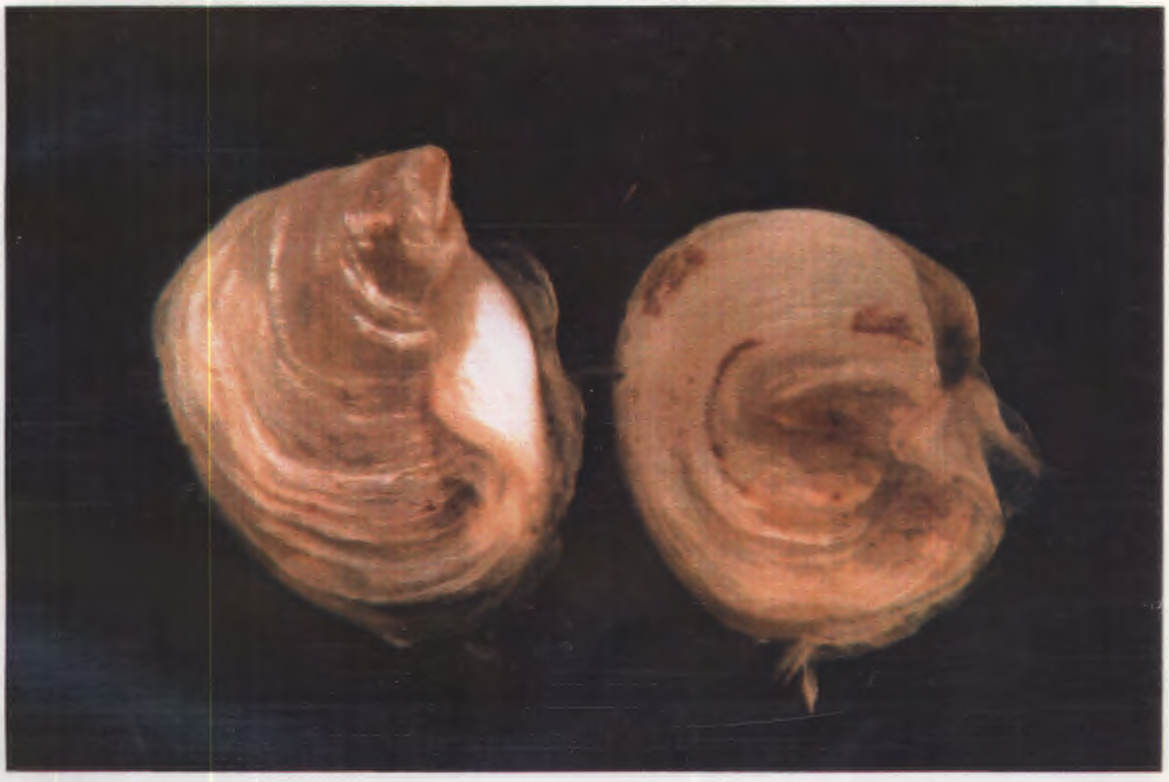
PHYSIOLOGICAL EFFECTS OF CADMIUM ON JUVENILE PACIFIC OYSTERS, *CRASSOSTREA GIGAS* , THUNBERG.

Rosalind Hand B.Sc. (Tas.)



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CONTENTS

ABSTRACT

1. "Small" *C. gigas* spat (2.8 mm) were more sensitive to acute cadmium exposure in seawater than larger spat (3.5 mm), with a 96 hour LC_{50} value of 10.29 and 28.43 mgL^{-1} total cadmium respectively.

2. Cadmium toxicity to juvenile *C. gigas* was more closely related to the free cadmium ion concentration than to the total cadmium concentration. The 96 hour LC_{50} in terms of free ion concentration was 0.25 and 0.27 mgL^{-1} for "small" and "large" spat respectively.

3. EDTA reduced the toxicity of cadmium to *C. gigas* spat by reducing the external free ion concentration.

4. Chronic exposure of *C. gigas* spat to sublethal concentrations of cadmium

-retarded growth of both shell and soft tissue;

-caused an apparent increase in shell abnormalities;

-affected heart contraction rate and caused cardiac arryhthmia;

-slowed the rate of filtration of microalgae (spat feed rates);

-slowed the behavioural response to tactile and light stimuli;

5. The cadmium bioaccumulation factors of spat exposed to 0, 10, 50 and 250 μgL^{-1} cadmium were 1.5×10^4, 3×10^3, 1.3×10^3 and 1.5×10^3 respectively. These values are similar to those previously reported by Ward (1983) for adult oysters (*Saccostrea commercialis*).

6. Accumulation of cadmium in spat soft tissue was linearly related to the external cadmium concentration. This is similar to the pattern of cadmium accumulation by other species of adult bivalves.

1. GENERAL INTRODUCTION

Concentrations of heavy metals above trace levels in natural waterways due to industrial activities have, in recent years, stimulated research into the effects of metals on the resident fauna. Particular emphasis has been placed on the invertebrate class Bivalvia due to their ability to accumulate heavy metals in their soft tissues at levels of up to ten percent of their dry weight (Thrower & Eustace, 1973).

Human consumption of food containing only 13 to 15 $\mu g g^{-1}$ cadmium has caused illness (MFIAC, 1950 IN Zaroogian & Cheer, 1976). Cadmium is a non-essential element that accumulates in animal tissues. Human excretion of cadmium is slow with a biological half-life of 10 to 30 years (Friberg *et al.*, 1974). Exposure to cadmium has a wide-ranging effect on human physiology including damage to lung, liver, renal, and skeletal tissue. Itai-itai disease, anaemia, emphysema, hypertension, proteinuria, amino aciduria and osteoporosis are amongst many diseases attributable to cadmium poisoning (Nilsson, 1970; Friberg *et al.*, 1974).

Crassostrea gigas (the Pacific oyster) is a commercially important bivalve species known to accumulate high tissue levels of heavy metals, including cadmium (Thomson, 1982; Frazier & George, 1983; Ward, 1983). *C. gigas* is farmed extensively throughout Tasmanian coastal waters. In 1990, production of the Pacific oyster in Tasmania was valued at approximately $6.2 million (Pollard, 1992). Elevated levels of heavy metals in natural waters are therefore of concern environmentally, commercially and for health reasons.

Numerous authors have investigated the toxicity of heavy metals to adult bivalves; however, as a more sensitive life-stage (Ringwood, 1990), studies concerning the responses of juvenile bivalves (spat) to metal exposure provide more relevent information on the impact of metal contamination on the population. In addition, chronic exposure of early developmental stages to heavy metals may result in subtle effects on the physiology (*eg.* growth) which are manifest in the adult stage. Exposure of adult bivalves to heavy metals above trace levels has been shown to increase mortality as well as affecting their general physiology. Cadmium, for example, causes abnormal and retarded growth (Sunila & Lindstrom, 1985), slows heart rate (Grace & Gainey, 1987), respiration and filtration (Patel & Anthony, 1991) and affects reproduction (Sunila, 1988). There are limited studies on the effects of heavy metals on juvenile bivalves; however, larval stages have received some attention for environmental biomonitoring. These younger life-stages have generally been found to be more sensitive to heavy metal exposure than adult bivalves.

The relative proportions of different forms of a metal influence its effect in the environment. Metal uptake by bivalves is from food, water and sedimentary sources. Despite frequently high levels of metals in marine sediments, uptake is predominantly from the surrounding water (Bryan *et al.,* 1985). Recommended limits for levels of trace metals in the environment are generally based on the total concentration. This is misleading where only specific forms of a metal are toxic and the concentration of those forms depend not only on the total metal concentration, but also on the physico-chemical conditions present. For example, the toxicity of cadmium to the grass shrimp *Palaemonetes pugio* is related to the free cadmium ion concentration rather than the total concentration (Sunda *et al.,* 1978). The free ion concentration of a metal in solution is highly dependent on the salinity of the water; *ie.* with decreasing salinity, the free ion concentration corresponding to a certain total cadmium concentration increases due to a reduction in the formation of chloro-complexes (Engel & Fowler, 1979). Cadmium in sea water is predominantly present as inorganic chloro-complexes, in particular, $CdCl_2^0$ and $CdCl^+$ with only a small proportion of the total cadmium present in the form of free cadmium ions (Zirino & Yamamoto, 1972).

In recent years, a widespread incidence of an abnormal shell growth of *C. gigas* spat, termed curly back, has been noted in the commercial shellfish industry. Curly back is characterised by an upward growth of the posterior, ventral shell valve; that is, growth is opposite to the "normal" direction of shell growth (pers. obs.). It is possible that chronic exposure to sublethal concentrations of metals may be responsible for this growth defect. A range of shell abnormalities has been reported in adult mussels, *Mytilus edulis*, in response to acute cadmium exposure (Sunila & Lindstrom, 1985). The observed growth abnormality as well as previous reports of high levels of heavy metals in Tasmanian waterways (*eg.* Bloom & Ayling, 1977; Tasmanian Department of Environment, 1987), prompted an interest in the effects of cadmium on the juvenile Pacific oyster.

In this thesis I will address two main areas of research:

1. lethal toxicity of cadmium to juvenile *C. gigas* (including the specific form of cadmium that is toxic to spat).

2. the effects of chronic sublethal exposure to cadmium on the physiology of *C. gigas* spat.

2. GENERAL EXPERIMENTAL METHODS

Outlined in the following chapter are materials and methods pertinent to experiments on both lethal and sublethal cadmium exposure. More specific materials and methods for each experiment are described in the relevant chapters.

2.1 EXPERIMENTAL SYSTEM

A static experimental system comprising a series of 400 mL vessels was used for tests of both lethal and sublethal effects of cadmium. Polyethylene treatment vessels were used to minimise loss of cadmium from solution by adsorption to the vessel walls. Gentle aeration promoted constant exposure of spat to the cadmium solution as well as reducing oxygen depletion and toxic metabolite accumulation in the vicinity of the spat. Temperature of treatment solutions was maintained at 20 ± 0.5 °C by means of a circulating water bath housed within a controlled temperature cabinet (Fig. 2.1). Salinity, temperature, dissolved oxygen and pH were monitored during all experiments. Treatment solutions were made by dilution (in oceanic water) of stock solutions of $1gL^{-1}$ $CdCl_2.2^1/2H_2O$ (AnalaR*). Stock solutions were made up in deionized water with 1mL/L HNO_3 (AnalaR*) to maintain cadmium solubility.

Exposure of spat to metals (other than experimental doses) was avoided at all stages of handling and treatment using standard bioassay procedures (APHA, 1989). Prior to use, all plastic and glassware (treatment vessels, sample bottles, aeration lines, volumetric glassware *etc.*) was washed, soaked for a minimum of 48 hours in 10% nitric acid then rinsed twice in deionised water to remove any contaminating metals. Oceanic water used throughout the experiment was stored in a clean tank and filtered to 0.45 µm before use. All water pipes and fittings were plastic.

Spat were obtained from commercial nursery facilities at Pipe Clay Lagoon, Tasmania. Spat had been pre-graded by size and were aged between 5 and 10 months. In the laboratory size was measured as the length from umbo to posterior valve margin (Fig. 2.2) using a light microscope with a pre-calibrated graticule. Experiments complied with the guidelines of the University Ethics Committee on animal experimentation.

Fig.2.1. Experimental system for maintenance of spat during experiments on lethal and sublethal effects of cadmium.

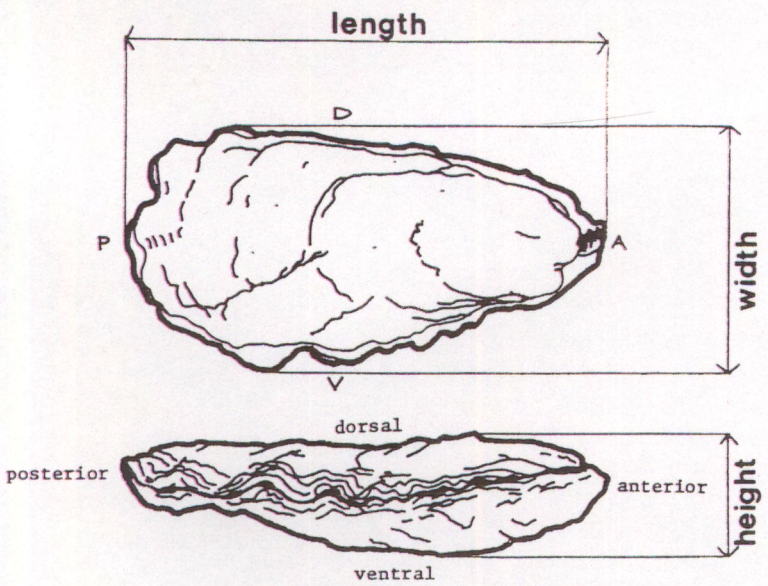

Fig. 2.2. Size criteria used for measurement of juvenile *C. gigas*.

2.2 WATER ANALYSIS

Concentrations of cadmium treatment solutions (for lethal exposure) and stock solutions were verified with flame atomic absorption spectrophotometry (flame AAS) using a Unicam SP 1900 Atomic Absorption Spectrophotometer. Treatment solutions were preserved with 10 mL/L HNO_3 and stored in 250 mL, acid soaked (see Section 2.1), polyethyene sample bottles at 5 °C until analysis by AAS.

Three replicates of each sample were measured for total cadmium (without digestion) by AAS using an air-acetylene flame. Dilution of more concentrated solutions was necessary for accurate determination of cadmium concentration. AAS operating conditions used for determination of cadmium concentration are shown in Table 1.1. Calibration curves were prepared from a series of cadmium standards of 0.5, 1, 2, 4 and 10 mgL^{-1} (as $CdCl_2.2^1/2H_2O$) in deionized water with 10 mL/L HNO_3.

Table 1.1 Flame AAS operating conditions for measurement of cadmium concentration in water.

WAVELENGTH	SLIT WIDTH	LAMP CURRENT	SENSITIVITY	OPTIMUM RANGE
228.8 nm	0.2 nm	3 mA	0.02 mgL^{-1}	0.2 - 1.8 mgL^{-1}

To avoid instrument drift, calibration was checked every 12 samples. Matrix interference was assessed using the method of known additions. Sample recovery was found to be between 98 and 102 % indicating that matrix interference was insignificant (APHA, 1989).

Limited variation (3.67 ± 1.58 %) occurred between estimated concentrations of cadmium in water and measured initial cadmium concentrations for all lethal exposure trials. Similarly, cadmium concentrations in exposure vessels varied by only 1.72 ± 0.66 % over the 48 hours between water changes.

Filtered oceanic (control treatment) water was measured for trace levels of cadmium by graphite furnace AAS (analysis courtesy of Dept. of Environment) using ammonium oxalate as a matrix modifier. Initial cadmium levels were found to be below 0.2 μgL^{-1} for all experiments.

2.3 MICROALGAL CULTURE

Microalgae are an essential food source required for long-term maintenance of bivalve molluscs in artificial systems. For experimental purposes feed rates, food quality and algal species composition may be controlled by feeding from laboratory monocultures of microalgae. The following section outlines the procedures used for culture of microalgae. Algae were provided to juvenile oysters during experiments on effects of exposure to sublethal cadmium concentrations and during assessment of spat feeding rates.

Spat were fed a mixed diet of *Isochrysis sp.* -clone T-ISO (Prymnesiophyceae) and *Nannochloris atomus* (Chlorophyceae). Both species are readily cultured under controlled conditions and are commonly used for bivalve nutrition. *Isochrysis sp.* is a particularly good source of nutrition for juvenile molluscs (Brown *et al.*, 1989).

2.3.1 Materials and Methods

Algal production was based on the batch culture methods of Guillard (1975). Axenic 150 mL stock cultures of algae were obtained from CSIRO Marine Laboratories, Hobart. Initial stock cultures were subcultured into 3 x 250 mL Erlenmeyer flasks containing approximately 150 mL of f/2 medium (see Appendix, Guillard, 1975). Auxilliary stock cultures were maintained by repeated subculturing into 250 mL flasks of f/2 medium every 14 to 20 days. Algal production was scaled up to 5 L Erlenmeyer flasks by inoculation with approximately 200 mL of stock culture. The routine for maintenance of algal cultures and culture vessel design is shown in Figures 2.3 and 2.4.

f/2 medium was made from nutrient stock solutions (Guillard, 1975) with oceanic water which had been pre-filtered through a series of filters to 0.4 µm. All media were autoclaved at 105 kPa and 121 °C for 30 minutes several days prior to inoculation with algae. Cultures were illuminated with fluorescent lights (Fig. 2.5) on a 12 hour day:night cycle. Aeration of the 2 and 5 L flasks was provided through silicon tubing with in-line 0.2 µm cellulose acetate membrane filters.

Being less susceptible to bacterial colonisation, *Nannochloris sp.* was cultured in 10 L open aerated culture vessels. Culture vessels with 10 L of f/2 medium were inoculated with 1 L of *Nannochloris sp.* culture every 7 to 14 days depending on culture densities and algal demand. All algae were harvested during the exponential growth phase and cell densities determined prior to feeding using a Neubauer haemocytometer.

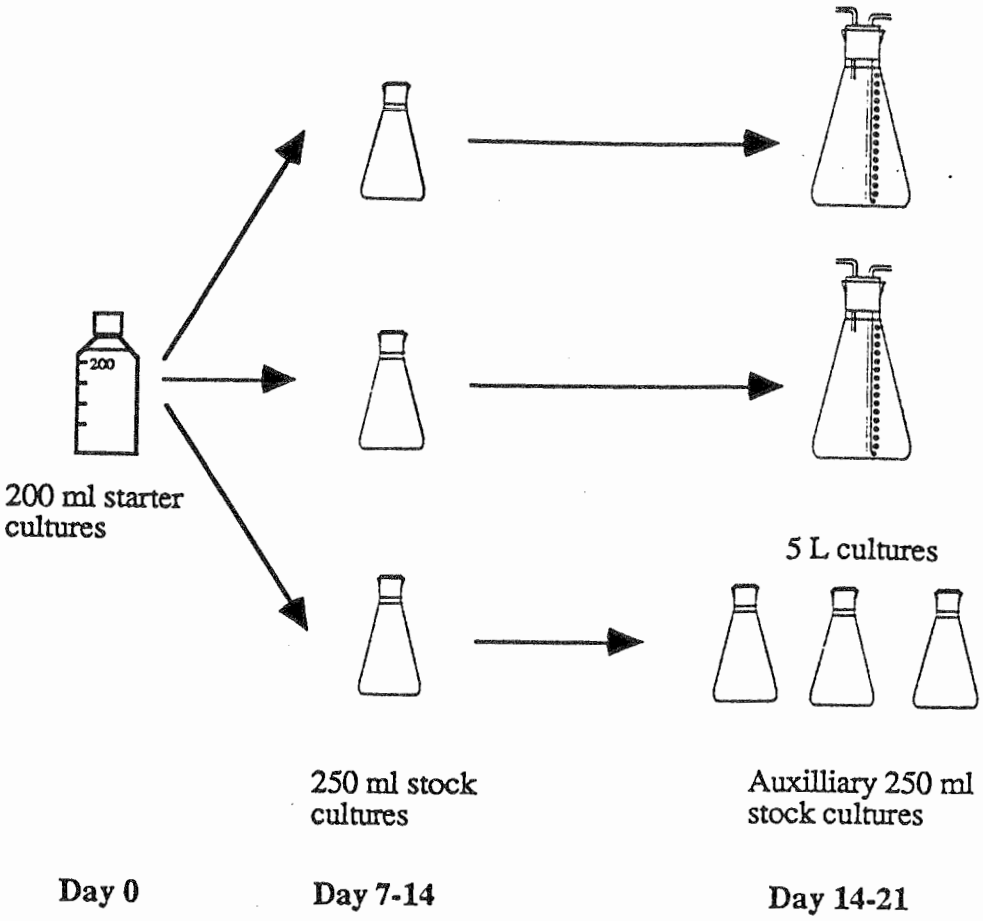

Fig. 2.3. Routine used for culture of micro-algae.

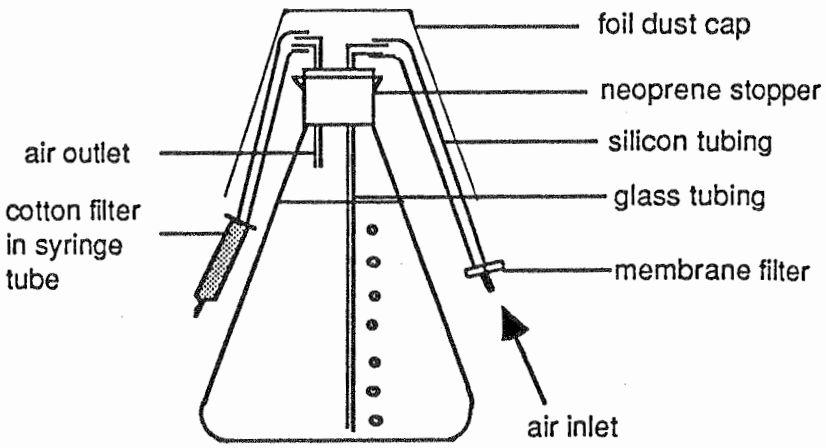

Fig. 2.4. Vessel design for culture of microalgae in 5L Erlenmeyer flasks.

Fig. 2.5. Micro-algae cultures in 250 mL Erlenmeyer flasks.

2.3.2 Results

Algae initially required a period of several weeks to adapt to the change in growth conditions (light, temperature *etc*); however,once cultures were acclimated good growth curves and cell concentrations were achieved for both species. Cell concentrations of up to 5.9 x 10^7 cells mL^{-1} of *Nannochloris sp.* and 1.04 x 10^7 cells mL^{-1} of *Isochrysis sp.* were measured 25 days following inoculation. Guillard (1975) suggests a range in cell densities of 10^6 to 10^7 cells mL^{-1} for small species (such as *Nannochloris sp.*) and 10^5 to 10^6 cells mL^{-1} for larger microalgae (such as *Isochrysis sp.*). Algae were generally harvested between 7 and 14 days post-inoculation (during the exponential growth phase) at between 5-8 x 10^6 cells mL^{-1} of *Isochrysis sp.* and 8-40 x 10^6 cells mL^{-1} of *Nannochloris sp.* Figures 2.6 and 2.7 show typical growth curves obtained for both microalgal species.

