STUDIES ON THE TOXICITY OF CADMIUM, COPPER AND ZINC TO THE BROWN SHRIMP, CRANGON CRANGON (L.).

being a Thesis submitted for the degree of Doctor of Philosophy in The University of Hull.

by

ROBIN KEVIN JOHN PRICE, B.Sc.

BEST COPY AVAILABLE.

VARIABLE PRINT QUALITY
TO MY PARENTS
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Chapter</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>GENERAL INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>GENERAL MATERIALS &amp; METHODS</td>
<td>10</td>
</tr>
<tr>
<td>CHAPTER 1. THE INFLUENCE OF SOME ABIOTIC FACTORS ON</td>
<td></td>
</tr>
<tr>
<td>THE TOXICITY OF CADMIUM, COPPER AND ZINC TO CRANGON CRANGON (L.).</td>
<td></td>
</tr>
<tr>
<td>Introduction</td>
<td>20</td>
</tr>
<tr>
<td>Materials &amp; Methods</td>
<td>24</td>
</tr>
<tr>
<td>Results</td>
<td>30</td>
</tr>
<tr>
<td>Discussion</td>
<td>35</td>
</tr>
<tr>
<td>CHAPTER 2. MOULT-STAGE DETERMINATION IN</td>
<td></td>
</tr>
<tr>
<td>CRANGON CRANGON (L.).</td>
<td></td>
</tr>
<tr>
<td>Introduction</td>
<td>42</td>
</tr>
<tr>
<td>Materials &amp; Methods</td>
<td>44</td>
</tr>
<tr>
<td>Results &amp; Discussion</td>
<td>47</td>
</tr>
<tr>
<td>CHAPTER 3. THE INFLUENCE OF SOME BIOTIC FACTORS ON</td>
<td></td>
</tr>
<tr>
<td>THE TOXICITY OF CADMIUM, COPPER AND ZINC TO CRANGON CRANGON (L.).</td>
<td></td>
</tr>
<tr>
<td>Introduction</td>
<td>51</td>
</tr>
<tr>
<td>Materials &amp; Methods</td>
<td>53</td>
</tr>
<tr>
<td>Results</td>
<td>56</td>
</tr>
<tr>
<td>Discussion</td>
<td>62</td>
</tr>
</tbody>
</table>
CHAPTER 4. THE UPTAKE OF CADMIUM, COPPER AND ZINC INTO VARIOUS TISSUES OF CRANGON CRANGON (L.).

Introduction
Materials & Methods
Results
Discussion

CHAPTER 5. THE EFFECTS OF CADMIUM, COPPER AND ZINC ON THE CARDIAC AND VENTILATORY ACTIVITIES OF CRANGON CRANGON (L.).

Introduction
Materials & Methods
Results
Discussion

GENERAL DISCUSSION

SUMMARY

ACKNOWLEDGMENTS

BIBLIOGRAPHY

All relevant Tables and Figs. are at the end of each Chapter.
GENERAL INTRODUCTION.

In conclusion, copper is an essential element for several living processes, having first been associated with life in studies of blood proteins of high purity (Kendall, 1941; cited in Greaves, 1976). Since then, the functional role of copper in the copper transport of the circulatory and intestinal hepatocytes has become well documented (Greaves, 1976). Copper is also seen to be an important constituent of certain enzymes such as tyrosinase and cytochrome oxidase (Scott & Major, 1975) and as an activator for certain catalysts (e.g., catalase, superoxide dismutase, SOD, and superoxide dismutase, SOD1). Furthermore, copper has been shown to be a necessary component for the successful growth of plants.
GENERAL INTRODUCTION.

During the last two decades, much public interest and concern has been expressed on the presence of metal ions in the environment and, particularly, in organisms of commercial significance. The concern has arisen because the amounts of such ions has, in local and sometimes general areas, increased to levels which are far higher than 'normal' background values.

The present thesis is concerned with studies on the effects of 3 metals on the brown shrimp, *Crangon crangon* - a species which is common in the inshore waters of the Yorkshire coast and which has a commercial significance to a number of local fisheries.

Of the metals selected for study, cadmium is known to be neither biologically essential nor beneficial to living organisms (Eisler, 1971; Thorpe & Lake, 1974; Bryan, 1976; Pascoe & Mattey, 1977). Consequently, this metal is absent, or present only in trace amounts, in organisms from unpolluted waters.

By contrast, copper is an essential element for several living processes, having first been associated with such in studies of blood proteins of *Helix pomatia* (Harless, 1847 - cited in Severy, 1923). Since then, its functional significance in the oxygen transport of crustacean and molluscan haemocyanins has became well documented (Redmond, 1955). Copper is known also to be an important constituent of certain enzymes such as tyrosinase and cytochrome oxidase (Scott & Major, 1972) and as an activator for certain others (e.g. malate dehydrogenase; Saliba & Krzyz, 1976). Furthermore, copper has been shown to be a necessary component for the successful accomplishment
of specific behavioural and physiological phenomena (e.g. the settling and metamorphosis of the oyster, *Crassostrea virginica*; Prytherch, 1931). Possibly, the ubiquitous distribution of this metal accounts for its wide biological functions in organisms.

Zinc also has been found to occur naturally in the tissues of many marine organisms (Bodansky, 1920) and, subsequently, has been shown to be an essential element in many metal-enzymes (Dixon & Webb, 1964). These enzymes include carbonic anhydrase (Keilin & Mann, 1940; Vallee, 1959; Coombs, 1972), alkaline phosphatase (Vallee, 1962; Wolfe, 1970; Coombs, 1972), carboxypeptidase (Vallee, 1962; Coombs, 1972), glutamate dehydrogenase, lactic dehydrogenase, alcohol dehydrogenase (Vallee, 1959) and $_\alpha$-D- mannosidase, (Coombs, 1972). Parker (1962) has suggested that the capacity of organisms to concentrate zinc reflects the biological function of this metal. However, as with copper, zinc is usually found in tissues in quantities far in excess of those required to satisfy the needs of enzymes. Coombs (1972), for example, used data of Vallee & Wacker (1970) to estimate that the oyster, *Ostrea edulis* used only 0.1% or less of its total zinc content for enzyme purposes.

Heavy metals are normal constituents of estuarine and marine environments and are usually present in trace amounts. Normally, they reach the sea via rivers following the erosion of rocks. However, with the advent of industrialization, man has contributed to the base levels of metals found in coastal waters. Cairns, Dickson, Sparks & Waller (1970) summarised the industrial attitude as "the production of wastes by industry is not related to the capacity of the ecosystem to absorb and transform these wastes, but rather to market demand."
Hence, large quantities of cadmium, copper and zinc, among other metals, have found their way in estuarine and coastal waters, mainly from copper and lead mines (McKee & Wolfe, 1963; Mount & Steven, 1967) and zinc smelting and electroplanting plants (Little & Martin, 1972; Jordan, 1975).

By virtue of their physiogeochemical nature, estuaries have the capacity (albeit to a limited extent) to 'detoxify' heavy metals by altering their biological availability. This is achieved by absorption to particulate material (Krauskopf, 1956) and by precipitation, chelation and sedimentation (Lewis, Whitfield & Ramnarine, 1972; Whitfield & Lewis, 1976; Batley & Gardner, 1978). However, those metal species which remain dissolved in seawater are likely to escape to the open sea (van Bennekom, Gieskes & Tijssen, 1975) and, if present in sufficiently high concentrations, are likely to be directly toxic to the fauna and flora.

Many organisms, especially sessile bivalves, can accumulate cadmium, copper and zinc and tolerate high concentrations of these in their tissues without any apparent signs of harm (Brooks & Rumsby, 1965). This suggests that such organisms have very efficient methods for preventing these metals from poisoning essential enzyme systems. Other organisms (e.g. Paratya tasmaniensis; Thorpe & Lake, 1974) do not have the ability to tolerate these metals and are killed by very low concentrations of them. There appear to be few reliable data available on the susceptibility of *C. crangon* to heavy metals and these studies were undertaken to provide comprehensive data on the toxicity of cadmium, copper and zinc to this species.
One widely used and accepted method for the assessment of the effects of pollutants to organisms, is that of toxicity testing (sometimes referred to as 'bioassays'). When death is used as the criterion of response in such studies, the method suffers serious disadvantages in that accuracy is limited because of the wide disparity of individual susceptibility. However, in toxicity studies, it is accepted universally that the most tolerant and the most susceptible individuals in a test group show greater variability of response than individuals near the median of this group. Consequently, the relevant studies in this thesis are concerned predominantly with the median responses to the test parameters (i.e. the responses of the average individual). The median lethal concentration (LC\textsubscript{50}) is the term used generally to describe the concentration at which 50% of the test population are killed (Alderdice, 1967; Brown, Jordan & Tiller, 1967; Sprague, 1969; Eisler, 1971). In situations where time is the effect parameter, the median lethal time (LT\textsubscript{50}) is used to describe 50% mortality value. Concentration and time, however, are here inextricably linked and to maximise their usefulness in comparative studies, LC\textsubscript{50} values need to be qualified by a prefixed time component. APHA (1965) has suggested that the time component may be expressed as hours, days or weeks, whichever is convenient in particular circumstances. Similarly, LC\textsubscript{50} values should be qualified by the concentration of the toxicant used. Median lethal concentrations and LT\textsubscript{50} values are determined by graphical means from plots of concentration or time respectively against the percentage mortality of the test population at specific times (LC\textsubscript{50}) or concentration (LT\textsubscript{50}).

Brown (1973) suggested that 'quantal' bioassays (using
concentrations and percentage mortalities) are superior to 'quantitative' bioassays involving exposure times and percentage mortalities. His reasoning is based on the fact that quantal bioassays yield mortality curves which are amenable to mathematical definition and thus enable confidence limits to be given in terms of units of concentration. On the other hand, quantitative bioassay mortality curves represent subjective estimates of effective concentrations. However, when experimental methodology imposes limitations to the design of experiments (e.g. in flow systems which permit but one concentration at a time to be tested) then quantitative methods offer an acceptable alternative.

A very important concept in toxicity studies is that of incipient lethal levels (ILL). Sprague (1969) defined an ILL as "that level of the environmental entity beyond which 50% of the population cannot live for an indefinite time." The same concept has been named the 'lethal threshold concentration' (Lloyd & Jordan, 1963) or the 'asymptotic LC50' (Ball, 1967a). These values or levels, however, should not be construed as safe levels as they represent the concentrations which would kill the average specimen of the test organism, on long exposure. In practice, the value for any particular toxicant is obtained from a composite plot of a series of LC50s at various exposure times - the ILL is the LC50 value at which the resulting plot becomes asymptotic to the exposure-time axis.

The standardisation of toxicity tests has been discussed by several authors. Brown (1973) has stated "as there is nothing 'standard' about any poison, animal or exposure in the environment, no 'standard' test can be advocated. The only standards to be applied in toxicity
testing are those of good experimental technique and sound scientific practice. The more standardised the test or the test organism, the less applicable the information is likely to become." For practical and comparative purposes, however, some standardisation of methodology is necessary. Therefore, the initial toxicity tests, described in Chapter 1 of this thesis represent an attempt to assess the effects of certain abiotic variables (e.g. static water, continuously flowing water, presence or absence of a sand substratum) which may affect the toxicity of cadmium, copper and zinc to C. crangon. The aim of these particular aspects of the present investigations was to maximise accuracy and convenience for subsequent toxicity tests.

Until recently, little attention seems to have been paid to the influence of environmental variables in toxicity testing of heavy metals to crustaceans. Although an extensive literature exists on temperature effects on the toxicity of pollutants to fish (for review, see Doudaroff & Katz, 1953) the results are equivocal and emphasise the need for short-term and long-term studies to be carried out on individual species.

Much attention has centred on the effects of salinity on various aspects of crustacean activities (e.g. Prosser, Green & Chow, 1955; Dehnel, 1960; Hagerman, 1970; Spaargaren, 1973). These reports, however, include little on the effects of salinity on the toxicity of pollutants and the few such reports that exist include those of Jones (1974, 1975) and Vernberg, Decoursey & O'Hara (1974). The effects of temperature and salinity on the toxicity of cadmium, copper and zinc to C. crangon have been investigated here and the results are described in Chapter 1 of this thesis.
The uptake, subsequent translocation and removal of pollutants in marine organisms will be allied closely to the metabolic disposition of the test organisms. Consequently, the rates of these activities will be affected not only by the environmental variables mentioned above, but also by the physiological status of the test organisms.

The moult cycle of crustaceans is known to be accompanied by profound changes to several aspects of the physiology of these organisms. Moulting can be taken to include not only the shedding of the old exoskeleton but also the physiological and behavioural changes that precede and succeed it. A convenient method for the determination of stage in the moult cycle of decapod crustaceans has been to follow the growth of new setae in the uropods. Several workers (e.g. Drach, 1944; Passano, 1960; Scheer, 1960; Drach & Tchernigovtzeff, 1967) have proposed certain criteria which distinguished the various moult stages in particular species of decapod. Although certain generalizations are common to this large taxonomic group, many inter specific differences exist. Furthermore, a full description of the various moult stages of *Crangon crangon* does not appear to exist and the aim of the work described in Chapter 2 of this thesis was to provide a clear description of the criteria which characterise the various moult stages in this species. To support this work, the opportunity was taken to assay the concentration of haemolymph total protein, and chloride ion and whole body water content of animals sampled at the various moult stages and data has been discussed in relation to similar, published information.

As *Crangon crangon* is a species which moults frequently throughout the year and, as the rate of the uptake of ions may vary according to moult stage, moult stage as a biotic variable which may affect the
toxicity of the test metals to this species, has been studied. This aspect of these studies, and the effects of other biotic variables (sex, size and reproductive condition) on the toxicity of the metals, are included in Chapter 3 of this thesis.

The literature includes reports which show that, within marine organisms, there are a number of modes of uptake and loss of heavy metals and that these vary even within taxonomic groupings. Certain species are known to rely on absorption from the external milieu for the uptake of ions (see Bryan, 1971; Penreath, 1973a; Bryan & Hummerstone, 1973) whilst other species ingest the metals with their food (see Bryan & Ward, 1965; Bryan, 1967; Young, 1974).

In decapod crustaceans, some regulation of essential metals such as copper (Zuckerkandl, 1960; Bryan, 1968; Djangmah, 1970) and zinc (Bryan, 1964, 1967) does occur. Regulation of metals requires both input and output elements and, as with metal uptake, ways that metals are lost varies between species - some species excreting excess ions via the gills (Wright, 1977a) and others voiding them via the urine or faeces (Bryan, 1967). Between the time of uptake and loss, excess metals are sometimes stored - usually in the form of granules in hepatopancreas tissue (Djangmah, 1968, 1970; Djangmah & Grove, 1970).

The aim of the work described in Chapter 4 of this thesis was to elucidate possible paths of uptake and internal translocation of copper, cadmium and zinc in _C. crangon_. The studies also aimed to determine whether the biologically non-essential metal, cadmium, is voided in this species. Within these studies, the opportunity was taken to examine the particular tissues associated with uptake and storage (gills and hepatopancreas respectively) to see whether the
metals caused any gross morphological or ultrastructural damage to them.

Oxygen consumption of crustacean gill tissue has been found to be depressed on treatment with some heavy metals (e.g. cadmium, Thurberg, Dawson & Collier, 1973; Collier, Miller, Dawson & Thurberg, 1973). However, no such depression has been found for whole animal oxygen consumption (Vernberg, Decoursey, Kelly & Johns, 1977; Collier et al, 1973). Recently, Cumberlidge (1977) and Dyer (1978) have modified impedance techniques so that they are suitable for continuous and simultaneous monitoring of cardiac and ventilatory activities in species like C. crangon. Chapter 5 of this thesis, describes the effects of acute exposures to high concentrations and chronic exposures to low concentrations of the 3 test metals to the qualitative and quantitative beating behaviour of the heart and scaphognathites of C. crangon. The use of cardiac and ventilatory responses as sensitive indicators of pollution stress was assessed and discussed in Chapter 5.
GENERAL MATERIALS & METHODS.

Both black populations were fed every other day on chopped 

Pt Kt 1,468.

generally, animals were left for at least one week before

experimentation, after which they were transferred to the experimental

conditions. They were then kept for a further 5 days without being fed

(unless otherwise stated). This second acclimation period was utilized
to facilitate the inclusion of control animals at the beginning of any

experiment.

2. Plastics.

Plastic "Fiji" aquariums (30 x 17 x 19.5 cm) were divided

into 12 compartments using "Fermap" partitions. In addition of 1/4 of

marked aquarium, each test during two a tank of 1.5 cm 1.5 cm by 1.5 cm.
GENERAL MATERIALS AND METHODS.

1. Animal husbandry.

Specimens of *Crangon crangon* (L) were collected throughout most of the year from Filey Bay, North Yorkshire (grid reference TA 122808). Collections were made within half an hour either side of low tide, using a hand beam trawl (1.5m across) equipped with two tickler chains. Animals were transported in oxygenated seawater in an insulated container to the seawater aquarium where they were kept in large, plastic aquaria (70 x 45 x 30cm) supplied with approximately 5cm layer of sand substratum and recirculating seawater. The water temperature and lighting regime were adjusted periodically, so as to correspond approximately to that pertaining in the natural environment.

This stock population was fed every other day on chopped *Mytilus edulis*.

Generally, shrimps were left for at least one week before experimentation, after which they were transferred to the experimental conditions. They were then left for a further 4 days without being fed (unless otherwise stated). This second acclimation period was utilized to obviate the inclusion of moribund animals at the beginning of any experiment.

2. Static tank bioassays.

Plastic 'Fiji' aquaria (39 x 17 x 19.5cm) were divided into 12 compartments using 'Perspex' partitions. On addition of 4 l of filtered seawater, each test shrimp had a tank surface area to volume
ratio of 1 : 7. An air stone on clear PVC line was supplied to the centre of each tank and preliminary tests had shown that this was sufficient to ensure full oxygenation of all parts of the tank. Oxygen levels (as % saturation) were monitored using an E.I.L. Portable oxygen meter, No. 1520.

Cadmium, was added as $3\text{CdSO}_4 \cdot \text{H}_2\text{O}$ (Analar grade); copper as $\text{CuSO}_4$ (Analar grade) and zinc as $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ (Analar grade). Mixing was tested using carmine dye and found to be constant throughout the tank. Metals were introduced at a calculated added concentration, and atomic absorption analysis of all test solutions was standard procedure in the final determination of these metal concentrations.

All the tanks were covered, and solutions were changed every 48 hrs.

3. Measurement of some allometric parameters of *C. crangon.*

(a) **Wet weight.** Excess moisture was removed by rolling the shrimp in paper tissue. Wet weight was then determined (nearest 0.1mg) on a Mettler P161 top-pan balance.

(b) **Dry weight.** Whole shrimps or single organs were dried to constant weight at 110°C for approximately 18 hrs (Poolsanguan, 1975). Dry weight was determined (nearest 0.1mg) on a Mettler P161 top-pan balance.

(c) **Length.** Length measurement (nearest mm) was taken from the tip of the rostrum to the end of the telson (Lloyd and Yonge, 1947).

(d) **Sexual dimorphism.** Sexes were separated on the basis of difference in morphology of the first and second pleopods. Tiews (1954) and Boddeke (1961), (both cited in Tiews, 1970)
maintain that the length of the endopodite of the first pleopod is not a definitive means of sexual differentiation in C. crangon below 40mm long. However, the author has found this criterion to hold true for specimens down to 30mm in length. Less convenient, is the microscopic examination of the endopodite of the second pleopod. In males, this appendage is biramous, the inside ramus being the appendix masculina (Nouvel, 1939).

(e) Moulting-staging. Crangon crangon were divided into twelve stages of the moult cycle; Postmoult A and B; Intermoult C, and C and Premoult D, D, D, D, D, D, D, as suggested by Scheer (1960). His criteria were modified and a complete description of each stage is given in a definitive study of the moult cycle in Crangon crangon (L) and forms the basis of Chapter 2.

4a) Atomic Absorption Spectrophotometry.

Atomic absorption spectrophotometry was carried out on a Perkin Elmer Model 103, coupled to a Perkin Elmer Model 56 chart recorder using the operating parameters given below:

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Wavelength (nm)</td>
<td>110 (228·8)</td>
<td>280 (324·8)</td>
<td>084 (213·9)</td>
</tr>
<tr>
<td>Slit Setting</td>
<td>0·7 nm</td>
<td>0·7 nm</td>
<td>0·7 nm</td>
</tr>
<tr>
<td>Light Source</td>
<td>Hollow Cathode Lamp</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flame Type</td>
<td>Air-acetylene Flame Oxidising, (lean blue).</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Stock standard solutions were obtained from Fisons Scientific Apparatus as:

(i) Cadmium, 1000mg l\(^{-1}\) (in N hydrochloric acid).
(ii) Copper, 1000mg l\(^{-1}\) (in N nitric acid).
(iii) Zinc, 1000mg l\(^{-1}\) (in N hydrochloric acid).

Dilute standards were made up, as and when required.

4b) **Determination of metals in seawater by chelation and concentration.**

Quality control checks were carried out on the seawater from collection sites and from the aquarium, using the following method:-

4ml of concentrated HCl were added to 50ml of seawater in a 500ml 'Quick-fit' distillation flask. The contents were evaporated to dryness and made up to 5, 10 or 15ml volumetrically, (depending on concentration of metal). Standards and blanks were made up in solutions containing salts (Analar grade) in the following concentrations:

- Sodium .......... 10,505mg l\(^{-1}\)
- Potassium .......... 382mg l\(^{-1}\)
- Magnesium .......... 1,356mg l\(^{-1}\)
- Calcium .......... 409mg l\(^{-1}\)

These solutions were then treated in the same way as the seawater samples.

4c) **Determination of metals in seawater, by direct aspiration.**

If concentrations of metals in contaminated seawater fell within the linear working range of the particular metals, seawater was aspirated directly into the atomic absorption spectrophotometer (AAS). If, however, the solutions were too concentrated they were diluted with
distilled water to an extent commensurate with the linear range of the metal in the AAS. Seawater solutions containing very low concentrations of metals were treated as above (Section 4b).

Standards and blanks were made up in pure, filtered seawater (or dilutions thereof).

This process is extremely rapid, in that no treatment (other than dilution) of samples is necessary. Hence, it was used extensively as the standard daily procedure for monitoring levels of metals in experimental tanks.

4d) **Background interference.**

Types encountered in this study were:

(i) **Condensed-phase (chemical) interference.**

This type of interference occurred in the analysis of calcium in tissue with high concentrations of phosphates. It was overcome by the addition of a releaser agent, such as 1% lanthanum, to all samples, standards and blanks and ensuring that the pH of all 3 remained constant.

(ii) **Matrix interference.**

In the analysis of tissue dissolved in small volumes of acid, the viscosity of standard and sample solutions differed. Erroneous results were minimised by the method of standard addition and subsequent calculation of the result, using the following formula:
\[ C_s = \frac{C_a \cdot R_s \cdot V_a}{V_s(R_a - R_s)} \]

where:

- \( C_s \) = concentration of sample element.
- \( C_a \) = concentration of element in standard solution, used to "spike" the diluted sample solutions.
- \( V_s \) = volume of original sample solution.
- \( V_a \) = volume of standard solution used (ml).
- \( R_s \) = reading obtained for diluted sample.
- \( R_a \) = reading obtained for 'spiked' sample.

In the analysis of seawater containing high concentrations of dissolved salts, matrix effects, (in this case, light scattering caused mainly by calcium and sodium atoms) cannot be corrected for, by standard additions (Billings, 1965). Slavin (1964) suggested correction by the subtraction of the measured signal on a non-absorbing spectral line near that of the absorption line of the tested element. Billings (1965) found no satisfactory non-absorbing lines for cadmium or zinc, so control effects adopted in this thesis, were by matching the concentrations of dissolved salts in samples, standards and blanks (section 4b).

The author appreciates that this method has it's shortcomings and possibly results err on the high side. However, this method was used throughout the work so results will be relative.

5. **Statistical treatment of data.**

(I) **Treatment of toxicity data.**

Data pertaining to toxicity studies has been treated.
according to the rapid graphical methods of Litchfield (1949), (Time-percent effect curves) and Litchfield and Wilcoxon (1949), (Dose-effect curves). Both these methods involve the plotting of arithmetic data (concentration/time and percentage mortality) directly onto logarithmic-probability paper (Chartwell graph data ref: 7508) and a straight line drawn through the points by eye.

Evaluation of LC50, LD50, 95% confidence limits and between curve comparisons were as follows:-

(A) **Dose-effect curves.**

(a) Heterogeneity (or goodness of fit) was calculated using \((\text{Chi})^2\).

The \((\text{Chi})^2\) of the line was obtained by

\[
\frac{\sum (O - E)}{N/K}
\]

where \(O\) = observed value.
\(E\) = expected value
\(N\) = number of animals
\(K\) = number of different concentrations
and \(n = K-2 = \text{degrees of freedom.}\)

(b) The LC50 was obtained directly from the graph, by reading the concentration at which 50% mortality was observed.

(c) Slope function (S), (the equivalent of the Standard Deviation) was calculated by:

\[
S = \frac{LC_{84}/LC_{50} + LC_{50}/LC_{16}}{2}
\]

(d) The factor for LC50 \((f_{LC50})\) is equal to the anti-logarithm of the Standard Error and is calculated as:

\[
f_{LC50} = \text{antilog} \left(2.77 \frac{s}{N_1}\right) = S^{2.77/N_1}
\]

where \(N_1 = \text{the total number of animals tested whose expected effects were between 16 and 84 percent.}\)
Thus the confidence limits of the LC50 are obtained by:

\[
\text{LC50} \pm f_{\text{LC50}} = \text{upper and lower limits for 95% probability.}
\]

(e) The factor for \( S \).

The concentration range (R) is as follows:

\[
R = \frac{[\text{highest}]}{[\text{lowest}]}
\]

using R and S (the Slope function) the value of A is obtained from a Nomograph (Litchfield, 1949).

Thus \( f_S \) is calculated as:

\[
(f) \quad f_S = \frac{10(K - 1)}{K/N^1}
\]

and hence the confidence limits of \( S \) are:

\[
S \times f_S = \text{upper and lower limits at 95% confidence limits}
\]

(f) Test for parallelism using the slope function ratio (SR)

\[
\text{SR} = \frac{S_1}{S_2} \text{ where } S_1 \text{ is the larger value.}
\]

Using \( f_{S_1} \) and \( f_{S_2} \), \( f_{SR} \) is obtained from a Nomograph (Litchfield, 1949).

If the value of SR exceeds \( f_{SR} \) then the curves deviate significantly \((P<0.05)\) from parallelism.

(g) Test for comparative potency using the potency ratio (PR).

\[
\text{PR} = \frac{\text{LC50}_1}{\text{LC50}_2} \text{ where } \text{LC50}_1 \text{ is the larger value.}
\]

Using \( f_{\text{LC50}_1} \) and \( f_{\text{LC50}_2} \), \( f_{PR} \) is obtained from a Nomograph (Litchfield, 1949).

If the values of PR exceeds \( f_{PR} \) then the 2 metals being compared differ significantly \((P<0.05)\) in potency.
(B) **Time-percent effect curves.**

Obtaining the parameters and confidence limits for these types of curves was essentially the same as for dose-effect curves i.e.

\[ \text{LT}_{50} \text{ read directly from graph} \]

\[ S = \frac{\text{LT}_{84}/\text{LT}_{50} + \text{LT}_{50}/\text{LT}_{16}}{2} \]

\[ f_{\text{LT}_{50}} = S \cdot 1.96/\sqrt{N} \]

\[ f_{S} = S \cdot 1.96/\sqrt{2N} - 1 \]

and the 95% confidence limits were obtained by multiplication and division of the parameter by their respective factors.

If, however, 100% of the test organisms did not react, then provision is made for degree of truncation by obtaining values from a Nomograph (Litchfield, 1949) so as to vary the values of \( \sqrt{N} \) and \( \sqrt{2N} - 1 \) to a degree commensurate with the degree of truncation.

The test for parallelism and reaction time ratios (RR) are exactly as for dose-effect curves.

Eisenberg (1952) compared the Litchfield and Wilcoxon methods to the much more involved probit analysis of Bliss (1935) and concluded that the median effective doses were in very good agreement. The 19/20 probability interval lines (95% confidence limits) were not in such close agreement, but those calculated by the method of Litchfield and Wilcoxon, in most cases, tended to err on the high side.

(II) **Comparison of 2 means.**

Testing of 2 sample means was carried out using the
following formula (from Zar, 1974).

(i) \[ t = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\frac{S_p^2}{n_1} + \frac{S_p^2}{n_2}}} \]

where

(ii) \[ S_p^2 = \frac{SS_1 + SS_2}{V_1 + V_2} \]

\( S_p^2 \) = pooled variance
\( SS \) = sum of squares
\( V \) = degrees of freedom

(III) Analysis of variance.

Single factor analysis of variance was calculated using the programme supplied by Hewlett Packard for use with HP 97 calculator.
CHAPTER 1.

THE INFLUENCE OF SOME ABIOTIC FACTORS ON
THE TOXICITY OF CADMIUM, COPPER AND ZINC
TO CRANGON CRANGON (L.).
INTRODUCTION.

In toxicity studies with aquatic animals the validity of using small, static volumes of test solutions has been questioned on the grounds that the animals themselves may alter their environmental conditions appreciably (e.g. by excretion of waste products or alteration of dissolved gas levels, Connor & Wilson, 1972, or by altering the pH, Pagenkopf, Russo & Thurston, 1974). It may be argued also that different toxicants would produce within and between species variability of reaction. Thus Lincer, Solon & Nair (1970) tested the toxicity of two insecticides to fish and found that Endrin was less toxic under static conditions than in a continuous flow system; but the converse effects pertained with DDT. Ahsanullah (1976) found the LC50 values for cadmium and zinc with Palaemon sp., Paragrapsus quadridentatus and Mytilus edulis planulatus were generally higher under continuous flow conditions than with static tank conditions. The effects of providing a suitable substratum (e.g. sand for C. crangon) as an element of experimental design does not appear to have received much attention from other workers, even though it is known that appropriate substrata satisfy thigmotropic requirements of many species, including C. crangon (Hagerman, 1970).

Many species of aquatic organisms, including C. crangon, are known to exhibit activity rhythms phased to the pertaining photoperiod (Naylor, 1960; Rodriguez & Naylor, 1972; Hörlyck, 1973). Thus C. crangon is predominantly a night active animal (Hagerman, 1970; Al-Adhub & Naylor, 1975) which spends much of the daylight period buried in the substratum.
Because the metabolic status of the animal will vary in direct accordance with the activity rhythm, there is a possibility that mortality due to toxicants may vary in a corresponding manner. Because of the absence of information on this aspect of toxicity studies, the present experiments were designed to assess possible diurnal variability in susceptibility.

Apart from the photoperiod changes, the seasons of the year in Britain are characterised also by marked seawater temperature changes. The major part of an extensive literature on temperature (usually as acclimation temperature) effects on the toxicity of pollutants, refers to work carried out on fish. In short-term tests of the effects of zinc to salmon, Sprague (1964) found that mortality was directly related to temperature. Subsequent work (Sprague, 1970) showed the converse relationship pertains when incipient lethal levels were used. However, it is clear that no generalisations can be made concerning temperature-and concentration-dependent responses of fish to toxicants, as the works of Pickering & Henderson (1966) and Brown, Jordan & Tiller (1967) show that other species of fish and other toxicants produce different combinations of concentration/temperature responses.

In toxicity studies with crustaceans, the general conclusion arising from short-term effects of heavy metals is that toxicity is directly related to temperature (Portmann, 1968; O'Hara, 1973a; Jones, 1975). However, McLeese (1974) on investigation of long-term effects of copper to Homarus americanus found that temperature had no effect on the incipient lethal level, although, in short-term tests, it was more toxic at 13°C than at 5°C.
Aquatic poikilotherms will operate optimally at their temperature of acclimation (Fry, 1947) and, in these studies, animals have been tested for temperature-dependent effects at times of the year when the chosen temperatures were ambient at the site of collection. The rationale for this design was to obviate possible error arising from incompletely acclimated animals.

Mercury (Jones, 1973), and copper (Jones, 1975) have each been found to react synergistically with salinity and temperature to result in increased toxicity of the metals. Evidence exists also that the toxicity of cadmium increases when salinity and temperature become an environmental stress (Eisler, 1971; Thurberg, Dawson & Collier, 1973; Thorp & Lake, 1974; Rosenberg & Costlow, 1976). Also, salinity-dependent tolerance to metals has been shown to vary according to ecological habitat in isopods; euryhaline species being less susceptible than fully marine forms (Jones, 1975).

**Crangon crangon** is a euryhaline, osmoregulating species which is known to undergo seasonal migrations from offshore waters in winter to warm, shallow, less saline inshore waters in spring and summer. Such behaviour can be interpreted as a means of maintaining at a constant value, the difference in osmotic pressure between the blood and the external medium (Verway, 1957). One of the physiological adaptations used by *C. crangon* to maintain its internal milieu hypertonic to the external medium is that of active uptake of inorganic ions to replace those lost by diffusion or via the urine. Inadvertently, this strategy may result in the uptake of toxic ions, should these be present in the external medium. This possibility was investigated in this section of the present thesis by assessing the differences in median lethal times obtained at different salinities, for each of the
three metals (cadmium, copper and zinc), when these were present in concentrations each corresponding to the respective 96 hr LC50 value.
1. **General procedures.**

Specimens of *C. crangon* were chosen at random from the holding tanks (see General Materials and Methods page 10). Twelve shrimps were transferred to each experimental and control tank where they were maintained for 4 days before test metals were added. Shrimps were not fed after their removal from the holding tanks. Each day the number of dead shrimps were recorded for each experimental and control tank, and any corpses were removed. Generally, experimental and control solutions were replaced every 48 hours.

2. **96 hour LC50 Determinations.**

These experiments were carried out using static tanks (see General Materials and Methods, page 10). The test metals, cadmium, copper and zinc, were added as the sulphates to the tanks so that the following concentrations were obtained in 4 l of test solution:

<table>
<thead>
<tr>
<th>Cadmium (mg l⁻¹)</th>
<th>Copper (mg l⁻¹)</th>
<th>Zinc (mg l⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.10</td>
<td>1.0</td>
<td>6.0</td>
</tr>
<tr>
<td>0.15</td>
<td>2.0</td>
<td>10.0</td>
</tr>
<tr>
<td>0.25</td>
<td>4.0</td>
<td>12.0</td>
</tr>
<tr>
<td>0.50</td>
<td>6.0</td>
<td>18.0</td>
</tr>
<tr>
<td>0.75</td>
<td>10.0</td>
<td>24.0</td>
</tr>
<tr>
<td>1.00</td>
<td>15.0</td>
<td>30.0</td>
</tr>
<tr>
<td></td>
<td>25.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>50.0</td>
<td></td>
</tr>
</tbody>
</table>

The experiments were performed at a water temperature (T) of 16°C; salinity, S, 34%, and photoperiod, LD 14:10 hr. Each test concentration was run in duplicate and duplicate control groups of 24 animals were set up for each metal.
Samples of each test solution were taken every 24 hours and analysed for metal concentrations (see General Materials and Methods, page 13).

3. Effects of experimental holding conditions on the acute toxicity of cadmium, copper and zinc.

Four combinations of experimental holding conditions were tested: static or recirculating water, with or without a sand substratum present.

The recirculating system comprised 3 polypropylene aquaria (60 x 35 x 25 cm) arranged one below the other and each containing 16 l of seawater. Seawater was siphoned from the top tank to the lower one via an external filter (Hykro of Denmark) containing glass wool and activated charcoal. Water was pumped from the bottom tank to the top tank by a rotary pump (Eheim model 381).

The flow rate was calculated at 2 l min\(^{-1}\) which gave 99% replacement in each tank every 40 minutes approximately (from information supplied by Hesner, cited in Sprague, 1969).

One of the three tanks was provided with a 2 cm layer of sand, which was previously washed in 2% EDTA and rinsed twice in clean seawater. All tanks were covered with 'Perspex' lids to reduce evaporation.

The tests carried out under static water conditions were as described in General Materials and Methods (page 10). A 2 cm layer of sand was provided in those tanks which were designed to have a substratum.
Twenty four *C. crangon* were used to test each holding condition and all animals were kept apart by perforated 'Perspex' partitions.

Metals were added to give final concentrations which corresponded to the previously determined 96 hour LC50 values i.e. 0.38, 6.0 and 11.0 mg l⁻¹ at 16°C for cadmium, copper and zinc respectively. The experiments were carried out at 16°C; salinity, S, 34% and LD, 14:10 hr. Daily analyses (by AAS) of the test solutions were made and metal deficiencies occurring in the recirculating system experiments were made good by the addition of the appropriate amounts of metal as sulphates.

