankton (Lenzi Grilli 1976; Eide *et al.* 1979; Jensen 1980); algae (Preston *et al.* 1972; Bryan and Hummerstone 1973a; Haug *et al.* 1974; Melhuus *et al.* 1978; Eide *et al.* 1980) and hydroids (Stebbing 1976; Stebbing and Pomroy 1978), to barnacles (Barbaro *et al.* 1978), gastropods (Navrot *et al.* 1974; Crothers 1978), bivalve molluscs (Goldberg 1975; Phillips 1976a,b, 1977c, 1978, 1979b; Majori *et al.* 1978b; Dunstan *et al.* 1980; Popham *et al.* 1980; Zaroogian 1980; Krieger *et al.* 1981; Phillips and Yim 1981) and fish (Dix *et al.* 1975; Schulz-Baldes and Cheng 1980; Westernhagen *et al.* 1980).

A number of methods for the assessment of the contamination of the marine environment have also been presented. The majority are only useful, however, for indicator surveys, as they tend to be non-specific as to the type of pollutant. Such techniques include: behavioural (Eisler 1979) and physiological effects (Bayne *et al.* 1979; Reichert 1979), population changes (Gray 1979) and skeletal deformities (Bengtsson 1979). Monitoring organisms, in general, possess a special characteristic that is pollutant-specific and easily measurable. This is the formation and concentration of residues of the pollutant(s) in the body. Such accumulation by both body tissue (Myklestad *et al.* 1978; Bryan 1979; Phillips 1979a) and, where present, shells (Romeril 1971a; Ferrell *et al.* 1973; Sturesson 1976, 1978; Carriker *et al.* 1980; Cotugno *et al.* 1980) provides readily measurable levels and has been suggested as the basis of a possible monitoring technique. The measurement of the tissue levels of a pollutant is also useful when considering the hazards to human health. It is this pollutant load that will be passed on if the

organism is consumed.

While the ability of marine organisms to accumulate the biologically available metal in seawater would appear useful in either an indicator or monitoring survey, their use also has its disadvantages. There are a number of extraneous factors that may interfere by affecting accumulation of the metals. These factors may be associated with the organism itself (species, size, weight, sex, reproductive cycle) or with its environment (temperature, salinity). Nonetheless it would appear that the savings in time, effort, cost and sensitivity gained by using a bioaccumulator may outweigh these disadvantages. Effects of some of these extraneous factors may even be overcome in the design of the programme.

In this present study I will be examining the possibility of utilising the accumulating ability of a marine organism to monitor four metals, cadmium, copper, lead and zinc, in open marine waters. These particular metals are selected as they are all deemed 'very toxic and relatively accessible' to biological systems, with copper and zinc biologically essential metals and cadmium and lead non-essential (Förstner and Wittman 1979). Apart from mercury, they are considered the metals of greatest overall hazard in the marine environment (Phillips 1980). They are also of local importance as pollutants (e.g. Bloom and Ayling 1977), have similar properties and occur naturally together.

No attempt is made in this thesis to review the vast amount of literature available on the association between heavy metals and marine organisms. A comprehensive review on the use of indicator organisms in aquatic systems was recently published by Phillips (1980), and the bulk of the literature in this thesis concerns that released subsequent to this review.

1.2 PROPERTIES OF A MONITORING ORGANISM

Before being able to select a suitable monitoring organism it is necessary to establish the attributes it must possess. These will of course depend on the type of information required from the monitoring survey. For example, it was mentioned in Section 1.1 that the total load of the heavy metal(s) in marine waters occurs in a wide variety of chemical forms. Nonetheless, it may be crudely divided into (a) metals in solution, (b) metals in suspension adsorbed to organic or inorganic particulate matter, and (c) metals in colloids or chelates which are difficult to assign to either soluble or particulate forms. Different organisms respond to different proportions of this total load. They may derive their supply of metal(s) by either one, or a combination of the following routes: (i) directly from solution, either via respiration (gills, skin surface) or by adsorption to body surface, (ii) by ingestion of food containing the metal(s), or (iii) by ingestion of particulate matter bearing the heavy metal(s) (Phillips 1977b). Thus when selecting a suitable monitoring organism an initial characteristic required must be:

1. That the kind of metal uptake exhibited by the selected organism is compatible with the form of the metal under investigation. If necessary a number of different organisms must be used in order to present a complete pollution profile.

This present study is concerned with the total metal load. Consequently, an ideal monitor would accumulate metals by all three pathways.

The monitoring organism selected for the present study is required to monitor the metal levels in open marine waters. For such an organism to be representative of the particular monitored locality, a further characteristic must be:

2. The organism must be able to be contained, and function normally, within a cage suspended in marine waters. It would, therefore, preferably be a sessile organism.

The fulfilment of these two characteristics is considered to be an essential feature of a possible monitoring organism for the present study. Further basic attributes were presented by Butler *et al.* (1971), and can be summarised thus:

3. The organism must be able to accumulate the metal(s) without any serious physiological effects from levels anticipated in the marine waters.

4. The organism should be locally abundant, of convenient size and easily collected.

5. There should be sufficient knowledge of the biology of the species to recognize its most sensitive life stage, its position in the trophic web, and its reaction to environmental changes other than those induced by the metal(s). The organism should be sufficiently long-lived so that one or more year classes will persist throughout the contemplated monitoring programme.

6. The species should have a broad distribution both ecologically and geographically.

7. The organism must be hardy and be able to adapt to handling and ~~transfer~~ transferance from one locality to another. (This must include survival under laboratory conditions.)

In addition the following basic requirements were presented by Haug *et al.* (1974):

8. The organism should exhibit a high concentration factor for the particular metal(s) under investigation, allowing direct analysis without a concentration step.

9. A simple correlation should exist between the metal content of the organism and the average metal concentration of the surrounding water.

Phillips (1977b) amended this final basic requirement as follows: That all organisms in a survey exhibit the same correlation between their metal contents and those in the surrounding water, at all locations studied, under all conditions.

This last amendment is highly desirable though it would appear to be too rigorous as a basic pre-requisite. Rather the influence of both environmental and physiological variables on the metal concentrations in the selected organism's tissue should be examined following selection. An investigator would then be able to predict under any circumstances likely to be encountered the response of the monitoring organism to the average metal concentration of the surrounding seawater. These, and other requirements suggested by Phillips (1980) will be discussed further in later chapters.

1.3 PRELIMINARY SELECTION OF A MONITORING ORGANISM

1.3.1 Phytoplankton

Phytoplankton, as mentioned in Section 1.1, have been suggested by a number of authors as possible monitors of heavy metals in marine waters. These organisms have received a considerable amount of attention recently in this context; e.g. Steeman Nielsen and Wium-Andersen (1970); Riley and Roth (1971); Topping (1974); Cossa (1976); Martin and Broenkow (1976); Greig *et al.* (1977); Sorentino (1979). Heavy

metals are reported to not only accumulate in phytoplankton (Table 1.1), but to cause decreases in growth,e.g. cadmium, copper and zinc (Braek *et al.* 1976, 1980). The levels of heavy metals in phytoplankton from polluted waters have been reported to be ten to one hundred times those from unpolluted waters (Knauer and Martin 1973; Skei *et al.* 1976; Eide *et al.* 1979).

Jensen *et al.* (1974, 1976) and Eide *et al.* (1979) have shown that both metal uptake and growth rate of marine phytoplankton can be examined in cage cultured organisms both in the laboratory and *in situ.* Other *in situ* studies have been reported by Sakshaug and Jensen (1978) and Jensen (1980).

Despite sharing some of the attributes listed above, phytoplankton are nonetheless unsuitable as monitoring organisms in the present study. They are at the very beginning of the food chain and so obtain their metal load directly from solution only. Hence they do not satisfy the initial requirement.

1.3.2 Macroalgae

Many reports show that marine macroalgae concentrate metals from the surrounding seawater (Table 1.1). Their tissue concentrations are also reported to reflect the seawater concentrations, which would appear to make them suitable candidates for monitoring purposes; e.g. Black and Mitchell (1952); Mullin and Riley (1956); Gutknecht (1965); Nickless *et al.* (1972); Fuge and James (1973, 1974); Haug *et al.* (1974); Morris and Bale (1975); Foster (1976); Myklestad *et al.* (1978); Phillips (1979a); Rice and La Pointe (1981) and Julshamn (1981e). The majority of this work deals with resident populations. However, Mykelstad and Eide (1978) successfully transplanted a species from one locality to another, with no deleterious effects. Despite apparent suitability, macroalgae, like phytoplankton, are responsive only to the soluble metal content of the surrounding seawater. This renders them unsuitable for the present study, as they do not reflect the total metal load.

1.3.3 Barnacles

The limited data available on the metal content of barnacles suggest that these organisms may be useful for monitoring copper and zinc levels (Clarke 1947; Ireland 1974; Stenner and Nickless 1975; Walker *et al.* 1975; Walker and Foster 1979; White and Walker 1981). As shown in Table 1.1, barnacles may accumulate high concentrations of both these metals. Walker *et al.* (1975) found that the levels of zinc in the soft tissues of *Balanus balanoides, Elminius modestus* and *Lepas anatifera* were closely related to the environmental levels. Furthermore, Barbaro *et al.* (1978) have shown that *Balanus amphitrite* concentrates copper to very high levels, along with lead and mercury. They also report that the accumulation of copper was in proportion to the level of copper in the water. Both laboratory (Pyefinch and Mott 1948) and field (Crisp and Patel 1961) studies have demonstrated the successful settlement of barnacles onto experimental panels. It is therefore feasible to prepare such panels with barnacles of a known age, and place them into suitably designed cages. The barnacle as a filter feeder obtains its metal load not only from solution but by ingestion of organic and inorganic particulates. It therefore reflects the total metal load present in the surrounding seawater. The biology of barnacles is relatively well understood, and they are sessile, hardy and abundant organisms. As they appear to possess the necessary attributes for a monitoring organism they are given further consideration in the present study.

1.3.4 Bivalve Molluscs

These organisms have been considered for some time now to be ideal indicators or monitors of heavy metals in marine waters. They have been successfully used in such areas as: the U.S.A. coastline (Goldberg 1975; Goldberg *et al.* 1978), in German waters (Karbe *et al.*

1978), the Bristol Channel in U.K. (Nickless *et al.* 1972) and Scottish waters (Davies 1976; Davies and Pirie 1977, 1978). Majori and Petronio (1973a, b, c, d) suggested that the mussel may possibly be used as a 'warning signal' of seawater contaminated by heavy metals. Similarly, Blackman *et al.* (1979) used oysters to reflect pollution problems. Other reports on the use of bivalves in monitoring heavy metals include: Crowley and Murphy (1976); Cunningham (1979); Bayne (1978a, b); Davies and Pirie (1980); Goldberg (1980); Gordon *et al.* (1980); Murray and Law (1980) and Julshamn (1981e). One reason for their popularity is their nutritional and economic importance to man. Another is that, as filter feeders they obtain their metal load not only from solution, but also from their food supply and by ingestion of inorganic particulates (Moore 1971). Their accumulation, therefore, reflects the total metal load biologically available. Adults of most bivalves are sessile and locally abundant. They are easily recognized and sampled along most coastlines. The more common bivalves, mussels and oysters, are also readily adaptable to laboratory conditions, and up to the present have received the most attention of all organisms as far as heavy metals are concerned.

The vast amount of work on mussels and oysters includes ample demonstration of the ability of these organisms to concentrate metals in their tissue to levels well in excess of those in the ambient seawater (Table 1.1). Laboratory studies include the examination of the effects of heavy metals on growth and reproduction (Brereton *et al.* 1973; Calabrese *et al.* 1973; Mandelli 1975; Beardmore 1978, 1980; Pesch and Stewart 1980),byssal production (Martin *et al.* 1975) and behaviour of the organism (Davenport 1977; Akberali and Black 1980; Akberali 1981). Tissue concentrations of the metals have been reported to be influenced by seasonal factors (Galtsoff 1964; Pringle *et al.* 1968; Frazier 1975; Zaroogian and Cheer 1976; Majori *et al.* 1978a; Romeril 1979; Orren *et al.* 1980; Behrens and Duedall 1981a; Boalch *et al.* 1981; Julshamn 1981a;

Ouellette 1981), reproductive cycle (Delhaye and Cornet 1975; Cossa *et al.* 1979), sex (Watling and Watling 1976), body size or weight (Romeril 1971b; Schulz-Baldes 1973; Boyden 1974, 1977; Ayling 1975; Mackay *et al.* 1975; Phillips 1976a, b; Harris *et al.* 1979; Simpson 1979; Julshamn 1981b), salinity (Hugget *et al.* 1973; George *et al.* 1978a; Phillips 1976a, 1977a) and depth (Nielsen 1974). Other workers have studied the tissue distribution (Romeril 1971a; Carmichael *et al.* 1979, 1980; Julshamn 1981c), and the storage and detoxification of the metals (Casterline and Yip 1975; Noël-Lambot 1976; George *et al.* 1978b, 1979; Schulz-Baldes 1978; Talbot and Magee 1978; Carpene *et al.* 1979; Lowe and Moore 1979a, b; Ridlington and Fowler 1979; Frankenne *et al.* 1980; Roesijadi 1981; Viarengo *et al.* 1980, 1981; Julshamn and Andersen 1981). Among these studies are some which describe successful transference and cage suspension of both mussels and oysters. Simpson (1979), for example, transferred mussels to new locations by placing clumps of mussels into rigid mesh cages. Young *et al.* (1976) and Harris *et al.* (1979) report the use of mesh bags, suspended from buoys. Other similar reports include: Thornton *et al.* (1975), Davies and Pirie (1978), Eganhouse and Young (1978), Davies (1979), Stephenson *et al.* (1979), Uysal (1978, 1979), Widdows *et al.* (1981), Behrens and Duedall (1981b), and Riisgård and Poulson (1981).

The bivalve molluscs appear to possess the necessary attributes required for a monitoring organism. Therefore bivalves, and mussels and oysters in particular, are given further consideration here.

1.3.5 Other Organisms

Other organisms suggested to be suitable for this type of study include limpets (Navrot *et al.* 1974), abalone (Steward and Schulz-Baldes 1976; Bryan *et al.* 1977) and tunicates (Eustace 1974; Papadopolow and Kanias 1977). Whilst they possess most of the desirable attributes

they were considered to be unsuitable for the present study. Tunicates appeared to be only useful for one of the test metals in this study, i.e. zinc. Limpets and abalone depend on the algae upon which they graze for both nutrition and part of their metal supply. This would provide practical problems in both the design of a suitable cage and in ensuring algae settled on the cage surfaces. Fish were also not considered in this study due to the practical problems of caging them in large numbers. They were also dismissed by Phillips (1977b) who concluded that their use was not yet justified for metals of interest to the present study (the exception for fish is mercury).

1.4 SELECTION OF A MONITORING METHOD

Schulz-Baldes (1974) suggested that the mussel *Mytilus edulis* may profitably be used to assess the degree of lead pollution in the marine environment. However,this was only possible if the mussel had reached equilibrium with the concentration of the metal in its environment; i.e. once the rates of accumulation and depuration of lead were equal. Following extensive laboratory experiments the author demonstrated that the rate of accumulation was linearly related to the external concentration, while the rate of depuration was linearly related to the tissue concentration. Therefore, if the two rates are equal, the tissue concentration is directly related to the external concentration. However, the use of this method appears limited. Firstly, it would be difficult to establish whether the mussel had reached equilibrium with its environment. Secondly, with regard to the present study, mussels suspended in cages may require a considerable amount of time to reach equilibrium. Schulz-Baldes (1974) acknowledged this by describing the 'extreme minimum time' to reach equilibrium as 230 days. Of course as the external metal concentration would be

unknown, so the time required to reach equilibrium would also be unknown. Hence it would be difficult to establish appropriate sampling intervals.

Majori and Petronio (1973e) reported that the accumulation of heavy metals from a constant concentration by *Mytilus galloprovincialis* exhibited a particular relationship with time. There was an initial linear accumulation phase, followed by an accumulation equilibrium (i.e. the accumulation rate decreases with time to zero). Like Schulz-Baldes (1974), the authors observed that the tissue concentration at which accumulation equilibrium occurred was directly related to the metal concentration in the water. For similar reasons to those presented above they expressed reservations about the use of this relationship in assessing the metal levels. Instead they recommended utilizing the initial rate of accumulation of the metal (i.e. the slope of the linear accumulation phase). This rate was observed to be directly related to the external concentration; except under extremely polluted conditions (Majori and Petronio 1973e).

From laboratory experiments, Schulz-Baldes (1974) described the accumulation of lead by mussels as linear, for up to 40 days exposure. Similar relationships were recorded over a wide range of external concentrations. Linear accumulation of heavy metals by mussels has been reported by many authors, e.g. Fowler and Benayoun (1974), George and Coombs (1977), D'Silva and Kureishy (1978), Westernhagen *et al.* (1978), Marletta *et al.* (1979) and Scholz (1980). Keerfoot and Jacobs (1976) and D'Silva and Qasim (1979) also found linear accumulations in oysters, although others have shown a curvilinear accumulation (Shuster and Pringle 1969; Zaroogian *et al.* 1979; Zaroogian 1980). White and Walker (1981) reported that the accumulation of zinc by the barnacle *Balanus balanoides* is linear with time. Pringle *et al.* (1968) were possibly the first to propose that the accumulation rate of a metal is dependent upon the external concentration. This was subsequently confirmed by such authors as Majori and Petronio (1973e), Schulz-Baldes (1974),

George and Coombs (1977), D'Silva and Kureishy (1978), and D'Silva and Qasim (1979). The relationship between rate of accumulation and external concentration will be investigated in later sections of this thesis.

CHAPTER 2

EXPERIMENTAL TECHNIQUES

The experimental techniques described below are relevant to all chapters of this thesis; any specific material or technique pertinent only to one particular experiment is described in the appropriate chapter.

2.1 GENERAL EXPERIMENTAL METHODS

All laboratory experiments were conducted under static conditions, with the test organisms held in aerated 20 litre capacity polyethylene tanks. The seawater was changed every 48 hours, unless a high rate of accumulation of the test metal(s) was expected, in which case the seawater was changed every 24 hours. The four test metals (Cd, Cu, Pb and Zn) were added to the control seawater (Section 2.2) in each experimental tank from either 10^5 or 10^6 $\mu g\ \ell^{-1}$ stock solutions to produce the specified concentrations. Stock solutions were prepared by dissolving the following analytical grade metal salts in glass distilled water:

Cadmium - $CdC\ell_2 \cdot 2\frac{1}{2}H_2O$

Copper - $CuSO_4$,

Lead - $Pb(NO_3)_2$,

Zinc - $ZnSO_4 \cdot 7H_2O$.

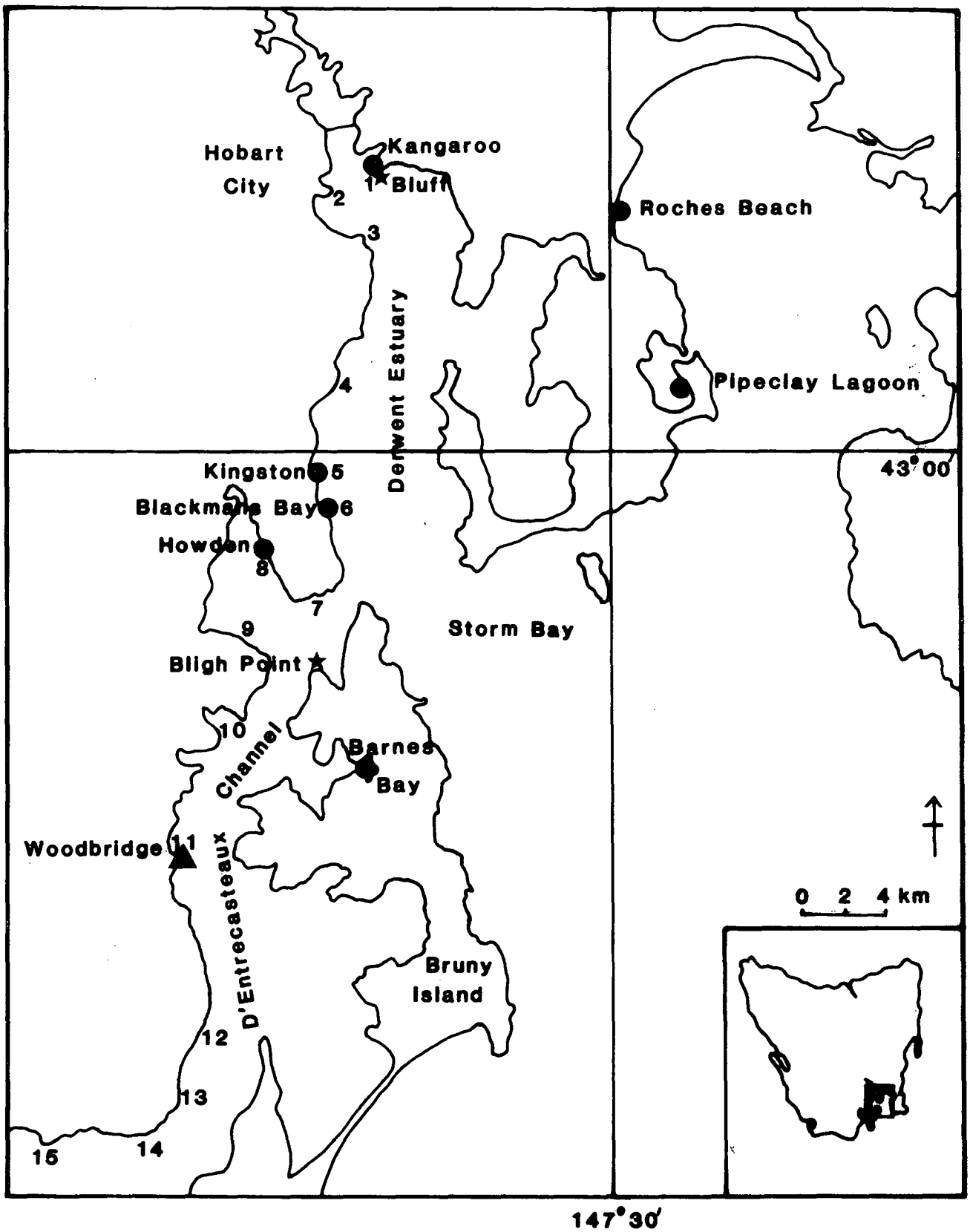
A biomass of not more than 6 g wet weight ℓ^{-1}, as recommended by O.E.C.D. Chemical Testing Programme, was maintained within the experimental tanks. The volume of seawater was adjusted as test organisms were removed from the tank to maintain this relationship approximately. In general, animals were withdrawn for analysis after 0, 4, 10 and 20 days from the start, and then at 10 or 20 day intervals, depending on the experiment. The number of individuals sub-sampled is discussed in Section 2.4. An acclimation period of between 24 and 168 hours, depending on the differential between ambient and

experimental temperature, was allowed prior to the start of an experiment. The number of individuals introduced at the start of an experiment always allowed for a possible loss of 20% due to mortality. The average loss over all experiments was 3% with a maximum loss of 12% for a completed experiment. Mortality usually occurred during the first two days due to damage incurred during collection.

The seawater was unfiltered and no additional food was added to the experimental tanks. No significant change in body weight of test organisms was recorded during the experiments (i.e. body condition was maintained). A group of organisms, exposed only to the background concentrations (Section 2.2), was set up in conjunction with each experiment and served as a control. The controls were sub-sampled at the same time as the experimental group. Both temperature and salinity of the seawater in the experimental tanks were measured daily. The salinity, measured using an American Optical Refractometer, ranged from 29‰ to 34‰, with an average value of 32‰. Unless otherwise stated, all experimental tanks were located in a constant temperature room and the seawater temperature ranged from 10°C to 14°C with an average of 11°C.

All test organisms were collected by hand from populations that were either continuously immersed or as close to the low tide mark as practically possible. The sites for the collection of all organisms are detailed in the description of each experiment, and are shown in Figure 2.1. Populations of barnacles were located on large rocks which were brought back to the laboratory and placed into the experimental tanks in their entirety. Only barnacles of shell diameter between 0.5 and 1.0 cm were used in experiments. The bivalves, collected individually, were categorised according to their maximum shell length. Individuals in an experiment were generally kept within a 1 cm shell length range. Before being introduced into an experimental tank, the outer surface of each individual bivalve was scrubbed with a wire brush to remove all algae and other encrustations. All byssal threads

Figure 2.1


Hobart
City
Kangaroo
Bluff
Roches Beach
Derwent Estuary
Pipeclay Lagoon
Kingston
Blackmans Bay
Howden
43°00'
Storm Bay
Bligh Point
Channel
Barnes
Bay
Woodbridge
D'Entrecasteaux
Bruny
Island
0 2 4 km
147°30'
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15

protruding through the mussel valves were removed. The rocks bearing barnacles were thoroughly washed to remove any algae and empty barnacle shells.

All experimental equipment and analytical glassware was soaked in 10% HNO_3 for at least 48 hours before use.

2.2 SEAWATER - COLLECTION AND ANALYSIS

To conduct laboratory experiments on various aspects of metal contamination, a supply of relatively pristine seawater was required. Bloom and Ayling (1977) reported that the Derwent Estuary was very seriously contaminated with heavy metals, especially mercury, cadmium, lead and zinc; some of the levels recorded were the highest reported in a marine environment. Subsequent reports by the Tasmanian Department of Environment (Director of Environmental Control 1978, 1979, 1980 and 1981) show that this problem still exists, particularly for areas close to the site of the University of Tasmania. Consequently, it was necessary to locate a suitable site some distance away, with ease of access and relatively stable low concentrations of heavy metals, from which a regular supply of seawater could be obtained.

A survey was conducted to examine the concentrations of the test metals in seawater from various locations along the shores of the Derwent Estuary and D'Entrecasteaux Channel. The sites sampled are shown in Figure 2.1, and the metal concentrations (Cd, Cu, Pb and Zn) recorded at each site are presented in Table 2.1; each value is the average of two samples. The site chosen for regular water sampling was Woodbridge, situated approximately 40 km from the University (Figure 2.1). Seawater from Woodbridge was collected every 5-10 days and was transported and stored in three 200 litre polyethylene-lined containers. This seawater is referred to hereafter as *control seawater*, and the metal concentrations are referred to as background. The water was pumped

TABLE 2.1 Metal concentrations ($\mu g\ \ell^{-1}$) in seawater samples taken from sites along the Derwent Estuary and D'Entrecasteaux Channel, 16th May 1979. Location of sites shown in Figure 2.1.

Site		Cadmium	Copper	Lead	Zinc
1.	Kangaroo Bluff	1	1	5	180
2.	Battery Point	3	2	7	175
3.	Sandy Bay	0.5	1	5	103
4.	Taroona	0.7	<1	<5	78
5.	Kingston	<0.5	<1	<5	83
6.	Blackmans Bay	<0.5	1	<5	95
7.	Tinderbox	ND	<1	<5	53
8.	Howden	ND	<1	<5	20
9.	Coningham	ND	<1	<5	38
10.	Oyster Bay	ND	<1	<5	10
11.	Woodbridge	ND	<1	<5	25
12.	Middleton	ND	<1	<5	45
13.	Gordon	ND	<1	<5	28
14.	Pensioners Bay	ND	1	<5	28
15.	Ninepin Pt.	ND	<1	<5	25

Analyses courtesy Tasmanian Dept. Environment Laboratories, Chemistry Dept.,University of Tasmania. ND - Not detected.

into the containers using a plastic lined centrifugal pump with aluminium hose fittings; no other contact with metal occurred. Samples for analysis were taken at each collection of seawater from Woodbridge and periodic samples were taken from the control stock in the laboratory. No evidence of any contamination during collection and storage was recorded. Metal levels in control and Woodbridge seawater varied at the most by *ca*. 18%. The background concentrations throughout the study were (mean in $\mu g\ \ell^{-1} \pm 95\%$ C.L.): cadmium - <0.50, copper - 2.01±0.81, lead <1.00 and zinc - 11.15±3.21.

The seawater samples for analysis were collected, and stored, in high density polyethylene bottles (1.2 litres), which were sterilised with 10% HNO_3 for at least 7 days before use (Batley and Gardner 1977). The samples were reduced to a pH <2 immediately on collection by the addition of 10 ml HNO_3 per litre. The bottles were then stored at 5 °C. Metal analysis by atomic absorption spectroscopy followed extraction of the metals from solution by a modified version of the chelation-extraction system described by Nix and Goodwin (1970). [Analysis was by courtesy Tasmanian Government Analyst Laboratory.] Samples were regularly taken from tanks during experiments. The variation between specified value and recorded value during this study was (average % ±95% C.L.): cadmium - 19.50±8.46, copper - 27.93±8.71, lead - 15.01±9.65, zinc - 14.66±5.92. The metal levels cited in each experiment refer to the concentration of metal added to background concentration. In other words, 100 $\mu g\ Zn\ \ell^{-1}$ implies that 100 µg zinc was added per litre of seawater in the tank. A number of authors, e.g. Robertson (1968), Gardiner (1974), Hennig and Greenwood (1981) and Massee *et al*. (1981), have reported metal loss from solution onto surfaces of solid objects, e.g. walls of containers. Thus a variation in the concentration of metal available to the test organism may occur. However, following the close approximation of recorded to specified concentrations and the fact that water was changed regularly, such losses were considered negligible in

this study.

In an effort to obtain metal levels lower than those in the control seawater, an artificial seawater was prepared. The seasalt used was 'Natura' (Waterlife Research Industries Ltd., Middlesex, England) and the trace elements provided separately to the salt were not added to the medium. Seasalt was dissolved in glass distilled water that had been aerated for at least 24 hours. The artificial seawater was then aerated for at least a further 24 hours prior to use. Sufficient seasalt was dissolved to obtain a specific gravity at 11°C water temperature of 1.022 - 1.024; this was consistent with levels recorded for control seawater. The background concentrations of 'Natura' were, however, not greatly different to those of the control seawater: (mean in $\mu g\ \ell^{-1} \pm 95\%$ C.L.): cadmium - <0.50, copper - 6.00±0.82, lead - <1.00, zinc - 9.25±4.10. The copper levels in 'Natura' were much higher than in the control seawater possibly because of contamination of the distilled water.

2.3 TISSUE SAMPLES - ANALYTICAL PROCEDURE

All bivalves required for analysis were opened using sterile, stainless steel, surgical blades to cut the adductor muscle. The tissues were rinsed with glass distilled water and allowed to drain for one minute before removal from the valves. Unless otherwise stated, the tissue refers to the whole body tissue of the bivalve. The byssus was removed from the mussels. Where barnacles were analysed whole animals (shell and tissue) were removed from the rock substrate, by means of a surgical blade, and then rinsed with glass distilled water. The wet weight of each sample was measured prior to drying at approximately 105°C for 16 hours. The dried tissue was weighed and then crushed to a fine powder. All dried tissue was stored in glass vials in desiccators.

Digestion of the dried tissue followed a standard nitric acid/perchloric acid digestion (e.g. Harris *et al.* 1979). Between 0.25 and 0.60 g of tissue was accurately weighed into a digestion flask and allowed to dissolve in 4 ml HNO_3 (AnalaR®) for at least 1 hour prior to heating (this prevented foaming which often occurred during the initial heating of HNO_3). The mixture was heated on a hot plate until near dryness (the flask was never allowed to boil dry), and the flask was then removed and allowed to cool before the addition of 2 ml $HClO_4$ (70%, Merck). The flask was again heated until a clear solution was obtained, which was then diluted to 10 ml with glass distilled water.

The specifications of the Atomic Absorption Spectrophotometer (AAS), employed for the analysis of cadmium, copper, lead and zinc levels in tissue samples, are shown in Table 2.2.

TABLE 2.2 Specifications of Atomic Absorption Spectrophotometer Type: Varian Techtron, model AA-5.

Metal	Wave-length Å	Slit width µm	Current mA	Sensitivity µg ml^{-1}	Conc. to give 0.25 absorbance µg ml^{-1}
Cadmium	2288.0	200	3	0.02	1.0
Copper	3247.5	100	3	0.04	2.0
Lead	2170.0	300	6	0.16	8.0
Zinc	2138.6	100	6	0.012	0.6

A Varian A-25 chart recorder was used to record the readings from the AAS. Calibration curves for each metal were prepared from aqueous standards at the commencement of each group of analyses. In order to reduce the effects of instrumental drift during an analysis sequence, calibration checks were made after every 10 samples.

Three digests per tissue sample were prepared, and two readings taken of each digest. Average variation between digests of a sample

during the study was (expressed as coefficient of variation = SD/mean%): cadmium - 5.45%, copper - 8.63%, lead - 9.35%, zinc - 9.14%. These values represent the coefficients of variation within homogenates. The magnitude of the coefficients between homogenates is discussed in Section 2.4 (Table 2.4).

Three reagent blanks were processed with each group of 50 - 60 digests, and a standard reference material was included, in triplicate, with each analysis group. Two reference materials were used, initially - NBS 1577, Bovine Liver and subsequently - NBS 1566, Oyster Tissue (U.S. National Bureau of Standards). The results obtained are comparable to the certified NBS values (Table 2.3). To evaluate the recovery efficiency following digestion, spiked samples were included in the analyses. Values between 97% and 101% recovery were consistently obtained for cadmium and lead, while copper and zinc were more variable. Copper values were between 89% and 108%, while zinc values were between 84% and 98% recovery.

2.4 SAMPLE SIZE AND HOMOGENIZATION

2.4.1 Inherent Variability and Sample Size

The results of numerous studies show that there is a considerable amount of variation in metal concentrations of the tissue between individual bivalves; even within small age, size or weight classes (e.g. Windom and Smith 1972; Karbe *et al.* 1977, 1978; Greig 1979; Harris *et al.* 1979; Phillips 1979b; Coleman 1980; and Tomlinson *et al.* 1980). As Boalch *et al.* (1981) commented from their results, the 'normal' values for the trace metal content of individual *Mytilus edulis* cover a wide range, approaching three orders of magnitude in some cases. They further stated that a mean value derived from 20 individuals may still have a standard deviation of *ca.* ±50% of the mean. Due to this

TABLE 2.3 Comparison of measured metal concentrations in Standard Reference Materials to those certified. Mean and 95% confidence limits provided. (Conc. - $\mu g\ g^{-1}$ dry wt.)

(a) Standard Reference Material 1577, Bovine Liver (U.S. National Bureau of Standards).

Metal	This Study	Certified Value
Cadmium	0.26 ± 0.04	0.27 ± 0.04
Copper	198.78 ± 3.64	193 ± 10
Lead	0.40*	0.34 ± 0.08
Zinc	131.83 ± 10.38	130 ± 10

(b) Standard Reference Material 1566, Oyster Tissue (U.S. National Bureau of Standards).

Metal	This Study	Certified Value
Cadmium	3.32 ± 0.06	3.5 ± 0.4
Copper	66.39 ± 2.34	63.0 ± 3.5
Lead	0.43 ± 0.01*	0.48 ± 0.04
Zinc	841.28 ± 7.05	852 ± 14

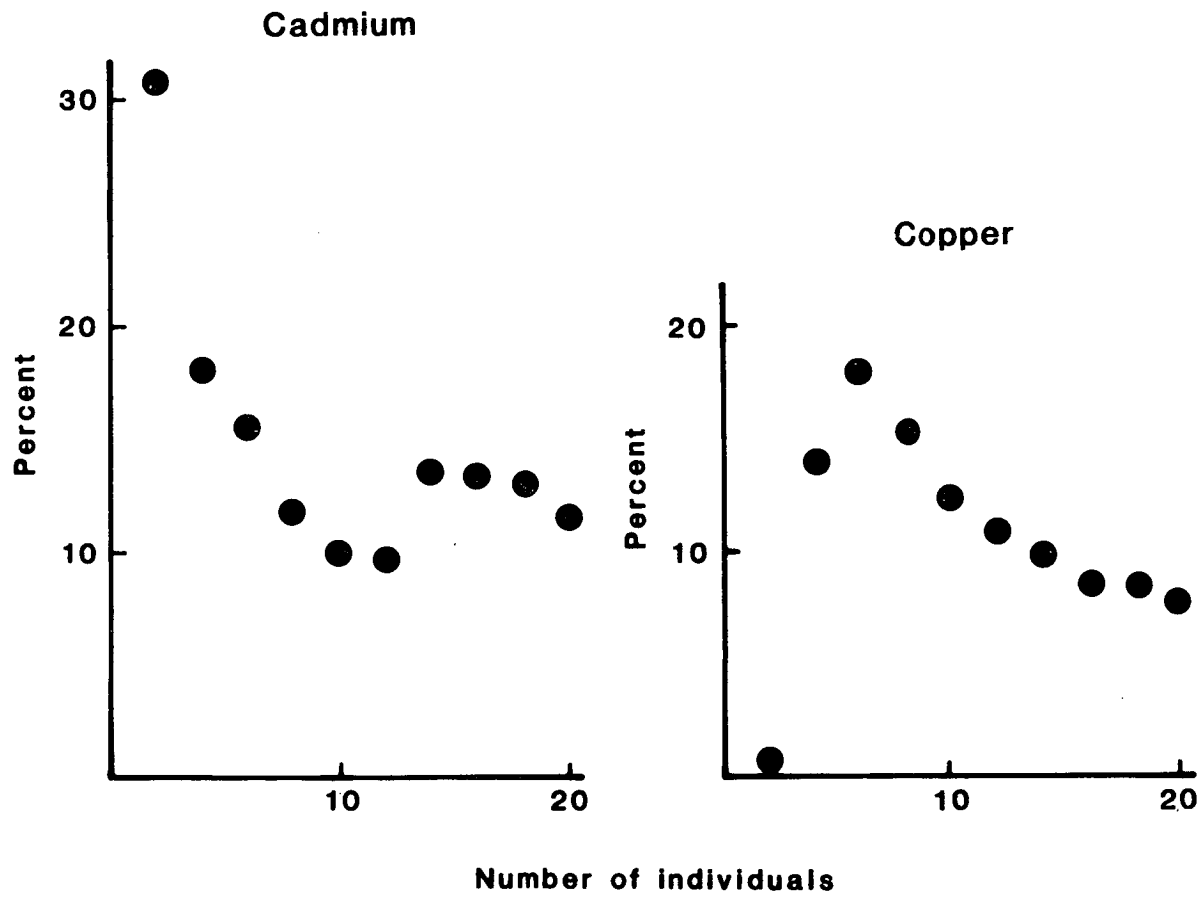
* Lead was detected in less than 30% of the digests, the level being below the detection range of the AAS described above.

individual variation it is necessary in any form of monitoring survey to determine the minimum number of individuals required to accurately represent the population or particular size class.

As already mentioned (Section 2.3) only barnacles with shell diameter between 0.5 and 1.0 cm were employed in the experiments. In order to obtain a sufficient dry weight for 3 digests, between 100 and 120 individuals were pooled per sample. Likewise for the bivalve *Brachidontes rostratus* 30 individuals (1.5 - 3.0 cm shell length) were required to provide sufficient dry tissue. In both cases the individuals were homogenised and the number used was considered sufficient to represent the population. Due to the difficulty of obtaining sufficient numbers of oysters it was possible to use only 5 individuals per sample; these too were homogenised.

For the mussel, *Mytilus edulis planulatus*, 10 individuals were used per sample. This figure was arrived at following the analysis for all four test metals of the tissue of 20 individual mussels (4.0 - 5.0 cm shell length, from Barnes Bay, Figure 2.1). The mussels were randomly grouped into sample sizes of 2, 4, 6-------20 individuals. The standard error of the mean metal concentration in each group was observed to decrease, as expected, with increasing numbers in the sample (Figure 2.2). It was apparent from Figure 2.2 that for all four metals an increase in sample size from 10 to 20 did not substantially reduce the variation of the mean concentration. With this in mind and in view of the logistics involved in collection, tank space, seawater required, analytical time and labour, a homogenate size of 10 individuals was adopted. The wisdom of this choice was verified by a further experiment: 50 mussels (3.0 - 4.0 cm shell length, from Howden, Figure 2.1) were individually analysed for each of the four test metals. The results, presented in Table 2.4, were randomly divided into 5 groups of 10, and analysed by a one-way ANOVA and Bartlett's Test for homogeneity. For each metal, the mean concentrations were similar and the variances

Figure 2.2


Cadmium
Copper
Percent
30
20
10
10
20
Number of individuals

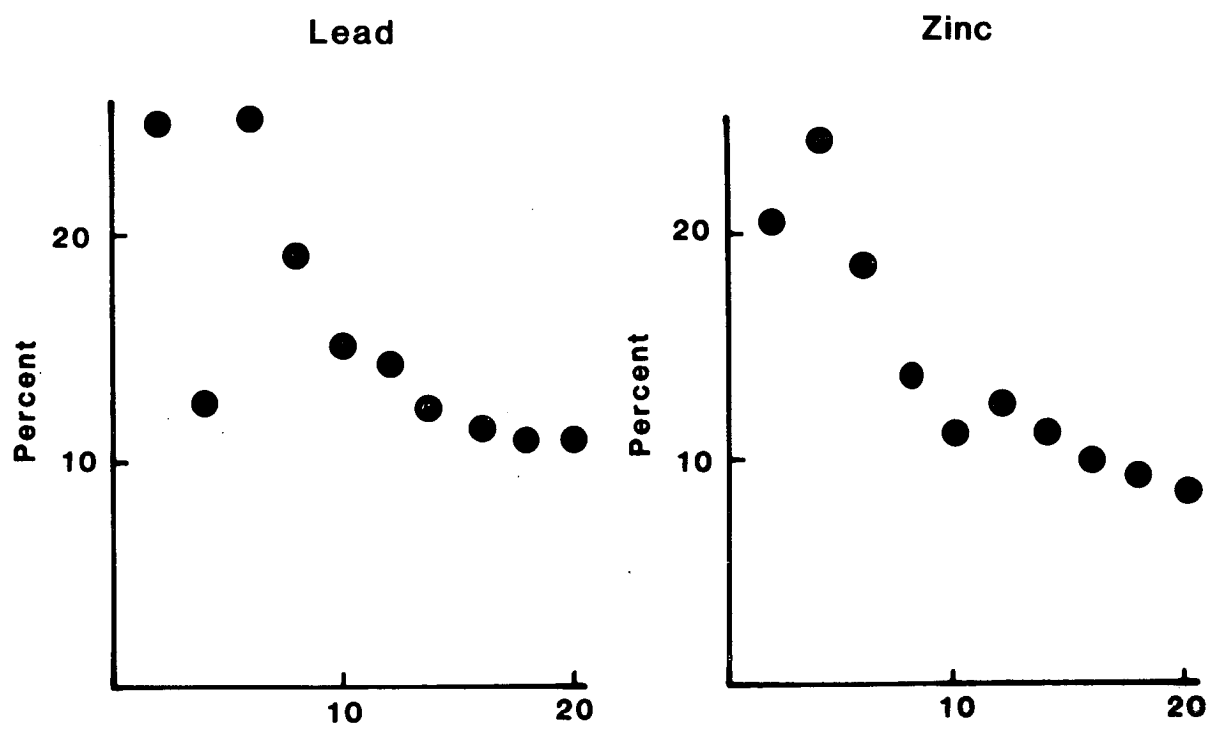

Lead
Zinc
Percent
20
10
10
20
Number of individuals

TABLE 2.4 Metal concentrations (μg g^{-1} dry wt.) in the tissue of 50 individual and five homogenates of 10 *M. e. planulatus*. Each homogenate value is the average of three digests. The results of Bartletts Test for homogeneity of variance and the F-value of the one-way ANOVA performed on the five random groups of 10 individuals are shown, along with 'h' - the adjusted individual ratio (see text for explanation). (nd: not detected, recorded as 0.01 in analyses.)

(a) Cadmium

Individuals	3.35	nd	3.33	2.76	4.40
	3.99	2.07	4.29	2.52	3.31
	nd	4.70	1.60	2.10	6.35
	3.09	2.18	3.85	3.56	1.35
	0.93	3.33	3.48	3.08	2.60
	2.97	3.61	2.75	2.69	1.83
	nd	3.53	5.69	nd	3.79
	1.32	2.72	nd	4.99	2.28
	1.55	nd	0.84	nd	3.17
	3.73	1.36	nd	2.82	3.35
$\bar{x}$	2.10	2.35	2.59	2.45	3.24
S^2	2.28	2.40	3.65	2.27	2.03
S	1.51	1.55	1.91	1.51	1.42
95% CL	±0.94	±0.96	±1.00	±0.93	±0.88
'h'	2.12	2.30	2.60	2.47	3.13

Coefficient of variation = 15.08%

Bartletts Test	χ^2 = 0.95	df 4	p>>0.05
F-statistic	0.73	df 4/45	p>0.05

Homogenate averages: 2.58, 3.37, 2.17, 3.00, 2.47
Coefficient of variation between homogenates = 17.28%

(b) Copper

Individuals	1.91	17.50	4.26	nd	9.20
	26.34	nd	1.70	4.00	nd
	nd	12.02	10.38	11.78	10.77
	nd	5.12	10.75	2.38	7.94
	2.16	21.70	4.57	10.27	4.32
	5.52	3.30	18.33	1.79	5.39
	4.98	1.41	4.56	12.40	10.20
	17.12	24.46	9.70	1.16	8.30
	16.42	12.85	nd	22.32	14.02
	19.06	3.99	11.55	15.30	12.40
$\bar{x}$	9.35	10.24	7.58	8.14	8.26
S^2	89.84	76.92	30.53	54.86	17.02
S	9.48	8.77	5.53	7.41	4.13
95% CL	±5.87	±5.44	±3.42	±4.59	±2.56
'h'	9.21	9.77	7.46	7.89	8.79

Coefficient of variation = 11.02%

Bartletts Test	χ^2 = 7.14	df 4	p>0.05
F-statistic	0.21	df 4/45	p>0.05

Homogenate averages: 6.81, 4.52, 6.86, 8.70, 7.78
Coefficient of variation between homogenates = 22.51%

TABLE 2.4 (continued)

(c) Lead

Individuals	nd	7.37	16.31	nd	nd
	6.34	nd	15.45	18.10	nd
	3.67	nd	nd	0.84	17.79
	11.49	9.20	5.37	9.00	13.20
	nd	ND	nd	nd	18.00
	nd	15.23	7.33	nd	15.10
	6.33	nd	14.50	nd	ND
	17.60	12.68	8.72	nd	3.68
	nd	8.03	13.67	nd	1.48
	6.93	11.16	11.55	nd	nd
$\bar{x}$	5.24	6.37	9.29	2.80	6.93
S^2	34.33	34.98	36.71	36.76	64.26
S	5.86	5.91	6.06	6.06	8.02
95% CL	±3.63	±3.67	±3.76	±3.76	±4.97
'h'	5.63	6.14	9.62	3.25	6.53

Coefficient of variation = 36.60%

Bartletts Test χ^2 = 1.35 df 4 p>0.05
F-statistic 1.36 df 4/45 p>0.05

Homogenate averages: 7.70, 4.22, 6.46, 6.81, 8.00
Coefficient of variation between homogenates = 22.43%

(d) Zinc

Individuals	291.59	390.79	280.19	327.19	326.18
	276.14	198.46	242.92	275.94	221.49
	396.60	286.34	353.78	269.36	364.61
	256.28	259.93	226.28	364.61	206.35
	253.78	248.06	436.42	325.46	316.61
	269.31	322.15	314.39	320.50	350.59
	300.50	193.86	324.22	161.49	186.23
	276.49	235.96	386.04	283.39	198.18
	302.92	331.06	250.42	143.66	260.52
	318.89	312.60	205.05	160.62	301.17
$\bar{x}$	294.25	277.92	301.97	263.22	273.19
S^2	1727.65	3905.47	5579.92	6367.06	4479.34
S	41.57	62.49	74.70	79.79	66.93
95% CL	±25.76	±38.73	±46.30	±49.46	±41.48
'h'	290.87	273.96	298.67	262.56	266.12

Coefficient of variation = 5.64%

Bartletts Test χ^2 = 3.81 df 4 p>0.05
F-statistics 0.56 df 4/45 p>0.05

Homogenate averages: 259.40, 342.45, 303.88, 262.15, 283.59
Coefficient of variation between homogenates = 11.8%

homogeneous (Table 2.4). It seemed reasonable, therefore, that the random selection of 10 individual mussels would provide a good representation of the population within a particular size class.

Three recent publications on the problems of inherent variability in bivalve molluscs all stress the point that this variation may be species, metal and population dependent (Martin 1979a; Gordon *et al.* 1980; Boyden and Phillips 1981). Thus guidelines set by one survey may not necessarily be valid for another. These authors suggest the use of between 10 and 30 individuals. In their paper, Boyden and Phillips (1981) provided a 'simple statistical method' for calculating the minimum number of individuals required per sample to accurately represent the population. For final confirmation the data for the 50 mussels analysed individually were also analysed by their method.

The method involves the random grouping of the individuals into sample sizes of 5, 10, 15, 20, 30, and 40 individuals. This was done five times per sample size. From these groups, five sets of coefficients of variation (calculated as SD/MEAN %) were obtained for each sample size. The variability of the data for each sample size was then established by calculating the coefficient of variation among the group coefficients. This variability is shown in Table 2.5 for each metal.

TABLE 2.5 Variability in coefficients of variation (%) per sample size for each metal concentration in tissue (μg g^{-1} dry wt.) of *M. e. planulatus*. (See text for explanation of statistical method).

	Sample Size					
Metal	5	10	15	20	30	40
Cadmium	43.90	18.56	9.64	9.10	17.58	7.37
Copper	52.75	22.18	17.10	10.11	7.80	5.01
Lead	43.89	22.59	28.59	29.81	16.77	9.59
Zinc	28.63	25.23	20.07	15.75	22.35	5.90

The results show that the largest decline in variability occurred between sample sizes of 5 and 10 individuals, and that using samples larger than 10 does not lead to substantial or consistently lower variability until samples of 40 are used. Samples of this size were impractically large.

2.4.2 Homogenization

To compromise between the large inherent variability in metal concentrations of the oyster *Crassostrea commercialis*, and the limitations of time and analytical effort, Mackay *et al.* (1975) regarded the analyses of homogenates as preferable to analyses of large numbers of individual oysters. They suggested that homogenization improved the precision of the results by damping the individual variations. These authors also pointed out that the mean metal concentration in a group of individually analysed organisms is given by:

$$\bar{x} = \sum_{i=1}^{n} \left(\frac{M_i}{W_i} \right) \Big/ n$$

where M_i = weight of metal in ith organism, and W_i = weight of ith organism. With an homogenate, however, a numerically different parameter (h) is measured:

$$h = \frac{\sum_{i=1}^{n} M_i}{\sum_{i=1}^{n} W_i}$$

To compare the use of 10 individual *M. e. planulatus* to an homogenate of 10, the mean values for the 5 groups of 10 individual mussels in Table 2.4 were recalculated using the equation above to give 'h' - the 'adjusted individual ratio' (Mackay *et al.* 1975) (Table 2.4). An additional 50 mussels of the same size collected at the same time were randomly divided into 5 equal groups and analysed as homogenates.

Their tissue concentrations are presented in Table 2.4 (the values are the average of 3 digests). These five homogenate values were then compared to the five recalculated 'h' values, for each metal, using unpaired t-tests. The two sets of values were similar for each metal ($p>0.05$).

Once the minimum number of individuals per sample has been determined, the decision on whether they should be analysed as individuals or as an homogenate depends on the requirements of the particular study. Limitations of manpower, time and effort as well as the level of precision required have to be considered. In the present study homogenization was selected.

2.5 STATISTICAL ANALYSES

Any statistical procedure employed in this study is described in the relevant section. However, in general, the results of accumulation experiments were analysed by family regression to establish the accumulation pattern. The slopes (coefficients) of linear regressions, representing the rate of accumulation, were compared by t-test. All statistical analyses were considered significant once the 5% level was attained. The use of 'significant' in the text implies a $p<0.05$ chance of the result being arrived at fortuitously. Symbols employed for significance in the figures and text are: ns - not significant, * - significant at 5% level, ** - 1% level and *** -0.1% level.

CHAPTER 3

SELECTION OF MONITORING ORGANISM AND METHOD

3.1 GENERAL INTRODUCTION

It was concluded following the discussion in Chapter 1 that bivalve molluscs and barnacles were the most suitable marine organisms for the present study. They were selected firstly, because they were well suited to cage suspension in open marine waters. Secondly, they obtain their metal loads not only from solution but also by ingestion of organic and inorganic particulates. Consequently, they are more likely to represent the total metal load that is biologically available.

The local species selected for further investigation were:

the Beaked mussel, *Brachidontes rostratus* (Dunker,1856);

the Commercial or Pacific oyster, *Crassostrea gigas* (Thunberg, 1793);

the barnacle, *Elminius modestus* Darwin, 1854;

the Common or Blue mussel, *Mytilus edulis planulatus* (Lamarck, 1819).

Goldberg *et al.* (1978) concluded from their results of the first year of the Mussel Watch programme in U.S.A. that oysters were more effective bioaccumulators of silver, zinc, copper and nickel than mussels, which were more effective for lead. Mussels also appear to be unsuitable for monitoring copper following the reports by Phillips (1976a), who described copper uptake by *Mytilus edulis* as erratic, and Davenport (1977), who described valve closure in the presence of copper for the same species. What little information there is on metal accumulation in barnacles is mainly concerned with copper and zinc (e.g. Walker 1975; Walker *et al.* 1975). In the light of all available evidence it seems reasonable to suspect that no one organism will be suitable for monitoring all metals.

As discussed in Chapter 1, numerous authors have reported the accumulation of metals by marine bivalves to exhibit a linear relationship with time (e.g. Schulz-Baldes (1974) for lead in *M. edulis*, and D'Silva and Qasim (1979) for copper in *Crassostrea cucullata*). The only similar work reported for barnacles is in agreement with the above authors (White and Walker 1981). Likewise, a number of authors, such as D'Silva and Kureishy (1978), have shown that the rate of accumulation of a metal (i.e. the slope of the linear relationship with time) is dependent on the external concentration. In the light of these observations and the suggestion by Majori and Petronio (1973e) it was concluded that, if an organism accumulates a metal linearly with time, then the rate of accumulation would be a simple means of assessing the external concentration.

In this chapter I have compared the ability of each of the above organisms to accumulate each of the four test metals, cadmium, copper, lead and zinc. Selection of a monitoring organism was followed by extensive laboratory experiments to provide preliminary data for a field trial. Finally the monitoring strategy was tested in the field using a cage suspended system.

3.2 COMPARISON OF ORGANISMS

3.2.1 Experimental Design

The 300 *M. e. planulatus* used in the following experiments were 4.0 - 5.0 cm shell length and collected from Barnes Bay (Figure 2.1). The 900 individuals of *B. rostratus* were 1.5 - 3.0 cm shell length and collected from Roches Beach (Figure 2.1). *C. gigas* was obtained from a commercial oyster farm at Pipeclay Lagoon (Figure 2.1); all 120 individuals used in the following experiments were 21 months old with a shell length of 7.0 - 8.0 cm. The barnacles were collected on rocks

from Blackmans Bay (Figure 2.1) and were of shell diameter 0.5 - 1.0 cm.

Samples of the four ~~organisms~~ species were divided into four equal groups and exposed for 40 days, at 11°C, to one of the following:

cadmium at 10 μg ℓ^{-1},

copper at 10 μg ℓ^{-1},

lead at 50 μg ℓ^{-1},

zinc at 100 μg ℓ^{-1}.

These metal concentrations are approximately 10 times higher than the concentrations recorded at the respective collection sites. The general experimental techniques and number of individuals sub-sampled for analysis are described in Chapter 2. Samples were periodically removed for tissue analysis during the 40 days exposure. The exposure time of 40 days was selected at random. However, it was considered to be long enough for a measurable accumulation to occur, and yet short enough that any possibility of a physiological change in the organism affecting metal accumulation might be minimized. A linear regression was fitted to the results of each experiment.

3.2.2 Results and Discussion

The accumulation of the four metals by each organism is shown in Figures 3.1 to 3.4. Only two relationships were observed to be non-linear. They were the accumulation of zinc by *E. modestus* (Figure 3.3d) and *B. rostratus* (Figure 3.4d). A comparison of the accumulating ability of the four organisms based on the results shown in Figures 3.1 to 3.4 is presented in Table 3.1. In the body of the table are given the initial tissue concentrations (i.e. Y-intercept of regressions) and the increase in the tissue concentration during the 40 days (i.e. 40 x regression coefficient). Also represented are the percentage increases

Figure 3.1

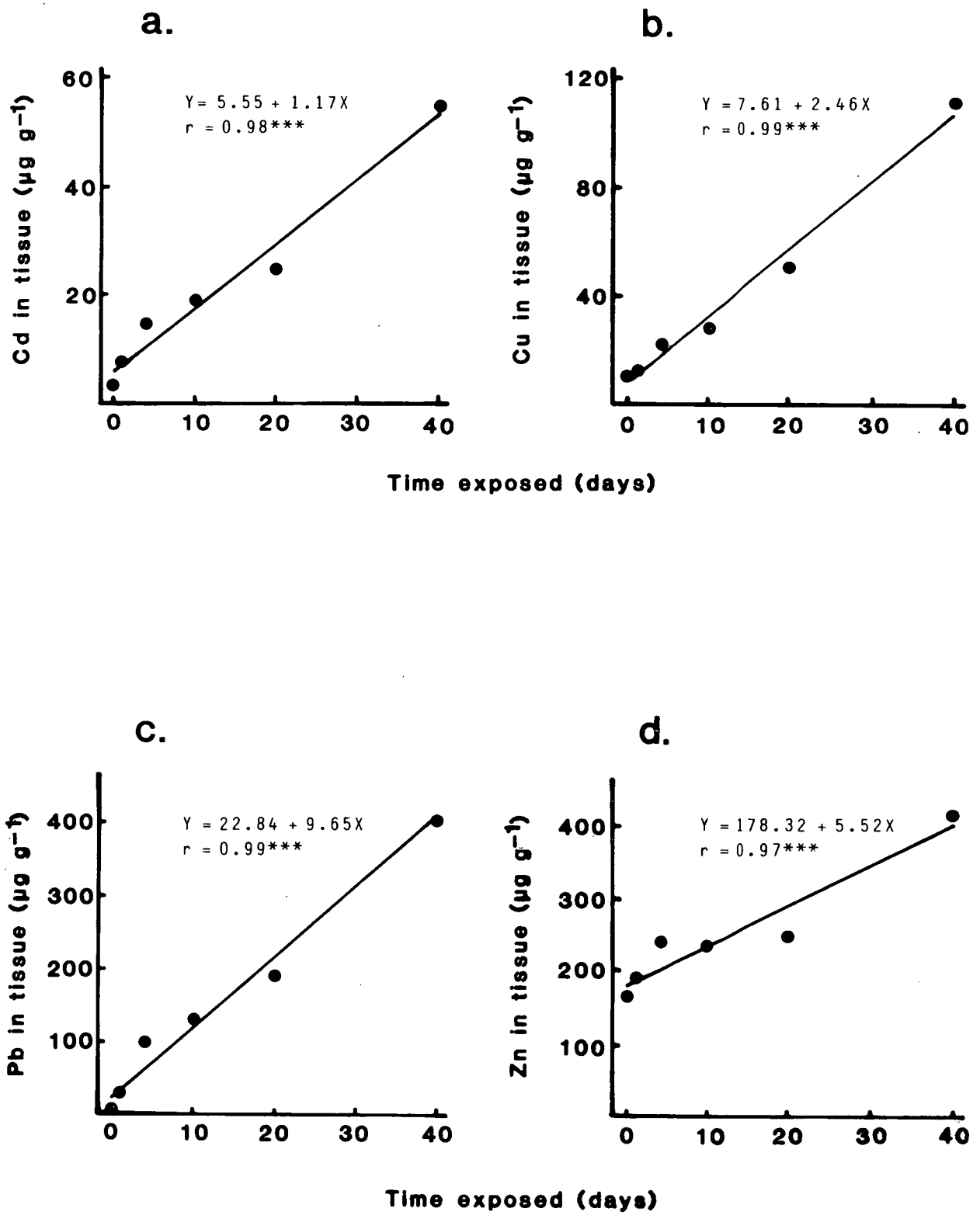

a.
b.
c.
d.
Y= 5.55 + 1.17X
r = 0.98***
Y = 7.61 + 2.46X
r = 0.99***
Y = 22.84 + 9.65X
r = 0.99***
Y = 178.32 + 5.52X
r = 0.97***
Cd in tissue (µg g⁻¹)
Cu in tissue (µg g⁻¹)
Pb in tissue (µg g⁻¹)
Zn in tissue (µg g⁻¹)
Time exposed (days)
Time exposed (days)

Figure 3.2

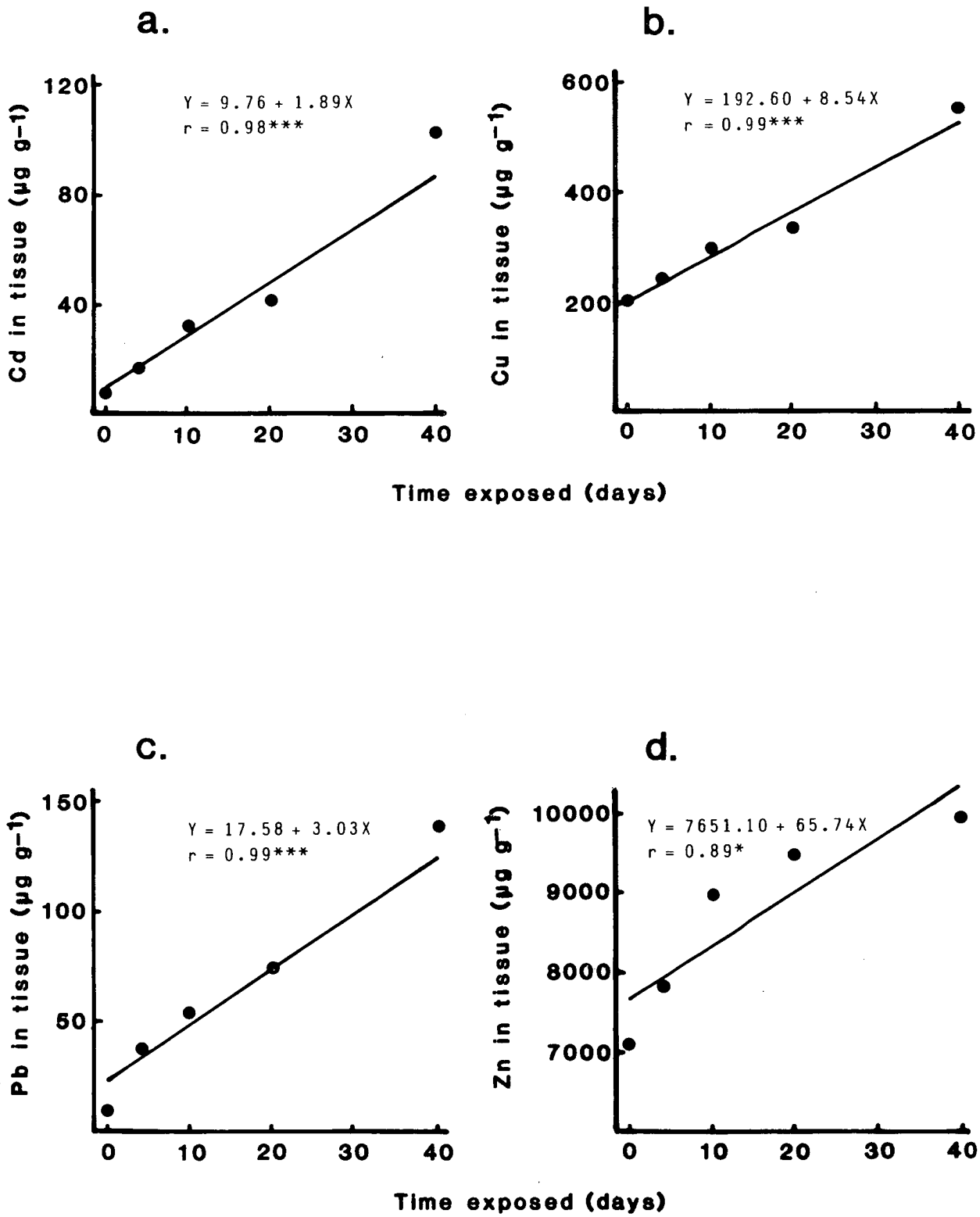

a.
b.
c.
d.
Y = 9.76 + 1.89X
r = 0.98***
Y = 192.60 + 8.54X
r = 0.99***
Y = 17.58 + 3.03X
r = 0.99***
Y = 7651.10 + 65.74X
r = 0.89*
Cd in tissue (µg g-1)
Cu in tissue (µg g-1)
Pb in tissue (µg g-1)
Zn in tissue (µg g-1)
Time exposed (days)
Time exposed (days)

Figure 3.3

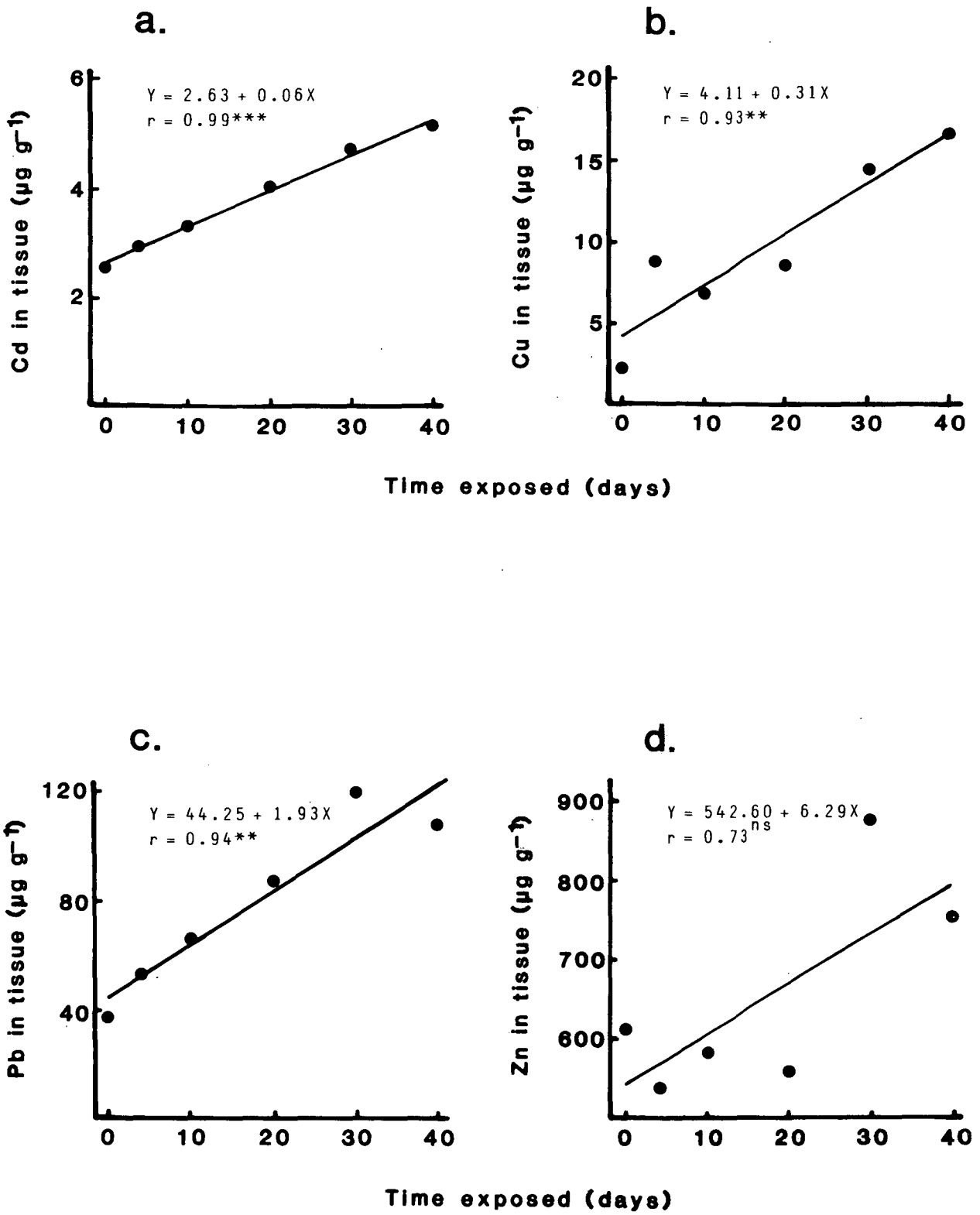

a.
Y = 2.63 + 0.06X
r = 0.99***
Cd in tissue (µg g⁻¹)
6
4
2
0
10
20
30
40
b.
Y = 4.11 + 0.31X
r = 0.93**
Cu in tissue (µg g⁻¹)
20
15
10
5
0
10
20
30
40
Time exposed (days)
c.
Y = 44.25 + 1.93X
r = 0.94**
Pb in tissue (µg g⁻¹)
120
80
40
0
10
20
30
40
d.
Y = 542.60 + 6.29X
r = 0.73ns
Zn in tissue (µg g⁻¹)
900
800
700
600
0
10
20
30
40
Time exposed (days)

Figure 3.4

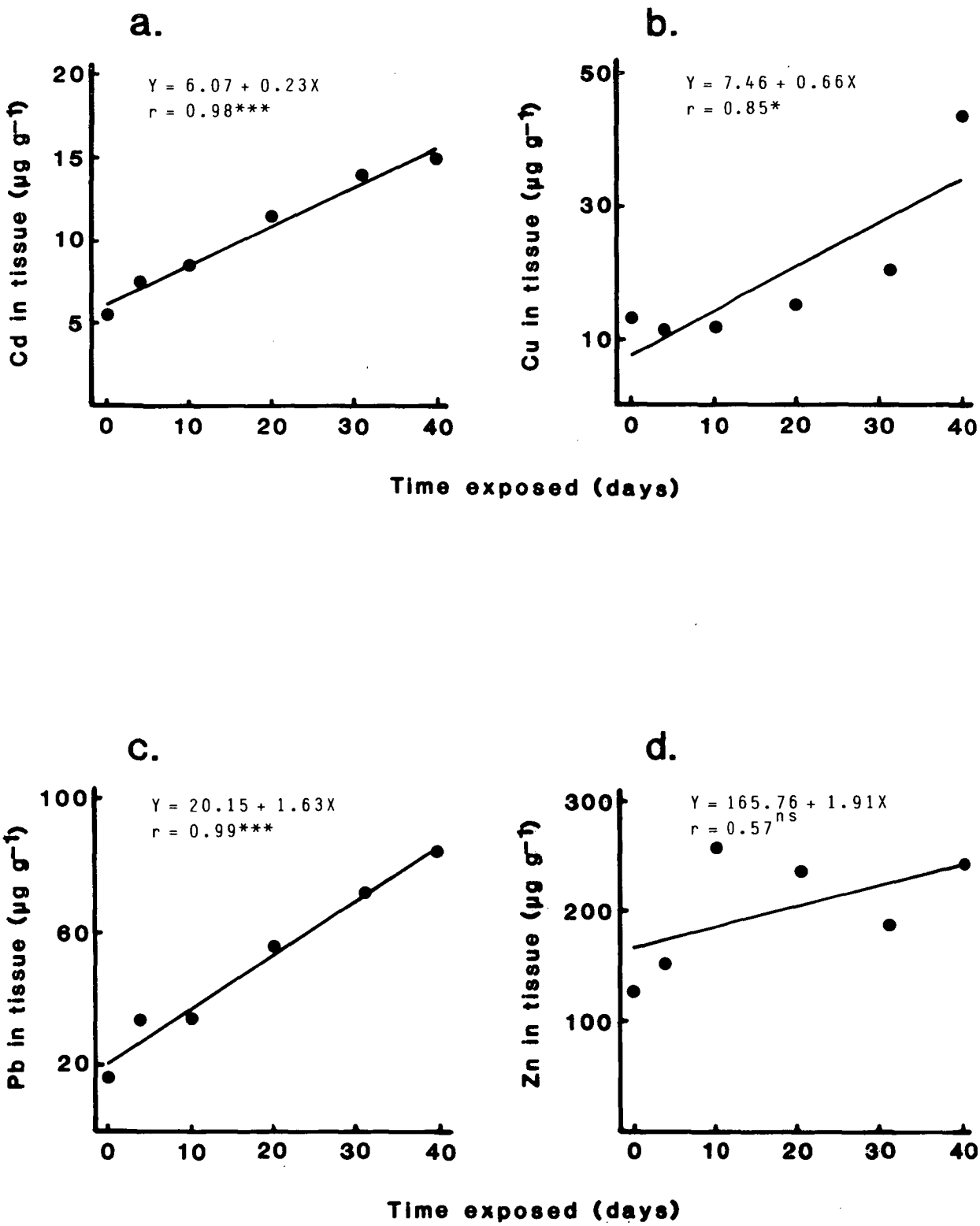

a.
Y = 6.07 + 0.23X
r = 0.98***
Cd in tissue (µg g⁻¹)
20
15
10
5
0
10
20
30
40
b.
Y = 7.46 + 0.66X
r = 0.85*
Cu in tissue (µg g⁻¹)
50
30
10
0
10
20
30
40
Time exposed (days)
c.
Y = 20.15 + 1.63X
r = 0.99***
Pb in tissue (µg g⁻¹)
100
60
20
0
10
20
30
40
d.
Y = 165.76 + 1.91X
r = 0.57ns
Zn in tissue (µg g⁻¹)
300
200
100
0
10
20
30
40
Time exposed (days)

TABLE 3.1 Comparison of heavy metal accumulated by the four test organisms based on the results shown in Figures 3.1 to 3.4. (See text for an explanation of Accumulation Factor.) (* - non-linear regressions.)