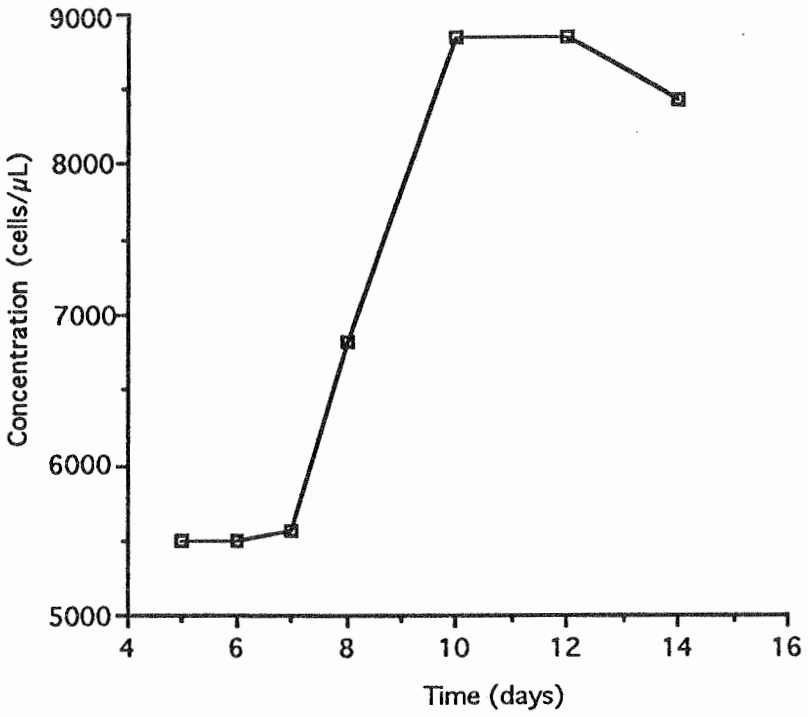

Fig. 2.6. Typical growth curve for *Nannochloris atomus* (5L flask culture)

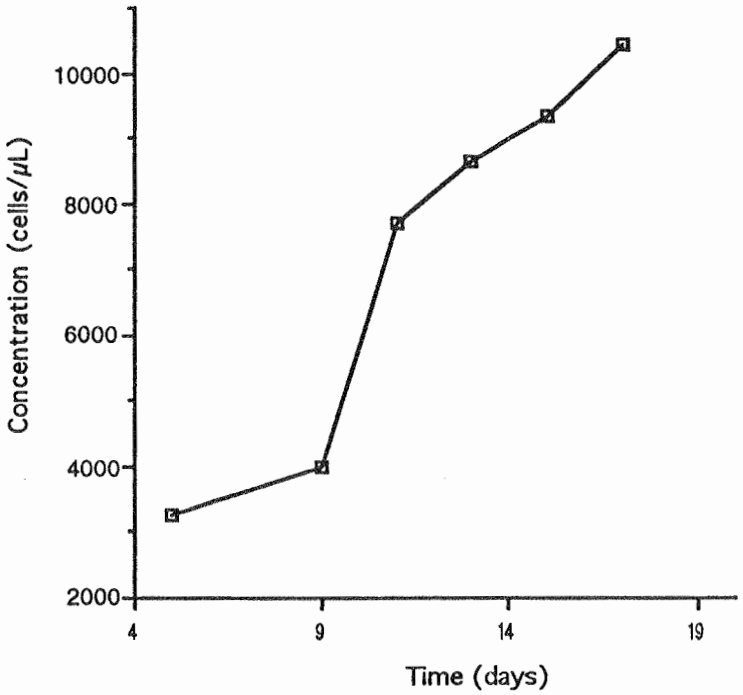

Fig. 2.7. Typical growth curve for *Isochrysis sp.* clone T-iso (5L flask culture)

2.3.3 Discussion

Jeffrey *et al* (1990) recommended harvesting of microalgae during the exponential growth phase at between 1-3 x 10^6 cells mL^{-1} for golden-brown flagellates (*eg. Isochrysis sp.*) and 1 x 10^6 cells mL^{-1} for green flagellates (*eg. Nannochloris atomus).* Cell densities achieved during culture were higher than these suggested values probably due to the small sizes of culture vessels (maximum 10 L). Growth rates and cell densities of both microalgal species were normal to high compared with published values (Guillard, 1975; Jeffrey *et al.*, 1990). Harvesting during the exponential growth phase maximises the culture's cell density whilst reducing the risk of contamination by bacteria, ciliates *etc.* as well as limiting toxic algal metabolites (Guillard, 1975). The biochemical composition of microalgae alters during the different phases of growth; generally the biochemical composition is more nutritious to bivalves during the early exponential growth phase (Whyte, 1987).

A mixed algal diet such as that used here is generally more nutritionally beneficial to mariculture species than feeding a single species of algae (Jeffrey *et al.*, 1990). For example, the two species used complement each other to provide a diet rich in the polyunsaturated fatty acids known to be essential to *C. gigas* spat (Langdon & Waldock, 1981). Overall, good algal culture growth rates and high culture concentrations combined with rapid algal clearance by control spat (Section 4.3.1) indicated that adequate microalgal culture techniques were employed.

3 LETHAL CADMIUM EXPOSURE

3.1 INTRODUCTION

Acute toxicity of heavy metals is conventionally assessed in terms of the "median lethal concentration" (LC_{50}), that is, the **total** concentration of the relevant metal that causes fifty percent mortality in a given exposure time (frequently 48 or 96 hours) (*eg.* Eisler, 1971; Calabrese *et al.*, 1973). More recently attention has been paid to the specific forms of metals that are toxic to various organisms (*eg.* Zamuda & Sunda, 1982; Patel and Anthony, 1991). Sunda *et al.* (1978) found that when exposed to cadmium the mortality of the grass shrimp *Palaemonetes pugio* was related to the free cadmium ion concentration rather than the total cadmium concentration. Similarly, accumulation of copper by the oyster *Crassostrea virginica* has been found to be related to the free cupric ion activity rather than the total copper exposure concentration (Zamuda & Sunda, 1982). For experimental purposes, chelating agents (*eg.* humic acid, nitrilotriacetic acid) allow variation of the free ion concentration of metals whilst maintaining a constant total metal concentration in solution (Sunda *et al.*, 1978).

LC_{50} values have limited use for direct assessment of the effects of toxicants in the environment where organisms are often chronically exposed to sublethal toxicant concentrations under fluctuating physico-chemical conditions. Generally, LC_{50}s are now used as a preliminary means of assessing toxicity before more extensive toxicity investigations such as chronic exposure experiments are used. Chronic exposure tests provide more ecologically relevant information of toxicity (Beaumont *et al.*, 1987). However, LC_{50}s do provide a valuable means of assessing relative toxicities between different toxicants or (in this case different forms of a toxicant) as well as the relative sensitivities between different species or life-stages.

Several methods are available for determination of LC_{50}s and their confidence limits. The more common analytical techniques are the arithmetic graph method, the logarithmic method and probit analysis (Reish & Oshida, 1986; APHA, 1989). The favoured, more precise method of LC_{50} determination is probit analysis which maximises use of the linear portion of the typical sigmoid dose-response curve, thus minimising the influence of extremes in tolerance to the relevant toxicant on the calculated LC_{50}.

The following aspects of acute toxicity of cadmium to two sizes of *C. gigas* spat were examined:

1) 96 hour LC_{50} determination using probit analysis.

2) the influence of free cadmium ion concentration on cadmium toxicity

3) general physiological responses to cadmium exposure

3.2 MATERIALS AND METHODS

3.2.1 Range assay

An initial range assay was conducted to determine the approximate test range required for determination of the 96 hour LC_{50} of *C. gigas* spat exposed to cadmium. Procedures were based on those outlined in APHA (1989). Spat were exposed to three concentrations of cadmium as $CdCl_2.2^1/_2H_2O$ in sea water in a log progression of 2, 20 and 200 mgL^{-1} with sea water as the control (control treatments were arbitrarily labelled 0 mgL^{-1} despite containing trace levels of cadmium (see Section 2.2)). The lowest treatment concentration was selected to correspond to the approximated 96 hour LC_{50} for juvenile *C. gigas* of 2 mgL^{-1} (Watling, 1978). Spat of commercial size grading "1500" were acclimated for 24 hours to experimental conditions of salinity and temperature, sized and placed in experimental vessels (section 2.1). Duplicate treatments of each exposure concentration were set up in 400 mL vessels with 50 spat in each vessel. Treatment solutions were renewed at 48 hours to avoid cadmium depletion and build-up of toxic metabolic products. Spat were not fed during periods of either acclimation or cadmium exposure. Mortality and morphological observations were recorded at 48 and 96 hours. Any dead spat were noted and removed from the treatment vessel after 48 hours of exposure to treatment solutions. Mortality was assessed using a light microscope by observing spat for the presence of heart contractions, filtering activity and behavioural responses to light and tactile stimuli (*eg.* shell gaping, valve adduction, *etc*). After 96 hours spat mortality was plotted against the log of cadmium exposure concentration and the approximate concentration corresponding to 50 % mortality was determined.

3.2.2 Definitive assay

Ninety-six hour LC_{50}s were determined for 2 sizes of spat of commercial grading "1500" and "1800"; these will subsequently be referred to as "small" and "large" spat.

Measurement of 200 spat in each size group, by light microscopy, gave mean sizes of 2.84 ± 0.03 mm and 3.46 ± 0.03 mm for "small" and "large" spat respectively. Prior to treatment, spat were acclimated to experimental conditions of salinity and temperature. Spat were not fed for two days prior to and during experiments.

An LC_{50} of approximately 10 mgL^{-1} for "small" spat was expected following analysis of the range assay data. Cadmium treatment concentrations were selected on an approximate log scale to encompass the expected LC_{50} of both sizes of spat. Triplicate treatment vessels of 0, 2, 5, 10, 20, and 50 mgL^{-1} cadmium were set up for each size group with 50 spat per 400 mL vessel (see Section 2.1).

To investigate the effect of free cadmium ion concentration on spat mortality the experimental system was replicated for both "small" and "large" spat with the addition of an artificial chelating agent *ie.* 5 x 10^5 M ethylene diaminetetraacetic acid (EDTA, di-sodium salt). EDTA enabled control of the free cadmium ion concentration. The program "MINEQL" (Westall *et. al.,* 1976) was used to determine free ion concentration in the resulting solutions (Table 3.1). This program accounts for interactions between the various ions present in water at equilibrium (including any added compounds *eg.* EDTA) and provides the final concentrations and forms of these ions. Figure 3.1 shows the relationship between free cadmium ion concentration and the total cadmium concentration used in the experimental systems. "pCd^{2+}" is the free ion concentration transformed to a scale analogous to the pH scale, where:

$$pCd^{2+} = -\log [Cd^{2+}].$$

Treatment solutions were renewed and any dead spat recorded and removed at 48 hours. Mortality and morphological observations were recorded at 48 and 96 hours.

Table 3.1: Concentration of free cadmium ions (Cd^{2+}) present at equilibrium corresponding to total cadmium concentration. (Data calculated using "Mineql" (Westall *et al.*, 1976))

[EDTA] (M)	Total [Cd] (mgL^{-1})	[Cd^{2+}] (mgL^{-1})	p[Cd^{2+}]
5×10^{-5}	0	5.6×10^{-9}	8.2
5×10^{-5}	2	1.8×10^{-4}	3.7
5×10^{-5}	5	2.3×10^{-3}	2.6
5×10^{-5}	10	1.3×10^{-1}	0.89
5×10^{-5}	20	2.6×10^{-1}	0.59
5×10^{-5}	50	2.9×10^{-1}	0.54
0	0	2.8×10^{-6}	5.6
0	2	6.0×10^{-2}	1.2
0	5	1.5×10^{-1}	0.82
0	10	2.5×10^{-1}	0.60
0	20	2.6×10^{-1}	0.58
0	50	3.0×10^{-1}	0.53

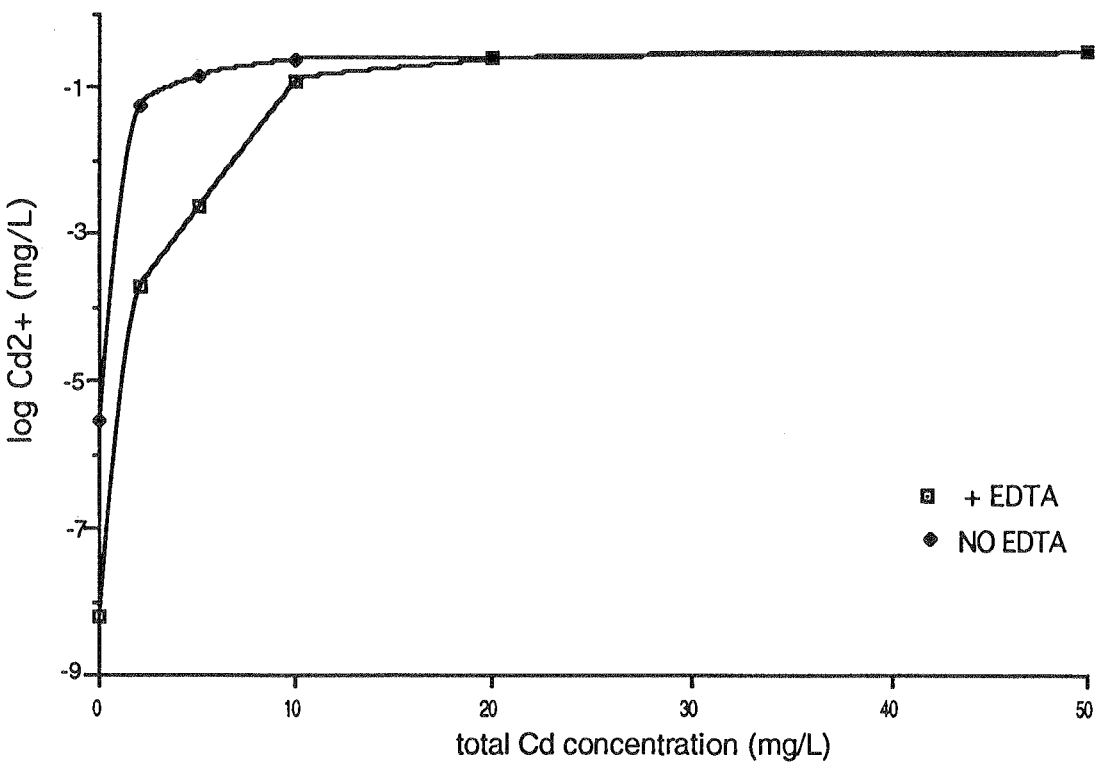

Fig. 3.1. Change in free ion concentration of cadmium with increasing total cadmium concentration with and without 5×10^{-5} M EDTA.

LC_{50}s were determined using probit analysis. Percent mortality was converted to probit values using a table of probits (Fisher & Yates, 1957) and plotted against the log of cadmium concentration to determine LC_{50}s. 95 % confidence limits to the LC_{50}s were determined from the slope function (S) of the data, where:

$$S = \frac{\frac{LC84}{LC50} + \frac{LC50}{LC16}}{2}$$

and

$$f_{LC50} = S^{\frac{2.77}{\sqrt{N}}}$$

f =95 % confidence limits of LC_{50}
S=slope function
N=No. of spat in treatments with 16 to 84 % mortality

Upper Limit $= LC_{50} \times f_{LC50}$
Lower Limit $= LC_{50} \div f_{LC50}$ (Reish & Oshida, 1986)

3.3 RESULTS

3.3.1 Range assay

Throughout the 96 hour exposure period pH ranged between 7-8.1, dissolved oxygen 7.0-9 mgL^{-1} and salinity remained at 35‰. Initial mean spat size was 2.78 mm ±0.02 (n=200). An initial exposure period of 48 hours was proposed; however, due to limited mortality at the tested concentrations after 48 hours it was decided to increase the exposure period to 96 hours.

Mean mortalities for the 48 and 96 hour exposure periods are shown in Table 3.2. A marked increase in spat mortality occurred between 48 and 96 hours at the lower cadmium exposure concentrations. Mortality increased from 3 to 14% at 2 mgL^{-1} and from 21 to 87% at 20 mgL^{-1} cadmium between 48 and 96 hours. All spat were dead after 48 hours in the 200 mgL^{-1} treatment. The soft tissue of these spat appeared pale in colour relative to spat in control water. Spat exposed to 2 and 20 mgL^{-1} cadmium also showed pale tissue colour as well as localised darkening of the tentacles. After 96 hours at 20 mgL^{-1} cadmium most live spat were gaping and slow

to respond to the microscope light or to mechanical stimulation whilst most control spat retracted into the shell and closed when subjected to the same stimuli. A similar but less dramatic response was observed after exposure to 2 mgL^{-1} cadmium.

Table 3.2. Mean percent mortality of spat after acute exposure to cadmium: range assay data.

Cadmium concentration (mgL^{-1})	% Mortality- 48 hours	% Mortality- 96 hours
0	2	4
2	3	14
20	21	87
200	100	100

Figure 3.2 shows the relationship of spat mortality to concentration for the 96 hour exposure period with a typical sigmoid dose-response curve (Hodsen *et al.*, 1976). 50 % mortality appears to correspond to approximately 10 mgL^{-1} cadmium.

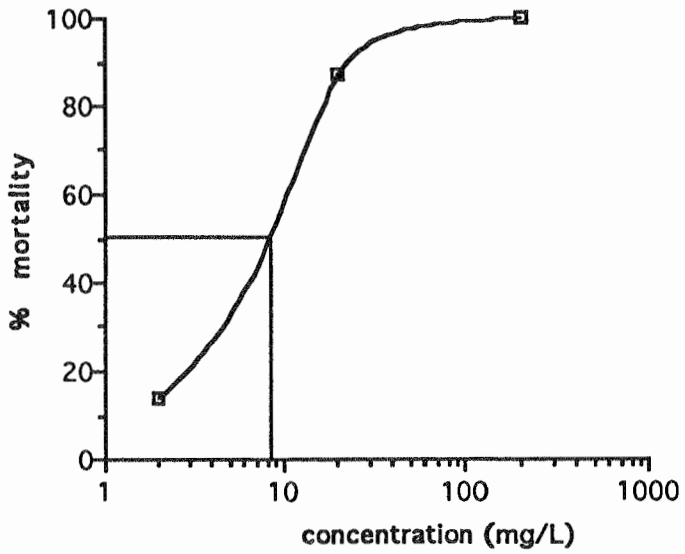

Fig.3.2. Mortality of spat following exposure to cadmium for 96 hours.

3.3.2 Definitive assay

Throughout all experimental trials pH ranged between 7.4-7.9, dissolved oxygen 6.1-9.0 mgL^{-1}, salinity 35‰ and temperature 20±0.5°C.

Mean spat mortality following 96 hour exposure to cadmium is shown in Table 3.3. In all treatments cadmium had relatively little effect on mortality after 48 hours; maximum mortality was only 14% of "small" spat at 50 mgL^{-1} (no EDTA) compared to 93.3% at the same exposure concentration after 96 hours. After 48 hours behaviour of "small" spat was affected from only 2 mgL^{-1} (no EDTA) with persistent retraction of the mantle by most spat. At higher doses (no EDTA) mantle retraction and valve adduction or,in more severe cases,valve gaping was observed. After 48 hours' exposure with EDTA present, activity of "small" spat was similar to that of controls at cadmium exposures up to 20 mgL^{-1}. A similar effect on "large" spat was observed at 48 hours. After 96 hours exposure to treatment solutions (no EDTA), both sizes of spat were gaping with slow response to stimuli and erratic heart contractions at all concentrations of cadmium. Addition of EDTA reduced the effect of cadmium on spat behaviour at the two lower cadmium concentrations (2 and 5 mgL^{-1}).

Figures 3.3 and 3.4 show a significant linear relationship between mortality (probits) and log of cadmium concentration for both spat sizes, with and without EDTA ($p<0.001$). Mortality of "large" spat tended to be lower (spat were more resistant) than that of "small" spat across the tested range of cadmium concentrations after 96 hours, particularly in the absence of EDTA. Addition of EDTA significantly reduced mortality of "small" spat when exposed to cadmium ($p<0.001$).

Cadmium	SMALL SPAT 48 hours		SMALL SPAT 96 hours		LARGE SPAT 48 hours		LARGE SPAT 96 hours	
(mgL^{-1})	EDTA	no EDTA	EDTA	no EDTA	EDTA	no EDTA	EDTA	no EDTA
0	2.7	7.3	8.0	9.3	3.3	0.7	3.3	1.3
2	4.0	4.6	5.3	8.7	4.0	2.0	5.3	10.0
5	8.7	4.0	15.3	14.7	2.0	1.3	2.7	14.7
10	10.0	6.7	30.7	46.7	2.0	3.3	32.0	25.3
20	10.0	13.3	63.3	80.0	2.7	4.0	42.7	44.7
50	8.0	14.0	80.0	93.3	4.7	12.0	66.7	70.7

Table 3.3. Mean mortality of two sizes of spat following exposure to cadmium for 48 and 96 hours.