4. Determination of Incipient Lethal Levels (ILL) of cadmium, copper and zinc.

Long-term (40-50 days) toxicity studies were carried out in static water tanks using the following range of concentrations of cadmium, copper and zinc:

<table>
<thead>
<tr>
<th>Metal</th>
<th>Concentration Range (mg l⁻¹)</th>
<th>Concentration Factor (x)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cadmium</td>
<td>0.001-100</td>
<td>10^5</td>
</tr>
<tr>
<td>Copper</td>
<td>0.1 -100</td>
<td>10^3</td>
</tr>
<tr>
<td>Zinc</td>
<td>1.0 -500</td>
<td>5×10^2</td>
</tr>
</tbody>
</table>

These experiments were performed during the summer (July-October) for copper (T=16°C; LD, 14:10 hr) and in the autumn and spring for cadmium and zinc (T=10°C; LD, 10:14 hr). In all cases the salinity was S, 34%.

Forty eight shrimps were used at each concentration of each of the 3 metals and 48 shrimps also were used as controls for each metal.
5. Effects of photoperiod variability on the acute toxicity of cadmium, copper and zinc.

The following photoperiod regimes and batches of experimental animals were used in these studies:

<table>
<thead>
<tr>
<th>Photoperiod (LD, hr)</th>
<th>Experimental (n)</th>
<th>Control (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>14:10</td>
<td>48</td>
<td>24</td>
</tr>
<tr>
<td>12:12</td>
<td>48</td>
<td>12</td>
</tr>
<tr>
<td>24:0 (LL)</td>
<td>48</td>
<td>12</td>
</tr>
<tr>
<td>0:24 (DD)</td>
<td>48</td>
<td>24</td>
</tr>
</tbody>
</table>

The experiments were performed at a seawater temperature of 12°C and a salinity of 34%.

Experimental aquaria (static tanks) were placed inside a light-proof box equipped with a daylight fluorescent strip light and a 15 W red light, both coupled to a time switch. The box was kept in a dark-room and in the DD experiments mortalities were recorded and the solutions replenished using red light illumination.

Metals were added to give test solutions with concentrations corresponding to the previously determined 96 hr LC50 values at 12°C (i.e. 0.5, 8.0 and 14.5 mg l⁻¹ respectively for cadmium, copper and zinc).

6. Seasonal/Temperature variation in toxicity of cadmium, copper and zinc.

(a) Acute Toxicity.

Collections of C. crangon were made in January (T=5°C; LD, 9:15 hr), May (T=10°C; LD, 12:12 hr), July (T=17°C; LD, 14:10 hr) and August (T=20°C; LD, 14:10 hr). In the laboratory the shrimps were maintained (at the temperature and photoperiod pertaining to the natural environment at the time of
collection) for one week before experimentation. Salinity was kept constant at S, 34%.

Acute toxicity tests were carried out at the different times of year, using the following concentrations:

<table>
<thead>
<tr>
<th>Cadmium (mg l⁻¹)</th>
<th>Copper (mg l⁻¹)</th>
<th>Zinc (mg l⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>1.0</td>
<td>4.0</td>
</tr>
<tr>
<td>0.15</td>
<td>2.0</td>
<td>6.0</td>
</tr>
<tr>
<td>0.25</td>
<td>4.0</td>
<td>12.0</td>
</tr>
<tr>
<td>0.50</td>
<td>6.0</td>
<td>18.0</td>
</tr>
<tr>
<td>0.75</td>
<td>10.0</td>
<td>24.0</td>
</tr>
<tr>
<td>1.0</td>
<td>15.0</td>
<td>30.0</td>
</tr>
<tr>
<td>3.0</td>
<td>25.0</td>
<td>60.0</td>
</tr>
<tr>
<td>5.0</td>
<td>50.0</td>
<td>100.0</td>
</tr>
<tr>
<td></td>
<td>100.0</td>
<td></td>
</tr>
</tbody>
</table>

Each concentration was presented to duplicate batches of n=12 shrimps for all experimental groups and duplicate batches of n=12 animals were used as controls for each metal at each temperature.

(b) Incipient Lethal Levels (ILL).

Cadmium, copper and zinc were added to static tanks (12 shrimps per tank; 4 tanks per metal at each temperature) at concentrations corresponding to their previously determined ILL (i.e. 0.005 mg l⁻¹ and 5.5 mg l⁻¹ for cadmium and zinc respectively at 10⁰C, and 0.75 mg l⁻¹ for copper at 16⁰C).

These experiments were carried out at the different times of year, and under the same experimental conditions as the acute toxicity tests (Section 6a, above).

Initially, experimental and control solutions were replaced every 48 hours, but, as metals losses were found to be <1% at these low concentrations, and as the solutions did not appear to be fouled
by excessive metabolic wastes, solutions were changed after every 96 hours.

7. Salinity-dependent effects on the acute toxicity of cadmium, copper and zinc.

Salinity-dependent changes in the acute toxicities of cadmium, copper and zinc were quantified from the variation in LT50 values obtained for each metal at concentrations corresponding to their 96 hour LC50's (i.e. 0.35, 5.5 and 11.0 mg l⁻¹ at 16°C for cadmium, copper and zinc respectively). The various salinities were obtained by dilution of normal seawater (S, 34%) with distilled water in proportions that would result in 4 l seawater per tank.

The salinities tested were:

<table>
<thead>
<tr>
<th>S%</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>34.0</td>
<td>100%</td>
</tr>
<tr>
<td>27.2</td>
<td>80%</td>
</tr>
<tr>
<td>23.8</td>
<td>70%</td>
</tr>
<tr>
<td>20.4</td>
<td>60%</td>
</tr>
<tr>
<td>17.0</td>
<td>50%</td>
</tr>
<tr>
<td>13.6</td>
<td>40%</td>
</tr>
<tr>
<td>10.2</td>
<td>30%</td>
</tr>
</tbody>
</table>

Specimens of *C. crangon* were allowed to acclimate to the various salinities for 7 days preceding the addition of the metals. The control and experimental group at the lower salinities were brought to these values via the following intermediate steps: - 2 days at S, 20%; 2 days at S, 17% and 3 days at either S, 13.6% or 10.2%.

At each salinity, 48 shrimps (4 batches of 12 animals) were used in the cadmium and copper experiments and 96 animals (8 batches of 12 animals) were used in the zinc experiments.

One control group (n=24) was used at each salinity.
RESULTS

1. Determination of 96 hour LC50 in cadmium, copper and zinc.

The calculated concentrations of each of the metals were seldom maintained exactly because losses occurred due to absorption, precipitation and aeration. Thus, although initial concentrations were accurate, daily analyses of experimental solutions were made to obtain the exact total concentrations of metals. Table 1 gives the relative percentage change in concentrations which occurred for the various solutions of each of the test metals, over the test period.

At low concentrations of all metals, variation from the calculated concentration did not exceed ca. 5% and all concentrations, when expressed graphically here, are given as the calculated concentration.

The cumulative mortality data of the test animals, at 96 hrs in each of the test solutions were transformed to a logarithmic-probability plot. Figures 1a,b,c, illustrate the plots produced for cadmium, copper and zinc respectively. In each case the plots are linear and in all cases, Chi² tests (P=0.05) showed homogeneity of response over the range of concentrations tested.

Table 2. gives data on the LC50 values, the slope functions, their respective 95% confidence limits and the calculated values of Chi². These data reveal that, in terms of LC50 values, cadmium is 16x more potent than is copper, which itself is ca. 2x as potent as zinc. There were no mortalities in the control groups over the time range tested here.
2. The effects of experimental holding conditions on acute toxicity of cadmium, copper and zinc.

Table 3 gives the data on relative change in concentration of each metal after 24 hours in the different types of experimental holding conditions employed: changes (usually losses) were greatest in the recirculating arrangement and least in the static, substratum-less system. Because the recirculating arrangement involved servicing both the with- and without-substratum tanks, it was not possible to assess the effects of substratum on metal loss, in this instance.

The cumulative mortality of animals in each of the experiments was plotted against exposure time as a logarithmic-probability plot. The data on LT50 values, the slope functions, their relative 95% confidence limits are given in Table 4a, b, c, and the significance of relevant comparisons of holding conditions are given in Tables 5a, b, c.

No significant differences (P>0.05) were found for any of the comparisons of slope functions or reaction time ratios. It is noteworthy, however, that the LT50 values were consistently higher under static test conditions (cf. circulating conditions) even though the concentrations of test metals were usually higher (less loss) than in the recirculating water system, (see Table 3.).

3. Determination of Incipient Lethal Levels (ILL) of cadmium, copper and zinc.

In these studies, LC50 values were obtained by plotting percentage mortality against concentration at times ranging from 12 to 996 hrs (1,200 hrs for cadmium). The resultant LC50's were then plotted against exposure time (hrs) as a bilogarithmic plot
to produce a toxicity curve for each metal. Figures 2, 3 & 4 show the toxicity curves for cadmium, copper and zinc, where each point and bar on the graph represents the mean and standard deviation respectively of four tests.

For copper (Fig. 3) and zinc (Fig. 4), the toxicity curves approach an asymptote with the time axis, giving an ILL between $0.7 - 0.8 \text{ mg} \cdot \text{l}^{-1}$ for copper and between $5.0 - 6.0 \text{ mg} \cdot \text{l}^{-1}$ for zinc. The apparently sharp inflections from acute to chronic action of these curves in Figs. 3 & 4 are a consequence of the bilogarithmic transforms and it must be realised that such changes are in fact gradual.

In the case of cadmium, the toxicity curve differed from those of copper and zinc in showing no indication of an ILL within the ranges of concentration and exposure times tested. However, the minimum time required to kill 50% of the test population at high concentrations ($100 \text{ mg} \cdot \text{l}^{-1}$) was approximately 18 hours.

4. The effect of photoperiod on the susceptibility of the animals.

Table 6 gives the details of the LT50 values, the slope functions and their respective 95% confidence limits, obtained from the plots of mortality v. time for C. crangon in each of the 3 metals at each of the photoperiod regimes used in these studies. Table 7 reveals that no significant ($P > 0.05$ in all cases) differences were found, for any of the metals, between the LD 14:10hr (the naturally pertaining photoperiod) mortality values and those at any of the other photoperiods. Control group mortality was unaffected by photoperiod - no animals died during the course of the experiment.
5. Seasonal/Temperature dependent changes in acute toxicity.

For convenience reasons only, the experiments undertaken at different times of the year are labelled here in terms of the seawater temperature pertaining at the time the animals were collected. The data obtained on the susceptibility of each sample in cadmium, copper and zinc are illustrated in Figs. 5a, 6a and 7a respectively. These curves show clearly that the samples varied in their susceptibility according to the seasonal temperature. Table 8 gives the details of the 96 hr LC50 values, the slope functions and their respective 95% confidence limits for each metal and Figs. 5b, 6b and 7b illustrate these data in terms of the test temperatures. Table 9 summarises the data on between-sample comparisons of slope function and potency ratio within each of the metals. These comparisons reveal that no significant (P>0.05) differences in slope function occurred for any of the metals, whereas the potency ratios were significantly (P<0.05) different in all comparisons except those of 17°C and 20°C in cadmium, and 16°C and 20°C in copper.

The ratio of the 96 hr LC50 values (potency ratio) for the extremes of the temperatures studied (5°C-20°C; corresponding to January and August collections) were calculated as 8.3 (cadmium), 5.6 (zinc) and 5.0 (copper). For each metal, the potency ratio between the 5°C and the 10°C samples was greater than that between 16°C and 20°C. No mortalities were recorded in the control groups at any of the temperatures.

6. Effects of season/temperature on ILL.

Figures 8a,b,c show the mortality data obtained after 996 hrs (in copper and zinc) and 1,200 hrs (in cadmium) for batches
(n=48) of *C. crangon*, exposed to the metals at temperatures corresponding to those of the seawater at the time of collection. Analysis of variance of these data are summarised in Tables 10a,b,c and show that no significant \((P>0.05)\) relationship between mortality and season/temperature, occurred in any of the metals. However, it is apparent from Figs. 8a,b,c that relative mortality is slightly higher at the higher temperatures.

7. The effect of salinity on 96 hour LC50 value.

In these experiments, 48 animals were used at each test salinity with cadmium and copper, and 96 animals were used at each salinity for zinc. At each salinity, the percentage mortality was plotted as a function of exposure time, to produce time-percent effect curves.

These curves were used to determine the LT50, slope function and 95% confidence limit data which are summarized in Table 11a,b,c. These data reveal that, for each metal, the LT50 values at the lower salinities tend to be lower (i.e. the shrimps were more susceptible) than those at the higher salinities (see Fig. 9,10,11).

Comparisons of the S, 34% LT50 values with those at the other salinities were made for each metal (Table 12a,b,c). These comparisons reveal that the LT50 values in salinities of S, 10% and S, 13% were significantly \((P<0.05)\) different (lower) than those at normal seawater salinity (S, 34%).

In the S, 10% control group a relative mortality of 8% was recorded. No other mortalities occurred in any other of the control groups.
Although it is generally accepted that cadmium, copper and zinc are highly toxic to aquatic organisms, there do appear to be differences in the hierarchy of potency according to the species of test animal studied. Doudaroff & Katz (1953) summarised the literature on the toxicity of those metals to fish and concluded that copper was more toxic than zinc or cadmium. Brown & Ahsanullah (1971) found that, at 1 mg l\(^{-1}\) concentrations, the hierarchy of potency to Artemia salina was copper > cadmium > zinc and to Ophyrotrocha labronica was copper > zinc > cadmium. However, Beisinger and Christensen (1972) showed a hierarchy of cadmium > copper > zinc to occur to Daphnia magna and the present results show the same hierarchy of potency occurs for *C. crangon*.

For *C. crangon*, the 96 hr LC\(_{50}\) of cadmium was 0.38 (0.3-0.47) mg l\(^{-1}\) at 16°C. This value compares well with the findings in several other reports of the potency of this metal to natantians (e.g. 96 hr LC\(_{50}\) values at 20°C and S, 20% of 0.32 and 0.42 mg l\(^{-1}\) respectively for *Crangon septemspinosa* and *Palaemonetes vulgaris*, Eisler, 1971; 120 hr LC\(_{50}\) value of 1.85 (1.32-2.59) mg l\(^{-1}\) for *Palaemon* sp., Ahsanullah, 1976). However, the much lower 96 hr LC\(_{50}\) value of 0.06 mg l\(^{-1}\) has been determined for the freshwater shrimp, *Paratya tasmaniensis* (Thorpe & Lake, 1974).

The 96 hr LC\(_{50}\) of copper to *C. crangon* was found, in these studies, to be 6.2 (4.7-8.1) mg l\(^{-1}\) at 16°C. Thus, *C. crangon* would appear to be more tolerant to this metal than some other crustacean species, as Pyefinch & Mott (1948) found the 12 hr LC\(_{50}\) of copper to the cyprids *Balanus crenatus* to be 5.9 mg l\(^{-1}\); and Winner & Farrell...
(1976) found 72 hr LC50 values of copper, to four species of *Daphnia*, to range between 0.47 and 0.59 mg l$^{-1}$. Hubschmann (1967, a) found a 96 hr LC50 of 3.4 mg l$^{-1}$ for the freshwater crayfish (*Orconectes rusticus*) but Portmann (1968) has recorded a very high 48 hr LC50 value of ca. 50 mg l$^{-1}$ for *C. crangon*, which suggests that his animals were much more resistant than those drawn from the Filey population.

In these studies, zinc was found to be the least toxic of the three metals to *C. crangon*, with a 96 hr LC50 of 11.0 (9.1-13.3) mg l$^{-1}$. This concentration is very similar to that found for the 96 hr LC50 of zinc to *Palaemon* sp. (Ahsanullah, 1976) but differs by an order of magnitude from the 48 hr LC50 value of 100 mg l$^{-1}$ of zinc to *C. crangon* (Portmann, 1968). *Paratya tasmaniensis* was found to be very susceptible to zinc- 96 hr LC50 of 1.21 mg l$^{-1}$ - (Thorpe & Lake, 1974) and the wide disparity of susceptibility found for various crustacean species may reflect genuine specific differences. Such explanation, however, does not account for the large difference found between the values recorded in the present study and those found by Portmann (1968) on the potency of copper and zinc to *C. crangon*. Some of the reported variation, however, may have arisen from differences in detail of the experimental conditions under which the experiments were performed.

The present results show that a sand substratum and/or the use of a water recirculation system made no significant ($P>0.05$) differences to the median lethal time for any of the 3 metals tested.

The methodologies employed here for the static tank bioassays (vigorous aeration, not feeding the animals and changing the test solution at frequent intervals), presumably, obviated many of the
'unfavourable' test conditions encountered by Connor & Wilson (1972) and Lincer et al. (1970). It is concluded that in toxicity studies on C. crangon, where mortality is the effect parameter, static tank bioassays are a valid proposition. The presence of a sand substratum does not affect mortality significantly but, as the metal losses due to such substrata contribute to error variability, there is no strong reason to include sand in the experimental design of such toxicity studies.

There is a general paucity of information concerning full evaluations of the acute toxicity of heavy metals to Crustacea. However, work on various fish species has shown that the time during which most metals exert their acute toxicity effects is usually within the first 100 hrs of exposure (Warner, 1967; Cairns, 1969). Hence, the usual 96 hr toxicity tests for such metals are appropriate as they are approximately inclusive of the total acute toxicity period. However, it has been suggested that 96 hrs is insufficient time to evaluate the acute toxic action of cadmium to aquatic organisms—principally fish (Doudaroff & Katz, 1953; Ball, 1967b; Shuster & Fringle, 1969; Eisler, 1971; Beisinger & Christensen, 1972). The present studies provide acute toxicity times of 160 hrs and 180 hrs for zinc and copper respectively; somewhat higher than the general figure of 100 hrs reported for fish species. Cadmium, however, within the 1,200 hrs of the experiment, failed to produce a threshold value. A similar lack of a threshold value has been found by Herbert, Elkins, Mann & Hemens (1957) in their tests of synthetic detergents to rainbow trout over a 12 week period and also by Jordan and Lloyd (1964) in 15 day tests of the effects of pH, on rainbow trout and roach.
A fundamental tenet of toxicology is that any substance (with the possible exception of carcinogenic ones) can occur at a concentration which will not be detrimental to the test organism. Because the constraints of concentration and time set by the worker are not sufficient to demonstrate such a threshold, does not signify that a threshold does not exist. In the present studies, the lowest test concentration of cadmium (0.001 mg l\(^{-1}\)) is approximately an order of magnitude greater than that found naturally in British coastal waters (Mullin & Riley, 1956). So it is possible that the IIL of this metal lies between these values. However, it is possible also that some aspect of the experimental methodology enhanced toxicity.

Other reports indicate that cadmium toxicity curves do not conform to those of most other metals. Concentration independence of the LT50 value has been found to pertain over concentrations of 0.01-1.0 mg l\(^{-1}\) in *Salmo gairdnerii* (Ball, 1967b) and at concentrations below 10 mg l\(^{-1}\) in *Gasterosteus aculeatus* (Pascoe & Mattey, 1977). No evidence of such independence of cadmium to *C. crangon* has been found here.

Of the few complete acute toxicity studies (other than short-term 96 hr studies) of copper to crustaceans, that of Hubschmann (1967, a) has found that 100% mortality of *O. rusticus* occurred within 10.5 days following a 24 hr exposure at 2 x the 96 hr LC50 value. This effect after a short a exposure to the metal complicates the exact definition of the acute toxicity of this metal. In the present studies, 24 hr exposure of *C. crangon* to the 96 hr LC50 value of copper, cadmium or zinc resulted, over the following 2 weeks, in mortalities which did not differ significantly (P>0.05) from those of control group animals.
in clean seawater. The difference between Hubschmann's (1967,a) results and those reported here may be a reflection of methodology differences or species variability.

The complex interrelationships of environmental variables that constitute a 'season' cannot be duplicated exactly under experimental conditions. So, although the animals used here were collected at different times during the year (to ensure that all shrimps had reasonable temperature acclimation), the only two environmental variables controlled under laboratory conditions were photoperiod and temperature. Photoperiod was found not to affect significantly (P>0.05) the toxicity values of the three metals. However, the data on short term (96 hr) experiments show that, for all 3 metals, there is a temperature-dependent variation in toxicity. The magnitude of the differences between the LC50s for any of the metals agree reasonably well with those recorded in studies by other workers. Thus the potency ratio (PR) of cadmium to C. crangon at 5°C/20°C was found here to be 8.3 - a value that compares well with the value of 8.24 for cadmium to Uca pugilator at 10°C/30°C (O'Hara, 1973b). Portmann (1968) showed that the tolerance of C. crangon to copper at 5°C was five times greater than that at 22°C - the same value as that found in this work. Little information is available to compare the present PR value of 5.6 for zinc to C. crangon (at 5°C/20°C) although Jones (1975) found that the mortality of isopods increased between 8°C and 10°C in the presence of zinc.

The shape of the toxicity curves of the metals to C. crangon (Figs. 5b, 6b, 7b) suggests that the trend of temperature-dependent response to the metals was an increased tolerance at low temperatures (as opposed to an increased susceptibility at high temperatures).
A similar type of curve has also been demonstrated by Portmann (1968) for the temperature-dependence of 48 hr LC50 of copper to *C. crangon*. Also, curves similar to the ones described here (Figs. 5b, 6b, 7b) would appear to fit the data on median survival times of rainbow trout in 5 and 10 mg l\(^{-1}\) zinc (Lloyd, 1960) even though this author has presented these as linear functions of temperature.

Analysis of variance, however, did not reveal significant (P>0.05) temperature-dependent differences of the mean percentage mortality for any of the metals, in the long-term tests. Thus it would appear that the IIL is temperature-independent. This finding implies that *C. crangon* does have some facility for temperature compensation although, in the presence of high concentrations of toxicants, this metabolic compensation is overwhelmed. McLeese (1974) found a similar phenomenon (temperature-dependence at acute levels of copper and temperature-independence at sub-lethal levels) for the lobster *Homarus americanus*. Together, these findings suggest such a pattern of events may be common amongst crustacean species. However, many studies have shown the non-validity of extrapolating from one species to another; long and short term effects of temperature on acute and chronic toxicities. Consequently, the above suggestion must remain tentative.

*Crangon crangon* is capable of using one of two osmoregulatory strategies depending on the salinity of the seawater of its environment. Towards the extreme salinities of its natural distribution range it maintains hypertonic haemolymph at low salinities and hypotonic haemolymph at high salinities. At intermediate salinities it behaves as a strong osmoregulator and gains the advantages of a relatively constant blood osmolarity (Weber & Spaargaren, 1970).
In terms of these physiological responses to external salinity, it is clear from the present results that the maintenance of a hypertonic haemolymph at low salinities is a disadvantage to *C. crangon* when the environment is polluted with ions of cadmium, copper or zinc. This has been observed as an increase in susceptibility to all 3 metals at salinities <S, 17%. Interestingly, the LT50 values for all 3 metals were unaffected by environmental salinity at values >S, 17%.

The present data tell nothing directly of the modes of toxic action but, when considered with what is known of the physiological responses of *C. crangon* to environmental changes, it is possible to make inferences of possible modes of toxic action. Thus, in dilute media, the compensatory increase in ion uptake (including toxic ions) may overwhelm the detoxification processes and enhance toxic action at the cellular level. The increased rate of ion transport across the gills may aggravate the deleterious effects of such ions to the gill epithelia (Bryan, 1967; Skidmore, 1970; Nimmo, Lightner & Bahner, 1977; Toseland & Nott, pers.comm.).

However, it should be recognised that the increased mortality which occurs in dilute media, may be the result of an increased biological availability of the metals (although concentrations for each metal were identical in all cases) due to increased solubility effects.
Table 1: Calculated concentrations (mg l\(^{-1}\)) and daily mean variations (% calculated value) for cadmium, copper and zinc.

<table>
<thead>
<tr>
<th>METAL</th>
<th>Calculated Concentration (mg l(^{-1}))</th>
<th>Mean % Variation</th>
<th>Calculated Concentration (mg l(^{-1}))</th>
<th>Mean % Variation</th>
<th>Calculated Concentration (mg l(^{-1}))</th>
<th>Mean % Variation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cadmium</td>
<td>0.10</td>
<td>1%</td>
<td>1.0</td>
<td>5</td>
<td>6.0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>0.15</td>
<td></td>
<td>2.0</td>
<td>5</td>
<td>10.0</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td></td>
<td>4.0</td>
<td>9</td>
<td>12.0</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>0.50</td>
<td></td>
<td>6.0</td>
<td>6</td>
<td>18.0</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>0.75</td>
<td></td>
<td>10.0</td>
<td>10</td>
<td>24.0</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>1.00</td>
<td></td>
<td>15.0</td>
<td>12</td>
<td>30.0</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>25.0</td>
<td>12</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>50.0</td>
<td>20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Copper</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zinc</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2: 96 hour LC50 values, slope functions and the respective 95% confidence limits and \(\chi^2\) value for each of the mortality curves given in Fig. 1a, b & c.

<table>
<thead>
<tr>
<th>METAL</th>
<th>LC50 (95% Confidence Limits)</th>
<th>Slope Function and (95% Confidence Limits)</th>
<th>(Chi)(^2) value (df)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cadmium</td>
<td>0.35 (0.31 - 0.47)</td>
<td>1.73 (1.47 - 2.04)</td>
<td>0.558 (4)</td>
</tr>
<tr>
<td>Copper</td>
<td>6.20 (4.77 - 8.06)</td>
<td>2.52 (2.02 - 3.15)</td>
<td>0.667 (5)</td>
</tr>
<tr>
<td>Zinc</td>
<td>11.00 (9.10 - 13.31)</td>
<td>1.94 (1.51 - 2.48)</td>
<td>6.26 (4)</td>
</tr>
</tbody>
</table>
Table 3: Mean % variation in concentration (mg l\(^{-1}\)) of cadmium, copper and zinc in the various experimental holding conditions, as measured 24 hours after the addition of the metals.

<table>
<thead>
<tr>
<th>Holding Condition</th>
<th>0.35 mg Cd l(^{-1})</th>
<th>6.2 mg Cu l(^{-1})</th>
<th>11.0 mg Zn l(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>static - sand</td>
<td>&lt;1%</td>
<td>5%</td>
<td>5%</td>
</tr>
<tr>
<td>static + sand</td>
<td>3%</td>
<td>8%</td>
<td>8%</td>
</tr>
<tr>
<td>recirculating (+ and - sand)</td>
<td>5%</td>
<td>12%</td>
<td>10%</td>
</tr>
</tbody>
</table>
Table 4a: Median lethal times (LT50), slope functions and their respective 95% confidence limits of mortality curves for C. crangon in (a) 0.38 mg Cd l^-1 at 16^\circ C (b) 60 mg Cu l^-1 at 16^\circ C and (c) 11.0 mg Zn l^-1 at 16^\circ C, under the various experimental holding conditions.

<table>
<thead>
<tr>
<th>Test Conditions</th>
<th>n</th>
<th>LT50 and 95% confidence limits (hours)</th>
<th>95% Slope function and Confidence limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>ST (-S)</td>
<td>24</td>
<td>100 (78-128)</td>
<td>1.78 (1.50-2.12)</td>
</tr>
<tr>
<td>ST (+S)</td>
<td>24</td>
<td>110 (84-144)</td>
<td>1.95 (1.61-2.36)</td>
</tr>
<tr>
<td>CF (-S)</td>
<td>24</td>
<td>92 (73-116)</td>
<td>1.75 (1.48-2.07)</td>
</tr>
<tr>
<td>CF (+S)</td>
<td>24</td>
<td>92 (70-120)</td>
<td>1.96 (1.61-2.39)</td>
</tr>
</tbody>
</table>

Table 4b:

<table>
<thead>
<tr>
<th>Test Conditions</th>
<th>n</th>
<th>LT50 and 95% confidence limits (hours)</th>
<th>95% Slope function and Confidence limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>ST (-)</td>
<td>24</td>
<td>100 (61-162)</td>
<td>2.88 (2.03-4.09)</td>
</tr>
<tr>
<td>ST (+)</td>
<td>24</td>
<td>115 (77-173)</td>
<td>2.54 (1.90-2.40)</td>
</tr>
<tr>
<td>CF (-)</td>
<td>24</td>
<td>70 (49-101)</td>
<td>2.31 (1.76-3.03)</td>
</tr>
<tr>
<td>CF (+)</td>
<td>24</td>
<td>90 (60-134)</td>
<td>2.47 (1.86-3.29)</td>
</tr>
</tbody>
</table>

Table 4c:

<table>
<thead>
<tr>
<th>Test Conditions</th>
<th>n</th>
<th>LT50 and 95% confidence limits (hours)</th>
<th>95% Slope function and Confidence limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>ST (-S)</td>
<td>24</td>
<td>94 (74-119)</td>
<td>1.81 (1.51-2.17)</td>
</tr>
<tr>
<td>ST (+S)</td>
<td>24</td>
<td>86 (70-106)</td>
<td>1.67 (1.43-1.95)</td>
</tr>
<tr>
<td>CF (-S)</td>
<td>24</td>
<td>84 (65-108)</td>
<td>1.89 (1.56-2.29)</td>
</tr>
<tr>
<td>CF (+S)</td>
<td>24</td>
<td>80 (65-98)</td>
<td>1.63 (1.41-1.89)</td>
</tr>
</tbody>
</table>

Footnote to Table 4:

ST = Static water tanks
CF = Continuous flow apparatus
(-S) = Without sand substratum
(+S) = With sand substratum
Table 5: Comparisons and significance of Slope function ratios and reaction time ratios of mortality curves for C. crangon in (a) 0.38 mg Cd l\(^{-1}\) at 16\(^\circ\)C (b) 6.0 mg Cu l\(^{-1}\) at 16\(^\circ\)C and (c) 11.0 mg Zn l\(^{-1}\) at 16\(^\circ\)C under various holding conditions.

**a)**

<table>
<thead>
<tr>
<th>Test Condition</th>
<th>Slope function Ratios and limits</th>
<th>P</th>
<th>Reaction time Ratio (and limits)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>ST(-S):ST(+S)</td>
<td>1.10 (0.85-1.42) N.S.</td>
<td></td>
<td>1.1 (0.77-1.56) N.S.</td>
<td></td>
</tr>
<tr>
<td>CF(-S):CF(+S)</td>
<td>1.12 (0.86-1.46) N.S.</td>
<td></td>
<td>1.0 (0.7-1.42) N.S.</td>
<td></td>
</tr>
<tr>
<td>ST(-S):CF(-S)</td>
<td>1.02 (0.80-1.31) N.S.</td>
<td></td>
<td>1.1 (0.79-1.54) N.S.</td>
<td></td>
</tr>
<tr>
<td>ST(+S):CF(+S)</td>
<td>1.00 (0.76-1.31) N.S.</td>
<td></td>
<td>1.20 (0.81-1.78) N.S.</td>
<td></td>
</tr>
<tr>
<td>ST(-S):CF(+S)</td>
<td>1.10 (0.85-1.43) N.S.</td>
<td></td>
<td>1.1 (0.77-1.57) N.S.</td>
<td></td>
</tr>
</tbody>
</table>

**b)**

Comparisons

| ST(-):ST(+)          | 1.17 (0.75-1.84) N.S.           |     | 1.15 (0.61-2.77) N.S.           |     |
| CF(-):CF(+)          | 1.07 (0.72-1.58) N.S.           |     | 1.29 (0.75-2.22) N.S.           |     |
| ST(-):CF(-)          | 1.24 (0.8-1.92) N.S.            |     | 1.43 (0.78-2.63) N.S.           |     |
| ST(+):CF(+)          | 1.03 (0.69-1.55) N.S.           |     | 1.28 (0.73-2.25) N.S.           |     |
| ST(-):CF(+)          | 1.21 (0.78-1.89) N.S.           |     | 1.11 (0.59-2.08) N.S.           |     |

**c)**

Comparisons

| ST(-):ST(+)          | 1.08 (0.85-1.37) N.S.           |     | 1.09 (0.81-1.46) N.S.           |     |
| CF(-):CF(+)          | 1.16 (0.91-1.48) N.S.           |     | 1.05 (0.76-1.45) N.S.           |     |
| ST(-):CF(-)          | 1.04 (0.81-1.34) N.S.           |     | 1.12 (0.79-1.59) N.S.           |     |
| ST(+):CF(+)          | 1.02 (0.83-1.25) N.S.           |     | 1.08 (0.81-1.45) N.S.           |     |
| ST(-):CF(+)          | 1.11 (0.89-1.39) N.S.           |     | 1.18 (0.86-1.63) N.S.           |     |

Footnote:

ST = Static water tanks  
CF = Continuous flow apparatus  
(-S) = Without sand substratum  
(+S) = With sand substratum
Table 6: Median Lethal times (LT50) slope functions and their respective 95% confidence limits of mortality curves for C. crangon in (a) 0.5 mg Cd l\(^{-1}\) (b) 8.0 mg Cu l\(^{-1}\) and (c) 14.5 mg Zn l\(^{-1}\) at 12°C under various photoperiod regimes.

(a) 0.5 mg l\(^{-1}\) Cadmium

<table>
<thead>
<tr>
<th>Photoperiod (hr)</th>
<th>n</th>
<th>LT50 and 95% Confidence limits</th>
<th>Slope function and 95% Confidence limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>14 : 10</td>
<td>48</td>
<td>100 (84-119)</td>
<td>1.86 (1.65-2.1)</td>
</tr>
<tr>
<td>12 : 12</td>
<td>48</td>
<td>90 (726-112)</td>
<td>2.13 (1.84-2.47)</td>
</tr>
<tr>
<td>24 : 0</td>
<td>48</td>
<td>94 (80-111)</td>
<td>1.78 (1.59-1.99)</td>
</tr>
<tr>
<td>0 : 24</td>
<td>48</td>
<td>88 (72-107)</td>
<td>2.01 (1.75-2.31)</td>
</tr>
</tbody>
</table>

(b) 8.0 mg l\(^{-1}\) Copper

<table>
<thead>
<tr>
<th>Photoperiod (hr)</th>
<th>n</th>
<th>LT50 and 95% Confidence limits</th>
<th>Slope function and 95% Confidence limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>14 : 10</td>
<td>48</td>
<td>105 (82-134)</td>
<td>2.41 (2.03-2.87)</td>
</tr>
<tr>
<td>12 : 12</td>
<td>48</td>
<td>94 (75-118)</td>
<td>2.26 (1.92-2.67)</td>
</tr>
<tr>
<td>24 : 0</td>
<td>48</td>
<td>86 (63-117)</td>
<td>2.94 (2.37-3.65)</td>
</tr>
<tr>
<td>0 : 24</td>
<td>48</td>
<td>82 (59-113)</td>
<td>3.10 (2.46-3.91)</td>
</tr>
</tbody>
</table>

(c) 14.4 mg l\(^{-1}\) Zinc

<table>
<thead>
<tr>
<th>Photoperiod (hr)</th>
<th>n</th>
<th>LT50 and 95% Confidence limits</th>
<th>Slope function and 95% Confidence limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>14 : 10</td>
<td>48</td>
<td>120 (105-137)</td>
<td>1.60 (1.45-1.76)</td>
</tr>
<tr>
<td>12 : 12</td>
<td>48</td>
<td>110 (93-130)</td>
<td>1.82 (1.61-2.06)</td>
</tr>
<tr>
<td>24 : 0</td>
<td>48</td>
<td>108 (93-125)</td>
<td>1.68 (1.51-1.86)</td>
</tr>
<tr>
<td>0 : 24</td>
<td>48</td>
<td>100 (83-120)</td>
<td>1.91 (1.68-2.18)</td>
</tr>
</tbody>
</table>
Table 7: Comparisons and significance of reaction time ratios of mortality curves for *C. crangon* in (a) 0.5 mg Cd l\(^{-1}\), (b) 8.0 mg Cu l\(^{-1}\), (c) 14.5 mg Zn l\(^{-1}\) at 12°C under various photoperiod regimes.

<table>
<thead>
<tr>
<th>Photoperiod (hr)</th>
<th>Reaction time Ratios and 95% Confidence limits</th>
<th>P = 0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>N.S.</td>
</tr>
<tr>
<td>(a) a : b</td>
<td>1.11 (0.84-1.47)</td>
<td></td>
</tr>
<tr>
<td>a : c</td>
<td>1.06 (0.83-1.35)</td>
<td></td>
</tr>
<tr>
<td>a : d</td>
<td>1.14 (0.88-1.48)</td>
<td></td>
</tr>
<tr>
<td>(b) a : b</td>
<td>1.12 (0.79-1.58)</td>
<td></td>
</tr>
<tr>
<td>a : c</td>
<td>1.22 (0.83-1.79)</td>
<td></td>
</tr>
<tr>
<td>a : d</td>
<td>1.28 (0.85-1.92)</td>
<td></td>
</tr>
<tr>
<td>(c) a : b</td>
<td>1.09 (0.88-1.35)</td>
<td></td>
</tr>
<tr>
<td>a : c</td>
<td>1.11 (0.91-1.35)</td>
<td></td>
</tr>
<tr>
<td>a : d</td>
<td>1.20 (0.96-1.50)</td>
<td></td>
</tr>
</tbody>
</table>
Table 8: 96hr LC50, slope functions and their respective 95% confidence limits for mortality curves for C. crangon in (a) cadmium (b) copper and (c) zinc at different seasons/temperatures of the year.