Metal	Metal Concentration ($\mu g\ \ell^{-1}$)	Organism	Initial tissue concentration ($\mu g\ g^{-1}$ dry wt.)	Accumulation in 40 days ($\mu g\ g^{-1}$ dry wt.)	Accumulation Factor (AF)	Percentage Increase
Cd	10	*M. e. planulatus*	5.55	46.8	117	843.2
		C. gigas	9.76	75.6	189	774.6
		E. modestus	2.63	2.4	6	91.3
		B. rostratus	6.07	9.2	23	151.6
Cu	10	*M. e. planulatus*	7.61	98.4	246	1293.0
		C. gigas	192.68	341.6	854	177.3
		E. modestus	4.11	12.4	86	301.7
		B. rostratus	7.46	26.4	66	353.8
Pb	50	*M. e. planulatus*	22.84	386.0	193	1690.0
		C. gigas	17.58	121.2	61	689.4
		E. modestus	44.25	77.2	39	174.5
		B. rostratus	20.15	65.2	32	323.6
Zn	100	*M. e. planulatus*	178.32	220.8	55	123.8
		C. gigas	7651.10	2629.6	657	34.4
		*E. modestus**	542.60	251.6	63	46.4
		*B. rostratus**	165.76	76.4	19	46.1

in tissue concentration and the Accumulation Factors (AF). This latter value was suggested by Majori and Petronio (1973e) as a useful measurement of an organism's accumulating ability. It is calculated by:

$$AF = \frac{\text{Accumulation}}{\text{Time x External conc.}} = \frac{\text{Rate of accumulation}}{\text{External conc.}}$$

The Accumulation Factor is proportional to the concentration factor (Majori and Petronio 1973e), and represents the efficiency with which an organism accumulates a metal at a particular external concentration. The AF values in Table 3.1 support the conclusions of Goldberg *et al.* (1978). The oyster (*C. gigas*) had the greatest efficiency (AF value) for the accumulation of copper and zinc, while the mussel (*M. e. planulatus*) had the greatest efficiency for lead. Both organisms recorded a similar value for the accumulation of cadmium. The other two organisms, *E. modestus* and *B. rostratus*, both exhibited very low AF values for all four metals. They, therefore, were rejected for the present purposes.

The apparent affinity for copper and zinc exhibited by *C. gigas* is also indicated by its relatively high initial tissue concentrations of these two metals (Table 3.1). With high initial levels the percentage increases in the tissue concentrations were low in comparison with those recorded for *M. e. planulatus*. Due to experimental, analytical and inherent variabilities that may occur during the measurement of the bioaccumulation of a metal, the greater the percentage increase in the tissue concentration, the greater the precision of the results. This greater percentage increase would represent a higher sensitivity to changes in metal levels. *M. e. planulatus* had the greatest percentage increase of all four metals (Table 3.1). Consequently, of the four species tested, this mussel appears to be the most suitable monitoring organism, with the ability to monitor all four test metals in this present study.

3.3 MONITORING METHOD

3.3.1 Experiments and Results

At the particular external concentrations examined, *M. e. planulatus* exhibited a linear accumulation of all four metals over 40 days. In light of the observations of Majori and Petronio (1973e) it was necessary to establish whether the body load of a metal would continue to increase, or would reach an equilibrium concentration under continued exposure. Groups of 100 mussels (4.0 - 5.0 cm shell length, from Barnes Bay) were exposed to separate solutions of relatively high concentrations of the test metals, *viz.* 50 µg Cd ℓ^{-1}, 100 µg Pb ℓ^{-1}, 200 µg Zn ℓ^{-1}, 50 and 20 µg Cu ℓ^{-1}. A copper concentration of 50 µg ℓ^{-1} was found to be highly toxic to the mussel, and the lower concentration was used subsequently. The results presented in Figure 3.5 show that the accumulation of each metal was linear; for up to 86 days exposure in the case of cadmium and lead.

To examine the relationship between rate of accumulation and external concentration of each metal, groups of 60 mussels (4.0 - 5.0 cm shell length, from Barnes Bay) were exposed, for 40 days at 11°C, to one of the following single metal exposures:

Cadmium: background concentrations (Control seawater and 'Natura' artificial seawater), 0.5, 5, 10, 20, 30 and 50 µg Cd ℓ^{-1};

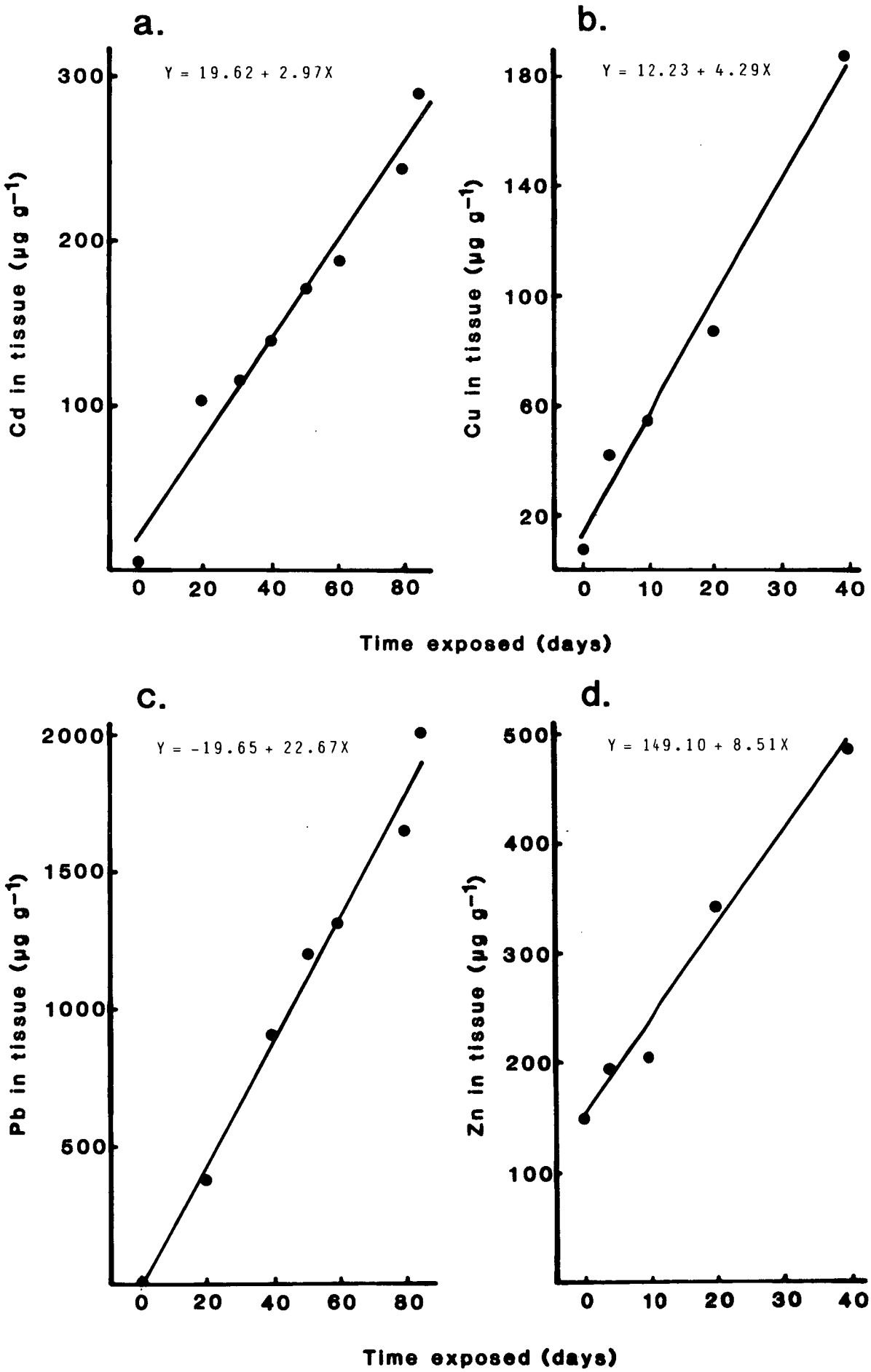
Copper: background concentrations (Control and 'Natura'), 5, 8, 10, 15 and 20 µg Cu ℓ^{-1};

Lead: background concentrations (Control and 'Natura'), 2, 10, 20, 35, 50 and 100 µg Pb ℓ^{-1};

Zinc: background concentrations (Control and 'Natura'), 15, 50, 100, 150 and 200 µg Zn ℓ^{-1}.

These concentrations represent a range equivalent to non-polluted to heavily polluted conditions. In each experiment 10 mussels were subsampled for tissue analysis on days 0, 4, 10, 20 and 40. A linear

Figure 3.5


a.
Y = 19.62 + 2.97X
Cd in tissue (µg g⁻¹)
300
200
100
0
20
40
60
80
b.
Y = 12.23 + 4.29X
Cu in tissue (µg g⁻¹)
180
140
100
60
20
0
10
20
30
40
Time exposed (days)
c.
Y = -19.65 + 22.67X
Pb in tissue (µg g⁻¹)
2000
1500
1000
500
0
20
40
60
80
d.
Y = 149.10 + 8.51X
Zn in tissue (µg g⁻¹)
500
400
300
200
100
0
10
20
30
40
Time exposed (days)

regression was fitted to the results of each experiment, all of which were highly significant ($p<0.01$). Rates of accumulation of the metals are given in Table 3.2. The rate of accumulation was then plotted against the respective external concentration to produce Figures 3.6 to 3.9. To obtain the relationships presented in these figures two assumptions were made:

1. that at an external concentration of zero there would be no accumulation of the metal;
2. the background concentrations were taken as: Control seawater - 0.5 µg Cd ℓ^{-1}, 2.0 µg Cu ℓ^{-1}, 1.0 µg Pb ℓ^{-1}, 11.0 µg Zn ℓ^{-1}; and 'Natura' - 0.5 µg Cd ℓ^{-1}, 6.0 µg Cu ℓ^{-1}, 1.0 µg Pb ℓ^{-1}, 9.0 µg Zn ℓ^{-1}.

Since the regressions shown in Figures 3.6 to 3.9 do not pass through zero, the lines have not been extended to the y axis to avoid the anomalous appearance of metal uptake at zero concentration. Possibly further experimentation and/or precision at the lower concentrations may correct this discrepancy, or in fact may show that the regressions curve to zero.

The relationships between the rate of accumulation and external concentration for copper (Figure 3.7), lead (Figure 3.8) and zinc (Figure 3.9) are all highly significant ($p<0.001$) linear relationships over the entire range of concentrations examined. Cadmium (Figure 3.6), on the other hand, exhibited an apparent levelling-off of the rate of accumulation at the highest external concentration. Nevertheless a highly significant ($p<0.001$) linear relationship exists up to a relatively high external concentration of 30 µg ℓ^{-1} (Figure 3.6).

The concentration factor, after 40 days accumulation, was plotted against the respective external concentration and the results are presented in Figure 3.10. These curves provide a measure of the accumulating efficiency of the mussel. The accumulation factor (AF), of Majori

TABLE 3.2 Rates of accumulation (μg g^{-1}dry wt.day^{-1}) recorded for *M. e. planulatus* exposed to different concentrations (μg ℓ^{-1}) of the four test metals.

Metal	Seawater Concentration (μg ℓ^{-1})	Rate of Accumulation (μg g^{-1}dry wt.day^{-1})
Cadmium	50	2.97
	30	3.57
	20	2.17
	10	1.17
	5	0.84
	0.5*	0.10
	Control seawater	0.00 - 0.09
	'Natura'	0.03
Copper	20	4.18
	15	3.59
	10	2.46
	8*	1.65
	5	1.51
	Control seawater	0.00 - 0.94
	'Natura'	1.11
Lead	100	22.63
	50	9.65
	35	8.11
	20	6.92
	10	3.57
	1*	1.20
	Control seawater	0.00 - 0.23
	'Natura'	0.31
Zinc	200	8.51
	150	7.22
	100	5.52
	50	2.41
	15*	2.44
	Control seawater	0.00 - 2.76
	'Natura'	1.80

* These experiments were conducted in 'Natura' artificial seawater.

Figure 3.6

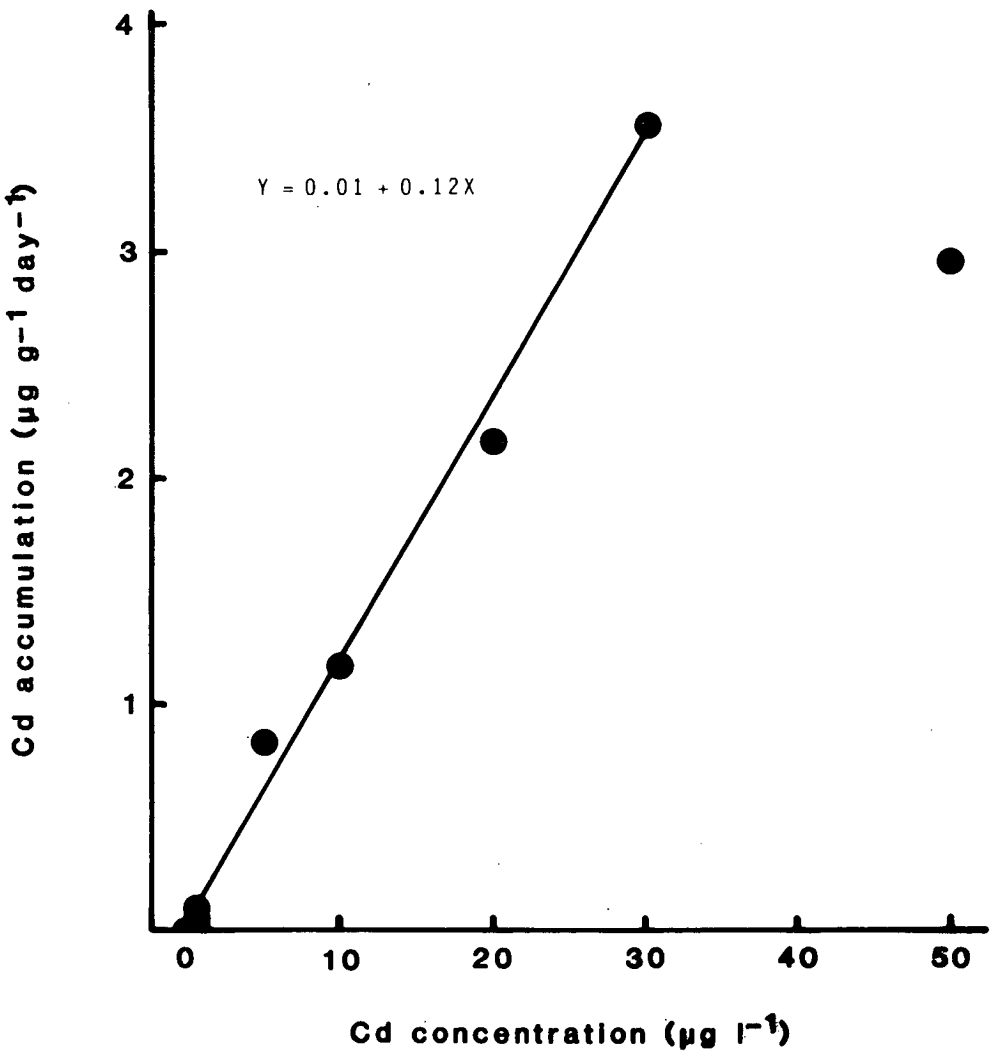

Y = 0.01 + 0.12X
Cd accumulation (µg g-1 day-1)
Cd concentration (µg l-1)
0
10
20
30
40
50
1
2
3
4

Figure 3.7

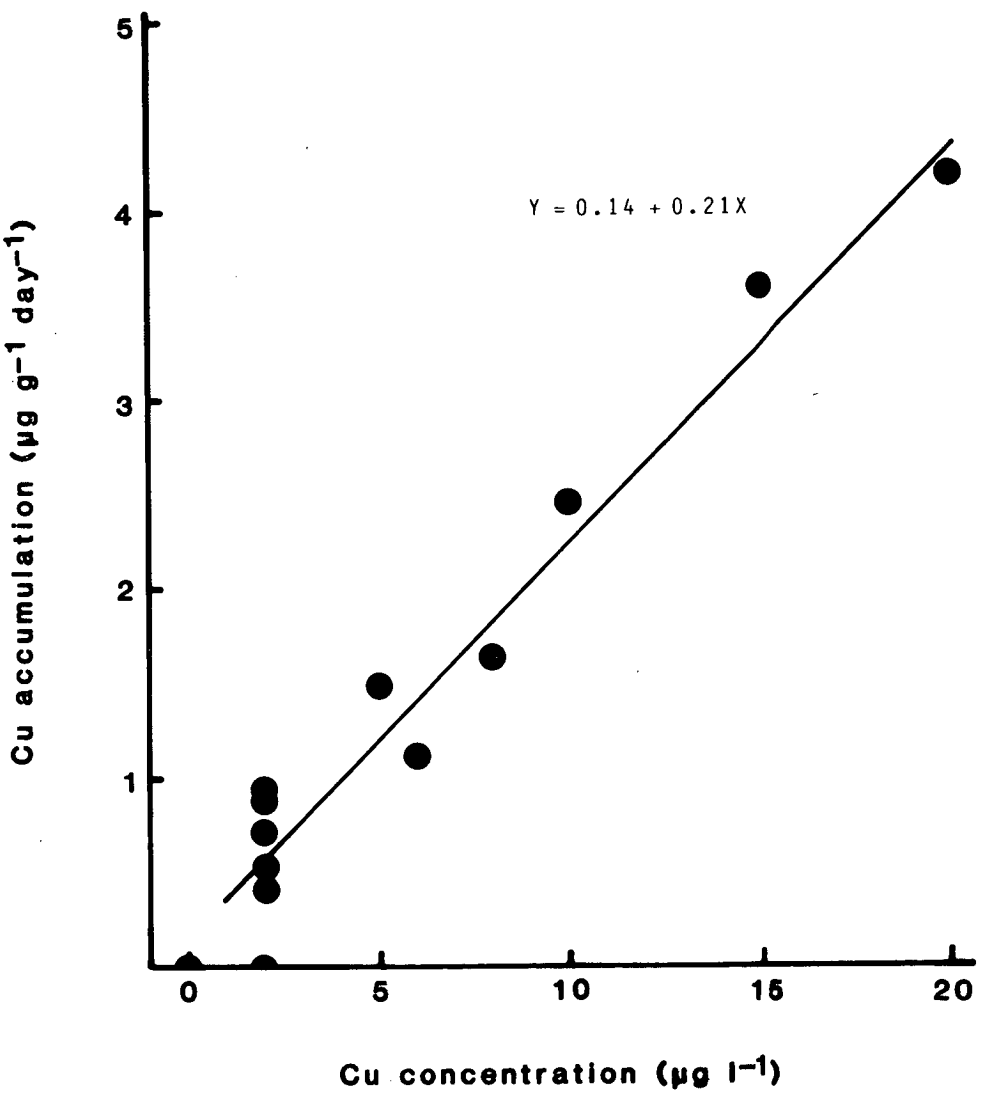

Y = 0.14 + 0.21X
Cu accumulation (µg g⁻¹ day⁻¹)
Cu concentration (µg l⁻¹)
0
5
10
15
20
1
2
3
4
5

Figure 3.8

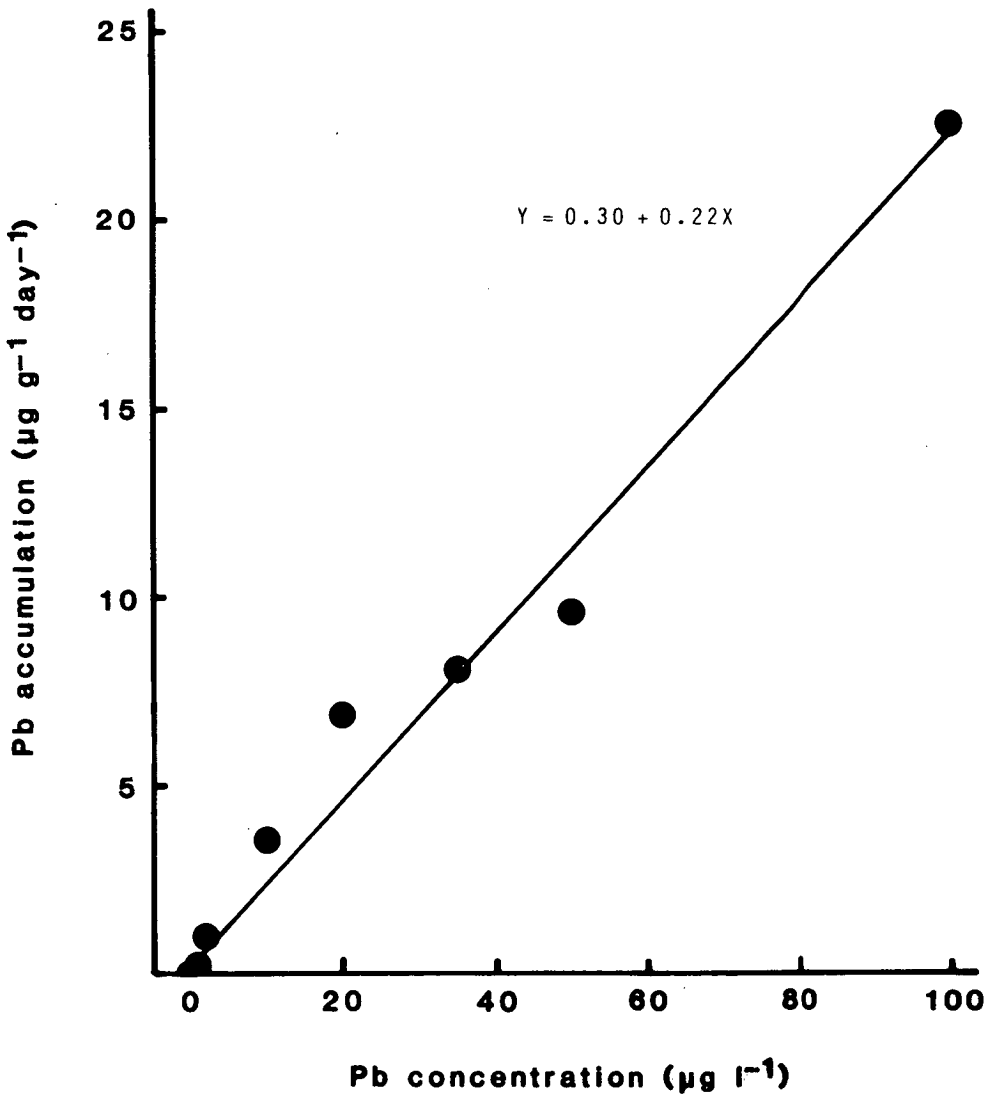

Y = 0.30 + 0.22X
Pb accumulation (µg g⁻¹ day⁻¹)
Pb concentration (µg l⁻¹)
25
20
15
10
5
0
20
40
60
80
100

Figure 3.9

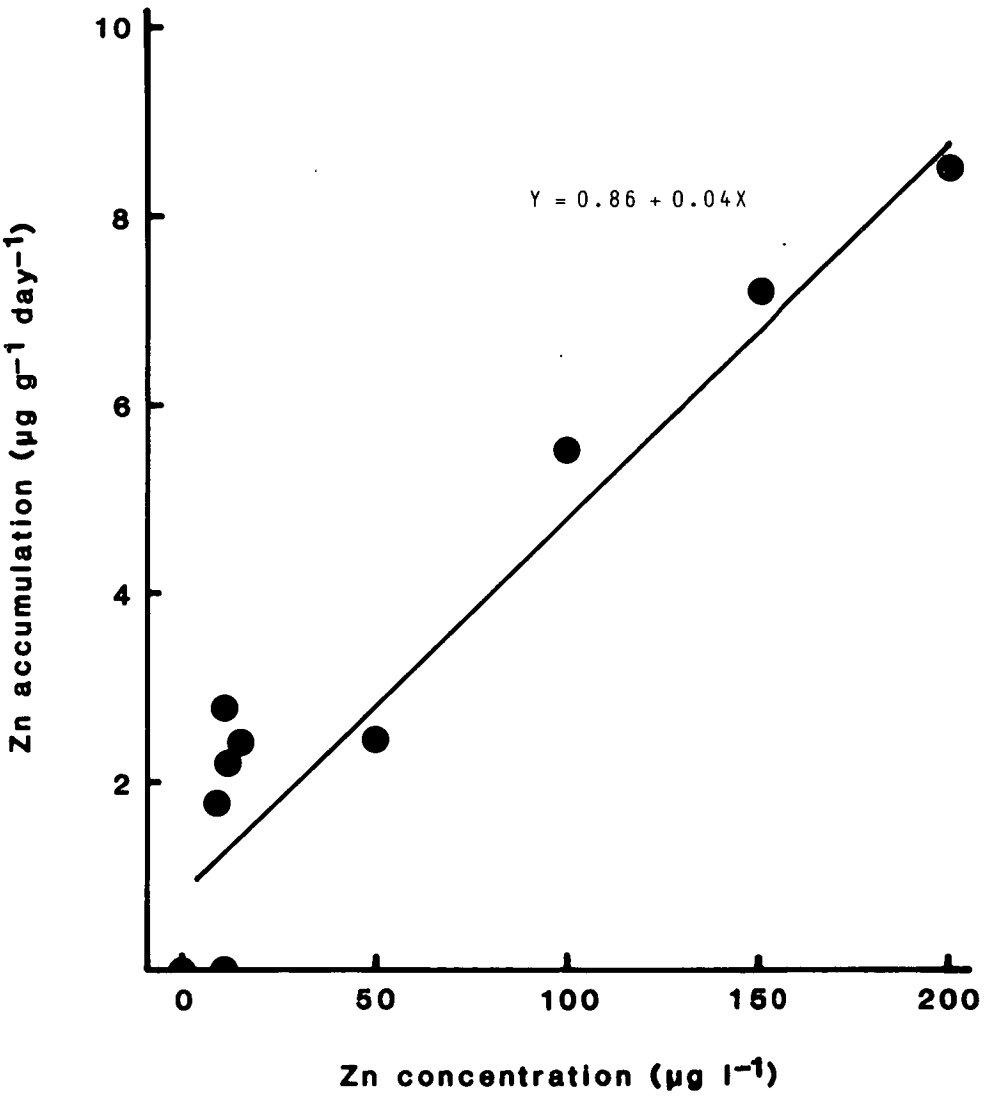
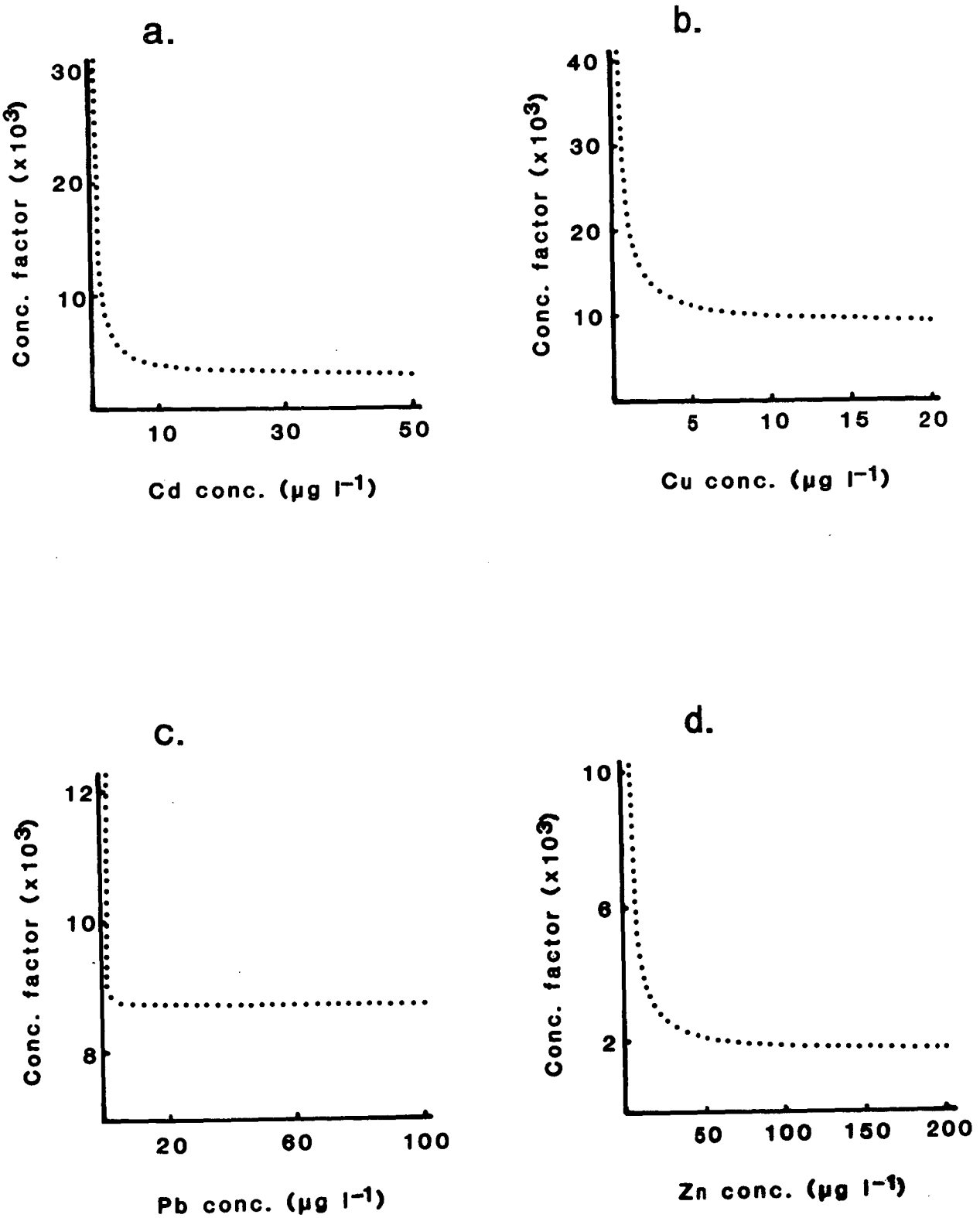

Y = 0.86 + 0.04X
Zn accumulation (µg g⁻¹ day⁻¹)
Zn concentration (µg l⁻¹)
10
8
6
4
2
0
50
100
150
200

Figure 3.10


a.
Conc. factor (x10³)
30
20
10
10
30
50
Cd conc. (µg l⁻¹)
b.
Conc. factor (x10³)
40
30
20
10
5
10
15
20
Cu conc. (µg l⁻¹)
c.
Conc. factor (x10³)
12
10
8
20
60
100
Pb conc. (µg l⁻¹)
d.
Conc. factor (x10³)
10
6
2
50
100
150
200
Zn conc. (µg l⁻¹)

and Petronio (1973e), would provide similar relationships. Concentration factors for all four metals, were considerably greater at the lower external concentrations. This may support the suggestion made above that the relationships between rate of accumulation and external concentration curve towards zero.

3.3.2 Discussion

The mussel *Mytilus viridis* is reported to accumulate both copper and zinc linearly over a 5 week period (D'Silva and Kureishy 1978). *M. edulis* exhibited a linear accumulation of both cadmium (George and Coombs 1977; Westernhagen *et al.* 1978; Scholz 1980) and lead (Schulz-Baldes 1974). Majori and Petronio (1973e) on the other hand, found that after an initial linear accumulation phase, *Mytilus galloprovincialis* reached an equilibrium tissue concentration. Jackim *et al.* (1977) interpreted the cadmium accumulation by the three bivalves employed in their study, one of which was *M. edulis*, as indicating that the initial rate of accumulation decreased with increasing body burden. In the present study *M. e. planulatus* exhibited linear accumulations of all four test metals over a wide range of external concentrations; in some cases for up to 86 days exposure. At no time was an equilibrium concentration reached.

Exposure to 50 µg Cu ℓ^{-1} proved to be lethal for *M. e. planulatus*, with 100% mortality by day 22 and 60% mortality by day 8. This particular concentration of copper was reported by D'Silva and Kureishy (1978) to be the threshold concentration above which the valves of *M. viridis* remained closed, and below which they remained open as in the control mussels. An LT50 of 2 days in 500 µg Cu ℓ^{-1} and 4-5 days in 250 µg Cu ℓ^{-1} was reported by Davenport (1977) for *M. edulis*. Total mortality in the same species occurred by day 15 of an exposure to 50 µg Cu ℓ^{-1} (Martin 1979b).

Pringle *et al.* (1968) proposed that the rate of accumulation of a metal is dependent upon the external concentration. The observations on mussels by a number of authors support this suggestion (e.g. Schulz-Baldes 1974; Fowler and Benayoun 1974; George and Coombs 1977; D'Silva and Kureishy 1978; Kumarugura and Ramamoorthi 1979; Marletta *et al.* 1979; George and Pirie 1980; Scholz 1980). Results obtained for *M. e. planulatus* indicated a relationship between rate of accumulation and external concentration, for the four metals tested. For copper, lead and zinc this relationship was linear over the wide range of concentrations examined. The rate of accumulation of cadmium, however, following an initial linear phase appeared to reach a peak rate, though possibly the results obtained at 50 µg Cd ℓ^{-1} were erroneous. Fowler and Benayoun (1974) for *M. galloprovincialis* and both George and Coombs (1977) and Scholz (1980) for *M. edulis* reported continued linear relationships at external concentrations above 50 µg Cd ℓ^{-1}. As the present study is concerned with open marine waters, concentrations above 30 µg Cd ℓ^{-1} would not be expected. Thus for the present purposes it may be concluded that the rate of accumulation of all four metals by *M. e. planulatus* is linearly related to the external concentration of the metal.

George and Coombs (1977) described the mussel *M. edulis* as being at its most efficient as an accumulator of cadmium at an external concentration of 700 µg Cd ℓ^{-1}. At this concentration this organism was also observed to exhibit the first signs of toxicity stress. The results for *M. e. planulatus* show that higher efficiency of accumulation occurred at the lowest seawater concentrations of the metals. At higher concentrations the concentration factor was greatly reduced. Similar inverse relationships were obtained by Majori and Petronio (1973e) for the accumulation of cadmium, copper and mercury by *M. galloprovincialis*. Only at high copper concentrations ($\geq$20 µg ℓ^{-1}) were toxic symptoms recorded for *M. e. planulatus*. The range of concentrations employed

for the other three metals appeared to be well below toxic levels.

3.4 FIELD TRIALS

Widdows *et al.* (1981) and Riisgård and Poulson (1981) used caged mussels successfully to study the physiological condition and scope for growth in *M. edulis* in polluted waters. Others, such as Young *et al.* (1976), Uysal (1978, 1979), Davies (1979), Harris *et al.* (1979), Simpson (1979), Behrens and Duedall (1981b) and Julshamn (1981d), have used a cage suspension system in the study of pollutant accumulation.

A simple cage suspension system was designed for the present study to enable field testing of the laboratory results on rate of accumulation of metals.

3.4.1 Experimental Design

Two sets of field experiments were conducted. In each case, 50 mussels (4.0 - 5.0 cm shell length, from Barnes Bay) were suspended in cages at two sites for approximately 9 weeks. Both sets were conducted during winter months when the seawater temperature ranged between 9.5°C and 12°C. The cages consisted of a PVC frame (25 cm x 15 cm x 9 cm) placed inside a synthetic fibre bag. The rigid frame allowed a free flow of water through the cage, and the dimensions allowed for a mussel density approximately equal to that of their natural population. The cages were suspended from a taut-line buoy system adapted from Young *et al.* (1976). Each was attached to a nylon line extending from a buoy floating 1 m below the water surface at low tide (Figure 3.11). The cages were suspended 0.5 m above the seafloor, corresponding to a depth of approximately 3 m below low tide.

The two sites selected for cage suspension were: Site 1, a known polluted site at Kangaroo Bluff in the Derwent Estuary and Site 2, a relatively 'clean' site at Bligh Point in the D'Entrecasteaux Channel

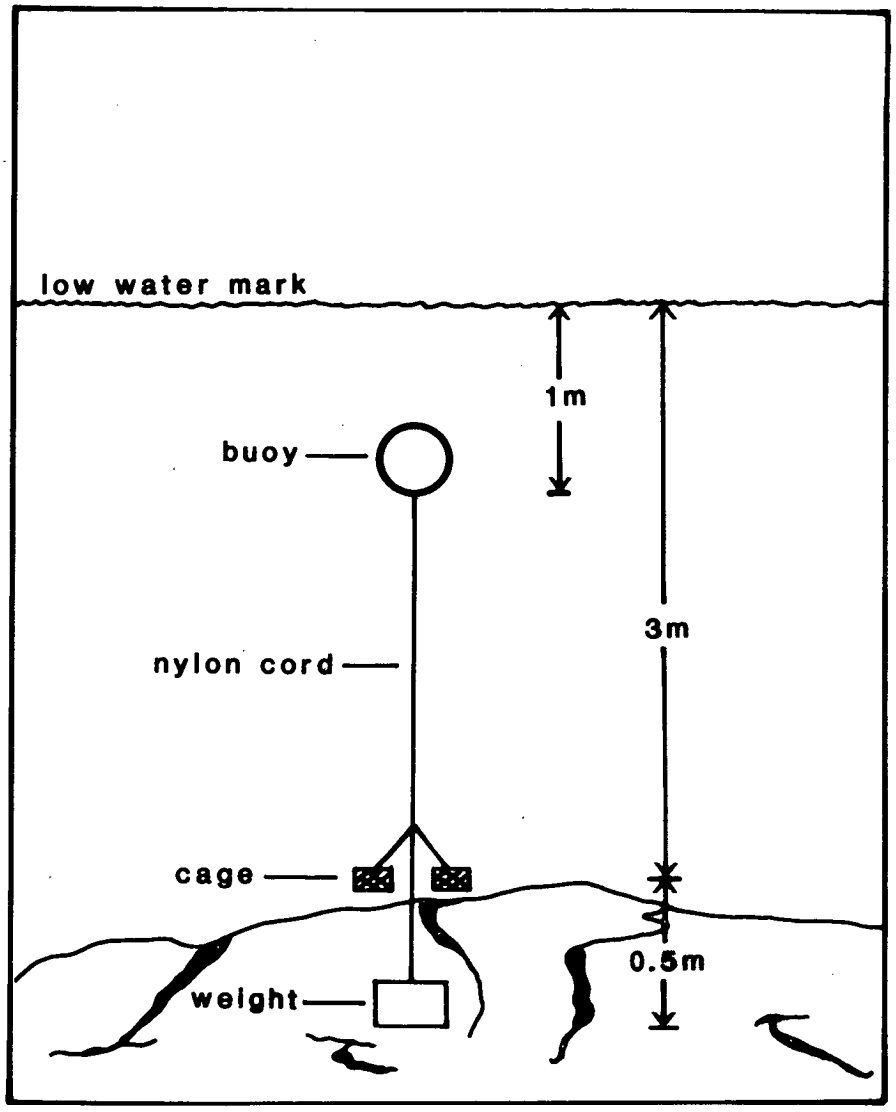


Figure 3.11 Diagrammatic representation of cage suspension system adapted from taut-line buoy system used by Young et al. (1976).

(Figure 2.1). The salinity at both sites was similar and consistently 31-33‰. The first trial was conducted between 16 April 1980 and 19 June 1980, with samples of 10 mussels removed from the cages after 6, 20, 40 and 64 days suspension. The second trial was conducted between 13 April 1981 and 12 June 1981 with samples removed after 21, 43 and 60 days. In each trial a day 0 sample was also taken. Seawater samples were collected from both sites as frequently as possible during the suspension period. These samples were analysed by conventional AAS technique described in Chapter 2. Shell length and dry tissue weights of the subsampled mussels were recorded as a measure of condition. The mussel tissues were then digested and analysed as described in Chapter 2.

The day 6 sample in the first trial was taken to ensure that the mussels were maintaining body condition (further discussion on growth in cages is presented in Appendix D) and that the suspension system remained viable. Previous suspension techniques had proved vulnerable to vandalism. In spite of precautions a third trial employing the submerged buoy system was lost due to a combination of vandals and storm weather. A maximum mortality of 10% was recorded in any one cage, and this was due predominantly to predation by starfish.

3.4.2 Results and Discussion

Body condition of the mussels was maintained throughout each experiment. The change in metal concentration in mussel tissue measured over a sampling period was recorded as the rate of accumulation (RA) of the metal ($\mu g\ g^{-1}\ day^{-1}$) (Tables 3.3 to 3.6). The RA value was then transformed to a seawater concentration of the metal ($\mu g\ \ell^{-1}$) by employing the relationships presented in Figures 3.6 to 3.9. These laboratory relationships were obtained using mussels from the same population, of equal size and in similar seawater temperature and salinity conditions.

TABLE 3.3 Results of field trial conducted at Site 1 - Kangaroo Bluff, in 1980. Relationship between the rate of accumulation (RA) of each metal by *M. e. planulatus* (μg g^{-1}dry wt.day^{-1}), and the seawater (SW) concentration of each metal (μg ℓ^{-1}) calculated by either (a) using the data in Figures 3.6 to 3.9, or (b) direct analysis by AAS.

	Cd	Cu	Pb	Zn
Day 0 - 20				
RA up to day 20	0.09	0.11	1.06	0.20
SW conc. (a)	0.67	-*	3.45	-*
Range of SW conc. (b)	<0.5 - 0.7	⩽1.0	⩽5	87 - 102
Day 0 - 40				
RA up to day 40	0.09	0.11	0.82	-1.18
SW conc. (a)	0.67	-*	2.36	-*
Range of SW conc. (b)	<0.5 - 1.0	⩽1.0	⩽5	65 - 102
Day 0 - 64				
RA up to day 64	0.15	0.16	0.75	0.81
SW conc. (a)	1.17	0.10	2.05	-*
Range of SW conc. (b)	<0.5 - 1.0	⩽1.0	⩽5	65 - 110
Day 20 - 40				
RA between days 20 and 40	0.10	0.11	0.58	-2.55
SW conc. (a)	0.75	-*	1.27	-*
Range of SW conc. (b)	<0.5 - 1.0	⩽1.0	<5	65 - 74
Day 40 - 64				
RA between days 40 and 64	0.24	0.24	0.63	4.13
SW conc. (a)	1.92	0.48	1.50	81.75
Range of SW conc. (b)	<0.5 - 1.0	⩽1.0	<5	80 - 110

* RA below y-intercept of linear relationship.

TABLE 3.4 Results of field trial conducted at Site 2 - Bligh Point, in 1980. Relationship between the rate of accumulation (RA) of each metal by *M. e. planulatus* (μg g^{-1}dry wt.day^{-1}), and the seawater (SW) concentration of each metal (μg ℓ^{-1}) calculated by either (a) using the data in Figures 3.6 to 3.9, or (b) direct analysis by AAS.

	Cd	Cu	Pb	Zn
Day 0 - 20				
RA up to day 20	0.08	-0.04	0.03	-0.1
SW conc. (a)	0.58	-*	-*	-*
Range of SW conc. (b)	<0.5	<1.0	<5	3 - 19
Day 0 - 40				
RA up to day 40	0.04	-0.05	0.21	-0.03
SW conc. (a)	0.25	-*	-*	-*
Range of SW conc. (b)	<0.5	<1.0	<5	3 - 19
Day 0 - 64				
RA up to day 64	0.02	-0.01	0.48	0.88
SW conc. (a)	0.08	-*	0.82	0.50
Range of SW conc. (b)	<0.5	<1.0	<5	3 - 19
Day 20 - 40				
RA between days 20 and 40	0.00	-0.06	0.39	0.05
SW conc. (a)	-*	-*	0.41	-*
Range of SW conc. (b)	<0.5	<1.0	<5	3 - 14
Day 40 - 64				
RA between days 40 and 64	0.00	0.06	0.92	2.38
SW conc. (a)	-*	-*	2.82	38.00
Range of SW conc. (b)	<0.5	<1.0	<5	14 - 18

* RA below y-intercept value of linear regression.

TABLE 3.5 Results of field trial conducted at Site 1 - Kangaroo Bluff, in 1981. Relationship between the rate of accumulation (RA) of each metal by *M. e. planulatus* ($\mu g\ g^{-1}$dry wt.day^{-1}), and the seawater (SW) concentration of each metal ($\mu g\ \ell^{-1}$) calculated by either (a) using the data in Figures 3.6 and 3.9, or (b) direct analysis by AAS.

	Cd	Cu	Pb	Zn
Day 0 - 21				
RA up to day 21	0.04	0.08	1.01	4.28
SW conc.(a)	0.25	-*	3.23	85.50
Range of SW conc.(b)	<0.5	<1	3-6	65-88
Day 0 - 43				
RA up to day 43	0.03	0.11	1.42	4.80
SW conc.(a)	0.17	-*	5.09	98.50
Range of SW conc.(b)	<0.5	<1	3-27	59-210
Day 0 - 60				
RA up to day 60	0.03	0.10	0.92	3.77
SW conc.(a)	0.17	-*	2.82	72.75
Range of SW conc.(b)	<0.5	<1	3-27	53-210
Day 21 - 43				
RA between days 21 and 43	0.03	0.10	2.42	5.53
SW conc.(a)	0.17	-*	9.64	116.75
Range of SW conc.(b)	<0.5	<1	6-27	59-210
Day 43 - 60				
RA between days 43 and 60	0.04	0.09	-0.33	1.14
SW conc.(a)	0.25	-*	-*	7.00
Range of SW conc.(b)	<0.5	<1	3-5	53-140

* RA below Y-intercept value of linear regression.

TABLE 3.6 Results of field trial conducted at Site 2 - Bligh Point, in 1981. Relationship between the rate of accumulation (RA) of each metal by *M. e. planulatus* ($\mu g\ g^{-1}$ dry wt.day^{-1}), and the seawater (SW) concentration of each metal ($\mu g\ \ell^{-1}$) calculated by either (a) using the data in Figures 3.6 and 3.9, or (b) direct analysis by AAS.

	Cd	Cu	Pb	Zn
Day 0 - 21				
RA up to day 21	0.02	0.05	0.33	1.43
SW conc.(a)	0.08	-*	0.14	14.25
Range of SW conc.(b)	<0.5	<1.0	<1.0	5-24
Day 0 - 43				
RA up to day 43	0.01	0.00	0.39	2.12
SW conc.(a)	0.00	-*	0.41	31.5
Range of SW conc.(b)	<0.5	<1.0	<1	5-35
Day 0 - 60				
RA up to day 60	0.03	0.01	0.37	1.81
SW conc.(a)	0.17	-*	0.32	23.75
Range of SW conc.(b)	<0.5	<1.0	<1.0	5-35
Day 21 - 43				
RA between days 21 and 43	0.00	-0.07	0.45	2.78
SW conc.(a)	-*	-*	0.68	48.00
Range of SW conc.(b)	<0.5	<1.0	<1.0	12-35
Day 43 - 60				
RA between days 43 and 60	0.03	0.06	0.33	1.02
SW conc.(a)	0.17	-*	0.14	4.00
Range of SW conc.(b)	<0.5	<1.0	<1.0	12-24

* RA below Y-intercept value of linear regression.

The estimated, time-integrated, seawater concentration is presented in Tables 3.3 to 3.6 as SW conc.(a).This value is compared in the tables with the range of seawater concentrations recorded by AAS analysis of the seawater samples (Range of SW conc.(b)) taken during the particular sampling period.

A number of the rates of accumulation were below the y-intercept value of the regressions (Figures 3.6 to 3.9). Consequently a seawater concentration could not be calculated, particularly for copper and zinc. Despite this, a comparison of the metal levels between sites was still possible. The RA values still provide a useful index of the metal levels for comparative purposes. For example: at Site 1 (Table 3.3) the rate of accumulation of copper ranged between 0.11 to 0.24, whilst at the less polluted Site 2 (Table 3.4) there was either a loss of copper or an RA value of only 0.06.

The disadvantage of using long monitoring periods (i.e. long intervals between samples) is shown in the comparison of zinc values in Tables 3.3 and 3.4. The RA value after 64 days at Site 1 (Table 3.3) was 0.81, while that at Site 2 (Table 3.4) was 0.88, indicating similar levels for both sites. However, if the 64 day period is broken down into approximately 20 day intervals, it can be seen that, particularly between days 40 and 64, Site 1 was subjected to higher zinc levels than Site 2.

Metal concentrations in the seawater obtained either by use of the biological monitor, or by direct seawater analysis exhibited marked similarities. The biological monitoring results (SW conc.(a)), however, reveal a more marked contrast between the two monitoring sites. This is particularly useful for metals at low seawater concentrations such as cadmium and copper, where the conventional analysis (AAS) results describe both sites as having values below a detection limit. The mussel is more sensitive to metal level variations than the analytical method employed and clearly separates the two sites. Use of a biological

monitor should also prove to be less costly, than direct water analysis, in terms not only of money but of time and analytical effort.

Although the results of the first trial (Tables 3.3 and 3.4) appeared unfavourable for using the mussel to monitor zinc levels, the second trial proved more successful (Tables 3.5 and 3.6). It was still possible, however, from the results of the first trial to successfully separate the two sites from each other by using the RA values. The mussel may, therefore, be successfully employed to monitor all four test metals both qualitatively and quantitatively.

3.5 METAL DEPURATION FROM MUSSEL TISSUE

3.5.1 Introduction

In field experiments mussels occasionally lost metal from the tissues. The amount of work conducted on depuration of metals from mussel tissue is limited, but does reveal that this process is a great deal slower than that of accumulation (Majori and Petronio 1973e; Calapaj 1974; Schulz-Baldes 1974; Simpson 1979; Taneeva 1979; Taneeva and Man'ko 1979; Scholz 1980). Both Schulz-Baldes (1974) and Scholz (1980) reported that the loss of a metal (lead and cadmium respectively) by *M. edulis* was dependent on the initial tissue concentration. The extent of depuration of the four test metals by *M. e. planulatus* was examined in the following 3 series of experiments.

3.5.2 Series I - Depuration in 'Natura'

Experimental Design

This series involved eight experiments, in each of which 50 mussels (3.0 - 4.0 cm shell length, from Howden, Figure 2.1) were first exposed to one of the following:

a. 5 µg Cd ℓ^{-1}, b. 50 µg Cd ℓ^{-1},

c. 5 µg Cu ℓ^{-1}, d. 30 µg Cu ℓ^{-1},

e. 5 µg Pb ℓ^{-1}, f. 50 µg Pb ℓ^{-1},

g. 50 µg Zn ℓ^{-1}, h. 500 µg Zn ℓ^{-1}.

This initial exposure was designed to increase the tissue concentration of the respective metal to a moderate or high level. Following 8 days exposure each group of mussels was transferred into 'Natura' artificial seawater in which all metals were at background concentrations (see Section 3.3.1). The mussels were subsampled for tissue analysis on days 0, 4, 8 and 12 after transfer into 'Natura'.

Results

The change in tissue concentration of the test metal for each experiment, during exposure to 'Natura', is shown in Table 3.7.

TABLE 3.7 The change in tissue concentration (µg g^{-1} dry wt.) of the test metal during exposure to the background concentrations in 'Natura' artificial seawater.

Metal	Expt.	Initial Exposure	Time (days) in 'Natura'			
			0	4	8	12
Cadmium	a	5 µg ℓ^{-1}	12.62	9.64	11.85	12.69
	b	50 µg ℓ^{-1}	91.03	74.02	67.59	82.65
Copper	c	5 µg ℓ^{-1}	21.71	28.54	27.71	32.24
	d	30 µg ℓ^{-1}	62.96	41.42	44.17	36.04
Lead	e	5 µg ℓ^{-1}	25.20	33.65	31.83	32.95
	f	50 µg ℓ^{-1}	171.12	170.63	162.94	164.73
Zinc	g	50 µg ℓ^{-1}	428.43	501.03	431.32	358.41
	h	500 µg ℓ^{-1}	723.25	686.78	503.30	508.08

Most of the depuration occurred when tissue concentrations were high, i.e. following exposure to high external concentrations (experiments b, d, f and h). Lead was the only metal which was not lost to any marked degree. The loss of both cadmium and copper appeared to be exponential, with the greatest loss occurring during the first 4 days. Following exposure to low initial levels, both copper and lead were accumulated from the background concentrations (experiments c and e). Cadmium was first lost from the tissue but then also accumulated. Zinc, on the other hand, first showed an increase and later a decrease (experiment g).

3.5.3 Series II - Depuration of Cadmium and Lead

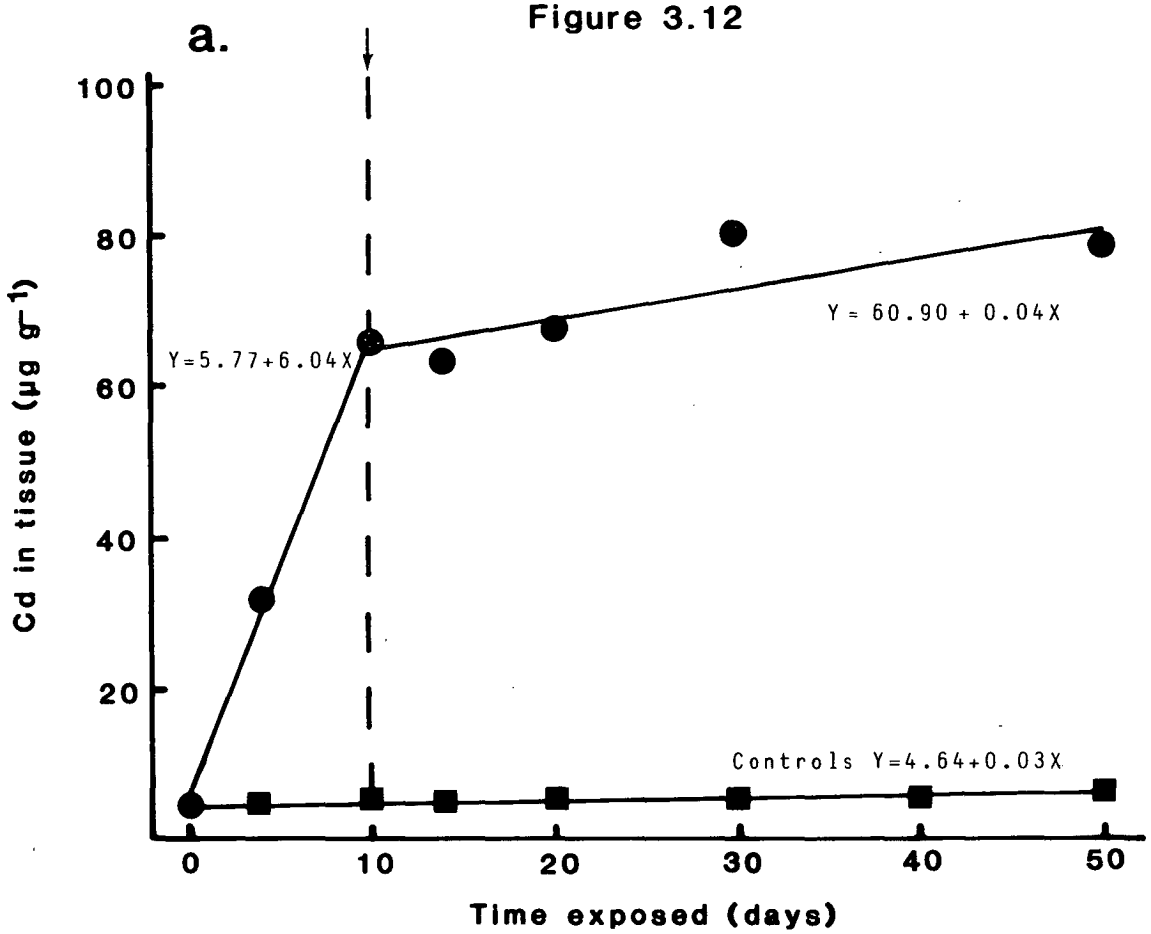
Experimental Design

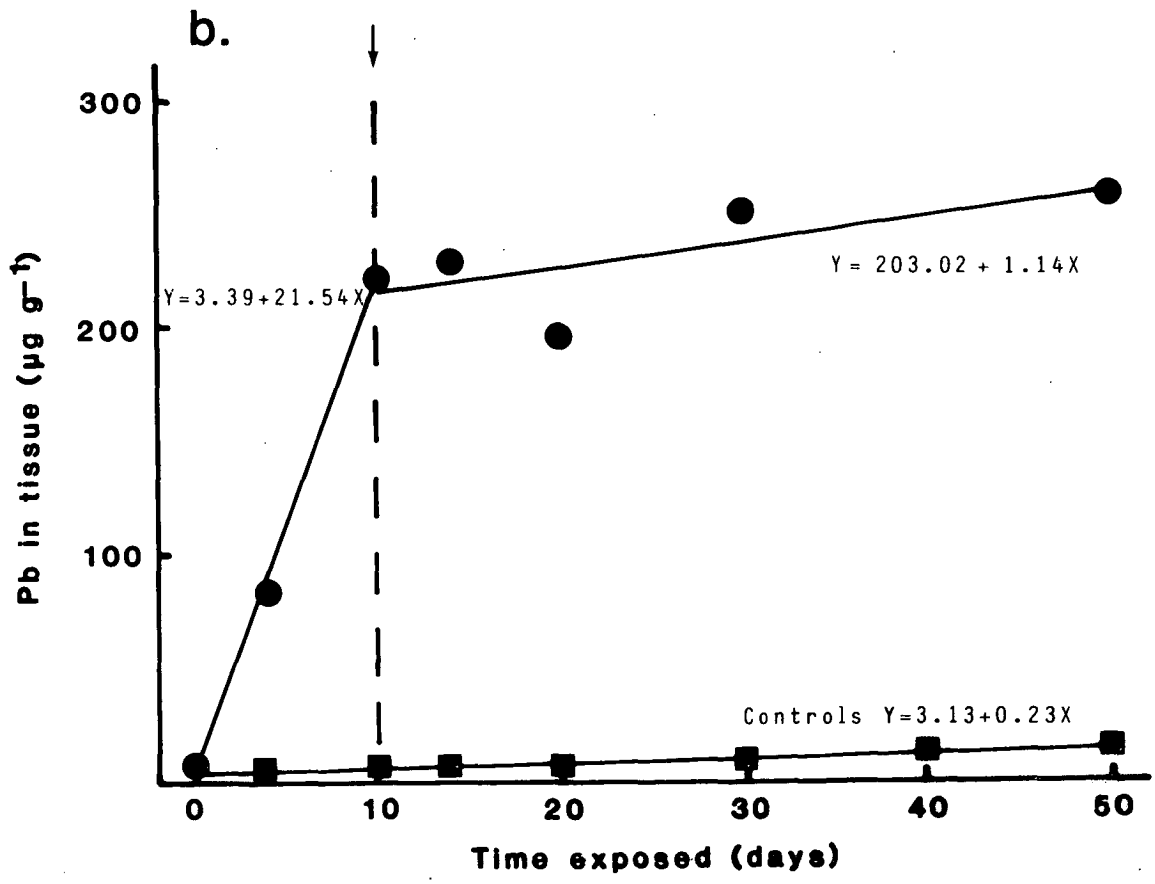
To examine further the possible depuration of cadmium and lead, 90 mussels (4.0 - 5.0 cm shell length, Barnes Bay) were first exposed for 10 days to the combination of 50 µg Cd ℓ^{-1} and 100 µg Pb ℓ^{-1}. They were then transferred into background concentrations of the Control seawater (see Section 3.3.1) for a further 40 days. Samples were removed for tissue analysis on days 0, 4 and 10 of the initial exposure, and then after 4, 10, 20 and 40 days in background levels. A control group of mussels was exposed to background levels only for 50 days, and sub-sampled at the same time as the other experimental group.

Results

Neither cadmium nor lead was lost from the mussel tissue during the exposure to background levels (Figure 3.12). In fact both metals were accumulated at rates greater than those recorded for the control mussels.

Figure 3.12


a.
100
80
60
40
20
Cd in tissue (µg g⁻¹)
Y=5.77+6.04X
Y = 60.90 + 0.04X
Controls Y=4.64+0.03X
0
10
20
30
40
50
Time exposed (days)


b.
300
200
100
Pb in tissue (µg g⁻¹)
Y=3.39+21.54X
Y = 203.02 + 1.14X
Controls Y=3.13+0.23X
0
10
20
30
40
50
Time exposed (days)

3.5.4 Series III - Depuration from Natural Levels

A group of 60 mussels (3.5 - 5.0 cm shell length) was collected from a population at Kangaroo Bluff (Figure 2.1). This population was known to be continuously exposed to metal levels in excess of those recorded in the Control seawater used in the laboratory (Section 2.2). Mussels were exposed in the laboratory, at 11°C (approximate temperature at the time of collection), for 40 days to the background metal levels in Control seawater. The tissue concentrations of each of the four test metals was recorded on days 0, 4, 10, 20 and 40 of the experiment (Table 3.8). The results clearly show that no loss of any metal occurred; all metals were accumulated from the background levels.

TABLE 3.8 Change in metal concentration (μg g^{-1} dry wt.) in tissue of mussels from Kangaroo Bluff exposed only to background concentrations in Control seawater in laboratory.

Day	Cadmium	Copper	Lead	Zinc
0	9.73	7.0	126	199
4	11.01	6.0	134	220
10	11.31	9.6	135	226
20	10.03	8.4	144	251
40	11.49	10.2	156	257

3.5.5 Discussion on Metal Depuration

M. e. planulatus exhibited a loss of cadmium, copper and zinc from its tissues, but only following accumulation to very high concentrations. No evidence of a depuration of lead was observed, in contrast to what has been reported for *M. edulis*. Schulz-Baldes (1974) recorded linear depurations of lead from tissue concentrations as low as 35 μg g^{-1} dry wt. in this species. On the other hand, *M. edulis* was found to

lose cadmium exponentially in a similar manner to that recorded for *M. e. planulatus* from tissue concentrations of *ca.* 100 μg g^{-1} (Scholz 1980). In both species there was either no loss or a very slow uptake of cadmium from low tissue concentrations. Both Schulz-Baldes (1974) and Scholz (1980) noted that the rate of depuration was dependent on the tissue concentration.

Unlike the other metals, the loss of zinc from the tissue of *M. e. planulatus* was more marked and irregular. This occurred not only in the laboratory experiments but also in field trials described in Section 3.4. Zinc levels may therefore be more susceptible to fluctuations in the external concentrations. Calapaj (1974) reported that *M. edulis* lost 50% of an equilibrium tissue concentration of zinc in 20 days, but only 20% of cadmium in 40 days. Slow loss of metals, such as lead and cadmium, from mussel tissue may be related to the strong permanent bonding of the metals within the tissue (Schulz-Baldes 1974; Sturesson 1978). Possibly zinc may not be as strongly bound as the other metals. This suggestion is supported by the observations of Coombs (1972) on the oyster *Ostrea edulis*. He reported that 95% of the tissue concentration of zinc was dialysable, compared to only 43% of copper.

Simpson (1979) reported that *M. edulis* transferred from a high to low contamination site, still had tissue levels of zinc and lead well in excess of the resident mussels after 3 months. Both metals exhibited an initial decrease in the first month, but then very little depuration in the latter 2 months. Julshamn (1981d),on the other hand, reported that this same species, following transfer for a year to a polluted site, lost the accumulated cadmium and lead within a year of being transferred back to the original site.

It does appear that depuration of metals is much slower than their accumulation by mussels, and is dependent on the tissue concentration. The loss of metal during a monitoring period would therefore only be

expected if (a) the mussels came originally from a heavily polluted environment, or (b) they were subjected for a short time to very high metal levels. Zinc levels appear to be more susceptible to external fluctuations than those of the other metals.

3.6 GENERAL CONCLUSIONS

It is proposed that *M. e. planulatus* is a suitable monitoring organism for cadmium, copper, lead and zinc in open marine waters. The direct relationship between rate of accumulation and the external concentration may be used to quantify the metal level in seawater. Meanwhile, the rate of accumulation, itself, may be used qualitatively in a monitoring programme.

CHAPTER 4

FACTORS INFLUENCING RATE OF ACCUMULATION

4.1 GENERAL INTRODUCTION

In Chapter 3 it was shown that rate of accumulation of certain heavy metals by *Mytilus edulis planulatus* is directly related to the external concentration of those metals. By using these relationships, the mussel may successfully be employed to quantify the metal concentration in seawater. The greatest disadvantage in using marine organisms as monitors of metal pollution is the possibility that extraneous factors may interfere in the accumulation of metal(s) by the organism (Phillips 1980). Such factors include those associated with the external environment (temperature, salinity, depth, form and discharge of pollutant) and those related to the organism itself (size, reproductive cycle).

In this Chapter I will examine some of the factors that have been reported to influence the rate of accumulation of heavy metals by mussels. The scope of the literature reviewed has been, in general, restricted to mussels and the heavy metals under consideration (Cd, Cu, Pb and Zn).

4.2 SEASONAL FACTORS

4.2.1 Introduction

Seasonal variations in metal concentrations in bivalve tissue have been reported on a number of occasions (e.g. Fowler and Oregioni 1976; Bryan and Uysal 1978; Majori *et al.* 1978a; Mojo *et al.* 1980; Orren *et al.* 1980 and Shiber 1980). Phillips (1976a) reported a spring maximum in the tissues of *Mytilus edulis* whilst Karbe *et al.* (1977) observed a late winter-spring maximum in the same species.

Despite the wealth of such observations it is unclear whether these changes are directly related to changes in metal accumulation rates. Other authors, e.g. Dare and Edwards (1975), have observed similar seasonal changes occurring in both the body weight and biochemical composition of mussels.