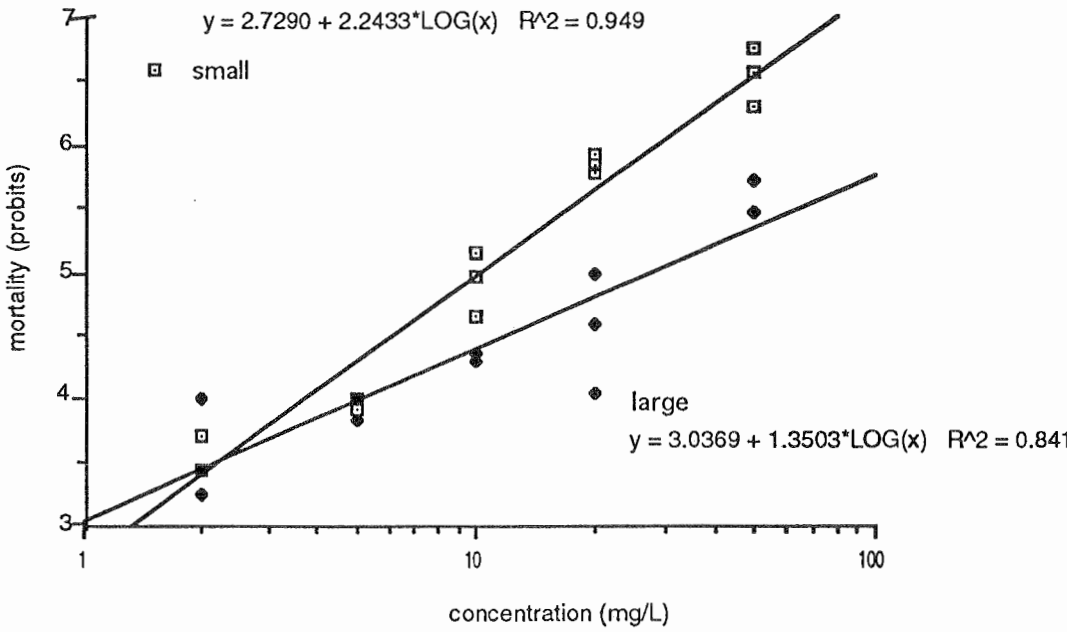

Fig. 3.3

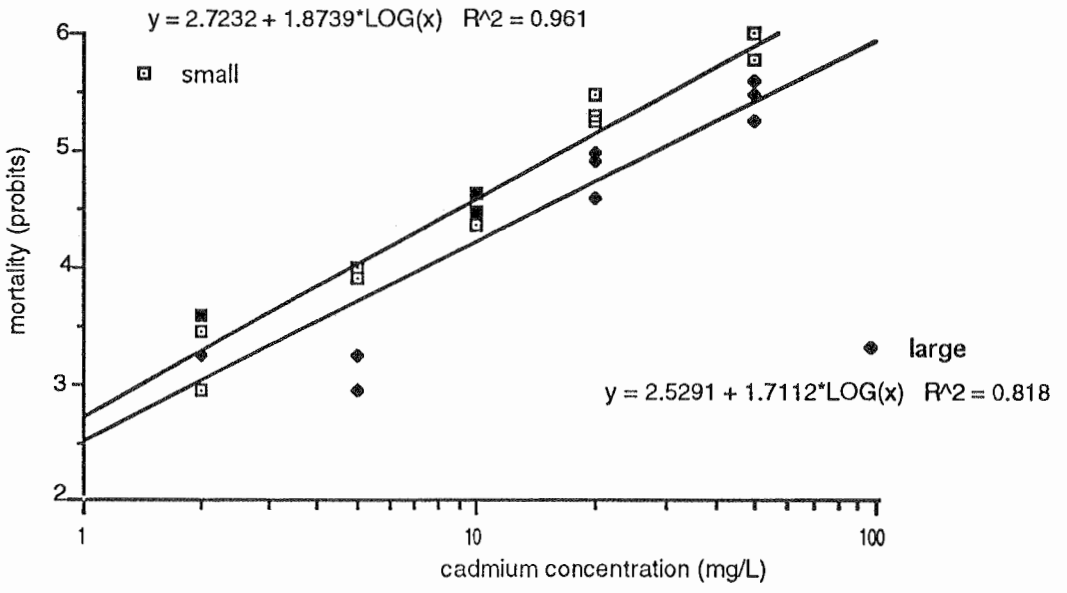

Fig. 3.4

Sensitivity (mortality expressed in probits) of "small" and "large" spat to cadmium. Fig. 3.3: no EDTA; Fig. 3.4: with addition of 5 x 10^5 M EDTA to the treatment solution

Calculated LC_{50}s with 95 % confidence limits are shown in Table 3.4. "Small" spat had a significantly lower 96 hour LC_{50} than "large" spat in both the presence and absence of EDTA. The LC_{50} for "small" spat was significantly increased from 10.29 to 16.41 mgL^{-1} on addition of the chelating agent; however, LC_{50}s of "large" spat were not significantly different with and without EDTA (27.79 and 28.43 mgL^{-1} respectively).

TABLE 3.4. 96 hour LC_{50} for two sizes of spat exposed to cadmium (mgL^{-1}) with corresponding LC_{50} in terms of free ion concentration (Cd^{2+}).

SIZE	EDTA	LC_{50} (95% C.L.)	S	Cd^{2+} LC_{50}	pCd^{2+}
SMALL	NO	10.29 (8.46, 12.53)	3.77	0.25	0.60
	YES	16.41 (15.19,17.72)	1.97	0.25	0.60
LARGE	NO	28.43 (22.04,36.54)	5.45	0.27	0.57
	YES	27.79 (23.33,33.10)	3.81	0.27	0.56

Figures 3.5 and 3.6 show the relationship between free cadmium ion concentration (Cd^{2+}) and mortality (in probits). Both sizes of spat show a marked relationship between mortality and free cadmium ion concentration irrespective of the presence or absence of EDTA. Spat appeared to tolerate cadmium free ion concentrations up to a threshold level (approximate pCd^{2+}= 0.8 for both sizes); at this concentration a rapid increase in mortality occurs. When LC_{50}s were then compared in terms of free ion concentration they were found to be virtually the same irrespective of the presence or absence of EDTA ie. 0.25 and 0.27 mgL^{-1} for "small" and "large" spat respectively.

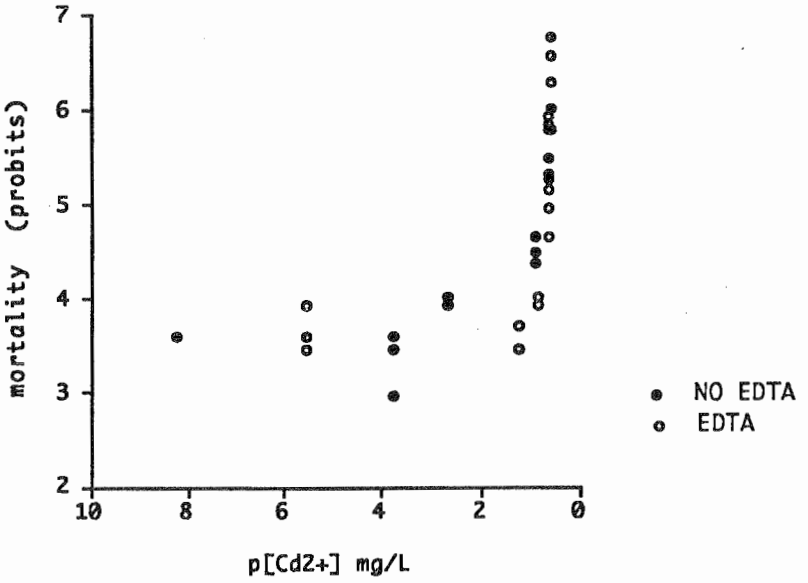

Fig. 3.5

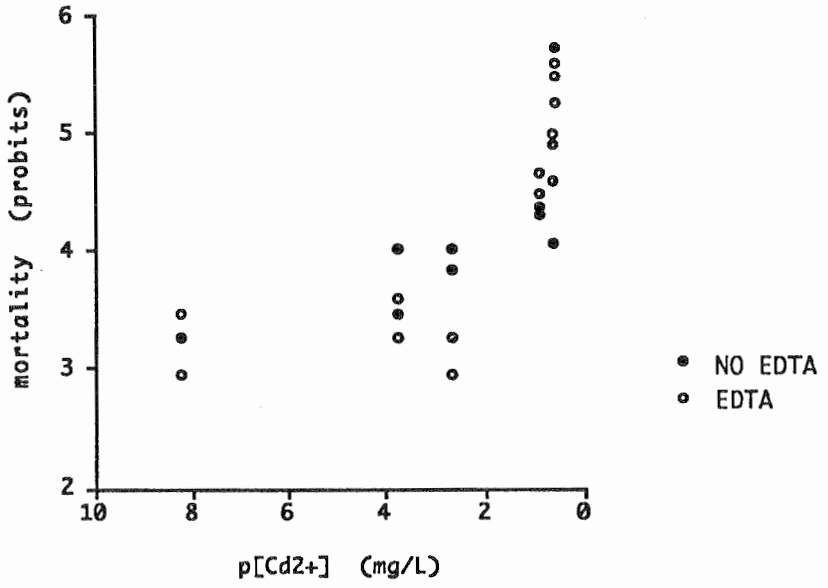

Fig. 3.6

Relationship of spat mortality to free cadmium ion concentration for "small" spat (Fig. 3.5) and "large" spat (Fig. 3.6).

3.4 DISCUSSION

Results of the range assay provided preliminary information on the effects and toxicity of cadmium to *C. gigas* spat. These results indicated an approximate 96 hour LC_{50} of 10 mgL^{-1} (total cadmium) for spat. Both range assay and definitive assay results indicated an adverse effect of exposure to cadmium on the behaviour and physiology of spat. Ninety-six hour exposure to as low as 2 mgL^{-1} cadmium resulted in impaired behavioural responses to light and tactile stimuli. General physiology was also affected with erratic heart contractions, persistent valve gaping and increased mortality. The effects of chronic cadmium exposure on spat physiology will be discussed in detail in Section 4. The cumulative effect of cadmium on physiology (*eg.* Ringwood, 1989) was illustrated by a dramatic increase in mortality between 48 and 96 hours compared to mortality between 0 and 48 hours.

Previously used criteria for mortality of bivalves may have overestimated mortality rate. For example, Watling (1978) assessed mortality of *C. gigas* as the number of oysters with gaping shells. The results presented here showed that following exposure to cadmium, live spat frequently showed persistent gaping of valves. Use of several criteria was necessary for precise measurement of spat mortality. A longer exposure period than the 96 hour criterion used here is recommended for more accurate determination of cadmium toxicity to *C. gigas* spat.

The difference in the toxicity of cadmium to the two spat sizes was surprisingly large. However, greater tolerance of larger spat compared with smaller spat to cadmium exposure is consistent with the findings of Watling (1978) who found greater tolerance of 64 day *C. gigas* spat (8.3 mm) than that of three month spat (4.1 mm). Conversely, Sunila (1981 in McLusky *et al.*, 1986) found greater resistance of small mussels (*Mytilus edulis*) to cadmium than larger ones. Variation in spat sensitivity due to size may be due to a combination of factors. Uptake of cadmium by adult *C. gigas* is inversely related to body size (Boyden, 1977); thus, the smaller spat were likely to have been subjected to higher tissue concentrations of cadmium. The faster metabolic rate rate of smaller spat and greater relative surface area would cause faster accumulation and therefore sensitivity to cadmium compared to larger spat. Previous metal exposure history of spat may also account for size-specific differences in sensitivity to cadmium. Exposure of bivalves to heavy metals causes induction of specific metal-binding proteins such as metallothioneins (Ringwood, 1992). Older spat are therefore more likely to possess higher levels of these proteins which allow detoxification of accumulated metal.

Ninety-six hour LC_{50}s in terms of total cadmium were 10.29 and 28.43 mgL^{-1} for "small" and "large" spat respectively. In terms of free cadmium ion concentration LC_{50}s were 0.25 and 0.27 mgL^{-1} respectively. These values are comparable to, although a little higher than previously published LC_{50} values for bivalves. The LC_{50} for *C. virginica* embryos is reported to be 3.8 mgL^{-1} total cadmium (48 hour LC_{50}) (Calabrese *et al.*, 1973) while that for the adult mussel *Mytilus edulis* is 25 mgL^{-1} (Eisler, 1971) or 1.62 mgL^{-1} for *M. e. planulatus* (Ahsanullah, 1976). Watling (1978) estimated a 96 hour LC_{50} for 3 month *C. gigas* spat of 2 mgL^{-1}; however, her results were intended only to illustrate relative tolerances of different life-stages and were not a precise measurement of acute toxicity. These values are not strictly comparable due to differences in experimental conditions, life-stages and species. Surprisingly, no short-term cadmium LC_{50} value for adult *C. gigas* could be found in the available literature.

Previously, acute toxicity of cadmium to bivalves has been assessed in terms of the total cadmium exposure concentration (*eg.* Watling, 1978; Ringwood, 1990). However, when percent mortality was related to the free cadmium ion exposure concentration they were found to be closely related. EDTA reduced mortality of both sizes of spat, by a reduction in the free cadmium ion concentration to which spat were exposed at a set total cadmium concentration. Indeed, a distinct threshold level of free ion (pCd^{2+}=0.8) above which mortality dramatically increased was evident for both sizes of spat irrespective of the presence or absence of EDTA. In addition, when the calculated LC_{50}s for both sizes of spat were expressed in terms of the corresponding free ion concentrations they were found to be virtually the same. Thus, as the free ion concentration is closely related to the total concentration of cadmium, measurements of acute toxicity in terms of total cadmium are misleading by masking the effects of free ions on an organism. This clear relationship between spat mortality and free cadmium ion concentration has important implications for both commercial and environmental situations. It is of particular relevance under conditions which promote high levels of free ion (*eg.* low salinity) despite levels of total cadmium below recommended limits.

4. EFFECTS OF SUBLETHAL CADMIUM EXPOSURE

4.1 INTRODUCTION

Studies concerning the effects of chronic exposure to sublethal levels of heavy metals provide more realistic information on their biological impact in the environment and on commercial operations than studies of acute toxicity. Levels of cadmium in natural waterways range from trace levels in oceanic waters of approximately 0.05 μgL^{-1} (Riley, 1971) to levels as high as 20-30 μgL^{-1} in the King River which flows into Macquarie Harbour, Tasmania (Tasmanian Department of Environment, 1987). The Tasmanian recommended limit for cadmium in marine water is 5 μgL^{-1} (Tasmanian Department of Environment, 1986). The USEPA (1986) chronic criterion for marine waters is 9.3 μgL^{-1} total cadmium. That is, the average concentration of total cadmium should not exceed 9.3 μgL^{-1} over a 4 day period more than once in three years. By their own admission, the USEPA (1986) suggest that the criteria for cadmium may be underprotective for sensitive species and at low salinities. Free cadmium ion concentrations in the environment vary according to the physico-chemical conditions, particularly pH, salinity, humic materials and total metal concentration (Mantoura *et al.*, 1978; Sunda *et al.*, 1978). Values for free cadmium ion concentration present in the environment are several orders of magnitude less than the total cadmium concentration. For example, Jackson & Morgan (1978) reported a typical seawater free ion concentration of 9.8×10^{-4} μgL^{-1} ($pCd^{2+}=3.0$) corresponding to a total cadmium concentration of 9.6×10^{-2} μgL^{-1}.

Reduced growth and shell abnormalities (*eg.* Sunila & Lindstrom, 1985) have been reported following acute exposure of adult bivalves to heavy metals; however, little is known of the effects of chronic exposure to metals on the juvenile oyster. Chronic exposure to sublethal doses of cadmium may exert a range of subtle effects on the physiology of juvenile bivalves which in turn affect their growth and survival. For example, acute exposure of *C. gigas* spat to lethal doses of cadmium (see Section 3.3) appeared to cause erratic heart contraction, reduced algal clearance rates and impaired behavioural responses. It is possible that long-term exposure to sublethal concentrations of heavy metals may have a chronic effect on these physiological processes which in turn affect growth and survival of spat in the environment. Chronic metal exposure may also be responsible for the shell growth abnormality termed "curly back" observed in the commercial oyster industry (Section 1).

The following hypotheses were investigated:

1. that chronic exposure to cadmium causes growth abnormalities in juvenile *C. gigas.*

2. that chronic exposure to cadmium produces a range of adverse, subtle effects on the physiology of juvenile *C. gigas.*

4.2 MATERIALS AND METHODS

4.2.1 Chronic exposure

Spat of a commercial sizing of 1500 µm (grading mesh size) were acclimated over 96 hours to experimental conditions of temperature and salinity. At the commencement of the experiment 50 spat were sized and placed in treatment vessels containing 0, 10, 50 and 250 μgL^{-1} total cadmium, in triplicate. The lowest dose was chosen to correspond to the USEPA (1986) chronic criterion for marine waters. Each vessel contained microalgae as 20 x 10^4 cells mL^{-1} *Nannochloris atomus* and 10 x 10^4 cells mL^{-1} *Isochrysis sp.* (T-iso). Spat were exposed to treatment solutions for a total of 32 days. Solutions and microalgae were replaced every 48 hours and dissolved oxygen, salinity and pH measured at each water change. Temperature was monitored daily.

Every four days spat were gently rinsed to remove faeces and other organic matter then each measured for size. Any dead spat were recorded and removed from the treatment vessel. On day 32 spat were measured for length and the percent with abnormal shell growth in each replicate determined. This was classed by an abnormal folding of the shell margin onto the ventral valve surface as seen in Figure 4.8. Spat were then transferred to untreated salt water for measurements of physiological effects following chronic cadmium exposure. Repeated measures analysis of variance (ANOVA) and Tukey-Kramer HSD comparison of the regression slopes of size over time for each exposure concentration were used to assess the effect of cadmium on length. The computer program, Systat (1992), was used for all statistical analyses.

4.2.2 Physiological effects

Unless otherwise indicated, all results were analysed using ANOVA and, where the null hypothesis was rejected, Tukey-Kramer HSD pairwise comparison of means.

Heart rate

Due to their small size, ventricular contraction of spat could be observed through the shell using a light microscope. The location of the heart in *Crassostrea* is shown in Figure 4.1. Spat were allowed to depurate for 24 hours in control salt water to remove any unassimilated algae or cadmium from the gut. Spat were then placed on one mL Sedgewick rafters in control salt water and left for 20 minutes by which time most were actively filtering. As heart contraction was not visible in all spat, heart rates of seven individuals were measured in each treatment replicate (21 spat per concentration) as the number of ventricular contractions during one minute.

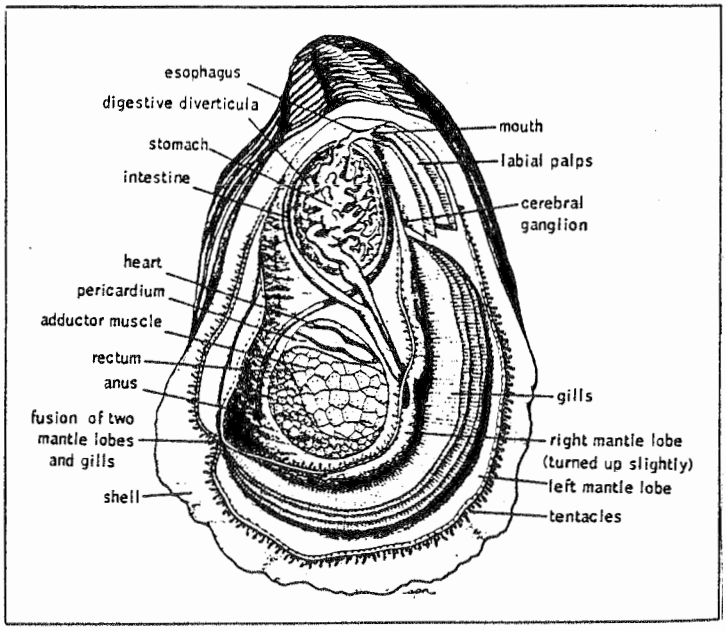

Fig. 4.1 Internal organs of the American oyster *Crassostrea virginica*, showing location of the heart (from Galtstoff in Barnes (1980)).

Valve adduction

Following a 24 hour depuration period spat were placed on one mL Sedgewick rafters in control salt water and left for 30 minutes by which time most were actively filtering. Individual spat were then exposed to concentrated light of 9×10^3 Lux and the time taken for complete valve adduction recorded for 10 spat in each replicate.

Feed rate

Feed rates were measured as rate of microalgal clearance. Prior to the experiment spat were allowed to depurate for 48 hours in control salt water. The microalgal species *Isochrysis sp.* (T-iso) was then added to each of the 12 culture vessels to achieve an initial algal concentration of 10 $x10^4$ cells mL^{-1}. Three counts of algal concentration were then measured for each vessel at regular intervals over 24 hours using a Neubauer haemocytometer. Gentle aeration maintained the algal cells in suspension throughout the experiment. Temperature was maintained at 20 ± 0.5 °C. Results were analysed using ANOVA for comparison of regression slopes of cell concentration over time.

Bioaccumulation

Following physiological tests spat soft tissue was dissected from the shells using surgical stainless steel forceps. Soft tissue and shells were rinsed in deionised water and dried for 96 hours at 60°C. Spat dry tissue was weighed on a microbalance in groups of ten spat (five groups per replicate). Following nitric acid digestion of soft tissue, bioaccumulation of cadmium by spat from each replicate was measured using graphite furnace AAS (analysis courtesy of Department of Environment). A comparable weight of standard oyster tissue (US National Bureau of Standards (NBS) #1566) was taken through the digestion procedure and analysed for cadmium content using AAS, as a calibration check. Due to difficulties in the acid digestion step shell was not analysed for cadmium content. However, accumulation of cadmium by the shell of bivalves is generally very low compared to soft tissue accumulation (*eg.* see Ringwood, 1991). The tissue concentration factor for spat over 32 days was determined from the ratio of tissue cadmium concentration to exposure concentration of cadmium.

4.3 RESULTS

Throughout the exposure period pH ranged between 7.8-8.3, dissolved oxygen 5.7-6.4 mgL^{-1}, salinity 35 ‰ and temperature 20 ± 0.5 °C. Spat mortality was low throughout the experiment with a mean mortality of 3.3%, 1.3%, 2.6% and 11.3% at 0, 10, 50 and 250 μgL^{-1} cadmium respectively. ANOVA indicated no significant effect of cadmium concentration on spat mortality ($p=0.278$).