<table>
<thead>
<tr>
<th>Seasonal Temperature</th>
<th>n</th>
<th>96hr LC50 and 95% Confidence limits</th>
<th>Slope and 95% Confidence limits</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2.4 (1.55-3.22)</td>
<td>2.07 (1.46-2.94)</td>
</tr>
<tr>
<td>5</td>
<td>24</td>
<td>0.66 (0.53-0.83)</td>
<td>2.20 (1.85-2.62)</td>
</tr>
<tr>
<td>17</td>
<td>24</td>
<td>0.35 (0.25-0.48)</td>
<td>1.76 (1.34-2.31)</td>
</tr>
<tr>
<td>20</td>
<td>24</td>
<td>0.29 (0.24-0.35)</td>
<td>1.67 (1.46-1.90)</td>
</tr>
<tr>
<td>55</td>
<td>24</td>
<td>22.5 (17.4-29)</td>
<td>2.43 (1.91-3.09)</td>
</tr>
<tr>
<td>10</td>
<td>24</td>
<td>10 (7.94-12.6)</td>
<td>2.50 (2.0-3.12)</td>
</tr>
<tr>
<td>17</td>
<td>24</td>
<td>6 (4.54-7.92)</td>
<td>2.68 (2.0-3.59)</td>
</tr>
<tr>
<td>20</td>
<td>24</td>
<td>4.5 (3.4-5.98)</td>
<td>2.41 (1.85-3.13)</td>
</tr>
<tr>
<td>5</td>
<td>24</td>
<td>56 (44.4-70.6)</td>
<td>1.80 (1.47-2.20)</td>
</tr>
<tr>
<td>10</td>
<td>24</td>
<td>18 (15-21.6)</td>
<td>1.91 (1.62-2.26)</td>
</tr>
<tr>
<td>17</td>
<td>24</td>
<td>12 (9.68-14.88)</td>
<td>1.70 (1.45-2.00)</td>
</tr>
<tr>
<td>20</td>
<td>24</td>
<td>10 (8.20-12.20)</td>
<td>1.66 (1.41-1.96)</td>
</tr>
</tbody>
</table>
Table 2: Comparisons and significance of slope function ratios and reaction time ratios of mortality curves for *C. crangon* in (a) cadmium (b) copper and (c) zinc at different seasons/temperatures of the year.

<table>
<thead>
<tr>
<th>Seasonal Comparisons</th>
<th>Slope function and 95% Confidence limits</th>
<th>Potency ratio and 95% Confidence limits</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>a)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5° : 10° C</td>
<td>1.06 (0.73-1.55)</td>
<td>N.S.</td>
</tr>
<tr>
<td>5° : 17° C</td>
<td>1.18 (0.76-1.83)</td>
<td>N.S.</td>
</tr>
<tr>
<td>5° : 20° C</td>
<td>1.23 (0.85-1.78)</td>
<td>N.S.</td>
</tr>
<tr>
<td>10° : 17° C</td>
<td>1.25 (0.91-1.73)</td>
<td>N.S.</td>
</tr>
<tr>
<td>10° : 20° C</td>
<td>1.32 (1.06-1.65)</td>
<td>N.S.</td>
</tr>
<tr>
<td>17° : 20° C</td>
<td>1.05 (0.78-1.42)</td>
<td>N.S. 3.64</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>b)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5° : 10° C</td>
<td>1.03 (0.75-1.42)</td>
<td>N.S.</td>
</tr>
<tr>
<td>5° : 16° C</td>
<td>1.10 (0.65-1.60)</td>
<td>N.S.</td>
</tr>
<tr>
<td>5° : 20° C</td>
<td>1.01 (0.71-1.43)</td>
<td>N.S.</td>
</tr>
<tr>
<td>10° : 16° C</td>
<td>1.07 (0.74-1.54)</td>
<td>N.S.</td>
</tr>
<tr>
<td>10° : 20° C</td>
<td>1.04 (0.74-1.47)</td>
<td>N.S.</td>
</tr>
<tr>
<td>16° : 20° C</td>
<td>1.11 (0.75-1.64)</td>
<td>N.S.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>c)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5° : 10° C</td>
<td>1.06 (0.82-1.38)</td>
<td>N.S. 3.11</td>
</tr>
<tr>
<td>5° : 17° C</td>
<td>1.06 (0.82-1.37)</td>
<td>N.S. 4.67</td>
</tr>
<tr>
<td>5° : 20° C</td>
<td>1.08 (0.83-1.40)</td>
<td>N.S. 5.6</td>
</tr>
<tr>
<td>10° : 17° C</td>
<td>1.12 (0.91-1.40)</td>
<td>N.S. 1.5</td>
</tr>
<tr>
<td>10° : 20° C</td>
<td>1.15 (0.91-1.46)</td>
<td>N.S. 1.8</td>
</tr>
<tr>
<td>17° : 20° C</td>
<td>1.02 (0.81-1.29)</td>
<td>N.S. 1.2</td>
</tr>
</tbody>
</table>

N.S. = Not Significant
Table 10: Analysis of variance of % mortality of C. crangon at different seasons/temperatures in (a) 0.05 mg Cd l⁻¹ (b) 0.75 mg Cu l⁻¹ (c) 5.5 mg Zn l⁻¹.

<table>
<thead>
<tr>
<th></th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>a)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
<td>451.95</td>
<td>3</td>
<td>150.65</td>
<td>3.07</td>
</tr>
<tr>
<td>Error</td>
<td>589.74</td>
<td>12</td>
<td>49.14</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1041.68</td>
<td>15</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>b) 0.75 mg/l Copper</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
<td>242.36</td>
<td>3</td>
<td>80.79</td>
<td>1.48</td>
</tr>
<tr>
<td>Error</td>
<td>656.12</td>
<td>12</td>
<td>54.68</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>898.48</td>
<td>15</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>c) 5.5 mg/l Zinc</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
<td>324.07</td>
<td>3</td>
<td>108.02</td>
<td>2.78</td>
</tr>
<tr>
<td>Error</td>
<td>466.68</td>
<td>12</td>
<td>38.89</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>790.74</td>
<td>15</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\[ F_{0.05,3,12} = 3.49 > F_{a,b,c} \]
Table 11: Median lethal times (LT50), slope functions and their respective 95% confidence limits of mortality curves for *C. crangon* in (a) 0.35 mg Cd l\(^{-1}\) (b) 5.5 mg Cu l\(^{-1}\) (c) 11.0 mg Zn 1-l at various salinities.

<table>
<thead>
<tr>
<th>Salinity</th>
<th>n</th>
<th>LT50 and 95% Confidence Limits</th>
<th>Slope and 95% Confidence Limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>34</td>
<td>48</td>
<td>91 (75-111)</td>
<td>1.98 (1.72-2.28)</td>
</tr>
<tr>
<td>27.2</td>
<td>48</td>
<td>94 (76-117)</td>
<td>2.13 (1.84-2.47)</td>
</tr>
<tr>
<td>23.8</td>
<td>48</td>
<td>93 (76-114)</td>
<td>2.03 (1.77-2.33)</td>
</tr>
<tr>
<td>20.4</td>
<td>48</td>
<td>75 (65-87)</td>
<td>1.74 (1.55-1.95)</td>
</tr>
<tr>
<td>17.0</td>
<td>48</td>
<td>65 (57-75)</td>
<td>1.64 (1.49-1.80)</td>
</tr>
<tr>
<td>13.6</td>
<td>48</td>
<td>56 (48-65)</td>
<td>1.71 (1.54-1.90)</td>
</tr>
<tr>
<td>10.2</td>
<td>48</td>
<td>52 (44-61)</td>
<td>1.75 (1.56-1.96)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Salinity</th>
<th>n</th>
<th>LT50 and 95% Confidence Limits</th>
<th>Slope and 95% Confidence Limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>34</td>
<td>48</td>
<td>100 (76-132)</td>
<td>2.64 (2.16-3.22)</td>
</tr>
<tr>
<td>27.2</td>
<td>48</td>
<td>96 (74-125)</td>
<td>2.51 (2.09-3.01)</td>
</tr>
<tr>
<td>23.8</td>
<td>48</td>
<td>105 (82-134)</td>
<td>2.40 (2.02-2.86)</td>
</tr>
<tr>
<td>20.4</td>
<td>48</td>
<td>89 (69-115)</td>
<td>2.47 (2.06-2.96)</td>
</tr>
<tr>
<td>17.0</td>
<td>48</td>
<td>69 (53-90)</td>
<td>2.63 (2.17-3.18)</td>
</tr>
<tr>
<td>13.6</td>
<td>48</td>
<td>62 (48-79)</td>
<td>2.40 (2.02-2.86)</td>
</tr>
<tr>
<td>10.2</td>
<td>48</td>
<td>58 (46-73)</td>
<td>2.27 (1.92-2.68)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Salinity</th>
<th>n</th>
<th>LT50 and 95% Confidence Limits</th>
<th>Slope and 95% Confidence Limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>34</td>
<td>96</td>
<td>96.5 (85-110)</td>
<td>1.91 (1.74-2.10)</td>
</tr>
<tr>
<td>27.2</td>
<td>96</td>
<td>110 (97-124)</td>
<td>1.83 (1.68-1.99)</td>
</tr>
<tr>
<td>23.8</td>
<td>96</td>
<td>84 (74-95)</td>
<td>1.84 (1.69-2.01)</td>
</tr>
<tr>
<td>20.4</td>
<td>96</td>
<td>78 (70-87)</td>
<td>1.75 (1.62-1.89)</td>
</tr>
<tr>
<td>17.0</td>
<td>96</td>
<td>81 (72-92)</td>
<td>1.81 (1.66-1.97)</td>
</tr>
<tr>
<td>13.6</td>
<td>96</td>
<td>71 (63-80)</td>
<td>1.88 (1.72-2.05)</td>
</tr>
<tr>
<td>10.2</td>
<td>96</td>
<td>62 (54-71)</td>
<td>1.94 (1.76-2.13)</td>
</tr>
</tbody>
</table>
Table 12: Comparisons and significance of reaction time ratios of mortality curves for *C. crangon* in (a) 0.35 mg Cd l⁻¹ (b) 5.5 mg Cu l⁻¹ (c) 11.0 mg Zn l⁻¹, between normal seawater (5.34%) and all other salinities.

(a) 0.35 mg/l Cadmium

<table>
<thead>
<tr>
<th>Salinity</th>
<th>Reaction Time Ratio</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(and 95% Confidence limits)</td>
<td></td>
</tr>
<tr>
<td>34:27.2</td>
<td>1.03 (0.77 - 1.38)</td>
<td>N.S.</td>
</tr>
<tr>
<td>39:23.8</td>
<td>1.02 (0.77 - 1.36)</td>
<td>N.S.</td>
</tr>
<tr>
<td>34:20.4</td>
<td>1.21 (0.93 - 1.57)</td>
<td>N.S.</td>
</tr>
<tr>
<td>34:17</td>
<td>1.40 (1.09 - 1.79)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>34:13.6</td>
<td>1.63 (1.25 - 2.12)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>34:10.2</td>
<td>1.75 (1.35 - 2.28)</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

(b) 5.5 mg/l Copper

<table>
<thead>
<tr>
<th>Salinity</th>
<th>Reaction Time Ratio</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(and 95% Confidence limits)</td>
<td></td>
</tr>
<tr>
<td>34:27.2</td>
<td>1.04 (0.71 - 1.53)</td>
<td>N.S.</td>
</tr>
<tr>
<td>34:23.8</td>
<td>1.05 (0.71 - 1.56)</td>
<td>N.S.</td>
</tr>
<tr>
<td>34:20.4</td>
<td>1.12 (0.77 - 1.64)</td>
<td>N.S.</td>
</tr>
<tr>
<td>34:17</td>
<td>1.45 (0.98 - 2.15)</td>
<td>N.S.</td>
</tr>
<tr>
<td>34:13.6</td>
<td>1.61 (1.10 - 2.35)</td>
<td>&lt;0.08</td>
</tr>
<tr>
<td>34:10.2</td>
<td>1.72 (1.19 - 2.49)</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

(c) 11 mg/l Zinc

<table>
<thead>
<tr>
<th>Salinity</th>
<th>Reaction Time Ratio</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(and 95% Confidence limits)</td>
<td></td>
</tr>
<tr>
<td>34:27.2</td>
<td>1.14 (0.96 - 1.36)</td>
<td>N.S.</td>
</tr>
<tr>
<td>34:23.8</td>
<td>1.15 (0.97 - 1.37)</td>
<td>N.S.</td>
</tr>
<tr>
<td>34:20.4</td>
<td>1.24 (1.07 - 1.48)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>34:17</td>
<td>1.19 (1.00 - 1.42)</td>
<td>N.S.</td>
</tr>
<tr>
<td>34:13.6</td>
<td>1.36 (1.14 - 1.62)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>34:10.2</td>
<td>1.56 (1.31 - 1.86)</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>
Fig. 1. Logarithmic-probability plots of concentration and cumulative % mortality of *C. crangon* after 96 hrs in (a) cadmium (b) copper (c) zinc at T=16°C.
Fig. 2. Toxicity curve for cadmium at 10°C. Concentration is plotted, as the LC50 value, against exposure time. Vertical bars represent 2 x standard deviation (arithmetic) of n=4 LC50 values at each exposure time.
Fig. 3. Toxicity curve for copper at 16°C.
Concentration is plotted, as the LC50 value, against exposure time. Vertical bars represent 2 x standard deviation (arithmetic) of n=4 LC50 values at each exposure time. The incipient lethal level (ILL) can be interpolated from the asymptote to the X (time) axis.
Concentration of Copper (mg l⁻¹) as LC50 (±S.D.)

Exposure Time (hours)

Fig. 3
Fig. 4. Toxicity curve for zinc at 10°C.

Concentration is plotted, as the LC50 value, against exposure time. Vertical bars represent 2 x standard deviation (arithmetic) of n=4 LC50 values at each exposure time. The incipient lethal level (ILL) can be interpolated from the asymptote to the X (time) axis.
Fig. 4

Concentration of Zinc (mg/l) as LC50 (± S.D.)

Exposure Time (hours)
Fig. 5b. 96 hr LC50's (mg Cd l⁻¹) and 95% confidence limits (from Table 8a) at 4 seasonal temperatures.

Fig. 5a. Logarithmic-probability plots of concentrations and cumulative % mortality of C. crangon after 96 hrs exposure to cadmium at different seasonal temperatures.
Fig. 6b. 96hr LC50's (mg Cu l⁻¹) and 95% confidence limits (from Table 8b) at 4 seasonal temperatures.

Fig. 6a. Logarithmic-probability plots of concentrations and cumulative % mortality of *C. crargon* after 96 hrs exposure to copper at different seasonal temperatures.
Fig. 6

Confidence limits (from Table 8) 96hr LC50 (mg/l) and 95% mortality.
Fig. 7b. 96hr LC50's (mg Zn l⁻¹) and 95% confidence limits (from Table 8c) at 4 seasonal temperatures.

Fig. 7a. Logarithmic-probability plots of concentrations and cumulative % mortality of *C. crargon* after 96 hrs exposure to zinc at different seasonal temperatures.
Confidence limits (from Table 8)
96h LC50 (mg/l) and 95%
Fig. 8. Relationship between % mortality and seasonal temperature when specimens of *C. crangon* were exposed to incipient lethal levels of cadmium, copper and zinc. (a) 0.005 mg Cd l$^{-1}$ for 1200 hrs (b) 0.75 mg Cu l$^{-1}$ for 996 hrs (c) 5.5 mg Zn l$^{-1}$ for 996 hrs. Vertical bars represent 2x standard deviation of 4 tests.
Fig. 8

(a) 
\[ y = 0.891x + 44.67 \]
\[ r = 0.987 \]

(b) 
\[ y = 0.633x + 39.7 \]
\[ r = 0.955 \]

(c) 
\[ y = 0.701x + 43.27 \]
\[ r = 0.93 \]

Temperature (°C)
Fig. 9. Median lethal times (LT50) and 95% confidence limits (from Table 1la) for C. crangon exposed to 0.35 mg Cd l⁻¹ for 96 hrs at T=16°c over a range of salinities.
Fig. 10. Median lethal times (LT50) and 95% confidence limits (from Table 11b) for *C. crangon* exposed to 5.5 mg Cu l$^{-1}$ for 96 hrs at T=16°C over a range of salinities.
Fig. 10

Median Lethal Time (LT50) (hours) and 95% Confidence Limits (from Table 11)

5.5 mg l⁻¹ Copper

Salinity (%)
Fig. 11. Median lethal times (LT50) and 95% confidence limits (from Table 11c) for C. crangon exposed to 11.0 mg Zn l$^{-1}$ for 96 hrs at T=16°C over a range of salinities.
Fig. 11

Median Lethal Time (LT50) (hours) and 95% Confidence Limits (from Table 11)

11.0 mg l⁻¹ Zinc
CHAPTER 2.

MOULT-STAGE DETERMINATION IN CRANGON CRANGON (L.).
INTRODUCTION.

The moult cycle as a concept was first proposed by Drach (1939). Subsequently, Drach (1944) was able to demonstrate the general applicability of this concept to decapod crustaceans and to describe 3 principal stages of the moult cycle: postmoult - a period of pronounced turgor following the absorption of water; intermoult - a phase of tissue growth and deposition of calcium on the exoskeleton, and premoult - a time of active preparation for the forthcoming moult or ecdysis when the old cuticle is shed.

Subsequently, a number of workers have detailed the various criteria which they have used to characterise the various moult stages in a variety of crustacean groups (e.g. Brachyura, Hiatt, 1948; Kincaid & Scheer, 1952; Anomura, Kurup, 1964; Natantia, Scheer, 1960; Macrura, Mills & Lake, 1975). Principally, these criteria have been concerned with the morphological changes which accompany setogenesis in these animals. On the basis of these reports, a few broad generalisations could be made about moult stage identification but Drach & Tchernigovtseff (1967) have pointed out that interspecific variability exists and should be evaluated before generalisations are made concerning large taxonomic groupings.

The facility of being able to segregate individual crustaceans into their particular moult stages has enabled studies to be made of the physiological and behavioural changes associated with moult. Such studies include those on the structure and metabolism of integumentary and hepatopancreatic tissue (Travis, 1955a, 1960; Skinner, 1962; Humphreys & Stevenson, 1973; Madhyastha & Ragneker, 1974), ionic regulation (Robertson, 1960; Hagerman, 1973), urine production
(Travis, 1955b; Hagerman & Larsen, 1977), oxygen consumption (Hagerman, 1976) and chemical changes to the composition of the haemolymph (Travis, 1955b; Djangmah, 1968; Barlow & Ridgeway, 1969; Djangmah & Grove, 1970; Elzen & Kamm, 1974; Spindler-Barth, 1976; Regnault & Luquet, 1978).

Despite a number of these studies pertaining directly to C. crangon, it appears that no clear description has been published of the details of the morphological criteria which characterise the moult in this species. Thus, Hagerman, (1973) and Hagerman & Larsen (1976) state that they have employed a modification of Passano's (1960) criteria, Djangmah (1968) and Price & Uglow (1979) used a modification of Scheer's (1960) criteria and Regnault (1977) used the criteria of Drach & Tchernigovtzeff (1967).

The aim of the present study was to provide a clear, unequivocal description of the various moult stages of C. crangon as identified by morphological changes during setogenesis of the uropods. The opportunity has been taken also to examine the concentrations of chloride ions and total protein concentrations in the haemolymph as well as the total water contents of batches of animals sampled at the various moult stages.
MATERIALS & METHODS.

Shrimps (Crangon crangon, L) were collected from Filey, Yorkshire during October. In the laboratory they were maintained in static seawater tanks each divided into 12 equally-sized compartments by perforated 'Perspex' partitions. Each tank was supplied with 1 l of seawater (S, 34%) and a 2cm deep layer of sand. The seawater temperature was maintained at 14°C and the photoperiod regime of the laboratory was set at LD 14:10 hrs. The water in the tanks was kept fully saturated with air.

The experimental animals comprised a batch of 12 individuals, each 45mm length (tip of rostrum to end of telson) and maintained one in each compartment of a single tank. A second batch (n=12) of animals, within the size range 25-65mm body length, were maintained in a similar manner in another tank. Animals were fed daily on chopped Mytilus edulis and the water in the tanks was changed every 48 hrs.

All animals were allowed to moult once, after they were examined after every 24 hrs for their morphological condition. Records of the morphological condition were kept as photographs. The animals were prepared for photography by restraining them on a thin, glass plate by placing a paper tissue, soaked in seawater, over the anterior two thirds of the body. Photographs were taken each day of the left uropod of each specimen. Two sites for photography were selected: a) the most posterior tip of the uropod and b) at a position approximately half-way along the exterior edge of the uropod. Photographs were taken on Panatomic X film (Kodak Ltd.), using a Leitz
Orthomat automatic exposure camera coupled to a Leitz Ortholux microscope. Such records were made over a period of 6 weeks, which corresponds to 2 complete moult cycles for the majority of the 45mm shrimps.

Once the various morphogenetic criteria for characterising the various stages of the moult cycle had been established, a further large batch of animals were collected and segregated according to their moult condition. For the relative water content determinations, groups of animals from each of the moult stages were damp-dried by rolling them in paper tissues and then each was weighed to the nearest 0.1 mg (Metler P161, top pan balance). Each specimen was placed in a preweighed specimen tube and dried to constant weight at 110°C. The water content of each animal was expressed as the proportion (%) of the wet weight.

Blood samples were obtained from specimens of C. crangon which had previously been segregated into the different moult stages. Such samples were obtained by capillary tube puncture of the pericardium. The individual blood samples were transferred, each to a clean cavity slide and a 25 μl sample of each was taken and blown into 100 μl of distilled water. Such samples were stored at -10°C until required (always within 24 hrs).

For the haemolymph protein analysis, 50 μl of diluted haemolymph were added to 500 μl of biuret solution (Boehringer Mannheim). The mixture was agitated and colour development allowed to proceed for 30 min at 25°C. Samples were read against a distilled water blank at 546 nm in a spectrophotometer (Cecil Instruments Ltd.). Protein standards (Boehringer Mannheim) were made up with distilled water at
concentrations of 3, 6 and 12 mg l⁻¹ and treated in the same manner as the test samples. Final protein concentrations were determined using the following formula:-

\[
\text{Concentration of protein} = \frac{\text{O.D. Sample}}{\text{O.D. Standard}} \times \frac{\text{Concentration of Standard}}{5^*}
\]

(* where 5 was the dilution factor).

The protein concentration was plotted against the particular moult stage of the animal from which the sample was drawn.

The remaining 50 µl of diluted haemolymph from each sample were used for the chloride ion determinations. Chloride ion concentrations (mEq l⁻¹) were obtained using a chloride titrator (Model CMT 10, Radiometer, Copenhagen). A five-fold dilution of the haemolymph was found to be convenient as this was sufficient to prevent protein precipitation when the samples were pipetted directly into the carrier solutions. Duplicate titrations (20 µl each) were taken for each sample. The resulting values were multiplied by 5 to obtain values for the undiluted haemolymph. Such values were plotted against the particular moult stage of the animal from which the sample was drawn.
RESULTS & DISCUSSION

The length of time which elapses between successive ecdyses of any particular decapod species varies with its age, size, environmental temperature and the feeding conditions (Roberts, 1957). Regnault & Luquet (1978) found that 26 mm long specimens of _C. crangon_ completed a moult cycle in 8-9 days at 16-18°C and Meixner (1966) found that larger shrimps (>60mm length) required about 31 days at 14°C. The present study showed that shrimps 45mm in length required 21 ± 2 days to complete a full moult cycle at 14°C.

A differential rate of setogenesis was observed to occur within the uropod. The setae at the posterior tip of the appendage show the most rapid growth whilst those at the anterior margins show a distinctly slower rate of development. As far as the author is aware, such variability within a single appendage has not been reported elsewhere and it illustrates the need to standardise the particular area of an appendage used to determine the moult stage of an individual animal. In the present study, the position approximately half-way along the posterior edge of the uropod was selected as the sequence of setogenesis in this area corresponded best with the sequence of events described by Scheer (1960) and Drach & Tchernigovtzeff (1967) for other natantians.

The morphological changes associated with setogenesis are best described by means of the illustrations and captions of Figs. 12-22.

It became obvious during the course of this study that setogenesis not only proceeded at different rates in different parts of the uropod, but differed also in detail between large and small animals. In small
shrimps (<40mm body length), completion of the internal cones was seen rarely to be achieved until the late premoult stages (D₂-D₄) and, in some cases, they were never completed before ecdysis (Fig. 23). In the large shrimps, however, the internal cones invariably were completed by late intermoult stage (Cᵣ) and, by this time also, the fibrous bundles (see Fig. 12) had disappeared. The shrimps with a body length of ca. 45mm showed an intermediate condition at moult stage (Cᵣ) and, as Fig. 12 shows, animals at this stage have the internal cones completed yet the fibrous bundles are still present.

Generally, the largest shrimps had heavier pigmentation in the uropods than smaller ones and this made precise identification of the early premoult stages (D₁',D₁ and D₃') difficult. In some cases the pigmentation can obscure completely the developing setae in the epidermis and thereby make difficult the identification of any stage after D₂.

The animals were observed to feed during the larger part of the moult cycle and feeding only ceased during the days preceding and succeeding ecdysis. Many specimens resumed active feeding within 12 hr of ecdysis and this resumption contrasts with the general statement that the intermoult stages are associated with the resumption of feeding (Carlisle, 1960). Ecdysis was seen to occur only whilst the animals were resting on the surface of the sand and, during these experiments, occurred only during the night.

During the moult, haemolymph mean protein levels rose gradually from minimum values of 4.8 ± 0.3 mg 100 ml⁻¹ at postmoult stage A to maximum values of 11.75 ± 0.81 mg 100 ml⁻¹ at middle premoult (stage D₂) - see Fig. 24. Between stages D₂ and D₄ the mean level dropped slightly
to 8.4 mg 100 ml\(^{-1}\). Analysis of variance of these data (Table 13d) revealed that the differences between the moult stages were highly significant (P<0.001) and between-mean comparisons (Newmann-Keul's multiple range test, Table 14a) revealed highly significant (P<0.001) differences between each successive moult stage except B and C\(_a\) and C\(_b\) and D\(_0\). The haemolymph protein concentrations obtained here are slightly higher than those recorded for C. \textit{crangon} by Djangmah (1970).

Figure 25. illustrates the mean chloride ion concentration of the haemolymph and Fig. 26. illustrates the mean body water content values, each set of data presented in terms of the various moult stages. Analyses of variance of these data (summarised in Table 13b,c) revealed that blood chloride and body water content varied significantly (P<0.001 and P<0.05 respectively) during the moult cycle. Comparisons of the means of successive stages, (Table 14b,c) however, failed to reveal any significant differences in relative water content (P>0.05 in all cases) but did reveal a significant (P<0.01) drop in haemolymph chloride concentration between stages B and C\(_a\) and a significant (P<0.01) rise in blood chloride between stages D\(_3\) and D\(_4\).

The present results would indicate that the nature of moult-dependent variation of haemolymph protein levels is such that this parameter can be used as a supplemental indicator of the moult condition of C. \textit{crangon}. Furthermore, the data on protein levels support Djangmah's (1970) contention that moult-dependent variation of such levels can mask any variation which may exist with sex or size in this species. Perhaps not surprisingly, the trends for variation of haemolymph chloride ion concentration and body water content with the moult, are very similar. Spaargaren (1972) found an average
water content of 73% of the fresh body weight for Wadden Sea populations of C. crangon and Poolsanguan (1975) recorded a value of 72.9% body weight for intermoult C. crangon from the same population as those used in the present studies. Here, even higher values (>75% body weight) have been recorded for the relatively brief stages of A, B, Cₐ, and D₄.

In conclusion it may be stated that, on the basis of setogenesis of the uropod, it is possible to segregate clearly specimens of C. crangon into the various arbitrary stages of the moult cycle. The criteria selected here to differentiate between the various moult stages have been derived from the analysis of animals at known stages of the moult, coupled with the interpretation of other reports in the literature (e.g. Scheer, 1960). Each of the stages ascribed had been accompanied by a photographic record and a written description. On the basis of the distinguishing features of the moult described here, the moult stage dependent variation of haemolymph protein and chloride ion levels and whole animal water content values are in accord with other reports in the literature.
Table 13: Summary of analyses of variance and levels of significance of (a) haemolymph total protein concentration (b) chloride ion concentration (c) body water content, associated with the moult stages of C. crangon.

<table>
<thead>
<tr>
<th>(a) Haemolymph total protein</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>281.01</td>
<td>7</td>
<td>40.14</td>
<td>48.60</td>
</tr>
<tr>
<td>Error</td>
<td>47.91</td>
<td>58</td>
<td>0.83</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>328.22</td>
<td>65</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

P < 0.001

<table>
<thead>
<tr>
<th>(b) Chloride concentration</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>12291.46</td>
<td>7</td>
<td>1755.92</td>
<td>10.29</td>
</tr>
<tr>
<td>Error</td>
<td>9899.07</td>
<td>58</td>
<td>170.67</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>22190.53</td>
<td>65</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

P < 0.001

<table>
<thead>
<tr>
<th>(c) Body water content (%)</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>50.11</td>
<td>7</td>
<td>17.16</td>
<td>3.09</td>
</tr>
<tr>
<td>Error</td>
<td>71.75</td>
<td>31</td>
<td>2.31</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>121.86</td>
<td>38</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

P < 0.05
Table 14: Summary of Newmann-Keul's Multiple Range Tests and levels of significance of comparisons of means of (a) haemolymph total protein (b) chloride ion concentration and (c) body water content associated with consecutive moult stages of C. crangon.

<table>
<thead>
<tr>
<th>Comparison</th>
<th>$(\bar{x}_1-\bar{x}_2)$</th>
<th>S.E.</th>
<th>q</th>
<th>p</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Haemolymph total Protein concentration (g/100ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A : C₁</td>
<td>1.71</td>
<td>0.38</td>
<td>4.5</td>
<td>2</td>
<td>P&lt;0.005</td>
</tr>
<tr>
<td>C₁ : C₂</td>
<td>0.34</td>
<td>0.34</td>
<td>1.0</td>
<td>2</td>
<td>P&gt;0.05</td>
</tr>
<tr>
<td>B : C₂</td>
<td>2.87</td>
<td>0.29</td>
<td>9.9</td>
<td>4</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>C₂ : D₀</td>
<td>0.73</td>
<td>0.33</td>
<td>2.2</td>
<td>2</td>
<td>P&gt;0.05</td>
</tr>
<tr>
<td>D₀ : D₂</td>
<td>1.31</td>
<td>0.33</td>
<td>3.97</td>
<td>2</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td>D₂ : D₃</td>
<td>2.24</td>
<td>0.31</td>
<td>7.23</td>
<td>4</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>D₃ : D₄</td>
<td>3.31</td>
<td>0.31</td>
<td>10.6</td>
<td>5</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>(b) Haemolymph Chloride ion concentration (mg 1⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A : B</td>
<td>0.83</td>
<td>4.40</td>
<td>0.19</td>
<td>2</td>
<td>P&gt;0.05</td>
</tr>
<tr>
<td>B : C₁</td>
<td>17.17</td>
<td>4.92</td>
<td>3.49</td>
<td>3</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td>C₁ : C₂</td>
<td>5.44</td>
<td>5.27</td>
<td>1.03</td>
<td>2</td>
<td>P&gt;0.05</td>
</tr>
<tr>
<td>C₂ : D₀</td>
<td>6.49</td>
<td>4.78</td>
<td>1.36</td>
<td>2</td>
<td>P&gt;0.05</td>
</tr>
<tr>
<td>D₀ : D₂</td>
<td>7.26</td>
<td>4.78</td>
<td>1.52</td>
<td>3</td>
<td>P&gt;0.05</td>
</tr>
<tr>
<td>D₂ : D₃</td>
<td>3.02</td>
<td>4.49</td>
<td>0.67</td>
<td>2</td>
<td>P&gt;0.05</td>
</tr>
<tr>
<td>D₃ : D₄</td>
<td>18.17</td>
<td>4.24</td>
<td>4.29</td>
<td>5</td>
<td>P&lt;0.005</td>
</tr>
<tr>
<td>A : D₄</td>
<td>16.00</td>
<td>4.55</td>
<td>3.52</td>
<td>3</td>
<td>P&lt;0.025</td>
</tr>
<tr>
<td>(c) Body water content %</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A : B</td>
<td>0.64</td>
<td>0.76</td>
<td>0.84</td>
<td>2</td>
<td>N.S.</td>
</tr>
<tr>
<td>B : C₁</td>
<td>0.38</td>
<td>0.76</td>
<td>0.50</td>
<td>3</td>
<td>N.S.</td>
</tr>
<tr>
<td>C₁ : C₂</td>
<td>2.64</td>
<td>0.69</td>
<td>3.83</td>
<td>6</td>
<td>N.S.</td>
</tr>
<tr>
<td>C₂ : D₀</td>
<td>0.58</td>
<td>0.65</td>
<td>0.89</td>
<td>2</td>
<td>N.S.</td>
</tr>
<tr>
<td>D₀ : D₂</td>
<td>0.46</td>
<td>0.65</td>
<td>0.71</td>
<td>2</td>
<td>N.S.</td>
</tr>
<tr>
<td>D₂ : D₃</td>
<td>0.08</td>
<td>0.60</td>
<td>0.13</td>
<td>2</td>
<td>N.S.</td>
</tr>
<tr>
<td>D₃ : D₄</td>
<td>1.32</td>
<td>0.74</td>
<td>1.78</td>
<td>2</td>
<td>N.S.</td>
</tr>
<tr>
<td>Comparison</td>
<td>$(\bar{x}_1 - \bar{x}_2)$</td>
<td>S.E.</td>
<td>q</td>
<td>p</td>
<td>Significance</td>
</tr>
<tr>
<td>------------</td>
<td>-----------------</td>
<td>------</td>
<td>----</td>
<td>----</td>
<td>--------------</td>
</tr>
<tr>
<td>A : $D_0$</td>
<td>3.08</td>
<td>0.72</td>
<td>4.28</td>
<td>7</td>
<td>N.S.</td>
</tr>
<tr>
<td>$D_0 : D_4$</td>
<td>1.86</td>
<td>0.78</td>
<td>2.38</td>
<td>4</td>
<td>N.S.</td>
</tr>
<tr>
<td>A : $D_4$</td>
<td>1.22</td>
<td>0.67</td>
<td>1.82</td>
<td>4</td>
<td>N.S.</td>
</tr>
</tbody>
</table>
Fig. 12  \( C_b \) (Late intermoult). Outer cuticular sheath (Sh) clearly visible. Internal cones (IC) completed and epidermis and cuticle beginning to separate. Distance (x) from the trichogens (Tg) to base of new setae is approximately 1/3 length of the internal cone. Note the fibrous bundles (FB) still present anterior to the completed internal cones. This stage corresponds to \( C_b \) in Scheer's (1960) criteria and to \( B_2 \rightarrow C \) in Drach & Tchernigovtzeff's (1967) criteria.

Fig. 13  \( D_0 \) (beginning of premoult). Characterised by the complete retraction of the epidermis ('apolysis', Jenkin & Hinton, 1966) from the cuticle. The distance (x) between the trichogens and base of the new setae is ca. 2/3 length of internal cone. Scheer (1960) describes the new setae as being at the base of the old cones. Here, the setae buds are still transparent and appear to be attached to the trichogens.

Figs. 12-23. Print mags. ca. \( \times 75 \).
Fig. 14. $D_1$ (Early premoult). Marks the beginning of development of the new setae. This stage is difficult to discern from $D_0$ unless stages are followed sequentially. The distance ($x$) between trichogens and base of new setae is $> \frac{2}{3}$ length of internal cone. Setae buds more discrete than $D_0$.

Fig. 15. $D_1$ (Early premoult). New setae clearly distinguished as discrete organs (no longer appear attached to trichogens). Bases of new setae invaginating into epidermis (arrowed) but their tips remain at the bases of internal cones (cf. Scheer, 1960).
Fig. 16. $D_{111}$ (Early premoult). New setae extend well into the epidermis (ca. 0.5-0.75 total length of setae; base of new setae arrowed). New setae extend partly into the old setae. Stage $D_1$ (not illustrated) is as Scheer (1960) describes it for other natantians, except the new setae are still at the bases of the old. In heavily pigmented specimens these stages ($D_1 - D_{111}$) are difficult to identify.