As pointed out by Phillips (1980), there are three inter-related factors that may contribute to an apparent seasonal effect, all of which may individually influence the ability of the mussel to accumulate metals:

(i) the rate of discharge of the pollutant to the environment,

(ii) the physiology of the mussel (reproductive cycle),

(iii) the change in seawater temperature.

4.2.2 The Discharge of the Pollutant

Hsu *et al.* (1979) reported that the seasonal variation in metal concentrations they observed in oysters and clams on the Taiwan coast was due in part to land drainage. The same cause was also proposed by Fowler and Oregioni (1976) and Boyden *et al.* (1979), to explain increased tissue concentrations during winter and spring. They suggested that this may be due to increased weathering and transport of the metals in the catchment areas of estuarine regions, resulting in increased concentrations in the marine environment.

There is one major problem associated with the discharge of pollutants, however, and that is that it is rarely consistent. Input of the pollutant may occur regularly with tides, or sporadically depending on climatic conditions (Ouellette 1981) or industrial discharge. Such periodic inputs of metals to the marine environment often provide the major stimulus for monitoring a particular area. The effects of changing exposure concentrations are examined in Sections 4.5 and 4.6.

4.2.3 Reproductive Cycle

The main physiological factor influencing metal accumulation in mussels seems to be the development of the gonads during the reproductive cycle (gametogenesis). The reproductive cycle is usually closely linked to seasonally varying environmental factors (Seed 1976). Likewise, body weight of mussels was reported by Chamber and Milne (1979) to follow a seasonal cycle, which Simpson (1979) suggested was related to the reproductive cycle. The body weight of mussels generally increases during gametogenesis reaching a peak prior to spawning, and a minimum immediately after it. Delhaye and Cornet (1975) reported that the spawning period coincided with an acceleration of copper accumulation by *M. edulis*, due to an increase in the organism's metabolism. The advent and length of reproductive events in many mussel species is not uniform; and in some cases there may be multiple or extended spawnings, so that the body weight would not change markedly during the year (Seed 1976). This variation may not only be species-dependent but also site-dependent (Seed 1976; Cossa *et al.* 1980).

A relationship between gonad development, body weight and metal concentration in bivalves has been reported by Frazier (1975), Fowler and Oregioni (1976), Phillips (1976a), Romeril (1979), Behrens and Duedall (1981a) and Julshamn (1981c). It appears that while seasonal changes in metal concentrations may occur, the total metal content remains stable. One explanation is that gonad tissues (gametes) are very low in metal content but form a relatively high proportion of the total body weight. In support of this, Pentreath (1973) and Schulz-Baldes (1974) have both shown that the gonads play a very minor role in the accumulation of metals.

Clearly changes in body weight due to the reproductive cycle may have a marked effect on the metal concentrations in mussel tissue. Such changes could confuse the interpretation of the results of a monitor-

ing survey which relied on the measurement of the accumulation rate. However, as Cossa *et al.* (1979) observed, such changes would occur only after the mussel had reached maturity. It is therefore suggested that in order to minimize the possible influence of the reproductive cycle:

(a) where possible only immature individuals should be used;

(b) if the use of mature mussels cannot be avoided, the monitoring period should be kept as short as possible;

(c) a measurement of body weight of each sample should be taken, and if possible a measurement of percentage weight of the gonad tissue as well.

4.2.4 Seawater Temperature

Introduction

Orren *et al.* (1980) reported that the seasonal trends apparent in the metal concentrations of *Choromytilus meridionalis* tissues were not related to gonad development but to the seawater temperature. This interpretation was based on Bryan's (1973) observation that metal concentrations tend to decrease in the warmer months because of the effect of temperature on the rate of excretion. Ouellette (1981) also found no relationship between concentration of metals in *Mytilus californianus* and reproductive condition, and suggested that any seasonal variations observed may be due in part to temperature of the seawater.

Very few laboratory studies on the effects of temperature on metal accumulation in mussels have been conducted. Fowler and Benayoun (1974) failed to find any influence of temperature on the accumulation of cadmium by *Mytilus galloprovincialis;* likewise Jackim *et al.* (1977) found no influence in *M. edulis* during the winter months. Phillips (1976a) reported that the accumulations of zinc and lead by *M. edulis* were also

unaffected by temperature. However, Jackim *et al.* (1977) found that cadmium accumulation was reduced during the summer months, as did Phillips (1976a), at low temperatures. In Phillips' experiments the effect only occurred at low salinities. Fowler *et al.* (1978) observed decreased mercury accumulation, and Ünlü and Fowler (1979) decreased arsenic accumulation in *M. galloprovincialis* at reduced temperatures. It is apparent that the influence of temperature on metal accumulation by mussels is still poorly understood. Accordingly I carried out the following investigation of the effect of temperature on accumulation in *M. e. planulatus*.

Experimental Design

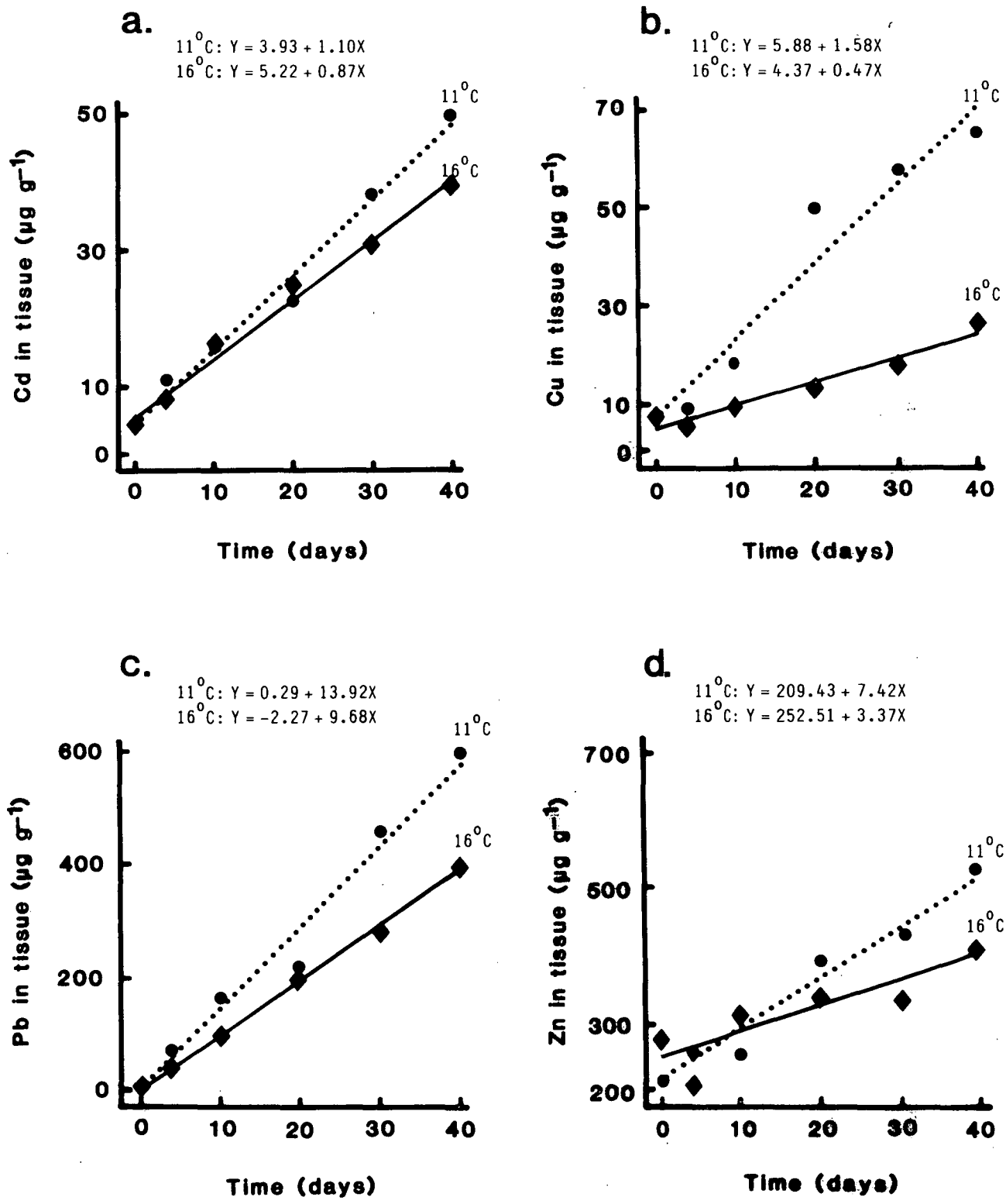
A collection of 140 mussels (4.0 - 5.0 cm shell length, from Barnes Bay) was divided into two equal groups and exposed at either 16(±1) °C or 11(±1)°C to the combination of 10 µg Cd ℓ^{-1}, 10 µg Cu ℓ^{-1}, 50 µg Pb ℓ^{-1} and 100 µg Zn ℓ^{-1}. Samples from both groups were removed for tissue analysis after 4, 10, 20, 30 and 40 days exposure. Experimental tanks were held in constant temperature rooms during the experiment. The mussels were collected during winter (seawater temperature 10°C) and allowed 7 days acclimation, before the experiment began.

Results and Discussion

The accumulation of each metal during the 40 day exposure period is presented in Figure 4.1. In each case mussels in the warmer conditions had lower rates of accumulation than those in the colder conditions. However, the difference was not significant ($p>0.05$) for cadmium accumulation.

While the results presented in Figure 4.1 do not support the conclusions reached by Phillips (1976a), Fowler *et al.* (1978) and Ünlü and Fowler (1979), they are consistent with the observations of Orren *et al.* (1980), Bryan (1973) and Karbe *et al.* (1977), who reported seasonal

Figure 4.1


a.
11°C: Y = 3.93 + 1.10X
16°C: Y = 5.22 + 0.87X
11°C
16°C
Cd in tissue (µg g⁻¹)
Time (days)
b.
11°C: Y = 5.88 + 1.58X
16°C: Y = 4.37 + 0.47X
11°C
16°C
Cu in tissue (µg g⁻¹)
Time (days)
c.
11°C: Y = 0.29 + 13.92X
16°C: Y = -2.27 + 9.68X
11°C
16°C
Pb in tissue (µg g⁻¹)
Time (days)
d.
11°C: Y = 209.43 + 7.42X
16°C: Y = 252.51 + 3.37X
11°C
16°C
Zn in tissue (µg g⁻¹)
Time (days)

maxima in the coldest months. However, the results of Jackim *et al.* (1977) suggest that a temperature response may be influenced by the fact that mussels may be winter or summer acclimatised, with a reduction in cadmium accumulation at low temperatures occurring only in summer mussels. The results in the present study may therefore be related to the fact that the mussels were winter acclimatised. In this context, Bayne *et al.* (1976) suggested that when gametogenesis is active in mussels, a temperature increase inflates the total metabolic rate due to an exponential increase in energy requirement. This results, by feedback, in a reduced ventilation rate and so limits the mussels' capacity to acclimate. The *M. e. planulatus* specimens used in the present study were mature and collected during winter when gametogenesis would be expected to be active (Wisely 1964; Wilson and Hodgkin 1967). Transfer of mussels into seawater at 16°C may have reduced the ventilation rate, as was found by Bayne *et al.* (1976), and so reduced accumulation of the metals. I suggest that the lack of a significant temperature influence on cadmium accumulation may be related to the fact that cadmium accumulation, unlike the other three metals, is independent of the ventilation rate.

Temperature has been shown to influence rate of accumulation of the metals in question. However for monitoring purposes this influence may be minimized by:

(a) transferring mussels between sites possessing similar temperature regimes;

(b) recording temperature regularly during the monitoring period, and keeping this period as short as possible;

(c) conducting appropriate laboratory experiments in order to be able to predict the effects.

4.3 THE INFLUENCE OF MUSSEL SIZE

4.3.1 Introduction

In the present study mussels have been classed according to their shell length rather than weight or age, as it was considered that size was both easier to measure and more reliable than the other two parameters. Most authors refer only to tissue concentrations of the metals varying with size (e.g. Schulz-Baldes 1973; Boyden 1974, 1977; Theede *et al.* 1979; Cossa *et al.* 1980; Kamimura 1980; Boalch *et al.* 1981; Julshamn 1981d; Strong and Luoma 1981), rather than to the influence of size on the rate of accumulation of the metals. Exceptions are the reports by Schulz-Baldes (1974) and Phillips (1976a,b) both on *M. edulis*, and Fowler *et al.* (1978) on *M. galloprovincialis*. The following investigation was carried out to examine the effect of size on rate of accumulation in *M. e. planulatus*.

4.3.2 Experimental Design

Two size classes of *M. e. planulatus* were selected:

(a) 'small', 3.0 - 4.5 cm shell length, and

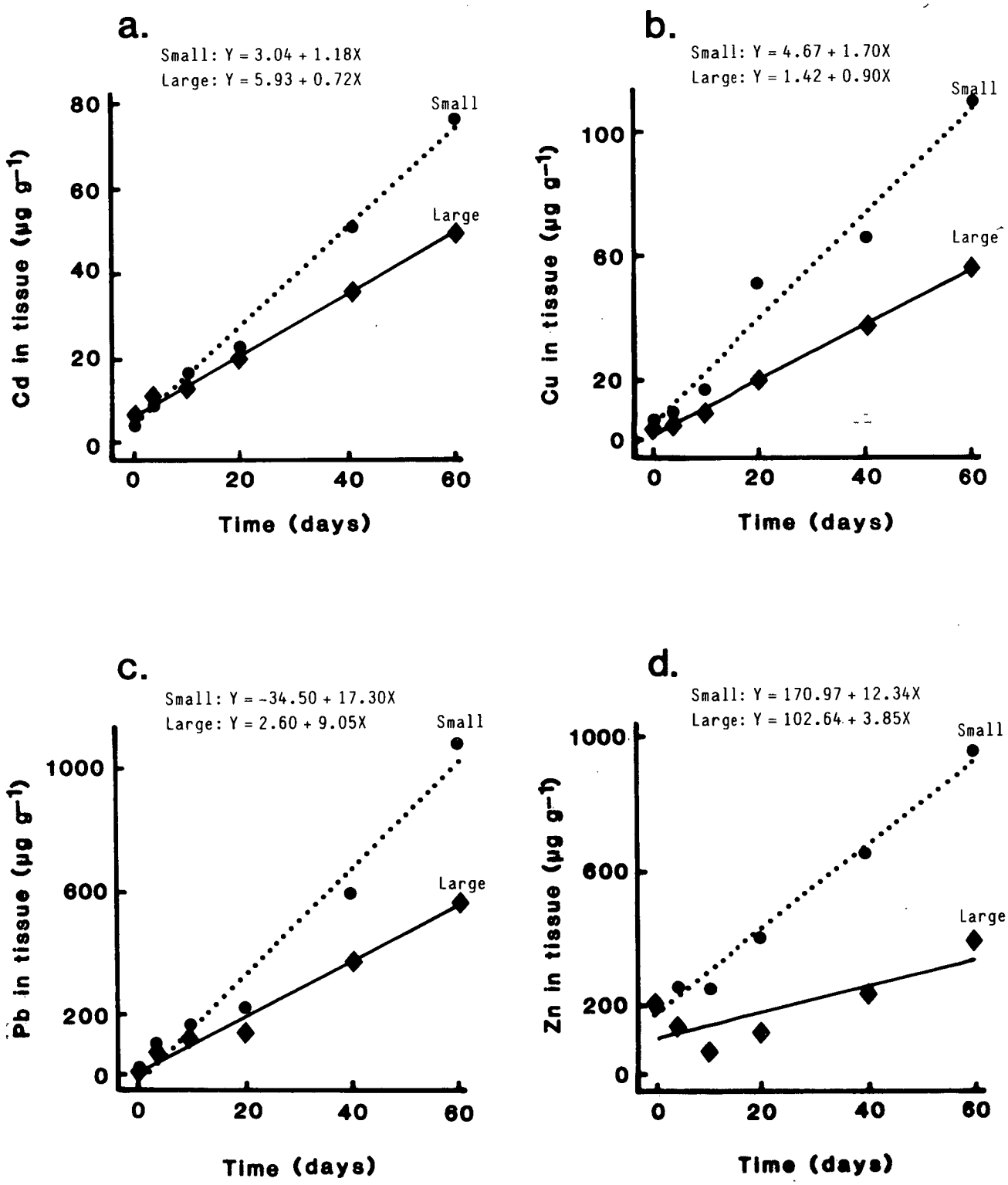
(b) 'large', 6.5 - 8.0 cm shell length.

Eighty mussels of both size classes were exposed, at 11°C, for 60 days to the combination of 10 µg Cd ℓ^{-1}, 10 µg Cu ℓ^{-1}, 50 µg Pb ℓ^{-1} and 100 µg Zn ℓ^{-1}. The mussels were all collected from the same population at Barnes Bay (Figure 2.1) and at the same time. Samples of 10 mussels for each size group were removed from the tanks for tissue analysis after 4, 10, 20, 40 and 60 days exposure.

4.3.3 Results and Discussion

Accumulation of each metal by the two size classes is shown in Figure 4.2. The rate of accumulation by the smaller mussels was

Figure 4.2


a.
Small: Y = 3.04 + 1.18X
Large: Y = 5.93 + 0.72X
Small
Large
Cd in tissue (µg g⁻¹)
Time (days)
b.
Small: Y = 4.67 + 1.70X
Large: Y = 1.42 + 0.90X
Small
Large
Cu in tissue (µg g⁻¹)
Time (days)
c.
Small: Y = -34.50 + 17.30X
Large: Y = 2.60 + 9.05X
Small
Large
Pb in tissue (µg g⁻¹)
Time (days)
d.
Small: Y = 170.97 + 12.34X
Large: Y = 102.64 + 3.85X
Small
Large
Zn in tissue (µg g⁻¹)
Time (days)

significantly greater than that by the larger mussels for all four metals ($p<0.05$). These results for *M. e. planulatus* are in agreement with those of other authors. Fowler *et al.* (1978), for example, found that small individual *M. galloprovincialis* had a faster rate of uptake of mercury, whilst both Schulz-Baldes (1974) and Phillips (1976a,b) reported greater uptake rates by smaller *M. edulis*.

A greater rate of accumulation of a metal means a greater change in tissue concentration over a given time. Consequently, if rate of metal accumulation is to be used as an index of external concentration, then smaller individuals should yield more satisfactory results. The greater change in tissue concentration would provide greater precision in both the analysis of the tissue and in the comparison of monitoring sites and time intervals. Smaller mussels would also be more likely to be immature thus satisfying another requirement noted in Section 4.2.3.

4.4 COMPARISON OF DIFFERENT POPULATIONS

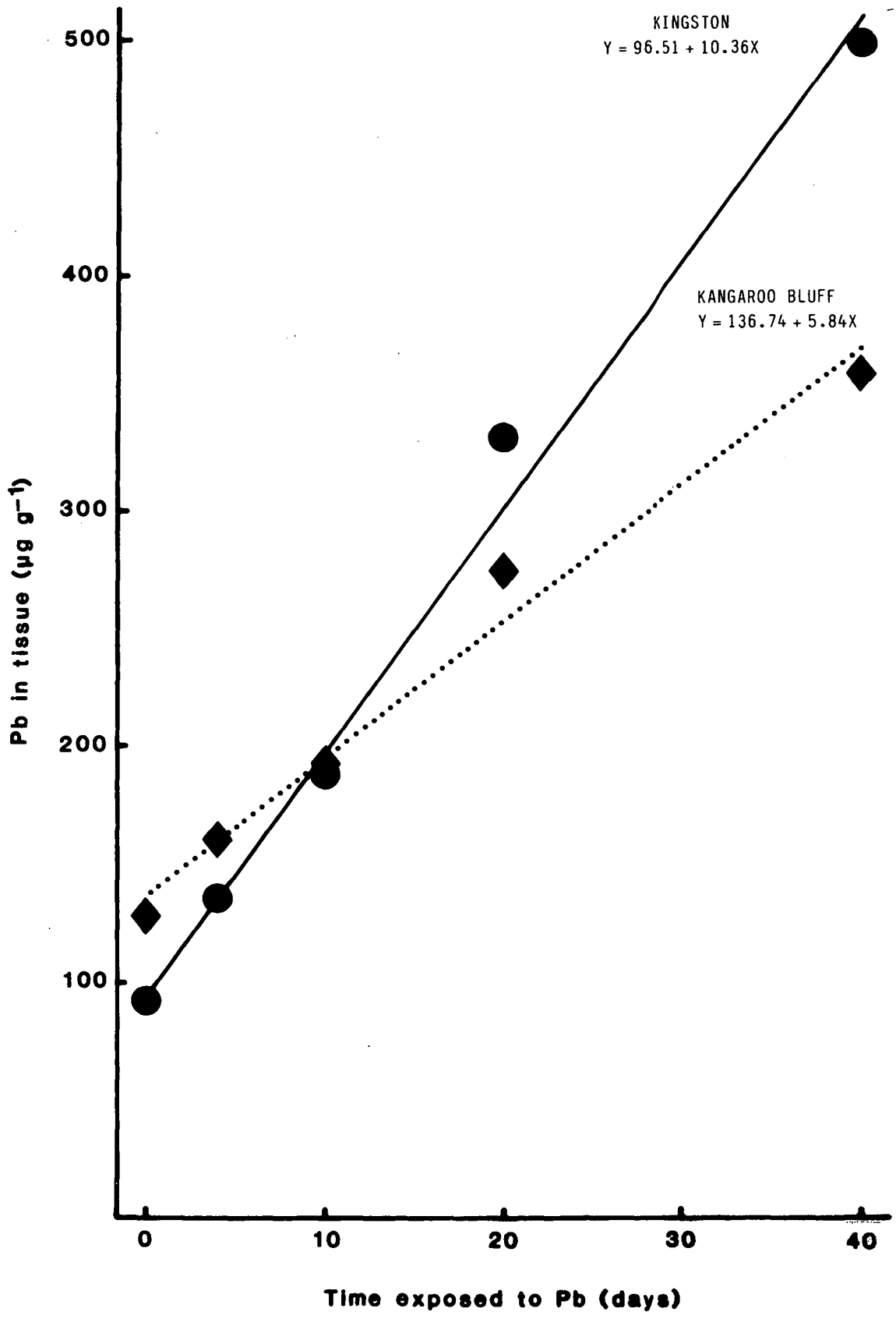
Gartner-Kepkay *et al.* (1980) considered that as genetic differences will alter the physiological responses of populations to various environmental variables, the differences between populations must be considered when using mussels as environmental monitoring organisms.

An example of the difference in rate of accumulation of a metal between two populations is presented in Figure 4.3, which shows the accumulation of lead by similar sized (3.5 - 4.0 cm shell length) mussels when exposed to 50 μg Pb ℓ^{-1} for 40 days. The mussels were collected from similar tidal levels, on the same low tide, from:

(a) Kangaroo Bluff, and (b) Kingston (see Figure 2.1).

Mussels from Kangaroo Bluff had a higher background tissue concentration of lead, but accumulated lead at a reduced rate compared to those from the Kingston population.

Figure 4.3


KINGSTON
Y = 96.51 + 10.36X
KANGAROO BLUFF
Y = 136.74 + 5.84X
500
400
300
200
100
Pb in tissue (µg g⁻¹)
0
10
20
30
40
Time exposed to Pb (days)

The rate of accumulation of lead by the 'small' mussels from Barnes Bay (Figure 4.2) was significantly greater (17.30 cf 10.36 and 5.84 $\mu g\ g^{-1}\ day^{-1}$) than that of either Kingston ($p<0.01$) or Kangaroo Bluff ($p<0.001$) mussels (Figure 4.3). The increase in accumulation rate might be related to the lower initial tissue concentration of lead in Barnes Bay compared to Kingston mussels, and similarly Kingston to Kangaroo Bluff mussels. Comparison with the results in Figure 4.2 may not be entirely valid, however, as these mussels were simultaneously exposed to elevated concentrations of cadmium, copper and zinc. Nevertheless the question still arises as to whether the variation in accumulation rates is due to population differences, i.e. genetical variation, or due to the mussels' history of exposure to the metal, i.e. initial tissue concentration.

Whatever the answer to this question, which is examined further in Section 4.5, it is suggested that individuals to be used in a monitoring programme should be obtained from a single population. In order to obtain a high sensitivity to concentration changes (i.e. a high rate of accumulation), they should come from a relatively pristine environment.

4.5 THE INFLUENCE OF A PREVIOUS EXPOSURE

The possibility that an elevated tissue concentration resulting from previous exposure to a metal may influence subsequent accumulation of that metal was examined by the following series of experiments:

4.5.1 Cadmium Accumulation

Experimental Design

A collection of 250 mussels (3.0 - 4.0 cm shell length, from Howden) was divided into five equal groups, each of which was exposed, at 11° C, to one of the following concentrations of cadmium for 6 days:

Cd_0 - background concentrations,

Cd_1 - 1 µg Cd ℓ^{-1},

Cd_2 - 5 µg Cd ℓ^{-1},

Cd_3 - 20 µg Cd ℓ^{-1},

Cd_4 - 40 µg Cd ℓ^{-1}.

At the end of the 6 day exposure each group was then exposed to 10 µg Cd ℓ^{-1} for a further 20 days. Mussels were sampled for analysis after the initial 6 day exposure and then after 4, 10 and 20 days subsequent exposure. The general experimental design, described above, also applies in Sections 4.5.2, 4.5.3 and 4.5.4 to follow.

Results

Linear regressions were calculated for each group of mussels, and are presented in Figure 4.4. A t-test comparison of the slopes of the regressions revealed that all five groups had a similar rate of accumulation of cadmium after the final 20 days, despite having different initial tissue concentrations.

4.5.2 Copper Accumulation

Experimental Design

Five groups of mussels were exposed for the initial 6 days to one of the following concentrations of copper:

Cu_0 - background concentrations,

Cu_1 - 5 µg Cu ℓ^{-1},

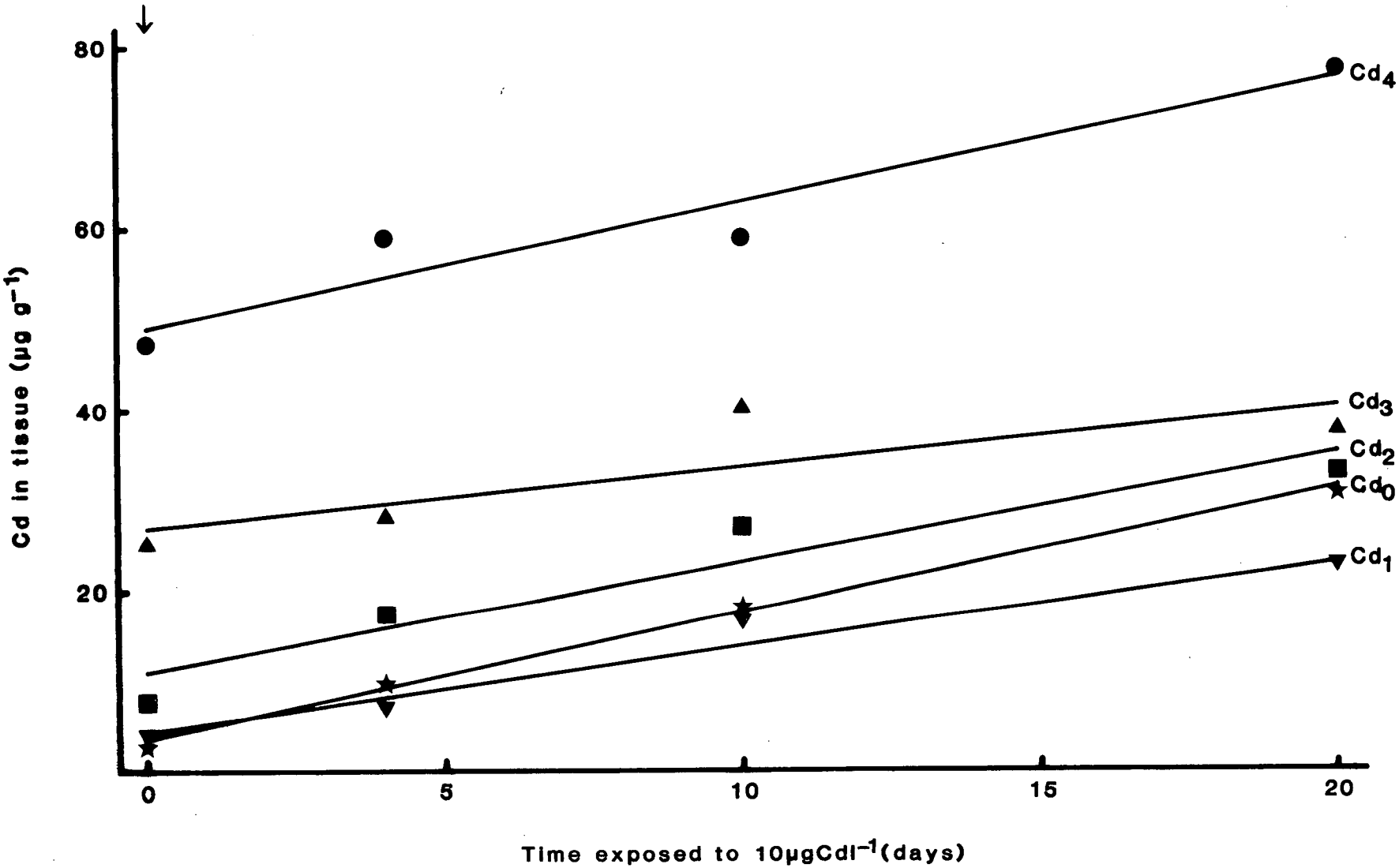
Cu_2 - 10 µg Cu ℓ^{-1},

Cu_3 - 20 µg Cu ℓ^{-1},

Cu_4 - 30 µg Cu ℓ^{-1}.

Each group was then exposed to 15 µg Cu ℓ^{-1} for a further 20 days.

Figure 4.4


80
60
40
20
0
5
10
15
20
Cd in tissue (µg g⁻¹)
Time exposed to 10µgCdl⁻¹(days)
Cd4
Cd3
Cd2
Cd0
Cd1

Results

The only group to display a linear accumulation of copper over the 20 day period was the group Cu_0, originally exposed only to background concentrations (Figure 4.5). As can be seen in Figure 4.5, the other four groups accumulated copper but only for the first 10 days exposure to 15 µg Cu ℓ^{-1}. After that time they either lost copper from the tissue or maintained a consistent tissue concentration.

This apparent saturation of the tissues by copper was unexpected as higher tissue concentrations of copper have been recorded in this mussel. It is therefore suggested that an initial exposure to copper, i.e. elevated tissue concentrations, may influence the accumulation of copper during a subsequent exposure.

4.5.3 Lead Accumulation

Experimental Design

Five groups of mussels were exposed for the initial 6 days to one of the following concentrations of lead:

Pb_0 - background concentrations,
Pb_1 - 5 µg Pb ℓ^{-1},
Pb_2 - 10 µg Pb ℓ^{-1},
Pb_3 - 30 µg Pb ℓ^{-1},
Pb_4 - 50 µg Pb ℓ^{-1}.

Each group was then exposed to 20 µg Pb ℓ^{-1} for a further 20 days.

Results

All five groups had a similar rate of accumulation of lead after the final 20 days, despite having different initial tissue concentrations (Figure 4.6).

Figure 4.5

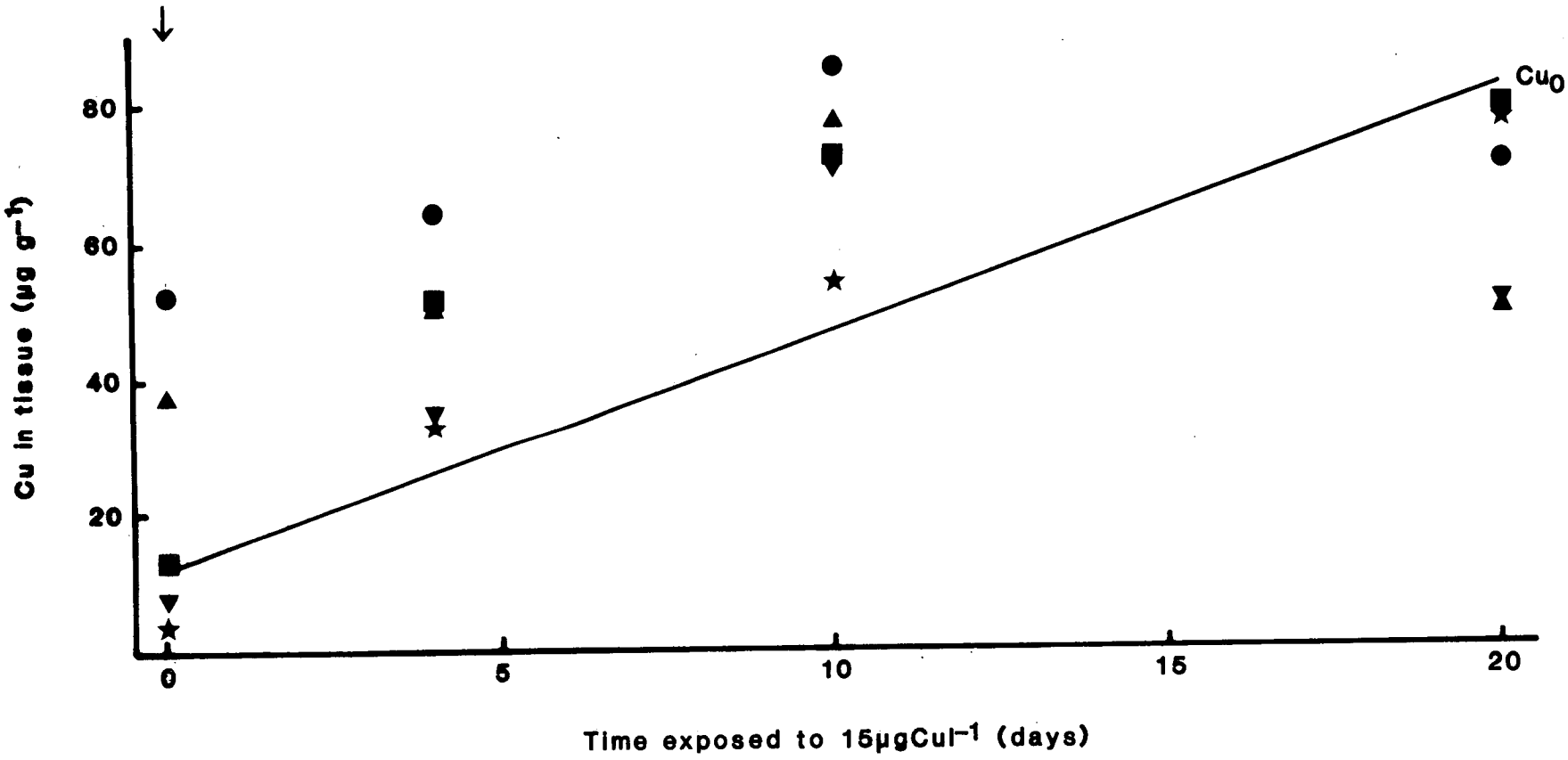
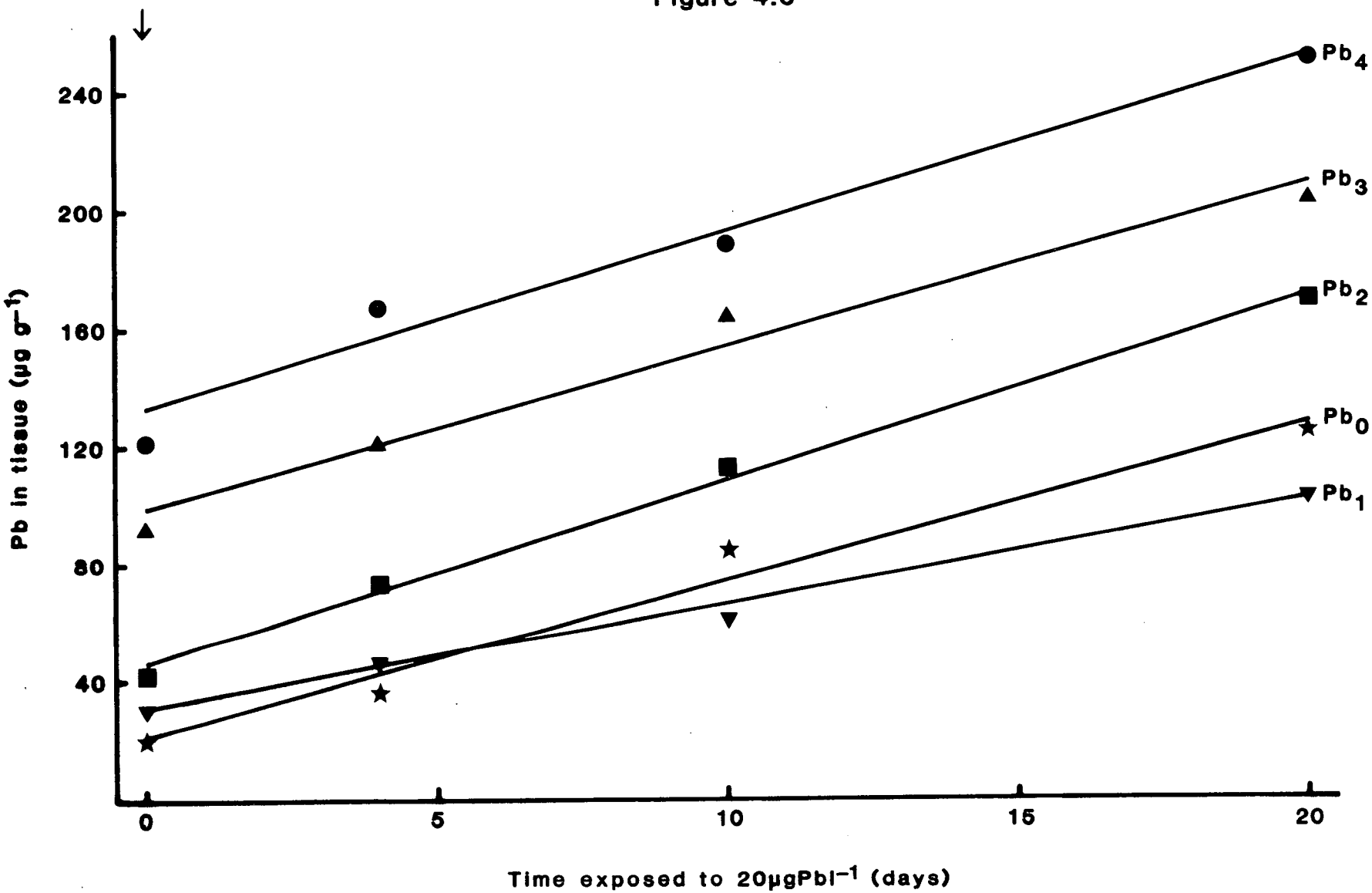

Cu in tissue (µg g⁻¹)
80
60
40
20
0
5
10
15
20
Cu0
Time exposed to 15µgCu l⁻¹ (days)

Figure 4.6


Pb in tissue (µg g⁻¹)
240
200
160
120
80
40
0
5
10
15
20
Time exposed to 20µgPbl⁻¹ (days)
Pb4
Pb3
Pb2
Pb0
Pb1

4.5.4 Zinc Accumulation

Experimental Design

Five groups of mussels were exposed for the initial 6 days to one of the following concentrations of zinc:

Zn_0 - background concentrations,
Zn_1 - 25 µg Zn ℓ^{-1},
Zn_2 - 50 µg Zn ℓ^{-1},
Zn_3 - 200 µg Zn ℓ^{-1},
Zn_4 - 400 µg Zn ℓ^{-1}.

Each group was then exposed to 100 µg Zn ℓ^{-1} for a further 20 days.

Results

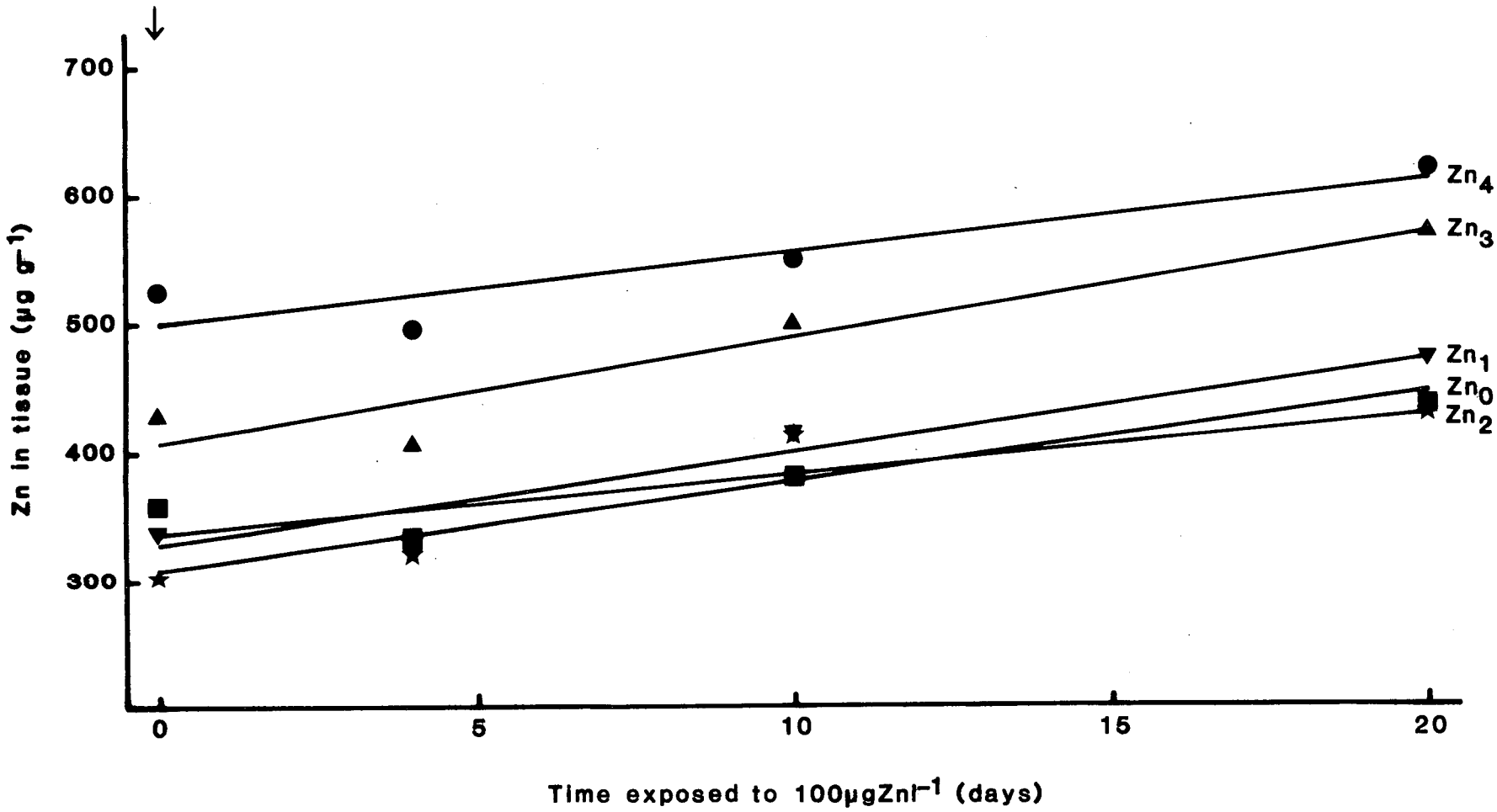
All five groups had a similar rate of accumulation of zinc after the final 20 days, despite having different initial tissue concentrations (Figure 4.7).

4.5.5 The Influence on Cadmium Accumulation of a Longer Previous Exposure

Experimental Design

This experiment consisted of three phases each of 20 days duration. During Phases I and III mussels were exposed to cadmium at 10 µg ℓ^{-1}, while during the intermediate Phase II they were exposed to background concentrations. A total of 240 mussels (4.0 - 5.0 cm shell length) were collected from Barnes Bay and divided into two equal groups. One group was exposed to the above experimental conditions and the other to background concentrations only for the 60 days (i.e. control mussels); both exposures were at 11°C water temperature. Ten mussels were sampled from both groups on days 4, 10 and 20 of each phase.

Figure 4.7


700
600
500
400
300
Zn in tissue (µg g⁻¹)
0
5
10
15
20
Time exposed to 100µgZnl⁻¹ (days)
Zn4
Zn3
Zn1
Zn0
Zn2

Results

The tissue concentrations of cadmium recorded during the experiment are presented in Figure 4.8. Accumulation of cadmium during the exposures to 10 μg Cd ℓ^{-1} (Phases I and III) was linear, and the regression slopes for Phase I and Phase III were similar, i.e. accumulation of cadmium in Phase III was not influenced by the mussels' previous 40 day history of exposure. During Phase II, however, the mussels also accumulated cadmium, from background concentrations, but at a rate greater than that recorded by the control mussels (Figure 4.8).

4.5.6 Conclusions

(i) An elevated tissue concentration had no influence on the rate of accumulation of cadmium, lead or zinc.

(ii) An elevated tissue concentration of copper may reduce the total accumulation of copper within mussel tissues.

(iii) Following an exposure to elevated concentrations of cadmium, an increased accumulation of cadmium from background concentrations may occur.

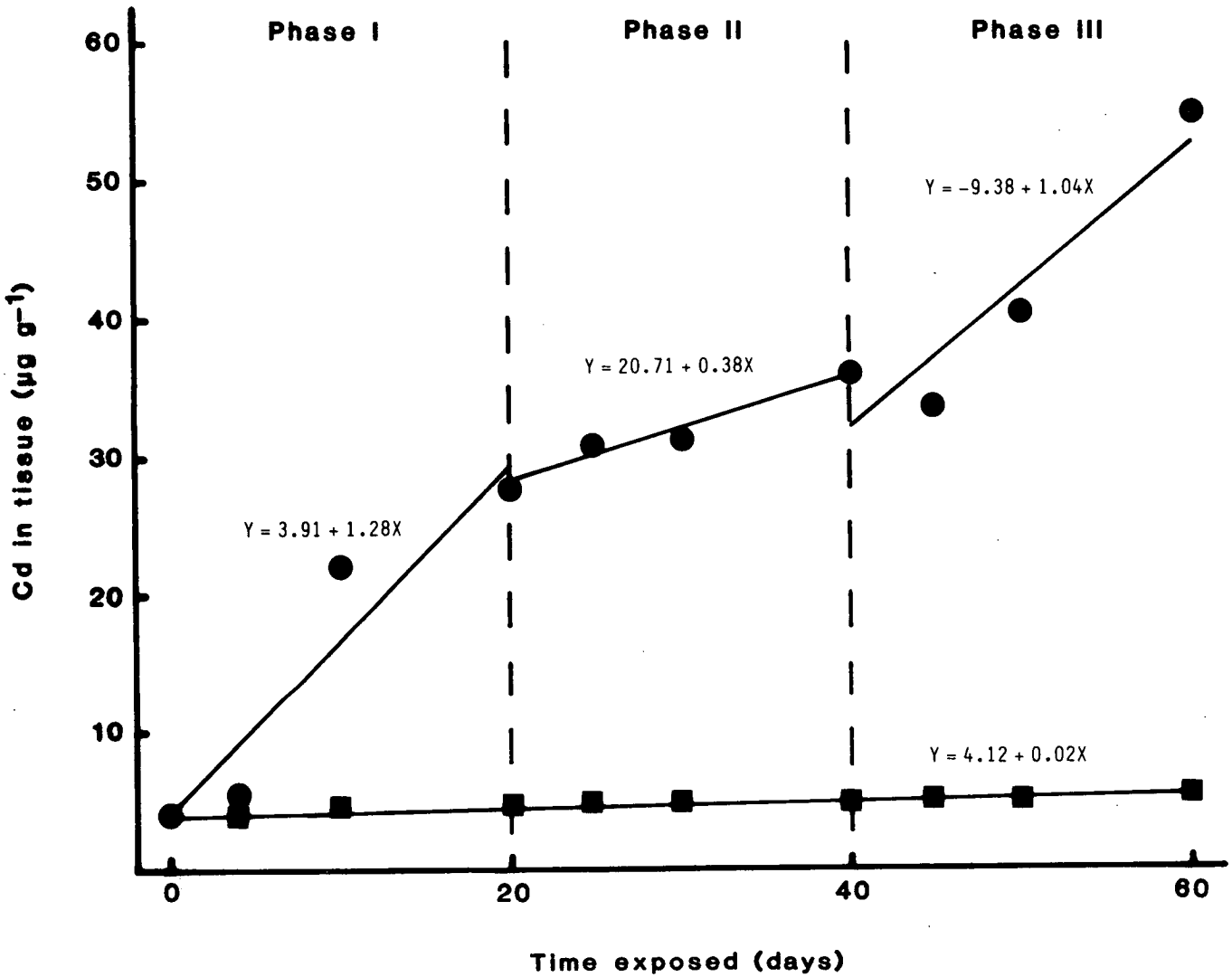
(iv) The population influence described in Section 4.4 was not due simply to elevated tissue concentrations. It is therefore assumed that either a genetic component is involved, or the long-term history (measured in months or years rather than days) of exposure has an influence on the rate of metal accumulation.

4.6 THE INFLUENCE OF A CYCLED EXPOSURE

4.6.1 Introduction

The delivery of heavy metal contaminants into marine environments may not be continuous, but may occur regularly with tides or both

Figure 4.8


Phase I
Phase II
Phase III
Y = 3.91 + 1.28X
Y = 20.71 + 0.38X
Y = -9.38 + 1.04X
Y = 4.12 + 0.02X
Cd in tissue (µg g⁻¹)
60
50
40
30
20
10
0
20
40
60
Time exposed (days)

regularly and irregularly from industrial discharge. One of the advantages of using marine organisms as monitors of metal pollution (Chapter 1) was the possibility that they would provide a time integrated measure of the metal concentration present in the seawater. This would depend on metal accumulation by the mussel being related to the exposure time. In this context, Brooks and Rumsby (1967) reported that the absorption (sic) of cadmium by oysters was discontinuous and occurred predominantly at night. Moreover, Coleman (1980) observed that mussels subjected to periods of emersion did not accumulate cadmium in proportion to the time spent immersed.

The following experiments were designed to examine whether the rate of accumulation of the four metals cadmium, copper, lead and zinc by *M. e. planulatus* is proportional to the time exposed to the elevated concentrations.

4.6.2 Experimental Design

In each of the following five experiments a collection of 150 mussels (4.0 - 5.0 cm shell length, from Barnes Bay) was divided into two equal groups and presented for 40 days with either:

(i) a continuous exposure to an elevated metal concentration;
or
(ii) an exposure alternating every 2 days between the elevated metal concentration and the background concentration.

A 2 day cycle corresponded to the routine seawater changes in the experimental tanks (Chapter 2). Mussels were sampled for tissue analysis after 0, 4, 10, 20 and 40 days. A sample was also removed on day 1 of the cadmium experiment, which was also continued for a further 10 days.

To analyse the results of the experiments it was assumed that, if the metal was accumulated in direct proportion to the time exposed

to the elevated concentration then:

$$\frac{b_2}{b_1} \text{ should be equal to } 0.5,$$

where b_1 = rate of accumulation by the mussels under continuous exposure i.e. regression coefficient, and b_2 = the rate of accumulation under cycled exposure. The two regression coefficients were compared by t-test to determine whether their ratio was equal to 0.5.

4.6.3 Cadmium

Experiment and Results

The elevated external concentration of cadmium used in this experiment was 10 µg ℓ^{-1}. Up to day 20 of the exposure period there was very little evidence of a difference in the accumulation of cadmium by the two groups (Figure 4.9). After day 20 the mussels under the cycled conditions exhibited a reduced accumulation of cadmium, and in fact appear to be able to regulate the concentration in their tissue.

Conclusion

Cadmium accumulation by *M. e. planulatus* was not in direct proportion to the time exposed to the elevated external concentration.

4.6.4 Copper

Experiment and Results

The copper level used in this experiment was 10 µg ℓ^{-1}. Both groups of mussels exhibited a linear accumulation of copper over the 40 day period, those under the cycled conditions having the lower rate of accumulation (Figure 4.10). However, the ratio of the rates of accumulation (i.e. regression slopes) was found to be significantly ($p<0.05$) greater than the ratio of exposure times: 0.82 cf 0.50.

Figure 4.9

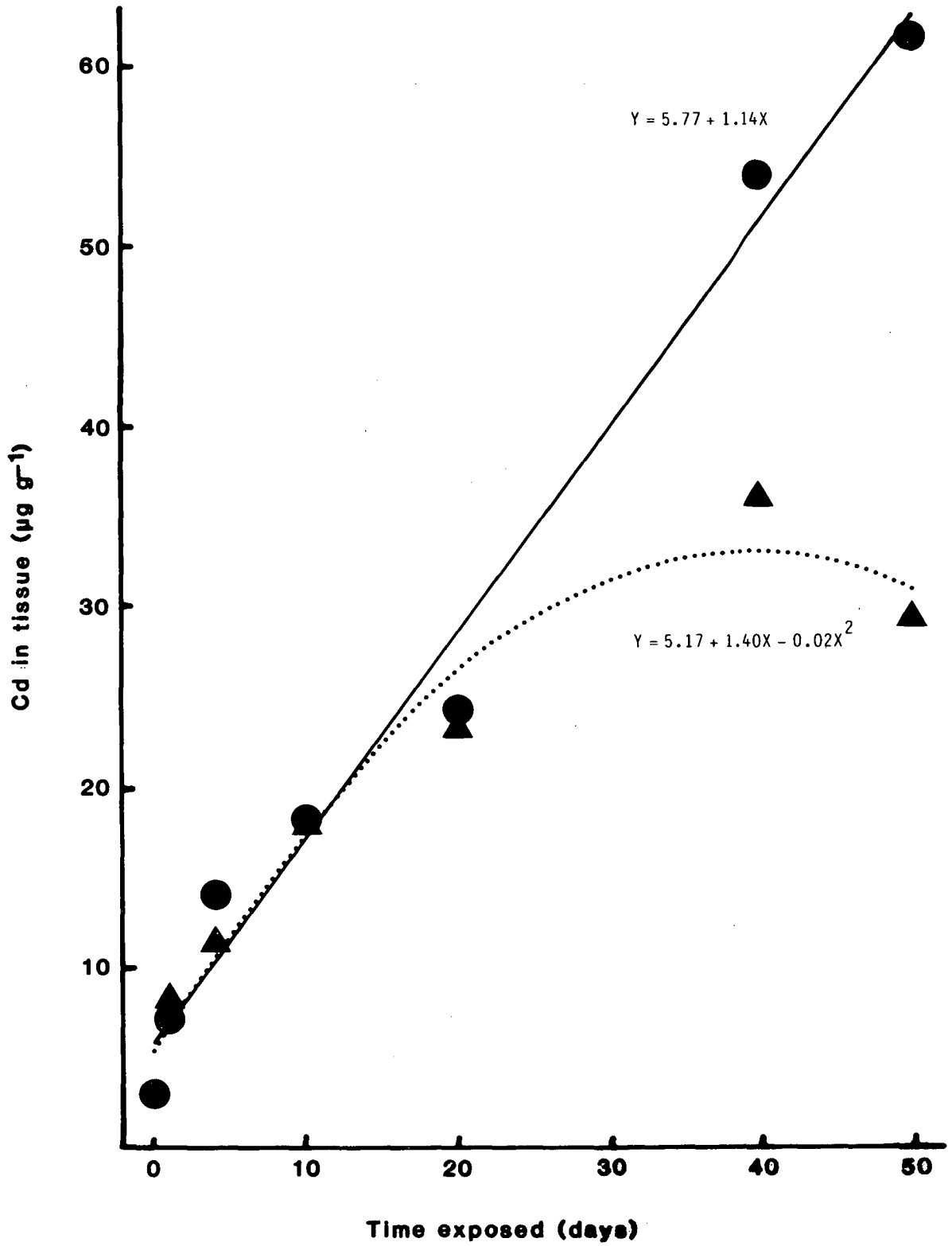
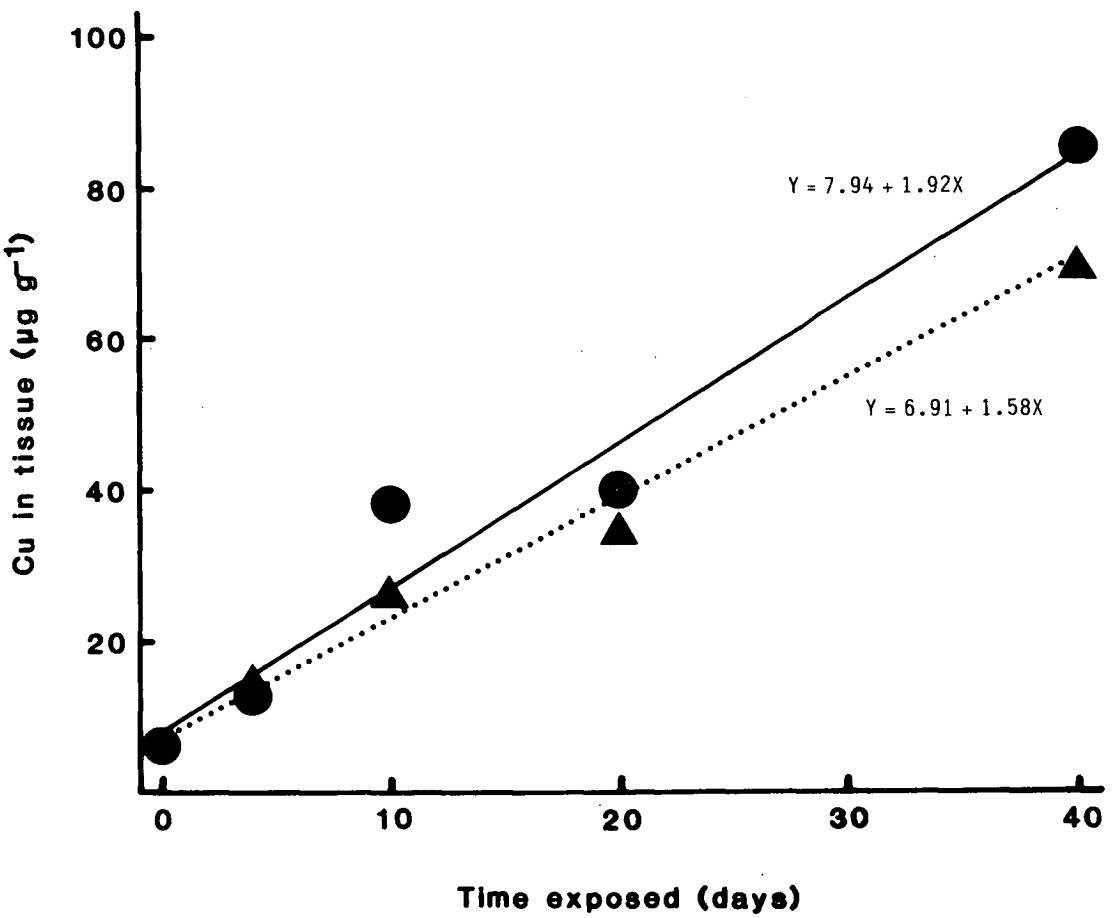

60
50
40
30
20
10
0
10
20
30
40
50
Y = 5.77 + 1.14X
Y = 5.17 + 1.40X - 0.02X2
Cd in tissue (µg g-1)
Time exposed (days)

Figure 4.10


100
80
60
40
20
Cu in tissue (µg g⁻¹)
Y = 7.94 + 1.92X
Y = 6.91 + 1.58X
0
10
20
30
40
Time exposed (days)

Conclusion

Copper accumulation by *M. e. planulatus* was not in direct proportion to the time exposed to the elevated external concentration.

4.6.5 Lead

Experiment and Results

The lead level used in this experiment was 20 µg Pb ℓ^{-1}. Both groups of mussels exhibited a linear accumulation of lead over the 40 day period, those under the cycled conditions having the lower rate of accumulation (Figure 4.11). The ratio of the rates of accumulation was not significantly ($p>0.05$) different from the ratio of exposure times: 0.48 cf 0.50.

Conclusion

Lead accumulation by *M. e. planulatus* was in direct proportion to the time exposed to the elevated external concentration.

4.6.6 Zinc

Experiment and Results

The zinc level used in this experiment was 200 µg ℓ^{-1}. Both groups of mussels exhibited a linear accumulation of zinc over the 40 day period, those under the cycled conditions having the lower rate of accumulation (Figure 4.12). The ratio of the rates of accumulation was not significantly ($p>0.05$) different from the ratio of exposure times: 0.60 cf 0.50.

Conclusion

Zinc accumulation by *M. e. planulatus* was in direct proportion to the time exposed to the elevated external concentration of zinc.

Figure 4.11

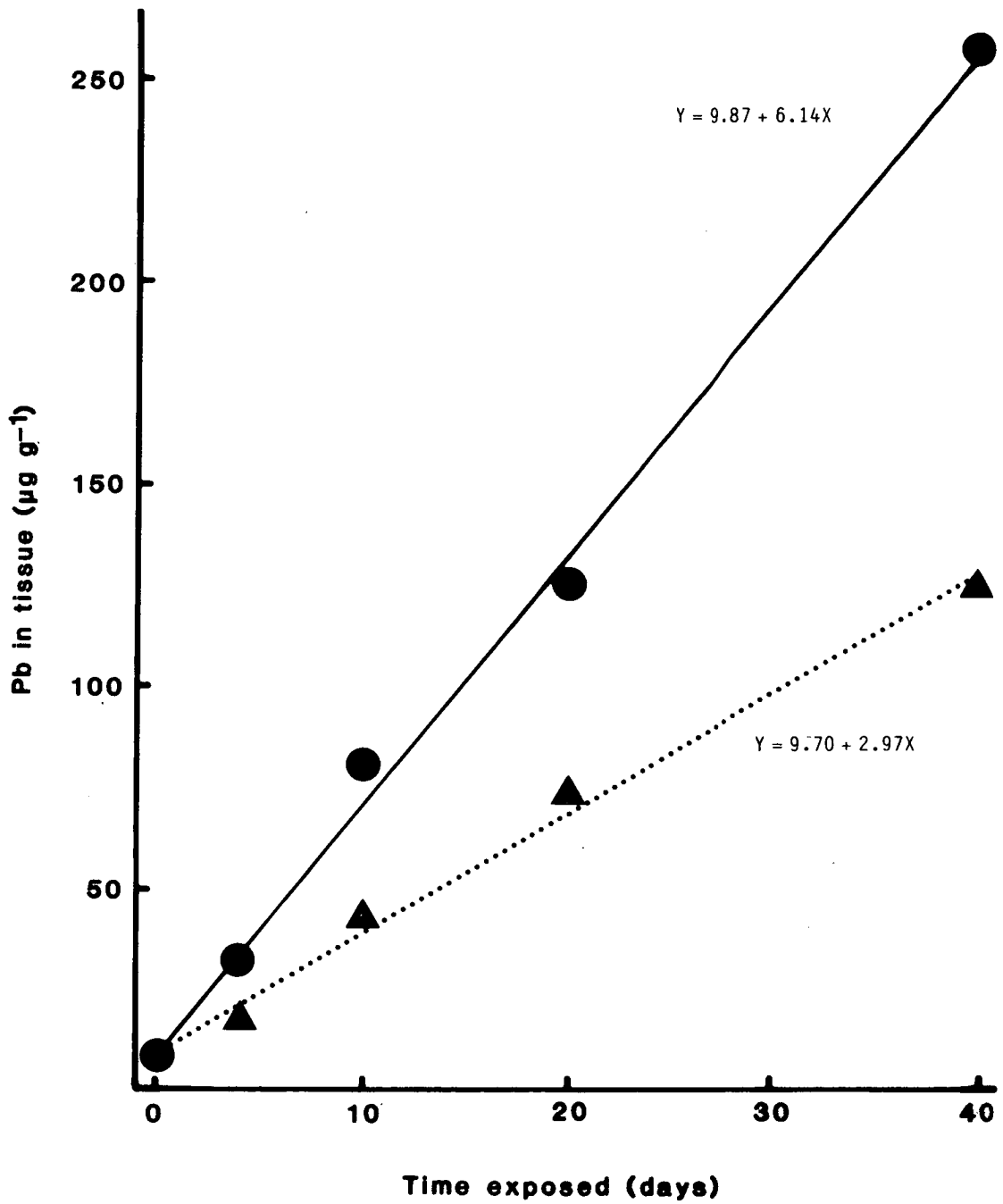
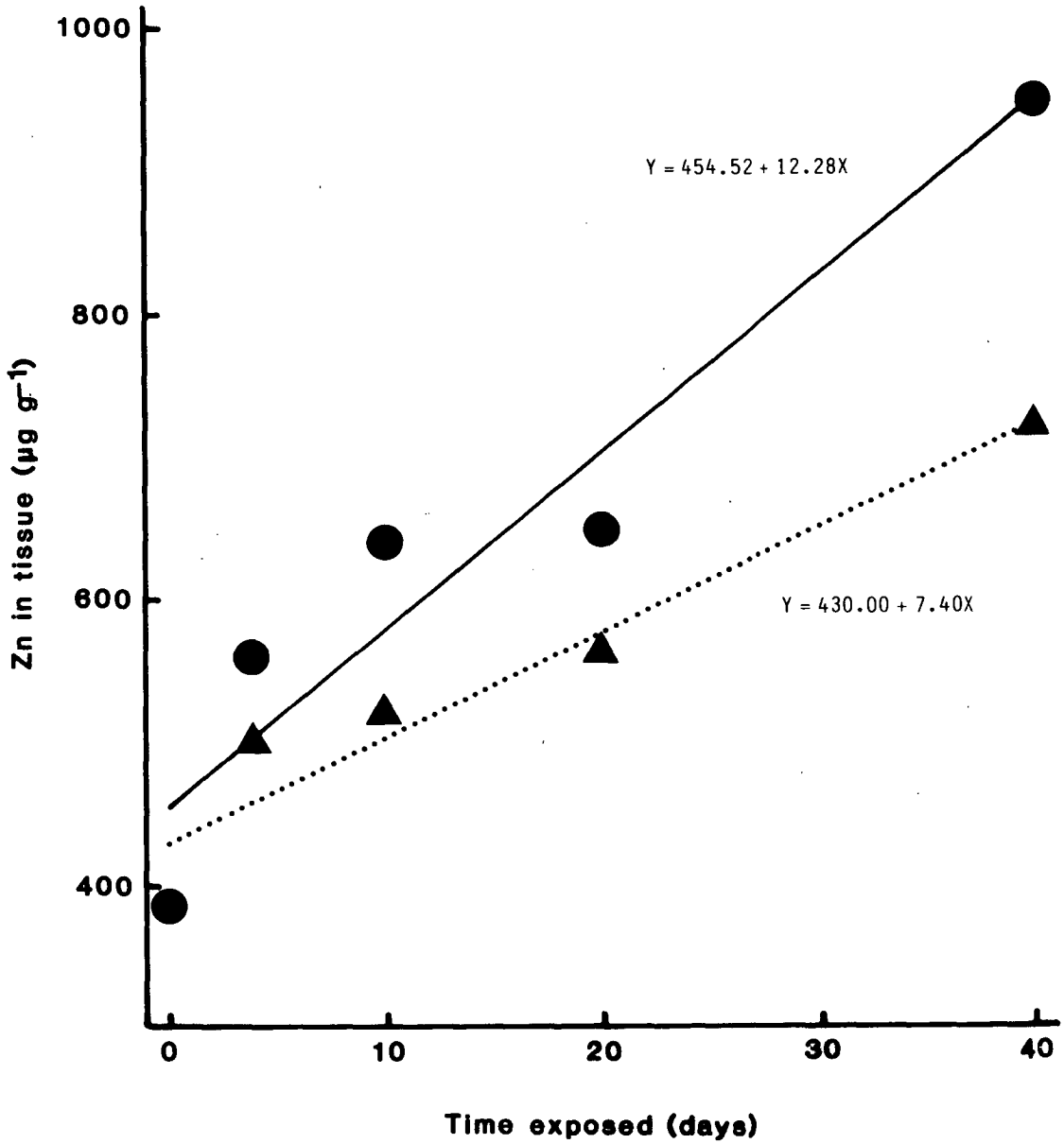

250
200
150
100
50
Pb in tissue (µg g⁻¹)
Y = 9.87 + 6.14X
Y = 9.70 + 2.97X
0
10
20
30
40
Time exposed (days)

Figure 4.12


1000
800
600
400
Zn in tissue (µg g⁻¹)
Y = 454.52 + 12.28X
Y = 430.00 + 7.40X
0
10
20
30
40
Time exposed (days)

4.6.7 Combination of All Four Metals

Experiment and Results

The elevated external concentration consisted of the combination: 10 µg Cd ℓ^{-1}, 10 µg Cu ℓ^{-1}, 20 µg Pb ℓ^{-1} and 200 µg Zn ℓ^{-1}. Both groups of mussels exhibited a linear accumulation of all four metals over the 40 day period, for each metal those under cycled conditions having the lower rate of accumulation (Figures 4.13 to 4.16). The ratio of the rates of accumulation was not significantly ($p>0.05$) different from the ratio of exposure times for both cadmium (0.57 cf 0.50, Figure 4.13) and lead (0.52 cf 0.50, Figure 4.15) accumulation. In the case of both copper (0.77 cf 0.50, Figure 4.14) and zinc (0.93 cf 0.50, Figure 4.16), rate of accumulation under cycled conditions was found to be significantly ($p<0.05$) greater than half that under continuous exposure.

Conclusion

When introduced to a combination of elevated metal concentrations the accumulation of both cadmium and lead by *M. e. planulatus* was directly proportional to the time exposed; however this was not the case with copper and zinc.