4.3.1 Chronic exposure

Growth

Figure 4.2 shows the sizes of spat over the 32 day exposure period. Growth curves indicate that growth of spat exposed to 50 and 250 μgL^{-1} cadmium was limited over the 32 day exposure period relative to growth of controls. Over 32 days spat grew by 192 and 180 μm at 0 and 10 μgL^{-1} respectively compared to only 89 and 56 μm at concentrations of 50 and 250 μgL^{-1} respectively. Differences between shell growth of control spat and spat from the highest cadmium concentration were visible by day 32 (Figures 4.3 and 4.4). From day four it was noted that the limited shell growth of spat exposed to cadmium was weak and fragmented, therefore susceptible to breakage; this may account for the fluctuations in size over time of spat exposed to cadmium. Spat at 0 μgL^{-1} grew rapidly to day 12 followed by a levelling off of growth rate to day 32. Control spat were observed to be active with even new growth and rapidly cleared added microalgae compared to spat exposed to cadmium. Activity of spat exposed to 10 μgL^{-1} appeared similar to that of controls over the 32 days with even new growth to approximately day 8. However, an increase in abnormal shell growth was apparent after further exposure (see results for "shell abnormalities"). At higher sublethal doses of cadmium (50 and 250 μgL^{-1}) spat appeared to be affected from as early as day four. Observations were noted of pale soft tissue, localised darkening of the tentacles, weak, fragmented shell growth and less faecal production when compared to control spat. Interestingly, after 24 days' exposure spat exposed to 50 μg cadmium/L appeared to develop unfragmented, even new shell growth. At 250 μgL^{-1} activity of spat appeared suppressed compared to that of controls. This was particularly obvious around day 28 with many spat showing persistent retraction of the mantle into the shell. Gut and soft tissue were pale in colour compared to spat at lower doses of cadmium. Repeated measures ANOVA indicated a significant difference in spat size with cadmium concentration ($p<0.01$) and over time ($p<0.001$). A significant time*concentration interaction ($p<0.001$) was also found *ie.* differences between the profiles of size over time (growth rates) at each exposure concentration. Comparison of regression slopes for growth at each cadmium concentration showed a significant difference between growth rate between 0 and 250 μgL^{-1} ($p<0.05$) and between 10 and 250 μgL^{-1} ($p<0.05$).

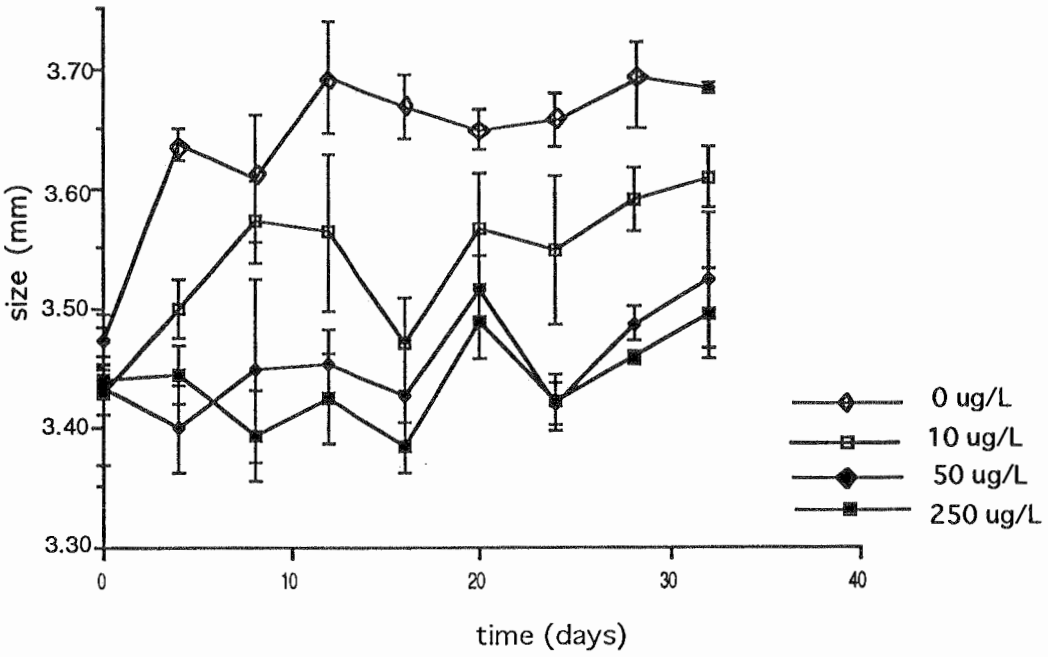

Fig. 4.2. Change in spat size (± SE) over 32 days' chronic cadmium exposure

Similar to shell growth, chronic cadmium exposure had an inhibitory effect on growth of spat in terms of weight. Tissue and shell dry weights of spat after 32 days' exposure to cadmium are shown in Figures 4.5 and 4.6. Both graphs show a trend of decreasing dry weight of both shell and soft tissue with increasing cadmium exposure concentration. Initial mean dry weight of spat was 0.063 ± 0.008 mg. The effect of cadmium concentration on tissue dry weight was highly significant ($p<0.001$) with the mean dry weight for control spat (0.245 ± 0.020 mg) over twice that of spat at 250 μgL^{-1} (0.114 ± 0.010 mg) following the 32 day exposure period. Tukey-Kramer HSD comparison of mean tissue dry weights showed significant differences ($p<0.05$) between all treatments except 0 and 10 μgL^{-1} ($p=0.598$) and 10 and 50 μgL^{-1} ($p=0.070$). Cadmium concentration also had a significant effect on shell dry weight ($p<0.05$). Comparisons of means showed a significant difference ($p<0.05$) between mean shell dry weights of spat from 0 and 250 μgL^{-1} treatments, *i.e.* 6.4± 0.3 mg and 5.1 ± 0.3 mg respectively.

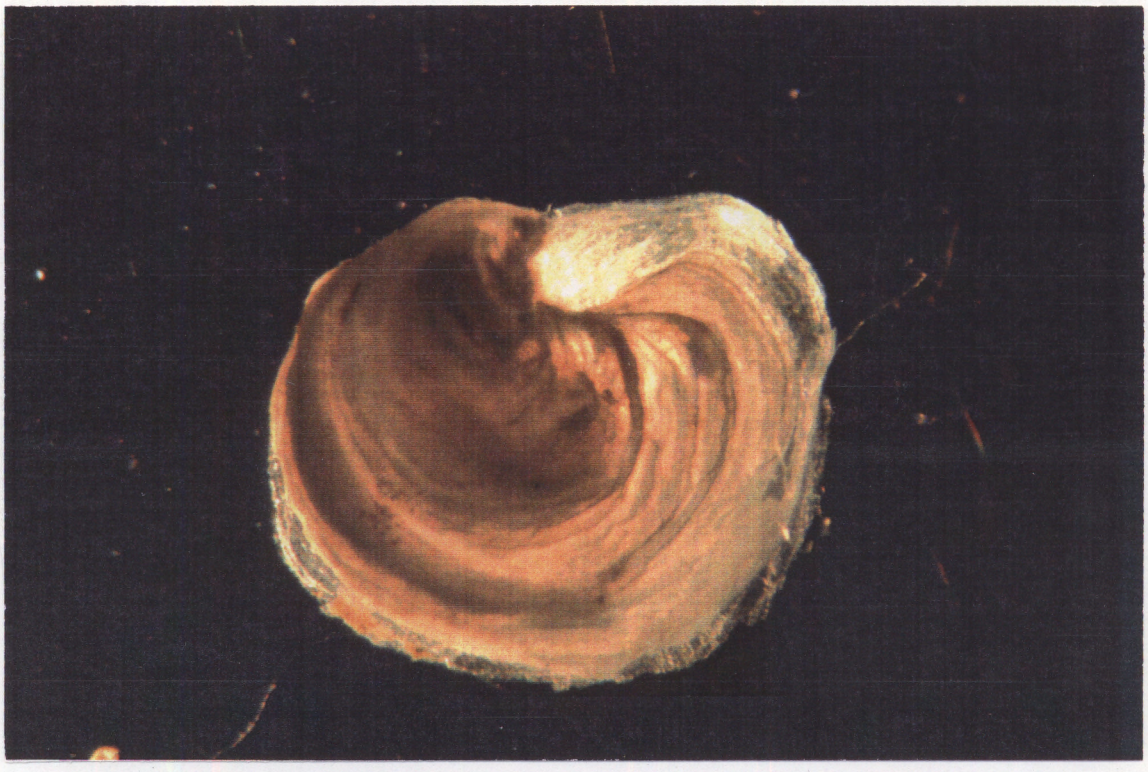

Fig. 4.3. *C. gigas* spat following 32 days in control sea water (note even, new growth on valve margin). x 20

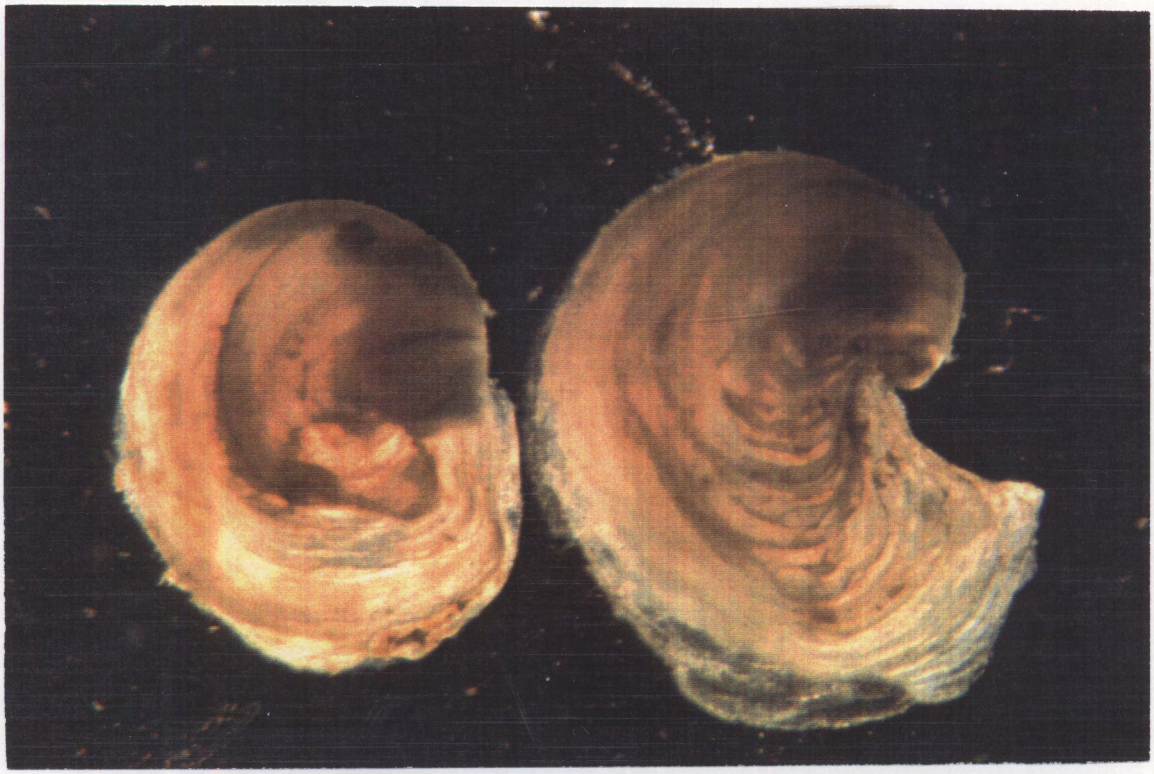

Fig. 4.4. *C. gigas* spat following exposure for 32 days to 250 μgL^{-1} cadmium (left) compared to spat from control sea water (right). x 20

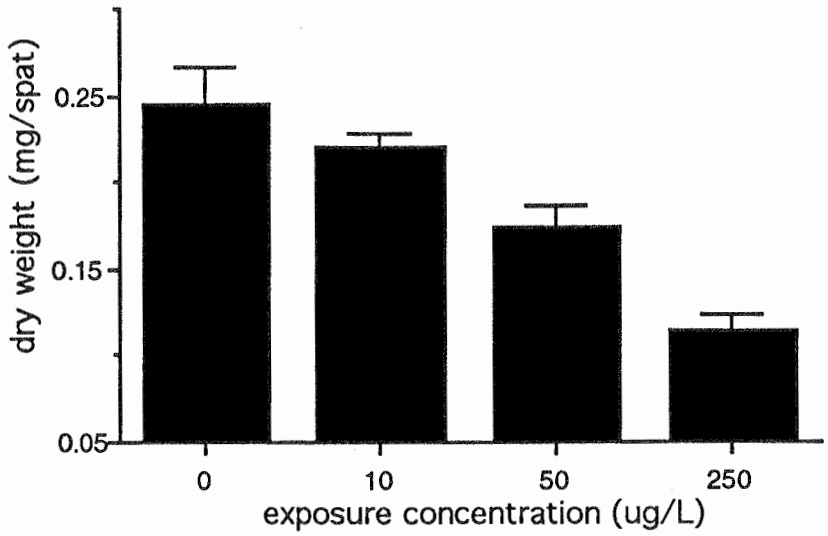

Fig. 4.5 Tissue dry weight (±SE) of spat following chronic cadmium exposure for 32 days.

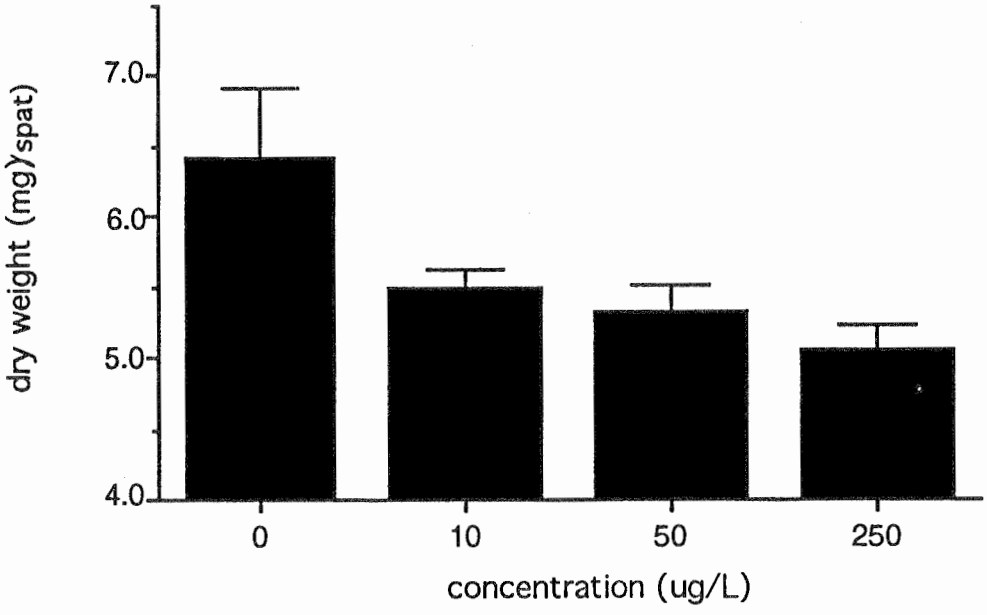

Fig. 4.6 Shell dry weight (±SE) of spat following chronic cadmium exposure for 32 days.

Shell abnormalities

Discrete differences in shell growth were observed within and between treatments (see Fig.s 4.7 & 4.8) allowing accurate determination of the percentage of spat with abnormal shell growth. The mean percentage of spat with shell abnormalities was increased in all treatments following chronic exposure to cadmium when compared to control spat. At the lowest cadmium concentration of 10 μgL^{-1} a notably greater proportion of spat with abnormal shell growth was evident compared to spat at the higher sublethal doses of 50 and 250 μgL^{-1}. Despite the increase in spat shell abnormalities following cadmium exposure the effect of cadmium concentration was not significant ($p=0.119$).

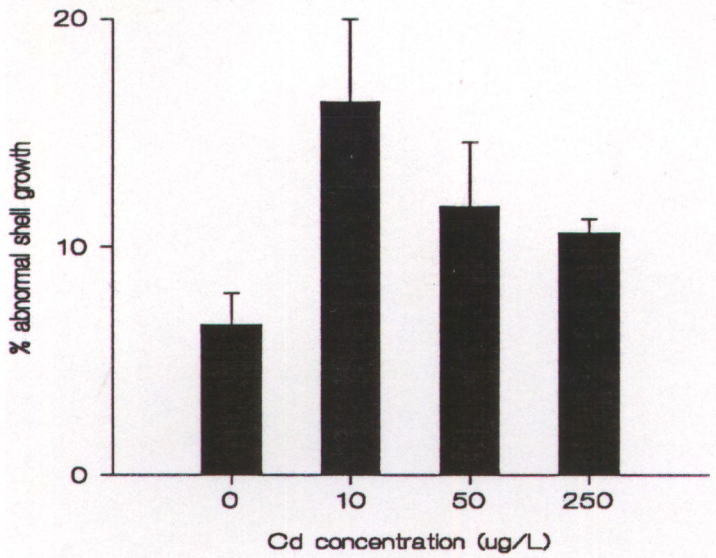

Fig. 4.7. Mean percentage (±SE) of spat with abnormal shell growth following chronic exposure to cadmium for 32 days

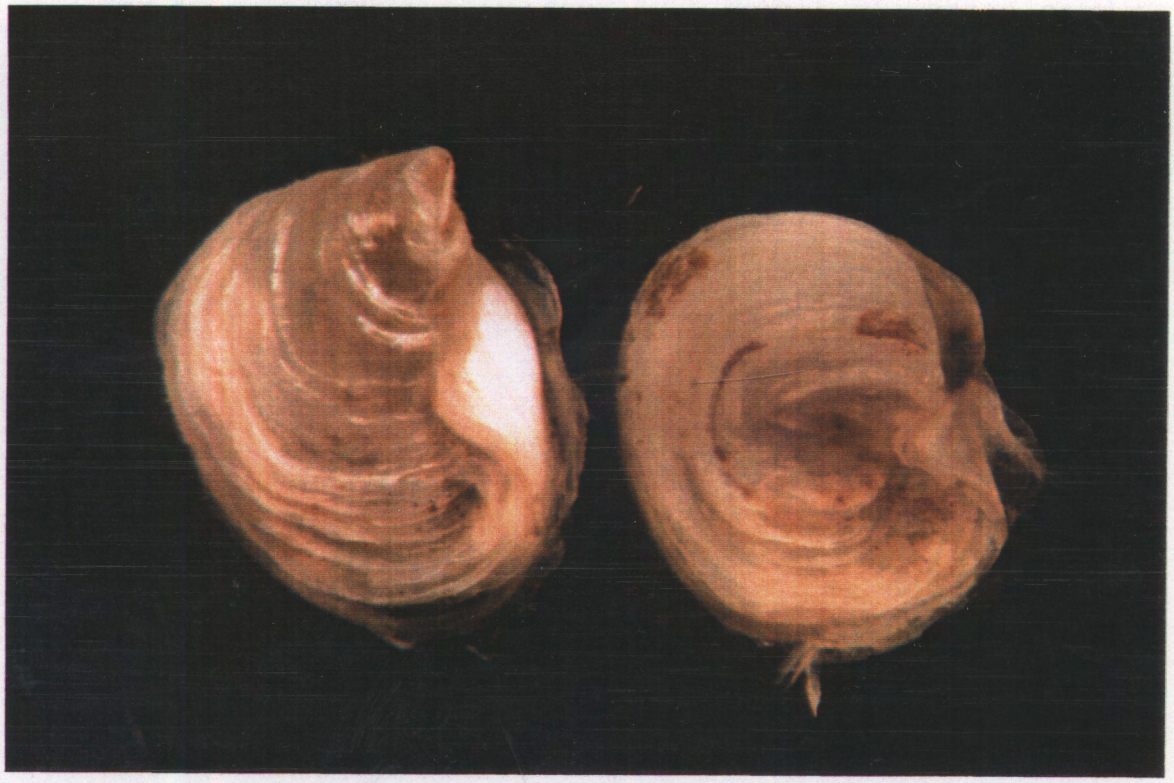

Fig. 4.8 Abnormal shell growth of spat following chronic exposure to cadmium (10 μgL^{-1}) for 32 days (note folding over of left, dorsal valve onto ventral surface, *ie.* right side of spat in picture)

4.3.2 Physiological effects

Heart rate

Measured heart rates of spat are shown in Figure 4.9. Heart rate appeared to be stimulated in spat following chronic exposure to the lowest cadmium dose whilst higher concentrations of cadmium, particularly at 250 μgL^{-1} slowed mean heart rates of spat relative to controls (mean heart rate was 43.1, 48.5, 37.6 and 31.3 bpm at 0, 10, 50 and 250 μgL^{-1} respectively). Spat from the control and 10 μgL^{-1} treatments had regular heart contraction. However, following exposure to both 50 and 250 μgL^{-1} cadmium, ventricular contraction of spat was erratic, typified by a series of beats followed by periods of no activity. Despite an apparent effect of cadmium concentration on heart rate it was not significant (p=0.063); this is probably due to the observed variability in ventricular contraction, particularly at 50 μgL^{-1}.

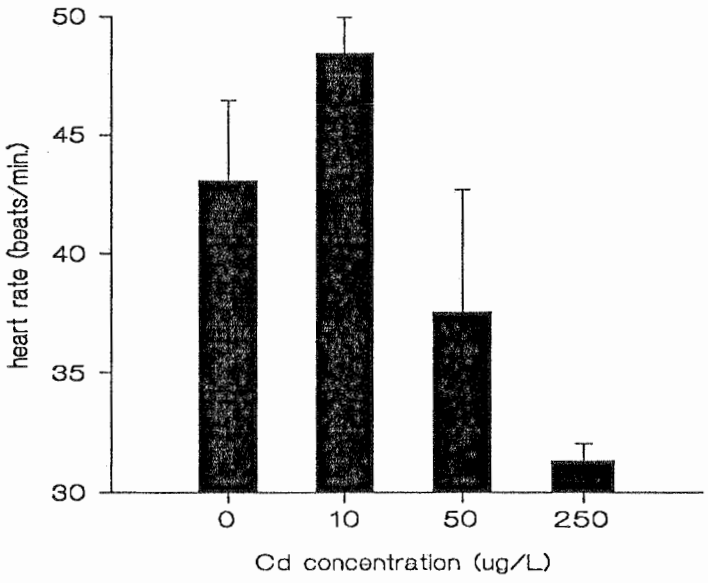

Fig. 4.9 Mean heart rates (±SE) of spat following chronic exposure to cadmium for 32 days.

Valve adduction

The behavioural response of spat to light was impaired following chronic exposure to cadmium at all concentrations tested (Fig. 4.10). Cadmium concentration had a significant effect on the time taken for complete valve adduction ($0.01<p<0.05$). Mean valve adduction time of controls was 5.35 sec ± 0.50. A significant increase in the time taken for complete valve adduction occurred following exposure to concentrations between 10 and 50 μgL^{-1} from 5.80 sec ± 0.72 to 10.58 sec ± 1.05 ($0.01<p<0.05$). However, there was no significant increase in the valve adduction time of spat exposed to higher cadmium levels; that is 12.04 sec ± 0.92 for spat from the 250 μgL^{-1} treatment ($p>0.05$). Valve adduction was spasmodic in spat from the 50 and 250 μgL^{-1} treatments, that is, complete adduction was achieved following a series of partial closures of the shell. Two spat from the 50 μgL^{-1} treatment failed to respond to the light stimulus during a three minute observation interval; these spat were not included for calculation of the mean valve adduction time. No significant difference was found between mean adduction time of spat from the 10 μgL^{-1} treatment and of control spat ($p>0.05$).