Fig. 17. $D_2$ (Middle premoult). New setae very clear, extend well into the old setae (arrow indicates approximate position of tip of new setae). Epidermis has deposited cuticular sheath around each developing seta.
Fig. 18. D₃ (Late premoult). New setae reach tips of the internal cones. Cuticular sheath not yet chitinised (cf. Scheer, 1960).

Fig. 19. D₄ (Final premoult). Approximately 12-24 hrs prior to ecdysis. Carapace very brittle and cuticular sheaths chitinised.
Fig. 20. A (Early postmoult). Photograph taken 12-16 hrs after ecdysis. Body very soft. Note the presence of internal matrix (m) of the new setae (absent in immediate postmoult animals) and the lack of trichogens.

Fig. 21. B (Late postmoult). Internal matrix disperses from distal portion of setae (arrowed) and begins to form fibrous bundle approximately half-way along setae. Internal cones not yet developing (cf. Scheer, 1960).
Fig. 22. $C_{a-b}$ (Mid-intermoult). Epidermis contracts approximately one sixth - one eighth way along the new setae and thus form the internal cones. Apolysis (usually associated with stage D0) is just commencing.

Fig. 23. Uropod of a 24mm body length C. crangon. Photograph taken 24 hrs prior to ecdysis. Note the chitinised cuticular sheaths and the well-developed new setae (characteristic of stage D4). Note, however, the poorly-developed internal cones cf. the D4 stage of 45mm body length shrimp (Fig.19).
Fig. 24. Haemolymph total protein values at the various moult stages of *C. crangon*. The moult stages are presented on a scale proportional to the temporal sequence of the moult at $1^\circ$C. Numbers in parenthesis correspond to numbers of animals sampled.
Fig. 25. Haemolymph chloride ion concentrations at the various moult stages of *C. crangon*. The moult stages are presented on a scale proportional to the temporal sequence of the moult at 14°C. Numbers in parenthesis correspond to numbers of animals sampled.
Fig. 25

Haemolymph Chloride Ion Concentration (± SE) (mEq⁻¹)

Stage in Moult Cycle

(8) A
(12) B
(6) C_a
(7) C_b
(9) D_0 - D_1
(9) D_2
(10) D_3
(9) D_4
Fig. 26. Relative water content of whole *C. crangon* at various stages of the moult cycle. The moult stages are presented on a scale proportional to the temporal sequence of the moult at $14^\circ$C. Numbers in parenthesis correspond to the numbers of animals sampled.
Fig. 26

Body Water Content (%) vs. Stage in Molt Cycle

- 77
- 76
- 75
- 74
- 73
- 72

Stage in Molt Cycle

- A
- B
- C
- D
- D0
- D1
- D2
- D3
- D4

(5) (6) (7) (8)
CHAPTER 3.

THE INFLUENCE OF SOME BIOTIC FACTORS ON
THE TOXICITY OF CADMIUM, COPPER AND ZINC

TO CRANGON CRANGON (L.).
**INTRODUCTION.**

*Crangon crangon* (L.) is a diecdysic species of shrimp, which mouls several times during the spring and summer months and enters a prolonged 'neuter' or intermoult period during the winter (Lloyd & Yonge, 1947). Because of the continuous succession from post-moult stages for much of the year, *C. crangon* does not have a 'normal' condition as defined by Carlisle (1960) for seasonally moulting genera (e.g. *Uca*, *Maia* or *Cambarus*). In addition to being a dynamic phenomenon, the moult cycle in this species is rapid, lasting as little as 8-9 days for a 26mm shrimp at 16°C-18°C (Regnauld & Luquet, 1978). Such rapidity makes standardisation of physiological condition within a batch of animals a difficult task. Such standardisation is often a desirable, if not essential, requirement when the animals are to be used in toxicity studies.

The ratio of the sexes is known to vary in *C. crangon* (Lloyd & Yonge, 1947; Tiews, 1970) and, between sexes, the size-class distributions differ. Thus, whereas females >50mm body length are common, very few males of this length are found. In addition to the presence of males and females in the population of *C. crangon*, a further grouping, ovigerous females, can be readily distinguished during the majority of the months of the year. Another biotic variable which reflects physiological differences within the population, is that of relative hepatopancreas size. Hepatopancreas relative size varies according to the dynamic processes of metabolic storage and mobilization, (Poolsanguan, 1975).

Despite all these apparent difficulties in standardisation of
samples drawn from populations, natantians are potentially useful animals in studies of the modes of toxicity of various substances to decapod crustaceans. In the present studies, experiments were designed primarily so that any moult-dependent mortality, or any metal-induced alteration in the moult cycle, would be revealed. The aim of these studies was to obtain data which would allow inferences to be drawn on the possible modes of toxicity and mortality, for each of the metals, to _C. orangon_. Opportunity was taken also to examine the data in terms of sex, size and relative hepatopancreas sizes.

Each metal was given at a final, determined concentration sufficient to produce 50% mortality in a 5-6 day period. This timing was considered to be the minimum necessary to establish any moult-stage dependency.

Certain copper and zinc were added (as the sulphates) to experimental diets in which gave final concentrations of 0.35, 1, and 6.4 ppm respectively. Samples of these experimental solutions were analysed every 5 days, using a Perkin-Elmer Model 103 atomic absorption spectrophotometer, in order to check the total metal concentrations. Every four hours after the metals were added, losses were noted to occur in some solutions. However, no solutions were charged every 48 nor were losses even suspected if in calcium, 20% in copper and 60% in zinc.

Each experimental and control series was inspected every morning and afternoon and the number of animals which were recorded (recovery of missing) as well as the total number of animals present. Dead
MATERIALS & METHODS.

Stock specimens of *C. crangon* (L.) were collected from Filey Bay, Yorkshire and maintained in large (77 x 52 x 30cm) plastic aquaria supplied with circulating seawater (10°C, Salinity (S), 34%), aeration and a sand substratum. The laboratory photoperiod approximated that occurring naturally (LD, 14:10). Stock animals (size range: 30-75mm body length) were held thus for at least 2 weeks before being used in experiments.

Experimental and control batches of animals were chosen randomly from the stock tanks and were transferred, 40 animals in each, to polypropylene aquaria containing 16 l of seawater, (S, 34%) under static conditions. The aquaria were brought to the experimental temperature (12°C) over a period of 24 hrs and the animals were allowed to acclimatize to this temperature for a further 3 days.

Cadmium, copper and zinc were added (as the sulphates) to experimental tanks in amounts which gave final concentrations of 0.35, 5.5 and 14.4 mg l⁻¹ respectively. Samples of these experimental solutions were analysed every 24 hrs, using a Perkin Elmer Model 103 Atomic Absorption Spectrophotometer, in order to check the total metal concentrations. Twenty four hrs after the metals were added, losses were found to occur in some solutions. However, as solutions were changed every 48 hrs these losses never exceeded 5% in cadmium, 10% in copper and 8% in zinc.

Each experimental and control tank was inspected every morning and afternoon and the number of moulted animals were recorded (recovery of exuviae) as well as the total number of animals present. Dead
specimens were removed, measured, for total length (tip of rostrum to end of telson, to the nearest mm) sexed (as in General Materials and Methods, page 11) blotted dry and weighed (to the nearest 0.1mg). All the eggs were removed from the ovigerous females before such animals were weighed. The hepatopancreas of each shrimp was removed, blotted dry, and weighed (nearest 0.1mg on Mettler H10 analytical balance) and the hepatopancreas index (organ index, Giese, 1969) was calculated as the relative (%) fresh body weight.

Hepatopancreases from the surviving shrimps were dried to constant weight (110°C), weighed to the nearest 0.01mg, and digested in 3 ml concentrated HNO₃ (Analar). Metal analyses were carried out on an AAS using working parameters as described in General Materials and Methods (page 12). The moult stage of each corpse was determined using the criteria described in Chapter 2 of this thesis.

After 14 days, the experiment was terminated and all experimental and control animals surviving the experiment were killed, measured, sexed, weighed and segregated into moult stages.

2. **Effect of cadmium, copper and zinc on feeding behaviour of C. crangon.**

Specimens of *C. crangon* were taken from the main-holding tanks, and placed, 40 in each, in compartmented experimental tanks under conditions as described above.

They were left thus for 10 days to ensure all stomach contents had been voided as faeces. Cadmium, copper and zinc (as sulphates) were added to the experimental tanks using the procedure described above. Experimental and control tanks were left for 48 hrs, as this
was observed to be the minimum time for the metals to inhibit feeding and moulting in previous experiments.

Sub-samples of 6 shrimps were taken from control and experimental tanks and the pyloric stomach of each animal was dissected out and checked for gut-contents using a stereo binocular microscope. The moult stage of each animal was noted also and this sampling was designated time 0. Food (chopped *Mytilus edulis*) was then presented to all shrimps (approximately 150mg g⁻¹ fresh wt. of shrimp), every 24 hrs.

Every 24 hrs, solutions were changed and a sub-sample of 6 shrimps were checked for stomach contents and moult stage. Any animals in stages A, B and D₃, D₄ were disregarded, as the normal behaviour of animals in these stages may preclude feeding.
RESULTS.

Mortalities within the control and experimental groups are shown in Fig. 27. as log v. probability plots. The characteristics of these approximately linear curves were determined by the methods of Litchfield (1949) and Litchfield & Wilcoxon (1949) and are summarised in Table 15, except for those of the control groups which were precluded because of the small number of mortalities. Comparison of the slope function ratios (SR) and the median reaction time ratios (RR) are summarised in Table 16. These show that, as predicted, the median estimated time (LT50) of each group fell within days 5-6 of the experiment and that no significant (P>0.05) differences in LT50 occurred between the groups. Although the groups shared a common LT50, deaths began to occur at times which differed between the groups, i.e. after 14, 31 and 45 hrs in the copper-, cadmium- and zinc containing groups respectively. These between-group differences persisted to yield mortality curves which differed significantly in slope (P<0.05) for all comparisons.

Comparisons (1-tailed, 2 sample 't' tests) of the mean hepatopancreas indices of all the animals, from the different metal solutions, that participated in the experiments (i.e. those that died and those that survived) are summarised in Table 17. These show that the mean hepatopancreas index of the copper-treated group was significantly (P<0.001) lower than those of the zinc- and cadmium-treated groups. No animals survived the zinc treatment but the animals that survived the cadmium treatment had hepatopancreas indices which were not significantly (P>0.05) different from those of the control group animals.
Comparisons of the mean hepatopancreas indices of the mortalities and survivors within each metal showed that, in all cases, the survivors had significantly \((P<0.0025)\) higher values than the animals that died. The copper-treated animals not only had relatively smaller hepatopancreases than any of the other groups but, the relative water content of this organ in the surviving copper-treated animals \((76.7 \pm 1.0\%)\) was significantly \((P<0.001)\) greater than that of the cadmium-treated groups \((70.0 \pm 0.8\%)\).

The data on hepatopancreas metal levels of the experimental and control groups showed that the levels in the cadmium-treated group were \(120.7 \pm 10.4 \mu g \text{ Cd g}^{-1} \) dry weight of tissue - approximately \(8\) x the value of the control group \((14.8 \pm 1.2 \mu g \text{ Cd g}^{-1} \) dry weight of tissue). The levels of copper in the copper-treated group were \(1,982 \pm 187 \mu g \text{ Cu g}^{-1} \) dry weight of tissue which corresponds to a \(3.5\) x increase over that of the control group \((564 \pm 44 \mu g \text{ Cu g}^{-1} \) dry weight of tissue).

The data pertaining to the animals of the various experimental and control batches were segregated into sexual (male, female, ovigerous female) groupings and resegregated into length \((31-40\text{mm}, 41-50\text{mm}, 51-60\text{mm} >60\text{mm})\) groupings.

Figure 28a,b,c shows the mortality curves obtained for the sex groupings in the 3 metals and the characteristics of these curves are given in Table 18. Relevant comparisons are given in Table 19 and show that, in the cadmium-treated groups, no significant \((P>0.05)\) differences between the 3 sex groupings were found. The ovigerous females in the copper-treated groups, however, had significantly \((P<0.05)\) different LT50 values than those of the non-ovigerous females.
In zinc, the non-ovigerous females were significantly (P<0.05) more susceptible than the males or ovigerous females. No significant (P>0.05) differences were found between the mean hepatopancreas indices of any of the sex groupings in zinc or copper, but in cadmium, the males had significantly (P<0.001) higher mean hepatopancreas indices than either of the female groupings (see Table 20).

Mortality data, as related to the various size groupings, are plotted for each of the metals in Fig 29a,b,c. The characteristics of these mortality curves are given in Table 21 and the relevant comparisons in Table 22. Comparisons of the slope function ratios of the cadmium-treated groupings show that the largest animals (group D) had significantly (P<0.05) different rates of mortality than the other size groupings.

Comparisons of the mean reaction time ratios (RR) showed that no significant (P>0.05) difference in LT50 value occurred between the size groupings of the cadmium-treated groups. In the copper-treated groups, the 41-50mm animals survived significantly (P<0.05) longer than the largest animals (>60mm) and, in zinc, the 51-60mm animals survived significantly longer than the >60mm group. Although no clear trend could be demonstrated statistically, it is noteworthy that in all metals the smallest and the largest size groupings invariably had the lowest LT50 values.

In addition to recording straightforward mortalities, the number of animals that had moulted and subsequently been cannibalised were recorded each day. These data are shown in Fig. 30a,b. and indicate that the rate of each of these phenomena in the control groups is more or less constant throughout the experiment whereas, in the
experimental groups, both processes had been all but abolished by day 3. It has been considered reasonable here to assume that the majority of mortalities which occurred in the first three days, in the experimental groups, were attributable more to ecdysis and subsequent cannibalism than to any deleterious effects of the metal solutions. However, the gradients of the mortality curves were calculated from the data pertaining between 16% and 84% mortality and, as 'n' is a reasonably large number in each group, such initial mortalities have been interpreted as not seriously affecting the values for the calculated gradients. Inspection of the control group data indicates that the principal cause of mortality is predation following ecdysis. This contention is supported by the data on the number of exuviae recovered during the last 2 days of the experiment and on the number of animals in post-moult stages A & B when animals were killed on day 14. The difference between these sets of data matched exactly the number of animals eaten during this period.

The presence of metals would appear to inhibit moulting and, either directly or indirectly, feeding also (see page 61). Thus the mortality curves shown in Fig. 27 are misleading because the deaths in the control group are due principally to moult-dependent predation whereas this source of mortality is absent in the experimental groups.

The possibility that animals, at certain stages of the moult cycle, were more susceptible to the metals was examined further. The initial sampling of experimental animals was made randomly from the stock population and it has been assumed that the mixture of moult stages (moult stage profile, MSP) in these experimental groups would be similar to that of the stock group at time 0. Figure 31 illustrates the MSP s of the stock population, the control and the experimental
groups. The data have been segregated into 3 broad subdivisions of the moult cycle; postmoult stages (A & B), intermoult (C_a & C_b) and premoult (D_3 - D_4) and, in the case of experimental and control groups, have been derived from examination of the corpses that were removed each day and of the survivors which were killed on day 14. The stock group MSP reflects the synchronization of a high incidence of precocious moulting which occurred after the animals were brought to the laboratory some weeks before. Thus a preponderance of intermoult animals occurred in the stock population, and persisted generally throughout the experiment. When batches of experimental animals were transferred to the experimental tanks a further increase in precocious moulting occurred as evidenced by the number of exuviae collected and the final MSP of the control group. The metal-treated groups, however, revealed interesting differences from the control group and between themselves. The MSP of the cadmium group showed little difference from that of the stock population and this, coupled with the number of exuviae collected from this group, suggests that moulting is inhibited by the metal at this particular concentration.

The zinc and copper groups produced MSPs which were similar to each other but different from those of the stock or control groups. Again, few exuviae were recovered from these groups which suggests that ecdysis was inhibited. However, the high proportion of premoult animals (47% and 44% in the zinc and copper treated groups respectively cf. 25% and 21% of the stock and control groups) indicates that progression from the intermoult to premoult condition was still possible.

Figure 32a, b, c. shows the mortality curves produced for each of the 3 broad divisions of the moult cycle and Table 23 summarises
the characteristics of these curves. Within each group, the curves show similar slopes and, as shown in Table 24, no significant \((P>0.05)\) differences in slope comparison were revealed. Comparisons of the RR values, however, revealed significant \((P<0.05)\) differences between the postmoult and the other stages for both the copper-and the zinc-treated groups. No significant \((P>0.05)\) differences between the premoult and intermoult stages were observed within any group.

The possible inhibition of feeding behaviour was examined further. At day 0 (corresponds to day 2 in metal solution) all animals had empty stomachs and were immobile. On presentation with chopped mussel, the control group animals immediately became active and the cadmium-and zinc-treated groups also reacted and fed readily. The copper-treated group remained immobile and would not feed.

Inspection on Day 1 revealed that 67% of the control and cadmium-treated groups, 30% of the zinc-treated groups and 0% of the copper-treated groups had full stomachs. Over the succeeding 4 days of the experiment the proportion of the groups that attained full stomachs rose to 100% in the control group, declined to 15% in the cadmium-and zinc-treated groups and attained 15% in the copper-treated group Fig. 33.
DISCUSSION.

The present data show that zinc, which was required at high concentrations to produce the required LT50 value, resulted in only a few mortalities in the first 45 hrs but, thereafter, the mortality rate in this metal increased rapidly so that 96% of the test animals died by the end of the experiment. This could suggest that the initial cellular tolerance was high or, perhaps more likely, that the detoxification mechanism initially was very efficient. The nature of the breakdown of this detoxification mechanism is not known but, possibly a combination of plasma protein saturation and gill epithelia damage (Bryan, 1968) reduces the facility to transport ions across the gills.

The results with copper indicate that more than one mode of toxicity may operate for this metal to C. crangon. Mortalities began to occur within 10 hrs of treatment but, after 2 weeks, only 77% of the test animals had died. Hubschman's (1967a) suggestion that 2 modes of toxic action may operate, each dependent on the metal concentration, may still be valid for C. crangon. However, as the concentration and other experimental conditions were constant in these studies, it is contended that the physiological condition of the test animals determines the mode of toxic action.

The present studies show also that cadmium is highly toxic to C. crangon as the concentrations needed to produce the 5-6 day LT50 were 20% and 2% of those for copper and zinc respectively. Clearly, exposure to copper produces a diminution of hepatopancreas size, as seen by the mean hepatopancreas indices of the fresh corpses and survivors of this treatment and those of the control and other
experimental groups. The survivors of the copper treatment also had higher mean hepatopancreas relative water content than the control or cadmium-treated groups. This latter finding suggests that the relative size of the hepatopancreas, in terms of protoplasm, became reduced even more than the gross hepatopancreas index would indicate. From this, it would appear reasonable to suppose that gross functional impairment would accompany such large allometric changes but, if this were the case, it is not evident from the mortality data as only 77% (cf. 84% in cadmium; 96% in zinc) of the copper-treated animals died during the test period.

Inspection of the data within each experimental group, revealed that survival was associated with a high hepatopancreas index (cf. those animals that died). This association may mean, simply, that animals with high metabolic reserves (i.e. high hepatopancreas index) are more likely to survive metal-treatment than 'weaker' shrimps with low metabolic reserves. Comparisons between the groups showed also that the survivors of the copper treatment had a significantly (P<0.001) lower mean hepatopancreas index than either the cadmium or the control group animals, indicating that copper has some degenerative effect upon hepatopancreas tissue.

The metal concentrations in the hepatopancreas of the surviving animals illustrate that, compared with the values in these control groups, _C. crangon_ can tolerate an 8-fold increase in stored cadmium or a 3.5-fold increase in stored copper (in this latter case, despite a significant (P<0.001) reduction in hepatopancreas size).

Although not designed to provide comprehensive information on the influence of sex and/or size on susceptibility to heavy metals, it
is possible to segregate the LT50 data into sex and size groupings and gain some impression of the possible effects of these variables. With the animals used in these studies, sex and size were not mutually exclusive. In the cadmium experiments, none of the sex or size groupings were significantly different from each other (P>0.05 for all relevant comparisons). Amongst the copper and zinc treated animals, however, ovigerous females were significantly (P<0.05) more tolerant than non-ovigerous females. In zinc, the males were also found to be more tolerant than non-ovigerous females. No ready explanation can be proposed to explain why egg-bearing females should be more tolerant than non-ovigerous females. Possibly a size-dependent variability of susceptibility to these metals operates in *C. crangon*, as inspection of the size groups within each metal showed a general trend of greater susceptibility for the largest and smallest size groupings. This finding, however, requires further investigation as the present samples did not include very large or very small shrimps.

It is well known that an alteration of the holding conditions may induce precocious moulting in certain decapod species (Passano, 1960, Kurup, 1964). The MSP of the stock population of the present studies reflects this with a strong bias towards intermoult shrimps as a result of the synchronization following their capture some weeks earlier. Subsequent removal to experimental conditions appeared also to induce precocious moulting as revealed by the MSP of the control group. In the cadmium-treated group, the MSP remained similar to that of the stock population which suggests that development in all stages of the moult cycle is inhibited. This contention is supported by the observation that postmoult shrimps were present in this group at the end of the experiment despite the lack of any ecdyses during the
previous 9 days. Under normal conditions, even the largest shrimps would have been expected to have progressed to the intermoult condition.

Similarly, inhibition of postmoult development was observed in the copper-and zinc-treated groups but these differed from the cadmium group showing an increased proportion of each batch developing into the premoult stages (cf. the stock population). However, as ecdysis was inhibited, this resulted in a build-up of numbers of premoult animals in the MSP s of these 2 groups.

It appears that the metal solutions not only inhibited the progression of the development of the moult cycle but also, in terms of mortality, acted specifically upon certain stages of the moult cycle. Thus, comparisons of the median reaction time ratios (Table 24) indicate that postmoult shrimps in copper and zinc were more susceptible than intermoult or premoult shrimps receiving the same treatment.

Establishment of a relationship between toxicant susceptibility and moult stage, coupled with knowledge of other aspects of the moult stage-dependent physiology, allows some implications to be made on the modes of toxicity. Immediately subsequent to ecdysis, *C. orangon* takes up an appreciable volume of water which dilutes the haemolymph (Djangmah & Grove, 1970). In the present studies, such uptake of contaminated water could be the instigation of the premature mortality (cf. other moult stages) observed in the postmoult stages of the copper- and zinc-treated groups. Because of the increased permeability of the cuticle at immediate postmoult, more work is required to maintain haemolymph isotonicity (Hagerman, 1976) and the presence of appreciable quantities of zinc and copper ions would add to this workload. Cadmium,
at a concentration of 0.35 mg l$^{-1}$, may not have constituted such an additional ionic stress. It is known also that the oxygen requirements during the postmoult stage greatly exceed those at other stages (Skinner, 1962) and this is supported by the oxygen consumption data for *C. crangon* given by Hagerman (1976). Tissue oxygen consumption, in many cases, has been shown to decrease in the presence of heavy metals (Kerkut & Munday, 1962; Skidmore, 1970; Burton, Jones & Cairns, 1972; Thurberg, Dawson & Collier, 1973). Thus, the presence of such metals in postmoult animals, may not only increase the workload, but may decrease also the efficiency of the regulating tissue. Again, the concentration of the cadmium ions used, was probably not high enough to induce mortality in this fashion.

The fact that copper tolerance was very high in intermoult *C. crangon* (also found in *O. rusticus* by Hubschman, 1967 b.), combined with the observation of high postmoult susceptibility of *C. crangon* to copper, substantiates the earlier suggestion that there may be 2 modes of toxicity for this metal, each dependent on the physiological status of the animal.

The results of the feeding experiments suggest that, at the concentrations used here, each of the metals caused a measure of inhibition of feeding in *C. crangon*. Copper caused a serious inhibition of feeding and no more than 15% of this group fed during the experiment. Both cadmium and zinc caused a more gradual inhibition of feeding as the number of fed animals in each group declined from a maximum value (although not all the animals fed) to low values by the end of the experiments. It is not suggested, however, that food deprivation was an additional source of mortality, as it had been shown in other experiments (page 31 ) that *C. crangon* (even when kept separate) can survive many weeks without being given food.
Table 15: Median lethal times (LT50), slope functions and 95% Confidence limits for *C. crangon* exposed to (a) 0.35 mg Cd l⁻¹, (b) 5.5 mg (c) 14.4 mg Zn l⁻¹.

<table>
<thead>
<tr>
<th>Metal</th>
<th>Concentration mg l⁻¹</th>
<th>n</th>
<th>Median lethal time and 95% Confidence Limits (hours)</th>
<th>Slope function and 95% Confidence Limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a)</td>
<td>Cadmium</td>
<td>0.35</td>
<td>76</td>
<td>148 (80 - 273)</td>
</tr>
<tr>
<td>(b)</td>
<td>Copper</td>
<td>5.5</td>
<td>86</td>
<td>130 (102 - 165)</td>
</tr>
<tr>
<td>(c)</td>
<td>Zinc</td>
<td>14.4</td>
<td>82</td>
<td>130 (116 - 145)</td>
</tr>
</tbody>
</table>
TABLE 16: Comparisons and significance of slope function ratios of mortality curves for *C. crangon* exposed to 0.35 mg Cd l^-1, 5.5 mg Cu l^-1 and 14.4 mg Zn l^-1.

<table>
<thead>
<tr>
<th>Metal</th>
<th>Slope Function Ratio (SR) and 95% Confidence Limits</th>
<th>P</th>
<th>Reaction Time Ratio (RR) and 95% Confidence Limits</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>CADMIUM : COPPER</td>
<td>1.40 (1.12 - 1.75)</td>
<td>&lt;0.05</td>
<td>1.14 (0.86 - 1.52)</td>
<td>N.S.</td>
</tr>
<tr>
<td>CADMIUM : ZINC</td>
<td>1.27 (1.18 - 1.37)</td>
<td>&lt;0.05</td>
<td>1.14 (0.91 - 1.42)</td>
<td>N.S.</td>
</tr>
<tr>
<td>ZINC : COPPER</td>
<td>1.77 (1.42 - 2.12)</td>
<td>&lt;0.05</td>
<td>1.00 (0.76 - 1.32)</td>
<td>N.S.</td>
</tr>
</tbody>
</table>
Table 17: Mean hepatopancreas indices and levels of significance, of C. crangon from (a) mortalities and (b) survivors of the experimental groups exposed to 0.35 mg Cd l\(^{-1}\), 5.5 mg Cu l\(^{-1}\) and 14 mg Zn l\(^{-1}\).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mortalities</th>
<th>Survivors</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>x H.I.</td>
<td>S.D.</td>
</tr>
<tr>
<td>Cadmium</td>
<td>48</td>
<td>0.024</td>
<td>±0.008</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Copper</td>
<td>53</td>
<td>0.016</td>
<td>±0.007</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Zinc</td>
<td>78</td>
<td>0.024</td>
<td>±0.007</td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

N.S. = Not Significant at 5% level; x H.I. = mean Hepatopancreas Index; S.D. = Standard Deviation.
Table 18: Median lethal times (LT50) slope functions and 95% confidence limits, of male, non-ovigerous and ovigerous groups of *C.* crangon exposed to (a) 0.35 mg Cd l\(^{-1}\), (b) 5.5 mg Cu l\(^{-1}\) and (c) 14.4 mg Zn l\(^{-1}\).

(a) Cadmium

<table>
<thead>
<tr>
<th></th>
<th>Median lethal times and 95% Confidence limits</th>
<th>Slope functions and 95% Confidence limits</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>95% Confidence limits</td>
</tr>
<tr>
<td>male</td>
<td>8</td>
<td>165 (87-312)</td>
</tr>
<tr>
<td>female</td>
<td>18</td>
<td>130 (103-164)</td>
</tr>
<tr>
<td>female +</td>
<td>42</td>
<td>165 (142-191)</td>
</tr>
</tbody>
</table>

(b) Copper

<table>
<thead>
<tr>
<th></th>
<th>Median lethal times and 95% Confidence limits</th>
<th>Slope functions and 95% Confidence limits</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>95% Confidence limits</td>
</tr>
<tr>
<td>male</td>
<td>8</td>
<td>100 (56-178)</td>
</tr>
<tr>
<td>female</td>
<td>18</td>
<td>96 (58-159)</td>
</tr>
<tr>
<td>female +</td>
<td>50</td>
<td>180 (134-241)</td>
</tr>
</tbody>
</table>

(c) Zinc

<table>
<thead>
<tr>
<th></th>
<th>Median lethal times and 95% Confidence limits</th>
<th>Slope functions and 95% Confidence limits</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>95% Confidence limits</td>
</tr>
<tr>
<td>male</td>
<td>10</td>
<td>148 (106-207)</td>
</tr>
<tr>
<td>female</td>
<td>17</td>
<td>92 (79-108)</td>
</tr>
<tr>
<td>female +</td>
<td>50</td>
<td>148 (135-163)</td>
</tr>
</tbody>
</table>
Table 19: Comparisons of slope function ratios and reaction time ratios of the mortality curves of male (non-ovigerous and ovigerous) groups of *C. crangan* exposed to (a) 0.35 mg Cd l\(^{-1}\) (b) 5.5 mg Cu l\(^{-1}\) and (c) 14.4 mg Zn l\(^{-1}\).

<table>
<thead>
<tr>
<th>Metal</th>
<th>Sex group comparison</th>
<th>Slope function ratio and 95% Confidence limits</th>
<th>P</th>
<th>Reaction time ratio and 95% Confidence limits</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cadmium</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>male : female-</td>
<td>1.42 (0.81-2.50)</td>
<td>N.S.</td>
<td>1.27 (0.64-2.50)</td>
<td>N.S.</td>
</tr>
<tr>
<td></td>
<td>male : female+</td>
<td>1.42 (0.82-2.47)</td>
<td>N.S.</td>
<td>1.00 (0.52-1.93)</td>
<td>N.S.</td>
</tr>
<tr>
<td></td>
<td>female- : female+</td>
<td>1.00 (0.81-1.23)</td>
<td>N.S.</td>
<td>1.27 (0.96-1.68)</td>
<td>N.S.</td>
</tr>
<tr>
<td></td>
<td>Copper</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>male : female-</td>
<td>1.29 (0.71-2.35)</td>
<td>N.S.</td>
<td>1.04 (0.48-2.25)</td>
<td>N.S.</td>
</tr>
<tr>
<td></td>
<td>male : female+</td>
<td>1.17 (0.72-1.91)</td>
<td>N.S.</td>
<td>1.80 (0.94-3.46)</td>
<td>N.S.</td>
</tr>
<tr>
<td></td>
<td>female- : female+</td>
<td>1.10 (0.69-1.76)</td>
<td>N.S.</td>
<td>1.88 (1.04-3.38)</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>Zinc</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>male : female-</td>
<td>1.24 (0.94-1.64)</td>
<td>N.S.</td>
<td>1.61 (1.11-2.33)</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>male : female+</td>
<td>1.23 (0.95-1.60)</td>
<td>N.S.</td>
<td>1.00 (0.70-1.42)</td>
<td>N.S.</td>
</tr>
<tr>
<td></td>
<td>female- : female+</td>
<td>1.01 (0.88-1.16)</td>
<td>N.S.</td>
<td>1.61 (1.34-1.93)</td>
<td>P&lt;0.05</td>
</tr>
</tbody>
</table>

N.S. = Not significant at the 5% level.
Table 20: Mean hepatopancreas indices and levels of significance of between-sex group comparisons of male, non-ovigerous and ovigerous *C. crangon* that died in 0.35 mg Cd l\(^{-1}\), 5.5 mg Cu l\(^{-1}\) and 14.4 mg Zn l\(^{-1}\).

<table>
<thead>
<tr>
<th></th>
<th>Cadmium</th>
<th>Copper</th>
<th>Zinc</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>x H.I. ± S.D.</td>
<td>x H.I. ± S.D.</td>
<td>x H.I. ± S.D.</td>
</tr>
<tr>
<td>male</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>6</td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>0.038 ± 0.01</td>
<td>0.018 ± 0.005</td>
<td>0.024 ± 0.007</td>
</tr>
<tr>
<td></td>
<td>P&lt;0.001</td>
<td>P&gt;0.5</td>
<td>P&gt;0.5</td>
</tr>
<tr>
<td>female−</td>
<td>15</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>0.21 ± 0.008</td>
<td>0.017 ± 0.009</td>
<td>0.025 ± 0.006</td>
</tr>
<tr>
<td></td>
<td>P&lt;0.001</td>
<td>P&gt;0.5</td>
<td>P&gt;0.5</td>
</tr>
<tr>
<td>female+</td>
<td>35</td>
<td>24</td>
<td>49</td>
</tr>
<tr>
<td></td>
<td>0.26 ± 0.008</td>
<td>0.016 ± 0.006</td>
<td>0.024 ± 0.007</td>
</tr>
</tbody>
</table>

− = non ovigerous, + = ovigerous, H.I. = hepatopancreas Index, S.D. = Standard Deviation
Table 21: Median lethal times (LT50), slope functions and 95% confidence limits for the various sizegroupings of *C. crangon*, exposed to (a) 0.35 mg Cd l⁻¹ (b) 5.5 mg Cu l⁻¹ and (c) 14.4 mg Zn l⁻¹.

<table>
<thead>
<tr>
<th>Metal</th>
<th>Size class (mm)</th>
<th>n</th>
<th>Median lethal time (days)</th>
<th>Slope function</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>95% Confidence limits</td>
<td>and 95% Confidence limits</td>
</tr>
<tr>
<td>a. Cadmium</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>31-40</td>
<td>4</td>
<td>118 (86-162)</td>
<td>1.38 (1.09-1.75)</td>
<td></td>
</tr>
<tr>
<td>41-50</td>
<td>36</td>
<td>160 (136-189)</td>
<td>1.63 (1.44-1.84)</td>
<td></td>
</tr>
<tr>
<td>51-60</td>
<td>18</td>
<td>170 (135-214)</td>
<td>1.62 (1.35-1.94)</td>
<td></td>
</tr>
<tr>
<td>61-70</td>
<td>11</td>
<td>120 (49-293)</td>
<td>4.23 (2.14-8.38)</td>
<td></td>
</tr>
<tr>
<td>b. Copper</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>31-40</td>
<td>6</td>
<td>98 (49-195)</td>
<td>2.37 (1.37-4.10)</td>
<td></td>
</tr>
<tr>
<td>41-50</td>
<td>42</td>
<td>170 (128-226)</td>
<td>2.43 (1.93-3.06)</td>
<td></td>
</tr>
<tr>
<td>51-60</td>
<td>19</td>
<td>170 (80-360)</td>
<td>4.75 (2.51-8.98)</td>
<td></td>
</tr>
<tr>
<td>61-70</td>
<td>11</td>
<td>76 (44-131)</td>
<td>2.44 (1.59-3.73)</td>
<td></td>
</tr>
<tr>
<td>c. Zinc</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>31-40</td>
<td>46</td>
<td>140 (120-164)</td>
<td>1.73 (1.54-1.94)</td>
<td></td>
</tr>
<tr>
<td>41-50</td>
<td>19</td>
<td>158 (140-179)</td>
<td>1.31 (1.20-1.43)</td>
<td></td>
</tr>
<tr>
<td>51-60</td>
<td>13</td>
<td>115 (93-143)</td>
<td>1.48 (1.26-1.73)</td>
<td></td>
</tr>
</tbody>
</table>
Table 22: Comparisons and significance of slope function ratios and reaction time ratios of the mortality curves of the various size groupings of *C. crangon* exposed to 0.35 mg Cd l⁻¹, 5.5 mg Cu l⁻¹ and 14.4 mg Zn l⁻¹.