4.6.8 Comparison of Combined and Single Exposure

Accumulation of Cadmium

Comparison of the results presented in Figures 4.9 and 4.13 indicates that the mussels under continuous exposure exhibited similar rates of accumulation of cadmium. However, accumulation of cadmium by mussels in cycled conditions was greatly reduced in the presence of the other three metals. The reduction is particularly evident during the first 20 days of the exposure. The most significant aspect of this reduction is the fact that under combined metal exposure accumulation

Figure 4.13

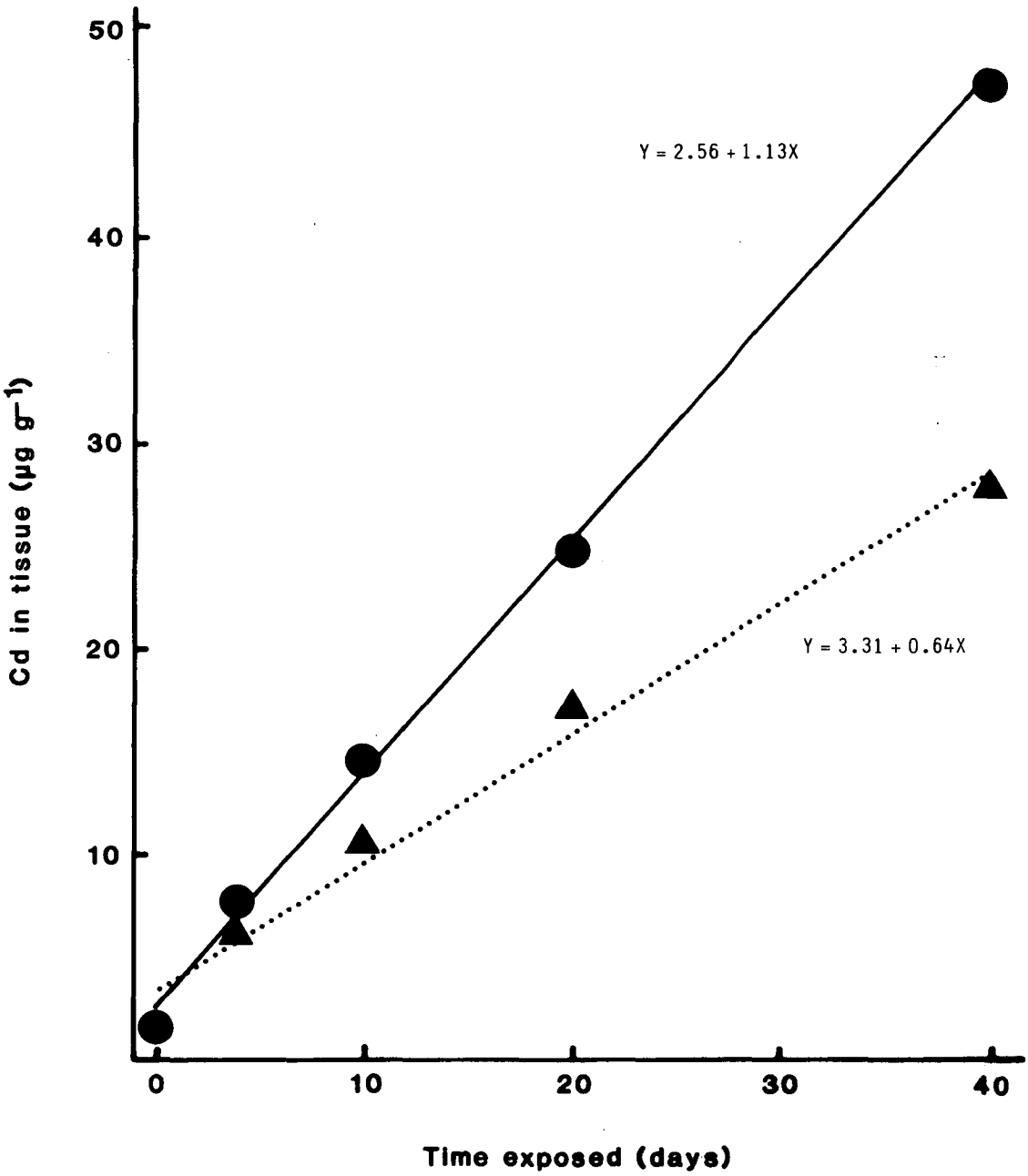

50
40
30
20
10
0
10
20
30
40
Cd in tissue (µg g⁻¹)
Time exposed (days)
Y = 2.56 + 1.13X
Y = 3.31 + 0.64X

Figure 4.14

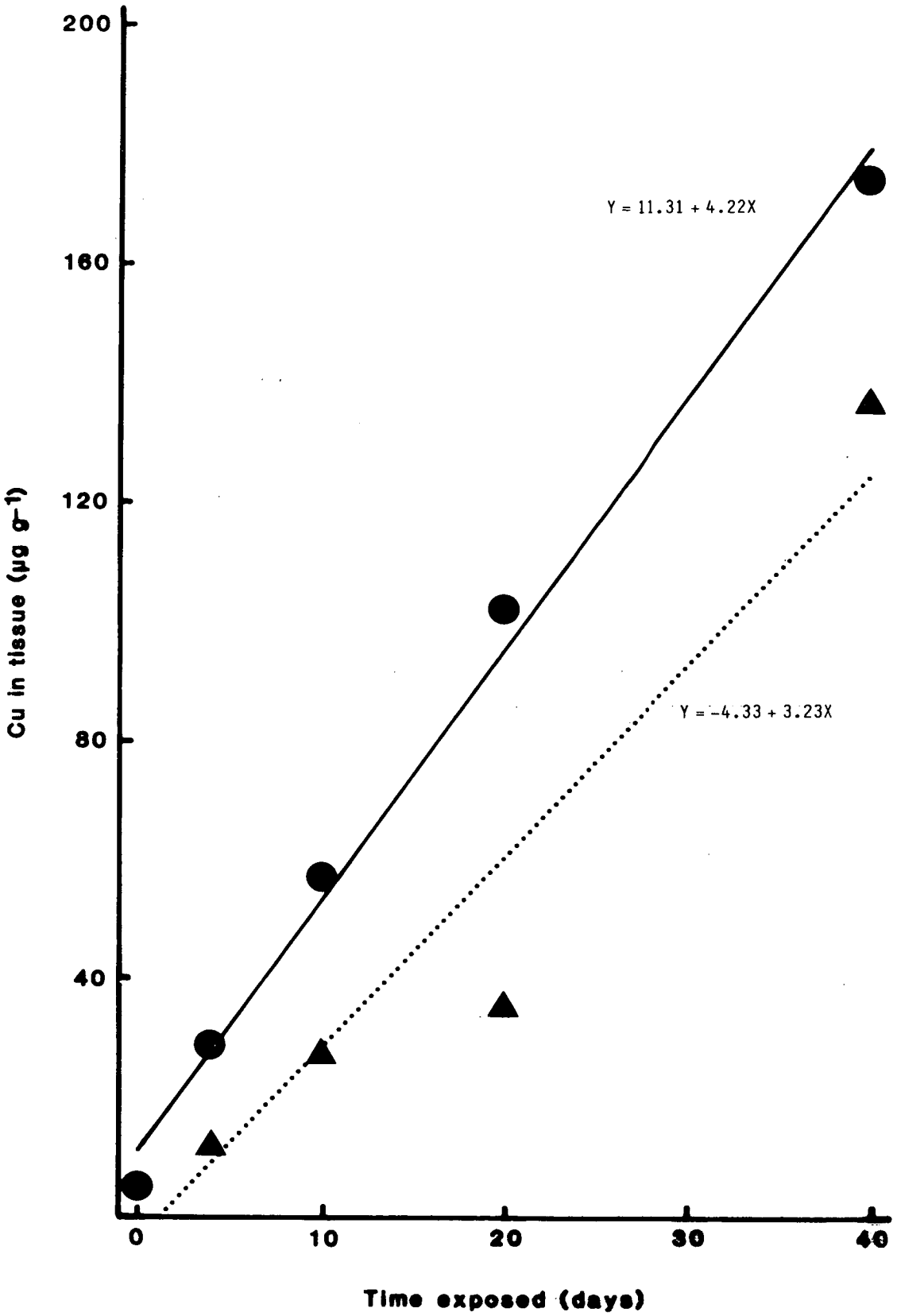

200
160
120
80
40
Cu in tissue (µg g⁻¹)
Y = 11.31 + 4.22X
Y = -4.33 + 3.23X
0
10
20
30
40
Time exposed (days)

Figure 4.15

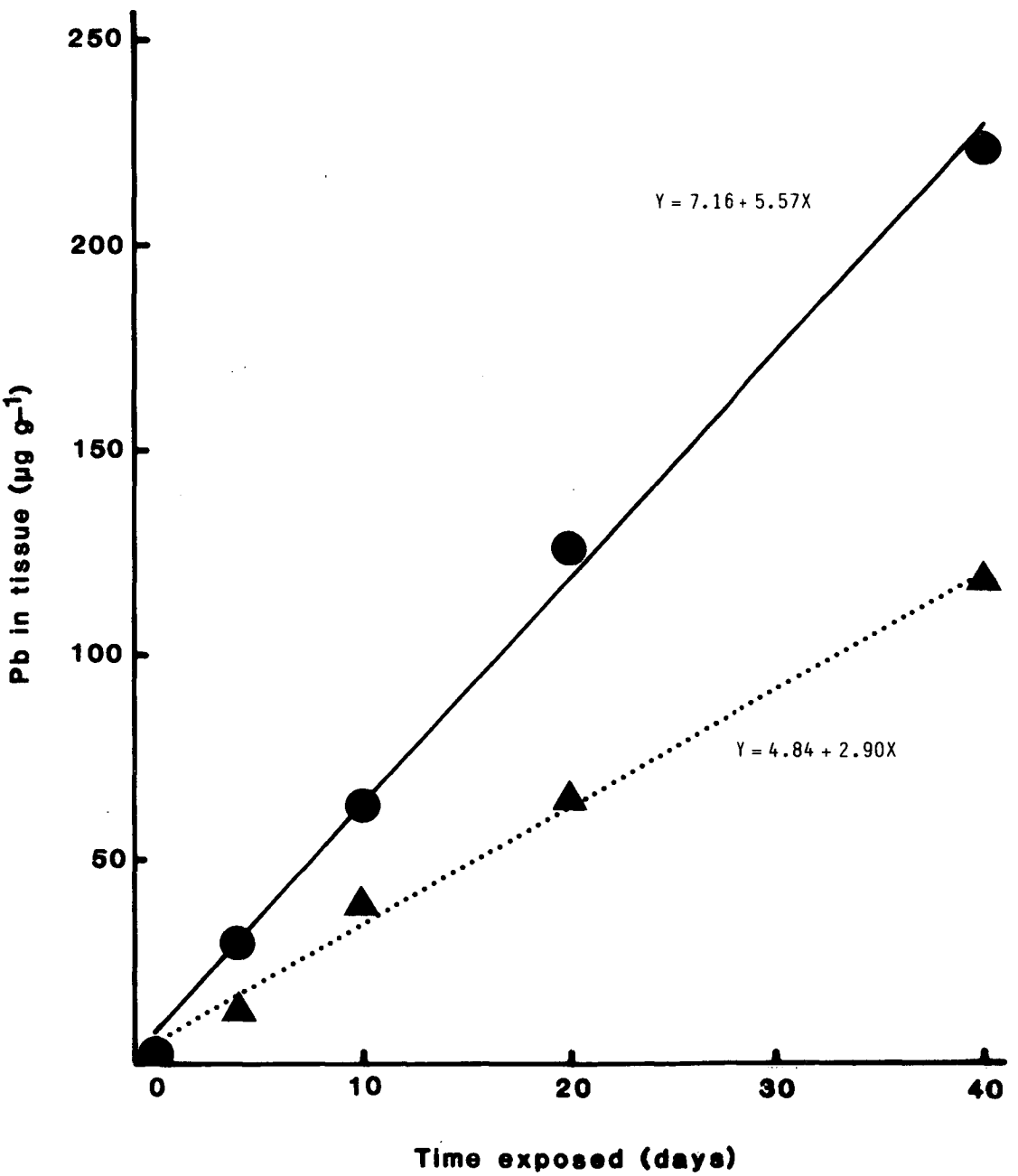
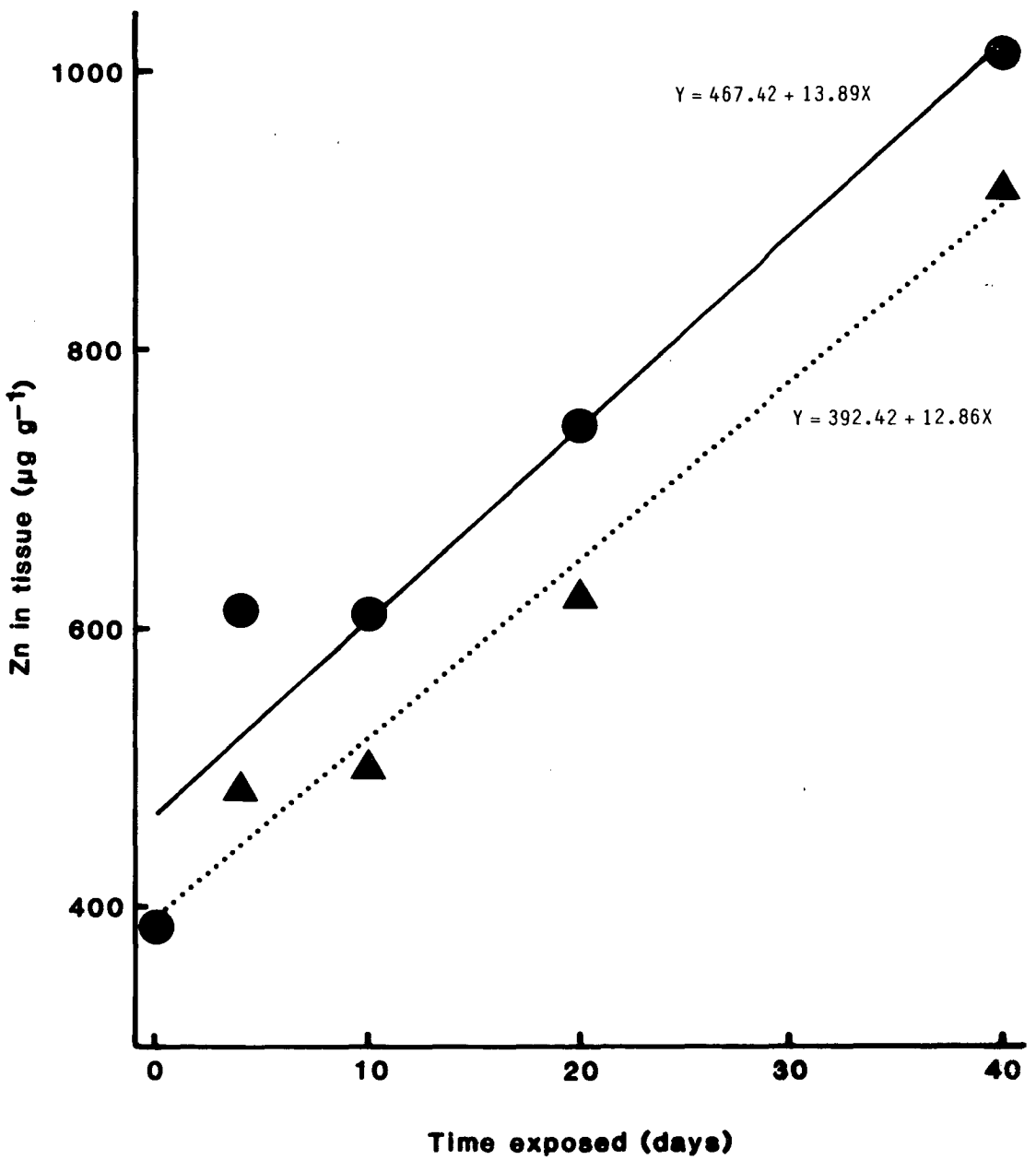

250
200
150
100
50
Pb in tissue (µg g⁻¹)
Y = 7.16 + 5.57X
Y = 4.84 + 2.90X
0
10
20
30
40
Time exposed (days)

Figure 4.16


1000
800
600
400
Zn in tissue (µg g⁻¹)
Y = 467.42 + 13.89X
Y = 392.42 + 12.86X
0
10
20
30
40
Time exposed (days)

of cadmium was directly proportional to the exposure time, which was not the case when the metal was presented singly.

Accumulation of Copper

Comparison of Figure 4.10 with Figure 4.14 indicates that the mussels under the cycled conditions in both experiments exhibited a similar reduction in accumulation compared to those continuously exposed; ratios of rates of accumulation were 0.82 and 0.77 respectively. The most significant aspect of the comparison of these two figures is the marked increase in the rates of accumulation of copper by both groups exposed to the combination of the four metals. Mussels under continuous exposure increased their accumulation rate from 1.89 to 4.22 $\mu g\ g^{-1}\ day^{-1}$, whilst those under cycled conditions increased their rate from 1.58 to 3.23 $\mu g\ g^{-1}\ day^{-1}$.

Accumulation of Lead

Comparison of Figures 4.11 and 4.15 reveals that the accumulation of lead was not influenced by the presence of the other three metals.

Accumulation of Zinc

The mussels under continuous exposure (Figures 4.12 and 4.16) exhibited a similar rate of accumulation of zinc despite the presence of the other three metals. On the other hand, accumulation under cycled conditions was greatly influenced by the presence of the other three metals. Comparison of Figures 4.12 and 4.16 reveals an increase in the rate of accumulation of zinc from 7.40 to 12.86 $\mu g\ g^{-1}\ day^{-1}$ when in a combined exposure. Importantly, while in a combined exposure, zinc accumulation is not directly proportional to the exposure time; it is when presented singly.

Conclusion

The accumulation of cadmium, copper and zinc by *M. e. planulatus* was influenced by the presence of elevated levels of the other metals; cadmium accumulation may be reduced, whilst copper and zinc may be increased.

4.6.9 Discussion

George and Coombs (1977) proposed that the transport of cadmium across the cell membranes of *M. edulis* is a carrier-assisted process; the metal must be first complexed to a carrier ligand associated with the cell membrane. A lag period therefore would be expected between time of exposure to cadmium and accumulation of the metal within the tissue. George and Coombs (1977) suggested that this period was 1.5 to 2 days for *M. edulis*. They further suggested that saturation of the binding capacity of the membrane may occur, providing hence an unbound cadmium component (Marshall and Talbot 1979).

If it is assumed that a similar mechanism occurs for the accumulation of cadmium by *M. e. planulatus*, then the following interpretation of the results shown in Figure 4.9 may be suggested: while exposed to 10 µg Cd ℓ^{-1}, cadmium ions were adsorbed onto the surface of cell membranes. These ions were transported across the membrane complexed to a carrier ligand associated with the membrane and this process facilitated adsorption of further cadmium ions to the surface. If this transfer was rapid then the mussels under the cycled conditions would be expected to exhibit a reduced rate of accumulation. However, the rate of accumulation of cadmium was not diminished up to day 20 despite the limited duration of exposure to 10 µg Cd ℓ^{-1} (Figure 4.9). Consequently it is suggested that the lag period for complexing to take place is at least 2 days. During the period of exposure to background concentrations, the mussels under the cycled conditions were still complexing cadmium adsorbed during the previous 2 days exposure to elevated levels.

Up to day 20, therefore, both groups of mussels were continuously complexing cadmium ions onto carrier ligands. After day 20 it is suggested that saturation of the binding capacity of the membranes was reached. After that time, unless the exposure to elevated cadmium was continuous, cadmium ions not complexed at the cell membrane were lost back into solution during the 2 days in background concentrations.

Under the combined metal exposure accumulation of cadmium was found to be directly proportional to the exposure time (Section 4.6.7). It might therefore be suggested that the binding capacity of the membrane was saturated due to the presence of the other metals and so the non-complexed cadmium ions were lost back into solution during the periods of exposure to background levels.

Copper accumulation by mussels under cycled conditions was reduced, but the reduction was not equal to the reduction in exposure time (Figure 4.10), in contrast to the results for lead and zinc (Figures 4.11 and 4.12). It is suggested, therefore, that copper may also require complexing to a carrier ligand before entry into a cell, but the lag period here is less than 2 days. Mussels under cycled conditions accumulated almost 20% less copper than those under continuous conditions. Consequently it is suggested that the lag period for copper is between 1 and 1.5 days. Unlike cadmium accumulation, there appears to have been no saturation of the binding capacity for copper, even under combined exposure conditions when marked increases in copper accumulation resulted (Figure 4.14).

Lead accumulation was directly proportional to the period of exposure, there being no lag period in its transfer into the cells (Figure 4.11 and 4.15). Apparently there was also no restriction on entry of zinc into cells (Figure 4.13). The ratio of rates of zinc accumulation was slightly greater than 0.50 (Section 4.6.6) and may have been due to a slow accumulation of zinc from background concentrations during the periods between exposure to elevated levels. Control

groups of mussels exposed only to background concentrations often exhibited a slow accumulation of zinc. Both lead and zinc, therefore, may enter a cell by passive diffusion, which in the case of zinc under cycled conditions was greatly increased by the presence of other metals.

Rates of metal accumulation recorded during previous experiments in which mussels were exposed to a combination of all four metals (e.g. Section 4.2.4), were comparable to those recorded when mussels were exposed to the metals individually (Section 3.3). Apparently there was no interaction occurring. The metal levels previously employed were, 10 μg Cd ℓ^{-1}, 10 μg Cu ℓ^{-1}, 50 μg Pb ℓ^{-1} and 100 μg Zn ℓ^{-1}. In the experiments reported in this section, lead concentration was 20 μg ℓ^{-1}, while zinc was 200 μg ℓ^{-1}. As the rate of lead accumulation was not influenced by the combined exposure, it is suggested that the increased zinc level produced the apparent metal interaction in the later experiment.

The accumulation pathways of metals into mussel tissue are very poorly understood as also are the interactions of metals during their accumulation. These processes must, of course, be related and will be examined in greater detail in Chapter 5.

4.7 CONCLUSIONS

Extraneous factors, either biological or associated with the external environment, may influence the rate of accumulation of a metal by mussels, and so confuse results for monitoring purposes. Some of the influences may, however, be overcome in the design of the monitoring programme, and to this end the following recommendations are made:

1. Mussels should be selected from a single population only, preferably from a relatively pristine environment;

2. Individuals should be of similar size, preferably small (see 3);
3. Individuals should preferably be immature;
4. The monitoring interval (i.e. time between samplings) should be as short as possible, especially if the mussels used are mature or if a change in seawater temperature is expected;
5. The body weight and condition of the individuals should be measured at each sampling;
6. If possible the influence of temperature should be established by laboratory experiments, so that any change during the monitoring interval may be compensated for.

There is one factor, however, that cannot be reduced or eliminated and that is the possible influence of metal interactions. Evidence for this phenomenon was reported in Section 4.6 and it is examined in greater detail in Chapter 5.

CHAPTER 5

METAL ACCUMULATION AND INTERACTION

5.1 GENERAL INTRODUCTION

The interpretation of the results from Section 4.6 (the effect of cycled exposure on metal accumulation) led to the hypotheses firstly, that the four metals cadmium, copper, lead and zinc, may be accumulated by different mechanisms. Secondly, these mechanisms may be susceptible to interference arising from the presence of more than one metal.

Very little information exists as to the precise manner in which metals are accumulated by marine organisms (Phillips 1980), and so interpretation of the results of the present study is ~~more~~ somewhat speculative. Before endeavouring to examine the extent of metal interaction within the mussel *M. e. planulatus*, the limited literature on mechanisms of metal accumulation by mussels was critically reviewed. In the light of the results of Section 4.6, possible pathways in the accumulation of cadmium, copper, lead and zinc by *M. e. planulatus* will be proposed.

5.2 METAL ACCUMULATION

5.2.1 Introduction

It is evident from reports such as those by Bryan (1979), Noël-Lambot *et al.* (1980) and Phillips (1980) that the pathways of metal accumulation by marine organisms are very much species and metal dependent. The accumulation of a metal by a mussel involves the combination of the uptake of the metal at the body surface and its subsequent storage within the mussel tissues. Bryan (1976) considered that the uptake of heavy metals by marine organisms in general is by passive diffusion. This involves an initial adsorption onto the cell membrane, followed by diffusion and, subsequently, binding to the intracellular components. Both Bryan (1979) and George (1980) suggested that the

net accumulation is controlled by the intracellular components. Bryan (1979) further suggested that the adsorptive phase is usually the most rapid.

5.2.2 Uptake

Cadmium

Compared to the other three metals (Cu, Pb and Zn) the mechanisms involved in the uptake of cadmium by mussels is relatively well understood. Fowler and Benayoun (1974) suggested that its uptake by *M. galloprovincialis* was not a metabolic process, as accumulation was independent of temperature. As discussed in Chapter 4 (Section 4.6), George and Coombs (1977) hypothesised that cadmium is not transported across the cell membranes of the tissues of *M. edulis* in the simple ionic form. The ions must be first complexed to carrier ligands associated with the cell membranes. The authors interpreted a lag period in cadmium uptake as representing the change of cadmium from ionic to complexed forms. The uptake of cadmium by the isolated gills of *M. edulis* was described by Carpene and George (1981) as being by passive diffusion, facilitated by intracellular binding.

According to Coombs and George (1978) the ions of pollutant metals in seawater are not present solely as free hydrated ions. They may be multi-complexed to inorganic or organic ligands. Prior complexing of cadmium with EDTA, humic and alginic acids or pectin was found by George and Coombs (1977) to double the rate of accumulation of cadmium by *M. edulis*. They suggested that this may result from the complexed cadmium reaching rapid equilibrium with the membrane associated carrier ligands. The cadmium ions, therefore, would not require prior complexing before transport across the membrane. This prior binding may represent an increase in the biological availability of the metal to mussels.

Lead

The results obtained by Schulz-Baldes (1978) for lead accumulation by *M. edulis* indicated an initial logarithmic rate followed by the more commonly observed linear uptake (e.g. Schulz-Baldes 1974). The initial logarithmic phase was interpreted as a reversible exchange of lead, and lasted for approximately 70 hours when mussels were exposed to 200 µg Pb ℓ^{-1}. The linear phase was interpreted as an irreversible phase.

The uptake of lead may well be by passive diffusion with the rate limiting step being the capacity for internal transport or storage of the metal (Schulz-Baldes 1978). Prior complexing may increase the transport or storage. Coombs (1977) reported that prior binding of lead to form a low molecular weight complex, e.g. with citrate, resulted in a 3-4 fold increase in accumulation by *M. edulis*. Binding to a high molecular weight complex, such as humic and alginic acids or pectin, resulted in a 1.5 - 2 fold increase.

Zinc and Copper

The uptake mechanisms for both zinc and copper are even less well understood than those for cadmium or lead, and the possibilities can only be speculated upon.

Wedemeyer (1968) suggested that the uptake of zinc, which was freely exchangeable in salmon eggs, is by passive diffusion. The kinetics of this process apparently differ from those of cadmium uptake by the gills of *M. edulis* (Carpene and George 1981). Coombs' (1972) results indicated that zinc may be present as a free ion within the tissues of the oyster *Ostrea edulis*. It is therefore suggested that the uptake of zinc is by passive diffusion without the assistance of a carrier system. If this were the case, the zinc ions would be present

in the free state on both sides of the membrane.

In contrast to zinc, Coombs (1972) suggested that copper was firmly bound within the oyster, as did Scott and Major (1972) for *M. edulis*. This may imply that copper uptake is by carrier assisted diffusion, as for cadmium.

5.2.3 Storage

Following the uptake of a metal there are two mechanisms by which it may be stored within the mussel tissue. These mechanisms involve either binding the metal to a low molecular weight metalloprotein, similar in structure to the metallothionein found in vertebrates, or containing it in a granular form within intracellular membrane-limited vesicles. Either alternative represents an effective means for detoxifying and transporting the metal.

Vesicles

Three of the four metals of interest here have been reported in the granular form within membrane-bound vesicles in mussel tissue. Lead was observed in this situation in the gills, gut and kidney by Coombs (1977) and Schulz-Baldes (1978), while Marshall and Talbot (1979) reported cadmium and lead in vesicles in the gills of mussels exposed to the respective metal. Cadmium, along with mercury was found in vesicles in the mid-gut gland by Janssen and Scholz (1979). Both cadmium (George and Pirie 1979) and zinc (George and Pirie 1980) were reported to be found in granular structures in the kidney of *M. edulis*. Although no record of copper occurring in this manner in mussels is known to the present author, this metal has been reported in similar structures along with zinc in oysters (George *et al.* 1978b). Copper in granules or vesicles along with lead and zinc, has also been found in other non-molluscan marine organisms (see review by Coombs and George 1978). The metal granules were found within circulating amoebocytes in the haemolymph. Metals may be transported to the kidney and other

tissues for storage and eventual excretion by means of endo- and exocytosis (Coombs and George 1978; George and Pirie 1980). George and Pirie (1979) report the possible presence of metallothionein in the granules of Cd-exposed mussels. The authors moreover proposed that metallothionein may be associated with particulate structures. The mechanism of the formation of membrane-limited granules is not known.

Metallothionein

The existence of low molecular weight metal-binding proteins collectively termed metallothionein, had been well established in vertebrates when Noël-Lambot (1976) reported the synthesis of a similar protein in *M. edulis*. This protein was induced by the exposure to elevated cadmium concentrations. Subsequently, similar low molecular weight proteins in mussels were found to be associated with and induced by copper, zinc and mercury (Talbot and Magee 1978; Viarengo *et al.* 1980 and 1981; Roesijadi and Hall 1981). Further reports on cadmium associated with metal-binding proteins have also been presented (Talbot and Magee 1978; George *et al.* 1979; Köhler and Riisgård 1982).

Talbot and Magee (1978) proposed that this metal-binding protein occurred naturally within *M. edulis* to regulate the uptake of zinc and copper. By coincidence it was also able to bind and detoxify cadmium. They based their proposal on the fact that although zinc and copper are essential elements, they are also highly toxic. Consequently, the protein also serves to detoxify any excess metal taken up. Roesijadi (1981) suggested that the naturally occurring metal-binding protein in marine organisms was involved in routine metabolism of such metals as zinc and copper.

The identity of this metal-binding protein in most marine invertebrates was regarded by Roesijadi (1981) to be still uncertain. Frankene

et al. (1980), however,demonstrated that the low molecular weight Cd-binding protein synthesised in *M. edulis* in response to a cadmium exposure was metallothionein.

A metal-binding protein similar in molecular weight to metallothionein but binding predominantly copper has been identified in mammalian tissue and termed Cu-chelatin (Winge *et al.* 1975). However, Suzuki and Yamamura (1979) concluded that in rats this protein is metallothionein. Recently, Ridlington *et al.* (1981) reported the finding of a similar Cu-binding protein in the tissues of marine animals.

No record of lead associated with these metal-binding proteins has been reported in the literature.

5.2.4 Properties of Metallothionein

Due to its structure, metallothionein is highly adapted to form metal complexes, and may bind up to seven gram-atoms of metal per mole of protein (Roesijadi 1981). In discussing cadmium and zinc interactions in the tissues of rats, Webb (1972a, b) reported that the binding protein induced by cadmium may also bind zinc. Moreover, cadmium may replace zinc in the Zn-pre-synthesised metallothionein. Suzuki *et al.* (1979) suggested that zinc was always present with cadmium in the metallothionein of the rat kidney. They also demonstrated that various Zn/Cd ratios on the metallothionein may in fact be experimentally manipulated by replacing zinc from Zn-thioneins with cadmium. The binding of zinc to metallothionein is weak compared to cadmium, which makes the replacement of cadmium by zinc rather difficult (Kägi and Vallee 1961; Suzuki *et al.* 1979).

While Suzuki *et al.* (1979) reported that zinc was always present in a cadmium-binding protein, Suzuki *et al.* (1977) reported that copper was the major element in a cadmium induced metallothionein in the rat kidney, but not in the rat liver. Suzuki (1979) noted that this high

copper content in the renal metallothionein was species dependent. The metallothionein has a stronger affinity for copper than either cadmium or zinc (Suzuki *et al.* 1977; Suzuki 1979). Mills (1980) in considering the antagonism between cadmium, zinc and copper, also in mammalian systems, reported that, as the tissue level of metallothionein increased as a consequence of cadmium or zinc presence, the proportion of bound copper also increased. This came about either because copper was incorporated during *de novo* synthesis of the protein, or because it displaced previously bound cadmium or zinc. In both cases metal complexes of metallothionein were formed (Mills 1980).

Parizek (1957) found that a pre-treatment with zinc prevented the destructive action of cadmium on rat testicular tissue. Webb (1972b) concluded that this preventative action was due to the pre-treatment stimulating production of metallothionein. The protein was then readily available to bind and detoxify the cadmium when introduced. This action overcame the normal lag period between cadmium introduction and the synthesis of metallothionein. Webb (1972b) further suggested that a small pre-treatment with cadmium itself might have the same effect.

It has been reported that in marine organisms cadmium may be initially bound to high molecular weight proteins before being bound to newly synthesised metallothionein (Noël-Lambot *et al.* 1980; Carpene and George 1981). In addition, Noël-Lambot *et al.* (1980) believed that there is a critical level of cadmium concentration in the tissue at which metallothionein production is induced. This induction increases the number of Cd-binding sites available. The accumulation of a metal was described in Section 5.2.1 as a combination of its uptake and storage, the net accumulation being dependent on the storage capacity. Thus the increase in the number of Cd-binding sites, representing an increase in the storage capacity, would permit a further uptake of cadmium.

This increase in uptake would in turn stimulate further production of metallothionein (Köhler and Riisgård 1982), so providing a mechanism for greatly increasing the storage capacity. Hence, as Bouquegneau *et al.* (1979) suggested, the induced production of metallothionein may be one of the most important mechanisms responsible for the continuous accumulation of cadmium (or other metals) in marine organisms.

5.3 ACCUMULATION IN *MYTILUS EDULIS PLANULATUS*

5.3.1 Introduction

Interpreting the results in Section 4.6 in the light of the discussion above (Section 5.2), I have proposed, in this section, mechanisms by which the metals cadmium, copper, lead and zinc are accumulated by *M. e. planulatus*. These proposed models are used later in Section 5.10 to aid the interpretation of the results of experiments on the interaction of metals observed during their accumulation.

It is reasonable to assume that the storage protein for the three metals cadmium, copper and zinc in *M. e. planulatus* is the low molecular weight metal-binding protein metallothionein. Synthesis of metallothionein may be induced by exposure to one of the metals. It is also considered that the metals may be present in the seawater in either the free hydrated ionic form or complexed to an external naturally occurring chelating ligand.

5.3.2 Proposed Mechanism for Cadmium Accumulation

In Section 5.2 it was concluded that accumulation of cadmium in *M. edulis* is by facilitated diffusion i.e. carrier assisted, followed by binding either to metallothionein or within membrane-limited vesicles. It is proposed that a similar process occurs for cadmium accumulation in *M. e. planulatus* (Pathway a, Figure 5.1). Cadmium is presumed to

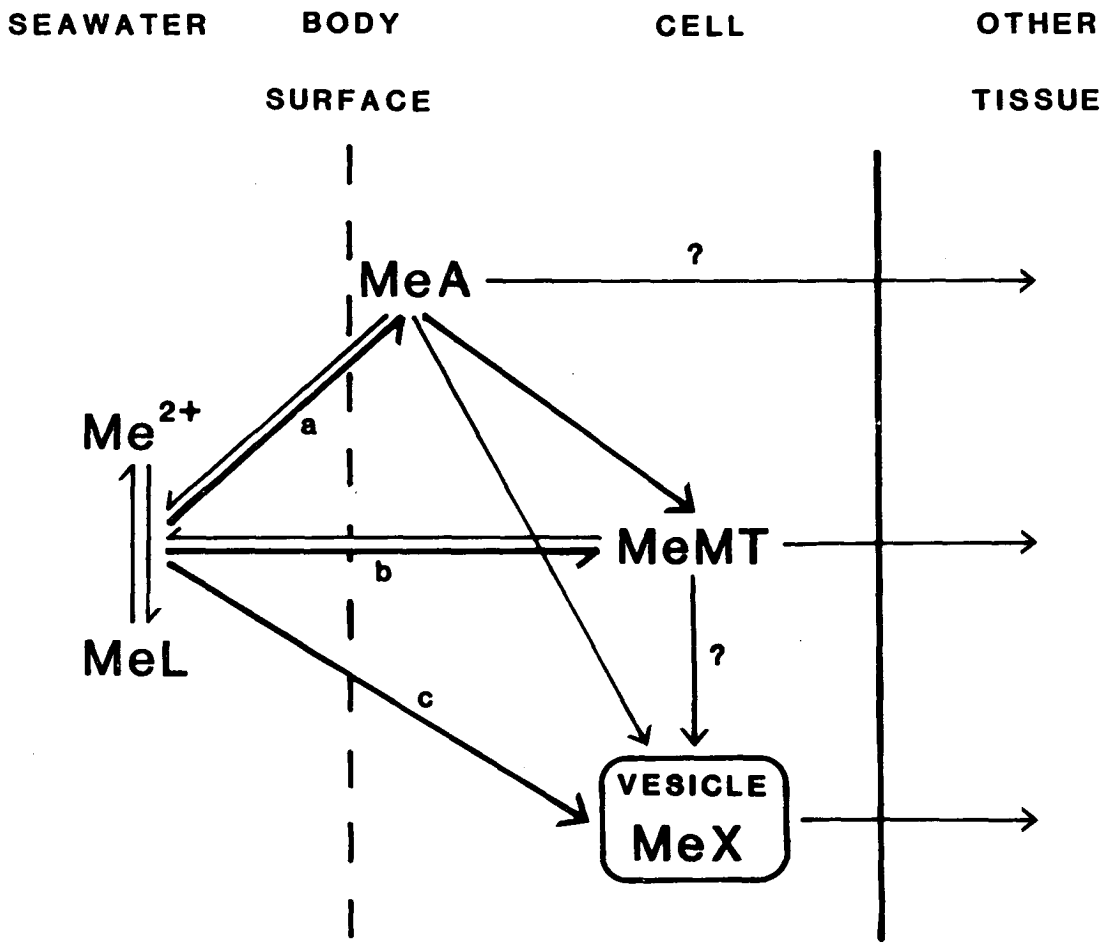


Figure 5.1 Schematic view of accumulation mechanisms proposed for metal accumulation in M.e. planulatus. The metal may be present in the seawater in either the ionic form (Me^{2+}) or complexed to an external chelating ligand (MeL). (Adapted from Carpene and George 1981). Me = metal, L = external chelating ligand, A = membrane associated carrier ligand, X = intracellular vesicle binding ligand, MT = metallothionein.

be adsorbed to the membrane surface prior to binding to the carrier ligand for transport across the membrane. The metal is then transferred to the storage protein, metallothionein. There exists a lag period between the cadmium adsorption and its binding to either the carrier ligand or the storage protein. The results from Section 4.6 support this proposal and indicate that the lag period is at least 2 days.

5.3.3 Proposed Mechanism for Copper Accumulation

The results from Section 4.6 indicated that copper accumulation by *M. e. planulatus* resembled that of cadmium. The rates of accumulation of both metals were not dependent upon the exposure period. Consequently, it is proposed that copper is also accumulated by facilitated diffusion followed by binding to the storage protein (Pathway a, Figure 5.1). When copper is presented by itself, this storage protein is considered to be a copper specific protein, similar to metallothionein. It may well be metallothionein that specifically binds copper due to its strong affinity for this metal. In a combined exposure copper may be bound preferentially to metallothionein that is also binding the other metals. The lag period between adsorption and binding is estimated, from the results presented in Figure 4.11, to be at least 1.5 days.

5.3.4 Proposed Mechanism for Zinc Accumulation

Coombs (1972) reported that in *Ostrea edulis* zinc was present either as a free ion or weakly bound and readily dissociated from proteins. The discussion in Chapter 3, on the results of experiments employing *M. e. planulatus* is in agreement with these conclusions; zinc appeared to be freely exchangeable, with rapid and marked fluctuations occurring in the tissue concentrations. Consequently it is proposed that zinc accumulation in *M. e. planulatus* occurs via passive diffusion

across the surface membrane followed by utilization of the biologically demanded zinc in metabolic processes. Excess zinc induces the production of metallothionein to which it is weakly bound, and from which it may be lost back into solution (Pathway b, Figure 5.1). This proposal for zinc accumulation is supported by the results from Section 4.6 (Figure 4.13). The accumulation of zinc was found to be dependent upon the duration of exposure to an elevated zinc concentration.

5.3.5 Proposed Mechanism for Lead Accumulation

The results from Section 4.6 (Figure 4.12) indicated that lead accumulation in *M. e. planulatus* is also dependent on the length of time exposed to the elevated lead concentration. Using the arguments proposed for zinc accumulation it is suggested that lead accumulation is also via passive diffusion in this species. The metal is, however, subsequently sequestered within intracellular vesicles (Pathway c, Figure 5.1), rather than associated with metallothionein. Other storage systems for lead may yet be described, but whatever the system the metal would appear to be firmly bound, as depuration is very slow (Section 3.5).

The mechanisms proposed for the accumulation of the four metals are supported by the speciation of the metals in seawater reported by Zirino and Yamamoto (1972). According to these authors zinc undergoes less complexation than the other metals studied, with 17% remaining uncomplexed at the average seawater pH (8.1); this may increase to 51% at pH 7.0. Cadmium and copper, on the other hand, exhibited only 2.5% and 1% uncomplexed ions respectively at pH 8.1 (copper may increase to 30% at pH 7.0). Cadmium is predominantly complexed to chloride ions, while the main copper species are $Cu(OH)_2$ and $CuCO_3$. The important zinc species at pH 8.1 is $Zn(OH)_2$. The major form of lead is as a carbonate (only 1.5% is uncomplexed at pH 8.1), in which form it was

also found in the tissues of *M. edulis* by Talbot and Magee (1978) and Marshall and Talbot (1979).

5.4 METAL INTERACTION

5.4.1 Introduction

As pointed out in Chapter 4 it is often possible to eliminate the effects of some factors that may confuse the interpretation of a monitoring survey at the time of sampling or in the design of the survey. However effects arising from the interaction of two or more metals cannot be selected for, and may lead to unjustified conclusions. Phillips (1980) considered that because interaction effects cannot be easily accounted for or eliminated, and indeed may not even be suspected, they represent the greatest potential for confusion in a monitoring survey.

It has been known for many years that metals in physiological concentrations may interact with each other. The majority of studies available have dealt with mammals, particularly rats, and most have been toxicity studies. For example, Smith and Larson (1946) found that the addition of dietary copper partially reduced an anaemia in young rats, usually caused by the presence of high levels of zinc. Similarly, Parizek (1957) reported that zinc counteracted the destructive effect of cadmium in the testes of rats.

The few studies on metal interactions in marine organisms have also concentrated largely on toxicity (i.e. survival). For example, synergistic effects between the toxicity of copper and zinc were observed by Sprague (1964) in salmon *Salmo salar*. Similarly, Braek *et al.* (1976) examined the effects of copper and zinc, and later the effects of cadmium and zinc (Braek *et al.* 1980), on the growth of phytoplankton. In both cases they reported both synergistic and antagonistic effects depending on species. Such studies are of limited value to an understanding of the problems of metal accumulation, as can be seen by

comparison of the results presented by Negilski *et al.* (1981) and those from Ahsanullah *et al.* (1981). For example, in the shrimp *Callianassa australiensis* the combination of cadmium and zinc resulted in enhanced accumulation of both metals (Ahsanullah *et al.* 1981). At the same time the observed toxic effects were higher than expected for cadmium alone, but similar to those expected for zinc (Negilski *et al.* 1981).

With regard to marine mussels, the interaction between cadmium and zinc has been examined by both Fowler and Benayoun (1974) and Jackim *et al.* (1977). Carpene and George (1981) examined the effects of copper, mercury, lead, iron and zinc, on the uptake of cadmium by isolated gill tissues. The accumulation of cadmium, copper, lead and zinc in combination was observed by Phillips (1976a), but the results were not conclusive. In general, the interaction of metals during accumulation by mussels is poorly understood. Similarly reports on the interaction of metals during their accumulation by other marine organisms are also sparse. In addition to the work by Ahsanullah *et al.* (1981), Bryan (1969) observed that cadmium, copper and manganese reduced the accumulation of ^{65}Zn by the alga *Laminaria digitata*. Ray *et al.* (1980) studied a cadmium-zinc interaction in the shrimp, *Pandalus montagui* , and the interactions during the uptake of toxic levels of cadmium, copper and zinc by the estuarine teleost, *Fundulus heteroclitus* were investigated by Eisler and Gardner (1973).

5.4.2 Sites of Possible Metal Interaction in *Mytilus edulis planulatus*

Following the arguments in Section 5.2, if the accumulation of one metal were to influence the accumulation of another, then such an interaction could occur at the site of uptake, the site of storage, or both. Interaction at the site of uptake is most likely when metals are competing for adsorption or binding sites. Interaction at the storage site would be expected when metals are competing for attachment

on the protein molecule. A consequence of the latter possibility might be an increase in the rate of accumulation of one or more metals following an increase in the rate of protein production.

Table 5.1 summarises the conclusions reached for the accumulation mechanisms for each metal (from Section 5.3).

TABLE 5.1 Summary of conclusions reached for the accumulation mechanism for each metal in *M. e. planulatus*.

Metal	Uptake		Storage	
	Passive diffusion	Facilitated diffusion	Binding protein	Vesicles
Cadmium		+	+	+
Copper		+	+	
Lead	+			+
Zinc	+		+	+

From this table the following predictions for interactions within *M. e. planulatus* can reasonably be made:

(a) cadmium, copper and zinc may all be expected to interact by competing for binding sites on the storage protein (metallothionein) molecule;

(b) cadmium and copper may also be expected to interact at the body surface by competing for adsorption sites or carrier ligands;

(c) lead would not be expected to interact with the other three metals.

5.4.3 Experiments

Metal interaction between cadmium, copper and zinc during accumulation in *M. e. planulatus* was investigated through the following four series of experiments:

Series I. This series involved a 3 x 3 x 3 factorial examination of the interaction of the 3 metals resulting from simultaneous exposure. Each metal was tested at three external concentrations, representing a range from the equivalent of non-polluted to heavily polluted seawater. The aim was to ascertain whether the metals interacted at all, and, if so, within what range of concentrations.

Series II. This series involved a number of experiments in which the accumulation of one metal at a single concentration was examined when mussels were simultaneously exposed to a second metal at one of six different concentrations. These experiments were aimed at further identifying the limits of any interactions.

Series III. This series involved the investigation of the accumulation of one metal at a single concentration by mussels which had previously been exposed, for 6 days, to a second metal at one of five different concentrations.

Series IV. This series involved mussels initially exposed for 20 days to one metal and then exposed either to a second metal alone, or to the combination of the first and second metals.

In each series of experiments the mussels employed were collected from Howden (see Figure 2.1) and were 3.0 - 4.0 cm shell length. Each experiment was conducted in a 10°C constant temperature room, and tissue concentrations of all three metals (Cd, Cu and Zn) were analysed in each sample taken. All other experimental procedures and conditions were as described in Chapter 2.

In the following four sections of this chapter each of the four series of experiments is presented separately. Each series is described in detail and the results presented. Some brief discussions and the conclusions reached regarding possible metal interactions are also presented. These possible interactions are then collectively considered in Section 5.9. In Section 5.10 the major conclusions are compared with the proposals presented in Section 5.3.

5.5 SERIES I

5.5.1 Experimental Design

A 3^3 factorial design was used to examine the effect of simultaneous exposure to one or two metals on the accumulation of a third. Each of the three metals, cadmium, copper and zinc, was examined at three concentrations, ranging from the equivalent of a non-polluted level to a relatively highly polluted level:

Cadmium - Cd_0: background concentration,
Cd_1: 10 μg Cd ℓ^{-1},
Cd_2: 20 μg Cd ℓ^{-1}.

Copper - Cu_0: background concentration,
Cu_1: 10 μg Cu ℓ^{-1},
Cu_2: 20 μg Cu ℓ^{-1}.

Zinc - Zn_0: background concentration,

Zn_1: 100 µg Zn ℓ^{-1},

Zn_2: 200 µg Zn ℓ^{-1}.

The design resulted in 27 treatments ($Cd_0Cu_0Zn_0$, $Cd_0Cu_0Zn_1$ ..... $Cd_2Cu_2Zn_1$, $Cd_2Cu_2Zn_2$), each of which was duplicated. Mussels were exposed to a particular treatment for 10 days. As all 54 experiments could not be conducted at the same time, the day 0 tissue concentrations varied. For this reason the *change* in each metal concentration of the mussel tissue over the 10 day exposure period was recorded, rather than the final tissue concentrations. A total of 15 mussels ~~were~~ was used in each experiment, from which 10 were sub-sampled after the 10 days exposure. These mussels were homogenized and 3 digests from each were analysed, as described in Chapter 2.

5.5.2 Statistical Analysis

The observed mean changes in tissue concentration of each metal following 10 days exposure to the particular combination of metal levels are shown in Table 5.2. Accumulation of each metal was analysed by a three-way ANOVA to identify possible sources of variation (Tables 5.3, 5.6 and 5.9). It is possible that one metal may influence the accumulation of another only at particular concentrations of that metal. Accordingly, the results were subjected to a further ANOVA. The influence of the other metals on the accumulation of the third metal was analysed at each concentration in turn of the third metal (Tables 5.4, 5.7 and 5.10). Where there was evidence ($p \leq 0.05$) of a possible effect the significance was tested by calculation of the l.s.d. (least significant difference) between treatment means (Tables 5.5, 5.8 and 5.11). Where the difference between a specific pair of treatment means exceeded the l.s.d. value, this difference was significant at $p = 0.05$.

TABLE 5.2 Mean changes in metal concentrations in mussel tissue (μg g^{-1}dry wt.), in each of two replicate experiments, following 10 days exposure to the metal combination shown. Mean day 0 concentrations (±95% CL) are also shown.

TREATMENT			CADMIUM		COPPER		ZINC	
Cd_0	Cu_0	Zn_0	-0.20	-0.12	4.15	7.85	34.75	23.15
Cd_0	Cu_0	Zn_1	0.21	0.35	6.43	5.78	96.51	79.47
Cd_0	Cu_0	Zn_2	0.22	-0.50	9.55	11.01	108.96	161.37
Cd_0	Cu_1	Zn_0	-0.14	-0.01	20.47	14.21	12.16	23.31
Cd_0	Cu_1	Zn_1	0.35	-0.51	46.29	56.65	162.77	129.58
Cd_0	Cu_1	Zn_2	0.90	0.83	72.96	51.69	178.04	166.08
Cd_0	Cu_2	Zn_0	0.24	0.15	47.56	45.42	165.30	111.85
Cd_0	Cu_2	Zn_1	0.67	0.54	77.82	79.25	224.15	169.95
Cd_0	Cu_2	Zn_2	1.17	1.00	71.68	77.82	288.21	301.32
Cd_1	Cu_0	Zn_0	13.23	15.16	5.28	-2.24	-59.94	15.24
Cd_1	Cu_0	Zn_1	17.61	18.22	3.82	5.22	56.92	72.83
Cd_1	Cu_0	Zn_2	12.08	14.29	-2.79	4.08	263.63	250.62
Cd_1	Cu_1	Zn_0	13.90	14.85	22.69	22.39	9.61	-7.95
Cd_1	Cu_1	Zn_1	18.44	16.15	30.80	29.53	166.32	121.83
Cd_1	Cu_1	Zn_2	10.74	11.59	28.47	32.11	339.12	322.19
Cd_1	Cu_2	Zn_0	17.89	16.65	36.27	39.25	157.66	165.91
Cd_1	Cu_2	Zn_1	19.56	20.42	101.42	103.62	291.44	227.86
Cd_1	Cu_2	Zn_2	13.22	10.18	116.44	122.87	302.85	354.11
Cd_2	Cu_0	Zn_0	37.17	38.52	6.98	4.45	-2.59	9.26
Cd_2	Cu_0	Zn_1	37.75	35.12	31.13	3.22	187.10	121.05
Cd_2	Cu_0	Zn_2	23.01	26.26	10.74	-1.67	307.88	210.12
Cd_2	Cu_1	Zn_0	31.15	34.81	11.92	14.83	-12.40	15.81
Cd_2	Cu_1	Zn_1	42.21	39.16	55.33	51.21	180.29	92.87
Cd_2	Cu_1	Zn_2	18.87	22.22	11.20	18.78	250.14	201.35
Cd_2	Cu_2	Zn_0	32.49	30.16	70.57	49.23	176.26	150.28
Cd_2	Cu_2	Zn_1	33.79	28.19	66.69	75.98	145.52	236.79
Cd_2	Cu_2	Zn_2	20.16	23.98	83.22	77.81	264.60	311.90
Day 0			3.40 ± 0.61		5.97 ± 1.32		265.39 ± 24.93	

5.5.3 Effects on Cadmium Accumulation

Results

The results of the three-way ANOVA on the mean changes in tissue concentration of cadmium are shown in Table 5.3. The further analyses at each particular cadmium level are shown in Table 5.4. The treatment means for each metal combination are shown in Table 5.5, together with the calculated l.s.d. values.

As anticipated the greatest source of variation was due to the change in cadmium concentration of the seawater (Table 5.3). Table 5.3 shows that there was also a considerable amount of variation due to the presence of zinc. There was little evidence of copper having affected the cadmium accumulation, except possibly at certain cadmium x copper levels.

Influence of Zinc

From Table 5.4 it can be seen that the presence of zinc influenced cadmium accumulation at both Cd_1 and Cd_2 levels. No effect was evident at Cd_0, as would be expected with no accumulation of cadmium occurring. From the Table of Means it can be seen that the major influence of zinc occurred at the Zn_2 level where there was a reduction in cadmium accumulation (Table 5.5). At the Zn_1 level, zinc influenced cadmium accumulation only at the Cd_1 level. The effect was to increase cadmium accumulation. The 'zinc means' at Cd_1 (Table 5.5b) show firstly, an increase from 15.3 to 18.4 μg Cd g^{-1} as zinc concentration increased to Zn_1, and then a decrease from 18.4 to 12.0 μg Cd g^{-1} as zinc concentration increased to Zn_2. The l.s.d. between 'zinc means' was 1.6, indicating that both differences were significant. At the Cd_2 level, after an initial insignificant increase from 34.1 to 36.0 μg Cd g^{-1}, there was a corresponding significant reduction to 22.4 μg Cd g^{-1} as

TABLE 5.3 Analysis of variance for changes in cadmium concentration in mussel tissue. Data as in Table 5.2.

Source of Variation	d.f.	Sum of Squares	Mean Square	F_o	$Pr(F>F_o)$
Cd	2	8400	4200	1701	0.000
Cu	2	9	5	2	0.16
Zn	2	413	207	84	0.000
Cd x Cu	4	77	19	8	0.000
Cd x Zn	4	360	90	36	0.000
Cu x Zn	4	20	5	2	0.12
Cd x Cu x Zn	8	77	10	4	0.003
Residual	27	66.67	2.47		

TABLE 5.4 Analysis of variance for changes in cadmium concentration in mussel tissue at each level of cadmium exposure.

a. Cadmium at Cd_0

Source of Variation	d.f.	Sum of Squares	Mean Square	F_o	$Pr(F>F_o)$
Cu	2	1.23	0.616	8.1	0.009
Zn	2	1.14	0.572	7.6	0.012
Cu x Zn	4	1.08	0.270	3.6	0.052
Residual	9	0.68	0.076		

b. Cadmium at Cd_1

Source of Variation	d.f.	Sum of Squares	Mean Square	F_o	$Pr(F>F_o)$
Cu	2	12.7	6.3	4.2	0.052
Zn	2	122.3	61.1	40.2	0.000
Cu x Zn	4	11.6	2.9	1.9	0.2
Residual	9	13.7	1.5		

c. Cadmium at Cd_2

Source of Variation	d.f.	Sum of Squares	Mean Square	F_o	$Pr(F>F_o)$
Cu	2	73.5	36.6	6.3	0.02
Zn	2	649.6	324.8	55.9	0.000
Cu x Zn	4	84.2	21.1	3.6	0.050
Residual	9	52.3	5.8		

TABLE 5.5 Mean changes in cadmium concentration in mussel tissue (μg g^{-1}dry wt.) following exposure to the metal combination shown. Values in the body of the table are the mean difference between initial and final cadmium levels (data as in Table 5.2). l.s.d.: least significant difference at p=0.05 (see text).

a. Cadmium at Cd_0

	Zn_0	Zn_1	Zn_2	Copper Mean
Cu_0	-0.2	0.3	-0.1	0.0
Cu_1	-0.1	-0.1	0.9	0.2
Cu_2	0.2	0.6	1.1	0.6
Zinc mean	0.0	0.3	0.6	

l.s.d. between: treatment means = 0.2, metal means = 0.4

b. Cadmium at Cd_1

	Zn_0	Zn_1	Zn_2	Copper Mean
Cu_0	14.1	17.9	13.2	15.1
Cu_1	14.4	17.3	11.2	14.3
Cu_2	17.3	20.0	11.7	16.3
Zinc mean	15.3	18.4	12.0	

l.s.d. between: treatment means = 0.9, metal means = 1.6

c. Cadmium at Cd_2

	Zn_0	Zn_1	Zn_2	Copper Mean
Cu_0	37.8	36.4	24.6	33.0
Cu_1	33.0	40.7	20.5	31.4
Cu_2	31.3	31.0	22.1	28.1
Zinc mean	34.1	36.0	22.4	

l.s.d. between: treatment means = 1.5, metal means = 3.1

zinc concentration increased to Zn_2 (Table 5.5c).

Influence of Copper

The only evidence of a copper influence on cadmium accumulation appears at the Cd_2 level (Table 5.4c). The high level of copper (Cu_2) decreased the accumulation of cadmium (Table 5.5c). The 'copper means' at Cd_2 decreased significantly from 33.0 to 28.1 μg Cd g^{-1}, when copper was increased from Cu_0 to Cu_2.

Influence of Zinc and Copper in Combination

The analysis reported in Table 5.3 indicated a possible effect on cadmium accumulation occurring due to the combination of all three metals (Cd x Cu x Zn). Table 5.4 shows that this effect occurred at the Cd_2 level. Examination of the treatment means at Cd_2 (Table 5.5c) reveals that the combination of copper and zinc at Cu_2 Zn_2, may have exaggerated the individual influence of each metal on cadmium accumulation, i.e. synergistic action.

Conclusions

The following possible interactions are suggested and a further investigation of these is presented in Section 5.6:

(i) the presence of zinc at high concentrations (>100 μg Zn ℓ^{-1}) may decrease the accumulation of cadmium;

(ii) zinc at low concentrations may increase the accumulation of cadmium, particularly at low cadmium concentrations;

(iii) the presence of high concentrations of copper may reduce the accumulation of cadmium from high cadmium concentrations;

(iv) the combined presence of zinc and copper at high concentrations may act synergistically to decrease the accumulation of cadmium.

5.5.4 Effects on Copper Accumulation

Results

Table 5.6 shows the results of the three-way ANOVA on the mean changes in tissue concentration of copper. Again, as anticipated the greatest source of variation was due to the change in copper concentration of the seawater. There was also a considerable amount of variation due to the presence of zinc, but cadmium alone showed little evidence of interference (Table 5.6). The results indicate, however, that the presence of zinc and cadmium together may have had a complex effect on copper accumulation. From Table 5.7 it can be seen that the presence of cadmium and zinc influenced copper accumulation at both Cu_1 and Cu_2 levels. No effect was evident at Cu_0.

Influence of Zinc

The Table of Means shows that the influence of zinc was to increase the accumulation of copper (Table 5.8). At the Cu_2 level the increase in zinc concentration from Zn_1 to Zn_2 did not significantly increase the effect on copper accumulation (Table 5.8c). However at the Cu_1 level the 'zinc means' indicate a significant decrease in the influence of zinc at the Zn_2 level, compared to that at Zn_1 (Table 5.8b). Yet this may be an artifact caused by the presence of cadmium. In the absence of cadmium (Cd_0), Table 5.8b shows that the Zn_2 level had a significantly greater influence on copper accumulation than the Zn_1 level.

Influence of Cadmium

The 'cadmium means' shown in Table 5.8b appear to indicate that the presence of cadmium at Cu_1 level resulted in a decrease in copper accumulation. However, Table 5.8c shows that at the Cu_2 level the presence of cadmium resulted in a significant increase in copper accumulation, but only at the Cd_1 level. However, these effects would appear from

TABLE 5.6 Analysis of variance for changes in copper concentration in mussel tissue. Data as in Table 5.2.

Source of Variation	d.f.	Sum of Squares	Mean Square	F_o	Pr $(F>F_o)$
Cd	2	141	70	2	0.2
Cu	2	42710	21355	475	0.000
Zn	2	5718	2859	64	0.000
Cd x Cu	4	2427	607	13	0.000
Cd x Zn	4	1037	259	6	0.002
Cu x Zn	4	3184	796	18	0.000
Cd x Cu x Zn	8	3734	467	10	0.000
Residual	27	1214	45		

TABLE 5.7 Analysis of variance for changes in copper concentration in mussel tissue at each level of copper exposure.

a. Copper at Cu_0

Source of Variation	d.f.	Sum of Squares	Mean Square	F_o	$Pr(F>F_o)$
Cd	2	156	78	1.3	0.3
Zn	2	82	41	0.7	0.5
Cd x Zn	4	153	38	0.6	0.6
Residual	9	530	59		

b. Copper at Cu_1

Source of Variation	d.f.	Sum of Squares	Mean Square	F_o	$Pr(F>F_o)$
Cd	2	1060	530	14	0.002
Zn	2	2303	1152	30	0.000
Cd x Zn	4	2019	505	13	0.001
Residual	9	348	39		

c. Copper at Cu_2

Source of Variation	d.f.	Sum of Squares	Mean Square	F_o	$Pr(F>F_o)$
Cd	2	1352	676	18	0.001
Zn	2	6517	3258	88	0.000
Cd x Zn	4	2600	650	17	0.000
Residual	9	335	37		

TABLE 5.8 Mean changes in copper concentration in mussel tissue ($\mu g\ g^{-1}$ dry wt.) following exposure to the metal combination shown. Values in the body of the table are the mean difference between initial and final copper levels (data as in Table 5.2). l.s.d.: least significant difference at p=0.05 (see text).

a. Copper at Cu_0

	Zn_0	Zn_1	Zn_2	Cadmium Mean
Cd_0	6.0	6.1	10.3	7.5
Cd_1	1.5	4.5	0.6	2.2
Cd_2	5.7	17.2	4.5	9.1
Zinc mean	4.4	9.3	5.2	

l.s.d. between: treatment means = 5.8, metal means = 10.0

b. Copper at Cu_1

	Zn_0	Zn_1	Zn_2	Cadmium Mean
Cd_0	17.3	51.5	62.3	43.7
Cd_1	22.5	30.2	30.3	27.7
Cd_2	13.4	53.3	15.0	27.2
Zinc mean	17.8	45.0	35.9	

l.s.d. between: treatment means = 4.7, metal means = 8.1

c. Copper at Cu_2

	Zn_0	Zn_1	Zn_2	Cadmium Mean
Cd_0	46.5	78.5	74.8	66.6
Cd_1	37.8	102.5	119.7	86.6
Cd_2	59.9	71.3	80.5	70.6
Zinc mean	48.1	84.1	91.6	

l.s.d. between: treatment means = 4.6, metal means = 8.0

closer examination of the treatment means in Table 5.8 to be an artifact caused by the influence of zinc. In the absence of zinc (Zn_0) at the Cu_1 level the presence of cadmium at Cd_1 increased the copper accumulation (Table 5.8b). At the Cd_2 level no significant effect occurred. At the Cu_2 level, in the absence of zinc, Cd_1 served to decrease copper accumulation and Cd_2 served to increase it (Table 5.8c). Following the results presented in Table 5.6, it is considered that neither effect is a true interaction.

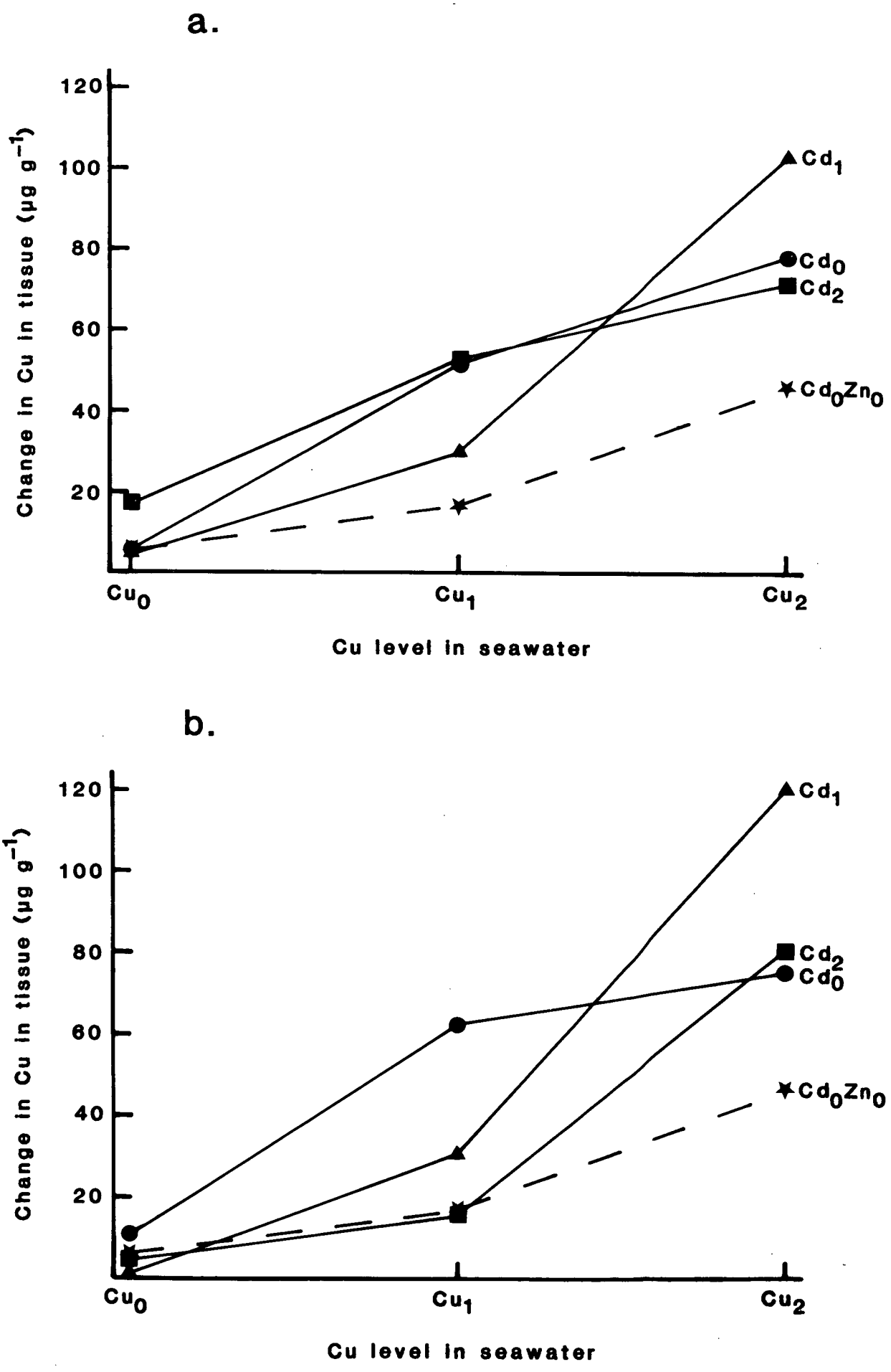
Influence of Zinc and Cadmium in Combination

As previously mentioned, very little variation in copper accumulation appears to have occurred at the Zn_0 level due to the presence of cadmium. If the treatment means shown in Table 5.8 are plotted for the Zn_1 and Zn_2 levels, as shown in Figure 5.2, the possibilities for the complex interaction of zinc and cadmium on the accumulation of copper can be seen. The general tendency was an expected increase in copper accumulation as the external copper concentration increased. In the absence of cadmium (Cd_0) the presence of zinc increased the accumulation of copper as previously described. When the cadmium exposure was increased to Cd_1 the enhancement effect of zinc at both Zn_1 and Zn_2 levels was decreased at the Cu_1 level, i.e. antagonistic action (Figure 5.2). When the copper concentration was at Cu_2 the presence of cadmium at Cd_1 resulted in increased copper accumulation, i.e. synergistic action. The high levels of cadmium (Cd_2) would appear from Figure 5.2 to influence the effect of zinc only at the Zn_2 level and Cu_1 level, where its presence antagonised the expected effect of zinc.

Conclusions

The following interactions appear to occur and a further investigation of these is presented in Sections 5.6 and 5.10:

Figure 5.2


a.
120
100
80
60
40
20
Change in Cu in tissue (µg g⁻¹)
Cd1
Cd0
Cd2
Cd0Zn0
Cu0
Cu1
Cu2
Cu level in seawater
b.
120
100
80
60
40
20
Change in Cu in tissue (µg g⁻¹)
Cd1
Cd2
Cd0
Cd0Zn0
Cu0
Cu1
Cu2
Cu level in seawater

(i) the presence of zinc may increase the accumulation of copper;

(ii) the presence of low concentrations of cadmium may increase the above zinc effect at high concentrations of copper;

(iii) at low concentrations of copper the presence of cadmium may decrease the above zinc effect on copper accumulation.