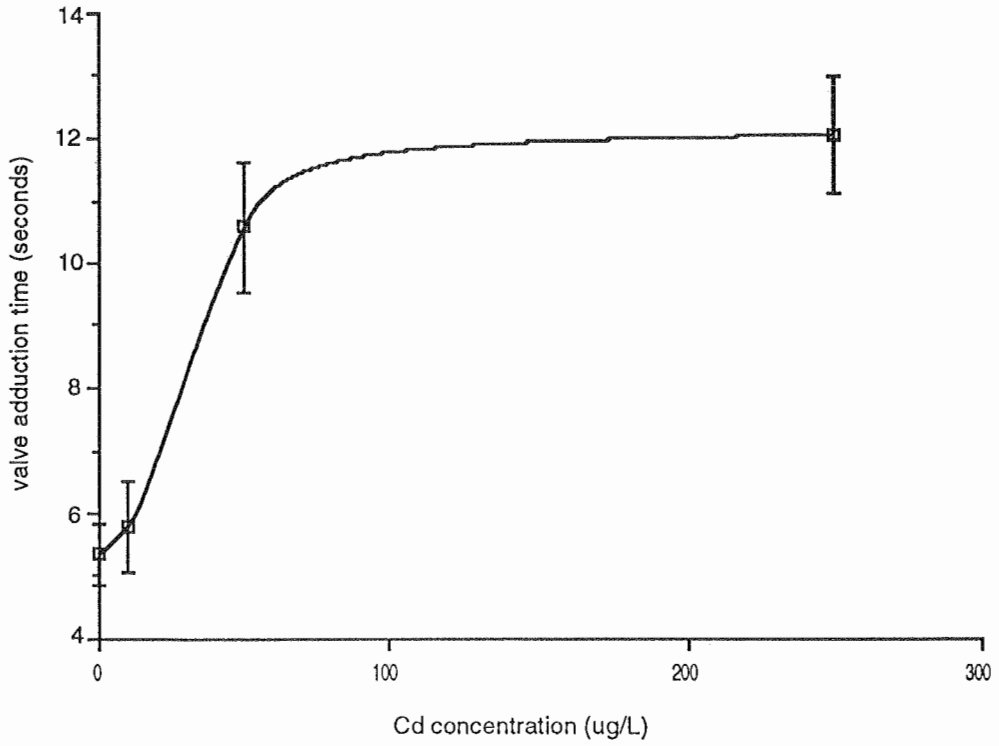

Fig. 4.10 Mean response to light (±SE) following chronic exposure to cadmium for 32 days

Feed rate

Exposure to cadmium had a significant effect on algal filtration rates of spat ($0.001<p<0.01$). Exposure at the lowest dose (10 μgL^{-1}) had little impact on spat feed rates relative to controls; however, spat from an exposure concentration of 50 μgL^{-1} cadmium had a significantly reduced rate of algal clearance relative to controls ($0.01<p<0.05$). Feed rate can be seen to be particularly reduced following exposure to 250 μg cadmium/L (see Figure 4.11). Most of the microalgae were cleared after ten hours following addition to the experimental systems except for spat from the highest cadmium treatment (250 μgL^{-1}). At ten hours the algal concentration of the control spat vessel was 0.25 x 10^4 cells mL-1 compared to 6.75 x 10^4 cells mL^{-1} in the vessel containing spat from the highest cadmium dose. These spat required 24 hours to reduce algal concentration in the vessel to the level in all other treatment vessels at only 10 hours (*ie.* below 1 x 10^4 cells mL^{-1}).

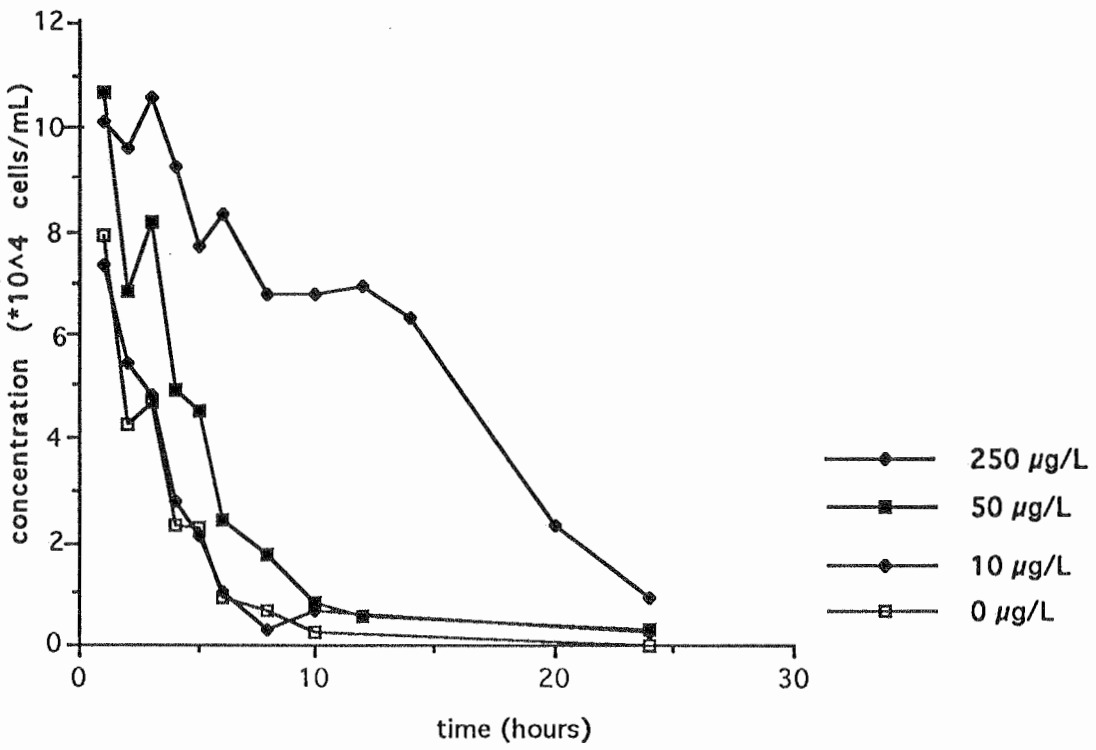

Fig. 4.11 Algal clearance by spat following chronic exposure to cadmium.

Accumulation

Analysis of standard oyster tissue for cadmium concentration confirmed the precision of the tissue digestion procedure and AAS analytical techniques. Measured tissue cadmium concentration was 4.2 μgL^{-1} compared to the NBS certified value (with 95 % confidence limits) of 3.5 $\mu gL^{-1} \pm 0.4$. Some variation of determined levels of cadmium occurred due to the low weight of oyster tissue; that is, results were susceptible to trace levels of contamination. Figure 4.12 shows the level of accumulation of cadmium in spat soft tissue after exposure to cadmium for 32 days followed by a three day depuration period. A highly significant linear relationship was found between tissue cadmium concentration and exposure concentration ($p<0.001$). Spat accumulated 30 $\mu gg^{-1} \pm 4.0$ after only 32 days at the lowest exposure concentration (10 μgL^{-1}). The concentration factor of spat at each exposure concentration is shown in Table 4.1 with published values for adult oysters at corresponding exposure concentrations. Bioconcentration factors of *C. gigas* spat were similar to those of adult bivalves, particularly at the lowest exposure concentration.

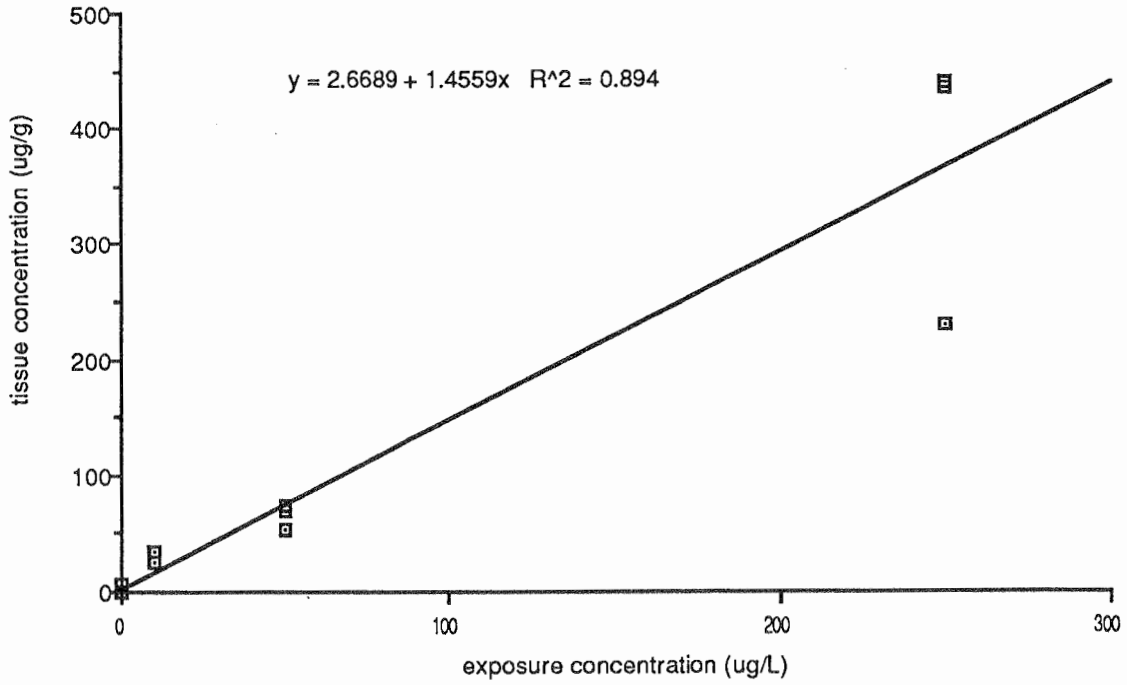

Fig. 4.12. Soft tissue accumulation of cadmium following chronic exposure for 32 days.

Table 4.1: Cadmium bioconcentration factors (C.F.) for oysters following chronic exposure. (* from Ward, 1983)

EXPOSURE [Cd] (μgL^{-1})	FREE ION [Cd] (μgL^{-1})	SPAT C.F. (*C. gigas*)	ADULT C.F.* (*Saccostrea commercialis*)
0	0.003	1.5E4	-
10	0.299	3.0E3	3.0E3
50	1.495	1.3E3	6.7E3
250(*150)	7.475	1.5E3	0.8E3

4.4 DISCUSSION

The mean mortality of control spat of 3.3 % over the 32 day exposure period is well below the APHA (1989) recommended maximum for toxicity tests of 10 %. The low overall mortality indicates that experimental conditions were suitable for maintenance of spat. Cadmium had no significant effect on mortality at the selected concentrations over the 32 day exposure period. It should be noted that growth rates were found to be slower than in previous studies. For example, Shuster and Pringle (1969) reported growth of control oysters (*C. virginica*) of 300 µm over a four week period compared to 192 µm in the present study. Slow shell growth of control spat was particularly obvious after day 12 following rapid initial growth. In any population of spat there are both fast and slow growing individuals. Spat available for these experiments were from healthy nursery graded stock known to have slow growth rates. In addition, these growth rates are not directly comparable to other studies where different species, ages and developmental stages of oyster are used. By day 32 it was evident that new growth of cadmium treated spat in these experiments had occurred predominantly on the left, anterior valve margin (see Fig. 4.8). Thus, the generally accepted size criterion for spat (length) did not account for much of the new shell growth. In future studies involving exposure to contaminants spat size should be measured in terms of shell width as well as length. The reduction in control growth rates after day 12 may have been due to an increased energy requirement due to greater size of spat. In these experiments algal feed rates were kept constant throughout the exposure period, rather than increased, for experimental purposes and may have limited growth of larger spat. Crowding of spat may have also limited growth rates. Further studies are required in order to determine the factors responsible for slow growth rates and optimal spat husbandry conditions for laboratory experiments.

Despite slow growth rates of control and treated spat, a significant effect of chronic cadmium exposure on growth was found. Over the 32 day exposure period cadmium significantly inhibited both shell growth (by length and weight) and soft tissue growth (by weight). The inhibitory effect of cadmium on spat growth (in terms of both length and weakness of shell) was more severe at higher concentrations of cadmium. This is consistent with the findings of Watling (1983) who reported reduced growth rates of *C. gigas* with increasing heavy metal exposure concentration. Even at 10 μgL^{-1} (approximately equal to the USEPA (1986) chronic criterion) spat grew more slowly than controls, with a greater percentage having abnormal shell growth. This has important implications for both environmental and commercial situations where spat may be exposed to such a level of cadmium (equivalent to a free cadmium ion concentration of 0.003 μgL^{-1}) particularly under conditions of low salinity.

Carbonic anhydrase and alkaline phosphatase are two enzymes believed to be involved in shell deposition in molluscs (Wagge, 1951; Wilbur & Saleuddin, 1983). The activity of alkaline phosphatase increases in *C. gigas* between 50 and 150 days post-settlement (George & Coombs, 1975). Spat used throughout the present experiments were aged between approximately 150 and 300 days. Thus, cadmium exposure may be inhibiting shell growth of spat by blocking the active site of metalloenzymes such as alkaline phosphatase involved in shell deposition. In addition, cadmium may be interfering with the normal increase in metalloenzyme activity of young spat. Indeed, Evtushenko *et al.* (1986) found inhibition of alkaline phosphatase (as well as other metalloenzymes) in adult scallops (*Mizuhopecten yessoensis*) following exposure to 0.5 mgL^{-1} cadmium for 60 days. Ringwood (1992) postulated that acute exposure to 20 μgL^{-1} copper for 24 hours interfered with zinc metallothionein synthesis in oyster embryos. She suggested that this affected activation of alkaline phosphatase and carbonic anhydrase thus blocking deposition of the larval shell. A similar mechanism may be involved in the observed effect of chronic cadmium exposure on *C. gigas* spat. The observed change in shell growth following 24 days exposure to 50 μgL^{-1} cadmium may be due to induction of cadmium metallothioneins; that is, spat developed the ability to detoxify accumulated cadmium. Chronic cadmium exposure also affects calcium homeostasis in bivalves (Regoli *et al.*, 1991); the concentration of calcium in the extrapallial fluid of molluscs is highly important to the amount and composition of deposited shell (Wilbur & Saleuddin, 1983).

Shell abnormalities have been reported in bivalve embryos (Ringwood, 1992) and adults (Sunila & Lindstrom, 1985) following exposure to heavy metals. The effect of cadmium on shell shape is probably mediated via a similar mechanism to the effect on shell growth (*eg.* by blockage of metalloenzyme active sites). Seed (1968) found that mussels (*M. edulis*) may grow more along the shell midline when exposed to adverse environmental conditions. I observed a similar effect on oyster spat exposed to cadmium. Seed proposed that this is due to retraction of the mantle thus exposing the free mantle edge to the shell midline resulting in localised shell secretion. The higher proportion of spat with shell abnormalities at the lowest cadmium concentration (10 μgL^{-1}) than at 50 and 250 μgL^{-1} was simply due to the very limited shell growth of spat at the higher concentrations. In order to determine if cadmium has a significant effect on shell abnormalities in spat, further experiments are required to investigate the effect of even lower concentrations of cadmium for a longer exposure period. The growth abnormality observed was similar (but more severe) to the condition referred to as "curly back" in commercial spat. "Curly back" is characterised by a more gradual growth of shell (in particular the posterior valve margins) in the direction of the ventral valve. No intermediate forms of the abnormal growth condition were apparent in this study.

Cadmium exposure caused pale tissue colour and localised darkening of spat tentacles. Pale tissue colour of oysters was also reported by Shuster and Pringle (1969) following chronic exposure to cadmium. However, localised darkening of the tentacles does not appear to have been previously noted in bivalves following exposure to heavy metals. These dark patches may correspond to cadmium detoxification sites (*eg.* amoebocyte or metallothionein-bound cadmium) in *C. gigas* spat. Thomson *et al.* (1985) reported localisation of zinc, calcium and iron (note that no analysis for cadmium was made by these authors) in membrane-limited vesicles at the "outer edge of the mantle". Further analysis of the tentacles following metal exposure is required before a valid explanation for these dark patches can be proposed.

Measurement of heart rate in adult bivalves generally involves removal of part of a valve for either direct observation of the heart (*eg.* Scott & Major, 1972) or for insertion of electrodes that are sensitive to heart contraction (Grace & Gainey, 1987; Grace & Kenyon, 1990). Such procedures are likely to cause some stress to the animal thereby influencing heart rate. Due to the thin shell, I was able to measure heart rates of oyster spat with minimal disturbance by direct observation using a light microscope. Heart rates of the juvenile oysters were higher than those reported for other adult bivalves. For example, control spat had a mean heart rate of 43 bpm (beats per minute) compared with that of adult *C. gigas* of 21 bpm (20-25 °C) (Uesaka *et al.*, 1987) and adult *M. edulis* of 24-26 bpm (17 °C) (Helm & Trueman, 1967). Heart rates of invertebrates are generally faster for smaller individuals (Vernberg & Vernberg, 1972). Chronic exposure of spat to sublethal cadmium concentrations appeared to cause bradycardia and erratic (arrhythmic) heart contraction. Similarly, short-term exposure of adult *M. edulis.* to copper causes a reduction in heart rate and "burst activity" (irregular heart contraction) (Scott & Major, 1972; Grace & Gainey, 1987; Gainey & Kenyon, 1990). At the lowest cadmium concentration mean heart rate appeared to be slightly higher than that of controls. A lack of studies on the effect of chronic metal exposure on heart rate in bivalves is apparent. Reduction of heart rate in response to metal exposure has previously been attributed to "avoidance behaviour" *ie.* valve closure which reduces heart rate and serves to reduce metal uptake by both avoiding the contaminated medium and reducing circulation (Davenport & Redpath, 1984; Gainey & Kenyon, 1990). Metal exposure may also inhibit cardio-regulatory chemicals such as adenylate cyclase (Gainey & Kenyon, 1990). Heart rate was measured in control water following chronic exposure to cadmium rather than in metal-contaminated water. Thus, the apparent effect of exposure to cadmium on heart contraction of spat was probably due to a more chronic physiological effect rather than a direct response to the metal (*eg.* valve closure).

Davenport & Manley (1978) found that following exposure to 20 μgL^{-1} copper for 10 days *M. edulis* exhibited a reduced behavioural response to further copper exposure. They described this effect as "acclimation" to raised levels of copper. It is more likely that this represented an impaired physiological response of spat rather than acclimation to conditions. Our results showed an impaired behavioural response of spat to light following chronic cadmium exposure. Cadmium appeared to have a distinct impact on either the photoreceptive detection or response mechanism of spat with no further effect following exposure to greater than 50 μgL^{-1}. The reduced behavioural response may be due to the influence of cadmium on calcium homeostasis in bivalves (Regoli *et al.*, 1991) which would interfere with adduction of the shell. Intracellular free calcium concentration is important in excitation and contraction of the adductor muscle in bivalves (Muneoka & Twarog, 1983). Alternatively, cadmium may interfere with the photoreceptive pathway of *C. gigas* spat; however, the former explanation is more plausible as spat were detecting the light stimulus. The mechanism by which oysters may be able to detect light is unclear. It has previously been suggested that the mantle tentacles of oysters may be able to detect light (Yonge, 1960). In addition, Paparo (1987) discusses photoreception in *C. virginica* by neuronal pigments. Further research is required for an understanding of the photoreceptive pathway of *C. gigas* .

Chronic exposure to cadmium had a dose-dependent inhibitory effect on spat feed rates, with a significant effect on algal clearance (relative to controls) following exposure to only 50 μgL^{-1} for 32 days. Similarly, exposure to 1200 μgL^{-1} cadmium for 96 hours reduced the filtration rate of clams (*Anadara granosa*) by at least fifty percent (Patel & Anthony, 1991). It should be noted here that algal feeding rates of bivalves are closely related but not strictly comparable to filtration rates. For example, the presence of algae stimulates filtration by *M. edulis* (Manley, 1983). There are few studies concerning the effects of heavy metals on bivalve feed rates. Inhibition of filtration by bivalves in response to heavy metal exposure may be due to either a behavioural response to the external metal (*eg.* valve adduction) which reduces filtration, or a physiological effect (*eg.* on gill structure or ciliary activity). In the presence of only 2 μgL^{-1} copper, valve movements and the activity of both the inhalant and exhalant siphons of *M. edulis* are affected which results in reduced ventilation rates (Manley, 1983). The laterofrontal and frontal cilia of lamellibranch gills (Jorgensen, 1966) are involved in trapping and transporting food particles, whilst the lateral cilia maintain a water current across the gill surface (Barnes, 1980). Thus, ciliary inhibition would reduce the rate of removal of microalgae from water. Chronic exposure of *M. edulis* to copper cause histopathological changes to the gill epithelium as well as inhibiting ciliary activity (Brown & Newell, 1972). The effect of copper exposure on filtration rates of *M. edulis* is probably due

mostly to a combination of damage to the gill interfilament junctions and neural inhibition of the lateral cilia (Sunila, 1981; Sunila & Lindstrom, 1985; Grace & Gainey, 1987).