<table>
<thead>
<tr>
<th>Metal</th>
<th>Size Class Comparison</th>
<th>Slope function Ratio and 95% Confidence limits</th>
<th>P</th>
<th>Reaction time Ratios and 95% Confidence limits</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(a) CADMIUM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A : B</td>
<td>1.18 (0.91-1.53)</td>
<td>N.S.</td>
<td>1.36 (0.94-1.96)</td>
<td>N.S.</td>
<td></td>
</tr>
<tr>
<td>A : C</td>
<td>1.17 (0.87-1.58)</td>
<td>N.S.</td>
<td>1.44 (0.97-2.13)</td>
<td>N.S.</td>
<td></td>
</tr>
<tr>
<td>A : D</td>
<td>3.07 (1.14-8.29)</td>
<td>P&lt;0.05</td>
<td>1.02 (0.40-2.60)</td>
<td>N.S.</td>
<td></td>
</tr>
<tr>
<td>B : C</td>
<td>1.01 (0.81-1.26)</td>
<td>N.S.</td>
<td>1.06 (0.79-1.43)</td>
<td>N.S.</td>
<td></td>
</tr>
<tr>
<td>B : D</td>
<td>2.60 (1.30-5.20)</td>
<td>P&lt;0.05</td>
<td>1.33 (0.59-2.99)</td>
<td>N.S.</td>
<td></td>
</tr>
<tr>
<td>C : D</td>
<td>2.61 (1.28-5.32)</td>
<td>P&lt;0.05</td>
<td>1.42 (0.62-3.24)</td>
<td>N.S.</td>
<td></td>
</tr>
<tr>
<td>(b) COPPER</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A : B</td>
<td>1.03 (0.57-1.85)</td>
<td>N.S.</td>
<td>1.73 (0.82-3.56)</td>
<td>N.S.</td>
<td></td>
</tr>
<tr>
<td>A : C</td>
<td>2.00 (0.86-4.62)</td>
<td>N.S.</td>
<td>1.73 (0.62-4.81)</td>
<td>N.S.</td>
<td></td>
</tr>
<tr>
<td>A : D</td>
<td>1.03 (0.52-2.06)</td>
<td>N.S.</td>
<td>1.29 (0.54-3.10)</td>
<td>N.S.</td>
<td></td>
</tr>
<tr>
<td>B : C</td>
<td>1.95 (0.99-3.84)</td>
<td>N.S.</td>
<td>1.00 (0.45-2.24)</td>
<td>N.S.</td>
<td></td>
</tr>
<tr>
<td>B : D</td>
<td>1.00 (0.62-1.61)</td>
<td>N.S.</td>
<td>2.44 (1.33-4.49)</td>
<td>P&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>C : D</td>
<td>1.95 (0.75-5.07)</td>
<td>N.S.</td>
<td>2.44 (0.98-6.40)</td>
<td>N.S.</td>
<td></td>
</tr>
<tr>
<td>(c) ZINC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B : C</td>
<td>1.39 (1.13-1.54)</td>
<td>N.S.</td>
<td>1.13 (0.93-1.38)</td>
<td>N.S.</td>
<td></td>
</tr>
<tr>
<td>B : D</td>
<td>1.17 (0.97-1.29)</td>
<td>N.S.</td>
<td>1.22 (0.94-1.59)</td>
<td>N.S.</td>
<td></td>
</tr>
<tr>
<td>C : D</td>
<td>1.13 (0.94-1.36)</td>
<td>N.S.</td>
<td>1.37 (1.08-1.74)</td>
<td>P&lt;0.05</td>
<td></td>
</tr>
</tbody>
</table>

N.S. = Not Significant at the 5% level; A = 31-40mm; B = 41-50mm; C = 51-60mm; D = 61-70mm
TABLE 23: Median lethal times (LT50), slope functions and 95% confidence limits for *C. crangon*, at 3 different stages of the moult cycle, exposed to (a) 0.35 mg Cd l−1 (b) 5.5 mg Cu l−1 (c) 14.4 mg Zn l−1

<table>
<thead>
<tr>
<th>Metal</th>
<th>Stage in Moult Cycle</th>
<th>n</th>
<th>Median Lethal time and 95% Confidence Limits (hours)</th>
<th>Slope Function and 95% Confidence Limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>a) CADMIUM</td>
<td>Postmoult</td>
<td>10</td>
<td>128 (88 - 185)</td>
<td>1.79 (1.34 - 2.40)</td>
</tr>
<tr>
<td></td>
<td>Intermoult</td>
<td>39</td>
<td>174 (150 - 202)</td>
<td>1.58 (1.41 - 1.77)</td>
</tr>
<tr>
<td></td>
<td>Premoult</td>
<td>21</td>
<td>148 (116 - 189)</td>
<td>1.69 (1.39 - 2.06)</td>
</tr>
<tr>
<td>b) COPPER</td>
<td>Postmoult</td>
<td>12</td>
<td>72 (42 - 122)</td>
<td>2.55 (1.73 - 3.75)</td>
</tr>
<tr>
<td></td>
<td>Intermoult</td>
<td>32</td>
<td>240 (164 - 350)</td>
<td>2.62 (1.88 - 3.64)</td>
</tr>
<tr>
<td></td>
<td>Premoult</td>
<td>34</td>
<td>155 (105 - 228)</td>
<td>3.07 (2.22 - 4.24)</td>
</tr>
<tr>
<td>c) ZINC</td>
<td>Postmoult</td>
<td>9</td>
<td>64 (48 - 86)</td>
<td>1.56 (1.26 - 1.93)</td>
</tr>
<tr>
<td></td>
<td>Intermoult</td>
<td>33</td>
<td>140 (125 - 157)</td>
<td>1.40 (1.30 - 1.51)</td>
</tr>
<tr>
<td></td>
<td>Premoult</td>
<td>37</td>
<td>152 (130 - 178)</td>
<td>1.61 (1.44 - 1.80)</td>
</tr>
</tbody>
</table>
Table 24: Comparisons and significance of slope function ratios and reaction time ratios of the mortality curves of moult stage groupings of *C. crangon* exposed to 0.35 mg Cd l\(^{-1}\), 5.5 mg Cu l\(^{-1}\) and 14.4 mg Zn l\(^{-1}\).

<table>
<thead>
<tr>
<th></th>
<th>Slope Function Ratio (SR) and 95% Confidence Limits</th>
<th>Reaction Time Ratio (RR) and 95% Confidence Limits</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SR: 95% Confidence Limits</td>
<td>RR: 95% Confidence Limits</td>
</tr>
<tr>
<td>a) CADMIUM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Postmoult : Intermoult</td>
<td>1.13 (0.81 - 1.58) N.S.</td>
<td>1.36 (0.91 - 2.04) N.S.</td>
</tr>
<tr>
<td>Intermoult : Premoult</td>
<td>1.07 (0.86 - 1.34) N.S.</td>
<td>1.18 (0.81 - 1.57) N.S.</td>
</tr>
<tr>
<td>Premoult : Postmoult</td>
<td>1.06 (0.76 - 1.48) N.S.</td>
<td>1.16 (0.74 - 1.81) N.S.</td>
</tr>
<tr>
<td>b) COPPER</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Postmoult : Intermoult</td>
<td>1.03 (0.62 - 1.70) N.S.</td>
<td>3.33 (1.75 - 6.33) &lt;0.05</td>
</tr>
<tr>
<td>Intermoult : Premoult</td>
<td>1.17 (0.74 - 1.85) N.S.</td>
<td>1.55 (0.91 - 2.64) N.S.</td>
</tr>
<tr>
<td>Premoult : Postmoult</td>
<td>1.20 (0.74 - 1.96) N.S.</td>
<td>2.15 (1.13 - 4.11) &lt;0.05</td>
</tr>
<tr>
<td>c) ZINC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Postmoult : Intermoult</td>
<td>1.12 (0.90 - 1.40) N.S.</td>
<td>2.19 (1.60 - 3.00) &lt;0.05</td>
</tr>
<tr>
<td>Intermoult : Premoult</td>
<td>1.16 (0.98 - 1.37) N.S.</td>
<td>1.09 (0.91 - 1.31) N.S.</td>
</tr>
<tr>
<td>Premoult : Postmoult</td>
<td>1.03 (0.80 - 1.32) N.S.</td>
<td>2.37 (1.69 - 3.32) &lt;0.05</td>
</tr>
</tbody>
</table>

Footnote: N.S. = not significant at 5% levels.
Fig. 27. Logarithmic-probability curve of time and cumulative % mortality for control and experimental *C. crangon*; □□□□□□□□ , control; \( \text{X-X , } 0.35{\text{Cu mg}}^{-1} \); ○○○○○○○○ , 5.5{\text{Cu mg}}^{-1} ; •••••••• , 14.4 mg Zn \( {\text{mg}}^{-1} \).
Fig. 28. Logarithmic-probability curves of time and cumulative % mortality for, X—X, male; O—O, non-ovigerous female and •—• ovigerous female groups of C. crangon exposed to (a) 0.35 mg Cd 1⁻¹ (b) 5.5 mg Cu 1⁻¹ (c) 14.4 Zn mg 1⁻¹.
Fig. 28

Cumulative Mortality (%)

Time (Days)
Fig. 29. Logarithmic-probably curves of time and cumulative mortality for, X——X, 31-40mm; O——O, 41-50mm; ⋅⋅⋅⋅⋅⋅⋅, 51-60mm; □——□, 61-70mm size groups of C. crangon exposed to (a) 0.35 mg Cd l$^{-1}$ (b) 5.5 mg Cu l$^{-1}$ (c) 14.4 mg Zn l$^{-1}$.
Fig. 30. Cumulative frequency of (a) moulting (b) predation in control and experimental groups of *C. crangon*; □——□, control; X——X, 0.35 mg Cd l\(^{-1}\); ○——○, 5.5 mg Cu l\(^{-1}\); •——• 14.4 mg Zn l\(^{-1}\).
Fig. 31. Moult stage profile histograms of stock, control and experimental groups of *C. crangon*. 1=Postmoult; 2=Intermoult; 3=Premoult.
Fig. 31

Stock Day 0

Population (%)

1 2 3

n = 60

Stock Day 14

Population (%)

1 2 3

n = 60

Control

Population (%)

1 2 3

n = 77

Cadmium

Population (%)

1 2 3

n = 76

Zinc

Population (%)

1 2 3

n = 82

Copper

Population (%)

1 2 3

n = 86
Fig. 32. Logarithmic-probability curves of time and cumulative % mortality for, ○——○ Postmoult; X——X, intermoult; and •——• premoult, groups of C. crangon exposed to (a) 0.35 mg Cd l\(^{-1}\) (b) 5.5 mg Cu l\(^{-1}\) and (c) 14.4 mg Zn l\(^{-1}\).
Fig. 32

Cumulative Mortality (%) vs. Time (Days)

A.

B.

C.
Fig. 33. Proportion (%) of samples of *C. crangon* with gut contents at various sampling times during exposure to 0.35 mg Cd l$^{-1}$; 5.5 mg Cu l$^{-1}$ and 14.4 mg Zn l$^{-1}$. 
Fig. 33

Control

Cadmium

Zinc

Copper

% of C. crangon with food present in pyloric stomach

Days of Feeding

Days exposed to metals

(3) (4) (5) (6) (7) (8)
CHAPTER 4.

THE UPTAKE OF CADMIUM, COPPER AND ZINC INTO VARIOUS TISSUES OF CRANGON CRANGON (L.).
INTRODUCTION.

It is well-established that a seasonal fluctuation of some biochemical constituents occurs in many species of marine organisms (see Barnes, Barnes & Finlayson, 1963; Ansell, 1974a,b; Ansell & Trevallion, 1967; Djangmah, 1970; Poolsanguan, 1975). It is known also that certain metals, such as copper and zinc, are associated with certain biochemical constituents and with various metabolic processes within animals. Therefore, it is reasonable to assume that seasonal fluctuations of biochemical constituents of the organism may lead to concomitant changes in metal concentrations within particular tissues and/or within whole animals. Cadmium, however, is not a biologically functional metal and is not regulated by 'regulating' species. Consequently, evidence of any seasonal fluctuations of this metal in tissues is likely to be a reflection of such variations in the environmental seawater concentrations of cadmium.

The first section of the present chapter of this thesis concerns an investigation of seasonal concentrations of cadmium, copper and zinc in whole animal preparations and in haemolymph, hepatopancreas and abdominal flexor muscle samples of *C. crangon*. The data obtained serve also to provide information on background levels of these metals in *C. crangon*.

In fish, metal ions have been found to accumulate primarily in the gills, gasterointestinal tract and the kidneys (Ohmono, Suzuki, Sumiya & Saiki, 1972; Cearly & Coleman, 1974; Rowe & Massaro, 1974; McCarty & Houston, 1976). In some molluscs (e.g. *Mytilus edulis*) the kidney is the site of maximum metal deposition (Hobden, 1967, 1969;
Penreath, 1973b; George, Pirie & Coombs, 1976) whilst in others (e.g. *Helix aspersa*) the digestive gland was found to be the most important metal store (Coughtrey & Martin, 1976). Although the hepatopancreas is generally accepted as a major storage organ of metal ions in crustaceans, comprehensive information of the dynamics of metal distributions in various tissues is not readily available for any species. The second section of this chapter is concerned with the uptake and accumulation of cadmium, copper and zinc into selected tissues (gills, haemolymph, hepatopancreas and abdominal flexor muscles) of *C. crangon* exposed to two salinities and using sublethal levels of the metals. Translocation of cadmium in the gills, hepatopancreas and branchiostegites in starved and fed groups of *C. crangon* was studied also - after exposure (1 week) to sublethal amounts of the metal. The object of these particular studies was to gain information which might help to elucidate the pathways of uptake, internal translocation and excretion of heavy metals in *C. crangon*.

The possibility that toxic ions can be 'mistakenly' taken up during processes designed for the regulation of other ions, has been suggested by a number of authors (Galtsoff, 1953; Brooks & Rumsby, 1965, 1967; Coombs, 1972; Martin & Flegal, 1975; Wright, 1977b). Such a possibility was investigated here by examining the concentrations of calcium, copper and zinc in various tissues of cadmium-treated *C. crangon*.

Ultimately, toxic ions will manifest their overt toxicological response either by reaction with cell membranes (altering the bioelectric potential and thus affecting ion transport mechanisms) or by reaction with the biochemical constituents within the cell. The last section of this chapter investigates the possibility that either of these
processes may alter the gross morphology or the ultrastructural integrity of gill and hepatopancreatic tissue of \textit{C. crangon} exposed to cadmium, copper or zinc.
\textbf{MATERIALS \& METHODS.}

\textbf{Seasonal variation in concentrations of cadmium, copper and zinc.}

Specimens of \textit{C. crangon} were collected from Filey Bay (see General Materials and Methods, page 10) at the first low tide of each calendar month.

In the laboratory, \( n = 40 \) shrimps were selected at random from the new catch; 20 shrimps each were used for whole animal and selected tissue metal analysis.

\textbf{I. Whole animal metal analysis.}

Shrimps were killed by cutting the body in half between the cephalothorax and 1\textsuperscript{st} abdominal segment. The body and cephalothorax of each animal was rinsed in 2\% EDTA and distilled water to remove any surface metals (Pourian, Smith, Gabrielian \& Baird, 1974) and dried to constant weight at \( 110^\circ\text{C} \). Each shrimp was ground to a fine powder (using pestle and mortar), weighed, (nearest 0·1 mg, using a Mettler H10 analytical balance) and digested in 10ml concentrated nitric acid (Analar) and 2·5ml perchloric acid (Analar).

The dissolved mixture was evaporated to an oily film (great care was needed to avoid charring), whereupon it was redissolved in 1-2ml concentrated HNO\(_3\) and made up to 25ml with deionized distilled water (Fisons deionizer). Standards and blank were made up in deionized distilled water and metal concentrations were determined using an AAS (see General Materials and Methods, page 12).
2. Metal analysis in selected tissues.

2a) Haemolymph.

Ten-twenty microlitres haemolymph were obtained from each shrimp, by inserting a fine pipette into the pericardium, between the cephalothorax and 1st abdominal segment. At certain times of year gonad maturation precluded the collection of blood samples from the pericardium. Under these circumstances, blood was obtained from the ventral surface, between the 1st and 2nd abdominal segments.

The haemolymph sample was blown into 3ml deionized, distilled water and solutions were aspirated directly into the AAS for metal determination. Standards and blank were made up in deionized, distilled water.

2b) Hepatopancreas.

The hepatopancreas was removed from each shrimp taking care not to puncture the organ or include the pyloric portion of the stomach. They were rinsed in 2% EDTA and distilled water, dried to constant weight at 110°C and weighed to the nearest 0.1mg. Hepatopancreases were digested in 3ml concentrated HNO₃ and warmed gently in a water bath to effect complete digestion. Standards and blank were made up in concentrated HNO₃.

2c) Abdominal flexor muscle.

The abdominal flexor muscle was dissected out by cutting along the lateral edge of the carapace just dorsal to the pleopods. Dorsal and ventral portions of the abdominal carapace were removed
exposing the muscle beneath. The muscle dorsal to the alimentary tract was then removed, washed thoroughly in 2% EDTA and distilled water and then treated for metal analysis as was the hepatopancreas (above).

3a) Uptake of cadmium, copper and zinc by selected tissues of C. crangon in metal-treated seawater at 2 salinities.

Specimens of C. crangon were removed from holding tanks and placed in experimental tanks containing 4 l seawater, at either S, 34% or S, 20%. After 2 days those in S, 20% were then placed in S, 10% seawater where they were left for a further 2 days at T=16°C and LD=14 : 10 hrs.

In S, 34%, 9 batches of 12 shrimps were used for each metal and 60 shrimps acted as controls for all 3 metals. In S, 10%, 12 batches of 12 shrimps were used for each metal and 60 shrimps were used as controls.

Cadmium, copper and zinc were added to final concentrations corresponding to 0.5 x 96 hr LC50 for each metal at 16°C i.e. 0.175 mg Cd l⁻¹, 2.1 mg Cu l⁻¹ and 5.6 mg Zn l⁻¹.

Eight live shrimps were taken from each metal and control group at Time 0 (before metals were added) and at 24 hourly intervals thereafter, for 1 week. Animals were not fed during the experiments; metal solutions were changed every 48 hrs and dead shrimps were removed daily.

3b) Metal determination in selected organs.

The gills were washed in situ, removed with fine forceps
and dried to constant weight at 110°C. A pooled sample of 8 pairs of gills was then weighed (nearest 0.1mg) and digested in 3 ml concentrated HNO₃. Standards and blank were made up in concentrated HNO₃.

Haemolymph, hepatopancreases and abdominal flexor muscles were treated for metal determinations as in sections 2a,b and c.

4. The uptake and loss of metals associated with exposure to cadmium for 1 week and subsequent return to uncontaminated seawater.

Specimens of *C. crangon* were taken at random from the main holding tanks and placed, 40 shrimps in each, in polypropylene aquaria supplied with 16 l seawater containing 0.175 mg Cd l⁻¹.

Two tanks, each (n=80 shrimps), were used for fed and starved groups.

The fed groups were given chopped mussels (ca. 150 mg g⁻¹ wet wt of shrimp) every 48 hrs. They were allowed to feed for approximately 3-5 hrs, after which all solutions were changed.

After 1 week, the cadmium-treated shrimps were returned to clean seawater, at 16°C, S, 34%, LD 14:10 hrs. On returning the shrimps to clean seawater, a subsample of 8 live shrimps was taken from each group and the gills, hepatopancreas and branchiostegites were dissected out for metal determinations. Subsequent subsamples of 8 live shrimps were taken on Days 2, 4, 12 and 21.

The branchiostegites were cut from each shrimp, washed thoroughly in 2% EDTA and distilled water, and dried to constant weight at 110°C. They were weighed (nearest 0.1mg), and digested in 2ml hot concentrated nitric acid and 1ml perchloric acid (Analar). Standards and blank...
were made up in 2:1 nitric/perchloric acids.

Hepatopancreases and gills were removed and treated for metal determination as in section 2a & b respectively.

5. Some histological and ultrastructural changes in gill and hepatopancreatic tissue of C. crangon exposed to cadmium, copper and zinc.

Ten static water tanks were used, each containing 4 l seawater at 16°C, S, 34%, ID 14:10 hr and 12 shrimps, separated by 'Perspex' partitions.

Two tanks each, (n=24 shrimps), were used for control/fed, control/starved and cadmium-, copper- and zinc-treated groups. The metal-treated groups were not fed during the course of the experiment.

Cadmium, copper and zinc were added to final concentrations of 0.35; 5.5 and 14.0 mg g⁻¹ respectively to each of the experiment tanks and solutions were changed every 48 hrs and any dead shrimps were removed daily.

After 14 days, surviving shrimps were sacrificed for histological preparation, and metal determination in gills and hepatopancreas.

5a) External gill morphology.

Gills were removed from each shrimp, mounted in seawater, on glass slides and photographed by a Leitz Orthomat camera coupled to a Leitz Ortholux Microscope.

5b) Electron Microscopy.

Gills and hepatopancreases were processed in the
following manner, as described by Manton & Parke (1965);

(1) Organs were removed and placed in 4% gluteraldehyde 0.1M sodium cacodylate for 2-5 hrs (not <2 hrs).

(11) Buffer wash 1. (1.0625g sucrose in 25ml 0.1M Na cacodylate) for ½ hr.

(111) Buffer wash 2. (0.5g " " " " " ) " " ".

(1V) Buffer wash 3. (0.25g " " " " " ) " " ".

(V) Fixed in osmium tetroxide (2% in 0.1M Na cacodylate) for ca. 1 hr.

(VI) Buffer wash (0.1M Na cacodylate).

(VII) Dehydrated in 30% alcohol for 1 hr.

50% " " " " ".
70% " " " " ".
85% " " " " ".

Absolute alc. I. " " ".
Absolute alc. II. " " ".

(3 : 1) Abs/LU resin " " ".
(1 : 3) " " " " ".

(VIII) Embedded in LU resin.

(IX) Embedded tissue was cured at 70°C for at least 8 hrs and sections were taken using a Huxley Mk. II Ultra-microtome. Thick sections (1μ) were also taken for light microscope studies and were stained in 1% Toluidine Blue in 1% Borax.

(X) Sections were placed on uncoated grids (200μ mesh) and electron micrographs were taken on a JEOL JEM 7A electron microscope.
RESULTS.

1. Seasonal variation of cadmium, copper and zinc concentrations in whole animals and in selected tissues of *C. crangon*.

1a) Whole animal metal concentrations.

Figure 34a illustrates the monthly mean concentrations (μg g⁻¹ dry wt) obtained for samples (n=20) of shrimps each month. The cadmium levels varied between 2.20 and 3.20 μg Cd g⁻¹, but showed no clear seasonal trend in variation. Both copper and zinc, however, showed concentration maxima and minima during January/February and June/August respectively. Generally, copper concentrations were higher and showed greater monthly variability than the zinc concentrations.

1b) Haemolymph metal concentrations.

In all instances the haemolymph cadmium concentrations were too low to be detected by AAS.

Figure 34b shows the haemolymph mean copper and zinc concentrations (μg ml⁻¹ ± S.E.) of the monthly samples (n=20) of shrimps. Clearly, these metals share a trend of temporal variability characterised by low winter values and high summer values. The ratio of mean maximum : mean minimum monthly values obtained here were 1.9 : 1 and 3.6 : 1 for copper and zinc respectively.

1c) Hepatopancreas metal concentrations.

Figure 34c illustrates the mean values of cadmium, copper and zinc (μg g⁻¹ dry wt ± S.E.) in the hepatopancreases of the healthy samples (n=20) of shrimps. All 3 metals share a similar trend.
of seasonal variability - characterised by low values in the summer months and high values in the winter months. The ratios of mean maximum : mean minimum monthly values obtained here were 1.9 : 1, 1.8 : 1 and 1.8 : 1 for cadmium, copper and zinc respectively.

ld) Abdominal flexor muscle metal concentrations.

In all instances, the muscle cadmium concentrations were too low to be detected by AAS.

Figure 34d shows the monthly mean copper and zinc concentrations (µg g⁻¹ dry wt ± S.E.) in the muscles of the monthly samples (n=20). This tissue differed from others examined in having higher levels of zinc than copper. The levels of both metals were maintained at more or less constant values throughout the year and no clear seasonal trend of quantitative variability was evident. Grand mean concentrations of 27.4 ± 1.8 and 7.7 ± 1.3 µg g⁻¹ dry wt. were recorded for copper and zinc respectively.

2 Uptake of cadmium, copper and zinc by selected tissues of C. crangon at two salinities: S, 34%o and S, 10%o.

2a) Gills.

Figure 35a,b,c. shows the daily metal concentrations obtained for gills from 8 animals. It is clear that copper is accumulated most rapidly during the first 24 hrs; cadmium most rapidly between days 3 and 4, and zinc between days 3 and 5. The maximum accumulation of cadmium (170 µg Cd g⁻¹) occurred by day 6 and, copper (ca. 1,700 µg g⁻¹) by day 5. The gills were not saturated with zinc by day 7.
Generally, metal accumulation occurred more rapidly in S, 10% water than in fully saline seawater, except in the case of copper (see Fig. 35b). As in S, 34% seawater, the cadmium-and copper-treated animals had ceased to accumulate the metals before the end of the experiment and the zinc-treated animals continued to accumulate the metal throughout the experiment.

2b) Haemolymph.

Figure 36a,b. shows haemolymph copper and zinc concentrations of copper-and zinc-treated groups respectively, for normal (S, 34%) and dilute (S, 10%) seawater. In normal seawater the temporal relationship of metal uptake is linear for copper and zinc although analyses of variance (Table 25a,b.) of these sets of data failed to reveal any significant (P>0.05) increase of either metal during the 7 day treatment. The mean haemolymph concentrations of the control groups also did not vary significantly, (P>0.5) for either of these metals (Table 26a,b.). In the S, 10% control groups, the haemolymph mean levels of copper and zinc were 64% and 68% of the respective values found in the S, 34% control groups. The haemolymph levels of copper and zinc in the respective experimental groups rose very rapidly during the first 24 hrs and less rapidly thereafter (Fig. 36a,b.). Analyses of variance of these sets of data (Table 27a,b) revealed highly significant (P<0.001) increases in haemolymph levels of copper and zinc.

Levels of cadmium in the haemolymph of all groups were below the level of detection.
2c) **Hepatopancreas.**

Figure 37a,b,c. shows the changes in hepatopancreas mean concentrations of cadmium, copper and zinc of experimental groups of animals in normal (S, 34%) and dilute (S, 10%) seawater. In the presence of each metal, the rate of increase of hepatopancreas concentrations was linear and was greater in dilute seawater. At no times did the hepatopancreas mean level of any metal vary significantly between the S, 34% and S, 10% control groups (P>0.05 in all cases).

Analyses of variance of the data from the control and experimental groups in normal and dilute seawater (summarised in Tables 28a,b,c. ; 29a,b,c. ; 30a,b,c.) revealed that the relevant hepatopancreas concentrations of each metal increased significantly (P<0.01) during the experiments, whereas concentrations remained unchanged in the relevant control groups.

2b) **Abdominal Flexor Muscle.**

No change in the levels of any of the metals occurred in the abdominal flexor muscles of any of the experimental groups in either of the experimental salinities.

3. **The uptake and loss of metals associated with exposure to cadmium for 1 week and subsequent return to uncontaminated seawater.**

Groups of fed and starved specimens of *C. crangon* were exposed to normal seawater (S, 34%) containing cadmium at 0.175 mg Cd·l⁻¹ for 1 week, after which they were returned to uncontaminated seawater. The concentrations of cadmium, copper, zinc and calcium were
measured in the gills, hepatopancreas and branchiostegites of samples of the experimental groups which were removed from metal enriched seawater after 1 week. Metal concentrations were measured also, in these tissues, at intervals during the subsequent 3 weeks in uncontaminated seawater.

3a) Gills.  

Figure 38a,b. illustrates the metal concentrations found in the gills of the fed and starved groups. Generally, metal concentrations after exposure to cadmium were higher in the gills of the starved group. Subsequently, the cadmium concentrations in the 2 groups remained more or less constant whilst the concentrations of copper, zinc and calcium tended to decrease progressively with time.

3b) Hepatopancreas.  

The data on the hepatopancreas levels of the test metals for the fed and starved groups are shown in Fig. 39a,b,c,d. One-tailed analyses of variance of these data (summarised in Tables 31a,b,c,d. and 32a,b,c,d.) revealed that cadmium and calcium values in the fed group increased significantly (P<0.05), whereas values of copper and zinc showed no significant change over the 3 week period. In the starved group, calcium concentrations showed a significant decrease over the 3 weeks but none of the other metals varied significantly (P>0.5 in all cases).

Comparisons of the mean concentrations of each metal between the fed and the starved groups, at each sampling time, are given in Table 33. These data reveal that metal levels in the starved animals were invariably higher than those in fed ones. These differences were not
all found to be significant at the 5% level except for the copper which, at all sampling times, was found in significantly (P<0.01) higher concentrations in the starved animals.

3c) Branchiostegites.

Figure 40a,b,c,d. shows the cadmium, calcium, copper and zinc concentrations found in the branchiostegites of fed and starved groups of C. crangon during these experiments. Copper and zinc concentrations remained fairly constant over the test period and analyses of variance (summarised in Tables 34a,b,c,d. and 35a,b,c,d.) reveals that no significant (P>0.5) differences in these metal concentrations were apparent in either fed or starved groups. Cadmium concentrations in the branchiostegites increased during the 3 weeks in the uncontaminated water, although analysis of variance showed that this increase was significant (P<0.05) only in the case of the fed group. During the period in uncontaminated water, branchiostegite calcium values fell. Again, this decrease in both fed and starved groups but analysis of variance revealed the change was significant (P<0.01) only in one of the groups - in this instance the starved group.

4 Some histological and ultrastructural changes in gill and hepatopancreas tissue of C. crangon exposed to cadmium, copper and zinc.

The concentrations of the 3 test metals found in the gills and hepatopancreas of the shrimps kept under the various test conditions (control/fed; control/starved, cadmium-copper and zinc-treated) are given in Table 36. These values are assumed here to be
representative of the values likely to have been present in the tissue samples taken for the histological and ultrastructural investigation. In the gills, little variation existed between the particular metal concentrations in the fed and starved groups, although copper and zinc levels were slightly lower in the starved animals. Interestingly, the ratio of concentration between the control groups and the 14 day metal-treated groups, for both fed and starved group comparisons, all produced similar values. Thus, for cadmium the ratio was $13-17 \times$; for copper, $13-15 \times$; and for zinc, $13-15 \times$. Despite this similarity, copper was the only metal to cause severe damage to the gills (see below, page 82).

The metal concentrations in the hepatopancreas showed more variability than those of gill tissue. The levels of the control/starved group were higher than those of the control/fed group ($2.5 \times$, $14 \times$ and $2 \times$ for cadmium, copper and zinc respectively). Apart from the copper-treated group, all other experimental had higher metal values than either of the control groups. In the copper-treated group, the final hepatopancreas concentration was $9 \times$ that of the control/fed group.

4a) **Gross appearance of the gills.**

Figure 41a shows a single gill from a shrimp kept in non-contaminated seawater and Fig. 41b shows a gill removed from a shrimp kept for 100 hrs in seawater enriched with $5.5 \text{ mg Cu l}^-1$. The copper treated specimens were, apparently, healthy but did show a black deposit coating the gill lamellae. Such specimens showed also a black deposit on the inner wall of the branchiostegites (see Fig. 41c) and this could be peeled off as a single layer. On inspection, the gills were found to be severely damaged with the lamellae fused into groups by the
black copper complex. Subjectively also, the gills appeared to be much more rigid than those of the control animals.

The gross appearance of the gills from shrimps treated with zinc and cadmium did not show any major, distinctive differences from those of untreated animals.

5. Electron Microscopy of the gills.

5a) Control Animals.

The outer cuticular layer of a gill lamella is illustrated in Fig. 42a. The cuticle of _C. crangon_ can readily be distinguished as 2 distinct layers (cf. 3 for cuticle of _Penaeus aztecus_; Foster & Howse, 1978) - the epicuticle and the endocuticle. The epicuticle comprises an electron-dense layer covered with a fine fibrillar layer orientated perpendicular to the lamella. Frequently, fine filamentous strands (Fig. 42b.) are seen to be associated with the fibrillar layer and possibly they comprise a product of the breakdown of the latter, as may be caused by contact with the rich flora and fauna (e.g. Fig. 43) associated with the gills as suggested by Koburger, Norden & Kempler (1975) and Couch (1978).

Beneath the epicuticle is the endocuticle which was observed to vary in appearance, apparently with the moult stage condition of the individual shrimp sampled. The endocuticle of shrimps in moult stages A-C (see Fig. 42a,b) may be described in the same manner as for the endocuticle of _Jaera nordmanni_; 'a layer of flocculent material which varies in thickness and compactness' (Bubel & Jones, 1974). The endocuticle of shrimps in moult stages D₀-D₄ comprises a series of
evenly spaced, electron-dense and opaque layers (Fig. 43). Beneath the cuticle are basal plaques which serve to bind the endocuticle to the epithelial (cell) layer beneath. In these studies, no subcuticular space was evident (cf. Caridina japonica; Nakao, 1974).

The gill epithelial cells were seen to contain the nucleus and organelles such as golgi apparatus, endoplasmic reticulum (smooth and granular), multivesicular bodies, myelin figures, vacuoles and microtubules.

The golgi apparatus was observed usually to be in the basal region of the epithelial cells (cf. Jaera nordmanni; Bubel & Jones, 1974) and comprised 5-8 parallel rows of cisternae. The multivesicular bodies and myelin figures also appear mainly in the basal portion of the cells.

The epithelial cells contain smooth and granular endoplasmic reticulum which is observed, usually, to be swollen with a flocculent material - possibly proteins. The cytoplasm contains many loose particles of a size comparable to ribosomes or β-glycogen particles. Numerous mitochondria are usually associated with the apical portion of the cell.

Occasionally, below the basal lamina, haemocytes can be seen (Fig. 44). In C. crangon these are spherical with a large, centrally-placed, electron-dense nucleus. Surrounding the nucleus, in the cytoplasm, are tightly-packed, large, electron-lucent granules (cf. J. nordmanni haemocytes, the granules of which are electron-dense; Bubel & Jones, 1974).
5b) Experimental Animals.

At the concentrations and the times used in these experiments, only slight structural damage to the gills occurred in the cadmium- or zinc-treated animals. However, changes were noted in the gills of animals treated for 1 week with 5.5 mg Cu l\(^{-1}\) (sample gill concentration = 2,310 μg g\(^{-1}\)). Sections through the gill lamellae revealed that the normally very straight line of the cuticle had been thrown into folds (Fig. 45). These undulations may constitute an artifact during sectioning but they may reflect a change in the properties of the gill lamellae brought about by treatment with copper.

The epicuticle was not as degenerated in copper-treated gills as light microscope studies would suggest. However, the filamentous strands and the fibrillar layer were no longer identifiable and the epicuticle became more electron-dense than those of the control specimens. The endocuticle appeared not to have suffered any damage but, often, the basal plaques were more electron-dense than those of the controls. Epithelial cells of copper-treated gill lamellae were always characterised by a very large number of membrane-bound vesicles, sometimes containing granules of flocculent material at the apical portion of the cell. Mitochondria in copper-treated gills were present also, in larger numbers than in the epithelial cells, of the control gills.

An interesting observation was that in all the sections of copper-treated gill lamellae, there was a complete absence of any of the microscopic organisms normally associated with the gills (see, for example, Fig. 43).

Unfortunately, no haemocytes were detected in any of the samples.
of the copper-treated lamellae.


6a) Control Animals.

Sections of the hepatopancreas were taken from approximately the middle of the organ, in order to standardise the region taken for analysis. Figure 46 gives a schematic representation of the main types of cells seen in the sections of the control hepatopancreas and these show both absorptive and secretary (digestive) cells. This would indicate that the sections were taken through B-cell and transition zones, as described for the crayfish (Loizzi, 1971).

Some individual cells were degenerate (possibly due to the exfoliation of the old cells) or had large vacuoles (possibly the result of the secretion of digestive enzymes). Other cells contained golgi apparatus, pinocytotic vesicles, lipid droplets, apical mitochondria and were typical of absorptive-type cells. Rough endoplasmic reticulum (granular) was present also in this type of cell (cf. hepatopancreas of Calanus helgolandicus; Ong & Lake, 1974).

No difference in gross morphological structures could be observed from the E.M. sections (not copper specific) of fed and starved hepatopancreases, although the starved group contained ca. 5 x as much copper as the fed group.

6b) Experimental Animals.

(i) Cadmium-treated hepatopancreases (14 days in 0.35 mg Cd 1⁻¹).

The cytoplasm of the cadmium-treated hepatopancreatic
cells was very granular in appearance. The granules being of approximately the same size as ribosomes or β-glycogen particles.

Mitochondria were present at the apical surface of many epithelial cells, in amounts 2-3 x those in the control animals. The granular endoplasmic reticulum comprised a large proportion of the cell volume (see Fig. 47). Electron-dense spherules could be seen also, but whether these were degenerate mitochondria or possibly spherules of cadmium complex, remains unclear.

No observable damage to the nucleus was detected in any of the sections of cadmium-treated hepatopancreas.

(ii) Copper-treated hepatopancreases (14 days in 5.5 mg Cu l⁻¹).

Hepatopancreatic cells from copper-treated animals were characterised by a general cellular breakdown. Nuclei were degenerate and the cytoplasm and cell organelles were amorphous and largely degenerate (Fig. 48).

(iii) Zinc-treated hepatopancreases (14 days in 14.4 mg Zn l⁻¹).

Hepatopancreatic cells of zinc-treated specimens of _C. crangon_ were also characterised by extensive cellular breakdown, though not to the same extent as seen in the hepatopancreases of copper-treated shrimps.
DISCUSSION.