5.5.5 Effects on Zinc Accumulation

Results

Table 5.9 shows the results of the three-way ANOVA on the mean changes in tissue concentration of zinc. The results show that apart from the anticipated effect of zinc concentration itself in the sea-water, interaction with copper is likely.

Influence of Copper

The results of the ANOVA at each level of zinc (Table 5.10), indicate that a copper interaction was present at all three levels of zinc. This is also shown in the Table of Means (Table 5.11). The presence of copper at Cu_2 increased zinc accumulation at all three levels. However, the 'copper means' in Table 5.11 indicate that copper at Cu_1 did not significantly increase the accumulation of zinc. This would appear to be an artifact caused by the presence of cadmium. Copper at Cu_1 significantly increased zinc accumulation at both Zn_1 and Zn_2 (Tables 5.11b and c). This increase occurred when cadmium was either absent (Cd_0) or low (Cd_1).

Influence of Cadmium

The only significant influence of cadmium on zinc accumulation occurred at the high level of zinc, Zn_2 (Table 5.10c). This can be seen as an increase in zinc accumulation by inspection of the 'cadmium

TABLE 5.9 Analysis of variance for changes in zinc concentration in mussel tissue. Data as in Table 5.2.

Source of Variation	d.f.	Sum of Squares	Mean Square	F_o	$Pr(F>F_o)$
Cd	2	10839	5420	4.7	0.02
Cu	2	138797	69399	60.4	0.000
Zn	2	358982	179491	156.4	0.000
Cd x Cu	4	11725	2931	2.5	0.06
Cd x Zn	4	23775	5943	5.2	0.003
Cu x Zn	4	14341	3585	3.1	0.03
Cd x Cu x Zn	8	19893	2487	2.2	0.06
Residual	27	31007	1148		

TABLE 5.10 Analysis of variance for changes in zinc concentration in mussel tissue at each level of zinc exposure.

a. Zinc at Zn_0

Source of Variation	d.f.	Sum of Squares	Mean Square	F_o	$Pr(F>F_o)$
Cd	2	689	344	0.5	0.6
Cu	2	89448	44724	74.9	0.000
Cd x Cu	4	3073	768	1.3	0.3
Residual	9	5378	598		

b. Zinc at Zn_1

Source of Variation	d.f.	Sum of Squares	Mean Square	F_o	$Pr(F>F_o)$
Cd	2	918	459	0.3	0.8
Cu	2	39877	19939	11.6	0.003
Cd x Cu	4	13519	3379	2.0	0.2
Residual	9	15470	1719		

c. Zinc at Zn_2

Source of Variation	d.f.	Sum of Squares	Mean Square	F_o	$Pr(F>F_o)$
Cd	2	33007	16504	14.6	0.001
Cu	2	23814	11907	10.5	0.004
Cd x Cu	4	15027	3757	3.3	0.06
Residual	9	10160	1129		

TABLE 5.11 Mean changes in zinc concentration in mussel tissue ($\mu g\ g^{-1}$ dry wt.) following exposure to the metal combination shown. Values in the body of the table are the mean difference between initial and final zinc levels (data as in Table 5.2). l.s.d.: least significant difference at p=0.05 (see text).

a. Zinc at Zn_0

	Cu_0	Cu_1	Cu_2	Cadmium Mean
Cd_0	29	18	139	62
Cd_1	-22	1	162	47
Cd_2	3	2	163	56
Copper mean	3	7	155	

l.s.d. between: treatment means = 18, metal means = 32

b. Zinc at Zn_1

	Cu_0	Cu_1	Cu_2	Cadmium Mean
Cd_0	88	146	197	144
Cd_1	65	144	260	156
Cd_2	154	137	191	161
Copper mean	102	142	216	

l.s.d. between: treatment means = 31, metal means = 54

c. Zinc at Zn_2

	Cu_0	Cu_1	Cu_2	Cadmium Mean
Cd_0	135	172	295	201
Cd_1	257	331	328	305
Cd_2	259	226	288	258
Copper mean	217	243	304	

l.s.d. between: treatment means = 25, metal means = 44

means' in Table 5.11c. In the absence of copper (Cu_0) the effects at Cd_1 and Cd_2 levels were not significantly different (Table 5.11c). The treatment means in Table 5.11b also show an increased accumulation of zinc at the Cd_2 level in the absence of copper (Cu_0).

Influence of Copper and Cadmium in Combination

There appears little evidence from Tables 5.9 and 5.10 of any combined influence of copper and cadmium on the accumulation of zinc.

Conclusions

The following interactions appear to occur and are considered further in Section 5.6:

(i) the presence of copper may increase the accumulation of zinc;

(ii) the presence of cadmium may increase the accumulation of zinc when either metal is at a high external concentration.

5.5.6 Conclusions from Series I Experiments

The conclusions regarding possible interaction of cadmium, copper and zinc are summarised in Table 5.12.

5.6 SERIES II

5.6.1 Introduction

The interpretation of the results from Series I experiments revealed inconsistencies in possible interactions between two metals at the three concentrations of each metal examined. In this second series of experiments, in which the metals were presented at a greater

TABLE 5.12 Possible interactions between cadmium, copper and zinc during simultaneous accumulation by *M. e. planulatus*.

Exposure	Interaction Effect
a. Influence on cadmium accumulation	
Cd + Zn (high conc.)	Cd accumulation may be decreased
Cd + Zn (low conc.)	Cd accumulation may be increased
Cd (high conc.) + Cu (high conc.)	Cd accumulation may be decreased
Cd + Zn + Cu (high conc.)	Cu and Zn may act synergistically in decreasing Cd accumulation
b. Influence on copper accumulation	
Cu + Zn	Cu accumulation may be increased
Cu (low conc.) + Zn + Cd	Cu accumulation may be increased, but Cd may act antagonistically with the expected enhancement effect of Zn (particularly at low Cd conc.)
Cu (high conc.) + Zn + Cd	Cu accumulation may be increased, and Cd may act synergistically with the expected enhancement effect of Zn (particularly at high Cd conc.)
c. Influence on zinc accumulation	
Zn + Cd	Zn accumulation may be increased when either metal is at a high conc.
Zn + Cu	Zn accumulation may be increased

number of concentrations, the aims were two-fold. Firstly, to prove whether the effects observed in Series I were true interactions, and secondly, to explore in more detail the concentrations at which these interactions were manifested. This involved six experiments each designed to examine two aspects:

1. the effect of a metal A, at six different concentrations, on the accumulation of a metal B, at one concentration; and
2. the effect of metal B, at one particular concentration, on the linear relationship between the rate of accumulation by the mussel and the external concentration of metal A.

5.6.2 Experimental Design

One hundred mussels were used in each experiment. The tissues of 10 were analysed at day 0; the other 90 were divided into 6 equal groups and exposed to various metal concentrations for 10 days. After this time their tissues were analysed for both test metals and any increase in tissue concentration was recorded. In each experiment the range of concentrations of metal A represented an environmental range equivalent to non-polluted to heavily polluted waters. The particular concentrations of metal B were selected following the Series I experiments, and were concentrations at which interactions might be expected to occur.

In preliminary experiments mussels were exposed to a single metal only for 10 days. Accumulation of each of the three test metals was examined at five concentrations, the range of which was equivalent to that for metal A. From the results linear regressions were obtained relating the rate of accumulation of each metal to the exposure concentration. These regressions were used as a measure of rate of accumulation of one metal in the absence of any others. The significance

of a change in the regression caused by the presence of another metal was tested by t-test. Standard deviations presented in Figures 5.3 to 5.8 were calculated using the coefficient of variation between homogenates, of samples of 10 similar sized mussels, established for each metal in Chapter 2.

5.6.3 Interaction Between Zinc and Cadmium: Part 1

Each of the six groups of mussels in this experiment was exposed to cadmium at 10 µg Cd ℓ^{-1} plus zinc at one of the following concentrations:

background, 50, 100, 150, 200, 250 µg Zn ℓ^{-1}.

Results

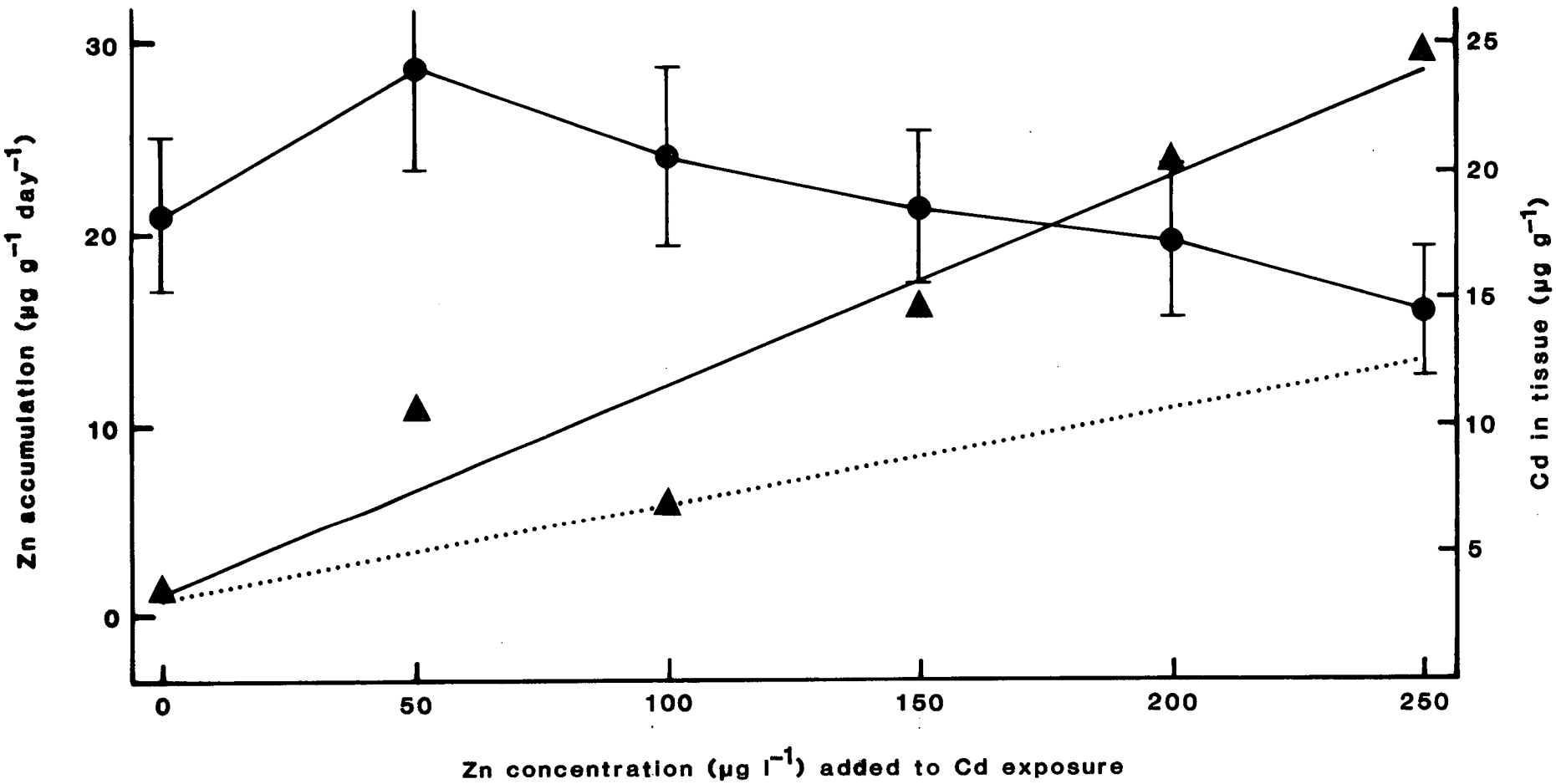
1. Interaction Effects at Different Concentrations

The data presented in Figure 5.3 suggest an increase of cadmium in mussel tissue at low concentrations of zinc followed by a decrease at high concentrations. However, these fluctuations in cadmium concentrations were not significant when considered in the light of the coefficient of variation between homogenates for cadmium.

2. Interaction Effects on Rate of Accumulation

Comparison of the regression coefficients for increase in the rate of accumulation of zinc with increase in external concentration, revealed that the presence of cadmium at 10 µg ℓ^{-1} significantly ($p<0.05$) increased the slope of the relationship (Figure 5.3). Cadmium at 10 µg ℓ^{-1} therefore increased the accumulation of zinc at all concentrations examined. The effect increased with increase in the external concentration of zinc (Figure 5.3).

Figure 5.3


Zn accumulation (µg g^{-1} day^{-1})
Cd in tissue (µg g^{-1})
30
20
10
0
25
20
15
10
5
0
50
100
150
200
250
Zn concentration (µg l^{-1}) added to Cd exposure

Conclusions

(i) The presence of cadmium at 10 μg ℓ^{-1} enhanced the accumulation of zinc.

(ii) The presence of zinc at concentrations up to 250 μg ℓ^{-1} had no influence on the accumulation of cadmium from an external concentration of 10 μg ℓ^{-1}.

5.6.4 Interaction Between Zinc and Cadmium: Part 2

Each of the six groups of mussels in this experiment was exposed to zinc at 200 μg Zn ℓ^{-1} plus cadmium at one of the following concentrations:

background, 1, 5, 10, 15, 20 μg Cd ℓ^{-1}.

Results

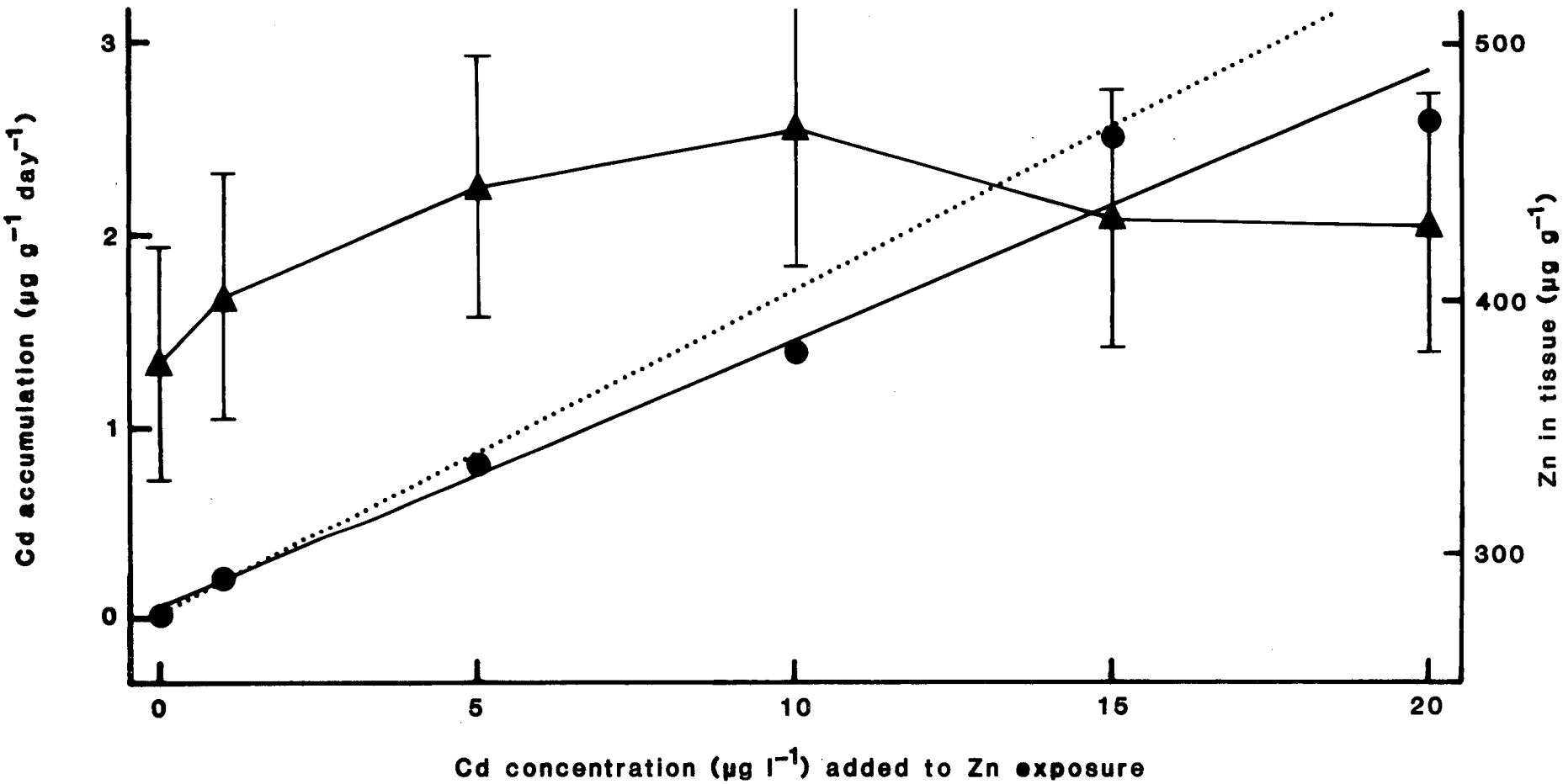
1. Interaction Effects at Different Concentrations

The data presented in Figure 5.4 indicate that the presence of cadmium did not significantly increase the accumulation of zinc from 200 μg Zn ℓ^{-1}.

2. Interaction Effects on Rate of Accumulation

The slope of the line relating rate of accumulation of cadmium to exposure concentration was significantly ($p<0.05$) decreased by the presence of zinc, as revealed by the comparison of the two regression coefficients (Figure 5.4). However, it can be seen that the decrease is due mainly to the data point at the highest cadmium concentration (20 μg ℓ^{-1}).

Figure 5.4


Cd accumulation (µg g^{-1} day^{-1})
Zn in tissue (µg g^{-1})
0
1
2
3
300
400
500
0
5
10
15
20
Cd concentration (µg l^{-1}) added to Zn exposure

Conclusions

(i) The presence of cadmium at concentrations up to 20 μg ℓ^{-1} had no significant effect on the accumulation of zinc from an external concentration of 200 μg ℓ^{-1}.

(ii) The presence of zinc at 200 μg ℓ^{-1} appears unlikely to influence the accumulation of cadmium except possibly at high cadmium concentrations (≥20 μg ℓ^{-1}).

5.6.5 Interaction Between Zinc and Copper: Part 1

Each of the six groups of mussels in this experiment was exposed to copper at 10 μg Cu ℓ^{-1} plus zinc at one of the following concentrations: background, 50, 100, 150, 200, 250 μg Zn ℓ^{-1}.

Results

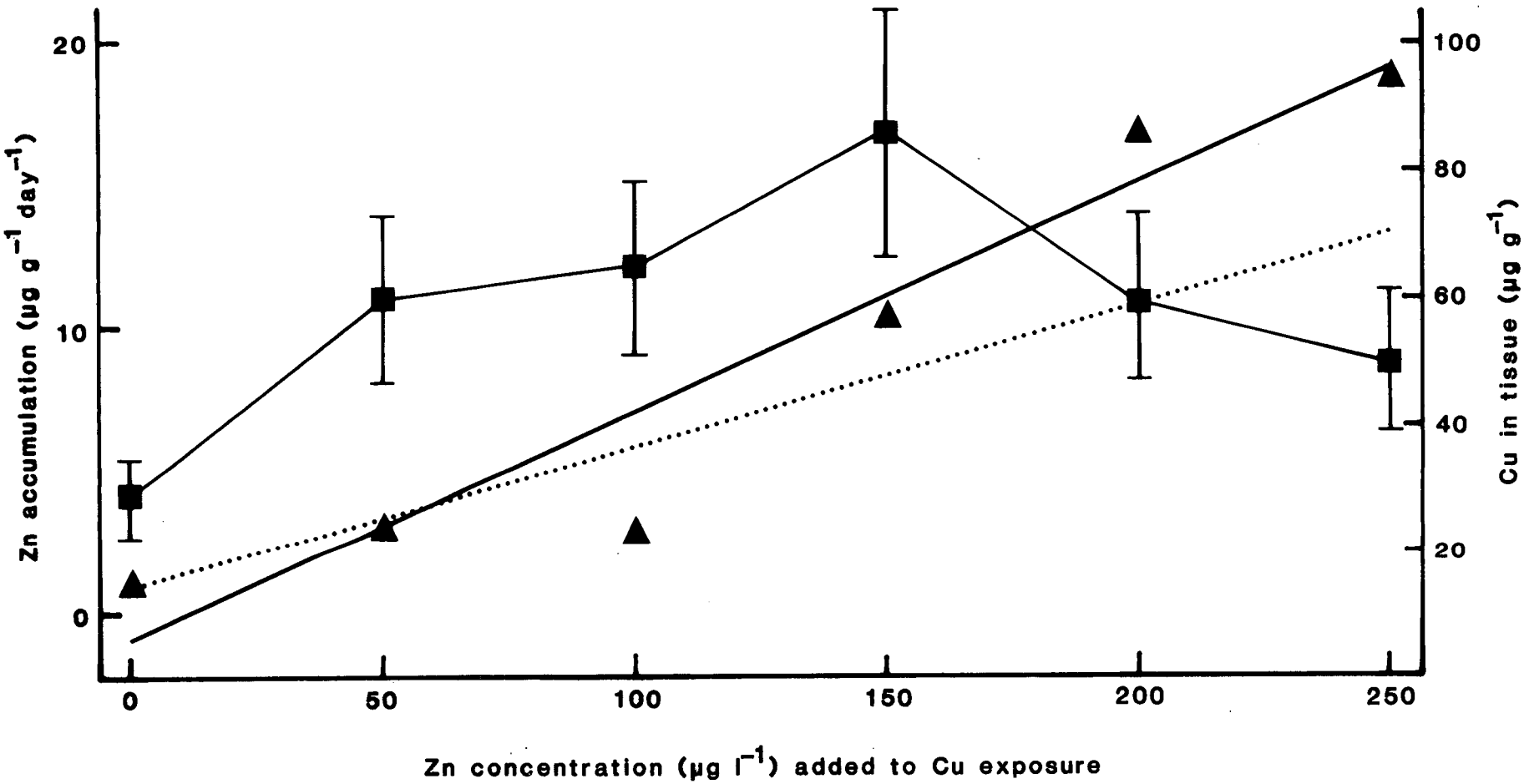
1. Interaction Effects at Different Concentrations

Figure 5.5 shows that the accumulation of copper was greatly increased by the presence of zinc. A peak in the effect of zinc occurred at a concentration of 150 μg Zn ℓ^{-1}.

2. Interaction Effects on Rate of Accumulation

The presence of copper appears to have increased the accumulation of zinc, particularly at high zinc concentrations (Figure 5.5). The slope of the regression line relating rate of accumulation and exposure concentration of zinc was increased by 50% (0.05 to 0.08) in the presence of copper. However, the two regression coefficients were not statistically ($p>0.05$) different. This may be due to the data point at 100 μg Zn ℓ^{-1} masking the true linear response.

Figure 5.5


Zn accumulation (µg g^{-1} day^{-1})
Cu in tissue (µg g^{-1})
20
10
0
100
80
60
40
20
0
50
100
150
200
250
Zn concentration (µg l^{-1}) added to Cu exposure

Conclusions

(i) The presence of zinc up to a level of 250 μg ℓ^{-1} greatly enhanced the accumulation of copper from a concentration of 10 μg ℓ^{-1}. A possible maximum enhancement effect may occur at 150 μg Zn ℓ^{-1}.

(ii) The presence of copper at 10 μg ℓ^{-1} appears unlikely to influence the accumulation of zinc, except possibly at high zinc concentrations (>100 μg ℓ^{-1}).

5.6.6 Interaction Between Zinc and Copper: Part 2

In this experiment each of the six groups of mussels was exposed to zinc at 200 μg Zn ℓ^{-1} plus copper at one of the following concentrations: background, 5, 10, 15, 20, 25 μg Cu ℓ^{-1}.

Results

1. Interaction Effects at Different Concentrations

At copper concentrations of 20 and 25 μg ℓ^{-1} the accumulation of zinc was increased (Figure 5.6). It is suggested that the relationship may be distorted by the data point at 15 μg Cu ℓ^{-1} masking an otherwise linear response. That data point apart, the interaction effect of copper increased with the external copper concentration.

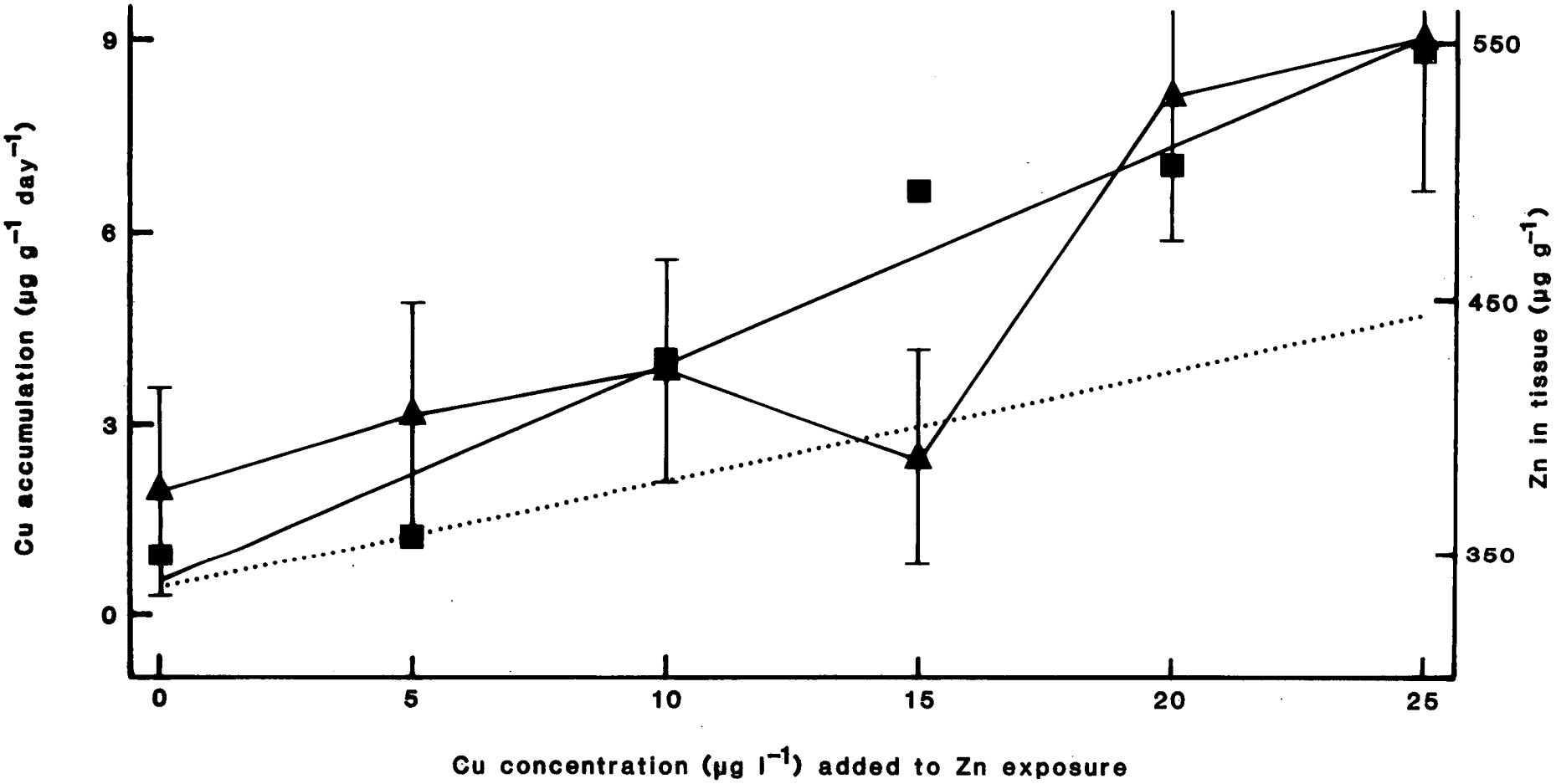
2. Interaction Effects on Rate of Accumulation

The data presented in Figure 5.6 also show clearly that the presence of zinc significantly ($p<0.01$) increased the rate of accumulation of copper over the range of copper concentrations tested.

Conclusions

(i) Copper, at least at concentrations >15 μg ℓ^{-1}, increased the accumulation of zinc from seawater containing 200 μg Zn ℓ^{-1}.

Figure 5.6


Cu accumulation (µg g^{-1} day^{-1})
9
6
3
0
Zn in tissue (µg g^{-1})
550
450
350
0
5
10
15
20
25
Cu concentration (µg l^{-1}) added to Zn exposure

(ii) Zinc at 200 μg ℓ^{-1} enhanced the accumulation of copper at all concentrations tested up to 25 μg Cu ℓ^{-1}.

5.6.7 Interaction Between Cadmium and Copper: Part 1

Each of the six groups of mussels in this experiment was exposed to copper at 10 μg Cu ℓ^{-1} plus cadmium at one of the following concentrations: background, 1, 5, 10, 15, 20 μg Cd ℓ^{-1}.

Results

1. Interaction Effects at Different Concentrations

The presence of cadmium at 15 and 20 μg ℓ^{-1} reduced the accumulation of copper from an external concentration of 10 μg ℓ^{-1} (Figure 5.7).

2. Interaction Effects on Rate of Accumulation

Figure 5.7 also shows that the exposure to 10 μg Cu ℓ^{-1} in combination with a range of cadmium concentrations had no effect on the rate of accumulation of cadmium. Regression lines describing cadmium accumulation in the presence and absence of copper had almost identical coefficients.

Conclusions

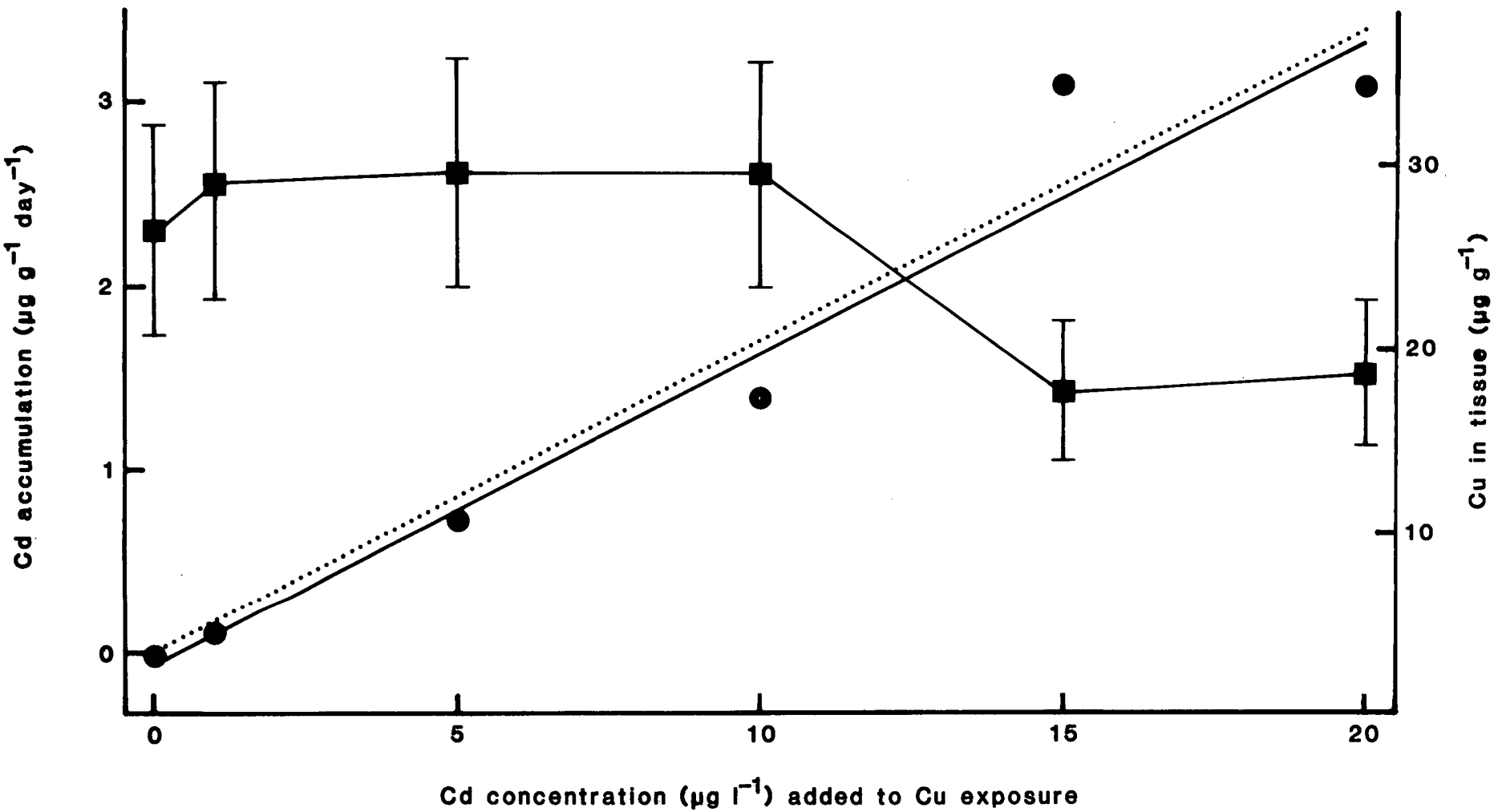
(i) The presence of cadmium concentrations ≥15 μg ℓ^{-1} reduced the accumulation of copper from an external concentration of 10 μg ℓ^{-1}.

(ii) The accumulation of cadmium was unaffected by the presence of copper at 10 μg ℓ^{-1}.

5.6.8 Interaction Between Cadmium and Copper: Part 2

In this experiment each of the six groups of mussels was exposed to cadmium at 10 μg Cd ℓ^{-1} plus copper at one of the following concentrations:

Figure 5.7


Cd accumulation (µg g^{-1} day^{-1})
Cu in tissue (µg g^{-1})
Cd concentration (µg l^{-1}) added to Cu exposure
0
1
2
3
10
20
30
0
5
10
15
20

background, 5, 10, 15, 20, 25 µg Cu ℓ^{-1}.

Results

1. Interaction Effects at Different Concentrations

Figure 5.8 shows that the accumulation of cadmium from 10 µg ℓ^{-1} was unaffected by the presence of copper at any of the concentrations tested.

2. Interaction Effects on Rate of Accumulation

The two regressions representing the rate of accumulation of copper in the presence and absence of cadmium had statistically ($p>0.05$) similar coefficients. Nonetheless at high external concentrations of copper (25 µg ℓ^{-1}) the presence of 10 µg Cd ℓ^{-1} may reduce the rate of accumulation.

Conclusions

(i) The presence of copper at concentrations up to 25 µg ℓ^{-1} had no effect on the accumulation of cadmium from an external concentration of 10 µg ℓ^{-1}.

(ii) Although the presence of 10 µg Cd ℓ^{-1} did not influence the accumulation of copper at low external concentrations, it may have reduced the accumulation at high concentrations (>20 µg ℓ^{-1}).

5.6.9 Conclusions from Series II Experiments

The conclusions reached from the six experiments in Series II on the possible interaction of cadmium, copper and zinc during accumulation by *M. e. planulatus* are summarised in Table 5.13.

Figure 5.8

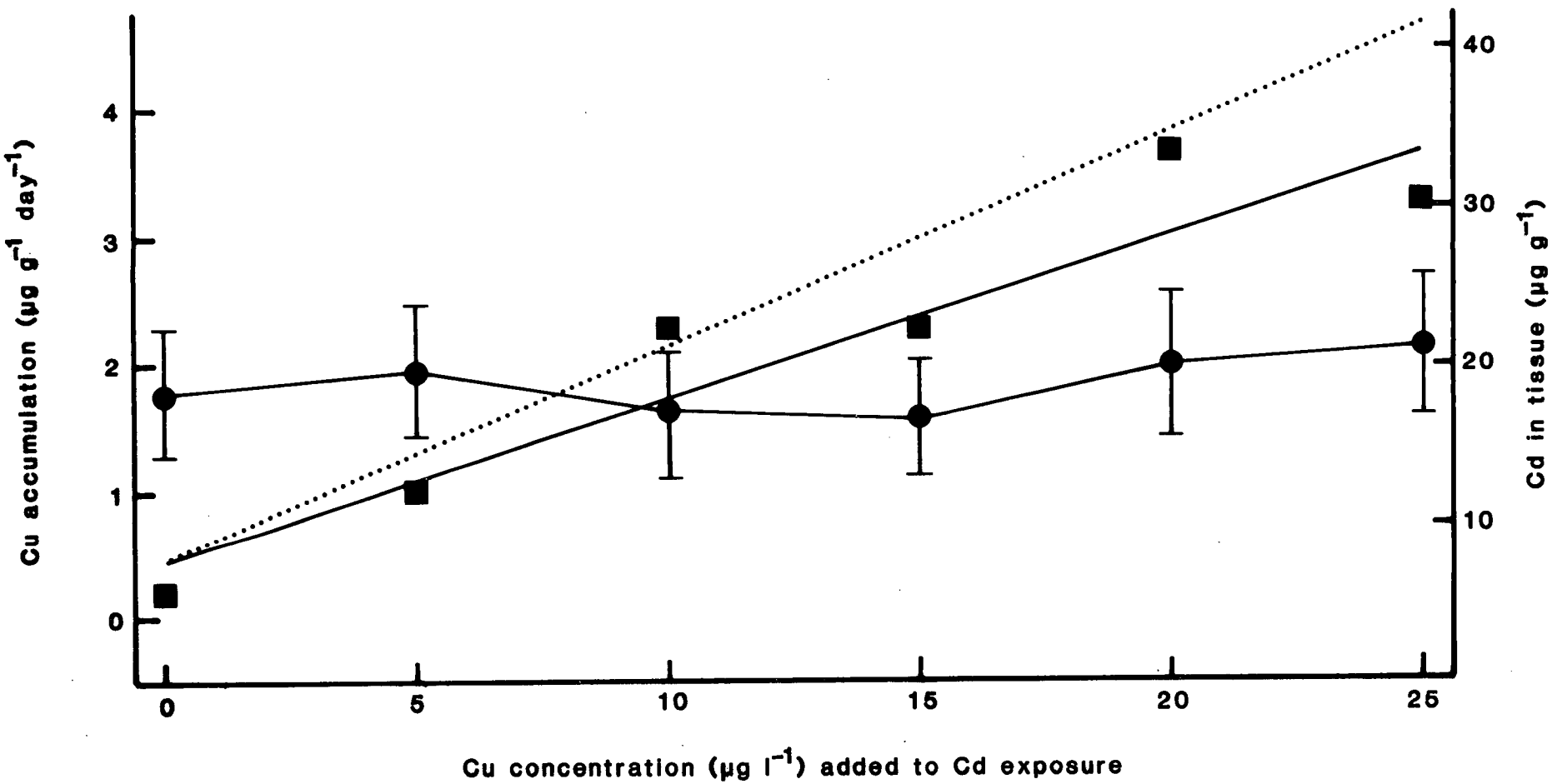

Cu accumulation (µg g^{-1} day^{-1})
Cd in tissue (µg g^{-1})
0
1
2
3
4
10
20
30
40
0
5
10
15
20
25
Cu concentration (µg l^{-1}) added to Cd exposure

TABLE 5.13 Interactions between cadmium, copper and zinc during simultaneous accumulation by *M. e. planulatus*.

Exposure	Interaction Effect
Cd + Zn	Zn accumulation is increased and Cd accumulation decreased when Cd >10 μg ℓ^{-1} and Zn >150 μg ℓ^{-1}
Cu + Zn	Cu accumulation is always increased, and Zn accumulation is increased when Cu >15 μg ℓ^{-1} and Zn >100 μg ℓ^{-1}
Cd + Cu	No influence on Cd accumulation, but Cu accumulation may be decreased when Cd >15 μg ℓ^{-1} and Cu >10 μg ℓ^{-1}

5.7 SERIES III

5.7.1 Experimental Design

In the following six experiments the influence of an initial six day exposure to one metal on the subsequent rate of accumulation of another, when the first metal was replaced by the second, was examined. The initial metal was presented at five concentrations, ranging from the equivalent of non-polluted to heavily polluted waters. The concentrations of the second metal were selected following the results obtained in Series I. They were considered to be concentrations at which an interaction might occur, and also concentrations likely to be observed in a polluted system. The second exposure period continued for 20 days following the transfer of the mussels from the initial exposure solution.

In each experiment 300 mussels were divided into five equal groups and exposed for 6 days to one of the five initial metal concentrations. These exposures were terminated after the 6 days and all five groups of mussels were introduced to the second metal solution at a single concentration. The mussels were sampled and analysed for all three test metals (Cd, Cu and Zn) on days 0, 4, 10 and 20 from the start of the second exposure period (i.e. subsamples of 10 mussels were removed on each occasion as described in Chapter 2). The data for the (second) 20 days exposure period for each group of mussels were first analysed by linear regression, the slope of the line representing the rate of accumulation of the metal. Rates of accumulation (i.e. regression coefficients) for the four groups initially exposed to elevated metal concentrations were then compared, by t-test, to that of the group exposed initially to the background (non-polluted) concentrations. This comparison indicated whether the initial exposure to the first metal had any influence on the rate of accumulation of the second metal.

5.7.2 Influence of an Initial Exposure to Cadmium

In Experiments 1 and 2 the five groups of mussels were first exposed to one of the following cadmium concentrations:

Cd_0 - background concentrations,
Cd_1 - 1 μg ℓ^{-1},
Cd_2 - 5 μg ℓ^{-1},
Cd_3 - 10 μg ℓ^{-1},
Cd_4 - 20 μg ℓ^{-1}.

These exposures were terminated after 6 days and all five groups in Experiment 1 were introduced into a 10 μg ℓ^{-1} copper solution for 20 days. At the same time the five groups in Experiment 2 were placed in a 200 μg ℓ^{-1} zinc solution for 20 days. In both experiments cadmium was present only at background concentrations during this 20 day period.

Results

Experiment 1: Subsequent Exposure to Copper

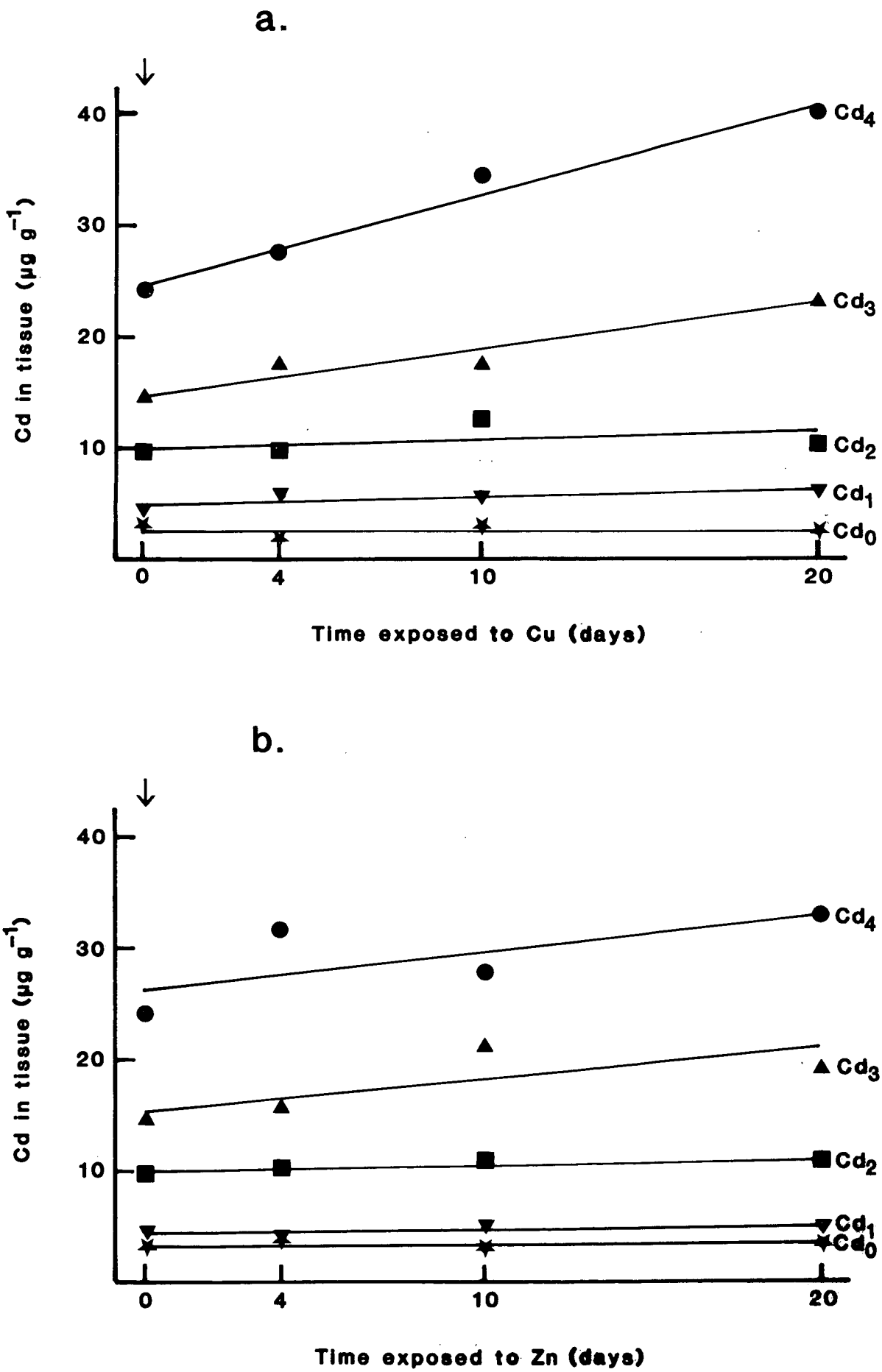
Cadmium Concentrations

The linear regressions shown in Figure 5.9a describing the accumulation of cadmium while exposed to copper, at 10 μg ℓ^{-1}, can be expressed as:

Cd_0 $Y = 2.52 - 0.00X$ $r = 0.04^{NS}$
Cd_1 $Y = 4.89 + 0.07X$ $r = 0.87^{*}$
Cd_2 $Y = 9.95 + 0.07X$ $r = 0.41^{NS}$
Cd_3 $Y = 14.73 + 0.41X$ $r = 0.95^{**}$
Cd_4 $Y = 24.62 + 0.82X$ $r = 0.99^{***}$

The three groups Cd_0, Cd_1 and Cd_2 showed very little change in their tissue concentrations of cadmium while exposed to copper. However, in groups Cd_3 and Cd_4 a considerable linear accumulation of cadmium from the background concentration occurred. The rates of

Figure 5.9


a.
Cd in tissue (µg g⁻¹)
40
30
20
10
0
4
10
20
Cd4
Cd3
Cd2
Cd1
Cd0
Time exposed to Cu (days)
b.
Cd in tissue (µg g⁻¹)
40
30
20
10
0
4
10
20
Cd4
Cd3
Cd2
Cd1
Cd0
Time exposed to Zn (days)

accumulation of these two groups were in proportion to their original exposure level. The Cd_3 group initially exposed to 10 µg Cd ℓ^{-1} recorded a rate of 0.41 µg Cd g^{-1} day^{-1}. The Cd_4 group initially exposed to 20 µg Cd ℓ^{-1} accumulated cadmium at a rate of 0.82 µg Cd g^{-1} day^{-1}. In both cases the rate of accumulation is considerably greater than that recorded for the Cd_0 group, initially exposed to only background concentrations.

Copper Concentrations

The regression equations describing the accumulation of copper by each group of mussels while exposed to 10 µg Cu ℓ^{-1} (Figure 5.10a) are given by:

Cd_0 $Y = 1.14 + 2.49X$ $r = 1.00^{***}$

Cd_1 $Y = 1.55 + 1.76X$ $r = 0.98^{**}$

Cd_2 $Y = 1.77 + 1.72X$ $r = 1.00^{***}$

Cd_3 $Y = -1.36 + 2.75X$ $r = 0.96^{**}$

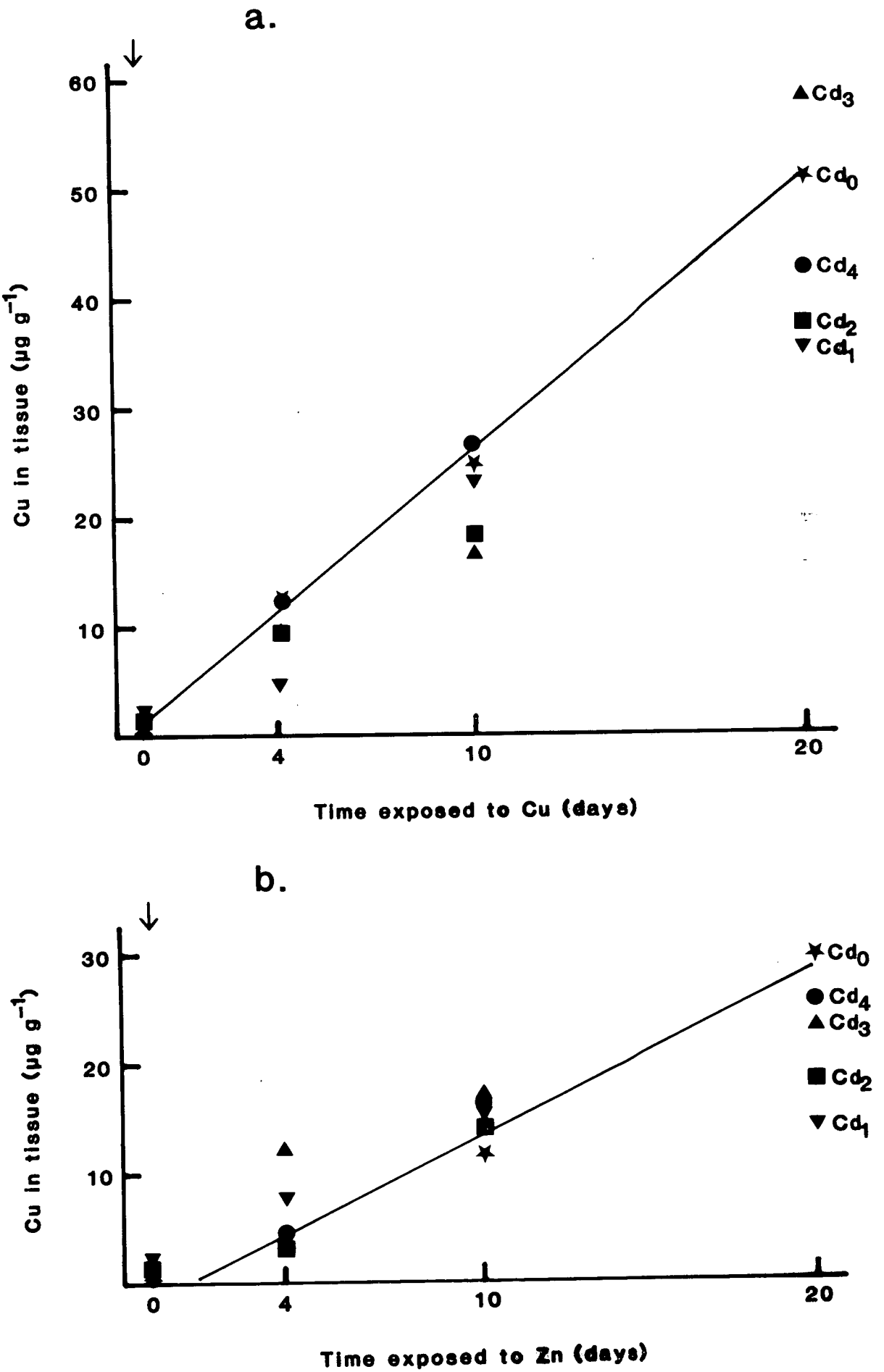
Cd_4 $Y = 3.49 + 2.04X$ $r = 0.99^{***}$

When the regression coefficients (rates of accumulation) for the four groups Cd_1 - Cd_4 were compared to that of Cd_0 group, only the Cd_2 group of mussels differed significantly ($p<0.05$). The rate of accumulation of copper by the Cd_2 group was reduced by the initial exposure to cadmium (5 µg Cd ℓ^{-1}). The rates for the other three groups were not influenced by their initial cadmium exposure.

Zinc Concentrations

The tissue concentration of zinc in all five groups of mussels did not change significantly during the 20 days exposure to copper.

Figure 5.10


a.
60
50
40
30
20
10
Cu in tissue (µg g⁻¹)
▲Cd3
★Cd0
●Cd4
■Cd2
▼Cd1
0
4
10
20
Time exposed to Cu (days)
b.
30
20
10
Cu in tissue (µg g⁻¹)
★Cd0
●Cd4
▲Cd3
■Cd2
▼Cd1
0
4
10
20
Time exposed to Zn (days)

Experiment 2: Subsequent Exposure to Zinc

Cadmium Concentrations

The linear regressions shown in Figure 5.9b describing the accumulation of cadmium while exposed to zinc at 200 μg ℓ^{-1}, can be expressed as:

Cd_0 $Y = 3.21 + 0.00X$ $r = 0.02^{NS}$

Cd_1 $Y = 4.23 + 0.03X$ $r = 0.57^{NS}$

Cd_2 $Y = 9.94 + 0.05X$ $r = 0.75^{NS}$

Cd_3 $Y = 15.30 + 0.28X$ $r = 0.73^{NS}$

Cd_4 $Y = 26.38 + 0.32X$ $r = 0.70^{NS}$

The only groups to show any change in the tissue concentrations of cadmium while exposed to zinc were Cd_3 and Cd_4. They showed similar increases over the 20 day exposure period, during which cadmium was at background concentrations.

Copper Concentrations

Despite the fact that they were exposed to background levels only of copper, during this experiment all five groups increased their tissue concentrations of copper while exposed to zinc (Figure 5.10b). The equations describing these increases are:

Cd_0 $Y = -1.43 + 1.50X$ $r = 0.99^{***}$

Cd_1 $Y = 5.22 + 0.58X$ $r = 0.82^{NS}$

Cd_2 $Y = 1.61 + 0.90X$ $r = 0.95^{**}$

Cd_3 $Y = 6.27 + 0.92X$ $r = 0.92^{*}$

Cd_4 $Y = 1.26 + 1.26X$ $r = 0.98^{**}$

The coefficients of these regressions, with the exception of Cd_2, did not differ significantly ($p>0.05$) from that of Cd_0. The

rate of accumulation of copper by the Cd_2 group, 0.90 μg Cu $g^{-1}day^{-1}$, represented a decrease following the initial exposure to cadmium (5 μg ℓ^{-1}). This decrease was significant only at the 0.05 level. As the other groups exposed to higher or lower initial cadmium levels exhibited no effect due to cadmium, the result for Cd_2 is regarded as an experimental anomaly.

Zinc Concentrations

All five groups increased their tissue concentrations of zinc while exposed to 200 μg Zn ℓ^{-1} (Figure 5.11). The regression equations for these relationships are given by:

Cd_0 $Y = 318.96 + 10.74X$ $r = 0.99^{***}$

Cd_1 $Y = 343.42 + 12.37X$ $r = 0.99^{***}$

Cd_2 $Y = 294.03 + 19.95X$ $r = 0.97^{**}$

Cd_3 $Y = 324.26 + 10.53X$ $r = 0.83^{NS}$

Cd_4 $Y = 303.80 + 14.04X$ $r = 0.98^{**}$

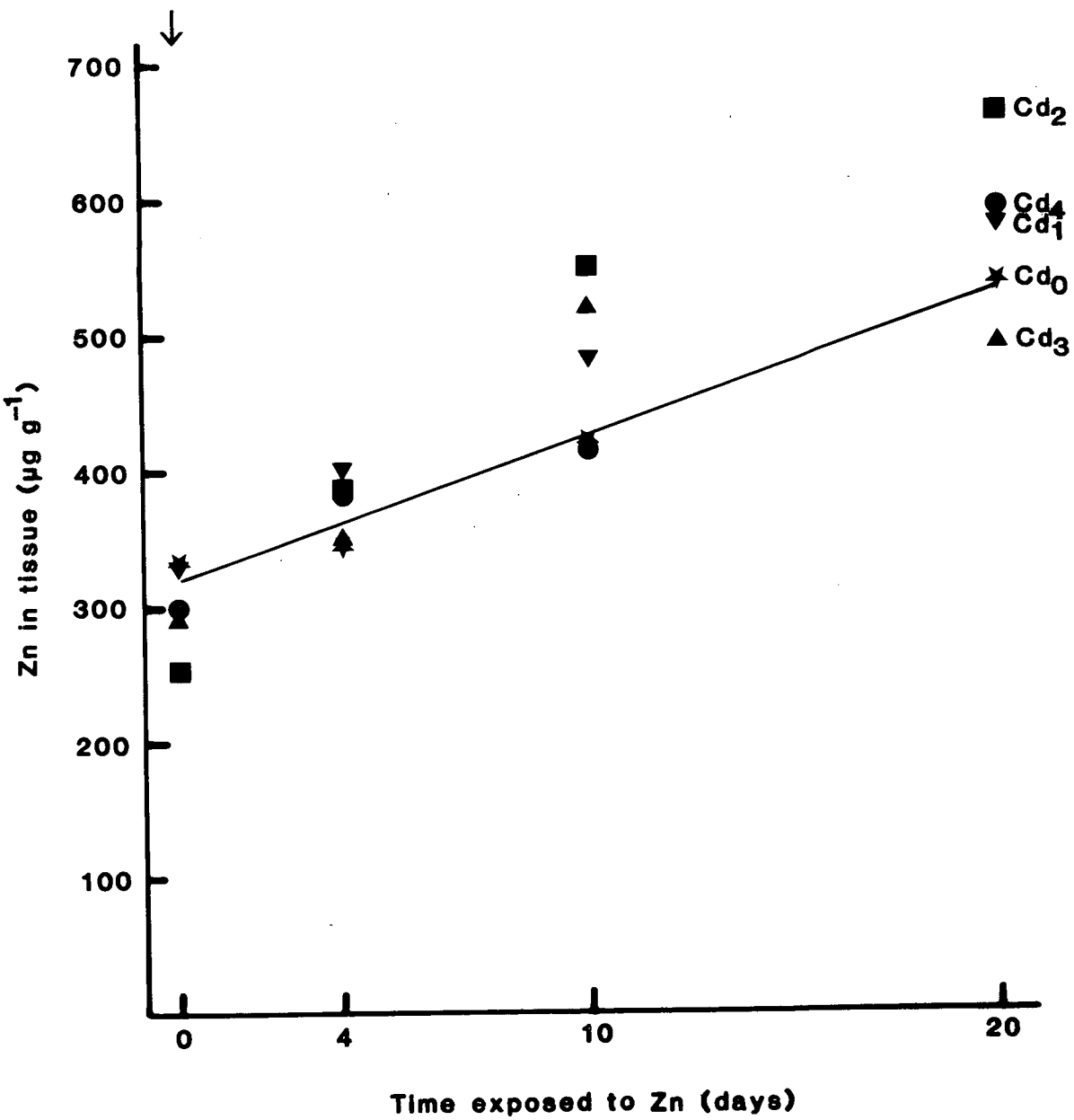
None of the regression coefficients differed significantly ($p>0.05$) from that of the Cd_0 group, exposed initially to background concentrations only. Therefore the initial exposures to cadmium had no influence on the rate of accumulation of zinc from a concentration of 200 μg ℓ^{-1}.

Conclusions

(i) An initial exposure to high external concentrations of cadmium (≥10 μg ℓ^{-1}) resulted in an elevated rate of accumulation of cadmium from background concentrations when the cadmium was replaced by copper or zinc.

(ii) Initial exposure to cadmium did not influence the subsequent rate of accumulation of either copper or zinc, when cadmium was replaced by either of these metals.

Figure 5.11


700
600
500
400
300
200
100
Zn in tissue (µg g⁻¹)
0
4
10
20
Time exposed to Zn (days)
Cd2
Cd4
Cd1
Cd0
Cd3

(iii) Mussels exposed to zinc for 20 days showed elevated tissue concentrations of copper. This was the result of an accumulation of copper from background concentrations.

5.7.3 Influence of an Initial Exposure to Copper

In Experiments 3 and 4 the five groups of mussels were first exposed to one of the following copper concentrations:

Cu_0 - background concentrations,
Cu_1 - 5 μg ℓ^{-1},
Cu_2 - 10 μg ℓ^{-1},
Cu_3 - 20 μg ℓ^{-1},
Cu_4 - 30 μg ℓ^{-1}.

These exposures were terminated after 6 days. All five groups of mussels in Experiment 3 were then placed in a solution of 200 μg ℓ^{-1} zinc for 20 days; whilst the five groups in Experiment 4 were introduced into 10 μg ℓ^{-1} cadmium for 20 days. In both experiments copper was present only at background concentrations during this 20 day period.

Results

Experiment 3: Subsequent Exposure to Zinc

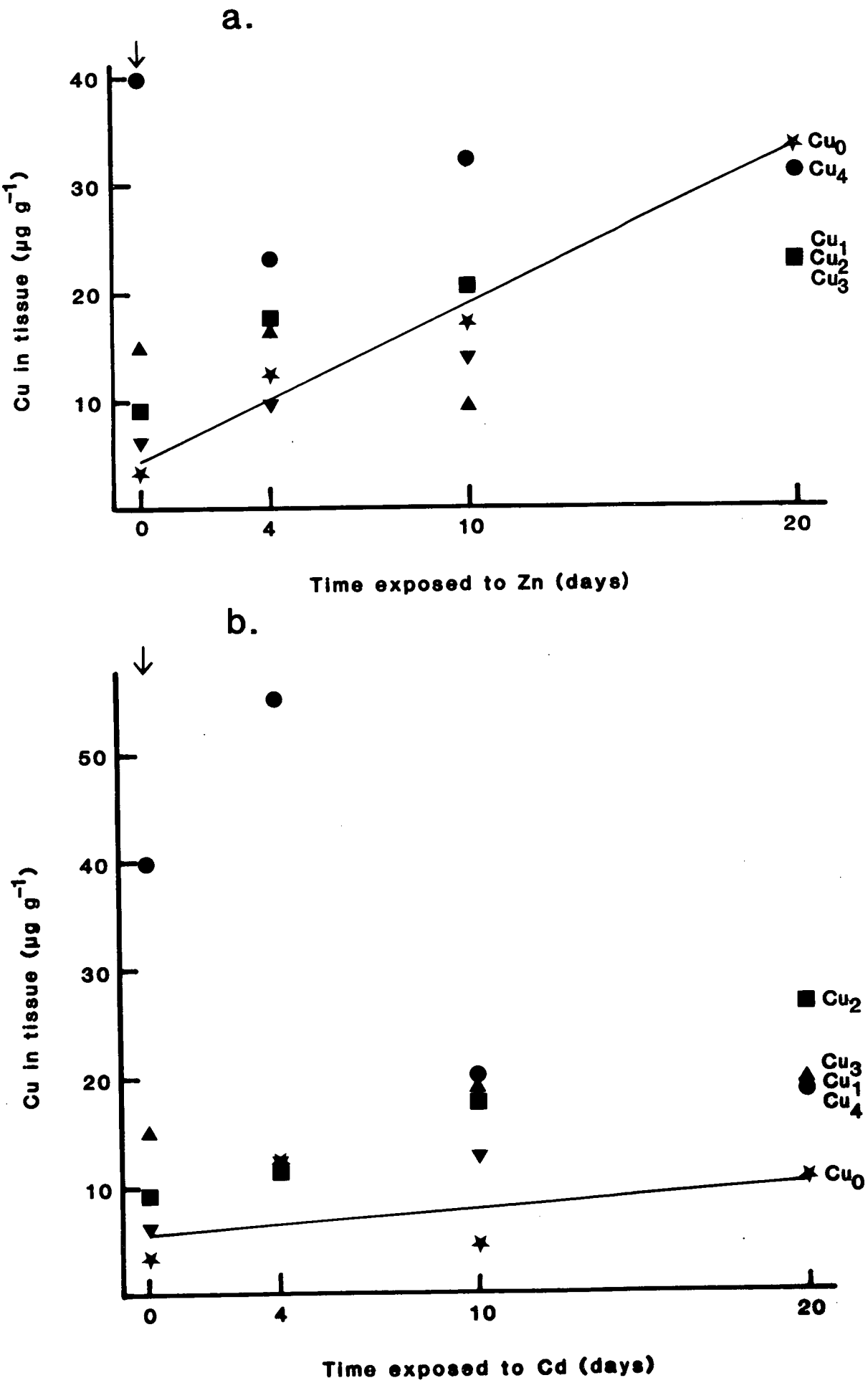
Cadmium Concentrations

The cadmium concentrations in the tissue of all five groups did not change significantly during the 20 days exposure to zinc.

Copper Concentrations

Only the Cu_4 group of mussels failed to increase their tissue concentrations of copper while exposed to zinc (Figure 5.12a). The other four each displayed an accumulation of copper which can be expressed as:

Figure 5.12


a.
40
30
20
10
Cu in tissue (µg g⁻¹)
Cu0
Cu4
Cu1
Cu2
Cu3
0
4
10
20
Time exposed to Zn (days)
b.
50
40
30
20
10
Cu in tissue (µg g⁻¹)
Cu2
Cu3
Cu1
Cu4
Cu0
0
4
10
20
Time exposed to Cd (days)

Cu_0 $Y = 4.43 + 1.45X$ $r = 0.99^{***}$

Cu_1 $Y = 6.27 + 0.83X$ $r = 1.00^{***}$

Cu_2 $Y = 12.55 + 0.60X$ $r = 0.87^{*}$

Cu_3 $Y = 13.21 + 0.31X$ $r = 0.52^{NS}$

The coefficients of these regressions were all significantly different ($p<0.05$). Rate of accumulation of copper decreased with the increase in initial exposure level. The Cu_4 group recorded a loss of copper from the tissue while exposed to zinc (Figure 5.12a).

Zinc Concentrations

The five groups of mussels all increased their tissue concentration of zinc while exposed to 200 µg Zn ℓ^{-1} (Figure 5.13). Regression equations for the relationships are given by:

Cu_0 $Y = 297.33 + 11.65X$ $r = 0.96^{**}$

Cu_1 $Y = 347.47 + 10.18X$ $r = 0.89^{*}$

Cu_2 $Y = 288.43 + 16.59X$ $r = 0.98^{**}$

Cu_3 $Y = 306.12 + 12.94X$ $r = 0.94^{*}$

Cu_4 $Y = 275.58 + 14.35X$ $r = 0.98^{**}$

Comparison of the regression coefficients revealed that all five groups of mussels recorded a similar rate of accumulation of zinc. This was irrespective of the copper concentration to which they had previously been exposed.