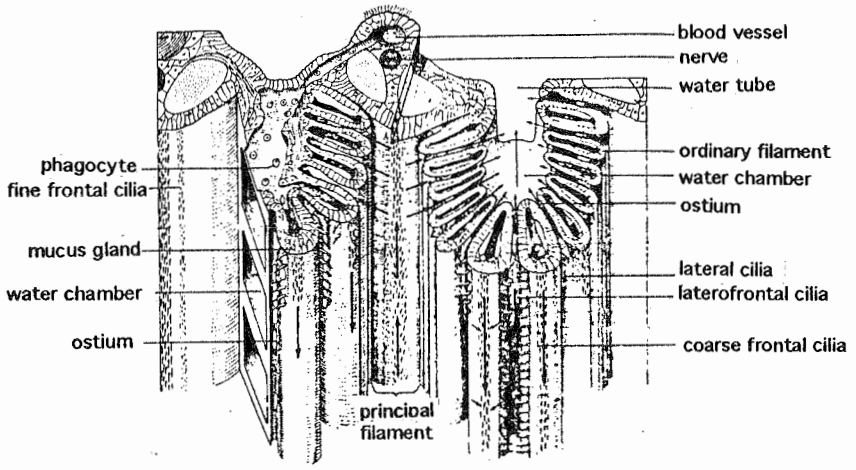

Fig. 4.13 Gill structure of *Crassostrea virginica* (from Jørgensen, 1966)

In these experiments, bioaccumulation of cadmium by *C. gigas* spat was linearly related to the exposure concentration of cadmium. Similarly, cadmium accumulation by adult bivalves is linearly related to the exposure concentration (Ritz *et al.*, 1982; Borchardt, 1983). Relative to other bivalve species, *C. gigas* rapidly bioaccumulates cadmium (*eg.* Frazier & George, 1983). This is probably due to high tissue levels of specific metal-binding proteins. For example, binding proteins with a molecular weight characteristic of cadmium metallothioneins have been detected in oyster larvae (Ringwood, 1992) and adults (Frazier & George, 1983) following exposure to cadmium. In addition, the American oyster *C. virginica* shows limited depuration of accumulated cadmium when transferred from cadmium contaminated water to sea water (Zaroogian, 1979). Cadmium accumulation by adult *C. gigas* is inversely related to size (Boyden, 1977; Thomson, 1982). However, similar bioconcentration factors were found for spat and adults, suggesting that similar uptake and storage mechanisms operate. Further research into the uptake and detoxification mechanisms of bivalve spat is required for a more complete understanding of the relative sensitivities of different life-stages. High levels of tissue cadmium may influence a number of physiological processes in spat such as those discussed above. The effects of cadmium exposure on spat growth probably result from both direct effects on growth as well as indirectly through a disruption of the general physiology (such as reduced feed rates and circulation).

In conclusion, chronic exposure to sublethal concentrations of cadmium had an effect on the general physiology of juvenile *C. gigas*. Relative to control spat, exposure to concentrations as low as 10 μgL^{-1} total cadmium (equivalent to a free cadmium ion concentration of 0.003 μgL^{-1}) for only 32 days had an effect on growth rate, heart contraction, algal filtration rate and behavioural response to light. Exposure to sublethal cadmium concentrations also caused an increase in the proportion of spat with abnormal shell growth. A growth abnormality similar but more severe than "curly back" was observed following exposure to only 10 μgL^{-1} cadmium for 32 days. Longer term exposure to lower cadmium concentrations (for example, the 5 μgL^{-1} limit allowed in Tasmanian waters) may well be responsible for the growth abnormality observed in the Tasmanian oyster industry.

5. GENERAL DISCUSSION

When compared to previously published LC_{50} values for other bivalve species, juvenile *C. gigas* were less sensitive to acute cadmium exposure than expected. *C. gigas* spat had LC_{50}s of 10.29 and 28.43 mgL^{-1} total cadmium for "small" and "large" spat respectively. The Hawaiian bivalve, *Isognomon californicum* has a 48 hour LC_{50} of 16.5 mgL^{-1} (Ringwood, 1990); however, 96 hour LC_{50}s for molluscs are generally lower than the values obtained here for *C. gigas* spat (see Table 5.1). A surprisingly large difference between total cadmium toxicity to the two sizes of spat was noted; however, when LC_{50}s were compared in terms of free ion concentration the difference was minimal (0.25 and 0.27 mgL^{-1} respectively). Addition of EDTA to cadmium treatment solutions, reduced the toxicity to spat by a reduction in the free ion concentration to which they were exposed. The toxicity of cadmium to juvenile Pacific oysters was found to be highly related to the free ion concentration as opposed to the total cadmium concentration.

Table 5.1: Acute toxicity of cadmium to several species of mollusc in terms of the 96 hour median lethal concentration.

SPECIES	LC_{50} (mgL^{-1})	SALINITY (‰)	TEMPERATURE (°C)	REFERENCE.
C. gigas spat	10.3-28.4	35	20	present study
C. virginica embryos	3.8 (48 hour)	25	26	Calabrese *et al.*, (1973)
I. californicum, spat adults	16.5 (48 hour) 27.5	34	24	Ringwood (1990)
Mya arenaria	2.2	20	20	Eisler (1971)
Mytilus. edulis	25	20	20	Eisler (1971)
M. e. planulatus	1.62	18.5	33	Ahsanullah (1976)
Nassarius obsoletus	10.5	20	20	Eisler (1971)
Urosalpinx cinerea	6.6	20	20	Eisler (1971)

The observed relationship between cadmium toxity to *C. gigas* spat and the free cadmium ion concentration may be understood through a knowledge of the mechanism of metal uptake by bivalves. If metal uptake is reduced, both tissue accumulation of the metal and physiological effects due to exposure (*eg.* mortality) will be reduced. Copper accumulation by the oyster, *C. virginica* is dependent on the free cupric ion concentration. Zamuda and Sunda (1982) suggested that this relationship is due to

equilibria between copper-carrying ligands at the cell surface and soluble copper in the external medium. If copper uptake into the cell is governed by the amount of copper bound to ligands at the surface, then it is also directly related to the free ion concentration in the external medium. Chelators (*eg.* EDTA) compete with these ligands for the available copper. A similar mechanism for cadmium would explain the relationship between free cadmium ion concentration and *C. gigas* mortality. Cadmium is predominantly present as chloride complexes in salt water (Riley, 1971); $CdCl_2$ passes through cell membranes 10^3 times faster than the free cadmium ion (Gutnecht, 1981 in Nelson & Donkin, 1985). Thus it is probable that cadmium uptake by *C. gigas* is facilitated. That is, the free cadmium ion concentration controls the rate of cadmium binding to ligand sites and thus the rate of facilitated uptake as opposed to controlling direct uptake of the free cadmium ion. Addition of EDTA reduced the free ion concentration and thus the cadmium available for binding at the cell surface of cadmium uptake sites (*eg.* gill and digestive gland epithelia).

The observed relationship between spat mortality and free cadmium ion concentration is of particular concern where total cadmium levels are within recommended limits whilst physico-chemical conditions promote high free ion concentrations. Commercial bivalve hatcheries frequently add EDTA to larval culture systems; however, there is limited control of metal levels in culture water for bivalves beyond metamorphosis. Cadmium has previously been shown to leach from black polyethylene and galvanised piping (Nilsson, 1970). Polyethylene piping, in particular, is frequently used to supply seawater to bivalve hatcheries and nurseries. Sources of cadmium contamination in the environment include pesticides, phosphate fertilisers, paints, anti-corrosion cadmium plating (including boat-fittings) and galvanised iron. Industrial activities such as mining and electrolytic processes (*eg.* the Electrolytic Zinc Company in Tasmania) contribute significant levels of cadmium to the environment (Nilsson, 1970; Friberg *et al.*, 1974).

The Tasmanian recommended limit for cadmium in marine water is 5 μgL^{-1} (Tasmanian Department of Environment, 1986); chronic exposure of oysters to levels of cadmium around 5 μgL^{-1} may well affect their appearance and physiology, particularly where conditions promote relatively high levels of free ions. The recently published Australian Water Quality Guidelines suggest a more conservative level for marine waters of 2 μgL^{-1} (ANZECC, 1992). Use of the ANZECC limit is recommended for Tasmanian waters, particularly in areas used for aquaculture. As I have shown, exposure to only 10 μgL^{-1} total cadmium (or a free ion concentration of 0.003 μgL^{-1}) for 32 days had an adverse effect on growth rate, heart contraction, algal filtration rate, behavioural response to light and caused an increase in spat with abnormal shell growth. Recommended environmental limits for heavy metals based on the total metal concentration provide an unreliable guide

for protection of waterways. Spat may well be exposed to free cadmium ion concentrations of around 0.003 μgL^{-1} in the environment, particularly in estuarine waters where lower salinity promotes a high free ion concentration relative to that in oceanic water. In environmental and commercial culture situations where oysters are exposed to such concentrations of cadmium for longer periods, from an earlier age and under a range of physico-chemical conditions these effects on the physiology of juvenile *C. gigas* would have important implications for their survival and growth.

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APPENDIX

f/2 Seawater Medium for culture of phytoplankton (from Guillard, 1975)

Major nutrients

$NaNO_3$	75 mg	
$NaH_2PO_4 \cdot H_2O$	5 mg	
$NaSiO_3 \cdot 9H_2O$	15-30 mg	(1.5-3 mg Si)

Trace metals

$Na_2 \cdot EDTA$	4.36 mg	
$FeCl_3 \cdot 6H_2O$	3.15 mg	(0.65 mg Fe)
$CuSO_4 \cdot 5H_2O$	0.01 mg	(2.5 µg Cu)
$ZnSO_4 \cdot 7H_2O$	0.022 mg	(5 µg Zn)
$CoCl_2 \cdot 6H_2O$	0.01 mg	(2.5 µg Co)
$MnCl_2 \cdot 4H_2O$	0.18 mg	(0.05 µg Mn)
$NaMoO_4 \cdot 2H_2O$	0.006 mg	(2.5 µgMo)

Vitamins

Thiamin.HCl	0.1 mg
Biotin	0.5 µg
B12	0.5 µg

Seawater — to one litre

PHYSIOLOGICAL EFFECTS OF HEAVY METALS ON BIVALVES, WITH PARTICULAR EMPHASIS ON JUVENILE STAGES

A review of the literature submitted to the Department of Zoology, University of Tasmania in partial fulfilment of the degree of Bachelor of Science with Honours.

CONTENTS

1. INTRODUCTION

The capacity of bivalves to bioaccumulate heavy metals is well documented (George and Coombs, 1975; Phillips, 1976; Cooper *et al.*, 1982). Concern over aquatic pollution from anthropogenic activities combined with the ability of bivalves to bioaccumulate metals has stimulated numerous studies into interactions between bivalves and heavy metals (George, 1980).

Research has focused on physiological effects (eg. Brown and Newell 1972; Manley, 1983), accumulation and detoxification (George, 1980) and biomonitoring (eg. Ritz *et al.*, 1982). Effects of heavy metals on growth and mortality have received particular attention. Recent studies have examined biochemical aspects of heavy metal uptake and detoxification by adult bivalves (Langston *et al.*, 1989; Weber, 1990).

Adult bivalves, particularly commercial species, have previously received considerable attention; however, little information is available on metal uptake by larval stages especially at chronic and/or sub-lethal exposure levels (Calabrese *et al.*, 1973; Ringwood, 1989). Nevertheless, developments in hatchery technology have ensured a reliable supply of juvenile bivalves heightening interest in studies of early life stages (Calabrese *et al.*, 1973). Hatchery reared bivalve larvae and embryos are convenient for controlled laboratory experiments due to size, availability and sensitivity (ASFA, 1976; Ringwood, 1991). The use of bivalve larvae and embryos for bioassays has received attention as a sensitive, efficient means of assessing contamination (ASFA, 1976).

This review discusses the physiological effects of heavy metals on marine bivalves and contributing factors, in particular, uptake, accumulation and depuration. Where information is available, emphasis is placed on juvenile bivalves. The effects of some of the more toxic heavy metals, especially mercury, cadmium, copper and zinc (McLusky *et al.*, 1986), are discussed in detail.

2. FACTORS AFFECTING HEAVY METAL UPTAKE

Relative to other invertebrates marine bivalves are tolerant to a range of environmental conditions due mainly to avoidance behaviour (Akberali and Trueman, 1985) and effective detoxification strategies (George, 1980); this accounts for their colonization success in estuarine and coastal environments (Akberali and Trueman, 1985). Bioavailability of metals is determined by their relative concentrations as ions, organic and inorganic complexes and combined with particulate matter (Engel *et al.*, 1981). Heavy metal uptake is controlled by both environmental conditions and biological factors (Simkiss and Mason, 1983). Different factors affect uptake of individual metals to varying degrees (George, 1980).

2.1 PHYSICO-CHEMICAL FACTORS

2.1.1 Metal source

Marine heavy metal uptake by bivalves is from three sources: water, food and sediments. The relative contribution of each source varies according to environmental conditions, metal form and bivalve species. For example, relative uptake of mercury from salt water, phytoplankton and suspended sediment by *M. edulis* is approximately in the ratio of 10:5:1. This ratio varies with environmental conditions (King and Davies, 1987). The source of a heavy metal also affects accumulation levels by different organs. Figure 1 shows the internal organs of a bivalve (*Geloina erosa).* The digestive gland and to a lesser extent the kidneys take up most of the metals obtained from phytoplankton feeding by *M. edulis*, sedimentary metals are taken up by the kidney and metal uptake from water is highest in the gills, kidney and digestive gland (King and Davies, 1987). Uptake pathways will be discussed in Section 3.

2.1.1.1 Water

Unlike secondary consumers such as the flounder (Riisgard and Hansen, 1990), the concentration effect of metal accumulation from food is less important than uptake from water, particularly for filter feeders (Mohlenberg and Riisgard, 1988). Filter feeding bivalves such as *Mytilus edulis* take up metals at an initial rate proportional to

water metal concentration (George,1980; George and Pirie, 1980; Borchardt, 1983). Schulz-Baldes (1974) reported an equal rate of uptake from food as from water by *M. edulis* but did not discuss the effect of algae on filtration rates. Janssen and Scholz (1979) accounted for increased cadmium uptake in the presence of algae due to greater ventilation rates.

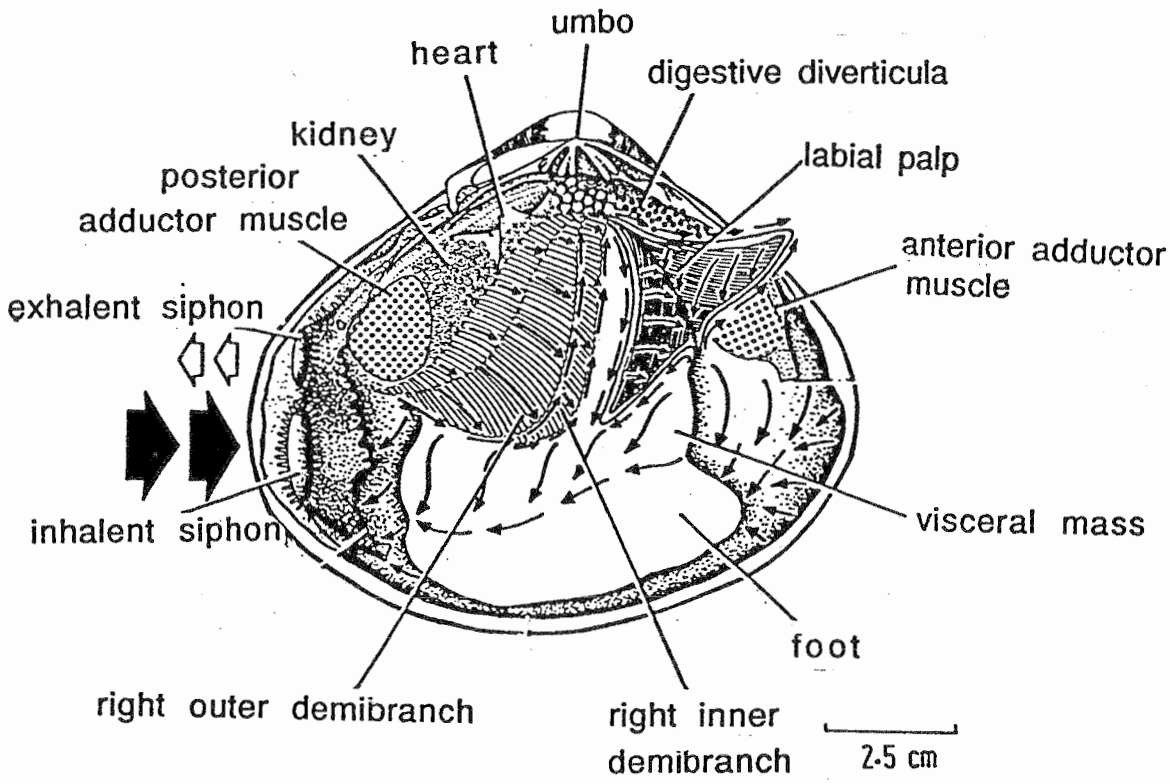

Fig. 1. ***Polymesoda*** **(*Geloina*)*erosa*. The internal organs and ciliary currents of the mantle cavity (from Morton, 1976).**

In contrast to filter feeders, zinc accumulation from solution by the deposit feeder *Scrobicularia plana* is negligible (Bryan, pers. comm. in George, 1980). Heavy metal uptake from water is discussed in further detail in section 2.1.2.

2.1.1.2 Food

Heavy metal uptake by *M. edulis* during feeding (ie. from phytoplankton) under normal conditions, is slower than uptake from water (King and Davies, 1987). Highly contaminated food may contribute significant amounts of trace metals to bivalves (Borchardt, 1983; Zhang *et al.*, 1990).

2.1.1.3 Sediments

Despite the high metal content of sediments relative to other sources (Bryan *et al.*, 1985) metal uptake from them by bivalves is generally low. Bivalves, in particular deposit feeders such as scallops and clams, may take up metals via the digestive gland from ingested sediments (George, 1980). The biogenic and interstitial water components of marine sediments provide the more bioavailable sources of sedimentary metal (Bryan, 1976; Bryan *et al.*,1985). Sediment composition may determine the availability of metals for accumulation (Cooke *et al.*, 1979). For example, the uptake of lead by the deposit feeder *S. plana* is related to the lead:iron ratio in surface sediments. High iron levels may increase binding of lead to iron oxide, thus decreasing lead uptake in the digestive tract (Luoma and Bryan, 1978). On the other hand, mercury uptake by *S. plana* and *Macoma balthica* is related to the mercury:total organic matter ratio of the surface sediments (Langston, 1982).

Filter feeders accumulate metals to a lesser extent from sediments. Greig and Wenzloff (1978) found copper levels in *Crassostrea virginica* were related to sedimentary levels. They suggested that uptake of copper occurs from sediments as well as from phytoplankton and water. Cadmium and silver, however, were not taken up directly from sediments. Brooks and Rumsby (1965) found uptake of chromium, iron, manganese and nickel is predominantly by ingestion of sedimentary matter of small particle size.

2.1.1.4 Gonads

Heavy metal levels in bivalve larvae and embryos are partially attributable to uptake by the parental gonads and gametes (Frazier, 1975; Stiles *et al.*, 1991). Copper, cadmium and zinc may be directly incorporated into the gametes of *C. virginica* (Frazier, 1975). Although low compared with total metal uptake, copper and zinc concentrations in *C. gigas* eggs may be comparable to those in the gills (Thomson *et al.*, 1985). Levels of contamination influence the gonad uptake of heavy metals by certain bivalve species. Greig *et al.*, (1975) found constant levels of copper and cadmium in the eggs of *C. virginica* adults from different environments and no transfer of silver, lead and zinc to the oocytes was detected. In contrast, Akberali and Trueman (1985) state that gamete metal uptake is stimulated by high metal levels. In a study by Roesijadi *et al.* (1982), *M. edulis* larvae from adults exposed to mercury, had greater cytosolic mercury concentrations than controls. The hard clam, *Mercenaria mercenaria,* exhibits

significantly different gonad metal concentration between polluted and relatively unpolluted environments (Stiles et al., 1991). Embryos from contaminated adults, cultured in reference sea water develop more chromosomal abnormalities than those spawned from uncontaminated adults. Thus metal uptake by prespawned gametes probably affected subsequent development of the fertilized embryos.

2.1.2 Chemical speciation

2.1.2.1 Dissolved species

Bioavailability of a dissolved metal species is a function of its activity (Riley, 1971). In bivalve studies metal uptake and toxicity are frequently expressed as a function of the total dissolved metal concentration (Brooks and Rumsby, 1965; Brereton *et al.*, 1973). However, in natural systems heavy metal accumulation is largely determined by free metal ion concentration. For example, an increase in salinity lowers cadmium toxicity by reducing the concentration of free ions (Engel *et al.*, 1981). Mantoura *et al.* (1978) found the trace metals zinc and manganese exist predominantly as free ions; however, free metal ion concentrations are generally low in marine environments (Nelson and Donkin, 1985). Metal ions are predominantly present as complexed species (Coombs and George, 1977). Table 1 shows levels of trace metals in the Derwent River estuary, Tasmania.

Inorganic metal speciation in salt water is largely determined by competitive binding with the major anions: Cl^-, CO_3^{2-}, OH^-, SO_4^{2-}, F^- (Riley, 1971) and Br^- (Kennish, 1989). Relative bioaccumulation of cadmium salts by bivalves is in the order of sulphate> iodide> acetate> chloride> nitrate> carbonate (Patel and Anthony, 1991). Simkiss (1983) proposed that inorganic complexes of heavy metals (including copper, zinc, cadmium and mercury) are more bioavailable than other metal species due to greater lipid solubility in cell membranes. Conversely, Bryan *et al.* (1985) describe the organic mercury species as more bioavailable than inorganic mercury complexes.
Dissolved organic metal complexes typically occur as metal-chelates, particularly in estuarine zones with high levels of dissolved organic matter (Riley,1971; Engel *et al.*, 1981). Organic complexes may provide a significant source of heavy metals to marine invertebrates; for example, Mohlenberg and Riisgard (1988) found increased uptake of organic mercury with age in *Cardium sp.* Conversely, organic chelators such as humic acid and the synthetic chelator EDTA (ethylenediaminetetraacetic acid) usually reduce heavy metal uptake (Knezovich *et al.*, 1981; Bryan *et al.*, 1985). However, George and

Coombs (1977) found that addition of EDTA doubled the rate of cadmium uptake in *M. edulis*. They proposed that cadmium requires prior binding before uptake occurs. An alternative explanation is an EDTA-mediated reduction in competition from other metals for tissue binding sites, resulting in increased cadmium uptake (Zamuda and Sunda, 1982). Assuming initial uptake, Riley (1971) states that marine organisms can obtain trace elements by breaking down metal-chelates.