Cadmium, copper and zinc are present in North Sea waters at concentrations approximating 0.00041, 0.0048 and 0.0046 mg l\(^{-1}\) respectively (Preston, Jeffries, Dutton, Harvey & Steele, 1972). Although seasonal variation in copper concentrations has been found in the English Channel (Atkins, 1953) and the Menai Straits (Foster & Morris, 1971), the large variation of concentrations found, at all sampling times, in whole shrimps, is considered here to be evidence that the seasonal variation in seawater metal concentrations from Filey Bay are not an important contribution to whole-animal level variability. The present studies do, however, show an apparently marked seasonal trend of copper and zinc in whole animal samples of *C. crangon*, with high winter and low summer values. Such a seasonal trend is not evident with cadmium in whole animal samples, but all 3 metals show the same seasonal trend in hepatopancreas levels. The cadmium levels of the haemolymph were below that of reliable detection but haemolymph copper and zinc levels both show a seasonal variation of high summer and low winter values. Cadmium was not detectable in the abdominal flexor muscles, and these tissues were found not to show any seasonal variation in copper or zinc levels.

However, the seasonal variability of metals within separate organs may not be a good indication of total metal flux in *C. crangon*, as metals (e.g. copper and zinc) are probably translocated within tissues (e.g. haemolymph and hepatopancreas) depending upon the metabolic status of the animal. Moreover, the low metal values found in the summer in the hepatopancreas may, to a certain extent, be an artefact of the increase in organic reserves (associated with feeding) 'diluting' the metals present. Therefore, seasonal trends are probably better
shown by the investigation of metals in whole shrimps.

Table 37 summarises extant data on metal levels in whole animal and tissue samples of *C. crangon* and includes those of the present studies. The copper and zinc values found here for the abdominal flexor muscles are in reasonable agreement with those found by Bryan (1968). However, the copper present in this tissue may be the result of contamination with haemolymph (Bryan, 1967, 1968; Djangmah & Grove, 1970). This assumption was based on the muscle/haemolymph copper relationship (Bryan, 1968) but no such clear-cut relationship was evident throughout the seasonal sampling in these studies. The author considers that the thorough washing of all tissues with chelating agents and distilled water was sufficient to minimise haemolymph contamination of the muscle and that the copper values reported represent the base levels of this tissue in *C. crangon*.

The hepatopancreas copper concentrations found by Bryan (1968) were higher than those obtained by Djangmah & Grove (1970) or those in the present study. However, it has been shown conclusively that hepatopancreas copper values will vary with stage in moult cycle and feeding status (Djangmah & Grove, 1970) and the value obtained by Bryan (1968) does, in fact, fall within the range of variability shown by this organ under the above conditions.

No other workers have investigated the tissue cadmium concentrations of *C. crangon*, but whole body concentrations agree reasonably well, apart from the value obtained by Hardisty, Huggins, Katar and Sainsbury (1974). In their study, samples of *C. crangon* from Oldbury-on-Severn had cadmium concentrations about 40 x greater than those found for samples of the Filey Bay population. This large difference in cadmium
concentration probably reflects a difference in the seawater concentrations of the 2 collection sites, as preliminary investigations (Abdullah, Royle & Morris, 1972; Butterworth, Lester & Nickless, 1972; Nickless, Stenner & Terrille, 1972; Preston, 1973) have shown that the Severn Estuary is polluted with many heavy metals, including cadmium.

Generally, the metals were accumulated in selected organs more rapidly in dilute seawater than in normal seawater. Such a salinity-dependent effect has been found for cadmium in some brachyurans (O'Hara, 1973a,b; Hutcheson, 1974; Wright, 1977a), although Wright (1977b) found also that cadmium uptake was dependent upon the external calcium ion concentration, regardless of salinity. However, under normal circumstances, dilution leads to a proportional reduction in calcium ion concentration.

After 24 hrs exposure to cadmium (0.175 mg l⁻¹), the concentration of this metal in the gills had doubled in the dilute seawater group but had increased by only half this amount in the normal seawater group. Hutcheson (1974) found also, that in Callinectes sapidus, after only 12 hrs of exposure, a significant difference in gill cadmium concentration could be shown between all experimental salinities and controls.

Maximum concentrations of ca. 170-180 μg Cd g⁻¹ dry wt were obtained in the gills after 6 days cadmium exposure, for the normal and dilute seawater groups. However, unlike O'Hara (1973b) who noted that fiddler crabs died shortly after obtaining maximum gill concentrations of 110 μg Cd g⁻¹ wet wt, the present values were not found to be critical for C. cragon. Wright (1977a) found gill cadmium levels of Carcinus maenas reached maximum values of 33.7 mg Kg⁻¹ wet wt after
2 weeks of uptake in 50\% seawater, and that any salinity effect on uptake was masked in this species after 48 hrs.

Zinc concentrations in the gills of zinc-treated animals in normal seawater showed a lag of 3 days before any significant accumulation occurred although in dilute seawater such a lag did not occur and uptake was proportional to exposure time. This finding suggests that salinity changes the zinc species to a more readily absorbing form, or, that the increased metabolic work, associated with dilution, increases non-specific ion uptake. No maximum concentrations of this metal were found at the concentrations and times tested here. This may be because the zinc was given at 0.5 x 96 hr LC50 value and that this fell below the ILL.

Copper accumulated in/on the gills of shrimps more rapidly in normal seawater than in dilute (cf. cadmium and zinc) and showed a 4-fold increase after the first 24 hrs. Any salinity-dependent effect was lost by day 5, when maximum concentrations of 1,700-1,800 µg Cu g\(^{-1}\) dry wt were reached. It is possible the high, early concentrations in normal seawater were the result of copper-precipitating from solution and becoming adsorbed to the surface of the gill lamellae. Such precipitation would be reduced in dilute seawater.

In normal salinity regimes, the haemolymph of many invertebrate species shows strict regulation of copper and zinc concentrations, (Bryan, 1964, 1967 & 1968: George et al, 1978). Even under conditions of very high seawater concentrations of copper (10-20 mg % = 100-200 mg l\(^{-1}\)), Djangmah & Grove (1970) found no significant increase in haemolymph copper concentrations in 24 and 48 hr exposed C. crangon. The present studies support these findings (and extend them to include
zinc) as no significant differences in levels of copper or zinc were detected over the 7 day exposure period.

In dilute seawater, the haemolymph concentrations of copper and zinc in the control group animals, at the end of the exposure period, were each approximately 65% of those found in normal seawater controls. Animals in the copper- and zinc-enriched, dilute seawater showed significant increases in haemolymph levels of these metals especially after the first 24 hrs of exposure. The trend of metal variability of the control groups suggest a measure of haemolymph dilution occurred in these groups. Consequently, the large increases of haemolymph copper and zinc found for the groups in metal-enriched seawater suggest that such dilution may be accompanied by a lessened specificity of ion uptake and a lessened efficiency of the detoxification mechanism.

The failure to detect cadmium in the haemolymph of groups exposed to normal or dilute seawater suggests that the detoxification mechanisms (nephrocytes in the gill epithelium; Toseland & Nott pers. comm., and translocation to the hepatopancreas) are able to cope with the concentrations used here, or that the volumes of haemolymph used here were too low. More work, using pooled blood samples, is needed before unequivocal statements can be made:

However, Wright (1977c) found that haemolymph cadmium of Carcinus maenas in 50% seawater was double that of animals in 100% seawater, after 14 days exposure to 20 μ-mol Cd l⁻¹ (≈2.248 mg Cd l⁻¹). This, he suggests, was because nearly all the cadmium was quickly bound to haemolymph proteins. Also, death was preceded by an increase in haemolymph cadmium which suggests that this detoxification process
Metal uptake in the hepatopancreas showed a marked salinity effect for all 3 metals studied. In normal and dilute seawater, a linear rise in hepatopancreas cadmium occurred over 7 days exposure, with the greatest rise in dilute seawater (Fig. 37a). Hutcheson (1974) showed a rise in Callinectes sapidus hepatopancreas cadmium after 96 hr exposure to 10ppm cadmium, but was not able to show any clear effects of salinity. Wright (1977a) showed that the hepatopancreas cadmium of Carcinus maenas rose to a maximum value (33.7 mg Kg\(^{-1}\) wet wt.) and then levelled off, but also, could not elucidate any salinity effect. As far as the author is aware, no comparable data is available giving salinity effects on the uptake of copper and zinc in decapod crustaceans. The increased concentration of these metals (and cadmium) found in the hepatopancreas of metal-treated shrimps in dilute seawater, may well be a result of the increased metabolic activity associated with the maintenance of a hyperosmotic internal milieu.

No change in cadmium, copper and zinc concentrations occurred in the abdominal flexor muscles after exposure to these metals. This finding is in accord with those of Eisler, Zaroorogian & Hennekey (1972) who found no such increase in lobster muscle cadmium concentrations, and Hutcheson (1974) who found no increase in claw muscles of Callinectes sapidus exposed to a thermosaline regime of cadmium solutions. Similarly, Bryan (1964) showed that injections of zinc into Homarus vulgaris failed to produce any change in muscle zinc concentrations.

As large increases in haemolymph copper were observed to occur
in dilute seawater treated animals, and as no concomitant increases
were observed to occur in abdominal muscle, these findings are taken
as further evidence of the lack of haemolymph contamination of muscle
samples.

After 7 days exposure to 0.175 mg Cd l\(^{-1}\), no significant (P>0.05)
differences in cadmium concentrations, for any of the tissues studied,
were observed between the fed and starved groups. This suggests that
the principal site of uptake of cadmium is via the gills and not via
food.

Of the other metals monitored (calcium, copper and zinc), in the
tissues, only hepatopancreas copper and zinc values showed any difference
between the fed and starved groups after 7 days exposure to cadmium.
Probably, these differences arose as a consequence of the deprivation
of food rather than as a direct result of exposure to cadmium. The
high hepatopancreas copper levels in starved animals are probably
the result of 2 physiological processes. Firstly, the utilization of
hepatopancreas organic reserves result directly in the diminution in size
of this organ (Stewart, Cornick, Foley, and Bishop, 1967; Djangmah,
1970) and secondly, catabolism of haemocyanins would result in the
storage of released copper (Djangmah, 1970). Possibly, similar
processes would account for the observed increases in hepatopancreas
zinc levels.

Hutcheson (1974) found no loss of cadmium occurred from Callinectes
sapidus subsequent to its removal from contaminated water and spending
96 hrs in uncontaminated water. Wright (1977b), however, found that
Carcinus maenas, loaded with cadmium after 37 days exposure, subsequently
showed a 50% drop in body cadmium levels after 11 days in uncontaminated
seawater. Losses from the gills and carapace were considered to be important components of this reduction. Fowler & Benayoun (1974) found that Lysmata seticaudata lost only 45% of accumulated $^{109}$Cd during an 8 month period in cadmium-free seawater. In these studies, changes of cadmium and other metals were studied in individual tissues, not whole animals.

No overall change in cadmium concentrations were detected in the gills after 3 weeks of the shrimp being returned to uncontaminated seawater although, in both the fed and starved groups, the gill levels of calcium, copper and zinc all tended to decline. These fluctuations may reflect the haemolymph metal concentrations, although haemolymph was not studied because of its indetectable cadmium levels.

The hepatopancreas cadmium and calcium concentrations rose in both starved and fed groups. This behaviour of cadmium suggests that its translocation from the gills via nephrocytes and haemolymph to the hepatopancreas is very slow. The concurrent increase in calcium cannot easily be explained. However, Wright (1977b) showed a similar rise in Carcinus maenas haemolymph calcium in the presence of cadmium ions and suggested there was some degree of competition for 'deposition' sites between these metals. Certainly, the ratio of the metals in the hepatopancreas would substantiate this suggestion. However, the opposite trend was found for branchiostegite metal level variability, i.e. the cadmium levels rose whilst the calcium levels declined. This finding suggests the preferential deposition of cadmium in the carapace, at the expense of the calcium ions. As C. crangon had been observed to feed on moulting exoskeletons, this method of 'loss' may serve to concentrate the metal (see Fowler & Small, 1967; Martin, 1970).
The gills of aquatic organisms are, by their nature and function, likely to be very vulnerable to damage through contact with toxic ions. Studies have shown that the epithelium of fish gills is damaged by zinc (Lloyd, 1960; Skidmore, 1970), copper (Crandall & Goodnight, 1963; Baker, 1969), cadmium (Gardner & Yevich, 1970) and by mixtures of cadmium, copper and zinc (Eisler & Gardner, 1973).

In the present studies, only copper produced any noticeable changes in the external morphology or the ultrastructure of *C. crangon* gill epithelium. Other workers, however, have shown that cadmium damages the gills of natantians (Nimmo et al. 1977; Thorpe & Lake, 1974; Toseland & Nott, pers comm). Damage to gill epithelia presumably impairs the osmoregulatory, ion exchange and oxygen exchange capabilities of the gills and, thereby, will enhance the susceptibility of the animals to any environmental changes. This has been shown to occur in fish (Lloyd, 1960; Skidmore, 1970; Burton, Jones & Cairns, 1972; McCarty & Houston, 1976) but such effects remain conjectural for crustacean species.

There is considerable evidence to suggest that the hepatopancreas (or some similar organ in other marine invertebrates) acts as a deposition site for unwanted metal ions. Studies using histological and/or X-ray microprobe analysis have located granules containing high concentrations of metals e.g. copper in hepatopancreas of *C. crangon* (Djangmah & Grove, 1970); copper and zinc granules in *Ostrea edulis* (George, Pirie, Cheyne, Coombs & Grant, 1978); copper granules in *Balanus balanoides* (Walker, 1977) and zinc granules in *B. balanoides* (Walker, Rainbow, Foster & Crisp, 1975). However, few reports exist on the possible damage caused to particular organs by the presence of excess metal ions, in crustaceans.
O'Hara (1973b) and Hutcheson (1974) found that the hepatopancreas of the crab species they studied, changed from a discrete, glandular organ into an amorphous, liquified state when the animals were exposed for 24-96 hrs to high concentrations of cadmium. In the present experiments, low concentrations and long exposures were tested (0.35 mg Cd l⁻¹ for 14 days). These conditions produced no overt change to the integrity of the hepatopancreas though some ultrastructural changes (cf. control organs) were noted. Together, these findings suggest that, for cadmium at least, the rate of accumulation determines the extent of the histological damage.

The hepatopancreases of copper-treated shrimps, however, did show similar gross morphological changes to those found for cadmium-treated crabs by O'Hara (1973b) and Hutcheson (1974). Electron micrograph studies of hepatopancreases taken from sacrificed animals, revealed that cellular degeneration was extensive (see Fig. 45). This finding, however, is enigmatic as starved animals from uncontaminated seawater could tolerate hepatopancreas copper concentrations far in excess of those attained by fed animals in copper-enriched seawater, and with no apparent damage to the organ. Therefore, absolute concentration per se does not appear to be the cause of tissue breakdown. Moreover, starved Crangon can accumulate copper in the hepatopancreas at a rate equivalent to that of fed animals in copper-enriched seawater; so rate of uptake, absolute concentration and anion effects are unlikely to be the potential causes of cellular breakdown. Although the present findings are not sufficient to resolve this enigma, it is possible that different processes exist for the translocation and storage of non-haemocyanin copper and haemocyanin released copper, and that the former process is the less efficient.
The electron micrographs of hepatopancreas tissue from zinc-treated *C. crangon* also showed the presence of considerable cellular disorder - though not to the extent shown in copper-treated specimens. In these instances, the damage may have been caused by the higher concentrations and the higher rates of metal accumulation of this metal into the hepatopancreas (cf. starved and fed control groups).
Table 25: Summary of analysis of variance and significance of (a) copper and (b) zinc concentrations in the haemolymph of *C. crangon* exposed for 7 days to 2.1 mg Cu l⁻¹ and 5.6 mg Zn l⁻¹ respectively in S, 34% seawater.

(a)

<table>
<thead>
<tr>
<th></th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>Sig</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>2139.73</td>
<td>7</td>
<td>305.68</td>
<td>1.64</td>
<td>P&gt;0.2</td>
</tr>
<tr>
<td>Error</td>
<td>10410.38</td>
<td>56</td>
<td>185.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>12550.11</td>
<td>63</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(b)

<table>
<thead>
<tr>
<th></th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>Sig</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>2133.50</td>
<td>7</td>
<td>304.9</td>
<td>2.31</td>
<td>P&gt;0.05</td>
</tr>
<tr>
<td>Error</td>
<td>7402.25</td>
<td>56</td>
<td>132.18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>9535.75</td>
<td>63</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 26: Summary of analysis of variance and significance of (a) copper and (b) zinc concentrations in the haemolymph of *C. crangon* exposed for 7 days to clean S, 34% seawater.

(a)

<table>
<thead>
<tr>
<th></th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>Sig</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>230.25</td>
<td>2</td>
<td>115.13</td>
<td>0.95</td>
<td>P&gt;0.5</td>
</tr>
<tr>
<td>Error</td>
<td>2555.75</td>
<td>21</td>
<td>121.70</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>2786.00</td>
<td>23</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(b)

<table>
<thead>
<tr>
<th></th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>Sig</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>24.25</td>
<td>2</td>
<td>12.13</td>
<td>0.13</td>
<td>P&gt;0.5</td>
</tr>
<tr>
<td>Error</td>
<td>1994.25</td>
<td>21</td>
<td>94.96</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>2018.50</td>
<td>23</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SS = sum of squares, df = degrees of freedom, MS = mean squares.
Table 27: Summary of analysis of variance and significance of (a) copper and (b) zinc concentrations in the haemolymph of C. crangon exposed for 7 days to 2.1 mg Cu l⁻¹ and 5.6 mg Zn l⁻¹ respectively in S, 10% seawater.

(a)  

<table>
<thead>
<tr>
<th></th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>Sig</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>15838.86</td>
<td>7</td>
<td>2262.69</td>
<td>11.85</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>Error</td>
<td>10690.63</td>
<td>56</td>
<td>190.90</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>26529.48</td>
<td>63</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(b)  

<table>
<thead>
<tr>
<th></th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>Sig</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>12107.36</td>
<td>7</td>
<td>1729.62</td>
<td>13.62</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>Error</td>
<td>7112.38</td>
<td>56</td>
<td>127.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>19219.73</td>
<td>63</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SS = sum of squares, df = degrees of freedom, MS = mean squares.
Table 28: Summary of analysis of variance and significance of the hepatopancreas cadmium concentrations of *C. crangon* exposed for 7 days to (a) 0.175 mg Cd \( \text{l}^{-1} \) in 34% seawater (b) clean S, 34% seawater and (c) 0.175 mg Cd \( \text{l}^{-1} \) in S, 10% seawater.

<table>
<thead>
<tr>
<th></th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>Sig</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Treatment</td>
<td>41007.23</td>
<td>6</td>
<td>6834.54</td>
<td>4.00</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td>Error</td>
<td>59766.95</td>
<td>35</td>
<td>1707.63</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>100774.18</td>
<td>41</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>Sig</th>
</tr>
</thead>
<tbody>
<tr>
<td>(b) Treatment</td>
<td>179.08</td>
<td>2</td>
<td>89.54</td>
<td>1.02</td>
<td>P&gt;0.5</td>
</tr>
<tr>
<td>Error</td>
<td>1840.75</td>
<td>21</td>
<td>87.65</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>2019.83</td>
<td>23</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>Sig</th>
</tr>
</thead>
<tbody>
<tr>
<td>(c) Treatment</td>
<td>139692.36</td>
<td>7</td>
<td>19956.05</td>
<td>82.07</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>Error</td>
<td>13617.13</td>
<td>56</td>
<td>243.16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>153309.40</td>
<td>63</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 29: Summary of analysis of variance and significance of the hepatopancreas copper concentrations of *C. crangon* exposed for 7 days to (a) 2.1 mg Cu l⁻¹ in S, 34% seawater (b) clean S, 34% seawater and (c) 2.1 mg Cu l⁻¹ in S, 10% seawater.

(a) \[
\begin{array}{lccccc}
  & SS & df & MS & F & Sig \\
  Treatment & 3125078.12 & 7 & 446439.73 & 5.73 & P<0.001 \\
  Error & 4361727.61 & 56 & 77887.99 & & \\
  Total & 7486805.73 & 63 & & & \\
\end{array}
\]

(b) \[
\begin{array}{lccccc}
  & SS & df & MS & F & Sig \\
  Treatment & 37313.09 & 2 & 18606.55 & 0.81 & P>0.5 \\
  Error & 484619.87 & 21 & 23077.14 & & \\
  Total & 521832.96 & 23 & & & \\
\end{array}
\]

(c) \[
\begin{array}{lccccc}
  & SS & df & MS & F & Sig \\
  Treatment & 10807603.80 & 7 & 1543943.40 & 16.97 & P<0.001 \\
  Error & 5096241.60 & 56 & 91004.31 & & \\
  Total & 15903845.40 & 63 & & & \\
\end{array}
\]
Table 30: Summary of analysis of variance and significance of the hepatopancreas zinc concentrations of C. crangon exposed for 7 days to (a) 5\(\cdot\)6 mg Zn l\(^{-1}\) in 34\% seawater (b) clean S, 34\% seawater and (c) 5\(\cdot\)6 mg Zn l\(^{-1}\) in S, 10\% seawater.

\[
\begin{array}{cccccc}
\text{SS} & \text{df} & \text{MS} & F & \text{Sig} \\
\hline
\text{Treatment} & 189802.86 & 7 & 27114.69 & 38.55 & P<0.001 \\
\text{Error} & 39386.88 & 56 & 703.34 & & \\
\text{Total} & 229189.73 & 63 & & & \\
\hline
\text{Treatment} & 446.00 & 2 & 224.00 & 0.70 & P>0.5 \\
\text{Error} & 6720.50 & 21 & 320.02 & & \\
\text{Total} & 7168.50 & 23 & & & \\
\hline
\text{Treatment} & 139692.36 & 7 & 19956.06 & 82.07 & P<0.001 \\
\text{Error} & 13617.13 & 56 & 243.16 & & \\
\text{Total} & 153309.48 & 63 & & & \\
\end{array}
\]

SS = Sum of squares; df = degrees of freedom
MS = mean squares.
Table 3: Summary of 1 tailed analysis of variance and significance of metal concentrations in the hepatopancreas of fed *C. crangon* over a 3 week period, following a 7 day exposure to 0.175 mg Cd l⁻¹.

(a) Cadmium

<table>
<thead>
<tr>
<th></th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>23681.03</td>
<td>4</td>
<td>5920.26</td>
<td>3.04</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>Error</td>
<td>44792.85</td>
<td>23</td>
<td>1947.52</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>68473.88</td>
<td>27</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(b) Calcium

<table>
<thead>
<tr>
<th></th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>180739.85</td>
<td>4</td>
<td>45184.96</td>
<td>4.11</td>
<td>P&lt;0.025</td>
</tr>
<tr>
<td>Error</td>
<td>241868.44</td>
<td>22</td>
<td>10994.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>422608.30</td>
<td>26</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(c) Copper

<table>
<thead>
<tr>
<th></th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>263331.73</td>
<td>4</td>
<td>65832.93</td>
<td>0.58</td>
<td>P&gt;0.5</td>
</tr>
<tr>
<td>Error</td>
<td>262251.60</td>
<td>23</td>
<td>114023.08</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>2885883.33</td>
<td>27</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(d) Zinc

<table>
<thead>
<tr>
<th></th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>5748.86</td>
<td>4</td>
<td>1437.21</td>
<td>0.71</td>
<td>P&gt;0.5</td>
</tr>
<tr>
<td>Error</td>
<td>46641.61</td>
<td>23</td>
<td>2027.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>52390.46</td>
<td>27</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SS = sum of squares, df = degrees of freedom, MS = mean squares.
Table 32: Summary of 1 tailed analysis of variance and significance of metal concentrations in the hepatopancreas of starved *C. crangon* over a 3 week period, following 7 days exposure to 0.175 mg Cd l⁻¹.

(a) **Cadmium**

<table>
<thead>
<tr>
<th></th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>33302.85</td>
<td>4</td>
<td>8325.71</td>
<td>1.15</td>
<td>P&gt;0.2</td>
</tr>
<tr>
<td>Error</td>
<td>116003.45</td>
<td>16</td>
<td>7250.22</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>149306.30</td>
<td>20</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(b) **Calcium**

<table>
<thead>
<tr>
<th></th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>19545059.72</td>
<td>4</td>
<td>4886264.93</td>
<td>3.01</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>Error</td>
<td>25966006.89</td>
<td>16</td>
<td>1622876.43</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>45511066.61</td>
<td>20</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(c) **Copper**

<table>
<thead>
<tr>
<th></th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>10153135.40</td>
<td>4</td>
<td>2538284.85</td>
<td>1.05</td>
<td>P&gt;0.5</td>
</tr>
<tr>
<td>Error</td>
<td>41110002.40</td>
<td>17</td>
<td>2418235.44</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>51263141.80</td>
<td>21</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(d) **Zinc**

<table>
<thead>
<tr>
<th></th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>4054.60</td>
<td>4</td>
<td>1013.65</td>
<td>0.33</td>
<td>P&gt;0.5</td>
</tr>
<tr>
<td>Error</td>
<td>55856.26</td>
<td>18</td>
<td>3103.13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>59910.86</td>
<td>22</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SS = sum of squares, df = degrees of freedom, MS = mean squares.
**Table 33:** Comparisons and significance of mean hepatopancreas metal concentrations of the fed and starved groups of *C. crangon,* over a 3 week period in clean seawater following 7 days exposure to 0.175 mg Cd l⁻¹.

Hepatopancreas metal concentration

(µg g⁻¹ ± S.E.)

<table>
<thead>
<tr>
<th>Day</th>
<th>n</th>
<th>Cd</th>
<th>Ca</th>
<th>Cu</th>
<th>Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Fed 8</td>
<td>115±11</td>
<td>265±24</td>
<td>607±76</td>
<td>149±12</td>
</tr>
<tr>
<td></td>
<td>Starved 8</td>
<td>144±23</td>
<td>4950±1691</td>
<td>2425±192</td>
<td>241±23</td>
</tr>
<tr>
<td></td>
<td>P&gt;0.20</td>
<td>P&lt;0.02</td>
<td>P&lt;0.001</td>
<td>P&lt;0.005</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Fed 8</td>
<td>91.5±11</td>
<td>478±36</td>
<td>728±55</td>
<td>145±13</td>
</tr>
<tr>
<td></td>
<td>Starved 8</td>
<td>151±3</td>
<td>1093±150</td>
<td>4180±201</td>
<td>238±22</td>
</tr>
<tr>
<td></td>
<td>P&lt;0.001</td>
<td>P&lt;0.002</td>
<td>P&lt;0.001</td>
<td>P&lt;0.005</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Fed 8</td>
<td>132±21</td>
<td>297±37</td>
<td>674±99</td>
<td>167±23</td>
</tr>
<tr>
<td></td>
<td>Starved 8</td>
<td>214±25</td>
<td>651±218</td>
<td>4128±597</td>
<td>223±23</td>
</tr>
<tr>
<td></td>
<td>P&lt;0.05</td>
<td>P&gt;0.1</td>
<td>P&lt;0.001</td>
<td>P&gt;0.1</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Fed 8</td>
<td>147±22</td>
<td>311±38</td>
<td>907±190</td>
<td>147±22</td>
</tr>
<tr>
<td></td>
<td>Starved 8</td>
<td>223±31</td>
<td>429±139</td>
<td>3775±717</td>
<td>260±15</td>
</tr>
<tr>
<td></td>
<td>P&lt;0.1</td>
<td>P&gt;0.2</td>
<td>P&lt;0.002</td>
<td>P&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>Fed 8</td>
<td>178±16</td>
<td>401±74</td>
<td>509±174</td>
<td>183±15</td>
</tr>
<tr>
<td></td>
<td>Starved 8</td>
<td>255±79</td>
<td>819±214</td>
<td>3992±1252</td>
<td>227±35</td>
</tr>
<tr>
<td></td>
<td>P&gt;0.2</td>
<td>P&lt;0.1</td>
<td>P&lt;0.02</td>
<td>P&gt;0.2</td>
<td></td>
</tr>
</tbody>
</table>
Table 34: Summary of 1 tailed analysis of variance and significance of metal concentrations in the branchiostegites of fed C. crangon over a 3 week period in clean seawater following 7 days exposure to 0.175 mg Cd l\(^{-1}\).

(a) **Cadmium**

<table>
<thead>
<tr>
<th></th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>Sig</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>264.77</td>
<td>4</td>
<td>66.19</td>
<td>3.76</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>Error</td>
<td>351.89</td>
<td>20</td>
<td>17.59</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>616.66</td>
<td>24</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(b) **Calcium**

<table>
<thead>
<tr>
<th></th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>Sig</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>6.77</td>
<td>4</td>
<td>0.19</td>
<td>1.49</td>
<td>P&gt;0.5</td>
</tr>
<tr>
<td>Error</td>
<td>2.59</td>
<td>20</td>
<td>0.13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>3.36</td>
<td>24</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(c) **Copper**

<table>
<thead>
<tr>
<th></th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>Sig</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>176.22</td>
<td>4</td>
<td>44.05</td>
<td>0.21</td>
<td>P&gt;0.5</td>
</tr>
<tr>
<td>Error</td>
<td>3749.53</td>
<td>18</td>
<td>208.31</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>3925.75</td>
<td>22</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(d) **Zinc**

<table>
<thead>
<tr>
<th></th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>Sig</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>429.39</td>
<td>4</td>
<td>107.35</td>
<td>0.70</td>
<td>P&gt;0.5</td>
</tr>
<tr>
<td>Error</td>
<td>2906.20</td>
<td>19</td>
<td>152.96</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>3335.59</td>
<td>23</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SS = sum of squares, df = degrees of freedom, MS = mean squares
Table 35: Summary of 1 tailed analysis of variance and significance of metal concentrations in the branchiostegites of starved *C. crangon* over a 3 week period in clean seawater following 7 days exposure to 0.175 mg Cd l⁻¹:

(a) **Cadmium**

<table>
<thead>
<tr>
<th></th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>Sig</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>145.47</td>
<td>4</td>
<td>11.37</td>
<td>1.26</td>
<td>P&gt;0.5</td>
</tr>
<tr>
<td>Error</td>
<td>135.60</td>
<td>15</td>
<td>9.04</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>181.07</td>
<td>19</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(b) **Calcium**

<table>
<thead>
<tr>
<th></th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>Sig</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>1.18</td>
<td>4</td>
<td>0.29</td>
<td>5.15</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td>Error</td>
<td>0.91</td>
<td>16</td>
<td>0.06</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>2.09</td>
<td>20</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(c) **Copper**

<table>
<thead>
<tr>
<th></th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>Sig</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>1109.02</td>
<td>4</td>
<td>277.25</td>
<td>1.50</td>
<td>P&gt;0.5</td>
</tr>
<tr>
<td>Error</td>
<td>2772.01</td>
<td>15</td>
<td>184.80</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>3881.03</td>
<td>19</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(d) **Zinc**

<table>
<thead>
<tr>
<th></th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>Sig</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>491.89</td>
<td>4</td>
<td>122.97</td>
<td>0.86</td>
<td>P&gt;0.5</td>
</tr>
<tr>
<td>Error</td>
<td>2289.43</td>
<td>16</td>
<td>143.09</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>2781.32</td>
<td>20</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SS = sum of squares, df = degrees of freedom, MS = mean squares.
Table 36: Mean concentrations (µg g⁻¹ Dry Wt.) of cadmium, copper and zinc in (a) gills and (b) hepatopancreas of C. crangon after 14 days exposure to 0.35 mg Cd l⁻¹, 5.5 mg Cu l⁻¹ and 11 mg Zn l⁻¹.

(a) Gills

<table>
<thead>
<tr>
<th>Metal</th>
<th>Control (fed)</th>
<th>Control (starved)</th>
<th>Experimental</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cadmium</td>
<td>11</td>
<td>15</td>
<td>195</td>
</tr>
<tr>
<td>Copper</td>
<td>210</td>
<td>149</td>
<td>2310</td>
</tr>
<tr>
<td>Zinc</td>
<td>150</td>
<td>128</td>
<td>1940</td>
</tr>
</tbody>
</table>

(b) Hepatopancreas

<table>
<thead>
<tr>
<th>Metal</th>
<th>Control (fed)</th>
<th>Control (starved)</th>
<th>Experimental</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cadmium</td>
<td>14±1.6</td>
<td>35.3±4.3</td>
<td>1750±20</td>
</tr>
<tr>
<td>Copper</td>
<td>340±98</td>
<td>4733±208</td>
<td>2830±390</td>
</tr>
<tr>
<td>Zinc</td>
<td>165±30</td>
<td>335±43</td>
<td>738±124</td>
</tr>
</tbody>
</table>
Table 37: Concentrations of cadmium, copper and zinc in whole body and selected tissues of *C. crangon* from various collection sites.

<table>
<thead>
<tr>
<th>Collection Site</th>
<th>Tissue</th>
<th>n</th>
<th>Cadmium (µg g⁻¹)</th>
<th>Copper (µg g⁻¹)</th>
<th>Zinc (µg g⁻¹)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oldbury-on-Severn</td>
<td>Whole body</td>
<td>6</td>
<td>-</td>
<td>-</td>
<td>(112)</td>
<td>Bryan (1968)</td>
</tr>
<tr>
<td></td>
<td>Blood</td>
<td>6</td>
<td>-</td>
<td>68¹</td>
<td>23¹</td>
<td>&quot;</td>
</tr>
<tr>
<td></td>
<td>Abdominal muscle</td>
<td>6</td>
<td>-</td>
<td>(15)</td>
<td>(49)</td>
<td>&quot;</td>
</tr>
<tr>
<td></td>
<td>Hepatopancreas</td>
<td>6</td>
<td>-</td>
<td>(1818)</td>
<td>(273)</td>
<td>&quot;</td>
</tr>
<tr>
<td>Milford Haven</td>
<td>Whole body</td>
<td>10</td>
<td>-</td>
<td>-</td>
<td>125.9±1.1</td>
<td>Hardisty et al (1974)</td>
</tr>
<tr>
<td>Lower Medway Estuary</td>
<td>Whole body</td>
<td>10</td>
<td>-</td>
<td>4.9±0.6</td>
<td>101.0±1.0</td>
<td>&quot;</td>
</tr>
<tr>
<td>Lymouth Power Station</td>
<td>Whole body</td>
<td>4</td>
<td>12.3</td>
<td>-</td>
<td>-</td>
<td>Wright (1976)</td>
</tr>
<tr>
<td></td>
<td>Muscle</td>
<td>2</td>
<td>-</td>
<td>(90)</td>
<td>-</td>
<td>&quot;</td>
</tr>
<tr>
<td></td>
<td>Exoskeleton</td>
<td>2</td>
<td>-</td>
<td>(24.5)</td>
<td>-</td>
<td>&quot;</td>
</tr>
<tr>
<td>Rhosneigr</td>
<td>Blood</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>58-200¹</td>
<td>Djangmah &amp; Grove (1970)</td>
</tr>
<tr>
<td></td>
<td>Hepatopancreas</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>870²</td>
<td>&quot;</td>
</tr>
<tr>
<td>Red Wharf</td>
<td>Blood</td>
<td>-</td>
<td>-</td>
<td>15-160¹</td>
<td>-</td>
<td>&quot;</td>
</tr>
<tr>
<td></td>
<td>Hepatopancreas</td>
<td>-</td>
<td>-</td>
<td>468²</td>
<td>-</td>
<td>&quot;</td>
</tr>
<tr>
<td>Weston-super-Mare</td>
<td>Whole body</td>
<td>-</td>
<td>(12)</td>
<td>-</td>
<td>(124)</td>
<td>Peden et al (1973)</td>
</tr>
<tr>
<td>Filey Bay³</td>
<td>Whole body</td>
<td>20</td>
<td>2.5-3.5</td>
<td>70-130</td>
<td>75-120</td>
<td>This study (1979)</td>
</tr>
<tr>
<td></td>
<td>Blood</td>
<td>20</td>
<td>-</td>
<td>59-130¹</td>
<td>20-83¹</td>
<td>&quot;</td>
</tr>
<tr>
<td></td>
<td>Abdominal flexor</td>
<td>20</td>
<td>-</td>
<td>5-9.8</td>
<td>25-31</td>
<td>&quot;</td>
</tr>
<tr>
<td></td>
<td>Hepatopancreas</td>
<td>20</td>
<td>14-27</td>
<td>510-950</td>
<td>145-275</td>
<td>&quot;</td>
</tr>
</tbody>
</table>

¹ = µg ml⁻¹
² = estimated as the mean of monthly means
³ = range of monthly mean values of n=20 shrimps
Fig. 34. Monthly, mean concentrations of cadmium, copper and zinc in (a) whole animal preparations (b) haemolymph (c) hepatopancreas (d) abdominal flexor muscles.
Fig. 35. Daily metal concentration found in gills of pooled samples of n=8 *C. crangon*, exposed to (a) 0.175 mg Cd l\(^{-1}\) (b) 2.1 mg Cu l\(^{-1}\) and (c) 5.6 mg Zn l\(^{-1}\), at 2 salinities, S,10% and S,34%.
Graphs a, b, and c show the accumulation of metal (Cu and Zn) in gills over time (days). The graphs are plotted on a log scale, with the x-axis representing time (days) and the y-axis representing the concentration of metal (μg Cu/g Dry Wt. gill or μg Zn/g Dry Weight). The graphs compare the accumulation at 10% and 34% concentrations. The results are labeled as "GILLS."
Fig. 36. Daily mean haemolymph concentration of specimens of *C. crangon* exposed to (a) 2.1 mg Cu l\(^{-1}\) and (b) 5.6 mg Zn l\(^{-1}\) at 2 salinities, S, 10% and S, 34%.
a. **HAEMOLYMPH**

- 10% Experimental
- 34% Experimental
- 34% Control
- 10% Control

Time (Days)

b. **HAEMOLYMPH**

- 10% Experimental
- 34% Experimental
- 34% Control
- 10% Control

Time (Days)

**Fig. 36**
Fig. 37. Daily, mean hepatopancreas metal concentrations of specimens of *C. crangon* exposed to (a) 0.175 mg Cd l\(^{-1}\) (b) 2.1 mg Cu l\(^{-1}\) and (c) 5.6 mg Zn l\(^{-1}\) at 2 salinities, S, 10%, and S, 34%.
Fig. 38. Concentrations of cadmium, calcium copper and zinc in samples of (a) fed and (b) starved *C. crangon*, tested during a 21 day recovery period in clean seawater, following exposure to 0.175 mg Cd l\(^{-1}\).
Fig. 38

Days in clean Sea-water

Days in clean Sea-water
Fig. 39. Mean hepatopancreas concentrations of (a) cadmium (b) calcium (c) copper and (d) zinc in fed and starved groups of C. crangon, tested during a 21 day recovery period in clean seawater, following 7 days exposure to 0.175 mg Cd l$^{-1}$. 
Fig. 39

**a.**

μg Cadmium g⁻¹ Dry Wt. Hepatopancreas

**b.**

μg Calcium g⁻¹ Dry Wt. Hepatopancreas

**c.**

μg Copper g⁻¹ Dry Wt. Hepatopancreas

**d.**

μg Zinc g⁻¹ Dry Wt. Hepatopancreas

Days in clean Sea-water

Days in clean Sea-water
Fig. 40. Mean branchiostegite concentrations of (a) cadmium (b) calcium (c) copper and (d) zinc in fed and starved groups of *C. crangon*, tested during a 21 day recovery period in clean seawater, following 7 days exposure to 0.175 mg Cd 1^{-1}.
Fig. 40

μg Copper g⁻¹ Dry Wt. Branchiostegite

μg Zinc g⁻¹ Dry Wt. Branchiostegite

μg Cadmium g⁻¹ Dry Wt. Branchiostegite

μg Calcium g⁻¹ Dry Wt. Branchiostegite
Fig. 41a. Single phyllobranchiate gill from a control (50mm long) specimen of *C. crangon*. Note the broad plate-like lamellae characteristic of this type of gill. *Print mag. x 35*

b. Single gill taken from a live, apparently healthy, 50mm long specimen of *C. crangon*, after 100 hrs exposure to 5·5 mg Cu l⁻¹. The lamellae appear to be fused with a black deposit of Cu complex, and, subjectively, appeared more rigid than control gills. The central axis, also, is reduced in width (cf. control gill). *Print mag. x 35*

c. External view of a live, apparently healthy, 50mm long specimen of *C. crangon*. The appearance of the black deposit on the inside of the branchiostegite, corresponds to the time of appearance of the blackened deposits on the gills, as seen above (Fig. 41b). *Print mag. x 3*
Fig. 42a. Outer cuticular layer of gill lamella of control *C. crangon*. Note the fibrillar layer orientated perpendicular to the gill lamella.