Experiment 4: Subsequent Exposure to Cadmium

Cadmium Concentrations

When exposed to 10 µg Cd ℓ^{-1} all five groups showed similar increases in tissue concentration of cadmium over the 20 days (Figure 5.14). Regression equations for these relationships are given by:

Figure 5.13

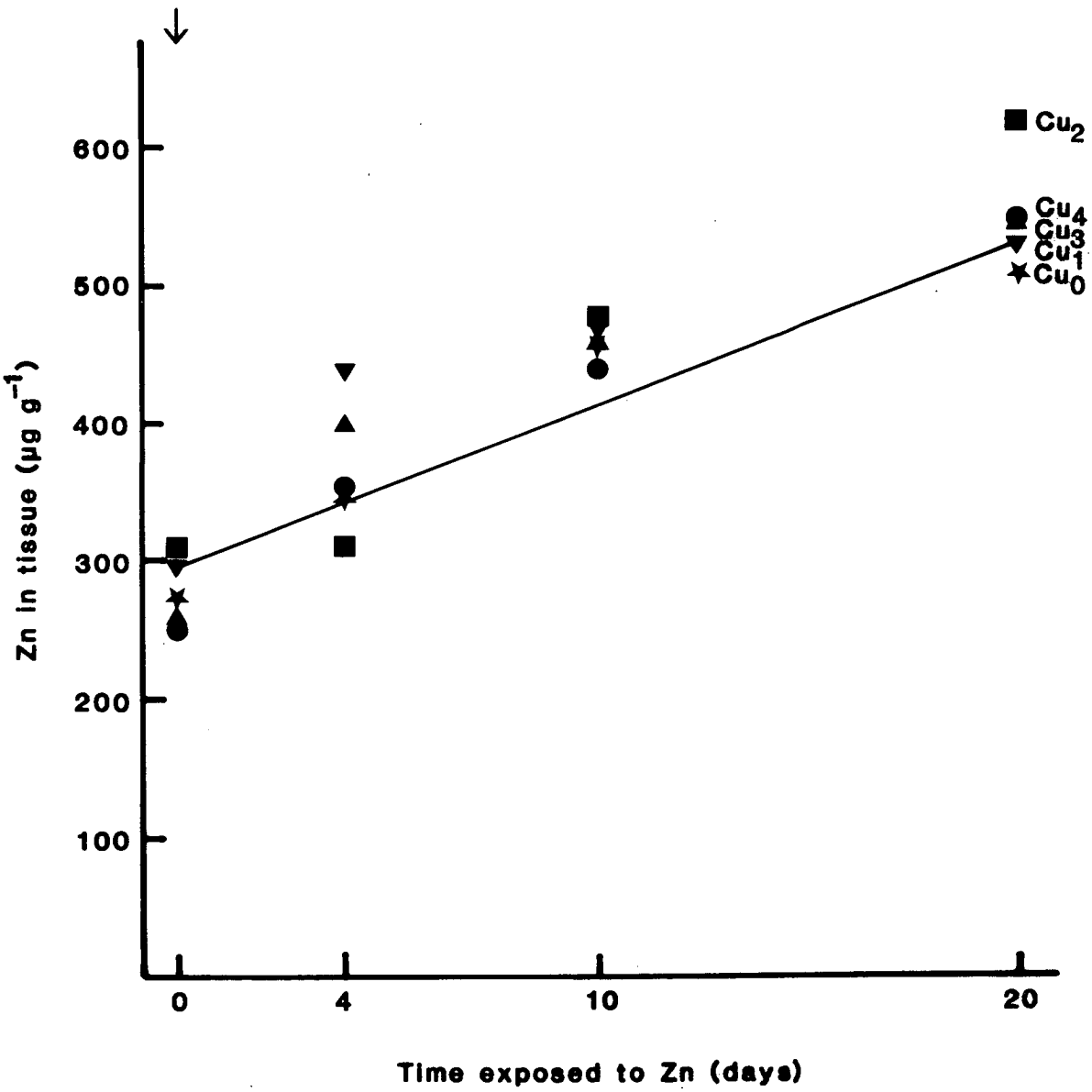
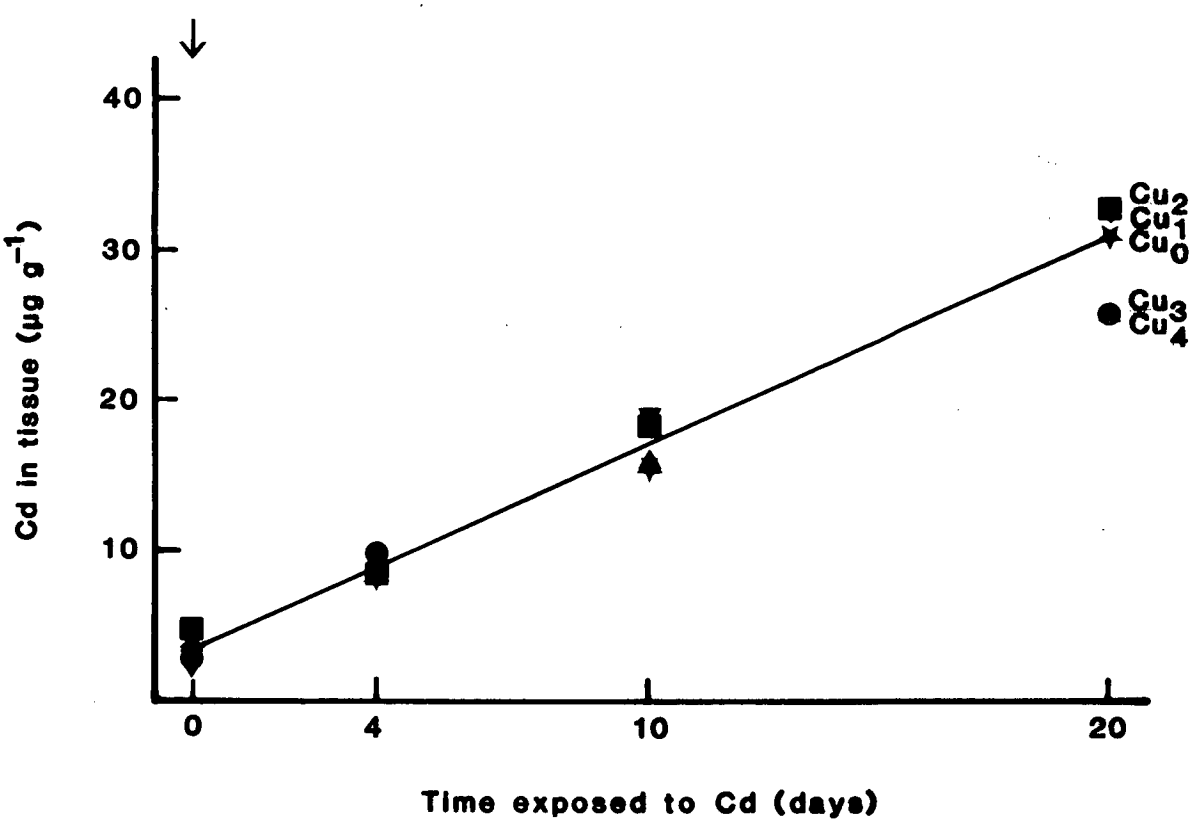

Zn in tissue (µg g⁻¹)
600
500
400
300
200
100
0
4
10
20
Time exposed to Zn (days)
Cu2
Cu4
Cu3
Cu1
Cu0

Figure 5.14


40
30
20
10
Cd in tissue (µg g⁻¹)
0
4
10
20
Time exposed to Cd (days)
Cu2
Cu1
Cu0
Cu3
Cu4

Cu_0 $Y = 2.95 + 1.36X$ $r = 1.00^{***}$

Cu_1 $Y = 2.88 + 1.51X$ $r = 1.00^{***}$

Cu_2 $Y = 3.76 + 1.44X$ $r = 1.00^{***}$

Cu_3 $Y = 4.09 + 1.10X$ $r = 1.00^{***}$

Cu_4 $Y = 4.63 + 1.13X$ $r = 0.98^{**}$

The coefficients of the five regressions were not significantly different ($p>0.05$), indicating that the rate of accumulation of cadmium was not influenced by the prior exposures to copper.

Copper Concentrations

As in Experiment 3, the Cu_4 group of mussels lost copper from the tissue, this time when exposed to cadmium (Figure 5.12b). The other four groups all increased their tissue concentrations of copper, according to the following regression equations:

Cu_0 $Y = 6.08 + 0.21X$ $r = 0.39^{NS}$

Cu_1 $Y = 7.84 + 0.56X$ $r = 0.95^{*}$

Cu_2 $Y = 8.78 + 0.88X$ $r = 1.00^{***}$

Cu_3 $Y = 13.74 + 0.33X$ $r = 0.73^{NS}$

These increases in copper concentrations resulted from accumulation of copper from background concentrations. The rates of accumulation by Cu_1 and Cu_2 were not significantly different ($p>0.05$).

Zinc Concentrations

The tissue concentration of zinc in all five groups did not vary significantly during the exposure to cadmium.

Conclusions

(i) An initial exposure to copper for 6 days has no influence on the subsequent rate of accumulation of zinc or cadmium.

(ii) Exposure to zinc or cadmium may result in an increased accumulation of copper from background concentrations; the increase being greater on exposure to zinc.

5.7.4 Influence of an Initial Exposure to Zinc

In Experiments 5 and 6 the five groups of mussels were first exposed to one of the following zinc concentrations:

Zn_0 - background concentrations,
Zn_1 - 50 μg ℓ^{-1},
Zn_2 - 100 μg ℓ^{-1},
Zn_3 - 200 μg ℓ^{-1},
Zn_4 - 400 μg ℓ^{-1}.

These exposures were terminated after 6 days. All five groups of mussels in Experiment 5 were then placed in a 10 μg ℓ^{-1} copper solution for 20 days. The five groups in Experiment 6 were exposed to cadmium at 10 μg ℓ^{-1} for 20 days.

Results

Experiment 5: Subsequent Exposure to Copper

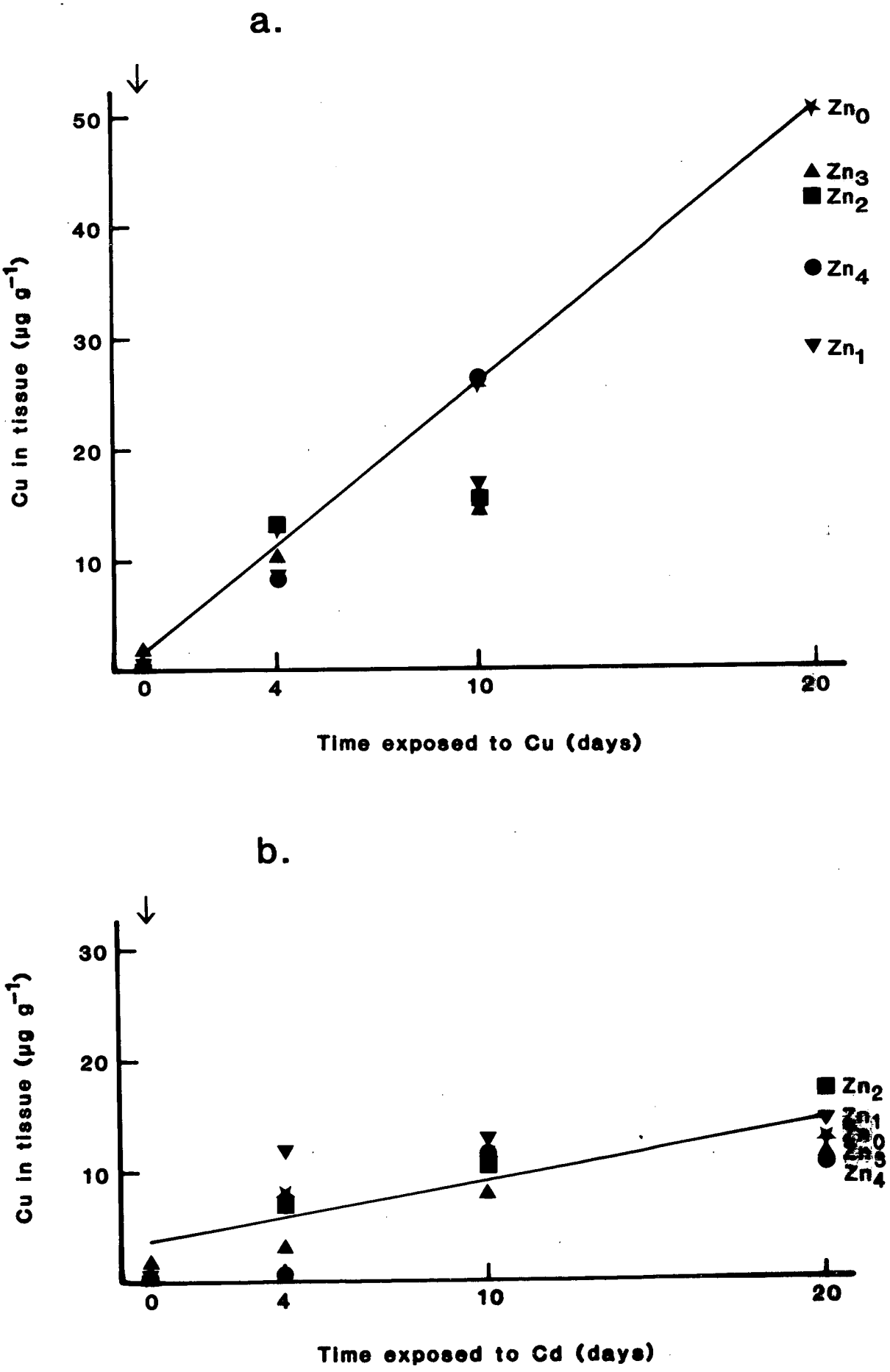
Cadmium Concentrations

No significant variations ($p>0.05$) in the tissue concentration of cadmium were recorded in any group during the 20 days exposure to copper.

Copper Concentrations

All five groups increased their tissue concentrations of copper while exposed to 10 μg Cu ℓ^{-1} (Figure 5.15a). Comparison of the regression coefficients revealed that the rates of accumulation of copper were not significantly different ($p>0.05$), irrespective of the

Figure 5.15


a.
50
40
30
20
10
Cu in tissue (µg g⁻¹)
Zn0
Zn3
Zn2
Zn4
Zn1
0
4
10
20
Time exposed to Cu (days)
b.
30
20
10
Cu in tissue (µg g⁻¹)
Zn2
Zn1
Zn0
Zn3
Zn4
0
4
10
20
Time exposed to Cd (days)

initial level of exposure to zinc. The regression equations for these relationships are given by:

Zn_0 $Y = 1.63 + 2.45X$ $r = 1.00^{***}$

Zn_1 $Y = 2.22 + 1.38X$ $r = 0.99^{***}$

Zn_2 $Y = 1.07 + 1.99X$ $r = 0.97^{**}$

Zn_3 $Y = 0.28 + 2.08X$ $r = 0.97^{**}$

Zn_4 $Y = 2.61 + 1.81X$ $r = 0.97^{**}$

Zinc Concentrations

At the end of 20 days exposure to 10 µg Cu ℓ^{-1} all five groups had similar tissue concentrations of zinc (Figure 5.16a). The Zn_3 and Zn_4 groups both decreased their tissue concentrations while the other three groups showed either a slight increase or no change. The regression equation for the Zn_0 group:

$$Y = 268.54 + 4.79X \quad r = 0.88^{*}$$

was the only significant relationship recorded.

Experiment 6: Subsequent Exposure to Cadmium

Cadmium Concentrations

When exposed to 10 µg Cd ℓ^{-1} all five groups showed similar increases in cadmium concentration of the tissue (Figure 5.17). Regression equations for these relationships are given by:

Zn_0 $Y = 3.21 + 1.26X$ $r = 0.99^{***}$

Zn_1 $Y = 2.99 + 1.49X$ $r = 0.99^{***}$

Zn_2 $Y = 4.77 = 1.30X$ $r = 1.00^{***}$

Zn_3 $Y = 1.36 + 1.15X$ $r = 0.99^{***}$

Zn_4 $Y = 2.11 + 1.14X$ $r = 1.00^{***}$

Figure 5.16

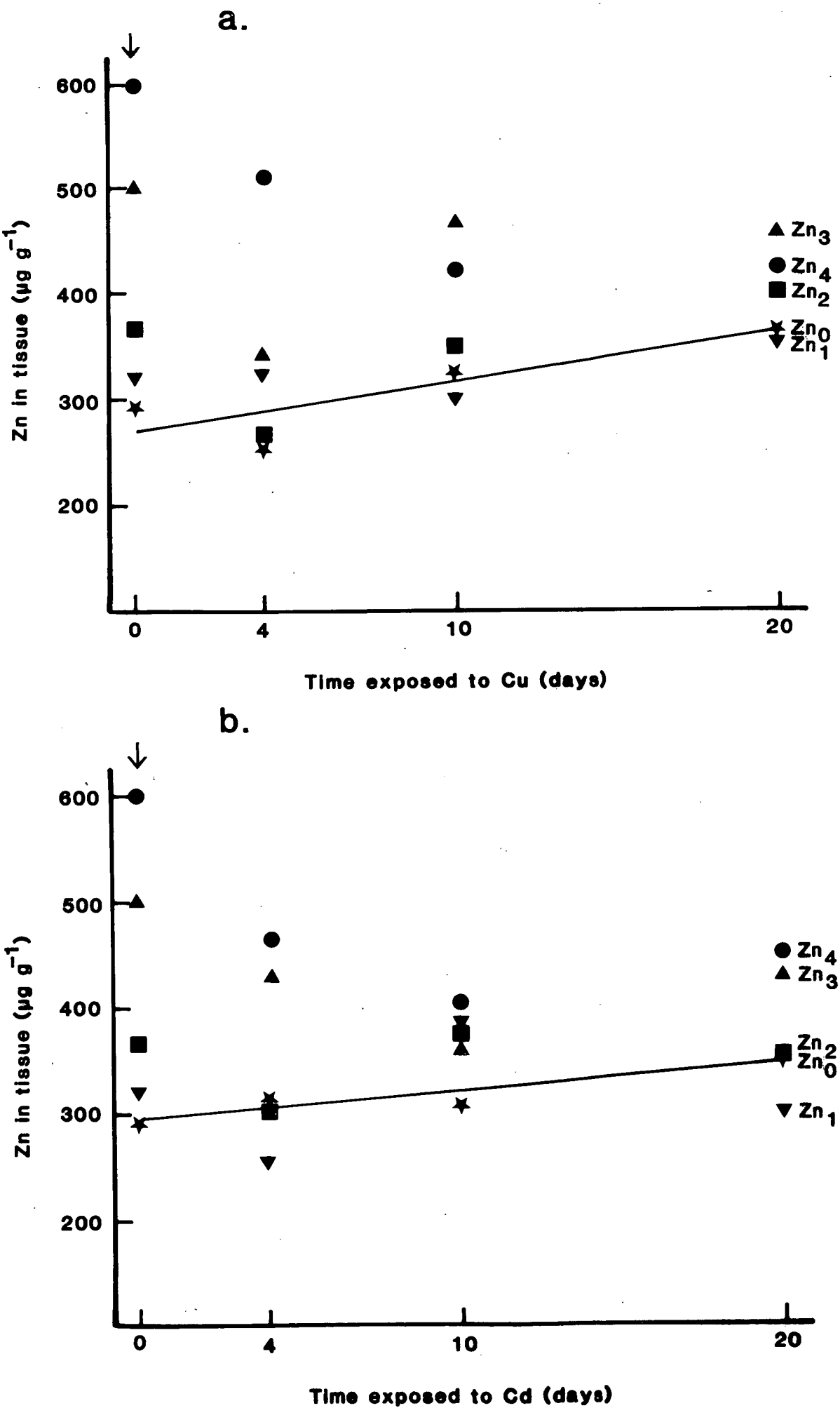
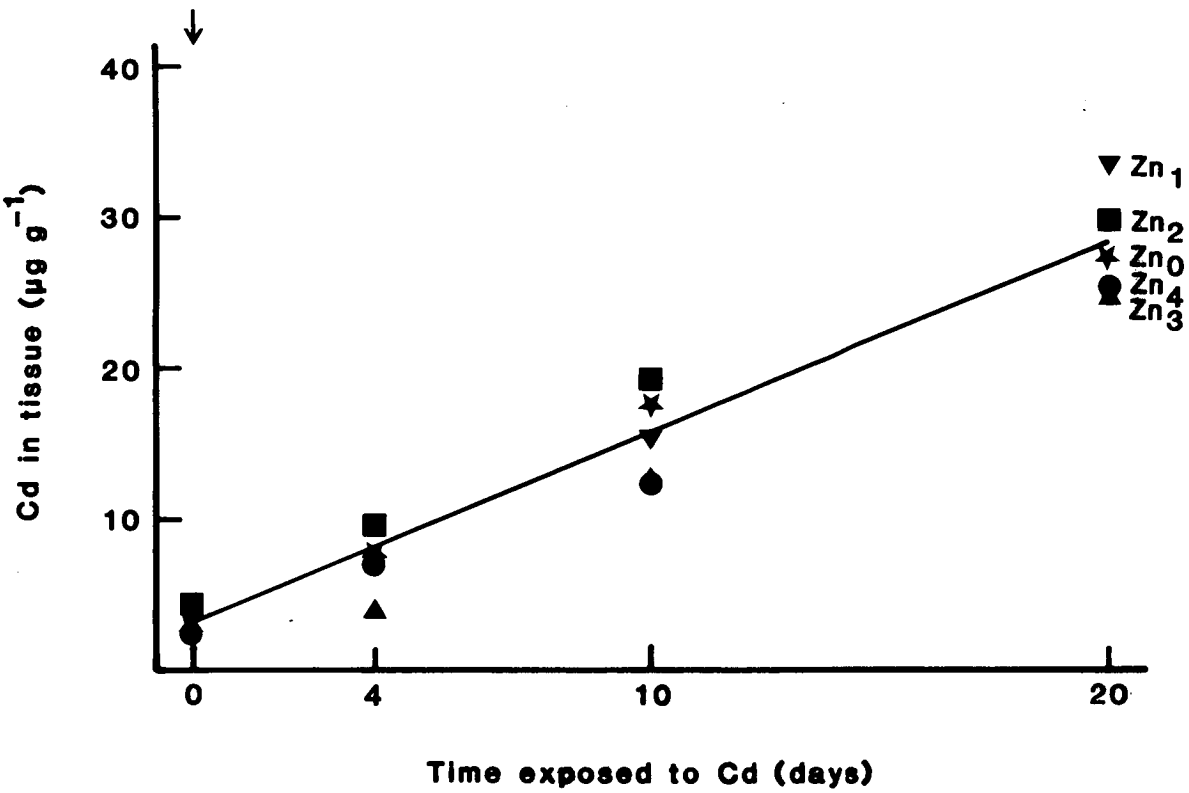

a.
600
500
400
300
200
Zn in tissue (μg g⁻¹)
Zn3
Zn4
Zn2
Zn0
Zn1
0
4
10
20
Time exposed to Cu (days)
b.
600
500
400
300
200
Zn in tissue (μg g⁻¹)
Zn4
Zn3
Zn2
Zn0
Zn1
0
4
10
20
Time exposed to Cd (days)

Figure 5.17


40
30
20
10
0
4
10
20
Cd in tissue (µg g⁻¹)
Time exposed to Cd (days)
Zn1
Zn2
Zn0
Zn4
Zn3

Comparison of the regression coefficients confirmed that the rates of accumulation were not significantly different ($p>0.05$). Thus the rate of accumulation of cadmium was not influenced by a prior exposure to zinc.

Copper Concentrations

When exposed to cadmium all five groups showed similar increases in copper concentration of the tissue (Figure 5.15b). The regression equations describing these relationships are given by:

Zn_0 $Y = 3.72 + 0.53X$ $r = 0.85^{NS}$

Zn_1 $Y = 5.36 + 0.56X$ $r = 0.97^{**}$

Zn_2 $Y = 2.07 + 0.79X$ $r = 0.97^{**}$

Zn_3 $Y = 2.04 + 0.48X$ $r = 0.99^{***}$

Zn_4 $Y = 1.11 + 0.58X$ $r = 0.83^{NS}$

The coefficients of these regressions were not significantly different ($p>0.05$), indicating that the rates of accumulation of copper from background concentrations were not influenced by the prior exposures to zinc.

Zinc Concentrations

Groups Zn_3 and Zn_4 lost zinc from the tissue while exposed to cadmium (Figure 5.16b). The tissue concentrations of the Zn_1 and Zn_2 groups were erratic but did not change significantly ($p>0.05$) during the 20 days (Figure 5.16b). On the other hand the Zn_0 group increased its zinc concentration while exposed to cadmium ($Y = 294.04 + 2.62X$, $r = 0.93^{*}$, Figure 5.16b).

Conclusions

(i) An initial six day exposure to zinc has no influence on the subsequent accumulation of copper or cadmium.

(ii) Mussels exposed to cadmium showed an increased accumulation of copper from background concentrations.

5.7.5 Conclusions from Series III Experiments

1. The accumulation of one metal is independent of a prior 6 day exposure to another metal.
2. Mussels exposed to high external concentrations of cadmium ($\geq$10 $\mu g\ \ell^{-1}$) may continue to accumulate cadmium from background concentrations when the cadmium is replaced by either copper or zinc.
3. Mussels may accumulate copper from background concentrations at an accelerated rate when exposed to elevated concentrations of either cadmium or zinc.

5.8 SERIES IV

5.8.1 Introduction

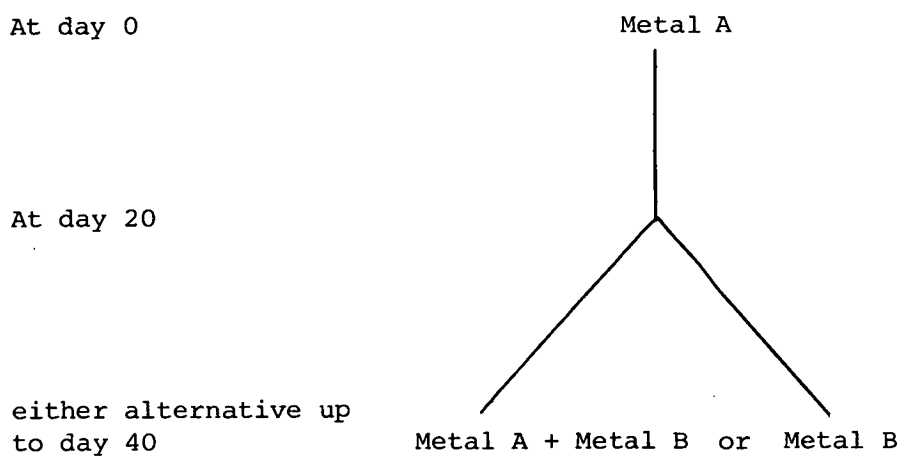
In this series of experiments two aspects of the possible interaction of the three metals are investigated. Both are extensions of the work described in the previous three series of experiments.

1. To investigate, following the lack of any observable interaction in Series III, whether a longer period of prior exposure to one metal would influence the subsequent accumulation of another.
2. To investigate whether the interactions observed in Series I and II, when mussels were exposed to two metals simultaneously, are influenced by a prior exposure to either of the two metals.

The length of time selected for the period of prior exposure in both aspects under investigation was 20 days.

5.8.2 Experimental Design

In each of six experiments, 130 mussels were exposed for 20 days to one metal (Metal A). These mussels were subsampled, and their tissue analysed for each of the three metals (Cd, Cu and Zn), on days 0, 4, 10 and 20. On day 20 the remaining mussels (approximately 90) were divided into two equal groups and exposed for a further 20 days to either a second metal alone (Metal B), or to a combination of Metals A and B. Samples were removed for analysis on days 24, 30 and 40 from the commencement of the experiment. This experimental design can be summarised as follows:



Six experiments were required in order to examine each double combination of the three metals:

Experiment No.	Metal A	Metal B
1	Cd	Zn
2	Zn	Cd
3	Cu	Zn
4	Zn	Cu
5	Cd	Cu
6	Cu	Cd

In conjunction with each pair of experiments, i.e. 1 and 2, 3 and 4, 5 and 6, an additional group of mussels was exposed to background concentrations only. This control group was subsampled at the same times as the experimental groups. Concentrations of all three metals were measured in each tissue analysis.

The three metals were presented at concentrations which were shown in Series I to be likely to produce interactions, viz:

Cadmium - 10 μg ℓ^{-1}, copper - 10 μg ℓ^{-1}, zinc - 200 μg ℓ^{-1}.

5.8.3 Experimental Analysis

The data for each 20 day phase of an experiment (i.e. day 0-20, day 20-40) were analysed by linear regression where appropriate. These regressions, however, were not included in Figures 5.18 to 5.24 as it was considered that an interpretation of these figures would be best aided by connecting the data points. Regression equations are presented within each figure for the respective data. The regression coefficients (i.e. rates of accumulation) were compared by t-test.

5.8.4 Experiments 1 and 2: Cadmium and Zinc

Results

Behaviour of Control Group

The control group of mussels associated with these two experiments exhibited uncharacteristic fluctuations in tissue concentration during the latter part of the experimental period (Figures 5.18, 5.19 and 5.20). The tissue analyses revealed increased concentrations of all three metals between days 20 and 40. These increases were greater than those previously recorded for control groups.

In view of the behaviour of the control mussels interpretation of the results of these two experiments must be treated with caution. However, the results are included because it is considered that the behaviour of the controls was due to unidentified contamination within

their tank, and not within the seawater supply. Periodic samplings of the seawater supply revealed no contamination (Chapter 2).

Cadmium Concentrations

The tissue concentrations of cadmium during Experiment 1 are shown in Figure 5.18a. Addition of 200 μg Zn ℓ^{-1} at day 20 had no effect on the accumulation of cadmium. Although a decrease in rate of accumulation from 1.06 to 0.89 μg Cd g^{-1} day^{-1} occurred, it was not significant ($p>0.05$) (Figure 5.18a). When cadmium was replaced at day 20 by zinc at 200 μg ℓ^{-1}, the rate of accumulation of cadmium declined and from day 24 onwards there was a loss of cadmium from the tissue (Figure 5.18a).

While exposed to 200 μg Zn ℓ^{-1}, in Experiment 2, the mussels did not accumulate any cadmium (Figure 5.18b). Once transferred to 10 μg Cd ℓ^{-1} at day 20, however, either alone or with continued zinc exposure, cadmium was accumulated. Comparison of the regression coefficients for cadmium accumulation between days 20 and 40, indicates that the rate of cadmium accumulation was independent of the presence of zinc.

While exposed to 10 μg Cd ℓ^{-1} alone in Experiment 1, cadmium was accumulated at a rate of 1.06 μg g^{-1} day^{-1} (Figure 5.18a). Following 20 days exposure to zinc in Experiment 2, the accumulation of cadmium remained at 1.06 μg g^{-1} day^{-1}. Thus the accumulation of cadmium was not affected by the initial 20 day exposure to zinc.

Zinc Concentrations

While exposed to 10 μg Cd ℓ^{-1}, in Experiment 1, the mussels did not accumulate zinc (Figure 5.19a). However, once the cadmium was replaced at day 20 by 200 μg Zn ℓ^{-1}, zinc was accumulated. Figure 5.19a further shows that the rate of accumulation of this zinc was independent of the presence of cadmium.

Figure 5.18

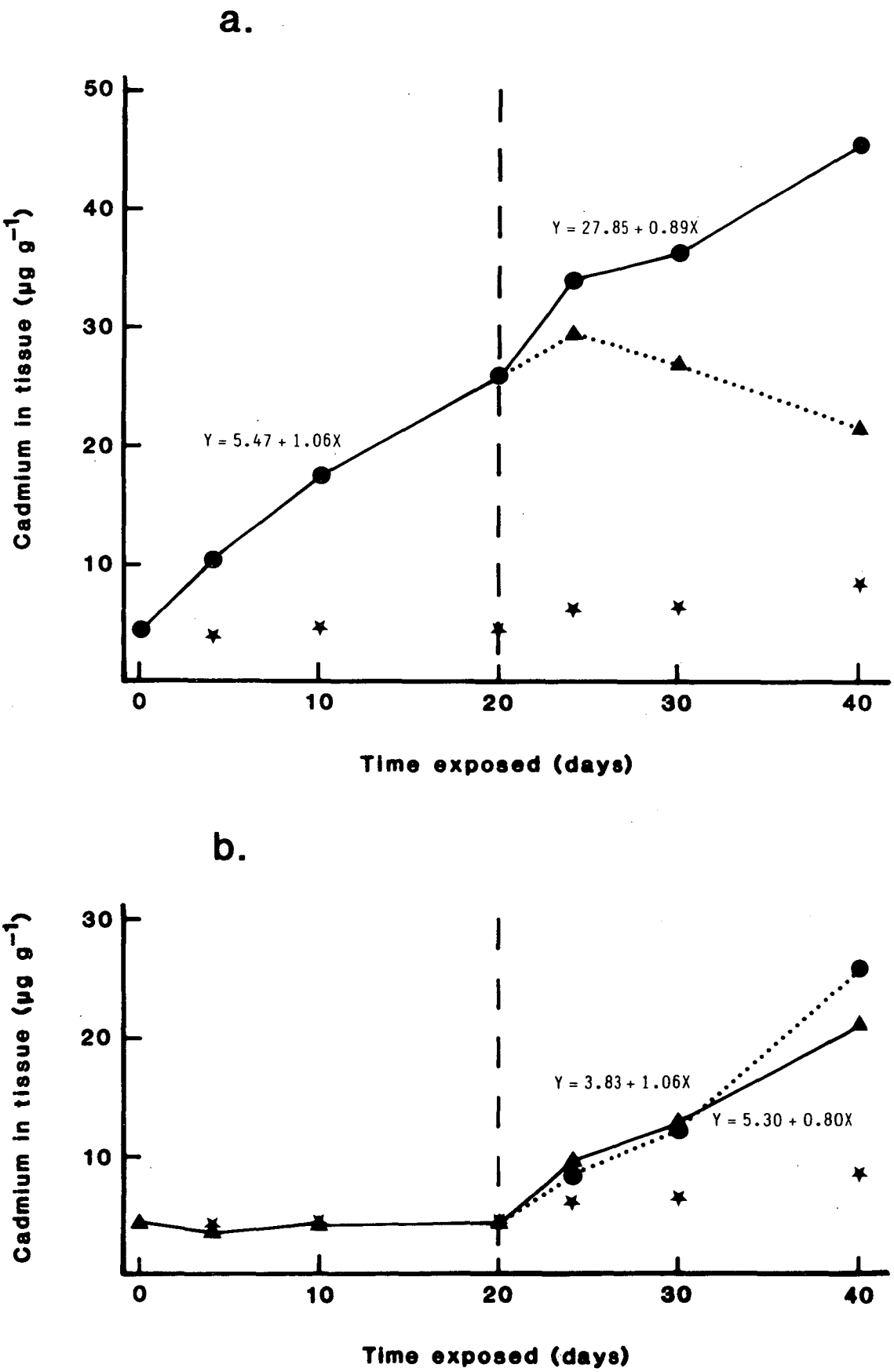
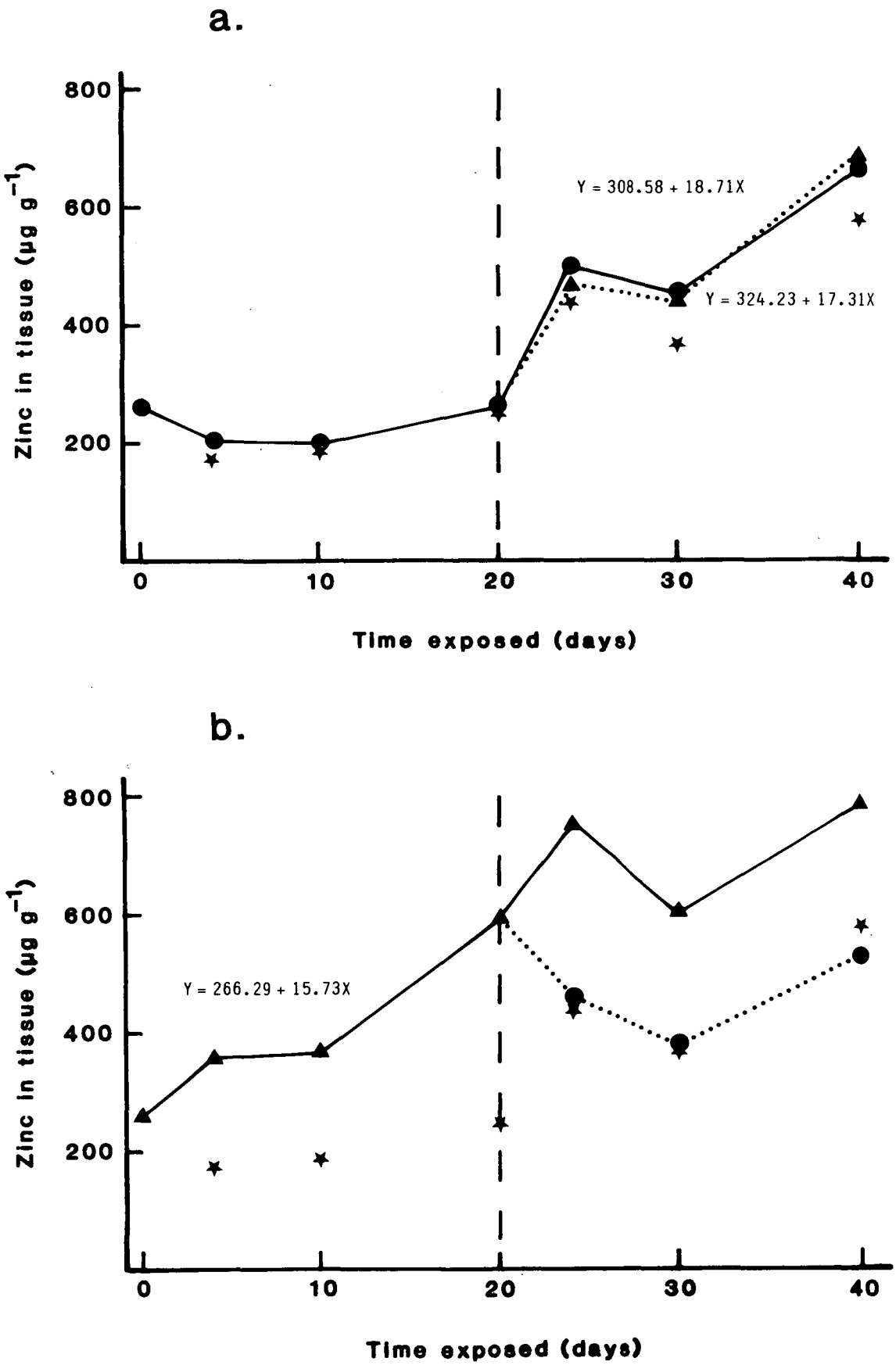

a.
Cadmium in tissue (µg g⁻¹)
50
40
30
20
10
Y = 27.85 + 0.89X
Y = 5.47 + 1.06X
0
10
20
30
40
Time exposed (days)
b.
Cadmium in tissue (µg g⁻¹)
30
20
10
Y = 3.83 + 1.06X
Y = 5.30 + 0.80X
0
10
20
30
40
Time exposed (days)

Figure 5.19


a.
800
600
400
200
Zinc in tissue (µg g⁻¹)
Y = 308.58 + 18.71X
Y = 324.23 + 17.31X
0
10
20
30
40
Time exposed (days)
b.
800
600
400
200
Zinc in tissue (µg g⁻¹)
Y = 266.29 + 15.73X
0
10
20
30
40
Time exposed (days)

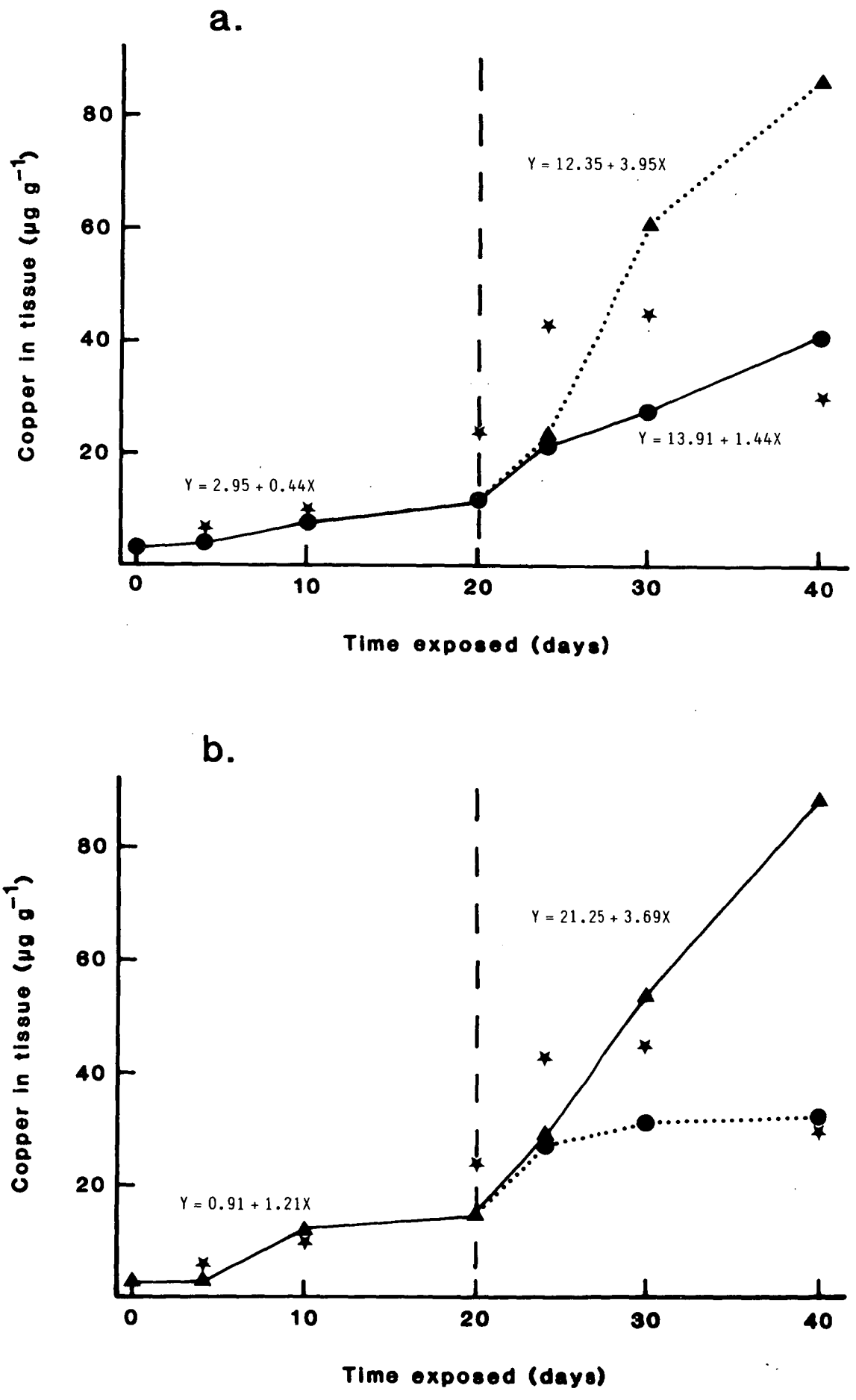
The accumulation of zinc from 200 μg Zn ℓ^{-1} during Experiment 2 was unaffected by the addition of 10 μg Cd ℓ^{-1} at day 20 (Figure 5.19b). When the zinc was replaced at day 20 by cadmium a loss of zinc from the mussel tissue resulted.

The rate of accumulation of zinc in Experiment 2 resulting from an exposure to 200 μg Zn ℓ^{-1} alone, was 15.73 μg g^{-1} day^{-1} (Figure 5.19b). Following 20 days exposure to cadmium in Experiment 1, the rate of accumulation of zinc by the mussels transferred to zinc was 18.71 μg g^{-1} day^{-1} (Figure 5.19a). These two rates were not statistically different ($p>0.05$). Thus the accumulation of zinc was not affected by the initial exposure to cadmium.

Copper Concentrations

While exposed to cadmium in Experiment 1, the tissue concentration of copper within the mussels increased slightly (Figure 5.20a). However, when the cadmium was replaced by zinc at day 20, a rapid accumulation of copper from background concentrations occurred. Where the mussels were exposed to cadmium and zinc together the rate of accumulation of copper also increased, but not to such a marked extent (Figure 5.20a). It therefore appears that the presence of cadmium antagonises the accelerated accumulation of copper induced by the presence of zinc. Figure 5.20b shows, however, that during exposure to 200 μg Zn ℓ^{-1} only a slow accumulation of copper from background concentrations occurred. This suggests that the accelerated accumulation of copper in the presence of zinc was stimulated by the initial exposure to cadmium. Nevertheless the addition of cadmium to zinc at day 20 in Experiment 2 also led to an increased accumulation of copper (Figure 5.20b). On the other hand replacement of zinc by cadmium led to only a slight increase in tissue copper.

Figure 5.20


a.
Copper in tissue (µg g⁻¹)
80
60
40
20
Y = 12.35 + 3.95X
Y = 13.91 + 1.44X
Y = 2.95 + 0.44X
0
10
20
30
40
Time exposed (days)
b.
Copper in tissue (µg g⁻¹)
80
60
40
20
Y = 21.25 + 3.69X
Y = 0.91 + 1.21X
0
10
20
30
40
Time exposed (days)

Conclusions

(i) Zinc either in an initial exposure or presented simultaneously with cadmium, had no influence on the rate of accumulation of cadmium.

(ii) Cadmium either in an initial exposure or presented simultaneously with zinc, had no influence on the rate of accumulation of zinc.

(iii) Exposure to zinc, either in combination with, or following, exposure to cadmium, resulted in accelerated rates of accumulation of copper from background concentrations.

5.8.5 Experiments 3 and 4: Copper and Zinc

Results

Cadmium Concentrations

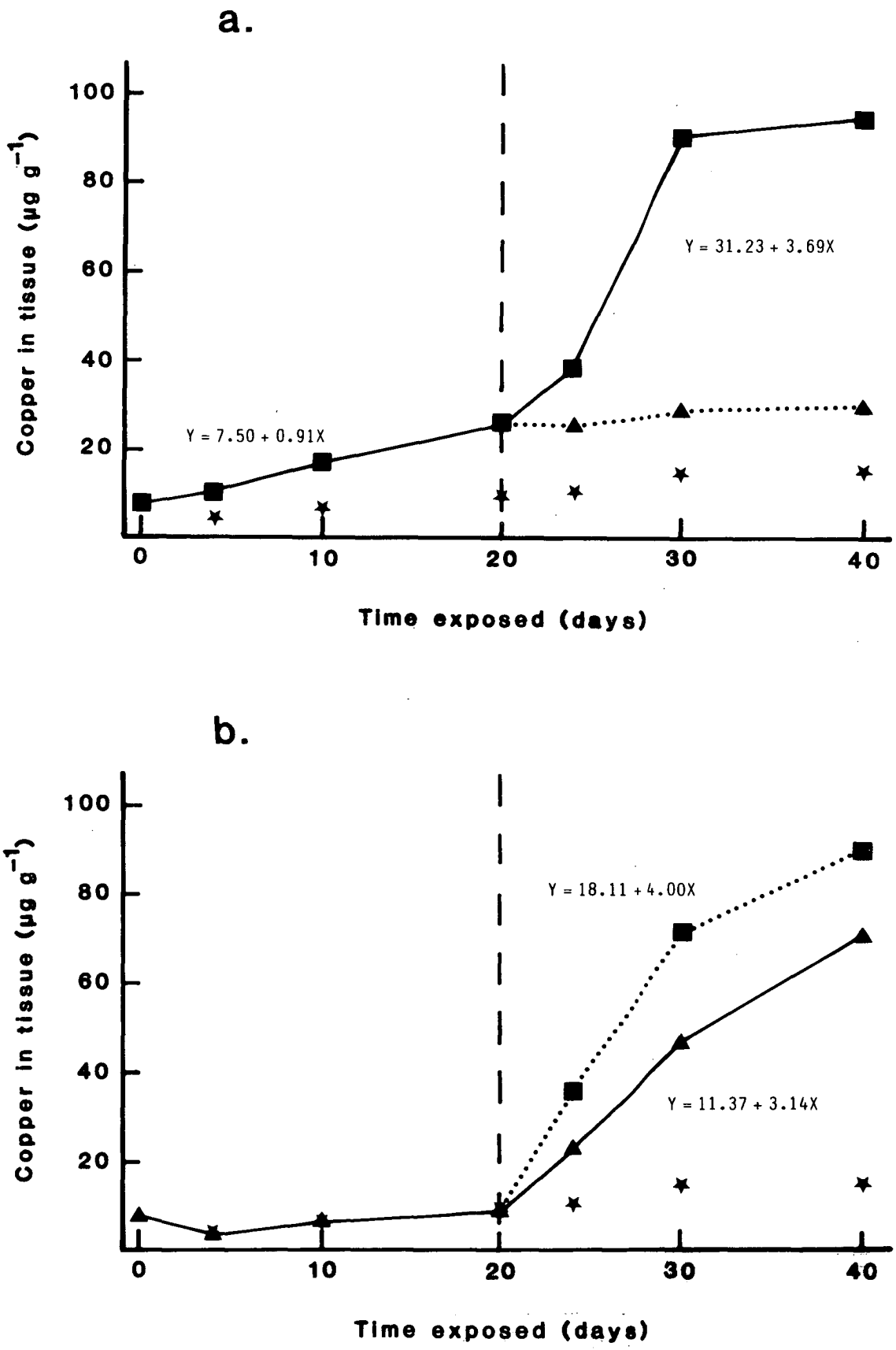
The tissue concentrations of cadmium recorded during both Experiments 3 and 4, did not vary from those of the control group of mussels exposed to background concentrations.

Copper Concentrations

The tissue concentrations of copper in the mussels during Experiment 3 are shown in Figure 5.21a. While exposed to 10 μg Cu ℓ^{-1} during the first 20 days, copper was accumulated at a rate of 0.91 μg g^{-1} day^{-1}. Addition of 200 μg Zn ℓ^{-1} to the copper resulted in a four-fold increase in rate of accumulation of copper to 3.69 μg g^{-1} day^{-1} (Figure 5.21a). When copper was replaced at day 20 by zinc, accumulation of copper was reduced to a rate similar to that shown by the controls.

Little change in tissue concentration of copper was observed when mussels were exposed to zinc (Figure 5.21b). However, the introduction of copper either in place of, or in combination with, zinc, resulted in a rapid accumulation of copper. Accumulation rate of copper was lower

Figure 5.21


a.
Copper in tissue (µg g^{-1})
100
80
60
40
20
Y = 31.23 + 3.69X
Y = 7.50 + 0.91X
0
10
20
30
40
Time exposed (days)
b.
Copper in tissue (µg g^{-1})
100
80
60
40
20
Y = 18.11 + 4.00X
Y = 11.37 + 3.14X
0
10
20
30
40
Time exposed (days)

when zinc and copper were presented together but the difference was not significant ($p>0.05$).

The rate of accumulation of copper during days 0 to 20 in Experiment 3 was 0.91 $\mu g\ g^{-1}\ day^{-1}$ (Figure 5.21a). The rate recorded between days 20 and 40 in Experiment 4 while exposed to copper alone was 4.00 $\mu g\ g^{-1}\ day^{-1}$. The increase in rate of accumulation of copper was due apparently to the 20 days prior exposure to zinc.

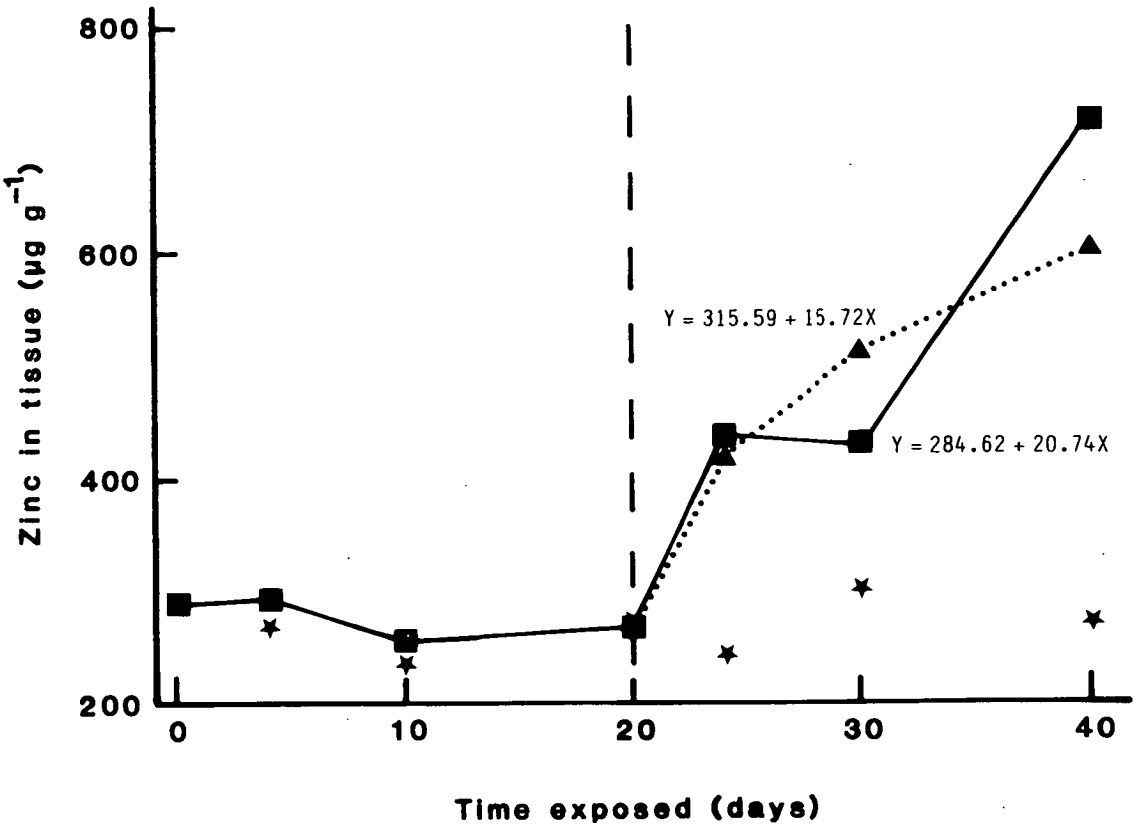
Zinc Concentrations

In Experiment 3, while exposed to copper the zinc concentrations within the tissue did not differ from those of the control group exposed to background concentrations only (Figure 5.22a). Upon the replacement of copper by 200 $\mu g\ Zn\ \ell^{-1}$ at day 20, zinc was accumulated at a rate of 15.72 $\mu g\ g^{-1}\ day^{-1}$. When copper and zinc were presented simultaneously the rate of zinc accumulation was 20.74 $\mu g\ g^{-1}\ day^{-1}$ (Figure 5.22a). These two rates were not significantly different ($p>0.05$). Accumulation of zinc was therefore independent of the presence of copper. Likewise the rate of accumulation of zinc between days 0 and 20 of Experiment 4 (Figure 5.22b) was not affected by the subsequent exposure to zinc and copper together. Although the rate increased from 9.76 to 13.99 $\mu g\ g^{-1}\ day^{-1}$, this increase was not statistically significant ($p>0.05$). When the zinc was replaced at day 20 by copper, zinc continued to be accumulated at a low rate from background concentrations.

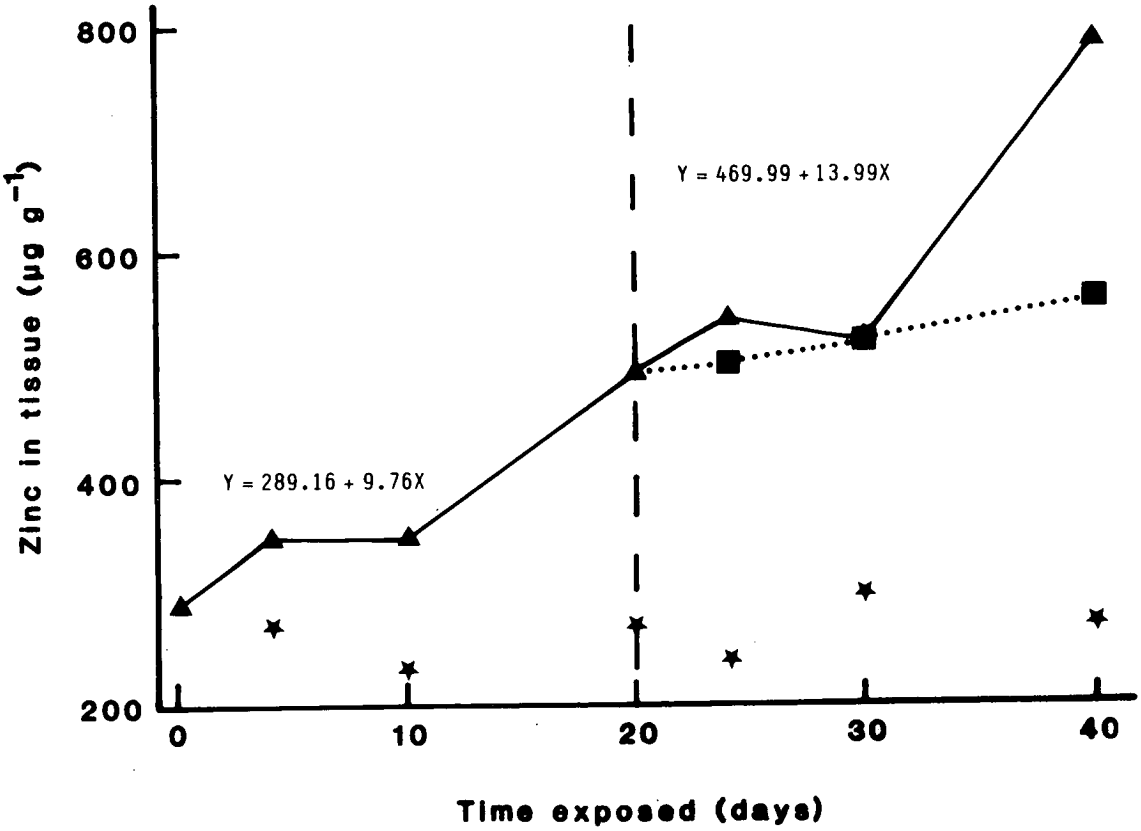
In Experiment 4, zinc was accumulated at a rate of 9.76 $\mu g\ g^{-1}\ day^{-1}$ from 200 $\mu g\ Zn\ \ell^{-1}$ (Figure 5.22b). In Experiment 3, the rate was 15.72 $\mu g\ g^{-1}\ day^{-1}$ following a prior exposure to 10 $\mu g\ Cu\ \ell^{-1}$ for 20 days (Figure 5.22a). These two rates were not significantly different ($p>0.05$). On the other hand, the simultaneous exposure to copper plus zinc in Experiment 3 resulted in an accumulation of zinc at 20.74 $\mu g\ g^{-1}\ day^{-1}$. This rate was significantly greater than the initial (9.76) rate recorded in Experiment 4 ($p<0.05$).

Figure 5.22

a.



b.



Conclusions

(i) The presence of zinc, either prior to, or in conjunction with, copper resulted in a greatly increased rate of accumulation of copper.

(ii) Exposure to copper and zinc together after a prior exposure to zinc did not influence the rate of accumulation of zinc.

(iii) An initial 20 day exposure to copper did not significantly alter the rate of accumulation of zinc; however, subsequent simultaneous exposure to copper and zinc may have increased the accumulation of zinc.

5.8.6 Experiments 5 and 6: Cadmium and Copper

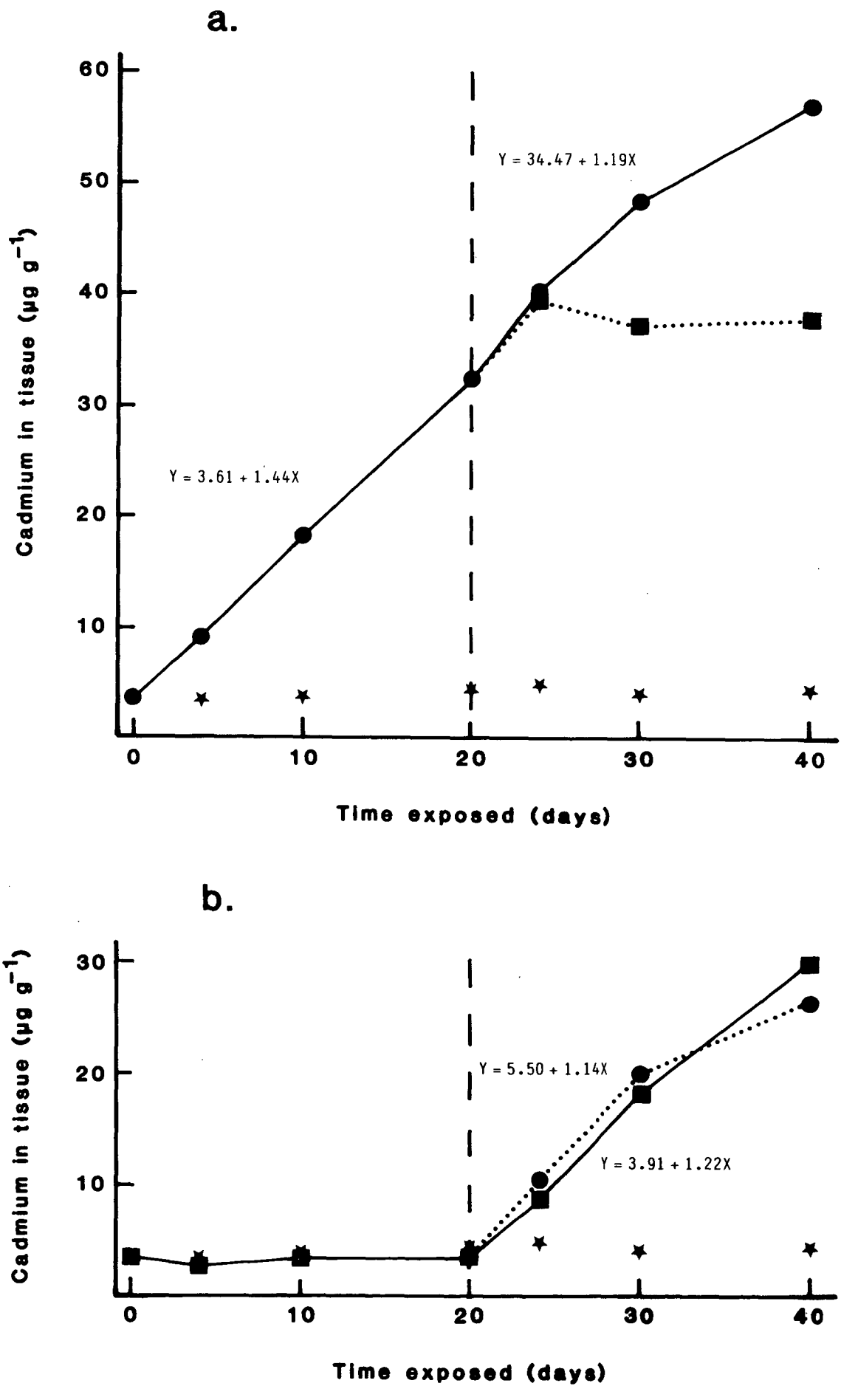
Results

Cadmium Concentrations

The tissue concentrations of cadmium during Experiment 5 are shown in Figure 5.23a. The addition of 10 µg Cu ℓ^{-1} at day 20 had no effect on the accumulation of cadmium. The decrease in rate of accumulation from 1.44 to 1.19 µg g^{-1} day^{-1} was not significant ($p>0.05$). When cadmium was replaced at day 20 by copper, the mussels continued to accumulate cadmium for approximately 4 days; subsequently the tissue concentration of cadmium remained steady. Likewise, while exposed to 10 µg Cu ℓ^{-1} in Experiment 6, the cadmium concentrations of the tissue did not vary (Figure 5.23b). However, when cadmium was added at day 20 a rapid accumulation of cadmium resulted. Exposure to copper in combination with cadmium did not alter the accumulation of cadmium.

The rate of accumulation of cadmium following 20 days exposure to copper was 1.14 µg g^{-1} day^{-1} (Figure 5.23b). This rate was not significantly different ($p>0.05$) from that recorded following the initial exposure to cadmium in Experiment 5, viz. 1.44 µg g^{-1} day^{-1} (Figure 5.23a).

Figure 5.23


a.
Cadmium in tissue (µg g⁻¹)
60
50
40
30
20
10
0
10
20
30
40
Y = 34.47 + 1.19X
Y = 3.61 + 1.44X
Time exposed (days)
b.
Cadmium in tissue (µg g⁻¹)
30
20
10
0
10
20
30
40
Y = 5.50 + 1.14X
Y = 3.91 + 1.22X
Time exposed (days)

Copper Concentration

Exposure to 10 μg Cd ℓ^{-1} during the first 20 days of Experiment 5, resulted in very little change in the copper concentration of the tissues. In contrast, the control group accumulated copper during this period (Figure 5.24a). When cadmium was replaced by copper at day 20 a rapid accumulation of copper resulted. A statistically similar accumulation occurred in the mussels exposed to cadmium and copper together (Figure 5.24a). Thus the presence of cadmium had no influence on the rate of accumulation of copper.

The rate of accumulation of copper increased significantly from 1.35 to 2.51 μg g^{-1} day^{-1} when cadmium was added at day 20 of Experiment 6 ($p<0.05$) (Figure 5.24b). On the other hand, when cadmium replaced copper a loss of copper from the tissues occurred (Figure 5.24b). Following 20 days initial exposure to cadmium, the rate of accumulation of copper recorded in Experiment 5 was 3.61 μg g^{-1} day^{-1} (Figure 5.24a). This rate was significantly greater ($p<0.05$) than that recorded in Experiment 6 for mussels exposed to copper alone (1.35 μg g^{-1} day^{-1}) (Figure 5.24b).

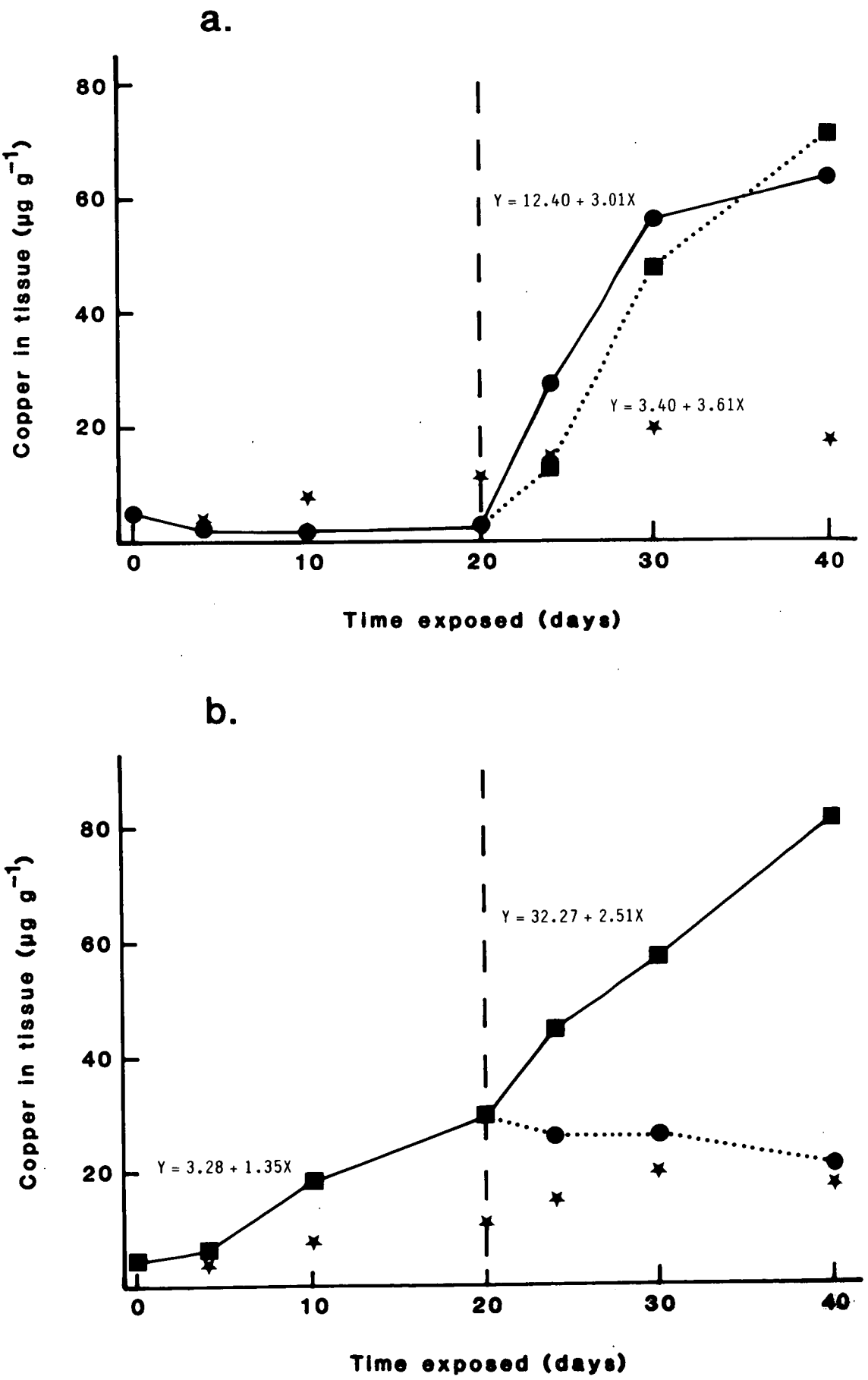
Zinc Concentrations

The tissue concentrations of zinc recorded during both Experiments 5 and 6 did not vary from those of the control group of mussels exposed to background concentrations.

Conclusions

(i) Copper either in an initial exposure or presented simultaneously with cadmium, had no influence on the rate of accumulation of cadmium.

Figure 5.24


a.
Copper in tissue (µg g⁻¹)
80
60
40
20
0
10
20
30
40
Y = 12.40 + 3.01X
Y = 3.40 + 3.61X
Time exposed (days)
b.
Copper in tissue (µg g⁻¹)
80
60
40
20
0
10
20
30
40
Y = 32.27 + 2.51X
Y = 3.28 + 1.35X
Time exposed (days)

(ii) Cadmium either in an initial exposure or presented simultaneously with copper, increased the rate of accumulation of copper.

5.8.7 General Conclusions from Series IV Experiments

The conclusions reached following the Series IV experiments are summarised in Tables 5.14 and 5.15.

5.9 DISCUSSION OF RESULTS FROM SERIES I-IV EXPERIMENTS

5.9.1 Interactions Involving Cadmium Accumulation

Bryan and Hummerstone (1973b) reported that increasing the external zinc concentration resulted in a decreased uptake of cadmium by the polychaete *Nereis diversicolor*. They recorded a 9% decrease of cadmium when zinc concentration was increased from 10 µg ℓ^{-1} to 100 µg ℓ^{-1}, and a 37% decrease when the exposure was increased to 1,000 µg Zn ℓ^{-1}. The decrease occurred over a wide range of external cadmium concentrations. However, these authors concluded that such effects would be difficult to detect under field conditions, except in extremely polluted situations.

Eisler and Gardner (1973) also observed that high concentrations of zinc (60,000 µg ℓ^{-1}) may have decreased the accumulation of cadmium (from a concentration of 10,000 µg Cd ℓ^{-1}) by the estuarine teleost *Fundulus heteroclitus*. On the other hand, copper (8,000 µg ℓ^{-1}) may have increased the accumulation of cadmium. In contrast, the accumulation of cadmium (from concentrations of 300-600 µg Cd ℓ^{-1}) by the shrimp *Callianassa australiensis* was found by Ahsanullah *et al.* (1981) to be increased in the presence of zinc (*ca.* 1,000 µg ℓ^{-1}). Copper (30 - 60 µg ℓ^{-1}) was found to have no effect on the cadmium accumulation in these experiments.

In marine mussels, Fowler and Benayoun (1974) reported that they could find no evidence that zinc influenced the accumulation of cadmium

TABLE 5.14 Metal interactions in *M. e. planulatus* during exposure to a combination of metals A and B following an initial exposure for 20 days to metal A only. n.e. - no effect.

Metal		Interaction Effect on Metal in Tissue		
A	B	Cadmium	Copper	Zinc
Cd	Zn	n.e.	Increased accumulation from background conc.	n.e.
Zn	Cd	n.e.	Increased accumulation from background conc.	n.e.
Cu	Zn	n.e.	Increased accumulation	n.e.
Zn	Cu	n.e.	Increased accumulation	n.e.
Cd	Cu	n.e.	Increased accumulation	n.e.
Cu	Cd	n.e.	Increased accumulation	n.e.