TABLE 1. Heavy metal concentrations (ug/L) in the Derwent River estuary, Tasmania, surface samples (Department of the Env., 1987)

SITE	Cadmium	Copper	Mercury	Zinc
Battery Pt	1.4	2	0.07	398
Kangaroo Pt	0.9	1	0.22	121
Cartwright Pt	3.9	1	0.08	102
Tryworks Pt	1.2	1	0.07	164

2.1.2.2 Particulate species

Marine particulate matter has substantial levels of heavy metals (Bryan *et al.*, 1985); however, this does not necessarily signify metal bioavailability (Bryan, 1976).

Inorganic particulate matter comprises minerals, skeletal remains and colloidal matter (Bryan *et al.*, 1985). Particulate metal uptake is predominantly from the colloidal and organic components of particulate matter in sea water. Phytoplankton, organic metabolic products (eg. faecal matter) and organic detritus take up metal species either by adsorption or complex binding (Riley, 1971; Bruland, 1983). Thus, feeding and filtration are important mechanisms for particulate metal uptake (Pentreath, 1973; George, 1980). The proportion of metal-bound particulate matter tends to be greater in coastal environments, particularly during upwelling periods. Knauer and Martin (1973) propose that upwelling produces an influx of decomposed planktonic matter binding heavy metals, especially cadmium and lead.

Sewage effluent may increase particulate metal speciation by flocculation of metals such as iron, nickel, copper and zinc with organic matter on estuarine mixing (Feely *et al.*, 1981). Hughes (1975) found that microorganisms in sewage effluent accumulate

zinc and cadmium. Sewage effluent reduced the toxicity of copper to the copepod *Euchaeta japonica* in a study by Lewis *et al.* (1972), probably by chelation.

Particulate metals vary in bioavailability according to type, concentration and source. Inorganic particulate metal complexes are generally less bioavailable than organic particulate species (eg. Riisgard & Hansen, 1990). Copper (Zamuda and Sunda, 1982) and cadmium (Engel *et al.*, 1981) uptake is normally related to the concentration of free ions. However, in a short term study Carpene and George (1981) reported 50% particulate cadmium uptake by diffusion across *M.edulis* gills.

Particulate species are arbitrarily defined as those which are retained on a 0.4um filter (Amdurer *et al.*, 1981). This by definition excludes colloidal species. Precipitates of colloidal metal hydroxides are taken up by molluscs by pinocytosis, particularly at the gill epithelium (George *et al.*, 1976; George, 1982).

2.1.3 Environmental interactions

Heavy metal uptake is influenced both directly and indirectly by environmental conditions such as temperature, salinity, pH, redox potential and other metals (George, 1980). Estuarine organisms, in particular, are affected by constant exposure to fluctuating environmental conditions (Jones, 1977). Environmental parameters interact with metal uptake by influencing metal bioavailability in the environment or by affecting the physiology and thus the uptake mechanisms of the organism itself (George, 1980).

2.1.3.1 Temperature

Elevated temperatures generally increase heavy metal uptake by bivalve molluscs (Bryan, 1976; Jackim *et al.*, 1977; Elliott, 1982). For example, Denton and Burdon-Jones (1981) examined the interaction of temperature and salinity with metal uptake in *Saccostrea echinata*. An increase in cadmium and mercury uptake due to elevated temperatures was augmented by concurrently lowering the salinity. Nevertheless, high temperature maintained this effect at higher salinities. *M. edulis* accumulation of cadmium is influenced by temperature at low salinities (Phillips 1976). Copper accumulation by *Donacilla cornea* is stimulated by high temperatures, whilst cadmium and manganese uptake is not significantly affected (Regoli *et al.*, 1991).

Conversely, low temperature may suppress metal uptake in sensitive life stages of bivalves even resulting in lead loss by *C. virginica* larvae (Zaroogian *et al.*, 1979). Accumulation of cadmium and copper by *C. virginica* (Zaroogian, 1979) and lead and zinc by *M. edulis* (Phillips, 1976) is not affected by low temperatures. Elliott (1982) reported lower metal uptake by *M. edulis planulatus* in warmer conditions. He concluded that the winter acclimatised mussels had a reduced capacity to accumulate metals at warm temperatures.

High temperature induced heavy metal uptake by bivalve molluscs may be a physiological response, for example, greater ventilation rates which increase uptake at the gills (Denton and Burdon-Jones, 1981) or increased solute movement across cell membranes (Cairns *et al.*, 1975). Alternatively, Simkiss and Watkins (1988) suggested that the effect of temperature on zinc uptake in *M. edulis* is a function of temperature fluctuations rather than the absolute temperature. A change in temperature causes zinc mobilization or detoxification resulting in additional uptake to replace "lost" zinc.

2.1.3.2 Salinity

Salinity has been shown to have an inverse relationship to heavy metal uptake in bivalves (Jackim et al., 1977). Cadmium uptake by *M. edulis* is stimulated by low salinity (Phillips, 1976; Engel et al., 1981). Phillips (1976) reported reduced lead uptake and constant zinc uptake in response to low salinity. This was explained by lowered filtration rates or changed ion flux. However, zinc uptake was affected by salinity in a study by Thomson (1982). As discussed in section 2.1.3.1 salinity also interacts with temperature to change heavy metal uptake rates.

High salinity reduces the more bioavailable free metal ions in marine systems by the formation of chloro-complexes (Mantoura et al., 1978; McLusky et al., 1986). George *et al.* (1977) proposed that low salinity may cause increased cadmium uptake by the gill surface due to increased ion flux or the swelling of the cell membrane exposing a carrier or membrane pore.

2.1.3.3 Other metals

Several examples of the influence of other metal species on heavy metal uptake are discussed in section 2.1.1.3. Uptake may also be influenced by competition between heavy metals for binding proteins; for example, cadmium, copper and zinc compete for binding sites on the metallothionein molecule (discussed in section 3). Exposure to either of the alternative metals increases copper and zinc uptake by *M. edulis planulatus* and inhibits cadmium uptake (Elliott, 1982; Patel and Anthony, 1991). The extent of metal interaction depends on the concentrations of the metals involved (Elliott *et al.,* 1986). Metal induction of metallothionein synthesis accounts for increased copper and zinc uptake. In contrast, Phillips (1976) found no change in net uptake of cadmium, lead and zinc by *M. edulis* exposed to other metals and no interaction between the metals. Copper uptake by *M. edulis* is very susceptible to environmental conditions, changing with exposure to other metals and changes in salinity and temperature (Phillips, 1976).

2.2 BIOLOGICAL FACTORS

2.2.1 Size

The relationship between age or weight and metal uptake varies between metals and bivalve species. Several authors have examined the dynamics of metal accumulation with respect to size. For example, cadmium uptake is inversely related to body size of the clam *Macoma balthica* (McLeese and Ray, 1984) and *C. gigas* (Boyden, 1977). Cadmium content is inversely related to dry weight of *M. edulis* (Cossa et al., 1980); in contrast cadmium content is directly related to dry weight of *C. virginica* (Zaroogian, 1980). Organic mercury content increases linearly with age and weight in cockles (Mohlenberg & Riisgard, 1988). No relationship exists between copper content and dry weight of the scallop *Argopecten iradians* (Zaroogian and Johnson, 1983) whilst Dermott and Lum (1986) reported greater accumulation of copper and zinc by *Elliptio complanata* during periods of fastest growth. Although metal content and concentration provide an idea of metal uptake by bivalves these values cannot strictly be compared to rates of uptake.

2.2.2 Binding proteins and ligands

George (1980) suggested that nett uptake of metals is regulated by intracellular binding ligands. Metal ions bound by ligands, possibly derived from mucus, increase metal uptake by *M. edulis.* Mucus sheets of *M. edulis* may affect the membrane permeability at uptake sites or serve to transport ions to the gut (Simkiss and Mason, 1983). Naturally, saturation of binding ligands reduces metal uptake (George and Pirie, 1980). Metal transport across membranes of *M. edulis* is probably by passive diffusion with little control of rates or metal type (George, 1980). Bivalves generally exhibit limited control of metal uptake. However, Bryan (pers. comm. in George, 1980) has observed control of zinc accumulation from solution by the deposit feeder *Scrobicularia plana.*

Relative levels of different metal binding proteins in bivalves vary between species and environment. In several species, exposure to certain heavy metals results in increased levels of low molecular weight binding proteins (including metallothionein-like proteins) and high molecular weight binding proteins (including metalloenzymes) (Harrison et al., 1988; Bebianno and Langston, 1991). The metallothionein-like molecule in *M. edulis* binds zinc, cadmium and copper with a greater affinity for cadmium (Bremner, 1977; Bebianno and Langston, 1991). Talbot and Magee (1978)

proposed that cadmium induction of metallothionein-like proteins in *M. edulis* aids the organism in control of cadmium, copper and zinc uptake. Interspecific differences in uptake may be due to metallothionein-like protein levels. For example, the gastropod *Littorina littorea* has naturally high metallothionein levels; metallothionein may be induced in *M. edulis* but is not produced by *Macoma balthica.* Cadmium is bound to high molecular weight proteins in *Macoma balthica* , this may account for relatively slow uptake compared to the rate of uptake by *M. edulis* and *L. littorea* (Langston *et al.*, 1989).

2.2.3 Bivalve Species

Differences in metabolic rate, binding proteins, behaviour and feeding strategies may account for interspecific differences in metal uptake. As discussed in section 2.2.2 differing metallothionein levels may account for different rates of metal uptake between *M. edulis, M. balthica* and *L. littorea* (Langston *et al.*, 1989). *C. gigas* has a greater rate of cadmium accumulation than *O. edulis* (Frazier and George, 1983). Patel and Anthony (1991) examined six bivalve species and found highest cadmium accumulation occurred in arcid clams, followed by venerid clams then mytilid mussels.

Feeding strategy, for example the filtration rate of filter feeders such as *M. edulis* and *C. gigas,* is an important factor affecting relative rates of metal uptake (Janssen and Scholz, 1979; George, 1980). As discussed in section 2.1.1.3, metal uptake by deposit feeders such as *S. plana* and *M. balthica* is affected by sediment composition.

2.2.4 Developmental stage

Bivalve embryos and larvae take up heavy metals at greater rates than adults. Ringwood (1991) found highest accumulation rates in embryos of *Isognomon californicum* followed by larvae then adults. She discounts the hypothesis posed in a previous paper (Ringwood, 1989) that larvae have superior accumulation rates to adults due to higher metabolic rates. Ringwood proposes that the greater exposed surface area:volume ratio of embryos and larvae is responsible for higher rates of uptake. For example, the gill buds and foot of *I. californicum* pediveligers may account for higher rates of metal uptake when compared to veligers (Ringwood, 1991). It is also likely that the faster filtration rates of juvenile stages contribute to differences in relative rates of metal uptake.

Adult bivalves change metal accumulation rates during and following the spawning period. Delhaye and Cornet (1975) reported increased copper uptake during the spawning period of *M. edulis* due to greater metabolic rates. Following spawning bivalves generally have suppressed metal accumulation (Zaroogian et al., 1979; Zaroogian, 1980; Zaroogian and Johnson, 1983).

3 MECHANISMS OF HEAVY METAL UPTAKE

3.1 EPITHELIAL UPTAKE

Bivalve heavy metal uptake occurs predominantly at the gill and digestive gland epithelia (Janssen and Scholz, 1979; George, 1980; Elliott, 1982). Uptake at other epithelial surfaces is generally slower (George, 1980). In an early study by Nakahara and Bevelander (1967), particulate metal uptake occurred in the pallial space of two bivalves across the epithelial mantle and by wandering amoebocytes. The site of epithelial uptake of heavy metals is largely determined by the metal species involved.

Particulate metal species are mostly taken up at the gut epithelium by endocytosis (Nakahara and Bevelander, 1967; George et al., 1980; Simkiss and Mason, 1983). Pinocytotic uptake of metal species has also been shown to occur at the gill epithelium (Coombs, 1977) and the mantle (George, 1980). In addition to endocytosis, particulate metals are transported across the digestive gland membrane by preferential chelation.

Soluble metal species, such as cadmium, zinc and lead, may be taken up either directly (by passive diffusion) or following adsorption onto mucus, usually at the gut or gill surface (Hobden, 1969; George, 1980; Elliott, 1982). Mucus production is inducible by metal exposure (Scott and Major, 1972; Sunila, 1984). It has also been suggested that soluble metal species, in particular cadmium and copper, may pass through the epithelium after being bound by a carrier molecule (Elliott, 1982; Zamuda and Sunda, 1982) for example, glycoproteins, phospholipids and metallothionein (George, 1982). Cadmium and copper compete for binding sites on carrier compounds (George and Coombs, 1977). Ringwood (1991) suggested cadmium uptake by *Isognomon californicum* pediveligers may occur at the foot and gill buds in addition to the velum.

The internal fate of heavy metals is similar irrespective of uptake mechanisms. High levels of metals are incorporated into membrane-bound vesicles (Janssen and Scholz, 1979) which may eventually be integrated into lysosomes (Nakahara and Bevelander, 1967; Ruddel and Rains, 1975; George *et al.*, 1980). Metals are exocytosed from the epithelial cell as vesicles or bound metal. They may then be transported in the haemolymph bound to proteins (George and Pirie, 1980; Robinson and Ryan, 1988) or to low molecular weight compounds (Coombs, 1974), or phagocytosed by amoebocytes (Coombs, 1977; George and Pirie, 1980; Simkiss and Mason, 1983).

3.2 INTERCELLULAR TRANSPORT

Janssen and Scholz (1979) showed that cadmium and mercury are transported predominantly in the blood plasma of *M. edulis* rather than in amoebocytes. In comparison, approximately half the haemolymph zinc in *M. edulis* is transported in amoebocytes and half bound to high molecular weight proteins. Such binding proteins tend to aggregate in the presence of metal ions, thus may serve to accumulate metals for endocytosis into storage tissue amoebocytes (George and Pirie, 1980). Similarly, metals are transported in the quahog clam *M. mercenaria* bound to high molecular weight proteins (Robinson and Ryan, 1988) whilst low molecular weight proteins bind 40% of the total zinc in *O. edulis*. Coombs (1974) postulated that low molecular weight proteins enable circulation of an exchangeable supply of zinc for metabolic processes, particularly enzyme systems. Binding proteins, especially metallothioneins, may be specific for metals as well as for deposition sites (Simkiss and Mason, 1983).

Amoebocytes transport heavy metals in the haemolymph from epithelial cells to various tissues, in particular the bivalve kidney, for storage and eventual excretion (George et al., 1980; George and Pirie, 1980). This transport mechanism is important for essential metals such as copper and zinc in *M. edulis* (George and Pirie, 1980). Ruddel and Rains (1975) reported significant levels of copper and zinc in *C. gigas* haemocytes. They proposed that the basophilic and acidophilic haemocytes transfer copper and zinc to traumatised tissue possibly to prevent pathogenic attack and to control the inflammatory response (Ruddel, 1971; Ruddel and Rains, 1975). Alternatively, amoebocyte transport enables removal of toxic metals by diapedesis across the epithelium (Ruddel, 1971).

SER coelomocyte cells in larval oysters (Elston, 1980) may possibly transport dissolved metals secreted into the visceral cavity from the gut, whilst phagocytes may take up particulate metals from the visceral cavity. However, although Elston (1980) examined transport of dissolved and particulate matter by larval coelomocytes, metal transport was not included.

3.3 DETOXIFICATION AND STORAGE

3.3.1 Binding proteins

In addition to heavy metal transport, proteins detoxify metals by forming stable protein-metal complexes. In particular, numerous authors have discussed the role of metallothionein-like molecules (subsequently referred to as metallothioneins) in metal detoxification by bivalve molluscs (Talbot and Magee, 1978; George *et al.,* 1979; Langston *et al.,* 1989). Bivalve metallothioneins are sulphur containing proteins with the capacity to bind specific metals, notably copper, cadmium, mercury and zinc (Roesijadi, 1980; Simkiss and Mason, 1983). Metallothioneins may be induced in certain species by exposure to metals. For example, cadmium induces metallothionein production in *M. edulis* but not in the clam *Macoma balthica* (Langston *et al.,* 1989). Howard and Nickless (1978) found no evidence of metallothionein production in the cockle *Cardium edule* or the scallop *Chlamys opercularis.* Amongst other species, metallothioneins have been detected in *M. edulis* (Noel-Lambot, 1976), *Protothaca staminea* (Roesijadi, 1980) and possibly *Mercenaria mercenaria* (Carmichael *et al.,* 1980) and *Ostrea sp.* (Casterline and Yip, 1975). A lack of literature concerning metal binding proteins in bivalve larvae is evident.

Heavy metal uptake may be affected by competition between cadmium, copper and zinc for binding sites on the metallothionein molecule (Elliott, 1982). Conversely, Bremner (1977) suggested that zinc actually stabilizes the copper metallothionein molecule in animals. Metal detoxification by metallothioneins is a saturatable process. For example, *M. edulis* binds mercury primarily to high molecular weight proteins, but under chronic exposure metallothionein accounts for further detoxification. This eventually becomes saturated so *M. edulis* reverts to high molecular weight protein binding of mercury (Roesijadi, 1982). High molecular weight protein binding of heavy metals is dependent on previous exposure, ambient metal levels and inherent metallothionein levels (Harrison *et al.,* 1988). High molecular weight (HMW) proteins, in particular ferritin, bind significant levels of copper and zinc in the bivalve *Saccostrea cucullata* (Webb *et al.,* 1985).

Low molecular weight compounds, for example taurine and homarine, reversibly bind copper and zinc in *C. gigas* and *O. edulis* (Coombs, 1974; Howard and Nickless, 1977) and cadmium in *M. mercenaria* (Langston *et al.,* 1989). The pattern of cadmium accumulation exhibited by both larval and adult *I. californicum* after cadmium exposure for 14 days suggests storage is by very low molecular weight (VLMW)

binding proteins (Ringwood, 1991). These compounds represent a more available store of metals to the organism than HMW compounds. Binding proteins occur in the haemolymph and tissues. Cellular binding proteins may be taken up by lysosomes and broken down to release the bound metal (George, 1982).

Unlike *I. californicum, O. edulis* larvae do not appear to accumulate metals. An increase in zinc metalloenzyme activity in older *O. edulis* spat, coincides with development of the ability to bioaccumulate zinc (George and Coombs, 1975). The different metal accumulation mechanisms of bivalve larvae compared to adults may contribute to their relatively higher rates of uptake (Ringwood, 1991)

3.3.2 Subcellular organelles

Bivalve blood amoebocytes and tissue cells sequester significant levels of heavy metals in subcellular organelles. The scallop *Pecten maximus* stores copper associated with mitochondria, lysosomes, microsomes, granules and cytoplasm (George *et al.,* 1980). Lysosomes may take up heavy metals from proteins and other organelles such as pinocytic vesicles (George, 1982). Subcellular storage and detoxification is predominantly as insoluble granules in membrane-bound vesicles formed from lysosomes (George and Pirie, 1980; Simkiss and Mason, 1983; Pirie *et al.,* 1984). In bivalves, a major proportion of these vesicles are located in the kidney (Carmichael *et al.,* 1980; Elliott, 1982). However, Thomson *et al.* (1985) showed that more than 90% of total copper and zinc in *C. gigas* is stored in amoebocyte vesicles. Metal detoxification varies between metals and tissues. For example, the gill epithelium of *C. gigas* stores less copper and zinc in granules than in the cytoplasm whilst calcium and iron are sequestered in membrane-bound granules (Thomson *et al.,* 1985). In *M. edulis* metals are stored temporarily in lysosomes of the digestive gland before excretion (Janssen and Scholz, 1979). By virtue of its essential role in enzyme systems zinc is more evenly distributed between tissues than other metals which tend to accumulate in the kidney (Carmichael *et al.,* 1980).

Placopecten magellanicus (Fowler *et al.,* 1988), *M. mercenaria* and *Argopecten irradiens* (Doyle *et al.,* 1931; Carmichael *et al.,* 1980) possess inorganic kidney granules of calcium and magnesium phosphates associated with trace metals (George, 1982). Fowler *et al.* (1988) found that the availability of these 'concretions' in *P. magellanicus* determines copper and cadmium sequestration by cadmium binding protein. Concretions are exocytosed from the kidney under high environmental copper

exposure, thus metal storage is restricted to cadmium binding proteins.

Metals are also deposited in bivalve shells and the byssal threads of *M. edulis* (George, 1980; Dermott and Lum, 1986). Davies and Paul (1986) suggested that tributyl tin (TBT) is stored in the adductor muscle of *Pecten maximus.*

3.3.3 Excretion

Bivalve metal excretion is mostly achieved via the following mechanisms:

1. Exocytosis (diapedesis): amoebocytes containing heavy metals migrate to bivalve epithelial surfaces where they may be exocytosed to the environment (George and Pirie, 1980). Diapedesis of phagocytes across the velar surface is an important excretory process in *C. virginica* larvae (Elston, 1980) although whether metal excretion occurs via diapedesis is unknown.

2. Urine: metals are excreted from the bivalve kidney in urine by vesicle extrusion or direct secretion (Schulz-Baldes,1977; George et al., 1980). For example, *M. edulis* excretes most heavy metals into the kidney lumen as particulate urine (George and Pirie, 1980).

3. Faeces: Janssen and Scholz (1979) estimated that 1/3 to 1/2 of the cadmium taken up in food by *M. edulis* is lost as granules or undigested algae in the faeces. Faeces account for excretion of significant levels of particulate and colloidal metal species (George, 1980).

4. Spawning: authors of several studies have discussed the loss of heavy metals during spawning (Cunningham and Tripp, 1975; Frazier, 1975; Lowe and Moore, 1979). Zaroogian (1980) and Zaroogian and Johnson (1983) reported a drop in metal content of adults after spawning. However, prespawned bivalve eggs have a low membrane permeability (Ringwood, 1991), thus, spawning probably plays a minor role in metal excretion.

4. PHYSIOLOGICAL EFFECTS OF HEAVY METALS ON BIVALVES

The physiological impact of a metal species on marine bivalves is a function of the uptake rates, which are in turn dependent on biological and environmental factors (section 2). In addition, metal accumulation and detoxification mechanisms (section 3) determine the effects on an organism's physiology.

Effects of metals on bivalves are frequently measured as median lethal concentrations (LC_{50} values) over 48 or 96 hour exposures, i.e. the concentration of a toxicant that results in 50 % mortality over a certain time period. However, sub-lethal or chronic measurements may provide more information about the action of metals in the environment on an organism's physiology (McLusky *et al.*, 1986; APHA, 1989).