Print mag. x21,070

Fb=Fibrillar layer, Ep=Epicuticle, En=Endocuticle.

b. Cuticle of gill lamella of control *C. crangon*, showing the filamentous strands associated with the bacterial flora. Note also, the endocuticle comprising flocculent material.

Print mag. x65,875

Bac=Bacterium.
Fig. 43. Electron micrograph showing the extensive bacterial flora and the cuticle of the gill lamella of a stage D11 shrimp. Note the electron-dense layers (EDL) in the endocuticle.

Print mag. x 49,657

BP=Basal plaques, M=Mitochondrion.
Fig. 43
Fig. 44. Electron micrograph showing the presence of haemocytes (H) in the blood sinus (BS) of a gill lamella of a control *C. crangon*. The nucleus (N) is electron-dense and surrounded by electron-lucent granules (ELG).

Print mag. x10,050

BL=Basal lamina.
Fig. 45. Electron micrograph of copper-treated gill lamella. The cuticle has been thrown into folds and the epicuticle is more electron-dense than from untreated shrimps. Many membrane-bound vesicles (V) are present; some containing flocculent material. No apparent damage to the nuclei is evident.

Print mag. x18,400

GA=Golgi apparatus.
Fig. 46. Schematic diagrams (adapted from Loizzi, 1971) of types of cells present in sections through the hepatopancreas of control _C. crangon_.

mag. _ca._ x3000

BI=Basal invaginations, ER=Endoplasmic reticulum
Gly=Glycogen, LD=Lipid droplets, Lu=Lumen
Mv=Microvilli, Pin=Pinocytotic vesicles
Sec=Surface enteric coat.
Absorptive Cells

Fig. 46
Fig. 47. Electron micrograph of hepatopancreatic cells of cadmium-treated *C. crangon*. Proliferation of mitochondria at the apical portion of the cell and an extensive endoplasmic reticulum are evident.

Print mag. x11,510
Fig. 48. Electron micrograph of hepatopancreatic cells of copper-treated C. crangon. These cells are characterised by degenerate nuclei and amorphous cell contents.

Print mag. ca. ×50,000
CHAPTER 5.

THE EFFECT OF CADMIUM, COPPER AND ZINC ON
THE CARDIAC AND VENTILATORY ACTIVITIES OF
CRANGON CRANGON (L.).

Recently, an interest has developed in the inclusion of cardiac and ventilatory activities as possible indicators of metabolic rate variability in decapod crustaceans. Reports so far, have been confined mainly to assessments of variation in heart frequencies associated with biotic (sex, size and molt condition) and abiotic (temperature, salinity, photoperiod) variables, but the possibility remains that...
INTRODUCTION.

Death is an unequivocal endpoint in toxicity studies but can be considered to be a crude index of stress in pollution studies. Many metals exert their toxic effects by acting as non-specific enzyme inhibitors (see Dixon & Webb, 1964). Consequently, it is reasonable to assume that an alteration in the metabolic status of an animal may be a logical and sensitive index of pollutant stress. In the goldfish, *Carassius auratus* and the crustacean, *Eriphia spinifrons*, D'Amelio, Russo & Ferraro (1974) used changes in rates of protein synthesis, and polyribosomal structures respectively as metabolic indicators of pollution stress. More commonly, oxygen consumption changes have been used as an index of the effects of pollutants. Brown & Newell (1972) and Scott & Major (1972) found that copper reduced oxygen consumption in whole specimens of *Mytilus edulis* but Collier, Miller, Dawson & Thurburg (1973) found that exposure of the mud crab, *Eurypanopeus depressus* to high levels of cadmium evoked no change in oxygen consumption. Vernberg, Decoursey, Kelly & Johns (1977) also failed to detect any change in oxygen consumption of *Palaemonetes pugio* when exposed to cadmium and they concluded that respiration rates (as oxygen consumption) were not 'predictable and reliable' indices of cadmium pollution.

Recently, an interest has developed in the inclusion of cardiac and ventilatory activities as possible indicators of metabolic rate variability in decapod crustaceans. Reports so far, have been confined mainly to assessments of variation in heart frequencies associated with biotic (sex, size and moult condition) and abiotic (temperature, salinity, photoperiod) variables, but the possibility remains that
such activities may show variations from 'normal' patterns when the animals are exposed to pollutants.

Many early reports on heart and scaphognathite activities were made by direct observations of the beating organs (Maynard, 1960; Thompson & Pritchard, 1969). Such methods require the animal to have a transparent exoskeleton or that it be immobilized. In many instances, surgery is required to render the organs patent. Such a method was used to observe the heart rate of Mytilus edulis exposed to copper (Scott & Major, 1972).

More recently, pressure transducers have been used to record scaphognathite activities (e.g. McMahon & Wilkens, 1972; Taylor, Butler & Sherlock, 1973; Hume & Berlind, 1976). However, these techniques also require considerable restraint of the test organism and are unsuitable for species as small as Carcinus maenas.

Impedance techniques have been used successfully to record heart and scaphognathite activities in Carcinus maenas (Cumberlidge, 1977; Cumberlidge & Uglow, 1977a,b., 1978) and Crangon crangon, (Dyer, 1978; Dyer & Uglow, 1977, 1978a,b.). The advantage of these techniques is that they allow cardiac and ventilatory activities to be monitored using non-invasive electrodes and with minimal restriction of the normal repertoire of whole animal movements.

The aim of the studies reported in this chapter, was to carry out preliminary investigations of the effects of cadmium, copper and zinc on cardiac and ventilatory behaviour of Carcinus maenas and to assess the potential use of such activities as indicators of pollution stress.

Dyer (1978), in a comprehensive study of the effects of various
biotic and abiotic factors on cardiac and ventilatory activities in
*C. crangon*, found that size and moult-stage differences (apart from immediate pre- and postmoult) did not affect beat frequencies of either organ to any significant extent. Therefore, in these studies, only animals in moult stages C₉, D₀, D₁ and within the size range 55-60mm body length, have been used. The larger shrimps are easier, technically, to wire up with electrodes and the intermoult period of larger animals is relatively longer than that of smaller ones. Dyer (1978) found also that *C. crangon* showed almost constant bilateral synchrony of scaphognathite movements and concluded that it was valid to monitor but one scaphognathite to obtain an accurate record of total ventilatory activity. Consequently, in these studies, the left scaphognathite only was monitored routinely.
1. **Electrodes and electrode implantation.**

Recording electrodes were made from shellac-coated copper wire (44 s.w.g.) which, for heart rate recording, had the last 3mm bared and, for scaphognathites, had the last 1mm bared. In both cases the last 2-3mm of the electrode were bent twice through 180°, the second bend being perpendicular to the plane of the first and 1mm posterior to the bared end.

Anchorage of the electrodes was effected by their adhesion onto the cephalothorax (previously roughened with 200 grade Emery paper) using low melting point black wax.

Heart electrodes were hooked over the posterior margin of the cephalothorax so that the bare end of the wire lay dorsal to the pericardium. Scaphognathite electrodes were hooked over the anterior margin of the branchiostegites so that their bared ends came to lie close to, but not touching, the anterior tip of the scaphognathite. Left scaphognathites only were monitored as these were technically easier to fit with electrodes than were the right scaphognathites.

Electrode attachment was carried out in 3 stages:

- **a)** The animal removed from water, the cephalothorax was roughened and a drop of black wax smeared on to the roughened cuticle.
- **b)** The heart electrode was affixed.
- **c)** The scaphognathite electrode was affixed.

Between each procedure stage, the animal was returned to seawater for 10 minutes. Such procedures reduced mortalities. Animals were left...
for 4 complete days to recover from the stresses of handling and electrode implantation, before any recordings were made of organ activities.

2. Heart and scaphognathite activity recording.

Organ activities were recorded using impedance techniques using the following systems:

a) The Impedance Pneumograph and the 'Physiograph' system (Narco Biosystems Inc.).

b) The Classic H7 Impedance Pneumograph (Scientific Instruments Centre) coupled to a 2-channel oscillograph (George Washington Ltd.).

Either of these systems were used to obtain pen traces of organ activity, usually in conjunction with an impedance detection system (developed at Hull) which monitored simultaneously the inputs of up to 10 impedance pneumographs. Inputs were monitored for preselected periods of time, at the end of which, the numbers of elapsed beats for each input were recorded as a digital print-out. (Roxburgh Electronics Ltd., RP 10-16 data printer). Inputs automatically resumed being monitored immediately after the command signal was given by the printer.

3. Experimental Animal Selection.

Specimens of *C. crangon* were selected to be of body length 55-60mm and of moult stages $C_b$, $D_0$, $D_1$. The rationale for these choices was because larger shrimps technically are more amenable to electrode implantation than are smaller shrimps, and large shrimps have a longer intermoult period than smaller shrimps. The latter
point is important as electrodes are dislodged with the old exoskeleton when the animals moult.

4. Acute organ responses to cadmium, copper and zinc.

These experiments were performed under conditions of static water at $T=17^\circ C$ and $S$, 34%. All tanks were sub-divided by perforated 'Perspex' partitions and contained a 2cm deep layer of sand and, initially, 2 l of aerated seawater.

Test metals were added by siphoning 2 l of test solution into the experimental tank so that the required concentration was achieved in a total of 4 l of seawater.

Cadmium, copper, zinc and sodium sulphate (for controls) were added to give final concentrations of 0.1, 1.0, 5.0 and 20.0 mg metal l$^{-1}$.

Simultaneous recordings of heart and scaphognathite activity of each test animal were taken as 6 x 5 minutes of recording prior to metal addition and from 5 minutes after metal addition. Data were calculated as the mean ± S.E. beats min$^{-1}$ for 30 min prior to, and immediately after, metal addition. Organ activities were subsequently recorded (10 minutes of recording for each organ) after 48 hrs post-addition of metals. Experimental and control shrimps were not fed during these experiments.

5. Organ responses to chronic exposure to cadmium, copper and zinc.

Animals were wired up for recording heart and scaphognathite activities and placed in experimental tanks
(as described above, page 104). After 4 days, the clean seawater was siphoned off and replaced with seawater contaminated with 0.005mg Cd l⁻¹ or 0.75mg Cu l⁻¹ or 5.5mg Zn l⁻¹. When the contaminated seawater was added initially, it was rapidly siphoned off and replaced with more, containing the same concentration of metal. This procedure was repeated 3-4 times as preliminary studies had shown, by AAS analysis of the seawater, that this was necessary to obviate dilution with the uncontaminated water remaining in the sand of the tank. During the 21 day course of the experiment, the experimental and control solutions were changed in the same manner, immediately after the organ activities had been recorded on days 4, 9, 13 and 17.

Experimental and control shrimps were not fed during these experiments.

Samples of recordings of heart activity were taken either as 10 min traces (the beats were subsequently counted) and/or as 10 min digital printouts. Recordings were taken at precisely the same time of day at each recording session.

6. **Organ activities during a 21 day recovery period in clean seawater after 3 weeks exposure to sublethal doses of cadmium, copper and zinc.**

Specimens of *C. crangon* were placed, 12 in each, into static water tanks containing 4 l of seawater with concentrations of 0.005mg Cd l⁻¹ or 0.75mg Cu l⁻¹ or 5.5mg Zn l⁻¹ at T=17°C, S, 34%. Tanks were provided with a 2cm layer of sand and perforated 'Perspex' dividers. Twenty four animals were used for each test metal and for
the control group. All solutions were changed every 96 hrs and any dead specimens were removed daily. The animals were not fed during this part of the experiment.

After the 3 week metal exposure period, heart and scaphognathite electrodes were attached to n=8, apparently healthy, shrimps selected from each group and these animals were transferred to tanks containing clean seawater.

Experimental and control groups were fed every other day with chopped *Mytilus edulis*. All animals were left undisturbed for 4 days, to allow recovery from handling and electrode implantation procedures, before organ activities were recorded. Recordings of organ activities were taken as 10 minute traces and as digital printouts of elapsed beats. Seawater was changed after the recordings were made on days 3, 7 and 14.
RESULTS.

Because of the considerable degree of individual variation in beat frequency shown by the hearts and scaphognathites of the normal (pre-treated) animals, the worth of using absolute rate data in comparative studies of acute response to metals has been considered here to be minimal. Instead, relative changes in rates ($\Delta f$), expressed as a percentage change from pretreatment rates ($f$), have been calculated as a measure of the response of each organ for each individual exposed to one or other of the metals. Figure 49 illustrates bilogarithmic plots of $\Delta f$ against $f$ for each organ type at 1 particular concentration for each of the 3 metals. The linearity of these curves allows the response of any organ to be calculated for an arbitrarily determined value of $f_s$ (standardised pretreatment frequency) thereby removing much of the complicating initial sampling error. For these studies, values for $f_s$(heart) and $f_s$(scaphognathite) have been selected as 70 and 100 beats min$^{-1}$ respectively, approximately mean values for a reasonably large sample of animals - to obtain the mean responses of $\Delta f_s$.

Plotting the values of $\Delta f_s$ obtained at different test concentrations of the metals against these concentrations produces response curves as shown in Fig. 50. Each organ responded to each of the metals with direct, linear increase of $\Delta f_s$ with concentration. The slopes of the plots are very similar for heart and scaphognathite exposed to any one particular metal and this is seen with ratios of scaphognathite : heart rates at the particular concentrations of the metals chosen (Table 38). Interestingly, none of the organ responses were sufficiently large to allow a reliable standard response.
curve to be drawn at concentrations of 0.1mg l\(^{-1}\) of any metal or for any of the sham treatments. Consequently, once the threshold for response is reached at some concentration <1mg l\(^{-1}\), an acute response of a substantial change in organ beat rate occurs.

Forty-eight hours after exposure to the metals, the frequencies of the hearts and scaphognathites of surviving shrimps were obtained and transformed to \(\Delta f\) values with reference to pretreatment values and immediate post-treatment \(\Delta f\) values.

Although the numbers of surviving animals were too small, in most cases, to allow reliable \(\Delta f\) values to be obtained, it is possible to comment on the trends of the changes that occurred. Table 39. summarises the mean \(\Delta f\) (± S.E.) of heart and scaphognathite rates as calculated from a) initial frequencies, and b) 30 minute post-treatment frequencies.

In all cadmium concentrations the frequencies of heart and scaphognathites had continued to increase over the values obtained after 30 minutes of exposure to this metal. After 48 hrs exposure to 1.0 or 5.0mg Cu l\(^{-1}\) or 1.0 and 5.0mg Zn l\(^{-1}\), the frequencies of hearts and scaphognathites had returned to approximately those pertaining in the pre-treatment animals. However, in 20mg Cu l\(^{-1}\), the rates of both organs had increased markedly over the 30 minute post-treatment values. In 20mg Zn l\(^{-1}\) the heart rates were not different from those of the 30 minute post-treatment values (ca. 60% higher than pretreatment values) whereas the scaphognathite frequencies had decreased by ca. 20% from post-treatment values.
Qualitative changes during the acute response of hearts and scaphognathites to exposure to cadmium, copper and zinc.

Figure 51a illustrates typical traces of the undisturbed heart and scaphognathite activities of C. crangon kept under the experimental conditions but before being exposed to the metals (i.e. static water T=16°C; S, 34% and in the presence of a sand substratum). Heart rates of this species are fairly constant and scaphognathite traces typically include pauses (apnoeas) which total approximately 5.5 min⁻¹ pumping time.

On exposure to low (0.1-5.0 mg l⁻¹) concentrations of the metals and on exposure to all the concentrations of Na₂SO₄ that were presented, the scaphognathite activity responded in 1 of 2 ways. Either they ceased beating completely for ca. 20-25 secs. and then resumed for several minutes at a higher rate than before, or, they displayed alternate periods of apnoea with high frequency bursts (5-15 beats) of beating (Fig. 51b). Such behavioural responses (rarely lasting >3 min) were not accompanied by any overt whole-animal activity changes, were common to all the control groups also (addition of seawater) and are interpreted here as responses to the mechanical stimulus of adding test solutions.

When high (20.0 mg l⁻¹) concentrations of metals were added, however, the large majority of test animals emerged from the sand and swam vigorously. This behaviour caused pen deflections to go off-scale and precluded the recording of the immediate reactions of heart and scaphognathites. After 3-4 minutes, most animals had ceased swimming and traces of organ activities could be obtained. Initially, with all metals, there was an abolition of arrhythmic events.
in the organ activities (see Fig. 51c) and beat rates were high (>450 and >200 beats min$^{-1}$ for scaphognathite and hearts respectively). However, such high rates rarely lasted for more than 10 minutes.

In the cases of copper- and zinc-treated animals, after the period of initial high rates had passed, the scaphognathites, typically, showed regular bursts of rapid beating (ca. 8-10 min$^{-1}$ see Fig. 51d) which persisted until the animals died. Dyer & Uglow (1978b) used a triple impedance electrode technique to demonstrate that such transient bursts of rapid beating were scaphognathite reversals. Patterns of scaphognathite activity which included very regular reversals occurred also, after 48 hrs exposure, in animals treated with 1.0 and 5.0 mg l$^{-1}$ copper.

The cadmium-treated animals maintained very arrhythmic patterns of heart and scaphognathite activities at all concentrations except 0.1 mg l$^{-1}$.

Only those animals exposed to 0.1 mg l$^{-1}$ cadmium, copper or zinc or 1.0 mg l$^{-1}$ zinc were ever observed to rebury in the sand, all the other test animals remained on the surface of the sand.

Organ responses to chronic exposure to cadmium, copper and zinc.

Ten minute samples of recordings were taken simultaneously for hearts and scaphognathites at various times over a period of 20 days. These recordings were made from animals in the following groups: - a) control, b) 0.005 mg Cd l$^{-1}$, c) 0.75 mg Cu l$^{-1}$ and d) 5.5 mg Zn l$^{-1}$. The metal concentrations chosen correspond to the incipient lethal levels of the metals at 16°C.
Figure 52a illustrates the data obtained for organ rates of the control group (n=8). Only one specimen died during the experiment but several others moulted so that, by the end of 20 days, only 3 shrimps were able to be monitored. Heart rates declined progressively over the course of the experiment whereas scaphognathite rates were not significantly (P>0.05) different from original values at the end of the experiment.

At the end of the experiment, the heart rates of the cadmium-treated group were not significantly (P>0.05) different from original values (Fig. 52b) but were significantly (P<0.001) greater than those of the control group at this time. This difference may have been even greater but the numbers of animals retaining their electrodes after day 12 was reduced to 3. The scaphognathite rates of this experimental group rose progressively between days 4 and 13. Thereafter, only 2 animals had retained their electrodes. Comparison of mean values showed the rates at Day 13 were significantly (P<0.01) higher than those at Day 1. Interestingly, no major qualitative changes to scaphognathite beat behaviour occurred at these concentrations (cf. the effects of higher concentrations, page 110).

Figure 52c illustrates the effects of exposure to 0.75mg Cu l\(^{-1}\) on organ activities. The mean rates of both organs showed a trend of progressively increased rate with exposure time and, in both cases, the final values were significantly (P<0.01) higher than original values. Only 1 animal died in this group but scaphognathite recordings could be made from only 3 animals after Day 12 because of the electrodes becoming 'plated' with copper and thus providing a 'noisy' signal. For the first 7 days of exposure, no qualitative...
differences in scaphognathite activities were evident - apart from the frequency changes. Subsequently, however, scaphognathite apnoeas were abolished and the occurrence of reversals increased to values of 5-10 min\(^{-1}\) - the same level as that shown by animals exposed to high concentrations (page 110). Coincident with this change in scaphognathite behaviour was the appearance of blackened cuticular lesions on the gills (see also Fig. 41b). No qualitative changes in heart beat behaviour were observed to occur.

Twenty days exposure to zinc resulted in heart rates that were significantly (P<0.01) greater than original values, although little change occurred within the first 11 days of exposure (Fig. 52d). Similarly, scaphognathite rates were little affected until day 11 but thereafter, the 2 animals which had retained electrodes, showed a marked increase in rates. These 2 animals also showed a marked increase in reversal rate after Day 11 and, by Day 20, were showing ca. 5 reversals min\(^{-1}\) (cf. <1 min\(^{-1}\) in a normal, undisturbed animal).

Heart and scaphognathite of C. crangon during a 21 day recovery period in clean seawater, after 21 days exposure to sub-lethal levels of cadmium, copper and zinc.

In these experiments, the first recordings (Day 1) were taken during the 5\(^{th}\) day in clean seawater. This delay was to allow animals to recover from the stress of handling and electrode implantation.

The control group animals displayed rates which did not vary significantly over the 3 week period (P>0.05 in both cases, Fig. 53a). Figure 53b illustrates the data obtained in the cadmium-treated group and shows that heart rates remained relatively constant at between
125-150 beats min\(^{-1}\) over the whole period - values that were significantly \((P<0.01)\) higher than those of the control group. The scaphognathite rates of this group, however, rose progressively over the period from an original mean value of ca. 190 beats min\(^{-1}\) (cf. 135 beats min\(^{-1}\) for the control group) to a final mean value of 280 beats min\(^{-1}\). The heart and scaphognathite traces showed an absence of any arrhythmic events such as cardiac pausing, apnoeas or reversals.

Figure 53c shows the data obtained for organ rates in the copper-treated group. Neither organ rate varied appreciably over the course of the recovery period and showed mean rates that varied between 200-236 beats min\(^{-1}\) and 122-141 beats min\(^{-1}\) for scaphognathites and hearts respectively. These values were significantly higher than those of the control animals \((P<0.001\) in both cases). However, scaphognathite reversals were reduced to final levels of 2-3 min\(^{-1}\) (cf. 5-10 min\(^{-1}\) on Day 1).

The behaviour of the organ beat frequencies in the zinc-treated animals differed from those described for any of the other groups (Fig. 53d). A gradual, progressive retardation of rates occurred for both organs and final mean rates were not significantly different from those of the control group \((P>0.05\) in each case). No evidence of increased reversal frequencies were seen in the Day 1 recordings of scaphognathite activity (cf. recordings of 21 days exposure to zinc, page 112) and, possibly, these were abolished during the post electrode implantation recovery period.

It is interesting to note that none of the shrimps recovering from sub-lethal exposures to any of the metals, moulted during the
experiment - despite being fed. Two (25%) of the control animals moulted during the same period.
DISCUSSION.

From these results it is apparent that there is a threshold concentration of cadmium, copper or zinc below which *C. crangon* either cannot detect the presence of the metals or shows no overt sign that it can detect them. Perhaps surprisingly, this threshold appears to lie between 0.1 and 1.0 mg l\(^{-1}\) for all 3 metals. All 3 metals at 1.0 mg l\(^{-1}\) concentration induced a very marked increase in both heart and scaphognathite beat frequency but this was not accompanied by any apparent change in the locomotory activity of the animals. At this concentration, and for the short time span of the experiment, all 3 metals would be completely in solution (i.e. no precipitates of particular metals) which suggests that the organ responses are evoked by chemical stimuli and not by mechanical stimuli such as particulate material in the gill chambers. Neither can the responses be attributed to an alteration of locomotory activity, which has been shown to be accompanied by heart and scaphognathite beat rate increases (Dyer & Uglov, 1977). At the highest concentrations of each metal used, however, the animals were observed to display greatly increased walking/swimming activities and this behavioural change may be contributory factor to the changes in organ rates that were observed.

The hierarchy of magnitude of organ responses was Cu>Zn>Cd which does not coincide with the hierarchy of toxicity of these metals (Cd>Cu>Zn, page 35). Possibly, copper and zinc were recognised by *C. crangon* to be toxic ions whereas cadmium, with a chemical configuration similar to that of calcium, was not recognised. However, after 48 hrs exposure to the metals, when these were at equal
concentrations, there resulted a decline of immediate post-treatment rates in the copper- and zinc-treated animals and an increase in the rates of cadmium-treated animals. At this time, the hierarchy of organ responses does correspond with that of the toxicity of the metals.

Although heart and scaphognathite rates were seen to increase with acute exposures to high concentrations and chronic exposures to low concentrations of the metals, certain qualitative changes of scaphognathite beat behaviour occurred also. This qualitative change differed between the cadmium-treated and copper- and zinc-treated groups. In the latter 2 groups, an increase in scaphognathite reversal occurrence was evident but this was not seen with cadmium-treated animals.

The function of scaphognathite reversals is controversial. Arudpragasam & Naylor (1964) have suggested they might serve to ensure adequate ventilation of the most posterior gills in *Carcinus maenas*. On the other hand, Mills (1972) suggests they may facilitate oxygen uptake by disturbing any stagnant diffusion barriers around the gills. A number of workers have suggested that they may play a role in gill-chamber sanitation by dislodging detritus from the gills (Borradaile, 1922; Arudpragasam & Naylor, 1966; McMahon & Wilkens, 1972; Cumberledge, 1977).

During the short-term experiments reported here, it would seem unreasonable that any oxygen deficiency caused by the presence of metals would be sufficient to evoke such an increase in reversal occurrence. More likely, is that the reversals were employed to remove from the gills the particulate metal-complex precipitates which would
be present in seawater solutions of 20mg l$^{-1}$ copper or zinc. At such concentrations, cadmium is very stable in seawater and would not precipitate out. This could explain why no increase in reversals was detected in the cadmium-treated groups.

The results of the chronic exposure experiments in relation to scaphognathite reversal function are more equivocal. Prolonged exposure to low concentrations of copper resulted eventually in blackened deposits on the gills and the branchiostegites and, at the same time, an increase in reversal occurrence. In this instance the reversals could still be fulfilling a sanitary function but, as the gill epithelium was damaged by the exposure to the metal, they may have been evoked to enhance oxygen uptake at the gill surface. On returning copper-treated animals to clean seawater, reversals and blackened copper deposits persisted so these results did not aid resolution of this problem.

In low concentrations of zinc, the increase in scaphognathite reversal frequency coincided with the time that scaphognathite beat rate increased rapidly. When the animals were returned to clean seawater, reversal numbers declined and scaphognathite rates gradually returned to pre-exposure values; these findings indicating possibly that the animals had 'recovered' from the metal treatment. In studies on fish, it was concluded that exposure to zinc caused tissue hypoxia through the lack of gas exchange at the damaged gill (Skidmore, 1970; Burton et al., 1972). Although these workers did not try to assess any possible recovery of Salmo gairdnerii, it would seem unlikely that recovery would occur. The finding that C. crangon does 'recover' after exposure to low concentrations of zinc, coupled with the findings that no detectable damage to gill tissue was evident in E.M. studies of
zinc-treated animals (Chapter 4, page 85), suggests that death of *C. crangon* in zinc, at the concentrations used, is not caused by tissue hypoxia resulting from extensive gill epithelial damage. If this assumption is correct then the increase of scaphognathite reversals would not be associated with the enhancement of oxygen uptake.

On returning *C. crangon* to clean seawater after exposure to 0.005mg Cd l⁻¹, the scaphognathite beat rates were observed to continue accelerating over the 3 week examination period. This continual and progressive increase in the ventilatory response suggests that the metal continues to exert a toxic effect during this time and that the metal which had been taken up was not rendered harmless by some detoxification process.

It is evident from the present findings, that cardiac and ventilatory activities are influenced strongly by the metals studied. Although the responses were not specific to these metals, when used with adequate control experiments, they appear to be sensitive and repeatable bioassays of the effects of exposing the animals to these metals. Thus, they contrast with oxygen consumption, which has been described as an unsatisfactory indication of pollution stress (Collier *et al.*, 1973; Thurberg *et al.*, 1973; Vernberg *et al.*, 1977). A qualifying statement to the above is that scaphognathite behaviour showed a greater response, both qualitatively and quantitively, than the heart activity, and ventilatory activity would appear to be the more sensitive indicator of pollution stress. This difference in magnitude of response, shown by heart and scaphognathites, may be due to the potential (but as yet unmeasured) variability of heart stroke volume. Variation in heart
stroke volume can effect a change in cardiac output without an alteration of beat rate, but the effective stroke volume of scaphognathites has been shown to be constant and frequency independent in at least some decapod species (Cumberlidge & Uglow, 1977b).
Table 38: Ratios of $\Delta f_s$(hearts) : $\Delta f_s$(scaphognathites) at the various concentrations of cadmium, copper and zinc for 30 min. post-treatment samples.

<table>
<thead>
<tr>
<th>Test Concentration Metal</th>
<th>Ratio of $\Delta f_s$(scaphognathite) : $\Delta f_s$(heart)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 mg $l^{-1}$</td>
</tr>
<tr>
<td>Cd</td>
<td>1.25</td>
</tr>
<tr>
<td>Cu</td>
<td>1.70</td>
</tr>
<tr>
<td>Zn</td>
<td>1.30</td>
</tr>
</tbody>
</table>
Table 39: Forty-eight hr Δf values of heart and scaphognathite rates calculated from the initial and the 30 min post-treatment frequencies for the various concentrations used. Values are presented as means ± Standard Errors.

<table>
<thead>
<tr>
<th>Metal</th>
<th>n</th>
<th>Initial</th>
<th>30 min post-treatment</th>
<th>Initial</th>
<th>30 min post-treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cadmium (mg 1⁻¹)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>+27±8</td>
<td>+28±8</td>
<td></td>
<td>+58±23</td>
<td>+43±17</td>
</tr>
<tr>
<td>6</td>
<td>+60±9</td>
<td>+40±5</td>
<td></td>
<td>+124±28</td>
<td>+61±10</td>
</tr>
<tr>
<td>4</td>
<td>+127±18</td>
<td>+76±18</td>
<td></td>
<td>+246±50</td>
<td>+107±33</td>
</tr>
<tr>
<td>2</td>
<td>+161±20</td>
<td>+56±3</td>
<td></td>
<td>+277±44</td>
<td>+37±10</td>
</tr>
<tr>
<td><strong>Copper (mg 1⁻¹)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>+4±4</td>
<td>-2±5</td>
<td></td>
<td>+25±13</td>
<td>+20±14</td>
</tr>
<tr>
<td>10</td>
<td>+8±4</td>
<td>-32±3</td>
<td></td>
<td>+8±6</td>
<td>-40±3</td>
</tr>
<tr>
<td>7</td>
<td>+14±4</td>
<td>-26±5</td>
<td></td>
<td>+8±6</td>
<td>-50±3</td>
</tr>
<tr>
<td>3</td>
<td>+279±44</td>
<td>+87±3</td>
<td></td>
<td>+144±10</td>
<td>+58±18</td>
</tr>
<tr>
<td><strong>Zinc (mg 1⁻¹)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>+2±7</td>
<td>+3±5</td>
<td></td>
<td>0±8</td>
<td>-2±2</td>
</tr>
<tr>
<td>11</td>
<td>0±3</td>
<td>-22±3</td>
<td></td>
<td>+9±6</td>
<td>-43±3</td>
</tr>
<tr>
<td>10</td>
<td>+5±5</td>
<td>-27±4</td>
<td></td>
<td>+5±7</td>
<td>-44±5</td>
</tr>
<tr>
<td>7</td>
<td>+60±12</td>
<td>+1±6</td>
<td></td>
<td>+80±23</td>
<td>-20±5</td>
</tr>
</tbody>
</table>
Fig. 49. Bilogarithmic transform of initial organ frequency ($f$) of (a) hearts and (b) scaphognathites against the percentage in frequency ($\Delta f$) after 30 min. exposure to $1.0 \text{ mg l}^{-1}$ for (i) cadmium (ii) copper and (iii) zinc.
Fig. 49

PERCENTAGE CHANGE IN ORGAN FREQUENCY ($\Delta f$)

a. 1.0 mg Cadmium l$^{-1}$

b. 1.0 mg Cadmium l$^{-1}$

1.0 mg Copper l$^{-1}$

1.0 mg Copper l$^{-1}$

1.0 mg Zinc l$^{-1}$

1.0 mg Zinc l$^{-1}$

HEARTS

SCAPHOGNATHITES

PRE-TREATMENT ORGAN FREQUENCY (BEATS MIN$^{-1}$)
Fig. 50. Magnitude-of-response curves of (a) hearts and (b) scaphognathites of *C. crangon* exposed to various concentrations of cadmium, copper and zinc.
\[ \Delta f_s \]

\[ \text{CONCENTRATION (mg l}^{-1}\text{)} \]

**a.**
- Zinc
- Cadmium
- Copper
- Control

**b.**
- Copper
- Zinc
- Cadmium
- Control

**HEARTS**

**SCAPHOGNATHITIDES**
Fig. 51. Typical impedance traces of heart and scaphognathite beating of C. crangon:

a. Undisturbed control animal. Note the variable beat frequency (particularly for the scaphognathites) and the occasional apnoeas on the scaphognathite trace.

b. Immediately following exposure to low (0.1 - 5.0mg l\(^{-1}\)) concentrations of cadmium, copper, zinc or sodium sulphate (sham). This type of high frequency beating rarely lasted >3 min.

c. 3 - 4 min. after addition of high (20mg l\(^{-1}\)) concentrations of test solutions. Note the complete abolition of arrhythmic events in the beating behaviour.

d. In high concentrations of copper or zinc or after chronic exposure to incipient lethal levels of these metals.