TABLE 5.15 Metal interactions in *M. e. planulatus* during exposure to metal B alone following an initial 20 day exposure to metal A only. n.e. - no effect.

Metal		Interaction Effect on Metal in Tissue		
A	B	Cadmium	Copper	Zinc
Cd	Zn	Lost from tissue	Increased accumulation from background conc.	n.e.
Zn	Cd	n.e.	n.e.	Lost from tissue
Cu	Zn	n.e.	n.e.	n.e.
Zn	Cu	n.e.	Increased accumulation	n.e.
Cd	Cu	Accumulation for 4 days from background conc.	Increased accumulation	n.e.
Cu	Cd	n.e.	Lost from tissue	n.e.

by *Mytilus galloprovincialis*. Similarly, Carpene and George (1981) observed that the uptake of cadmium by isolated gills of *Mytilus edulis* was unaffected by the presence of either zinc, copper, mercury or iron. Phillips (1976a) suggested that his results for the accumulation of metals in combination by *M. edulis* were consistent with the hypothesis that no interaction occurred between cadmium, zinc and lead. Yet his results, reproduced in Table 5.16, do not fully support his interpretation. Table 5.16 shows that the mussels in Tank 4 exposed to cadmium at 40 μg ℓ^{-1} had a similar tissue concentration of cadmium to the mussels in Tanks 2 and 3 exposed to only 20 μg Cd ℓ^{-1}. The concentration of zinc was also higher in Tank 4. This may suggest that zinc at 400 μg ℓ^{-1} decreased the accumulation of cadmium. This interpretation of Phillips' results is in accordance with the observation of Jackim *et al.* (1977), who found that in both *M. edulis* and *Mulinia lateralis* the accumulation of cadmium was reduced in the presence of zinc. However, in *M. edulis*, the effect only became marked at high concentrations of zinc, *viz.* 500 μg ℓ^{-1}. It must be noted that Fowler and Benayoun (1974) had used a maximum zinc concentration of 100 μg ℓ^{-1} when they failed to demonstrate any significant effect on the uptake of cadmium. It appears, therefore, from these few reports that the presence of zinc may well decrease the accumulation of cadmium by mussels, but the interaction is only apparent at high external concentrations of zinc.

In the present investigation it was concluded, following both Series I (Section 5.5.6) and Series II (Section 5.6.9) experiments, that the presence of high concentrations of zinc (≥200 μg ℓ^{-1}) resulted in decreased accumulation of cadmium by *M. e. planulatus*. The results of Series II further suggested that a threshold concentration of 10 μg Cd ℓ^{-1} is required before zinc has any marked effect on cadmium accumulation. This may well explain the conclusion from Series III and IV (Sections 5.7.5 and 5.8.7 respectively) that zinc had no influence on cadmium accumulation, i.e. in these experiments either or both metals were below

TABLE 5.16 Mean concentrations and standard deviations (μg g^{-1} wet wt.) of zinc, cadmium, lead and copper in whole soft parts of mussels, *Mytilus edulis* exposed for 35 days to four different combinations of metal in solution. Data have been weight-normalised where appropriate. (Taken from Phillips (1976a)).

Tank	Exposure concentrations (μg ℓ^{-1})				Concentration in mussels (μg g^{-1} wet wt.)			
	Zn	Cd	Pb	Cu	Zinc	Cadmium	Lead	Copper
Control	0	0	0	0	21.2 ± 5.8	0.47 ± 0.13	1.80 ± 0.68	BDL*
1	100	10	10	10	33.0 ± 13.4	3.11 ± 0.57	10.7 ± 1.9	0.22 ± 0.09
2	200	20	20	20	56.4 ± 21.8	6.13 ± 1.21	13.7 ± 3.5	1.24 ± 0.47
3	200	20	40	40	52.3 ± 15.0	6.02 ± 1.08	20.1 ± 7.6	7.42 ± 2.31
4	400	40	20	20	73.7 ± 38.7	6.68 ± 2.50	11.1 ± 4.0	0.53 ± 0.15

* BDL: below detection limit

the threshold concentration at which observable interaction occurs. This is exemplified by the results of Experiments 1 and 2 of Series IV presented in Figure 5.18. In both experiments, the rate of accumulation of cadmium was lower (though not significantly so) during the combined exposure to zinc and cadmium, than that recorded during the exposure to cadmium alone. It is equally possible that the initial exposure period to either metal (6 days in Series III and 20 days in Series IV) may in some way reduce or even prevent the interaction previously observed during simultaneous presentation. Both possible interpretations are discussed further in Section 5.10, when the possible mechanisms involved in interactions are considered.

The data from Phillips (1976a) shown in Table 5.16 are consistent with the observations of Carpene and George (1981) that copper did not affect the accumulation of cadmium by *M. edulis*. In the present study it was also concluded from the Series II experiments (Table 5.13) that copper had no influence on the accumulation of cadmium by *M. e. planulatus*. However, Series I results indicated that at high concentrations of both copper and cadmium there was a reduction in cadmium accumulation. It is suggested, therefore, that copper may reduce the accumulation of cadmium by mussels when both metals are at high external concentrations (≥ 20 µg ℓ^{-1}). As with zinc, the Series IV experiments (Experiments 5 and 6, Figure 5.23) revealed non-significant reductions in the cadmium accumulation rate in the presence of copper. Both metals were apparently at concentrations below the observable interaction level. It was concluded from both Series III and IV experiments (Sections 5.7.5 and 5.8.7 respectively) that, again as with zinc, an initial exposure to copper had no influence on the subsequent accumulation of cadmium. Although both metal concentrations were again below the apparent interaction threshold, it is proposed that an initial exposure to copper would not be expected to influence the subsequent accumulation of

cadmium (for further discussion see Section 5.10).

5.9.2 Interactions Involving Zinc Accumulation

From their toxicity studies Ahsanullah *et al.* (1981) reported that both copper and cadmium increased the accumulation of zinc by *Callianassa australiensis*. Eisler and Gardner (1973), on the other hand, reported that, while copper appeared to have no effect, cadmium reduced the accumulation of zinc by *Fundulus heteroclitus*. The accumulation of zinc by the alga *Laminaria digitata* was observed by Bryan (1969) to be reduced by the presence of either cadmium or copper.

The results of Phillips (1976a), shown in Table 5.16, indicate that copper had no apparent effect upon the accumulation of zinc by *M. edulis* (compare Tanks 2 and 3). However, the rather small increase in tissue concentration of zinc between Tank 2 and Tank 4, may have been the result of a cadmium inhibition. The cadmium exposure concentration was the only one, apart from that of zinc, to have been increased in Tank 4.

In the present investigation it was concluded from both Series I (Table 5.12) and Series II (Table 5.13) experiments that zinc accumulation by *M. e. planulatus* was increased in the presence of either cadmium or copper, but only at high external concentrations of both metals. Both series indicated that either cadmium had to be above 10 µg Cd ℓ^{-1} or zinc above 150 µg Zn ℓ^{-1} for any significant increase in zinc accumulation to result. The influence of copper was considerably greater than that of cadmium. Copper at 20 µg ℓ^{-1} increased zinc accumulation at all external concentrations. At 10 µg Cu ℓ^{-1}, interaction occurred only when zinc was above 100 µg Zn ℓ^{-1}. The combined exposure to copper and zinc in Series IV experiments (Experiments 3 and 4) also resulted in increased rates of accumulation of zinc (Figure 5.22). However, this increase was only significant following initial exposure

to copper (Figure 5.22a). Whether the lack of a significant increase following prior exposure to zinc is a consequence of that exposure is discussed in Section 5.10. Likewise, the lack of any influence of cadmium on zinc accumulation in Experiments 1 and 2 of Series IV is also discussed in Section 5.10.

A preliminary exposure to either cadmium or copper was found to have no influence on the subsequent rate of accumulation of zinc (Sections 5.7.5 and 5.8.7. As with the accumulation of cadmium, it is not clear whether this resulted from the metals being at external concentrations that were below the interaction threshold, or, as discussed in Section 5.10, the initial exposure was truly inconsequential to any subsequent accumulation.

5.9.3 Interactions Involving Copper Accumulation

Ahsanullah *et al.* (1981) observed that both zinc and cadmium reduced the accumulation of copper by *Callianassa australiensis*. Results of Phillips' (1976a) experiments with *M. edulis* may also indicate a reduction in copper accumulation due to zinc and/or cadmium (Table 5.16), though Phillips himself did not draw this conclusion.

In Series I, II and IV experiments in the present investigation, zinc was found to have a marked synergistic effect on copper accumulation (Tables 5.12 - 5.15). It was only in the Series III experiments that zinc failed to influence the accumulation of copper.

Cadmium, on the other hand, whilst showing a synergistic effect on the rate of accumulation of copper in the Series IV experiments (Tables 5.14 and 5.15), also exhibited antagonistic effects at high concentrations in Series II experiments (Table 5.13). The results of Series I and III experiments indicated no apparent effect on copper accumulation resulting from the presence of cadmium. In contrast, cadmium in combination with zinc did have an influence on the accumulation

of copper in the Series I experiments (Figure 5.2 and Table 5.12). The synergistic effect of zinc at low external concentrations of copper may be reduced by high external concentrations of cadmium, i.e. Cd acts antagonistically. At the same time, low cadmium concentrations may increase the synergistic effect of zinc at high copper concentrations, i.e. Cd acts synergistically. In summary, under particular circumstances, high concentrations of cadmium ($\geq$20 $\mu g\ \ell^{-1}$) may reduce copper accumulation, whilst low concentrations of cadmium (<20 $\mu g\ \ell^{-1}$) may increase copper accumulation.

It was also observed during both the Series III and IV experiments that mussels, while accumulating zinc or cadmium from elevated external concentrations, also accumulated copper at an accelerated rate from background concentrations. The rate of accumulation of copper was greater in the presence of zinc.

5.9.4 Summary of Interactions Occurring in *M. e. planulatus*

1. Simultaneous exposure to cadmium and zinc produced an interaction when both metals were at high external concentrations. A decrease in the accumulation of cadmium and an increase in zinc accumulation resulted.
2. Simultaneous exposure to copper and zinc resulted in an increase in the accumulation of both metals. The increase in zinc accumulation, however, occurred only at high external concentrations of either metal. A 20 day initial exposure to zinc also resulted in an increase in the subsequent accumulation of copper.
3. Simultaneous exposure to cadmium and copper resulted in an antagonistic interaction at high external concentrations of both metals. However, at low concentrations, following a 20 day preliminary exposure to cadmium, the subsequent accumulation of copper was increased. Likewise the addition of cadmium following a prior

copper exposure also increased the accumulation of copper.

4. Simultaneous exposure to elevated concentrations of all three metals generally resulted in a reduction of cadmium accumulation and an increase in both copper and zinc accumulation.

5.10 INTERPRETATION OF METAL INTERACTIONS

In this section I have attempted to interpret possible interactions of the three metals cadmium, copper and zinc during accumulation by *M. e. planulatus*, by means of the accumulation pathways proposed in Section 5.3.

5.10.1 Review of Accumulation Pathways and Possible Sites for Interaction

Cadmium. The uptake of cadmium into a mussel requires a carrier ligand to transport the metal ions across the surface membrane. From this carrier the cadmium ions are transferred to a storage protein, termed metallothionein. The synthesis of this protein is induced by the presence of cadmium within the cells.

Copper. A carrier ligand is required to transport the copper ions across the surface membrane. Once inside the cells, copper is transferred to a storage protein. This storage protein may be either metallothionein or a copper specific protein termed Cu-chelatin. Its synthesis is induced by the presence of copper.

Zinc. Zinc crosses the cell membrane by passive diffusion without the involvement of a carrier system. The rate of diffusion is dependent on the external concentration. Within the cells, metal ions induce the production of metallothionein to which they are weakly bound.

If the metals are indeed accumulated via these proposed pathways then interactions may occur, as was proposed in Section 5.4, in one of three ways:

(i) Cd and Zn may interact by competing for binding sites on the metallothionein molecule;

(ii) Cu and Zn may interact by competing for binding sites on the metallothionein molecule;

(iii) Cd and Cu may interact either by competing for carrier ligands or by competing for binding sites on the metallothionein molecule.

All three metals may therefore compete for sites on the storage protein. Apparently synthesis of this protein is induced by all three metals. Metallothionein is known to form metal complexes containing all three metals in various ratios, though one would predominate when presented at an elevated external concentration. Synthesis of a copper specific protein may also be induced by exposure to elevated external copper levels. Metallothionein is reported to have a stronger affinity for copper than for other metals (Suzuki 1979). It would therefore be expected to bind copper in preference to cadmium or zinc.

5.10.2 Interactions During Simultaneous Exposure

Hill and Matrone (1970) suggested that metals, such as cadmium, copper and zinc, with similar physical and chemical properties would act antagonistically to each other in biological systems. The only antagonistic interaction observed during this present study was between cadmium and copper at high external concentrations where accumulation of both metals was reduced. The suggested sites for interaction between these two metals differ from those between other pairs in that they may compete for the carrier ligands. It is reasonable to assume that cadmium and copper share the same carrier system, a system that has a finite binding capacity (George and Coombs 1977). Thus it is proposed that at high external concentrations of both metals competition for the limited number of binding sites occurs. The uptake of both metals would be reduced as a result.

At low external concentrations of cadmium and copper sufficient carrier binding sites would be available for both metals to gain entry into the cell. However, if both metals are separately inducing the synthesis of metallothionein, which has a stronger affinity for copper, it would seem reasonable to expect that the extra production of protein due to the presence of cadmium would provide extra binding sites for copper. This would result in increased accumulation of copper, and possible reduction, or no effect, on cadmium accumulation. No such interaction was observed in *M. e. planulatus*. It is therefore suggested that the synthesis of metallothionein induced by cadmium and copper is controlled by the total number of metal ions associated with the carrier system, and not by the individual metals.

When mussels are introduced to elevated levels of copper alone, the protein induced may be copper-specific. On the other hand, in a combined exposure with cadmium, the metallothionein binds both metals, and possibly also zinc. Zinc is reported to be always present in a cadmium-induced metallothionein molecule (Suzuki *et al.* 1979). It is suggested that this is due to the free unbound state of the zinc ion within a cell. This contrasts with cadmium (and copper) which would be bound to its carrier ligand. The zinc ions are therefore more readily available for binding to newly synthesised protein.

When a combined exposure includes zinc as one of a metal pair it is suggested that two distinct stimuli for metallothionein induction exist: one from the carrier system of cadmium or copper, and the other in response to the increase in free zinc ions in the cell. This 'double' synthesis would provide extra binding sites with strong affinities for copper. Hence, as shown in Series I and II experiments, copper accumulation is increased in the presence of zinc. The increased storage capacity for copper would promote a greater accumulation. At low concentrations of zinc there would be sufficient binding sites for the free

zinc ions, and zinc accumulation would not be influenced. Yet it is possible as suggested by the results in Series I, that the rapid induction of protein synthesis in response to high copper levels may increase the accumulation of zinc from low concentrations. The free form of the zinc ions may allow binding of zinc to the copper induced non-specific metallothionein, with increase in zinc storage resulting in an increased accumulation.

At high concentrations of zinc in a combined exposure with copper, both zinc and copper accumulation is increased. It is suggested that at high external concentrations the rate of passive diffusion of zinc may outstrip the rate of metallothionein synthesis induced by the zinc, so providing a surplus of free zinc ions. This surplus may then begin to monopolise the binding sites available on both zinc-induced protein and that induced by copper. As a result there would be an increased storage of zinc and so an increased accumulation. As copper is strongly favoured for binding to the protein its accumulation would still be increased but not to such a great extent. Figure 5.5 shows that zinc enhancement of copper accumulation may be maximal at a concentration of 150 μg Zn ℓ^{-1}. It is proposed that at extremely high external concentrations of zinc, the accumulation of copper (like cadmium) may in fact be reduced, because of zinc ions swamping the binding sites on the metallothionein. This may have been the case in Tank 4 of Phillips' experiment (1976a), Table 5.16. The reduction in cadmium accumulation and increase in zinc accumulation, observed in the present study at high external concentrations, may be due to this swamping effect of the free zinc ions. Unlike copper, the cadmium ions are not strongly favoured for binding, and therefore the surplus zinc ions can more readily exclude them from binding to the cadmium-induced protein. The reduction in storage capacity for cadmium would result in a reduced accumulation. At low external concentrations of both zinc and cadmium, sufficient binding sites are available for both

metals, and so no interaction occurs.

During the Series III and IV experiments it was observed that mussels exposed to cadmium or zinc accumulated copper rapidly from background concentrations. Following the comments of Mills (1980), discussed in Section 5.2, it is suggested that copper is readily incorporated into the metallothionein synthesised in response to the other metals. It is assumed that the carrier ligands are constantly transporting copper for metabolic purposes, and this copper may readily be transferred to the metallothionein.

5.10.3 Interactions Following a Preliminary Exposure to One Metal

The protection of rat testes against cadmium toxicity by pre-treatment of the rats with zinc, was suggested by Webb (1972b) to be related to the synthesis of metallothionein. Pre-treatment with zinc induces the synthesis of metallothionein in the livers of these animals. This induction increases the subsequent accumulation of cadmium by the liver, so preventing the cadmium from reaching the testes. Webb (1972b) further suggested that a pre-treatment with small amounts of cadmium would produce the same effect. The initial induction of metallothionein overcomes the normal lag period between cadmium introduction and protein synthesis. This explanation may well apply to the increased cadmium accumulation from background concentrations observed in Experiments 1 and 2 in Series III.

In the light of the above observations by Webb it would be expected that a prior exposure to either the same metal or another metal would increase the rate of accumulation of a metal subsequently introduced. Yet, with only 2 exceptions, no such interaction effects were observed in the experiments in either Section 4.5 or those of Series III and IV in this chapter. The exceptions are the increased accumulation of copper following 20 days prior exposure to either cadmium or zinc,

observed in the Series IV experiments. In most cases lack of any observed interaction may be due to the metal concentrations employed being below an interaction threshold. As discussed with simultaneous exposure interactions, certain effects were only apparent at particular external concentrations. In this context, Webb (1972b) and Kagi and Nordberg (1979) reported that the pre-treatment effect of zinc on cadmium toxicity requires the dosage of zinc to be one or two orders of magnitude higher than that of cadmium, for an effect to occur. Whilst this explanation may well hold for the lack of effects on cadmium and zinc accumulation, it does not seem plausible for the lack of an effect on copper accumulation in Series III experiments. Not only was a wide range of concentrations used in these experiments, but they were the same concentrations as employed in Series IV experiments, in which an interaction occurred. It would appear therefore that the interaction effect on copper accumulation may be related to the exposure time (20 days *cf.* 6 days). The longer exposure period possibly resulted in a greater synthesis of metallothionein.

Following 20 days exposure to either cadmium or zinc, metallothionein synthesis would be readily induced. On the introduction of copper, not only would the copper specific protein be induced but also further metallothionein. The result of the 'double' protein production would tend to increase the accumulation of copper, as both proteins have a strong affinity for copper ions. Likewise in Series IV experiments the simultaneous exposure to low concentrations of copper and cadmium following prior exposure to copper, resulted in increased copper accumulation. No interaction at low concentrations of these two metals was expected following the arguments in Section 5.10.2. However, during the prior exposure to copper, production of the copper specific protein would be likely. The subsequent exposure to cadmium and copper would then induce the production of both metallothionein and copper specific protein. Hence extra binding sites for the copper ions and so an increased

accumulation would result. The production of the copper specific protein during exposure to copper would also explain why the subsequent exposure to cadmium or zinc did not result in increased accumulation rates of these two metals. The lag period for metallothionein production had apparently not been exceeded and no extra supply of binding protein was available.

5.11 CONCLUSIONS ON THE INTERACTION AND ACCUMULATION OF METALS

The three metals cadmium, copper and zinc have been shown to interact during their accumulation by *M. e. planulatus*. These interactions resulted in marked differences in the rates of accumulation of the metals. However, it was observed that these interactions occurred predominantly at extremely high external concentrations of the metals, i.e. at the equivalent of severe pollution levels. Thus, the interpretation of the results of a monitoring study, such as the one proposed in this thesis, would not be affected unless heavily polluted conditions were experienced. If this were to occur, then such conditions would register in the tissues of monitoring organisms and confirmation could be sought by direct analysis of water from the monitoring site.

Whilst it may be said that the accumulation mechanisms proposed initially for the metals aided in the interpretation of the results of the Series I - IV experiments, it may equally be said that these results appear to fully support the initial proposals. The interactions observed in the accumulation of cadmium, copper and zinc by *M. e. planulatus* and the proposed mechanisms by which they occur are summarised by Table 5.17.

TABLE 5.17 The observed interactions between cadmium, copper and zinc during their accumulation by *M. e. planulatus*, and the mechanisms proposed to explain them.

Observed Interaction	Proposed Site of Interaction	Interpretation of Interaction
Simultaneous exposure to high concs. of Cd and Zn led to decreased Cd and increased Zn accumulation.	Intracellular storage site: metallothionein.	Both Cd and Zn induce metallothionein production. Zn at high concs. monopolises binding sites. The reduction in Cd binding sites reduces Cd accumulation.
The presence of Zn simultaneously with Cu increased Cu accumulation.	Intracellular storage site.	Both Cu and Zn induce production of metallothionein with strong affinity for Cu; Cu therefore has extra binding sites.
At high concs. of Cu and Zn, Zn accumulation was also increased.	Intracellular storage site.	At high concs. Zn monopolises more binding sites on the metallothionein.
Simultaneous exposure to high concs. of Cd and Cu resulted in decreased accumulation of both metals.	Membrane surface: carrier ligands.	Competition for a finite number of carrier ligand binding sites.
An initial exposure to Cd or Zn increased the subsequent Cu accumulation.	Intracellular storage site.	Cd and Zn induce metallothionein production, which continues in presence of Cu. Production of Cu-specific protein induced by exposure to Cu alone. The result is extra binding sites for Cu.
Addition of Cd or Zn after prior exposure to Cu, increased Cu accumulation.	Intracellular storage site.	Cu alone induces production of Cu-specific protein; when Cd or Zn added non-specific metallothionein is also produced together with Cu-specific protein. Both proteins have strong affinity for Cu.
Cu was accumulated from background concs. at increased rate when mussels exposed to Cd or Zn.	Intracellular storage site.	Metallothionein induced by Cd or Zn has strong affinity for Cu which is bound during protein synthesis.

CHAPTER 6

METAL DISTRIBUTION IN DIFFERENT TISSUES OF *MYTILUS EDULIS PLANULATUS*

6.1 INTRODUCTION

In biomonitoring of heavy metal pollution whole body concentrations of the metals are in general determined, as it is usually the total body load that is under investigation. The costs in terms of time and effort favour the analysis of whole animals rather than individual tissues. It was concluded earlier (Chapter 5) that a knowledge of the mechanism by which each metal is accumulated in the mussel was important in understanding metal interactions during accumulation. Equally important is a knowledge of the sites of metal uptake and storage, and a study of metal distribution in individual tissues can throw some light on this.

It was proposed that copper and cadmium shared a similar uptake mechanism and, along with zinc, a similar storage system in *M. e. planulatus* (Chapter 5). Lead was considered to be separate from the other three metals in this respect. Nair and Andersen (1972) found the highest concentrations of copper and lead in the gills of *Mytilus edulis*, whilst cadmium and zinc were most concentrated in the visceral mass. Julshamn (1981c), on the other hand, found that copper, zinc and cadmium were all present in greatest quantities in the digestive system of *M. edulis*, with lead predominantly in the gills. In an earlier study, Brooks and Rumsby (1965) reported lead, zinc and copper at high concentrations in the visceral mass of *Mytilus edulis aoteanus*, with copper also at a high concentration in the gills.

The present study concerns the distribution of cadmium, copper, lead and zinc in the tissues of *Mytilus edulis planulatus*. Their distribution is examined both at natural levels in different populations, and also following accumulation and depuration experiments in the laboratory.

6.2 MATERIALS AND METHODS

The general experimental techniques followed were as described in Chapter 2. Following collection, all algae and encrustations were cleaned from the mussel valves, and all protruding byssal threads were cut. The mussels were then allowed 168 hours acclimation at the experimental temperature of 11(±1) °C.

The natural distribution of metals within mussels was examined in three populations: Barnes Bay, Howden and Kangaroo Bluff (Figure 2.1). Two experiments were conducted to examine the distribution following accumulation and depuration:

Experiment 1. 50 mussels (4.0 - 5.0 cm shell length) from Barnes Bay were exposed for 20 days to the metal combination: 10 µg Cd ℓ^{-1}, 10 µg Cu ℓ^{-1}, 50 µg Pb ℓ^{-1} and 100 µg Zn ℓ^{-1}. Subsamples of 10 mussels were removed for individual tissue analysis on days 0, 4, 10 and 20.

Experiment 2. 180 mussels (3.0 - 4.0 cm shell length) were collected from Howden, and 150 were exposed initially for 10 days to the metal combination: 10 µg Cd ℓ^{-1}, 10 µg Cu ℓ^{-1}, 50 µg Pb ℓ^{-1} and 100 µg Zn ℓ^{-1}. The mussels were then transferred for a further 18 days into background levels of the four metals. Subsamples of 20 mussels were removed on days 0, 4, 10, 14, 20 and 28. Ten were analysed for whole tissue metal levels and ten for individual tissues. The other original 30 mussels were exposed continually to the background levels for the 28 days and samples of 10 mussels were removed for individual tissue analysis on days 10 and 28.

Mussels required for tissue analysis were opened with a sterile stainless steel surgical blade. Dissection of the individual tissues was carried out with stainless steel scissors and plastic forceps. Tissues isolated were (Experiment 1 and Kangaroo Bluff mussels): foot (including byssal gland, and byssal threads for Experiment 1); gills

(including palps); muscle; gonads and mantle; and digestive tissue (including stomach, liver and kidney). In Experiment 2, two of these tissues were dissected further by separating as individual tissues the byssal threads and kidney.

The individual tissues from each of the ten mussels per sample were pooled and dried at 105°C. As described in Chapter 2, all dried tissue was crushed to a fine powder and acid digested. All tissues were analysed for cadmium, copper, lead and zinc.

6.3 RESULTS

6.3.1 Natural Distribution

Cadmium

The distribution of cadmium within the tissues of the mussels from the three populations examined is presented in Table 6.1.

TABLE 6.1 Cadmium in tissues of *M. e. planulatus* from three populations. (Conc.: μg g^{-1} dry wt.; %: metal in tissue as percentage of total in mussel); n.a.: not analysed.

Tissue	Barnes Bay		Howden		Kangaroo Bluff	
	Conc.	%	Conc.	%	Conc.	%
Byssal threads	n.a.	-	18.0	*2.91*	n.a.	-
Foot	0.9	*0.97*	0.2	*0.16*	1.5	*0.68*
Gills	8.1	*17.34*	1.4	*6.79*	11.6	*16.81*
Muscle	2.6	*17.11*	1.9	*12.28*	5.1	*9.24*
Gonads & Mantle	2.8	*39.94*	1.2	*17.77*	9.3	*48.82*
Digestive	5.8	*24.64*	5.9	*30.53*	13.5	*24.46*
Kidney	n.a.	-	45.8	*29.56*	n.a.	-

In all three populations over 60% of the body load was present in the digestive tissue (including kidney) and gonads and mantle. The mussels from Barnes Bay and Kangaroo Bluff both displayed high concentrations in gills and digestive tissue, while Howden mussels exhibited high concentrations in kidney, digestive tissue and the byssal threads. Compared to the other two populations, the Kangaroo Bluff mussels, known to be exposed to higher natural levels of metals in seawater (Chapters 2 and 3), had higher concentrations in all tissues.

Copper

The gills of the Kangaroo Bluff mussels were found to have both the highest concentration and content of copper, while the digestive tissue contained the highest levels in the other two populations (Table 6.2). The gills of the Howden mussels, however, also exhibited a high metal level, while no copper was detected in the kidney, byssal threads or foot of these mussels. The latter finding was unexpected

TABLE 6.2 Copper in tissues of *M. e.planulatus* from three populations. (Conc.: $\mu g\ g^{-1}$ dry wt.; %: metal in tissue as percentage of total in mussel) n.a.: not analysed, ND: not detected.

Tissue	Barnes Bay		Howden		Kangaroo Bluff	
	Conc.	%	Conc.	%	Conc.	%
Byssal threads	n.a.	-	ND	-	n.a.	-
Foot	5.5	*13.62*	ND	-	6.7	*2.83*
Gills	0.4	*2.04*	23.6	*24.33*	35.7	*48.28*
Muscle	ND	-	0.9	*1.21*	2.8	*4.73*
Gonads & Mantle	0.6	*6.66*	5.6	*19.84*	5.1	*25.01*
Digestive	7.8	*77.67*	49.6	*54.61*	11.8	*19.10*
Kidney	n.a.	-	ND	-	n.a.	-

following the relatively high concentrations found in the foot of the mussels from the other two populations. Compared to the other two populations, the Kangaroo Bluff mussels did not exhibit the expected higher levels of copper in all tissues.

Lead

As with cadmium, all tissues of the Kangaroo Bluff mussels displayed higher lead concentrations than those of the mussels from the other two populations (Table 6.3). The highest concentration and content (70%) in the Kangaroo Bluff mussels was in the gonads and mantle, although a high concentration was also present in the gills. The Barnes Bay mussels had relatively low lead levels which were predominantly found in the gonads and mantle and digestive tissue. Lead in the Howden

TABLE 6.3 Lead in tissues of *M. e. planulatus* from three populations. (Conc.: μg g^{-1}dry wt.; %: metal in tissue as percentage of total in mussel). n.a.: not analysed, ND: not detected.

Tissue	Barnes Bay		Howden		Kangaroo Bluff	
	Conc.	%	Conc.	%	Conc.	%
Byssal threads	n.a.	-	270	*19.22*	n.a.	-
Foot	1.54	*3.92*	15.5	*7.76*	51.9	*0.99*
Gills	1.6	*8.31*	8.7	*18.58*	270	*16.50*
Muscle	ND	-	4.0	*11.39*	81.3	*6.21*
Gonads & Mantle	1.4	*48.55*	0.6	*3.91*	316	*70.01*
Digestive	3.8	*39.21*	12.5	*28.47*	82.7	*6.28*
Kidney	n.a.	-	37.5	*10.68*	n.a.	-

mussels was relatively evenly distributed with the highest proportion in the digestive tissue. A high concentration was found in the kidney though the highest was found in the byssal threads.

Zinc

The kidney exhibited the highest concentration of zinc recorded in an individual tissue (Table 6.4). In general, zinc was relatively evenly distributed with each population containing a higher proportion in a different tissue. However, high concentrations were generally found in the gills and foot (or byssal threads) in all three populations.

TABLE 6.4 Zinc in tissues of *M. e. planulatus* from three populations. (Conc.: µg g^{-1} dry wt.; %: metal in tissue as percentage of total in mussel). n.a.: not analysed, ND: not detected.

Tissue	Barnes Bay		Howden		Kangaroo Bluff	
	Conc.	%	Conc.	%	Conc.	%
Byssal threads	n.a.	-	370	*0.73*	n.a.	-
Foot	201	*4.36*	ND	-	519	*9.28*
Gills	320	*14.21*	316	*18.69*	608	*34.78*
Muscle	136	*18.58*	329	*25.95*	111	*7.94*
Gonads & Mantle	118	*34.96*	96	*17.42*	158	*32.77*
Digestive	316	*27.88*	199	*12.57*	213	*15.23*
Kidney	n.a.	-	3125	*24.65*	n.a.	-

6.3.2 Accumulation and Depuration

Cadmium

During exposure to elevated metal levels cadmium was accumulated by all tissues, the digestive tissue and in particular the kidney exhibiting the highest rates of accumulation (Figures 6.1 and 6.2). The gills were the other major site of accumulation, while the foot was the lowest despite the high concentrations found in the byssal threads. The cadmium in the digestive tissue not only increased in concentration

Figure 6.1

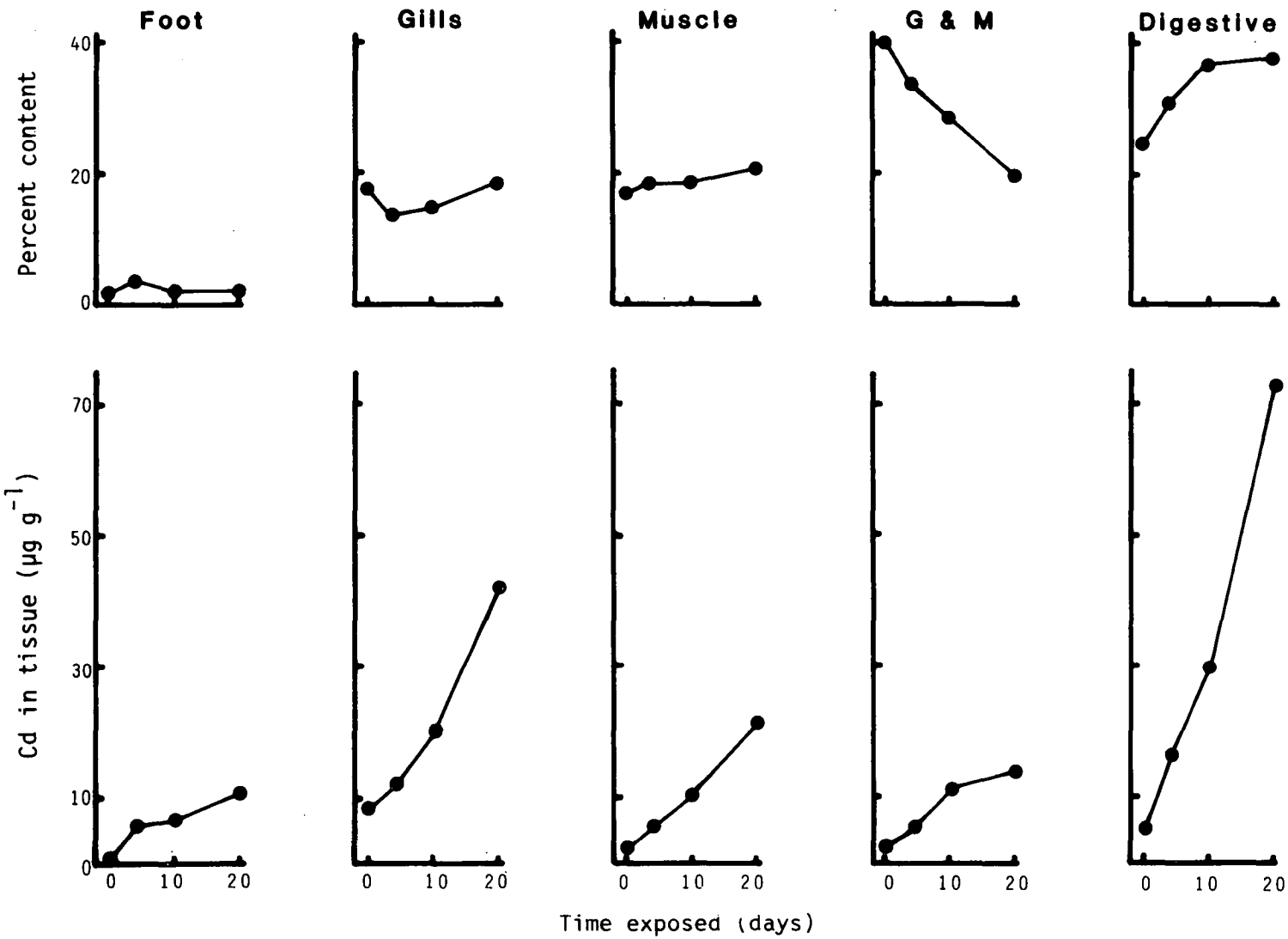
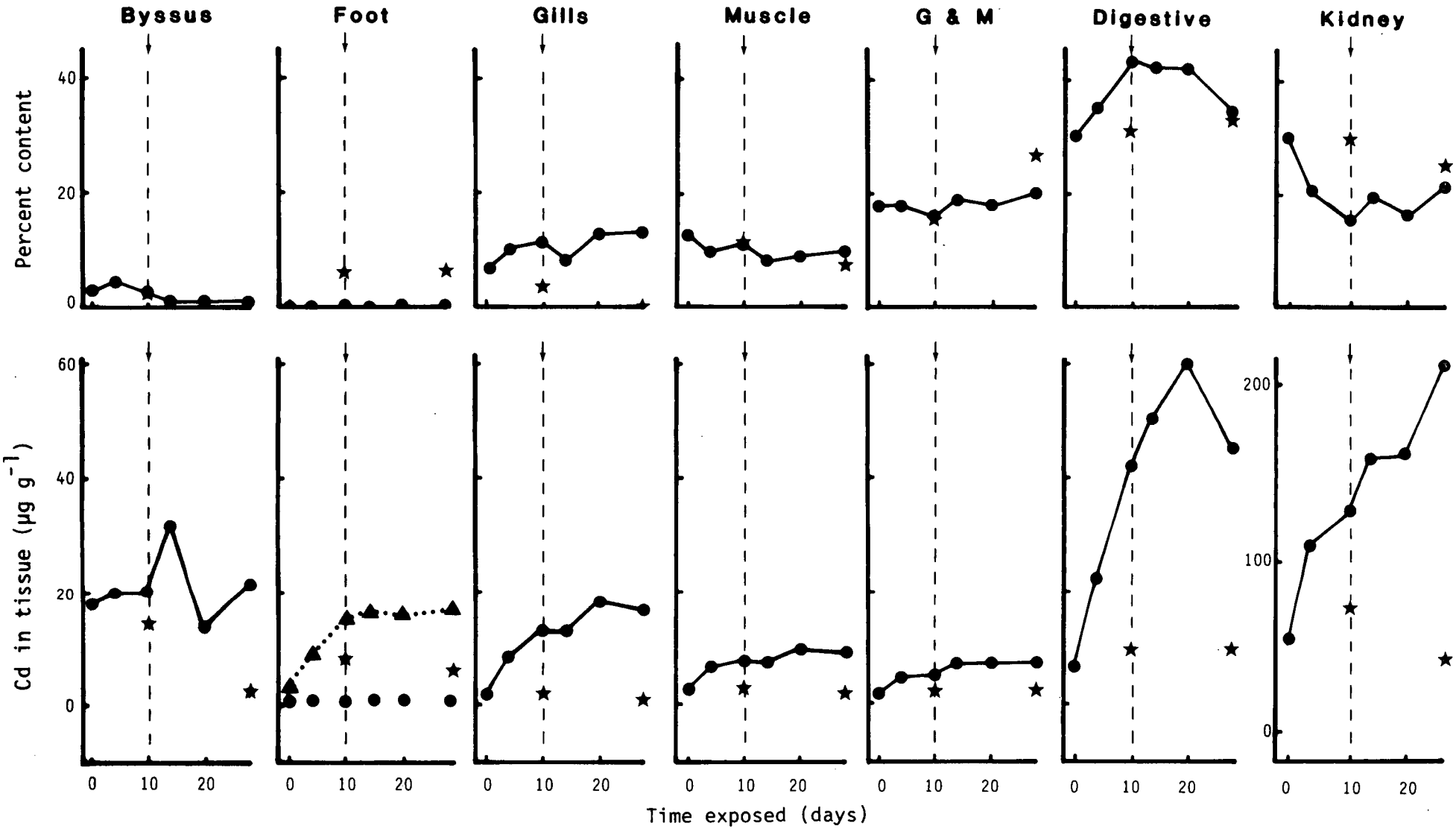

Foot
Gills
Muscle
G & M
Digestive
Percent content
40
20
0
Cd in tissue (µg g⁻¹)
70
50
30
10
0
0 10 20
Time exposed (days)

Figure 6.2


Byssus
Foot
Gills
Muscle
G & M
Digestive
Kidney
Percent content
Cd in tissue (µg g⁻¹)
Time exposed (days)
0
10
20
40
60
100
200

but also in proportion to the total body load. In Experiment 1 (Figure 6.1) the percentage of cadmium in digestive tissue was inversely related to that in the gonads and mantle, however in Experiment 2 (Figure 6.2) the proportion contained in the kidney decreased as that in the digestive tissue increased. The proportion in the other tissues up to day 10 in Experiment 2 remained relatively stable, except that compared to the controls the foot was low in cadmium while the gills were high.

After transfer into background levels in Experiment 2 (at day 10) the whole soft tissues displayed a slight accumulation of cadmium (Figure 6.2). This was reflected by a continued increase in concentration in most tissues, but particularly the gills, kidney and digestive tissue. The digestive tissue, however, showed a decline in percentage content to a level equal to that of the controls, particularly after day 20 when a loss in concentration was also recorded. This loss may have been a redistribution of metal to other tissues. By day 28 the distribution of cadmium, in the experimental mussels compared to controls, indicated a higher load in the gills with corresponding decreases in such possible storage tissues as foot, gonads and mantle and kidney. The byssal threads while not increasing in percentage content had a concentration well in excess of the controls.

Copper

The major sites for copper accumulation were the gills and foot, or in particular the byssal threads, with very little accumulation in other tissue (Figures 6.3 and 6.4). The digestive tissue exhibited no accumulation of copper and in both experiments declined from being the major copper bearing tissue to one of the lowest. No copper was detected in the kidney and only very low levels were present in muscle tissue.

Figure 6.3

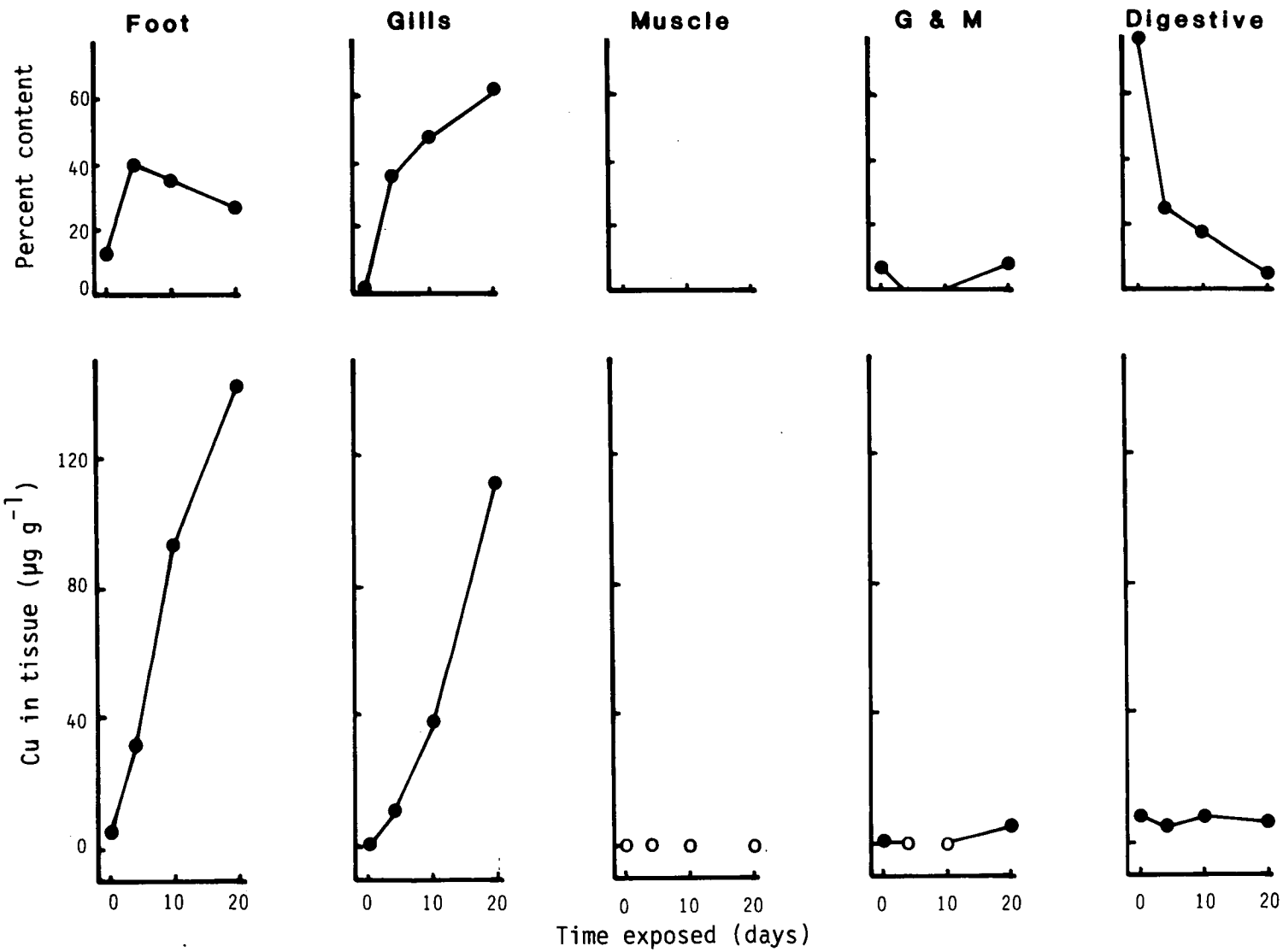
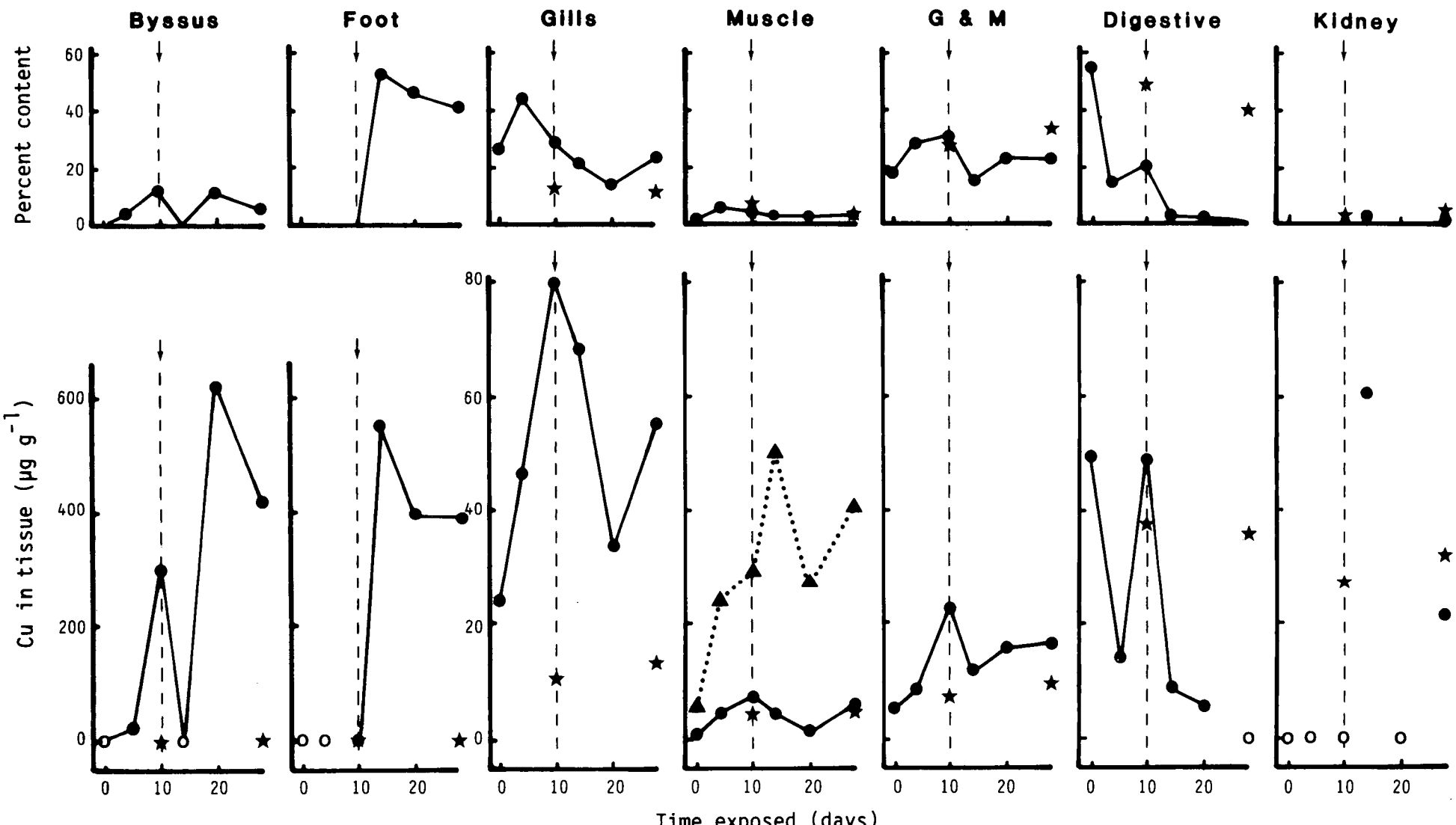

Foot
Gills
Muscle
G & M
Digestive
Percent content
60
40
20
0
Cu in tissue (µg g^{-1})
120
80
40
0
0
10
20
Time exposed (days)

Figure 6.4


Byssus
Foot
Gills
Muscle
G & M
Digestive
Kidney
Percent content
Cu in tissue (µg g^{-1})
Time exposed (days)

After transfer into background levels (Experiment 2, day 10) the whole soft tissues continued to accumulate copper, although erratically (Figure 6.4). Unlike cadmium, this accumulation was not reflected in the individual tissues, where a redistribution of the metal was more apparent. A marked accumulation of copper in the foot was accompanied by an equally marked loss from both gills and digestive tissue both in terms of concentration and percentage content (Figure 6.4). The gonads and mantle also lost metal during this period.

The digestive tissue of the control mussels in Experiment 2 still contained 40% of the total body load at day 28, while copper was not detected in this tissue from the experimental mussels (Figure 6.4). On the other hand, the foot of the experimental mussels contained 43% of the total load while the metal was undetected in this tissue from the controls. Similarly the byssal threads had a high concentration in the experimental mussels but no detectable copper in the controls.

Lead

Like cadmium, all tissues, with the exception of the byssal threads which already had a high concentration, were found to accumulate lead during the exposures to elevated metal levels (Figures 6.5 and 6.6). The kidney had the highest metal concentration and displayed the highest accumulation rate. The gills were the other major accumulation site. In Experiment 1, the gills and muscle were found to increase their proportion of the total body load (combined percentage increased from 8% to 59%) while that of gonads and mantle and digestive tissue decreased (Figure 6.5). However, in Experiment 2, the proportion of lead in the gonads and mantle increased (from 4% to 24%) as did that of the kidney. At the same time the percentage in gills and muscle in this experiment remained constant while that in the digestive tissue again decreased (Figure 6.6).

Figure 6.5

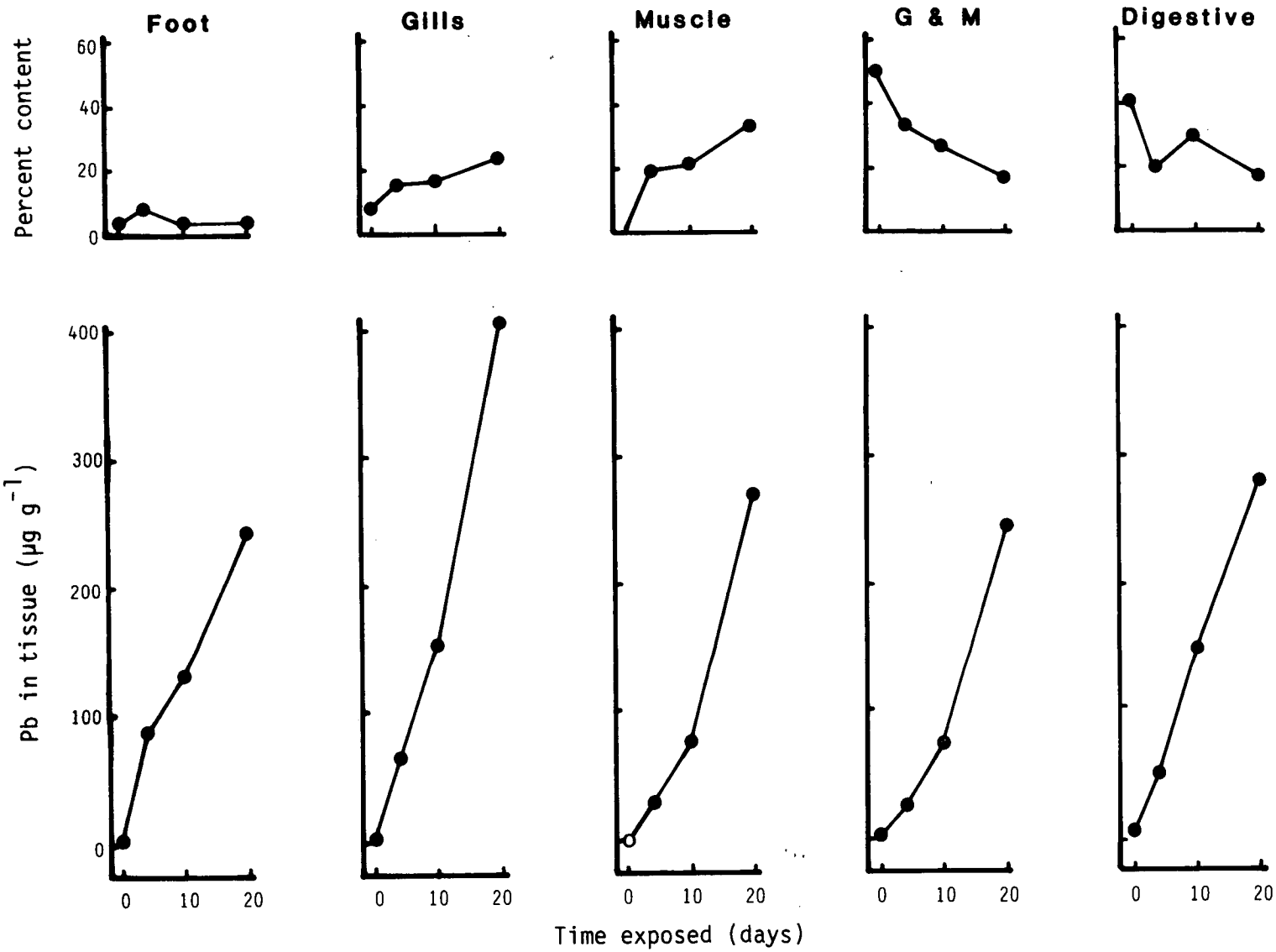
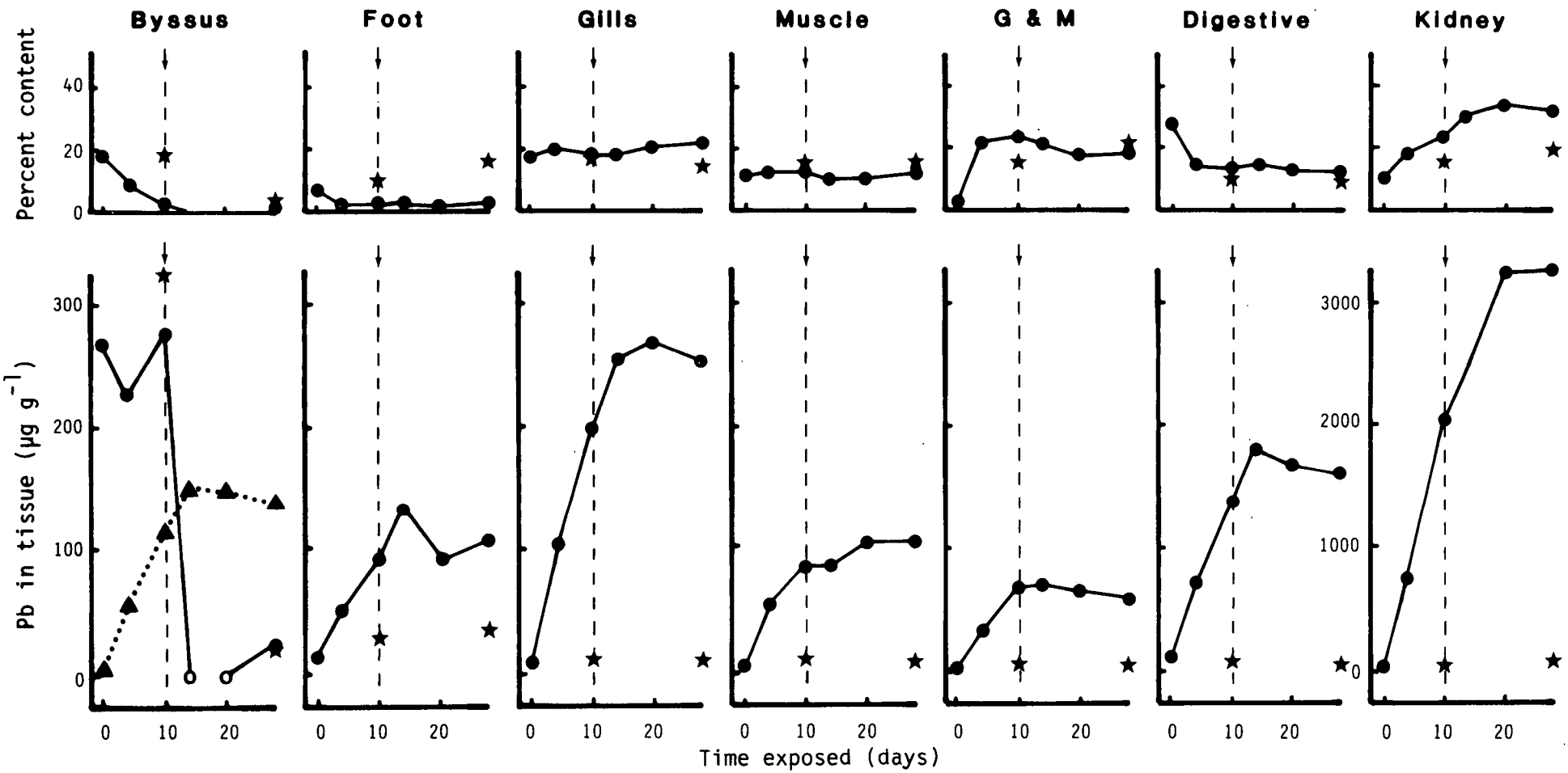

Foot
Gills
Muscle
G & M
Digestive
Percent content
60
40
20
0
Pb in tissue (µg g⁻¹)
400
300
200
100
0
0
10
20
Time exposed (days)

Figure 6.6


Byssus
Foot
Gills
Muscle
G & M
Digestive
Kidney
Percent content
Pb in tissue (µg g⁻¹)
Time exposed (days)

After transfer into background concentrations the whole soft tissues continued to accumulate lead for the first 4 days before remaining at a fairly constant tissue concentration (Figure 6.6). This trend was reflected by most individual tissues, except the kidney, which continued to increase in concentration for 10 days. This continued accumulation possibly represents a redistribution of the metal from other tissues, and by day 28 this tissue, and the gills, were the only tissues with higher percentage contents than control mussels. The foot had a very low proportion of the body total compared to the controls, despite a relatively high concentration (Figure 6.6).

Zinc

During Experiment 1 all tissues accumulated zinc, although the gonads and mantle and digestive tissue did so rather erratically (Figure 6.7). The foot and muscle were found to have the highest rates of accumulation, and the muscle also increased its body proportion at the expense of the gonads and mantle and digestive tissue. The marked accumulation by the foot appears from Experiment 2 to be predominantly in the byssal threads, with no zinc detected in the foot during metal exposure (Figure 6.8). Likewise, the kidney which was found to have both a high tissue concentration and high proportion of the total body load, may have accounted for the greater proportion of the zinc in the digestive tissue of Experiment 1. However, in Experiment 2 the muscle tissue lost zinc during exposure to high metal levels, while the byssal threads, kidney, gills and digestive tissue exhibited accumulation (Figure 6.8). This loss from the muscle was, however, regained when the mussels were transferred into background levels at day 10. This may have been a redistribution of metal from gills, gonads and mantle and kidney. The pattern of accumulation exhibited by the whole soft tissue was not reflected by the individual tissues, except that levels

Figure 6.7

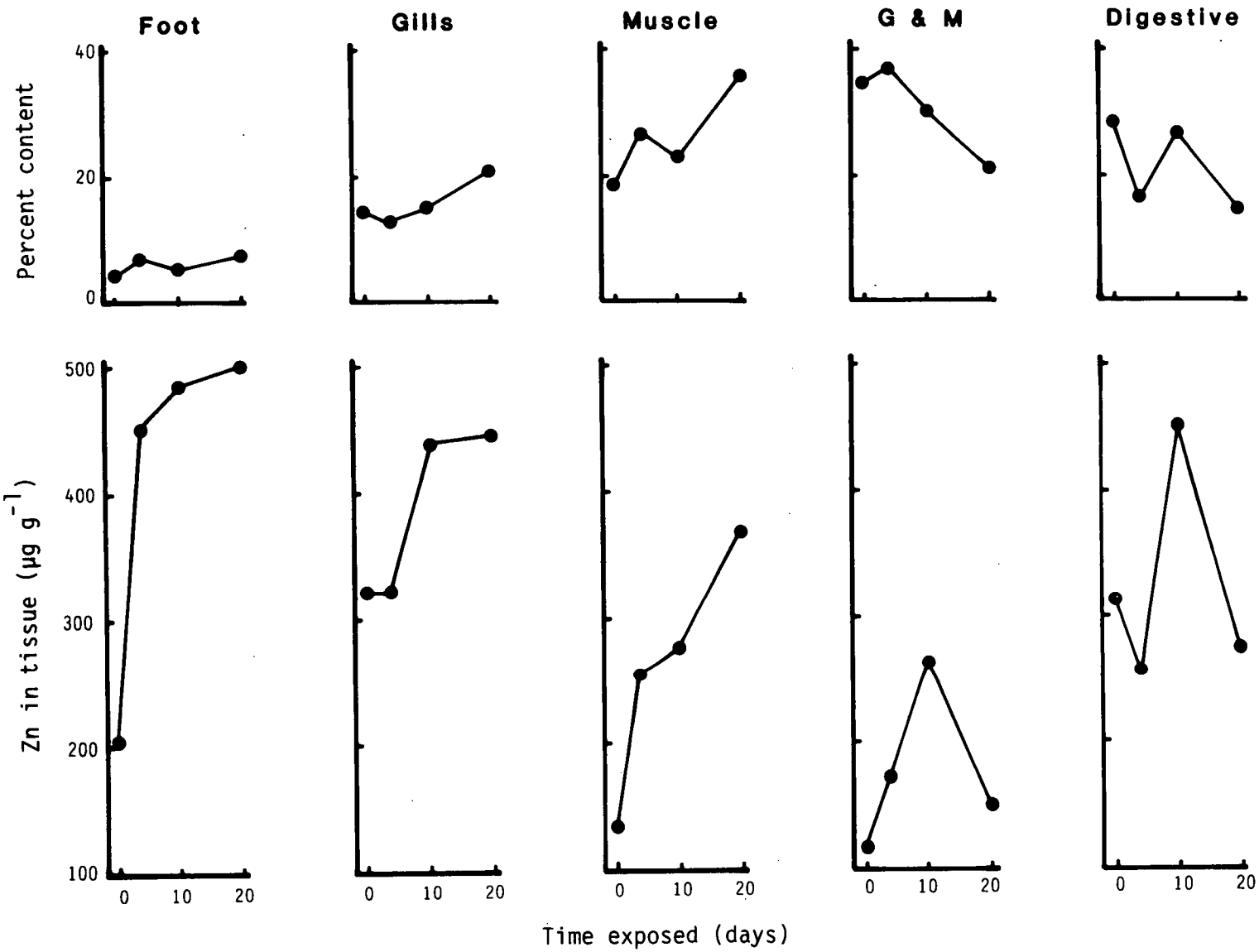
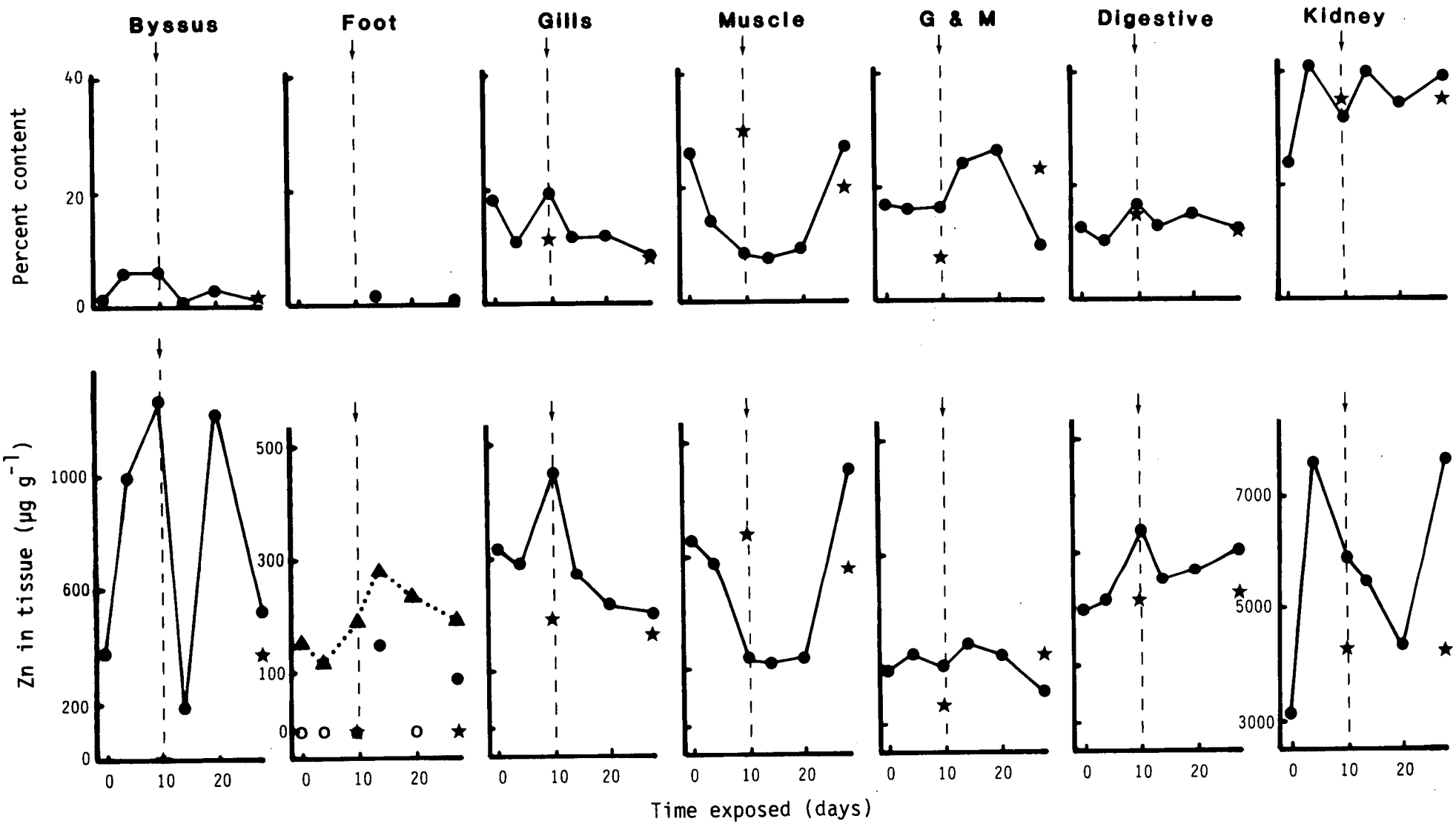

Foot
Gills
Muscle
G & M
Digestive
Percent content
40
20
0
Zn in tissue (µg g⁻¹)
500
400
300
200
100
0
10
20
Time exposed (days)

Figure 6.8


Byssus
Foot
Gills
Muscle
G & M
Digestive
Kidney
Percent content
Zn in tissue (µg g⁻¹)
Time exposed (days)

were rather erratic (Figure 6.8).

6.4 DISCUSSION

Cadmium

The results indicate that the major cadmium-bearing tissue in *M. e. planulatus* is the digestive tissue, the kidney exhibiting the highest concentration. Such findings have been reported for *M. edulis* by Nair and Andersen (1972), Janssen and Scholz (1979), Theede *et al.* (1979) and Julshamn (1981c). All tissues in *M. e. planulatus* accumulated cadmium, with the gills, digestive tissue and kidney exhibiting the greatest increases. George and Coombs (1977) also reported high cadmium accumulation in the mussel kidney. Once this metal is taken up by the gills and digestive tissue, it is possibly stored not only in the kidney and digestive tissue but also in the gonads and mantle, which contained between 20% and 49% of the total body load. The byssal threads contained relatively high levels of cadmium, and may possibly be an excretory system for the metal in addition to the kidney.

Copper

The greatest concentration of copper in mussels has been found in the gills of *M. edulis* (Nair and Andersen 1972; Delhaye and Cornet 1975) and *M. e. aoteanus* (Brooks and Rumsby 1965). Brooks and Rumsby (1965) also found high concentrations in the viscera and intestine, while Julshamn (1981c) recorded 50% of the copper in *M. edulis* present in the digestive system. The digestive tissue in *M. e. planulatus* from Howden and Barnes Bay, was found to contain 55% and 78% respectively, of the total body load. However, the Kangaroo Bluff mussels exposed naturally to higher metal levels in the water, contained 48% of their copper in the gills. In both accumulation experiments the gills were

found to be a major accumulating tissue, with no such accumulation apparent in the digestive tissue. Sutherland and Major (1981) also found the gills of *M. edulis* to be the major accumulation site for copper, but they also recorded accumulation in the kidney in contrast to the results for *M. e. planulatus*. The muscle tissue was very low in copper, as was also reported by Julshamn (1981c) and Delhaye and Cornet (1975). The lamellibranch liver is reported to concentrate copper considerably (Vinogradov 1953, cited in Nair and Andersen 1972) which suggests that the high copper levels in the digestive tissue in *M. e. planulatus* at the time of collection may represent storage in the liver. The concentration of copper in digestive tissue did not increase during exposure to high metal levels, but copper was lost during the period in background levels (Figures 6.3 and 6.4).