4.1 EFFECTS ON ADULT BIVALVES

4.1.1 Lethal effects

Metals are involved in a number of physiological processes; in particular, copper and zinc are essential metals involved in bivalve enzyme systems, respiratory proteins and redox systems (Simkiss and Mason, 1983). Substitution or displacement of an essential metal by an influx of another metal may have toxic effects due to enzyme inhibition or protein denaturation (Alzieu *et al.*, 1982; Simkiss and Mason, 1983). Regoli *et al.* (1991) reported disruption of calcium homeostasis in the bivalve *Donacilla cornea* due to copper and cadmium accumulation. An increase in calcium will affect a number of metabolic processes; notably membrane transport, muscle contraction and respiration (Bayne, *et al.*, 1976; Stryer, 1981). Mortality of *M. edulis* is an exponential function of the initial copper concentration, with a critical concentration between approximately 0.3 and 1 mg/L. Delhaye and Cornet (1975) found the median lethal time for mussels during the spawning period decreased from 9 days at 0.3 mg/L to 3 or 4 days at 1 mg/L. Mortality was also heightened during the reproductive period

The 24 hour LC_{50} for *M. edulis* exposed to cadmium (4 mg/l) is 10 times that for copper (0.4 mg/l) (Lewis *et al.*, 1972). Cadmium (a non-essential element) competes, under high concentrations, with copper in metalloproteins (George and Coombs, 1977). Above trace levels both metals are highly toxic to bivalves such as *Mya arenaria*

(Eisler, 1977) and *C. virginica* (Schuster and Pringle, 1969).

Toxicity is generally heightened by an increase in temperature or a decrease in salinity (McLusky *et al.,* 1986). This is particularly important for bivalves exposed to low metal concentrations; for example, survival of the scallop *Pecten maximus* at low mercury concentrations decreases significantly under conditions of low salinity and high temperature (Paul and Davies, 1986). Similarly, *M. arenaria* survival increases at colder temperatures when exposed to zinc or copper (Eisler, 1977). This effect may partially explain the observation that mortality of oysters exposed to high environmental copper concentrations is greatest during Summer and Spring (Mandelli, 1975).

4.1.2 Sublethal effects

4.1.2.1 Growth

Heavy metals may affect growth directly or by initially affecting another aspect of metabolism such as filtration rates and ventilation (Manley, 1983; Sunila and Lindstrom, 1985). Direct metal accumulation probably accounts for longer term disruption of shell growth (Manley *et al.,* 1984). Heavy metals, including copper, cadmium and zinc, above trace levels generally suppress growth of adult bivalves (eg. Schuster and Pringle, 1969; Manley *et al.,*1984; Sunila and Lindstrom, 1985). However, exposure to low levels of copper (Paul and Davies, 1986) and cadmium (Stromgen, 1982) may enhance bivalve growth.

4.1.2.2 Filtration and respiration

Copper, cadmium, mercury and zinc have all been shown to reduce oxygen consumption in marine invertebrates (Brown and Newell, 1972; Vernberg and Vernberg, 1972; Patel and Anthony, 1991). This may be a response to metal-suppressed filtration rates (Brown and Newell, 1972; Manley, 1983; Patel and Anthony, 1991) or to disruption of mitochondrial processes (Vernberg and Vernberg, 1972; Akberali *et al.,* 1984). Metal exposure may affect mitochondrial metabolism by reducing the number of mitochondria (Vernberg and Vernberg, 1972), disrupting ATP levels or mitochondrial membrane transport (Akberali and Earnshaw, 1982; Akberali *et al.,* 1984).

Abel (1976) reported a 50% reduction in filtration rate of *M. edulis* following exposure to 0.15 mg/l $CuSO_4$. However, short-term exposure of*M. edulis* to 0.5 mg/l Cu inhibited respiration but allowed ciliary movement in the gills (Delhaye and Cornet, 1975). Nonetheless, chronic metal exposure or high environmental levels may inhibit ciliary movement (Brown and Newell, 1972). Oxygen consumption may also be influenced by behavioural responses to metal exposure such as sealing the valves and mantle tissue or exhalent siphon restriction (Manley, 1983).

4.1.2.3 Metal accumulation and induction of protein synthesis

Heavy metal storage is discussed in section 3.4.2. Metal exposure influences subsequent metal accumulation by affecting initial epithelial uptake (sections 2 and 3) and internal processing such as availability of metal binding sites, internal membrane transport and binding protein concentrations.

Formation of another series of proteins, the "heat shock" or "stress" proteins, is induced by acute metal exposure in the gills and haemocytes of *M. edulis* (Steinert and Pickwell, 1988). These proteins generally suppress normal protein synthesis and confer stress resistance on the organism (Lindquist, 1986; Steinert and Pickwell, 1988). Further research into the formation and effects of stress proteins in bivalves is required.

The respiratory protein haemoglobin is induced by cadmium exposure in *Scapharca inaequivalvis* under anoxic conditions (Weber *et al.,* 1990).

4.1.2.4 Reproduction

Exposure to heavy metals generally reduces gonad condition in bivalves (eg. His and Robert, 1987). In a study on the effect of copper and cadmium exposure on *M. edulis* Sunila (1988) described a mechanism for the impact of heavy metals on the bivalve gonad. Following sub-lethal metal exposure (to water containing 0.8 mg/l Cu or 2 mg/l Cd) granular haemocytes entered the male gonad follicles and "phagocytosed ripe sperm". Eventually haemocytes were observed crossing the epithelium by exocytosis leaving irregular, necrosed gonad tissue (Sunila, 1984, 1988). Similarly, exposure of *M. edulis* to high concentrations of copper or cadmium results in vacuolisation of the ova (Sunila, 1984). Maung Myint and Tyler (1982) observed suppression of oocyte growth and development and gamete lysis following exposure to low levels of copper and zinc. Elevated cadmium levels suppress follicle development in *M. edulis* but conversely, stimulate spawning frequency ,thus have a limited impact on gamete production (Kluytmans *et al.,* 1988).

Heavy metal tissue concentration increases during the spawning period due to faster metabolic rates (and therefore uptake rates) (Delhaye and Cornet, 1975) and a decrease in weight, thus concentrating the higher levels of metals present in the gill and mantle (Zaroogian, 1980).

4.1.2.5 Behaviour

Bivalve molluscs exhibit "testing" behaviour when exposed to adverse conditions such as high metal levels or low salinity (Manley and Davenport, 1979; Manley, 1983). That is, bivalves adduct the valves to avoid adverse conditions combined with periodic opening of the valves as a "testing" response. The frequency and period for which the valves open increases with more suitable conditions. Thus, intermittent exposure to elevated levels of metals allows the organism to avoid toxic concentrations whilst maintaining respiration. Constant metal exposure has more severe effects due to less frequent testing behaviour thus increased oxygen demand (Davenport, 1977). For example, Sunila and Lindstrom (1985) found continuous exposure of *M. edulis* to 0.2-0.4 mg/l Cu or 1.25-2.5 mg/l Cd resulted in prolonged valve closure. *M. edulis* also restricts flow through the inhalent and exhalent siphon in response to metal exposure (Manley, 1983).

4.1.2.6 Abnormalities

The development of green pigmentation in oysters in response to copper exposure has been reported by several authors (eg. Schuster and Pringle, 1969; Davies and Paul, 1986). The role of copper in producing these "green-sick" oysters has been recognised as far back as 1713 (Johnston in O'Shaughnessy, 1866). Schuster and Pringle (1969) found copper exposure increased mantle colour whilst cadmium exposure decreased pigmentation of the mantle edge and digestive gland.

Shell deposition is also affected by exposure to high levels of metals. This appears to be a response to disruption of several physiological processes rather than a single process (Sunila and Lindstrom, 1985). Sunila and Lindstrom, (1985) provide detailed descriptions of abnormal shell form in *M. edulis* exposed to copper and cadmium. They reported an increase in abnormal shell formation at an intermediate copper concentration of 0.2 mg/l compared to relatively higher concentrations. At toxic levels all mussels exhibited abnormal shell growth.

Exposure to certain heavy metals has been shown to cause inflammatory lesions in *C. virginica* and *M. edulis* (Austin-Farley, 1988; Sunila, 1988). Sunila (1988) observed haemocyte movement to the mantle haemolymph vessels followed by the appearance of gill and kidney lesions, ulcers and digestive tract hemorrhages after exposure of *M. edulis* to copper. The immunity of *C. virginica* is suppressed following exposure to cadmium or copper ions. Both ions inhibit release of enzymes, which are involved in immune responses, from the haemocytes to plasma (Cheng, 1990).

4.2 EFFECTS ON LARVAL BIVALVES

4.2.1 Lethal effects

In an early study, Wisely and Blick (1966) postulated that bivalve larvae exposed to heavy metals exhibit greater survival rates relative to other invertebrate larvae due to their protective shell. Metal toxicity to the various bivalve life stages is generally: embryos> pediveligers> veligers> spat> adults (embryos being the most sensitive stage) (Boyden *et al.*,1975; His and Robert, 1982; Ringwood, 1990, 1991). In contrast, Calabrese *et al.*, (1977) found that copper is more toxic to *M. mercenaria* larvae than to embryos. Table 2 shows the 48 hour median lethal concentrations for different life-

stages of several bivalve species. Possible explanations for differences in toxicity are greater uptake rates (Ringwood, 1989), efficiency of detoxification mechanisms, or indirect effects such as food availability or quality (His and Robert, 1985). Metal toxicity is generally linearly related to exposure concentration (eg. Conner, 1972). The action of metals on bivalve physiology varies between metals; for example, nickel is less toxic relative to other metals but causes severe effects on growth rates and abnormalities in larval *M. mercenaria* (Calabrese *et al.*, 1977). Cadmium is more toxic than zinc to *C. gigas* larvae and spat (Watling, 1978); in contrast, relative metal toxicities to *C. virginica* larvae are Hg> Cu> Zn> Cd (Calabrese *et al.*, 1973).

TABLE 2. 48 hour LC_{50}'s for different life-stages of bivalves (µg/L)

SPECIES	COPPER	CADMIUM	ZINC	MERCURY	AUTHOR
C. gigas			250	5.7 (EC_{50})	
embryos/					from Deslous-
D-larvae	5.3	611	119	6.7	Paoli (1982).
C. virginica					Calabrese
embryos	103	3800	310	5.6	*et al.*(1973)
M. mercenaria					Calabrese
embryos/	-	-	166	4.8	& Nelson
D-larvae					(1974)
M. edulis					Martin *et al.*
embryos/	5.8	1200	175	5.8	(1981)
D-larvae (EC_{50})					
adults	320	25000	-		Sunila &
					Lindstr. ('85).
M. arenaria					
adults	5000	3400	52000	-	Eisler (1977)

4.2.2 Sublethal effects

4.2.2.1 Growth

Exposure to sublethal concentrations of heavy metals may slow growth of bivalve larvae to the extent that recruitment to the adult population is reduced. Calabrese *et al.*

(1973) suggested that larval bivalves in natural waterways are more susceptible to mortality from factors such as predation and disease than adults.

Walne (1970) observed reduced growth rates in *Ostrea edulis* following exposure to dissolved zinc levels as low as 0.5 ug/l. Similarly, exposure for 7 days to 20 ug/l Cd decreased the growth of 5 day old *C. gigas* larvae by 20% compared to controls (Watling, 1978). Similar observations for the effects of copper on larval size have been observed (eg. His and Robert, 1982; Robert *et al.*, 1982; Beaumont *et al.*, 1987).

4.2.2.2 Metamorphosis and development

In an early study, Prytherch (1934) observed the settlement response of *C. (O.) virginica* to sublethal copper exposure. Of 10 metals tested (Al, Mn, Fe, Ni, Cu, Zn, Ag, Sn, Ba & Pb) only copper elicited this response. Exposure to between 0.05 and 0.6 mg/l Cu induced settlement behaviour, byssal gland secretion and ultimately settlement in mature larvae. Above 0.6 mg/l Cu settlement was induced but abnormal development resulted in increased mortality

In more recent studies, the inhibition of larval development by metal exposure above trace levels has been examined. Zinc sulphate has no effect on *C. gigas* development at 50 ug/l but retards growth at concentrations over 100 ug/l (Brereton *et al.*, 1973). Elevated cadmium levels (> 50 ug/L) reduce settlement of *C. gigas* larvae and those that do settle are younger and less viable than controls (Watling, 1978). McInnes and Calabrese (1979) found copper exposure above 30 ug/L reduced the tolerance of *C. virginica* to suboptimal conditions of temperature and salinity.

Artificial induction of settlement in *C. gigas* larvae by epinephrine heightens sensitivity of spat to sublethal zinc exposure (Robers and Bonar, 1986).

4.2.2.3 Metabolism

There is a lack of studies concerning the effects of metal exposure on physiological processes such as filtration and respiration. Thurberg *et al.*, (1975) found exposure of the clam *Spisula solidissima* to $AgNO_3$ stimulated oxygen consumption of larvae and adults (Deslous-Paoli, 1982).

As with adult bivalves, larval accumulation of heavy metals is probably related to the exposure concentration (Ringwood, 1989). Metal induction of metallothioneins in larval bivalves has been suggested but as yet remains unverified (Beaumont *et al.*, 1987; Ringwood, 1991).

4.2.2.4 Behaviour

Trace level exposure of bivalve larvae to heavy metals generally results in avoidance behaviour. For example, Prytherch (1934) observed intermittent swimming behaviour, retraction of the velum and finally shell closure by *C.(O.) virginica* larvae exposed to elevated copper concentrations. Similarly, *M. edulis* larvae retract into the shell when exposed to copper (Wisely, 1963). Exposure of *Mytilus* and *Crassostrea* to sublethal mercury and copper concentrations results in withdrawal of the foot, turning movements and general avoidance behaviour (Wisely, 1963). Settlement behaviour such as crawling is inhibited by exposure of *C. gigas* pediveligers to zinc; however, larvae that do settle show no adverse effects (Boyden *et al.*, 1975). As discussed in section 4.2.2.2 low copper exposure induces settlement behaviour in *C.(O.) virginica* (Prytherch, 1934).

4.2.2.5 Abnormalities

Larval abnormalities following exposure to heavy metals have been reported at the tissue (Brereton *et al.*, 1973; His and Robert, 1982) and subcellular levels (Stiles *et al.*, 1991). The proportion of abnormal larvae increases with exposure concentration (Brereton *et al.*, 1973; Knezovich, *et al.*, 1981). Perhaps the most obvious abnormality is deformed shell growth. For example. Eyster and Morse (1984) exposed surf clam embryos to various concentrations of $AgNO_3$ immediately following fertilization. After 72 hours larvae from 9.5 ug/l treatments exhibited slowed shell growth and metal-treated larvae had shell valves considerably smaller than larval soft tissue. In contrast, normal D-larvae (the second stage of larval development) developed in control treatments after 24 hours. Exposure of *S. solidissima* gametes to 6.4 ug/L silver resulted in increased morphological abnormalities.

Brereton *et al.* (1973) reported damage to cilia as well as deformed shell growth in *C. gigas* exposed to100 ug/l zinc for 5 days. According to Bayne (1965) the velum is the primary organ affected by copper exposure.

Larval abnormalities may also be due to a high metal body burden of the parent bivalves rather than direct exposure of early life-stages (Zaroogian and Morrison, 1981). In a recent study, *M. mercenaria* cultured in reference sea water but from contaminated parents exhibited more chromosomal abnormalities (eg. polyploidy and mosaic embryos) and retarded larval growth than those from relatively uncontaminated adults. Mutation by prespawned gametes probably only occurs in highly contaminated environments, whilst reversible chromosome abnormalities (eg. chromosome stickiness) occurred following fertilization in embryos reared in less polluted site water (Stiles *et al.*, 1991).

4.3 Effects on gametes and embryos

4.3.1 Lethal effects

Metal toxicity to bivalve embryos is generally in the following order Cu & Hg> Ag> Zn> Cd (Martin *et al.*, 1981). Following hatching, embryos are generally more susceptible to toxicants than larvae. The embryonic membrane is impermeable to metals and thus confers protection on embryos (Calabrese, 1972; McInnes and Calabrese, 1979; Ringwood, 1990, 1991). Similarly, embryonic accumulation of cadmium by *I. californicum* does not occur until post-hatching (Ringwood, 1990). Spawned female gametes are generally more sensitive to heavy metals than ovarian gametes or post-fertilization life-stages (eg. Eyster and Morse, 1984).

Different metal salts may influence the toxicity of a metal to juvenile bivalves; for example, $CuSO_4$ is less toxic than $CuCl_2$ to *C. gigas* embryos (His and Robert, 1982).

As with larval and adult bivalves, metal exposure alters the temperature and salinity tolerance of embryos. However, in direct contrast to larvae, low salinity is more toxic than high temperature to embryos at low copper concentrations (McInnes and Calabrese, 1979). Copper and zinc toxicities to *C. virginica* embryos are additive at 20, 25 and 30 °C (McInnes and Calabrese, 1977).

4.3.2 Development

Copper slows embryonic cell division and development to the D-larval stage (His and Robert, 1982; Johnson, 1988). For example, development of *C. gigas* embryos is slowed above 5ug/l (Knezovich *et al.,* 1991) and most cell division is arrested at 500ug/l Cu (His and Robert, 1982).

Exposure of adult *M. edulis* to copper (0.05 ug/L) suppresses gametogenesis, zinc (0.2 ug/L) is less toxic but nevertheless suppresses oocyte development and cadmium (0.05 mg/L) only influences early gonad development (Maung Myint and Tyler, 1982). Akberali *et al.* (1984) reported significant stimulation (47%) of respiration by *M. edulis* oocytes at 0.5mM Cu^{2+} and inhibition (49%) of sperm respiration by Zn^{2+}.

The cytogenetic effects of metals on bivalve embryos are discussed briefly in section 4.2.2.5.

5. CONCLUSIONS

The physiological responses of bivalves to heavy metal exposure is determined by both environmental and biological factors. Initial metal uptake and accumulation by bivalves largely depends on the chemical form of the metal (particularly the free ion concentration) as well as its physical source ie. water, food or sediments (Riley, 1971; George, 1980). The latter is particularly important to uptake by different species and life-stages with different feeding strategies. Metal speciation and source also affects the specific sites of accumulation. Dissolved metal species mostly accumulate in the gills and kidney, whilst particulate species are taken up predominantly by the digestive gland and stored in the kidney. Bivalve metal accumulation from phytoplankton is generally considered to be relatively minor compared to dissolved metals (King and Davies, 1987); however, high levels of phytoplankton contamination have resulted in significant uptake via feeding (eg. Zhang *et al.*, 1990).

The amount of metal accumulation by bivalves generally increases with adult size but bivalve embryos and larvae take up metals at superior rates to adults (Ringwood, 1991). A lack of knowledge concerning chronic accumulation of metals by larval bivalves is evident. Metals are known to accumulate in adult bivalves in intracellular vesicles located in tissues or haemocytes (George and Pirie, 1980). Specific binding proteins and other compounds sequester metals within cells or in the haemolymph (Simkiss and Mason, 1983). Bivalve metal excretion is predominantly via urine and faecal production as well as by granulocyte diapedesis across the epithelial surfaces (George, 1980; George and Pirie, 1980).

Heavy metals above trace level concentrations exert a range of adverse effects on the physiology of bivalve molluscs. Exposure to sub-lethal concentrations, particularly to essential metals such as copper and zinc, may be beneficial to the growth and development of bivalves (eg Paul and Davies, 1986). However, chronic exposure generally counterracts any short term stimulus to bivalve physiology (eg. Watling, 1978).

Toxic effects of heavy metals on bivalves are generally measured as 48 or 96 hour LC_{50}'s. A general order of decreasing metal toxicity to bivalves is Cu> Hg> Zn> Cd (see Table 1). This order varies slightly between bivalve species and life-stages, probably due to differences in metabolism and feeding strategies.

Sub-lethal effects provide more specific information about the action of heavy metals

on organism metabolism. Limited information is available on the chronic impact of sub-lethal exposure to heavy metals. This may have particular relevence to the commercial shellfish industry and to environmental pollution studies.

Essential metals such as zinc and copper are involved in a range of metabolic functions; in particular, enzyme activation, respiratory proteins, induction of protein synthesis and redox systems (Stryer, 1981; Lindquist, 1986). Interference with these systems occurs when an organism is exposed to excess metal concentrations particularly copper and zinc as well as to toxic metals which either compete with or displace essential metals (Simkiss and Mason, 1983). By disrupting such metabolic processes accumulation of heavy metals causes measurable responses in bivalve physiology generally suppressing filtration, growth, respiration, mortality and reproduction as well as influencing behaviour. Physical and cytogenetic abnormalities are particularly obvious in the sensitive larval stages of bivalves exposed to heavy metals. The rapid developmental responses of bivalve embryos to metal exposure has been frequently employed in bioassay tests for water quality.

Due to metal-induction of binding proteins, physiological responses are generally a function of metal exposure levels and the exposure history of the individual. Storage and detoxification mechanisms in adult bivalves have been discussed by numerous authors; however, a lack of information concerning metal accumulation and detoxification mechanisms in juvenile bivalves is evident.

6. RESEARCH PROPOSAL

Although numerous authors have examined bioaccumulation of heavy metals and their physiological impact on bivalves, the majority have been restricted to acute effects, particularly on adult bivalves. Studies of larval bivalves have mostly concerned the acute effects of metals on mortality, growth and development. However, chronic studies provide more information on the action of toxic metals on organism physiology and are more ecologically realistic (Beaumont *et al.*, 1987).

The proposed study will be an investigation of the effects of cadmium exposure on the juvenile stage (spat) of the commercially important bivalve species *Crassostrea gigas*. Cadmium has been shown to be highly toxic to oyster larvae (Watling, 1978) and to cause abnormalities in growth and development in adult bivalves. Initial chronic dose-response curves will be established for the effect of cadmium on spat survival. The influence of constant exposure to environmentally realistic (see table 1) salt-water concentrations of cadmium will then be examined. Growth, physiology and survival in response to chronic cadmium exposure will be assessed. In addition, cadmium accumulation levels will be determined and compared to values for adult bivalves from previous literature.

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