Delhaye and Cornet (1975) and Brooks and Rumsby (1965) both recorded relatively high copper concentrations in the mussel foot. This tissue and the byssal threads appear to be of great importance as storage and possibly excretory organs for copper in *M. e. planulatus*. The storage of copper in the byssal threads, which are secreted by glands in the foot, and then the subsequent discarding of the byssus would be a very efficient method for excretion of unwanted metal. George *et al.* (1976) reported such a process for iron in *M. edulis* where the percentage of radioactive iron in the byssus increased in 7 days from 7% to 37%. In Experiment 2 of the present study the copper content of byssal threads increased from zero to 13% of the total body load during 10 days. For all metals, except cadmium in Experiment 2, there was a marked decline in concentration in the byssal threads immediately after transfer into background levels (Figures 6.4, 6.6 and 6.8). This suggests that byssal threads may have been discarded to excrete previously accumulated metal.

Lead

Lead was reported by Schulz-Baldes (1978) to be taken up by the gills and viscera of *M. edulis* and then distributed by the blood to be stored in membrane-bound vesicles within the kidney. Julshamn (1981c) found the gills of this same species to be the only tissue accumulating lead, and to contain 37% of the body load. Nair and Andersen (1972) and Brooks and Rumsby (1965) also recorded high concentrations in the gills, although the latter authors found the highest concentration in the intestine of *M. e. aoteanus*. The gonads and mantle in two of the mussel populations of *M. e. planulatus* examined, contained the highest content of lead. In the other population (Howden) the metal was evenly distributed with a very high concentration in the byssus.

Lead, like cadmium, was accumulated within all tissues, with the gills and kidney exhibiting the greatest increases. In *M. e. planulatus* the metal appears to be taken up by the gills and digestive tissue, as suggested by Schulz-Baldes (1978), and then is stored in the kidney, as well as the gonads and mantle, muscle and byssal threads.

Zinc

Brooks and Rumsby (1965) and Nair and Andersen (1972) found the highest concentrations of zinc in the viscera and gills of mussels, while Julshamn (1981c) reported an even distribution with high levels in the digestive system, gills and muscle. In *M. e. planulatus* zinc was also relatively evenly distributed, the highest natural concentrations being found in the kidney, gills and byssal threads/foot. While the gills appear to be the major uptake site, zinc is stored predominantly in the kidney but also in most other tissues. For example, the gonads and mantle and muscle appear to be important storage systems, both containing high natural levels. Again byssal threads appear to be an important excretory system.

6.5 CONCLUSIONS

The gills were a major site for uptake of all four metals, while the digestive tissue was important for all except copper. The digestive tissue of the mussels from all three populations had relatively high natural metal loads when collected. Its importance as a storage site declined, however, except for cadmium, during the accumulation experiments. This may be due to a natural redistribution of these metals, following accumulation, into such tissue as kidney and liver which were included in the digestive tissue. Alternatively it may be due to the fact that a major source of these metals is by ingestion of organic and inorganic particulates and not just from solution, as would be the case in these experiments.

The kidney was the major storage site for all metals except copper, and is possibly the major site of excretion for these metals. However, the byssal threads appear to be the major site for excretion of copper, although the liver may be important. The loss of metal via the byssus was also important for zinc, and may be a secondary system for cadmium and lead. This subject is considered further in Appendix C.

The uptake, storage and possible excretory systems for each metal are summarised in Table 6.5. While particular aspects of the accumulation processes are common to all four metals, some individual differences are evident in storage and excretory pathways, particularly for copper. However, it appears that more detailed micro analysis of the accumulation of metals within individual tissues is necessary before a complete understanding of metal accumulation and interaction in mussels is possible.

TABLE 6.5 Suggested accumulation and excretory pathways of cadmium, copper, lead and zinc in *M. e. planulatus*.

Metal	Uptake	Storage	Excretion
Cadmium	All tissues, but gills and digestive tissue main sites.	Kidney, digestive tissue, gonads and mantle.	Kidney (byssus)
Copper	Gills	Foot and byssus, digestive tissue (liver), gonads and mantle.	Byssus (liver)
Lead	All tissues, but gills and digestive tissue main sites.	Kidney, muscle, gonads and mantle.	Kidney (byssus)
Zinc	Gills and digestive tissue.	All tissues but mainly kidney, muscle and byssus/ foot.	Kidney and byssus

CHAPTER 7

CONCLUSIONS

7.1 MONITORING PROGRAMME

The mussel *Mytilus edulis planulatus* has been shown to be a very sensitive qualitative and quantitative monitor for detecting levels of cadmium, copper, lead and zinc in seawater. A monitoring strategy utilizing these properties has been suggested. This system has a number of advantages over the more commonly used 'mussel watch' technique (e.g. Goldberg *et al.* 1978; Goldberg 1980; Murray and Law 1980). Firstly, the system described can be used to monitor levels in the strict sense rather than just to indicate local 'hot spots' of pollution. Secondly, it may be employed in open marine waters and also in areas where the particular monitoring organism may not be naturally abundant or may even be absent. The influence of population differences in response to metal levels is also eliminated by the use of the one population only. Its main advantage, however, is the ability to quantify the metal levels present in the water.

This form of monitoring and quantifying the metal levels would be readily adaptable for use in underdeveloped areas, where technical skills and the more sensitive and modern analytical devices are not readily available. Even with the introduction of specific cage and buoying systems, such as the 'mussel motel' and submersing-reel assembly unit designed by Major *et al.* (1981), the monitoring strategy is both easy to use and will return reliable and useful information from a minimum of input in terms of capital cost and labour.

7.2 FUTURE NEEDS

Monitoring an area by such a method as that presented in this study would provide an estimate of the 'biologically available' metal, while a system such as that devised by Davey and Soper (1975), for concentrating heavy metals from seawater *in situ* using chelex resin,

would provide an estimate of the total metal load. Whilst it is understood that it is the 'biologically available' metal that may be hazardous to marine organisms, it is important for researchers to establish what metal species are 'biologically available' and to which particular organisms.

Using a biomonitoring programme of the type described, the levels of metals that are available to the mussel can be determined. However, whether these levels are hazardous to the organisms is a quite separate problem. For example, this technique tells us nothing about the possible sublethal effects of this level of the metal on the individual, the population or the ecosystem. To date numerous 'respectable' environmental limits have been set using the LC50 values of, say, 96 hours exposure. Such levels are inappropriate in environmental management, as extrapolation back from toxic levels would not provide any information on the possible short or long-term effects on reproductive capabilities, genetic stability, physiological or behavioural aspects that may occur at lower metal levels, hence 'the need for sublethal studies' (Perkins 1979). The need for such information has been amply documented recently during working groups (GESAMP 1980) and symposia (Cole 1979a) held on the topic. Because of the diverse nature of the possible sublethal studies some standardization is required, as noted by Eagle (1981). However, this area of research presents considerable problems. The long-term effects of sublethal levels of a metal both at individual and population level, are difficult to disentangle as numerous other factors may also be involved. Nonetheless, sublethal studies are urgently needed to be able to set environmental limits. Continual surveillance to ensure that these limits are not exceeded could then be by a system such as that designed in the present study.

It was emphasised in this thesis that there is a general lack of knowledge of the pathways of the metals within an organism, its tissues

and the individual cells. Such knowledge, as previously reported, is essential to the understanding of the interaction of metals. This area of research into the association of the metals with metal-binding proteins and the granular structures within membrane-limited vesicles, requires further examination of metal accumulation at the ultrastructural level, For example, via X-ray microanalysis (e.g. Marshall and Talbot 1979) and scanning electron microscopy (e.g. Pirie and George 1979). This is required to fully understand metal uptake and storage, and sublethal effects within an organism and within the food web. For example, does the detoxification of a metal by binding to metallothionein, or formation of a granular structure, render this metal detoxified when the organism is consumed by a predator? When an organism dies naturally, is the stored metal still 'biologically available', and is that metal incorporated into such structures as the shell or byssal threads also still 'available' or is it permanently detoxified? From the point of view of environmental management such areas must be addressed with some urgency, or the results obtained from biomonitoring programmes will be of limited use.

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APPENDIX A

MONITORING MERCURY CONTAMINATION

Introduction

Mercury is considered to be a non-essential but highly toxic element to living organisms (Förstner and Wittman 1979). It has very similar properties to cadmium and zinc, and along with cadmium,copper, lead and zinc is considered of greatest overall hazard in the marine environment (Phillips 1980).

The presence of mercury at elevated levels in the tissues of mussels has been reported by such authors as Raymont (1972), Leatherland and Burton (1974), Davies (1976) and Eganhouse and Young (1976). Majori and Petronio (1973) found that *Mytilus galloprovincialis* was a rapid accumulator and useful indicator of mercury levels in seawater. *Mytilus edulis* suspended in cages was successfully used to monitor mercury levels by Davies and Pirie (1977), and Eganhouse and Young (1978). Bloom and Ayling (1977) concluded that the mussel *Mytilus edulis planulatus* would be a valuable indicator of mercury contamination.

In Chapter 3 it was demonstrated that *M. e. planulatus* could be used to monitor levels of cadmium, copper, lead and zinc in seawater. The relationship between rate of accumulation and external concentration of mercury is examined in this section to test whether mussels can be used to monitor this metal in the same way.

Materials and Methods

Four groups of 60 *M. e. planulatus* (4.0 - 5.0 cm shell length, from Barnes Bay) were exposed in the laboratory, under static conditions, to one of the following: background concentrations, 0.1, 0.2 or 0.4 µg Hg ℓ^{-1}. Mercury was added to the experimental tanks from a 10^3 µg ℓ^{-1} stock solution prepared by dissolving Hg $C\ell_2$ in glass distilled water. The experiment was conducted at a water temperature of 11(±1)°C and continued for 40 days.

On days 0, 4, 10, 20 and 40 of the experiment 10 mussels were subsampled from each concentration for tissue analysis. All experimental equipment and procedures were as described in Chapter 2, except the tissue digestion and analysis.

Mussels were opened using a sterile, stainless steel, surgical blade. The tissue was rinsed with glass distilled water and allowed to drain for 1 min. before being removed from the valves. Byssal threads were removed. The whole wet tissue of the 10 mussels was homogenised in a Sorvall Omni-Mixer (Du Pont Instruments) and stored frozen prior to digestion. Tissue digestion followed a cold acid digestion procedure modified from that of Hatch and Ott (1968), to produce mercury vapour that was analysed using a Precision Devices Model Hg-3 (Precision Devices, Hobart).

The digestion procedure was as follows: between 2.0 and 2.5 g wet weight of tissue was placed into a digestion flask and 10 ml HNO_3/H_2SO_4 (3:2) added. The flask was packed in ice and allowed to stand for over 24 hours, prior to the addition of 40 ml $KMnO_4$. Then the flask was again packed in ice and allowed to stand for 48 hours before the addition of 25 ml $NH_2OH.HC\ell$, to clear the solution. Stannous chloride was added immediately before analysis. The results were converted to a dry weight concentration using a dry/wet weight ratio of 15.27%, obtained during previous experimentation. Analysis of seawater sampled regularly from experimental tanks was also by means of a cold acid permanganate digestion (Tasmanian Government Analyst Laboratory).

Results and Discussion

The results of the analyses of the seawater samples revealed that mercury levels in the experimental tanks were as specified. Accumulation of mercury at each concentration was linear over the 40 day exposure period (Figure A.1). Rate of accumulation was also directly proportional to the external concentration (Figure A.2). The background concentration was

Figure A.1

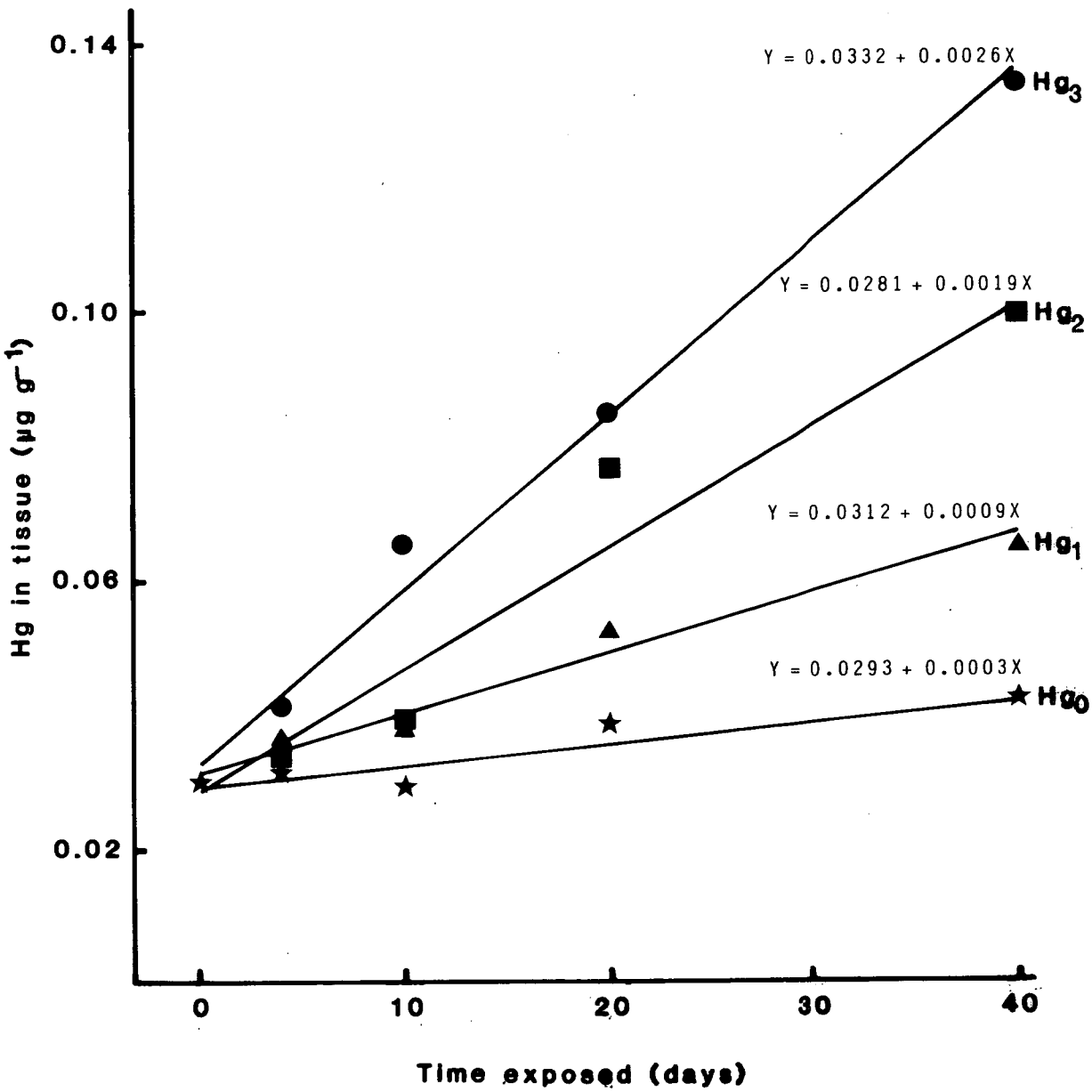
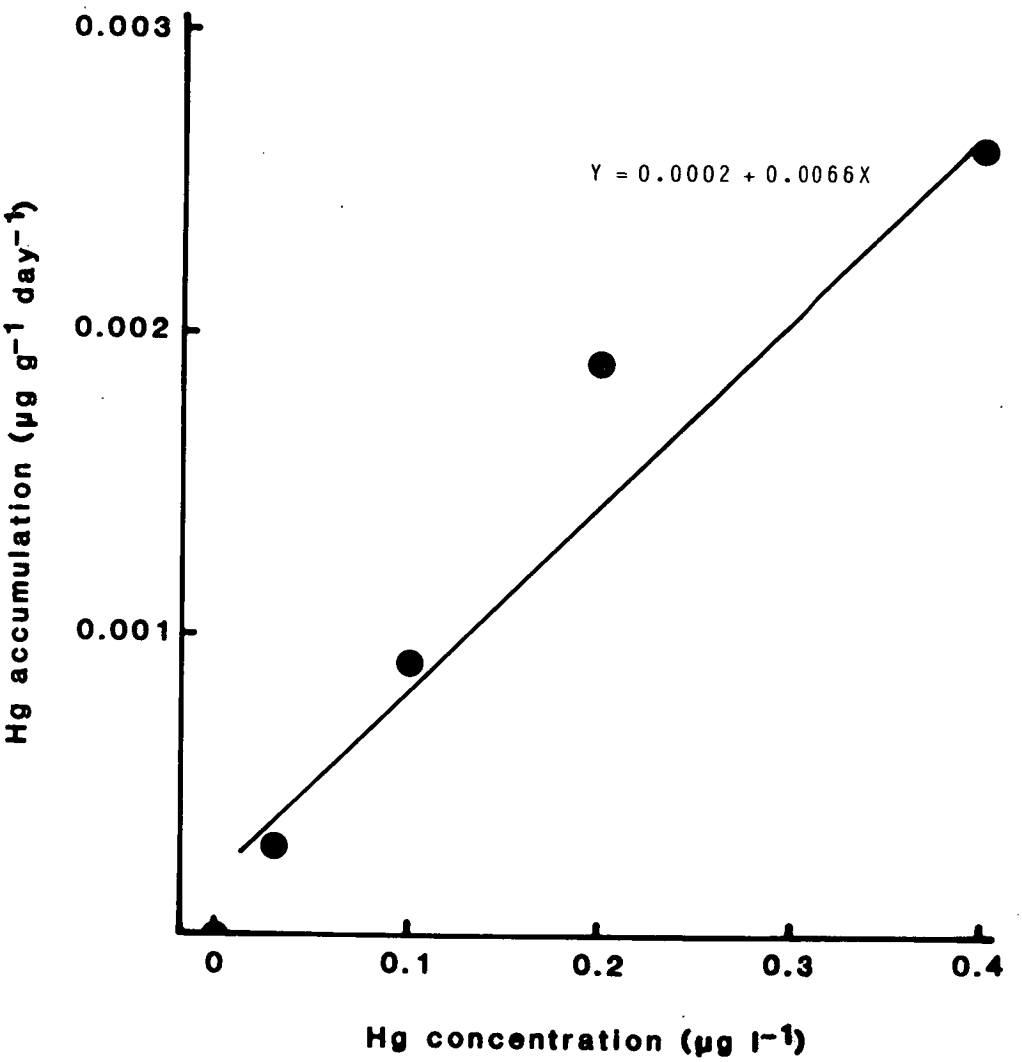

0.14
0.10
0.06
0.02
Hg in tissue (µg g⁻¹)
Y = 0.0332 + 0.0026X
Hg3
Y = 0.0281 + 0.0019X
Hg2
Y = 0.0312 + 0.0009X
Hg1
Y = 0.0293 + 0.0003X
Hg0
0
10
20
30
40
Time exposed (days)

Figure A.2


0.003
0.002
0.001
Hg accumulation (µg g⁻¹ day⁻¹)
Y = 0.0002 + 0.0066X
0
0.1
0.2
0.3
0.4
Hg concentration (µg l⁻¹)

taken as 0.03 μg ℓ^{-1} (mean 'seawater', Brewer 1975). Also a zero accumulation was assumed at a zero external concentration. Since the regression in Figure A.2 does not pass through zero, the line has not been extended to the y axis to avoid the anomalous appearance of metal uptake at zero concentration.

Lakshmanan and Nambisan (1979) reported the rate of uptake of mercury by the mussel *Perna viridis* increasing with increased concentrations in the water during laboratory experiments. The mercury content of caged *M. edulis* was found to be closely related to the external concentration after 20 days exposure under natural conditions (Davies and Pirie 1977). The relationship, however, deteriorated with a longer exposure time. The highest concentration factors for *P. viridis*, indicating greater uptake efficiency, occurred at the lowest mercury concentrations (Lakshmanan and Nambisan 1979). Concentration factors after 40 days exposure calculated from the regression in Figure A.2 reveal a similar efficiency in *M. e. planulatus*, *viz*:the concentration factor at 0.01 μg ℓ^{-1} = 1.07, 0.03 μg ℓ^{-1} = 0.53; 0.1 μg ℓ^{-1} = 0.34; 0.2 μg ℓ^{-1} = 0.30 and 0.4 μg ℓ^{-1} = 0.28. A similar relationship was recorded for cadmium, copper, lead and zinc (Chapter 3).

Unfortunately field trials for assessing the value of *M. e. planulatus* as a monitoring organism for mercury levels failed due to a combination of vandalism and storm weather. However the laboratory results appear to suggest that the mussel may be as sensitive a monitor of mercury levels as it is of cadmium, copper, lead and zinc.

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APPENDIX B

COPPER AND ZINC IN THE BARNACLE

ELMINIUS MODESTUS, DARWIN

Introduction

The ability of barnacles to accumulate the biologically essential metals, copper and zinc, from seawater has previously been reported (Clarke 1947; Ireland 1974; Walker and Foster 1979). The zinc content of the tissues was correlated with the level in the surrounding water, which led to the suggestion that the barnacle could be used as an indicator of zinc contamination (Walker *et al.* 1975a). Copper and zinc have both been found in distinctive granular forms in the tissues of *Balanus balanoides* (Walker *et al.* 1975a, b; Walker 1977).

The present study was part of an initial investigation into the possible use of the local barnacle, *Elminius modestus*, as a monitor of copper and zinc levels in seawater. It deals specifically with the relationship between these two metals in the barnacle tissue.

Materials and Methods

Barnacles

Specimens of *E. modestus* were collected on small rocks at low tide level from Blackmans Bay, Southern Tasmania (Figure 2.1). On return to the laboratory the rock surface was cleaned of all algae and encrustations, as well as dead barnacle shells. The rocks containing the barnacles were then placed into polyethylene tanks containing 16 ℓ aerated seawater at 11(±1)°C. As described in Chapter 2 the seawater was changed every 2 days. The animals were allowed 120 hours acclimation time before the start of an experiment. Only barnacles between 0.5 to 1.0 cm shell diameter were used in the experiments. A biomass of less than 6 g wet weight per litre was maintained in each tank.

Experiments

Two experiments were conducted, each lasting 90 days and consisting of three phases. In Phase I, days 0 - 40, barnacles were exposed to an

elevated single metal concentration and subsamples of barnacles were removed for analysis on days 0, 4, 10, 20 and 40. In Phase II, days 41 - 80, the barnacles were exposed to background metal levels of the laboratory seawater, and subsamples were removed on days 45, 50, 60 and 80. In Phase III, days 81 - 90, the barnacles were again exposed to the elevated level of the original metal. Subsamples were removed on days 85 and 90. The metal levels were: Experiment 1, copper at 10 μg ℓ^{-1}; Experiment 2, zinc at 100 μg ℓ^{-1}.

Metal Analysis

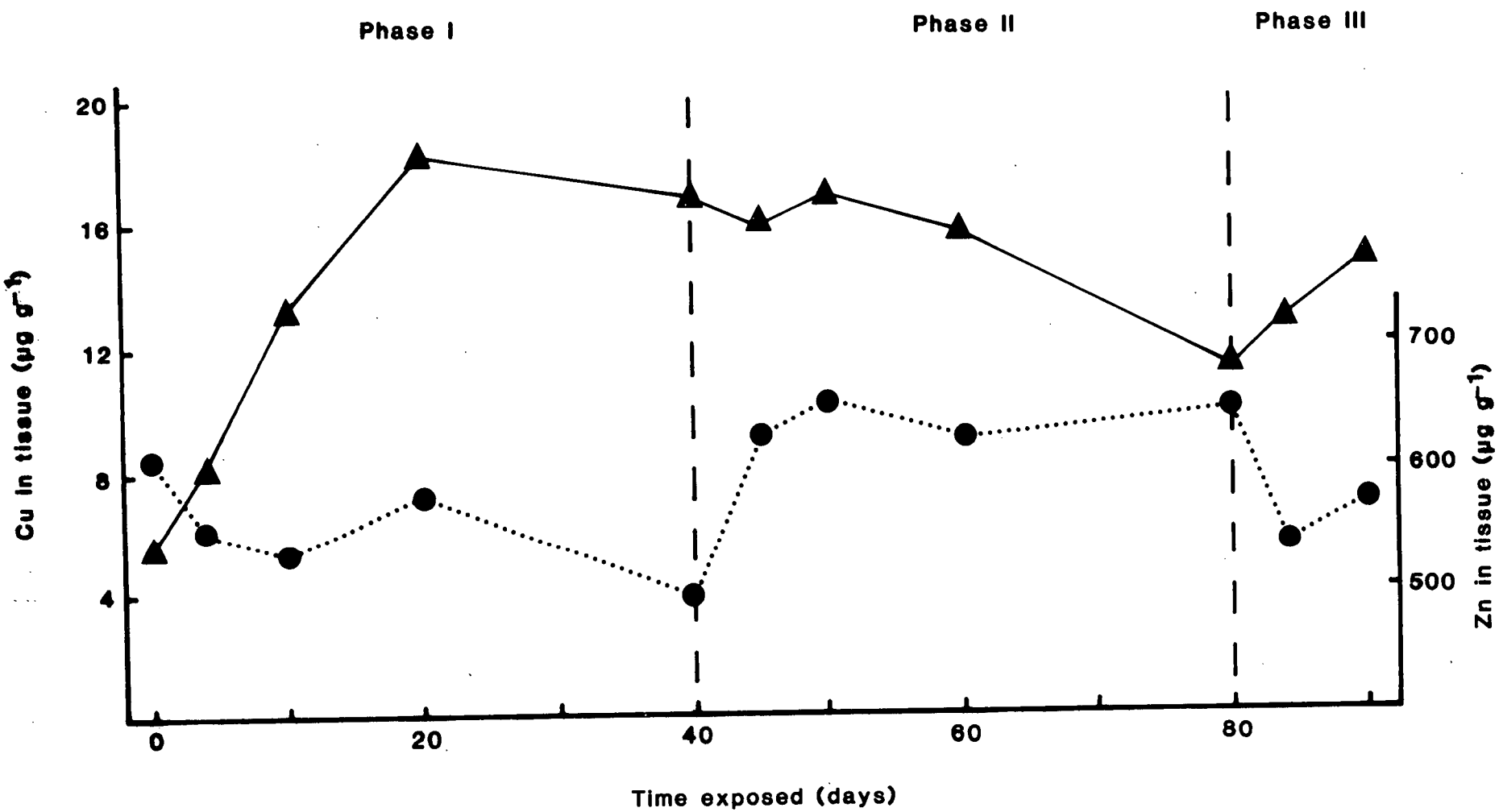
Barnacles for analysis were removed from the rock surface using a sterile stainless steel surgical blade, after the shell surface of the barnacles had been rinsed with glass distilled water. Between 100 and 120 individuals were required per subsample in order to provide sufficient dry tissue (soft body tissue plus shell) for three digests. The pool of barnacles was weighed and then dried at 105 °C to a constant weight. The dried tissue was then crushed into a fine powder.

Three samples of at least 0.25 g dry wt. were placed into separate flasks and digested in a $HNO_3/HC\ell O_3$ mixture as previously described (Chapter 2). The final solution was analysed for copper and zinc using a Varion Techtron AA-5 atomic absorption spectrophotometer. Aqueous standards were used for calibration and NBS 1577, Oyster tissue, was employed as a standard reference material.

Results

While exposed to 10 μg Cu ℓ^{-1} (Phase I, Experiment 1), accumulation of copper occurred up to day 20, after which an apparent equilibrium tissue concentration was reached (Figure B.1). Transfer into background levels at day 40 (Phase II) resulted in a loss of copper from the tissue. Approximately 50% of the previously accumulated copper was lost over the

Figure B.1


Phase I
Phase II
Phase III
20
16
12
8
4
Cu in tissue (µg g⁻¹)
700
600
500
Zn in tissue (µg g⁻¹)
0
20
40
60
80
Time exposed (days)

subsequent 40 days (Figure B.1). Copper was again accumulated upon re-exposure to 10 µg Cu ℓ^{-1} at day 80 (Phase III).

During the exposure to, and accumulation of copper, zinc was apparently lost from the barnacle tissue (Phases I and III, Figure B.1). On removal from the high copper level (Phase II), zinc was rapidly accumulated, from background concentrations, back into the tissues. The level of zinc reached was similar to that recorded at day 0 and possibly represents an equilibrium level at the background concentration of the seawater.

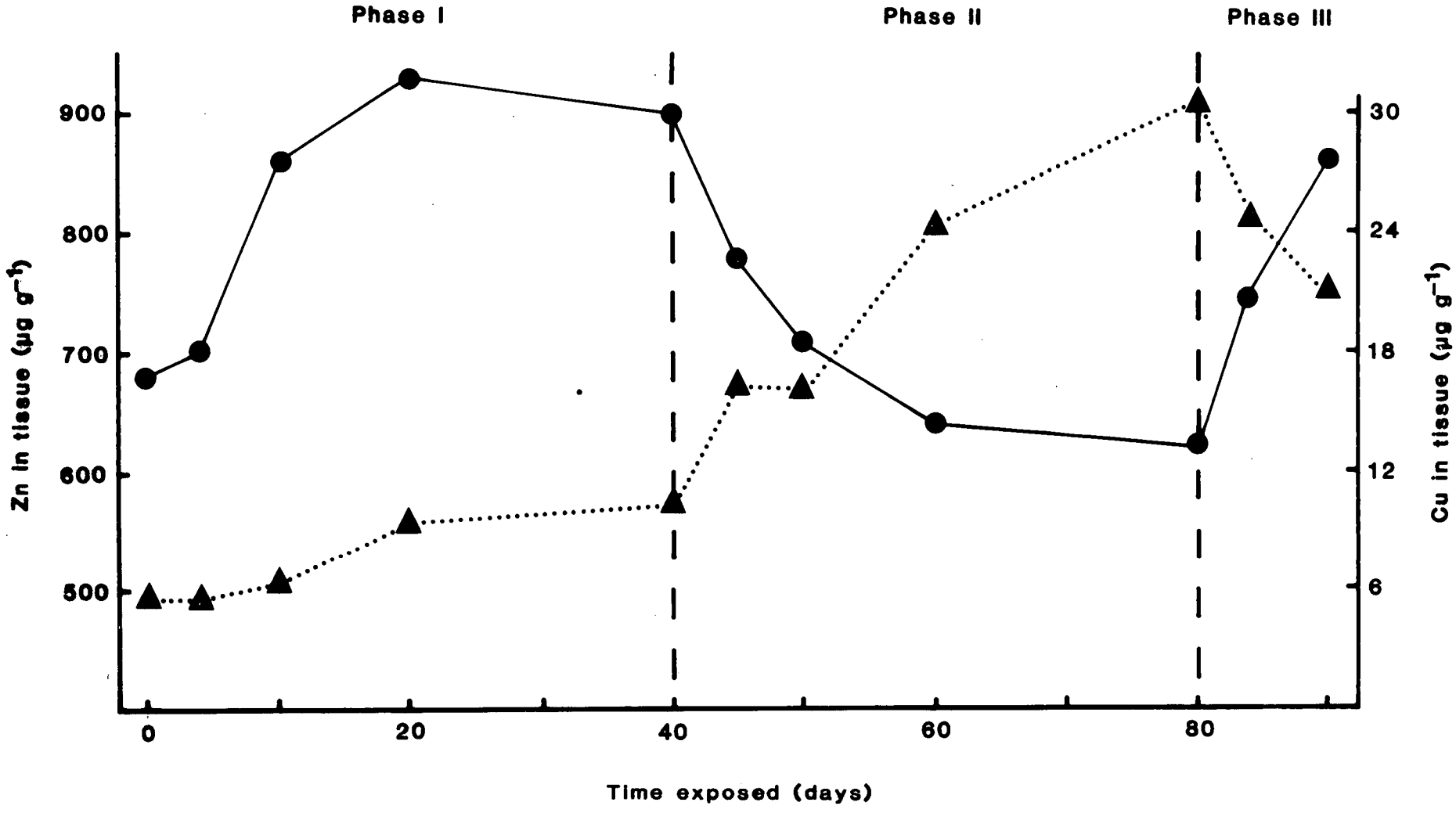
Exposure to 200 µg Zn ℓ^{-1} (Experiment 2) resulted in a linear accumulation of zinc for 20 days until an equilibrium tissue concentration was reached (Phase I, Figure B.2). Removal from the high zinc level (Phase II) resulted in an exponential loss of the accumulated zinc from the barnacle tissues. Re-exposure to zinc (Phase III) again resulted in an accumulation of the metal. During Phase I a slow accumulation of copper from background levels occurred (Figure B.2). This accumulation was markedly increased on removal from the high zinc level at day 40 (Phase II). While zinc was lost from the tissue, copper was rapidly accumulated from background levels. Subsequent exposure to zinc resulted in a loss of copper.

Discussion

Both metals appeared to reach equilibrium concentrations in the tissue after 20 days exposure. For zinc the value recorded (900 - 950 µg g^{-1} dry wt.) was similar to that found in other (unpublished) experiments as a saturation level. However continued exposure to copper in these other experiments resulted in greater tissue concentrations of copper.

A definite relationship exists between the tissue levels of copper

Figure B.2


Phase I
Phase II
Phase III
900
800
700
600
500
Zn in tissue (µg g⁻¹)
30
24
18
12
6
Cu in tissue (µg g⁻¹)
0
20
40
60
80
Time exposed (days)

and zinc in *E. modestus*. Accumulation of copper was accompanied by a loss of zinc, and at high levels of copper in the tissue the reverse also occurred, i.e. they act antagonistically. Hill and Matrone (1970) proposed that as these two metals have similar physical and chemical properties, they would act antagonistically to each other in biological systems. This appears to be the case in *E. modestus* when one metal is present in the external medium at an elevated level. Simultaneous exposure to both metals resulted in synergistic uptake in *M. e. planulatus* (Chapter 5).

This interaction between copper and zinc within the tissues of *E. modestus* casts doubt on the suitability of this organism for monitoring purposes.

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APPENDIX C

METAL ACCUMULATION IN BYSSAL THREADS

Introduction

Mussels, particularly *Mytilus edulis*, are amongst the most important fouling animals in seawater cooling culverts and are often controlled by chlorination, which weakens their byssal attachment (Roberts 1976). These collagenous threads secreted by exocrine glands in the foot (Waite and Tanzer 1981) are of supreme importance to the mussels (Yonge 1976). Their production has been found to be influenced by factors such as temperature and salinity (Glaus 1968), exposure (Van Winkle 1970) and population density (Martella 1974), as well as pollutants such as oils (Linden 1977; Carr and Reish 1978; Linden and Foberg 1980) and heavy metals (Martin *et al.* 1975). Swedmark *et al.* (1973) suggested that byssal production may in fact be a more sensitive biological function than valve closure in pollutant detection.

George *et al.* (1976) reported that a major portion of iron absorbed by *M. edulis* was excreted by transfer to the byssal threads. The byssus may readily be discarded and new threads produced. Experimentation has shown that high concentrations of cadmium, copper, lead and zinc may be found in the byssus of *Mytilus edulis planulatus* (Chapter 6). Miramand *et al.* (1980) found the highest concentration of vanadium in *Mytilus galloprovincialis* to be present in its byssus.

The present study was designed to investigate the extent of metal accumulation in the byssal threads of *M. e. planulatus*, and whether their production was influenced by the metal levels in the seawater.

Materials and Methods

The general experimental techniques described in Chapter 2 apply to the present study with two modifications. Firstly, when mussels were subsampled during an experiment for tissue analysis, the byssus, once cut away from the soft tissue, was not discarded. Instead the

byssal threads of the 10 mussels per sample were pooled and rinsed with glass distilled water. They were then dried at 105°C, acid digested and analysed in a similar manner to the soft tissue. Special care was also taken when the mussels were removed from the experimental tanks to ensure the entire byssus was removed with the mussel. Secondly, in a number of experimental tanks, the mussels were placed on glass sheets which were easily removed from the tanks, thereby allowing the counting of the threads produced without disturbing the mussel.

Metal levels in the byssus were analysed during the following metal exposures: background levels i.e. laboratory seawater; cadmium at 5, 30 and 50 μg ℓ^{-1}; copper at 5 and 20 μg ℓ^{-1}; lead at 20, 35, 50 and 100 μg ℓ^{-1}; zinc at 50 and 150 μg ℓ^{-1}; and the combined exposure of 10 μg Cd ℓ^{-1}, 10 μg Cu ℓ^{-1}, 50 μg Pb ℓ^{-1} and 100 μg Zn ℓ^{-1}. Metal concentration in the soft tissues and byssal threads were recorded regularly during the experiments.

Results and Discussion

The respective accumulation of the metals in the soft tissues and byssal threads of *M. e. planulatus* during each experiment are presented in Figures C.1 - C.5. In the majority of cases the accumulation of metal by the byssus paralleled that of the soft tissue.

The only significant accumulation of a metal within either the byssus or soft tissue during exposure to background levels was for copper (Figure C.2a). One striking aspect of the results was that the concentrations of copper and zinc were 5 to 10 times higher in the byssus than the soft tissues (Figures C.2, C.4 and C.5). On the other hand cadmium and lead levels were more comparable (Figures C.1, C.3 and C.5). This supports the suggestion in Chapter 6, that the byssus is a more important organ for excretion of copper and zinc than the other two metals.

Figure C.1

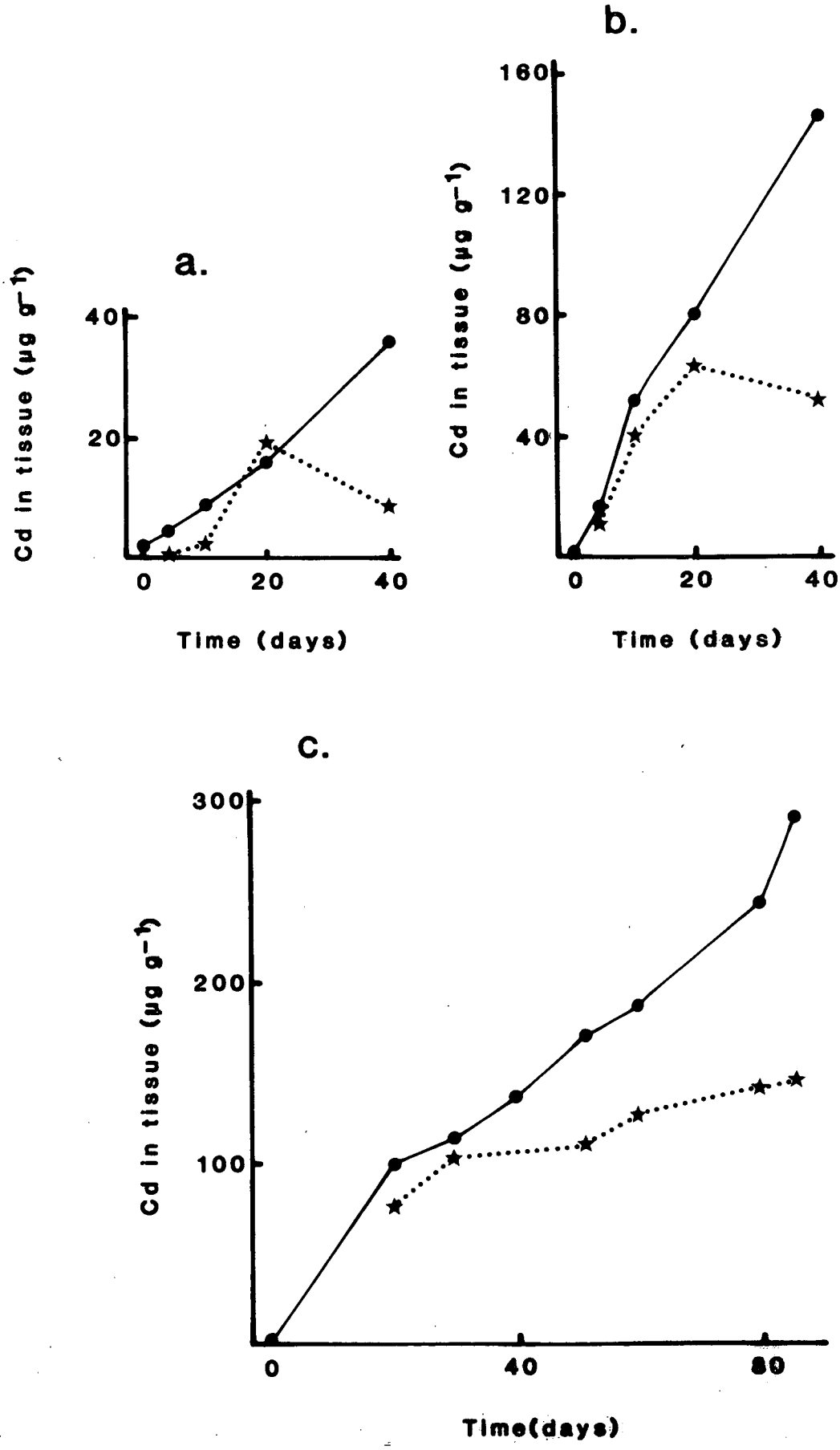

a.
b.
c.
Cd in tissue (µg g⁻¹)
Time (days)
Time(days)
0
20
40
80
100
120
160
200
300

Figure C.2

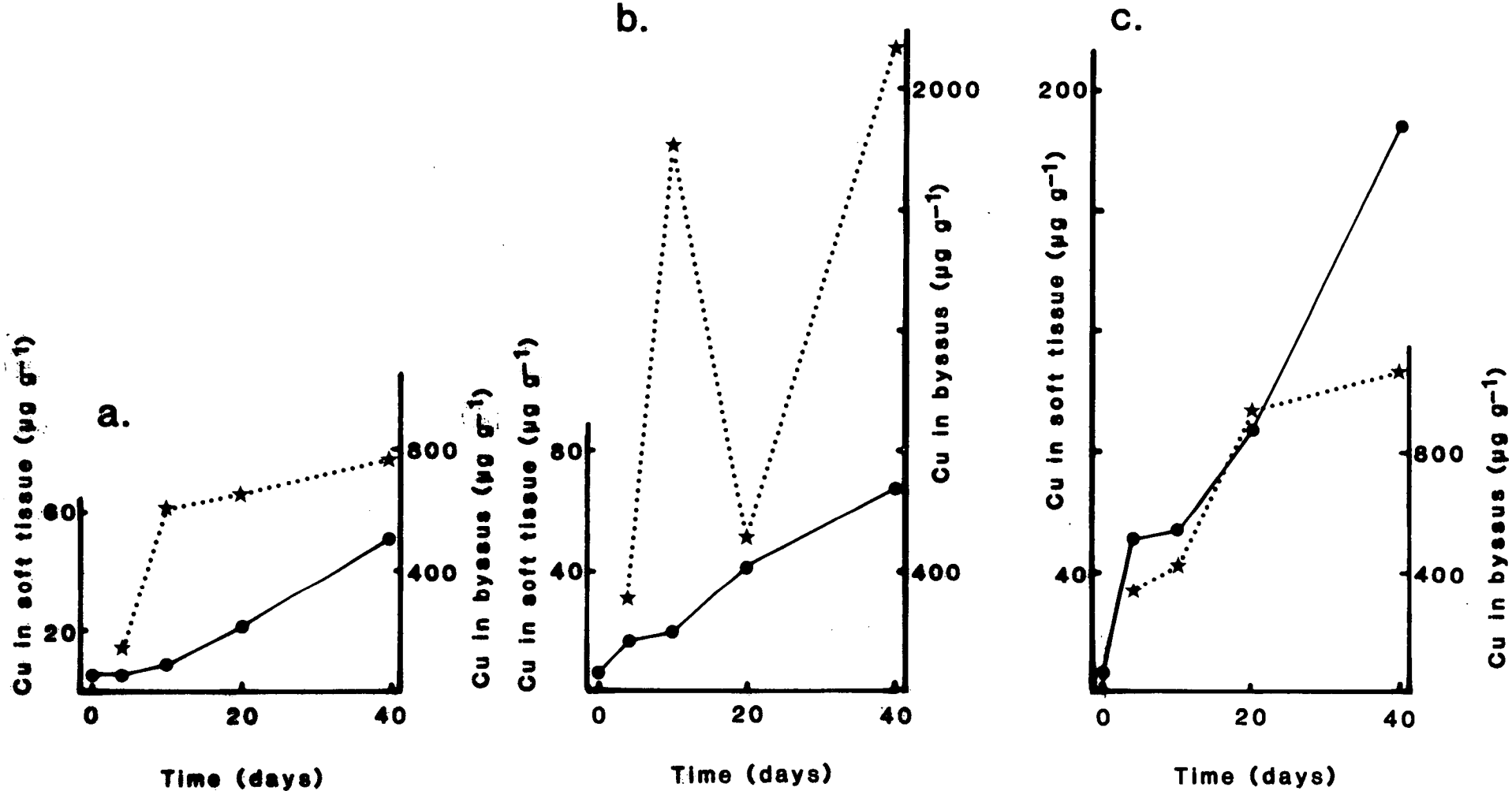

a.
b.
c.
Cu in soft tissue (µg g⁻¹)
Cu in byssus (µg g⁻¹)
Time (days)
0
20
40
60
20
800
400
80
40
2000
200

Figure C.3

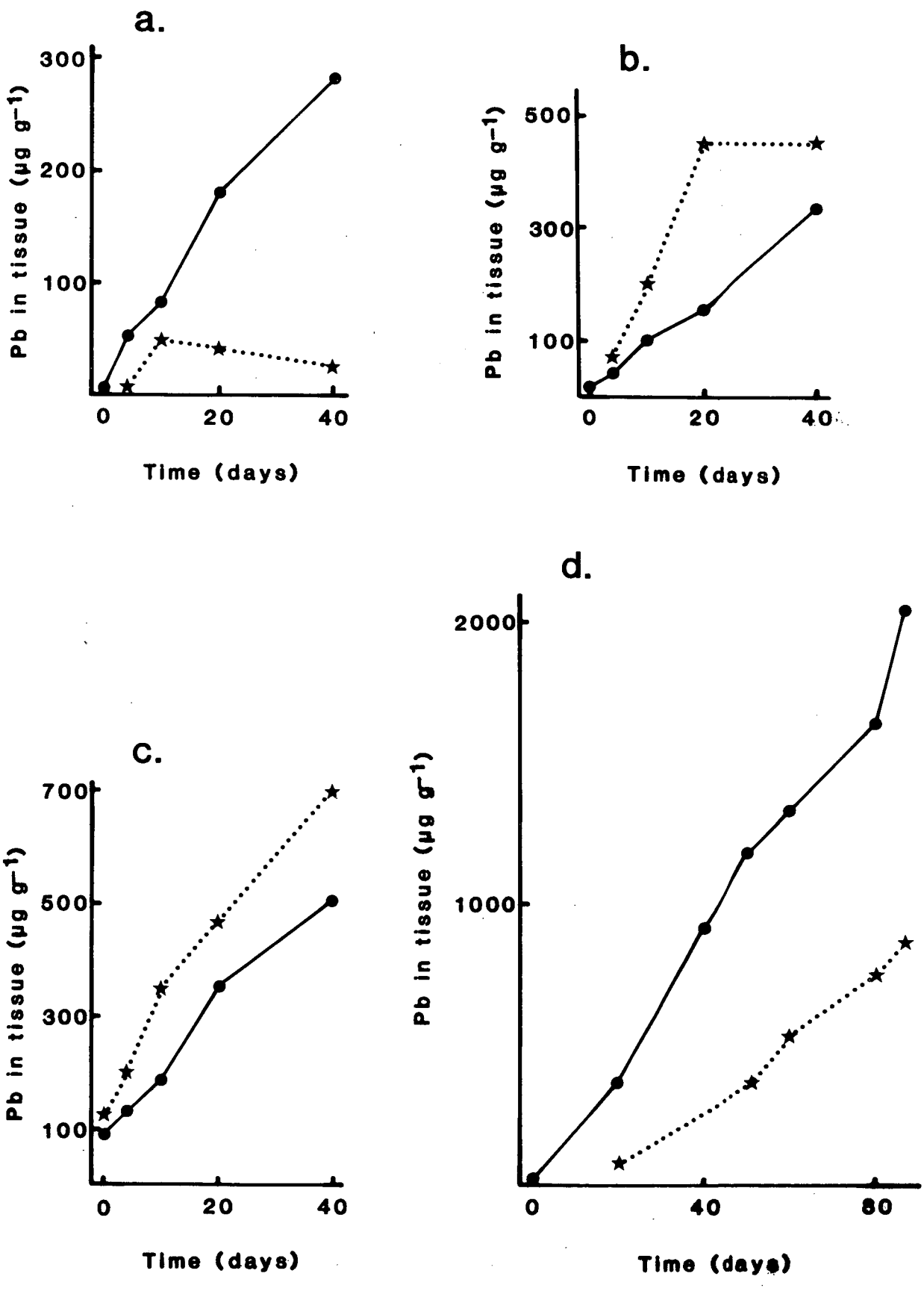

a.
300
200
100
Pb in tissue (µg g⁻¹)
0
20
40
Time (days)
b.
500
300
100
Pb in tissue (µg g⁻¹)
0
20
40
Time (days)
C.
700
500
300
100
Pb in tissue (µg g⁻¹)
0
20
40
Time (days)
d.
2000
1000
Pb in tissue (µg g⁻¹)
0
40
80
Time (days)

Figure C.4

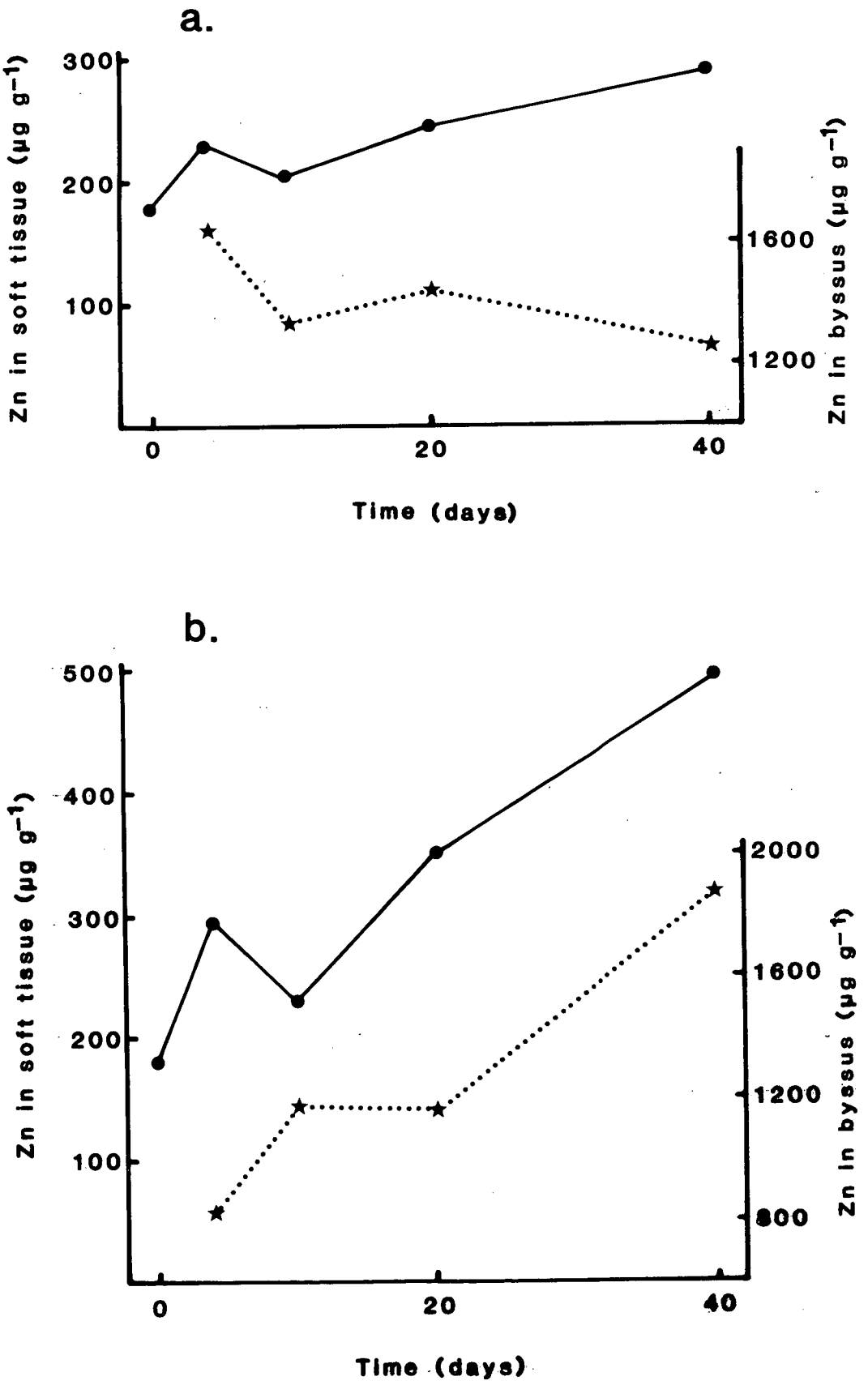
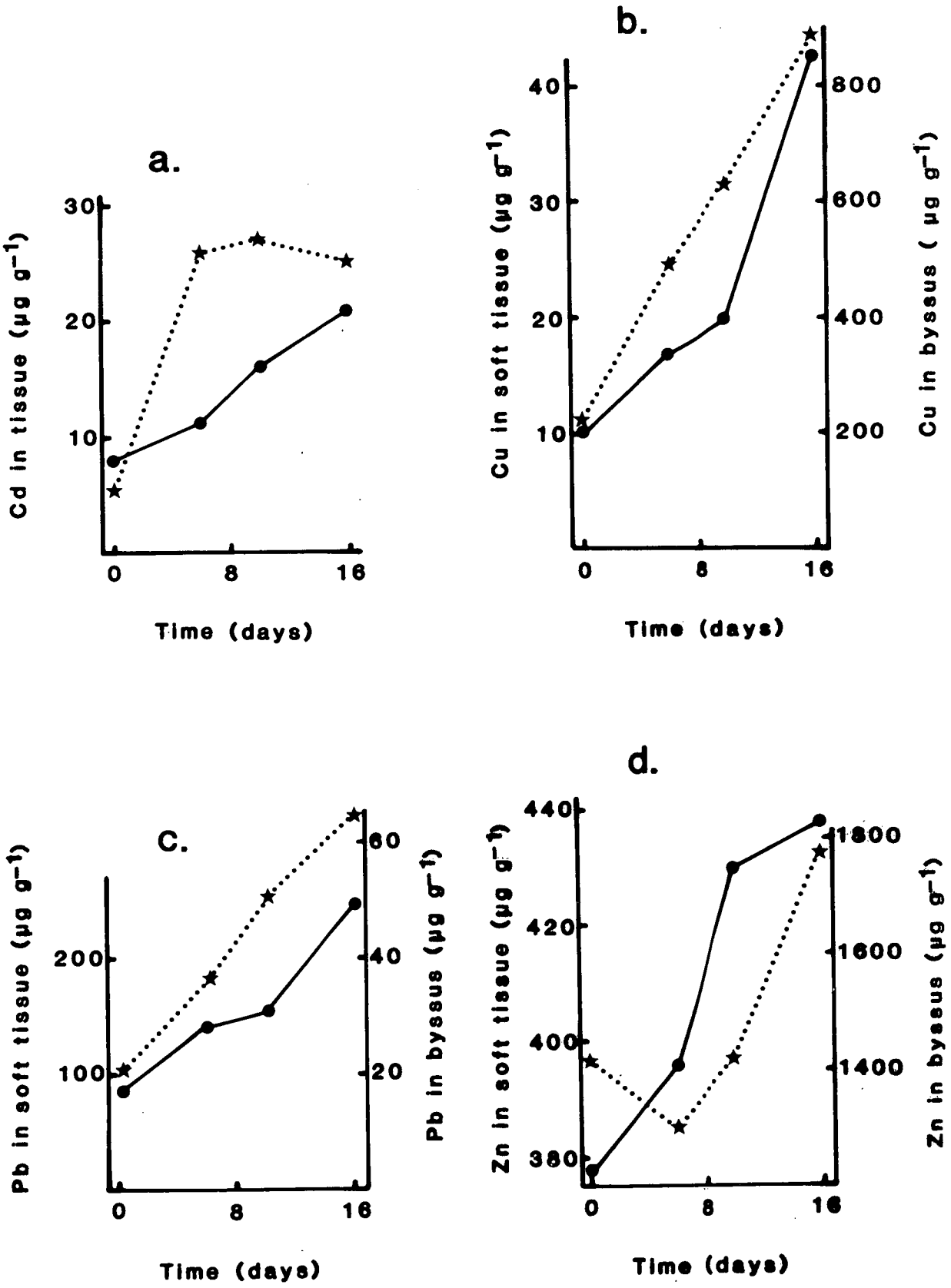

a.
300
200
100
Zn in soft tissue (µg g⁻¹)
1600
1200
Zn in byssus (µg g⁻¹)
0
20
40
Time (days)
b.
500
400
300
200
100
Zn in soft tissue (µg g⁻¹)
2000
1600
1200
800
Zn in byssus (µg g⁻¹)
0
20
40
Time (days)

Figure C.5


a.
Cd in tissue (µg g⁻¹)
30
20
10
0
8
16
Time (days)
b.
Cu in soft tissue (µg g⁻¹)
40
30
20
10
800
600
400
200
Cu in byssus (µg g⁻¹)
0
8
16
Time (days)
c.
Pb in soft tissue (µg g⁻¹)
200
100
60
40
20
Pb in byssus (µg g⁻¹)
0
8
16
Time (days)
d.
Zn in soft tissue (µg g⁻¹)
440
420
400
380
1800
1600
1400
Zn in byssus (µg g⁻¹)
0
8
16
Time (days)

Two difficulties arise in the analysis of the byssus of mussels. Firstly, there is only a small tissue weight (average for 10 mussels 0.065 g, range 0.01 - 0.14 g), which may lead to inaccurate analyses. Secondly, the mussels have the ability to discard the byssus and produce new threads. This may be a convenient method for the mussel to excrete metal from its body, but causes problems of interpretation since, firstly newly secreted threads may contain very different levels of metal, and secondly the discarded byssus is not easily paired to a particular mussel. It was found that each individual *M. e. planulatus* discarded its byssus on average every 5 days during the laboratory experiments.

Counting the number of byssal threads secreted during laboratory experiments revealed no significant (5% level) variation in production that could be attributed to metal levels in the water. There was, as reported by Martella (1974), considerable individual variation. The lack of any significant variation seems reasonable as the EC_{50} values (concentrations at which there was a 50% reduction in byssal production), for 168 hours at 17°C, recorded in *M. edulis* by Martin *et al.* (1975) were at 500 µg ℓ^{-1} for cadmium, 250 µg ℓ^{-1} for copper, 2500 µg ℓ^{-1} for lead and 1800 µg ℓ^{-1} for zinc. These concentrations were well in excess of those employed in this present study. Average production by *M. e. planulatus* in the first 24 hours at 11°C was 5.4 threads per mussel. Martin *et al.* (1975) recorded control levels of 4 to 7 threads per day per mussel. However, over a 4 - 6 day period, an average *M. e. planulatus* produced 1.7 threads per day plus discarding a byssus every 5 days. A discarded byssus contained between 3 and 45 threads.

Lead, and possibly cadmium, may not be transferred to the byssus during a combined metal exposure with copper and/or zinc. This is exemplified by comparison of the lead concentrations in the byssus in Figures C.3c and C.5c. In both experiments lead was at 50 µg ℓ^{-1} but in Figure C.5c the mussels had also been exposed to elevated cadmium,

copper and zinc. Although the soft tissue concentration of lead was not affected there was a reduction in lead level in the byssus.

Conclusions

It does not seem possible to monitor metal levels in seawater by the number of byssal threads produced. However, the results of the accumulation experiments indicated that the byssus is an important storage site, and possible means for excretion of all four metals, but in particular copper and zinc. The metal levels in the byssus in general reflected those in the soft tissue. It is suggested that the quantity of a metal incorporated into a new byssal thread is proportional to that present in the soft tissues, and may therefore reflect the concentration in the surrounding water.

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APPENDIX D

SHELL SHAPE AS A POLLUTION INDEX

INTRODUCTION

Mussels (*Mytilus edulis planulatus*) collected from different populations in the Derwent Estuary and D'Entrecasteaux Channel were found to exhibit wide variations in shell morphology. These mussels were from similar population densities, tidal levels, and temperature and salinity regimes. Heavy metal levels in the water were, however, known to vary between sites.

Seed (1968) examined at some length the variation in shell morphology of *Mytilus edulis* and found it to be essentially phenotypic. The observations of Freeman and Dickie (1979) indicated a genetic influence involved in growth rates. The growth of mussels was described by Riisgård and Randløv (1981) as a sensitive parameter of the 'suitability' of the environment.

The following study was designed to investigate whether heavy metal contaminants in the water exert any influence on shell shape in mussels.

POPULATION SURVEY

Mussels were collected from four sites, *viz.* Kangaroo Bluff, Kingston, Howden and Barnes Bay (Figure 2.1), which were known to have widely different heavy metal concentrations (Chapter 2). Shell length was used as a standard measurement in all previous experiments and all mussels in the present study were within the length size range 3.0 to 4.0 cm. The numbers per site used in the analysis ranged from 27 to 42, and were all collected on the same day, from similar tidal level and population density. On return to the laboratory each mussel was measured to the nearest 0.002 cm, using vernier calipers, in three dimensions - length, height and width (Figure D.1).

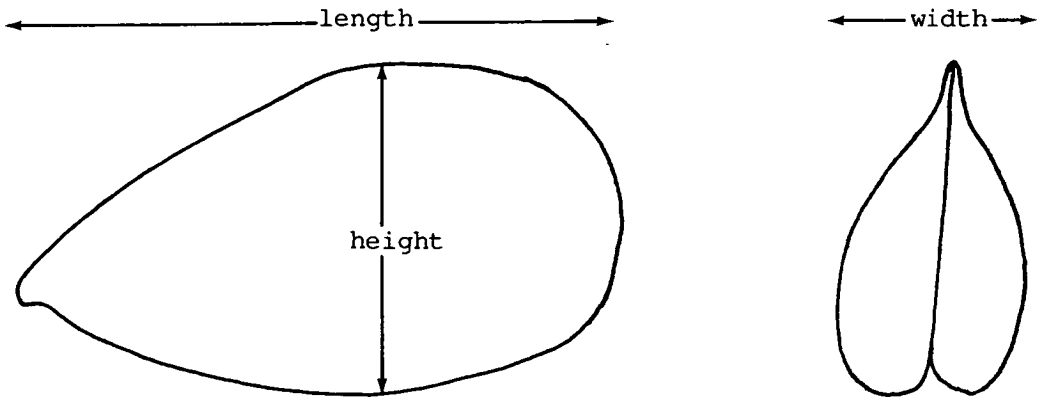

FIGURE D.1 Terminology of mussel shell measurements.

The four sites may be separated using allometric growth measurements (Table D.1); separation of the sites occurring through the width measurement. It is clear therefore that mussels of similar shell length taken from different populations can be separated by their width measurement.

TABLE D.1 Shell dimensions (average ±s.d., cm) of *M. e. planulatus* from four populations.

	Length (L)	Height (H)	Width (W)	$\frac{L}{H}$	$\frac{L}{W}$	$\frac{H}{W}$
Kangaroo Bluff	3.51±0.25	1.96±0.21	1.88±0.19	1.79	1.87	1.04
Kingston	3.48±0.24	2.00±0.15	1.52±0.12	1.74	2.29	1.32
Howden	3.51±0.26	2.01±0.15	1.49±0.15	1.75	2.36	1.35
Barnes Bay	3.45±0.28	2.11±0.22	1.27±0.15	1.64	2.72	1.66

The metal levels in mussel tissue and seawater at each site are presented in Table D.2. The separation between Kangaroo Bluff and other sites is as marked in metal levels (particularly lead and zinc) as it is on the basis of mussel shell shape ($\frac{L}{W}$ and $\frac{H}{W}$, Table D.1). However, in terms of metal levels there is a greater difference between Kingston and Howden than between the latter and Barnes Bay, which is

in contrast to the relationship shown in Table D.1. It is therefore unclear whether metal levels are the factors influencing shell shape differences.

TABLE D.2 Metal concentrations in mussel tissue (μg g^{-1} dry wt.) and seawater (μg ℓ^{-1}) at four separate populations.

	Cadmium		Copper		Lead		Zinc	
	tissue	water	tissue	water	tissue	water	tissue	water
Kangaroo Bluff	9.7	<0.5	7.2	<1	126	4	342	140
Kingston	6.1	<0.5	5.4	<1	93	1	270	60
Howden	2.9	<0.5	6.4	<1	3.0	<1	115	7
Barnes Bay	3.4	<0.5	6.6	<1	5.4	<1	212	12

TRANSFER EXPERIMENTS

I. Barnes Bay to Bligh Point and Kangaroo Bluff

Field experiments were described in Chapter 3 in which *M. e. planulatus* (4.0 - 5.0 cm shell length) from Barnes Bay was suspended in cages at two sites: a polluted site - Kangaroo Bluff, and a 'cleaner' site - Bligh Point. The average size (length, height and width) of 10 individuals at each site was measured at the start of the experiment and after 43 days suspension. The results were compared by unpaired t-test for any growth and site differences (Table D.3).

The mussels at the 'cleaner' site, Bligh Point, exhibited higher average growth in all three dimensions (Table D.3). However, mussels from the two sites did not differ significantly ($p>0.05$) in their width measurement after 43 days exposure. While all mussels increased in size in all three dimensions, those at Kangaroo Bluff had a reduced growth in length and height. This result supports the findings of the population survey, where mussels of equal length had a wider shell at Kangaroo Bluff compared to other sites.

TABLE D.3 Average (±s.d.) shell dimensions of *M. e. planulatus* before and after transfer from Barnes Bay. Measurements with same superscript do not differ significantly ($p>0.05$).

	Length (cm)	Height (cm)	Width (cm)
At collection	4.27±0.33	2.59±0.22[a]	1.57±0.15
After 43 days suspension at:			
Kangaroo Bluff	4.63±0.30	2.70±0.18[a]	1.81±0.23[b]
Bligh Point	5.00±0.37	2.94±0.18	1.91±0.17[b]

II. Transfer Between Howden and Kangaroo Bluff

A random collection of 64 mussels with shell length between 1.5 and 4.5 cm was made from both Howden and Kangaroo Bluff intertidal populations. On return to the laboratory each mussel was individually identified by cutting a small number in the left valve (Freeman and Dickie 1979), and then measured to the nearest 0.002 cm in three dimensions (Figure D.1). Half the mussels from both sites were then placed in cages, suspended 0.5 m above the sea floor (Figure 3.11), at each collection site. After 48 days the cages were recovered and each mussel remeasured. The percentage increase in each dimension for each individual was recorded (Table D.4). While the mussels suspended at Howden appeared to have a greater average growth, the difference between sites was not significant ($p>0.05$). There was considerable individual variation in growth (Table D.4), as was also reported for *M. edulis* by Freeman and Dickie (1979).

LABORATORY EXPERIMENTS

The growth, in three dimensions, of mussels used in previously described laboratory experiments (Chapter 3), was examined, using

TABLE D.4 Percentage increase in shell dimensions of *M. e. planulatus* during transplantation experiment. Standard deviation (s.d.) and coefficient of variation (cv%) are also shown.

	Kangaroo Bluff mussels transferred to		Howden mussels transferred to	
	Kangaroo Bluff	Howden	Kangaroo Bluff	Howden
Length:				
mean	13.39	15.77	17.27	20.75
s.d.	14.96	11.66	12.85	14.97
cv%	111.73	73.94	74.41	72.14
Height:				
mean	13.57	15.68	17.11	18.24
s.d.	17.08	12.90	13.33	15.69
cv%	125.87	82.27	77.91	86.02
Width:				
mean	14.77	8.99	19.79	20.33
s.d.	21.18	6.19	16.50	14.06
cv%	156.94	68.85	83.38	69.16

average and individual growth over 40 day exposures to various metal levels. As expected, growth rate in the laboratory, where no food source was added to the seawater supply (Chapter 2), was lower than that recorded in caged mussels. For example, mussels in the Transfer Experiment I (Barnes Bay to Bligh Point and Kangaroo Bluff) increased in length by 169 and 84 μm day^{-1}, respectively. In the laboratory the maximum growth rate recorded was 30 μm day^{-1}.

The only significant ($p<0.05$) changes in growth rate which could be attributed to exposure to a metal were recorded in two experiments

using 500 µg Zn ℓ^{-1} and once using 100 µg Pb ℓ^{-1}. In both instances for zinc the rate of growth in length was decreased (to <18 µm day^{-1}). In both the lead and one zinc experiment a reduction in rate of growth in width and an increase in height was recorded. Again marked variation in individual growth was recorded.

CONCLUSIONS

While it is possible that very high metal levels in seawater may reduce mussel growth, this factor cannot account for the shape differences observed between the different populations. Other factors which might be implicated are nutritional supply or exposure, the former being the most likely. Freeman and Dickie (1979) concluded that an environmental index using growth and mortality rates of *M. edulis* may well provide a sensitive measure of relative productivity between areas.

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