H = Heart; S = Scaphognathite.
A = Apnoea; R = Reversal.
Fig. 52. Heart and scaphognathite frequencies (beats min$^{-1}$ ±S.E.) of C. crangon obtained at intervals during 20 days exposure to (a) clean seawater (b) 0.005 mg Cd l$^{-1}$. (c) and (d) overleaf.
Fig. 52. Heart and scaphognathite frequencies (beats min\(^{-1}\))
S.E.) of _C. crangon_ obtained at intervals during
20 days exposure to (c) 0.75mg Cu l\(^{-1}\).
(d) 5.5mg Zn l\(^{-1}\).
Fig. 53. Heart and scaphognathite frequencies (beats min$^{-1}$ ±S.E.) of C. crangon obtained at intervals during 21 days in clean seawater subsequent to exposure to (a) clean seawater (b) 0.005 mg Cd l$^{-1}$ (c) 0.75 mg Cu l$^{-1}$ and (d) 5.5 mg Zn l$^{-1}$ for 20 days.
Recovery Period in clean sea-water (days)

**Q.**

- a. CONTROL
- b. 0.005 mg l⁻¹ CADMIUM
- c. 0.75 mg l⁻¹ COPPER
- d. 5.5 mg l⁻¹ ZINC

Fig. 53
GENERAL DISCUSSION.

In addition to the other elements of our study, an attempt was made to outline the problem concerning the influence of the species at all stages of the life history of molluscs through successive generations.

The breeding facilities in the Zoology Department, University of Texas, provide a situation well suiting our needs. The species in question include Physa gyrina, although delicately, these stages of the life cycle have not included in this study program.

The events which orderly the adult cycle of the species were classified as physiological, behavioral, and ecological changes observed for these species. From the aspect of experimental design, this is particularly true for the species which are frequently within any one year and which have an extended breeding season which may include two broods within it. In many studies, on species such as Anomia, these variations in physiological status may be considered to affect the magnitude of type of response monitored, and the measure of physiological status should be an integral part of the experimental design. It is for this reason that particular attention has been paid in these studies to describing and illustrating the particular criteria which have been used to segregate the experimental animals into their various stages of the adult cycle.
GENERAL DISCUSSION.

The body of data in this thesis comprise a comprehensive study of the toxicity of cadmium, copper and zinc to adult individuals of *Crangon crangon*.

The information gained, however, is not intended to be suitable for predicting 'safe' concentrations of these metals or for setting water quality standards, as each of these is complicated by the necessity to account for the sensitivity of the species at all stages of its life history - preferably through several generations.

The existing facilities in the Zoology Department, University of Hull are not suitable for hatching and rearing the eggs and larvae of *C. crangon* and, although desirable, these stages of the life cycle were not included in the study programme.

The events which underly the moult cycle of decapod crustaceans govern many of the patterns of physiological behavioural change observed for these animals. From the aspect of experimental design, this is particularly true of diecdysic species which moult frequently within any one year and which have an extended breeding season which may include two broods within it. In many studies, on species such as *C. crangon*, where variation in physiological status may be suspected to affect the magnitude or type of response monitored, some measure of physiological status should be an integral part of the experimental design. It is for this reason that particular attention has been paid in these studies to describing and illustrating the particular criteria which have been used to segregate the experimental animals into their various stages of the moult cycle. *Crangon crangon*
is a readily available species in many coastal areas of Europe and has been used widely in many types of study. It is intended that the moult stage descriptions of this species included here will enable future workers to segregate their test animals into the various moult stages.

Vernberg et al (1974) have stressed that conditions of toxicity testing should, as closely as possible, parallel the conditions pertaining in the natural environment of the test species. However, such conditions are not always practical to reproduce in the laboratory and, in the present studies, the validity of using static-water tank conditions was investigated. The finding that mortalities of *C. crangon* were not significantly different in static water conditions (cf. circulating water systems) makes this species a particularly convenient test organism in pollution studies of this nature.

The seasonal effects of cadmium, copper and zinc toxicity were here discussed in terms of temperature. Brown (1973) suggested that, in poikilothersms, high temperatures (within the normal tolerance range) would increase metabolic rates and thereby favour detoxification processes. However, in poikilothersms, any change in environmental temperatures will not only bring about an overall change in metabolism, but, more importantly, may lead to sharply differential effects on various components of metabolism (Bullock, 1955; Somero & Hochachka, 1976). However, the increasing tolerance at low temperatures (as opposed to increasing susceptibility at high temperatures) seen here in the seasonal acute toxicity tests (Chapter 1) may be a result of the uptake and the detoxification processes having different temperature responses.
It should be emphasized, however, that temperature comprises only one component of many seasonal variables. A point considered, but not included in the experimental design, is that the population structure may also vary with season to show different ratios of sex and moult stages. Thus, Poolsanguan (1975) showed that ovigerous, intermoult females would be expected to predominate in the winter and spring collections and that a high proportion of the late summer/autumn catches would comprise non-ovigerous females. Subsequent experiments (Chapter 3) showed that sex, sexual condition and moult stage condition were variables affected to different extents by acute exposures to copper or zinc (not to cadmium). The ovigerous condition for females, and the intermoult stage of all animals were conditions that favoured tolerance to the metals. Non-ovigerous females, generally, were more susceptible than any of the other sex groupings to copper and zinc. Such differential responses, shown by animals of different physiological condition, will undoubtably have contributed to the characteristics of the toxicity curves produced for cadmium, copper and zinc (Figs. 5b, 6b, 7b) although these curves remain valid for the representative samples tested.

It is reasonable to assume that those environmental and physiological factors which affect the overt susceptibility of *C. crangon* to the 3 test metals, do so *via* their influence on the rates of uptake, detoxification and excretion of these toxicants. Thus, animals in environmental or physiological conditions that necessitate an alteration of the osmoregulatory status of the animals (e.g. at low salinities or during the immediate post-moult stage of the moult cycle) will, by doing so, increase the rate of uptake of toxic ions.
More detailed work (e.g. using labelled metals) is needed before specific pathways of uptake and loss of these 3 metals in *C. crangon* can be elucidated. However, from knowledge of the physiological conditions of the test organisms and of the experimental conditions, coupled with available information in the literature, tentative suggestions on uptake, internal translocation and loss of cadmium in *C. crangon* can be made from the present findings.

Wright (1977b) found that cadmium uptake in *Carcinus maenas* was closely related to the external calcium ion concentration - regardless of salinity. This implies either that cadmium was being taken up, as a case of 'mistaken identity' between similar ions, or that there is competition for binding sites by the 2 metals. In the present studies, all 3 test metals were taken up at a greater rate in dilute seawater than in 'normal' seawater. On removal to clean seawater, cadmium was found to become deposited in the exoskeleton which implies that, for this metal at least, uptake and translocation are by active processes. O'Hara (1973b) holds this view, and other workers also have shown that certain 'non-regulated' metals have been regulated along with species of metals (Galtsoff, 1953; Brooks & Rumsby, 1965, 1967; Coombs, 1972). These findings suggest that active processes are involved and that they are not as specific as Robertson (1960) has suggested (Spaargaren, pers comm).

Generally, metals have an affinity for specific ligands (carboxyl, imidazole and sulphhydryl) which are associated with proteins (Rothstein, 1959). This property allows them to be detoxified by binding to haemolymph proteins (Bryan, 1971). The increased susceptibility seen with the postmoult shrimp treated with copper or zinc, may be a result
of such 'dilution' of the detoxification mechanism (see haemolymph total protein concentrations at various moult stages, page 48). This finding, that differential mortality between the moult stages was not observed to occur with the cadmium-treated shrimps, is difficult to explain. It may imply that protein-binding is not the primary mode of detoxification of this metal. Toseland and Nott (pers. comm.) have found that cadmium in the gills of *C. crangon* is pinocytosed by nephrocytes.

Loss of metals from the test organism can be via the gills, urine or faeces (Bryan, 1964, 1967; Boothe & Knauer, 1972) or the exoskeleton (Benayoun, Fowler & Oregioni, 1974) depending on the type of metal or the particular species of test organism. It is contended that *C. crangon* loses cadmium via the exoskeleton.

Heavy metals can exert their toxic effects by their interaction and damage at the cell surface (e.g. Skidmore, 1970; Burton et al., 1972) or possibly, at lower concentrations, by their interaction with ligands, particularly those associated with special arrangements of amino acid residues. Because of this propensity to proteins, heavy metals are potent, non-specific enzyme inhibitors (Dixon & Webb, 1964). The effects of heavy metals can vary also with their concentration (Hubschman, 1967a), with high concentrations likely to cause damage at the cell surface. In these studies, cadmium and zinc were not found to cause damage at the gill epithelial surface but black lesions were observed to occur with high, and eventually with low, concentrations of copper. In cases where lesions occur, death may be due to asphyxia (as in fish suffering from exposure to zinc, Burton et al., 1972), but when environmental conditions impose an osmotic stress also, then this
added stress may accelerate the rapid demise of the shrimps (see also Thurberg et al, 1973).

Copper was found also to cause extensive cellular damage in the hepatopancreas - the principal storage organ for this metal. The results obtained in the metal uptake experiments, however, appeared to be anomalous. Starved _C. crangon_ were found to increase copper levels (from the catabolism of haemocyanins, Djangmah, 1968; Uglow, 1969) in the hepatopancreas at approximately the same rate as fed, copper-treated shrimps. Furthermore, it was found that starved shrimps could store much higher concentrations in their hepatopancreases, without apparent ultrastructural damage, than could copper-treated animals. As anion effects were eliminated as a potential cause of this damage, the possibility remains that different pathways may operate for the internal translocation of haemocyanin copper and non-haemocyanin derived copper. Such a possibility deserves further studies aimed specifically at resolving these differences.

Cadmium was found to be extremely toxic to _C. crangon_ and the estimated incipient lethal level of this metal was approximately 1100 x and 150 x less than that of zinc or copper respectively. Moreover, cadmium was found to be lethal regardless of the physiological status (moult stage) of the test animals, although its lethality was modified by certain abiotic factors (e.g. salinity, season/temperature). Cadmium exerts its toxic effects at low concentrations and possibly this is the reason why it appeared to cause few overt deleterious effects (other than death); certainly no apparent damage at the cellular or sub-cellular level was evident.

Of the 3 metals tested, zinc was found to be the least toxic to
C. crangon. This may be a reflection of the efficient regulation of this metal. In the toxicity tests a lag of ca. 48 hrs was found to elapse before mortalities began to occur in this metal and, in the zinc uptake experiments, this period of exposure time coincided with that of a sharp increase in the uptake of zinc by the gills. In the uptake experiments, no apparent difference in the haemolymph zinc concentrations was found, although hepatopancreas concentrations rose linearly with exposure time. These findings indicate that the detoxification of zinc by C. crangon, is an efficient process and that the toxic effect may have been exerted partly by the impairment of ion/gas exchange at the gill surface. However, little gross morphological damage was evident at the gill surface (cf. copper-treated shrimps) so, possibly, death was a result of osmoregulatory impairment due to the bioelectric potential at the gill surface caused by zinc ions. Again, further detailed research into the exact mechanisms is required before unequivocable statements can be made concerning modes of toxicity.

Cumberlidge (1977), Dyer (1978) and the data presented in Chapter 5 of this thesis, have shown that cardiac and ventilatory activities (the latter, particularly) are strongly influenced by environmental variables. That these activities are associated with changes in the metabolic rate, however, still awaits corroborative evidence based on blood gas tensions and oxygen consumption. Whatever the functional significance of heart and scaphognathite behaviour, the present findings indicate that their use in providing supportive evidence of changes induced by the 3 test metals, is a valid proposition. Hitherto, the large variability of beat frequency shown by these organs in 'normal'
animals, has limited their adoption as indices of pollution stress in decapods. The use of a standardized response, based on the percentage change from initial, pretreatment organ frequency has proved to be an effective means of overcoming this problem. These preliminary studies provide evidence of the need to extend the development of this technique which shows promise of being a useful and sensitive means of measuring the magnitude of pollution stress, by particular toxicants, to decapod crustaceans.
SUMMARY.

1. In acute toxicity tests to *Crangon crangon* (L.), cadmium was found to be 16x more potent than copper, which itself was 2x as potent as zinc.

2. The acute toxicity of the 3 metals was found to be unaffected by the experimental holding conditions (ie. presence or absence of a sand substratum; static or recirculating seawater), or by the photoperiod. However, salinities > 17% increased the toxicity of the metals. Seasonal/temperature differences were found to manifest their effects by causing, at low temperatures, an increased tolerance of *C. crangon* to the metals.

3. The incipient lethal levels of the 3 metals were found to be 0.005 mg Cd l\(^{-1}\), 0.75 mg Cu \(l^{-1}\) and 5.5 mg Zn \(l^{-1}\) at 10°C, 16°C and 20°C respectively. Mortality at the incipient lethal levels of the metals was not significantly (P>0.05) affected by differences of season/temperature.

4. A pictorial sequence of the stages in the moult cycle of *C. crangon* has been presented. This was designed to clarify the 'modifications' of the criteria, used by other workers to describe moultng of *C. crangon*, which exist in the literature. Opportunity was taken to corroborate the various designated moult stages with some physiological parameters (haemolymph total protein and chloride ion concentrations and relative whole body water content) which are known to vary with the progress of the moult cycle.
5. Animals in the postmoult condition were found to be most susceptible to copper and zinc. Cadmium was equally toxic at all stages of the moult cycle.

6. Non-ovigerous female *C. crangon* were more susceptible to copper and zinc than were males or ovigerous females. No differences in susceptibility to cadmium were found between animals of different sex or sexual condition.

7. Susceptibility to the 3 metals was unaffected (statistically) by size, although a trend of increased susceptibility was noted for small (40mm body length) and large (60mm body length) animals.

8. Survival after 14 days exposure to cadmium, copper or zinc was always associated with a relatively high (cf. dead specimens) hepatopancreas index.

9. Acute levels of cadmium, copper and zinc inhibited moulting totally after 3 days exposure, and also reduced the feeding level to 15% of that found in the control groups. Exposure to incipient lethal levels of the test metals did not affect moulting or feeding behaviour.

10. Mean copper and zinc concentrations in whole-animal preparations showed a seasonal trend of high winter values and low summer values. No such seasonal trend was found for cadmium concentrations. Haemolymph copper and zinc concentrations were highest in the summer samples and
lowest in winter whereas hepatopancreas concentrations of these metals showed the converse trend (ie. maxima and minima in winter and summer months respectively). No seasonal trends of metal concentrations were found in the studies on abdominal flexor muscles.

11. Metal accumulation in/on the gills was generally more rapid in dilute (S, 10%) than in normal (S, 34%) seawater, although the converse was true for copper. Maximum concentrations of ca. 170 mg Cd l\(^{-1}\) and 1700 mg Cu l\(^{-1}\) after 6 and 5 days exposure to 0·175 mg Cd l\(^{-1}\) and 2·1 mg Cu l\(^{-1}\) respectively. No maximum value was obtained during the 7 days exposure to 5·5 mg Zn l\(^{-1}\).

12. Haemolymph copper and zinc concentrations were unaffected by exposure to levels of these metals (given in 11 above) in S, 34% seawater. However, in S, 10% seawater, haemolymph copper and zinc concentrations showed a significant \( (P<0.001) \) increase during the exposure period.

13. Concentrations of cadmium, copper and zinc in the hepatopancreas increased linearly with exposure time on exposure to 0·175 mg Cd l\(^{-1}\), 2·5 mg Cu l\(^{-1}\) or 5·6 mg Zn l\(^{-1}\). This rate of increase was greater in S, 10% seawater than in S, 34% seawater.

14. After 1 weeks exposure to low (0·175 mg Cd l\(^{-1}\)) of cadmium, the concentration of this metal in the gills of \textit{C. crangon} did not decrease following their subsequent return to clean seawater. However, cadmium levels were shown to decrease in the hepatopancreas and increase in
in the branchiostegites. It is suggested that 1 of the methods
C. crangon uses to excrete this metal is via the exoskeleton at
exuviation.

15. Cadmium and zinc, at acute levels, caused little gross morphological
change at the gill surface. Exposure to acute levels of copper
resulted in blackened deposits on the gill surface.

16. Acute levels of cadmium caused little ultrastructural damage to
gill epithelial cells, but increased numbers (cf. untreated
animals) of mitochondria were evident in the apical portions of
absorptive cells. Copper ultimately caused complete cellular
degeneration.

17. The responses of the heart and scaphognathites to acute levels of
cadmium, copper and zinc, have been considered in terms of their
relationship to pre-treatment beat frequencies. The magnitude of the
response ($\Delta f$) of either organ, for any individual, was found to be
related to the original frequency of that organ. The standard
response ($\Delta f_s$) for any organ at any concentration was determined for
preselected frequencies of 70 beats min$^{-1}$ (hearts) and 100 beats min$^{-1}$
(scaphognathites). For any organ and any metal, a direct, linear
association of $\Delta f_s$ to test concentration was found.

18. Cadmium caused the abolition of arrhythmic events characteristic of
normal scaphognathite behaviour; copper and zinc evoked an increased
occurrence of scaphognathite reversals.

19. During chronic exposure to cadmium, copper or zinc at their respective incipient lethal levels, organ beat frequencies increased to levels above those found in untreated C. crangon.

20. During chronic exposure to copper or zinc, an increased incidence of scaphognathite reversal frequencies (cf. normal animals) was evident.

21. During a 21 day recovery period in clean seawater, following 20 days exposure to incipient lethal levels of cadmium, copper or zinc, only the zinc-treated animals appeared to recover (ie. organ beat frequencies returned to those shown by the control group animals). In the copper treated shrimps, scaphognathite beat frequencies continued to increase during the recovery period.

22. The findings in this thesis have been discussed in terms of their functional significance and in relation to information extant in the literature.
ACKNOWLEDGMENTS.

It gives me great pleasure to thank my Supervisor Dr. Rog Uglow, for his constant help, encouragement and friendship throughout the course of these studies. I am indebted also, to Professor J.G. Phillips for providing the research facilities in the department.

I would like to thank Jan Mundy for her invaluable technical assistance in the preparation of material for the electron microscopy studies, and Roland Wheeler-Osman, for the photographic work involved in the preparation of this thesis.

I would like also to extend my gratitude to all the other members of the department who have helped me in one way or another, during the course of this study - particularly, Chris Parke and Chrissie Ware.

Especial thanks are due to my wife, Janet, who's first (laborious) attempts at typing, were in the typing of this thesis.

Finally, I would like to acknowledge my gratitude to the Science Research Council for the provision of a research studentship which allowed me to undertake this work.
BIBLIOGRAPHY.


Heavy metal concentrations in coastal waters.


Acute toxicity of cadmium and zinc to seven invertebrate species from Western Port, Victoria.


Emergence rhythms and tidal migrations in the brown shrimp, Crangon crangon (L).


The detection and measurement of water pollution - Biological assays.


Seasonal changes in the biochemical composition of the bivalve Chlamys septemradiata from the Clyde Sea area.


Seasonal changes in the biochemical composition of the bivalve Nucella sulcata from the Clyde Sea area.


Studies on Tellina tenuis Da Costa. 1. Seasonal growth and biochemical cycle.

APHA, (1965).

Standard Methods for the Examination of Water and Waste water Including Bottom Sediments and Sludges.

Gill ventilation and the role of reversed respiratory currents in Carcinus maenas (L).


Patterns of gill ventilation in some decapod crustacea.
J. Zool. Lond., 150 : 401-411.


The seasonal variation in the copper content of seawater.


Histological and electron microscopical observations on copper poisoning in the winter flounder (Pseudopleuronectes americanus).


The relative susceptibilities of some species of freshwater fish to poisons I. Ammonia.


The toxicity of cadmium to rainbow trout (Salmo gairdnerii, Richardson).


Changes in serum protein during the moult and reproductive cycles of the American lobster (Homarus americanus).


The seasonal changes in body weight, biochemical composition, and oxygen uptake of two common Boreo-Artic cirripedes, Balanus balanoides and B. balanus.


A study of copper, lead and cadmium speciation in some estuarine and coastal marine waters.

Effects of various metals on survival, growth, reproduction and metabolism of Daphnia magna.


Flux of cadmium through Euphausiids.


Eutrophication of Dutch coastal waters.

BILLINGS, G.K., (1965).

Light scattering in trace element analysis by atomic absorption.


The calculation of the Dosage-Mortality curve.

BODANSKY, M., (1920).

Biochemical studies on marine organisms II. The occurrence of zinc.


The possible importance of fecal material in the biological amplification of trace and heavy metals.
Límno/ and Oceanogr., 17 : 270-274.


On the mouth parts of the shore crab.
J. Linn. Soc. (Zool), 35 : 115-143.


The biogeochemistry of trace element uptake by some New Zealand bivalves.
Límno/ and Oceanogr., 10 : 521-527.

Studies on the uptake of cadmium by the oyster Ostrea sinuata (Lamark).


Effect of heavy metals on mortality and growth.


Concepts and outlook in testing the toxicity of substances to fish.
Bioassay Techniques and Env. chemistry (Ann Arbor Sci. Pub. Inc.).


The effect of temperature on the acute toxicity of phenol to rainbow trout in hard water.


The effect of copper and zinc on the metabolism of M. edulis.

BRYAN, G.W., (1964).

Zinc regulation in the lobster Homarus vulgaris I. Tissue zinc and copper concentrations.

BRYAN, G.W., (1967).

Zinc regulation in the freshwater crayfish (including some comparative copper analyses).

BRYAN, G.W., (1968).

Concentrations of zinc and copper in the tissues of decapod crustaceans.

BRYAN, G.W., (1971).

The effects of heavy metals (other than mercury) on marine and estuarine organisms.

Some aspects of heavy metal tolerance in aquatic organisms.


Adaptations of the polychaete Nereis diversicolor to estuarine sediments containing high concentrations of zinc and cadmium.


The absorption and loss of radioactive and non-radioactive manganese by the lobster, Homarus vulgaris.


Fine structure of the gills of Jaera nordmanni (Rathke) (Crustacea ; Isopod).


Compensation for temperature in the metabolism and activity of poikilotherms.


Acute zinc toxicity to rainbow trout (Salmo gairdnerii): confirmation of the hypothesis that death is related to tissue hypoxia.


Distribution of heavy metals in the Severn Estuary.


Fish bioassays - Reproducibility and rating.
Revista de Biologia; 7 : 7-12.

A preliminary report on rapid biological information for water pollution control.


Moultting cycles in crustacea.


Cadmium toxicity and bioconcentration on large mouth bass and bluegill.


Physiological response of the mud crab Eurypanopeus depressus to cadmium.


A continuous-flow apparatus for assessing the toxicity of substances to marine animals.


The distribution of zinc in the oyster, Ostrea edulis and its relation to enzymic activity and to other metals.


Diseases, parasites and toxic responses of commercial penaeid shrimps of the Gulf of Mexico and South Atlantic coasts of North America.


The distribution of Pb, Zn, Cd and Cu within the pulmonate Mollusc, Helix aspera (Miller).
Oecologia (Berl), 23 : 315-322.


The effects of sublethal concentrations of several toxicants to the common guppy, Lebistes reticulatus.

Studies on the activity of the heart and scaphognathites of the shore crab, Carcinus maenas (L).
Ph. D. Thesis (Hull).


Heart and scaphognathite activity in the shore crab, Carcinus maenas (L).


Size, temperature and scaphognathite frequency-dependent variations of ventilation volumes in Carcinus maenas (L).


Heart and scaphognathite activity during the digging behaviour of the shore crab, Carcinus maenas (L).


The effect of heavy metals on protein synthesis in crustaceans and fish.


Effect of temperature and salinity on the oxygen consumption of two intertidal crabs.


The Enzymes.


Studies on the blood proteins of Crangon vulgaris (Fabr.).
Ph.D. Thesis (University of Wales).


The effects of feeding and starvation on copper in the blood and hepatopancreas, and on the blood proteins of Crangon vulgaris (Fabricius).

Blood and hepatopancreas copper in *Crangon vulgaris*.  


Critical review of literature on the toxicity of industrial wastes and their components to fish. II. The metals, as salts.  
Sewage Ind. Wastes, 25 : 802-839.

DRACH, P., (1939).

*Mue et cycle d'intermue chez les crustacés decapods*.  

DRACH, P., (1944).

*Étude préliminaire sur le cycle d'intermue et son conditionnement hormonal chez Leander serratus* (Pennant).  


*Sur la méthode de détermination des stades d'intermue et son application générale aux crustacés*.  
Via et Milieu, 18 : 595-610.


Studies of the cardiac and ventilatory behaviour of the brown shrimp, *Crangon crangon* (L).  
Ph.D. Thesis (Hull).


On a technique for monitoring heart and scaphognathite activity in Natantia.  


Gill chamber ventilation and scaphognathite movements in *Crangon crangon* (L).  


Heart and scaphognathite beat behaviour in laboratory held *Crangon crangon* (L).  
A comparison of Litchfield-Wilcoxon and Bliss estimates.
Biometrics, 8 : 120-121.

Cadmium poisoning in Fundulus heteroclitus (Pisces: Cyprinodontidae) and other marine organisms.

Acute toxicology to an estuarine teleost of mixtures of cadmium, copper and zinc salts.

Cadmium uptake by marine organisms.

Haemolymph DNA concentrations during the moult cycle of the freshwater crayfish, Procambarus clarkii.

A morphological study on gills of the brown shrimp, Penaeus aztecs.
Tissue and Cell, 10 : 77-92.

The seasonal variation of dissolved ionic and organically associated copper in the Menai Straits.

Experimental studies on cadmium flux through marine biota.
I.A.E.A. Proceedings of Symposium on Nuclear Techniques in Comparative Studies of Food and Environmental Contamination.
Otaniemi, Finland. pp. 159-178. Vienna : IAEA.

Moulting of Euphausia pacifica as a possible mechanism for vertical transport of zinc-65 in the sea.
GALTSOFF, P.S., (1953).
Accumulation of manganese, iron, copper and zinc in the body of the American oyster, Crassostrea virginica.

Histological and hematological responses of an estuarine teleost to cadmium.

The kinetics of accumulation and excretion of ferric hydroxide in Mytilus edulis (L) and its distribution in the tissues.

Detoxification of metals by marine bivalves : an ultrastructural study of the compartmentation of copper and zinc in the oyster Ostrea edulis.

A new approach to the biochemical composition of the mollusc body.

The oxygen consumption of Crangon vulgaris (Fabricius) (Crustacea, natantia) in relation to salinity.
Ophelia, 7 : 283-292.

Ionic regulation in relation to the moult cycle of Crangon vulgaris (Fabr.) (Crustacea, Natantia) from brackish water.
Ophelia, 12 : 141-149.

The respiration during the moult cycle of Crangon vulgaris (Fabr.) (Crustacea, Natantia).
Ophelia, 15 : 15-21.

The urinary flow in *Crangon vulgaris* (Fabr.) (Crustacea, Natantia) during the moult cycle.

Ophelia, 16 : 143-150.


Ecological implications of heavy metal in fish from the Severn Estuary.


Toxicity of synthetic detergents to rainbow trout.


HIATT, R.W., (1948).


HOBDEN, D.J., (1967).


Iron metabolism in *Mytilus edulis*. II Uptake and distribution of radioactive iron.


Seasonal and diel variation in the rhythmicity of *Idotea baltica* (Pallas) and *Idotea granulosa* (Rathke).

Ophelia, 12 : 117-127.


Effects of copper on the crayfish *Orconectes rusticus* (Girard) I. Acute Toxicity.

Crustaceana, 12 : 33-42.


Effects of copper on the crayfish, *Orconectes rusticus* (Girard) II. Mode of toxic action.

Crustaceana, 12 : 141-150.

Heart and scaphognathite rate changes in a euryhaline crab, *Carcinus maenas*, exposed to dilute media.


Changes in epidermal DNA, Protein and protein synthesis during the moult cycle of the crayfish *Orconectes saborni* (Faxon).
Comp. Biochem. Physiol., **44**: 1121-1128.


The effect of temperature and salinity on cadmium uptake by the blue crab, *Callinectes sapidus*.


Apolysis in arthropod moulting cycles.
Nat., Lond., **211**, 871.


Survival and oxygen consumption in various salinities of three species of *Idorea* (Crustacea, isopoda) from different habitats.


Synergistic effects of salinity, temperature and heavy metal on mortality and osmoregulation in marine and estuarine Isopods (crustacea).


The resistance of rainbow trout (*Salmo gairdneri* Richardson) and roach (*Rutilus rutilus* L.) to alkaline solutions.
Int. J. Air Water Pollut., **8**: 405-409.


Effects of zinc smelter emissions and fire on a Chestnut-Oak woodland.
Ecology, **56**: 78-91.
KEILIN, T. & MANN, T., (1940).

Carbonic anhydrase. Purification and nature of the enzyme.


The effect of copper on the tissue respiration of the crab, Carcinus maenas.


Hormonal control of metabolism in crustaceans. IV. Relation to
tissue composition of Hemigrapsus nudus to intermoult cycle and
sinus gland.


The microbial flora of the rock shrimp - Siconia brevirostris.
J. Milk Food Technol., 38: 747-749.

KRAUSKOPF, K.B., (1956).

Factors controlling the concentrations of thirteen metals in
seawater.


The intermoult cycle of an anomuran, Petrolisthes cinctipes
Randall (Crustacea ; Decapoda).


Some particulate and soluble agents affecting the relationship
between metal toxicity and organism survival in the calanoid
copepod, Euchaeta japonica.


DDT and Endrin fish toxicity under static versus dynamic bioassay
conditions.

The biology of Crangon vulgaris (L.) in the Bristol Channel and Severn Estuary.

LLOYD, R., (1960).

The toxicity of zinc to rainbow trout.


Predicted and observed toxicities of several sewage effluents to rainbow trout.

LITCHFIELD, J.T., (1949).

A method for rapid graphical solution of time/percent effect curves.
J. Pharmac. exp. Ther., 97 : 399-408.


A simplified method for evaluating dose-effect experiments.


A survey of Zn, Pb and Cd in soils and natural vegetation around a smelting complex.


Interpretation of crayfish hepatopancreatic function based on fine structural analysis of epithelial cell lines and muscle network.


Effects of exposure to sublethal doses cadmium upon water electrolyte status in the goldfish, Carassius auratus.


Water quality criteria.
Toxicity of copper at two temperatures and three salinities to the American lobster (*Homarus americanus*).

Simultaneous apnoea and bradycardia in the lobster, *Homarus americanus*.

Observations on the moult cycle of the prawn, *Metapenaeus monoceros* (Fabricius). Part II - Cyclic histological changes in the hepatopancreas.
Broteria, 13 : 135-149.

Observations on the fine structure of two species of *Platymonas* with special reference to flagellar scales and the modes of origin of the theca.

The possible transport of trace metals via moulded copepod exoskeletons.

High copper concentrations in squid livers in association with elevated levels of silver cadmium and zinc.

Heart rate and body size in the spiny lobster.

Setal development and moult staging in the crayfish, *Parastacoides tasmanicus* (Erichson), Decapoda, Parastacidae).
MILLS, P.J. (1972).

Respiration in the Invertebrates.
Macmillon Press, pp 212.


The effects of food supply on moulting, growth and spawning in the shrimp Crangon crangon (L.)
ICES Shellfish Committee, M1-21.


A method for detecting cadmium poisoning in fish.
J. Wildl. Manage., 31 : 168-172.


The occurrence of cadmium in seawater and in marine organisms and sediments.


Electron microscopic study of the open circulatory system of the shrimp Caridina japonica.


Locomotory rhythms in Carcinus maenas (L.) from non-tidal conditions.


Distribution of cadmium, lead and zinc in the Bristol Channel.


Effects of cadmium on the shrimps Penaeus duorarum, Palaemonetes pugio and Palaemonetes vulgaris.
NOUVEL, L., (1939).

Observation de l'accouplement chez une espèce de crevette Crangon crangon.

O'HARA, J., (1973a).

Cadmium uptake by fiddler crabs exposed to temperature and salinity stress.

O'HARA, J., (1973b).

The influence of temperature and salinity on the toxicity of cadmium to the fiddler crab, Uca pugilator.


Studies on accumulation, excretion and distribution of iodine-131 and cadmium-115 in freshwater fish and marine fish.


The ultrastructural morphology of the midgut diverticulum of the calanoid copepod, Calanus helgolandicus (Claus) (Crustacea).


Effects of complexation on toxicity of copper to fishes.


Zinc in a Texas bay.


Studies on the toxicity of cadmium to the three spined stickleback Gasterosteus aculeatus (L.)
PASS → overleaf

Cadmium in the marine environment of the United Kingdom.

British Isles coastal waters: the concentration of selected heavy metals in the seawater suspended matter and biological indicators - a pilot survey.

Some effects of certain heavy metals on development and mortality within the moult cycle of Crangon crangon (L.).
Mar. Env. Pollut. (in press).

Ionic and osmotic concentrations in blood and urine of Pachygrapsus crassipes acclimated to different salinities.

PRYTHERCH, H., (1931).
The role of copper in the settling, metamorphosis and distribution of the American oyster, Ostrea virginica;

The sensitivity of barnacles and their larvae to copper and mercury.

The respiratory function of haemocyanin.

Variations quantitatives de l' acide déoxyribonucléique (ADN) au cours du cycle de mue, dans les téguments, le muscle et l'hépatopan créas de la crevette Crangon crangon (L.).

ROE → cont. 1 page forward

Moulting and its control.


Heavy metals in Somerset organisms.

PENREATH, R.J., (1973a).

The accumulation and retention of $^{65}$Zn and $^{54}$Mn by the plaice, Pleuronectes platessa (L.).

PENREATH, R.J., (1973b).

The accumulation from water of $^{65}$Zn, $^{54}$Mn, $^{58}$Co, $^{59}$Fe by the mussel, Mytilus edulis.


The acute toxicity of some heavy metals to different species of warmwater fishes.


Studies on the quantitative changes of some biochemical constituents in the blood and tissues of Grangon vulgaris (Fabricius).

PORTIMANN, J.E., (1968).

Progress report on a programme of insecticide analysis and toxicity testing in relation to the marine environment.
Helgolander wiss Meeresunters, 17: 247-256.


Evaluation of digestion techniques for the AAS determination of metal in kelp.
Unpublished report presented before the Division of Environmental Chemistry American Chemical Society, Los Angeles, California March 31-April 5, 1974.

Thermal acclimation of metabolism in the crab Pachygrapsus crassipes Randall. I. The influence of body size, starvation and moulting.


ROBERTSON, J.D., (1960).

Osmotic and Ionic Regulation.


Behavioural rhythms in littoral prawns.


Synergistic effects of cadmium and salinity combined with constant and cycling temperature on the larval development of two estuarine crab species.


Cell membrane as site of action of heavy metals.


Cadmium uptake and time dependent alterations in tissue levels in the white catfish, Ictalurus catus (Pisces : Ictaluridae).

Bull. Environ. Contam. Toxicol., 11 : 244-249.


Acclimation and tolerance of Artemia salina to copper salts.


SCHERR, B.T., (1960).

Aspects of the Intermoult cycle of Natantians.


The effects of copper (II) on the survival, respiration and heart rate in the common blue mussel Mytilus edulis.

SEVERLY, W., (1923).

The occurrence of copper and zinc in certain marine animals.


Trace metal accumulation by the american eastern oyster, Crassostrea virginica.


Respiration and osmoregulation in rainbow trout with gills damaged by zinc sulphate.


The structure and metabolism of a crustacean integumentary tissue during a moult cycle.

SLAVIN, W., (1965).

Comments on 'Light scattering in trace-element analysis'.


Biochemical adaptions to temperature.


Determination of the extracellular volume in the shrimp, Crangon crangon (L).


The effect of salinity and temperature on the heart-rate of osmoregulating and osmoconforming shrimps.


Changes in the chemical composition of the common shore crab, Carcinus maenas, during the moult cycle.

Lethal concentrations of copper and zinc for young Atlantic salmon.


Measurement of pollutant toxicity to fish. I. Bioassay methods for acute toxicity.


Measurement of pollutant toxicity to fish. II. Utilizing and applying bioassay results.


Muscle weight relationship to serum proteins, haemocytes and hepatopancreas in the lobster, Homarus americanus.


The respiratory and cardiovascular changes associated with the emersion response of Carcinus maenas (L) during environmental hypoxia, at three different temperatures.


Respiratory adoptions of two burrowing crustaceans, Callianassa californiensis and Upogebia pugettensis (Decapoda : Thalassiuidea).


Toxicity bioassays of cadmium on selected freshwater invertebrates and the interaction of cadmium and zinc on the freshwater shrimp, Paratya tasmaniensis Rick.


Effects of copper and cadmium on osmoregulation and oxygen consumption in two species of estuarine crabs.
Synopsis of biological data on the common shrimp *Crangon crangon* (Linnaeus, 1758).
FAO Fisheries Synopsis No. 91: 1167-1224.

TRAVIS, F., (1955a).
The moultng cycle of the spiny lobster, *Panulirus argus* Latreille. II Pre-ecdysial histological and histochemical changes in the hepatopancreas and integumental tissues.

The moulting cycle of the spiny lobster, *Panulirus argus* Latreille. III Physiological changes which occur in the blood and urine during normal moulting cycle.

Haemolymph protein concentrations in portunid crab II The effects of imposed fasting on *Carcinus maenas*.

Biochemistry, Physiology and Pathology of zinc.

VALLEE, B.L., (1962).
Zinc.

Metallo-proteins.

Multiple environmental effects on the physiology and behaviour of the fiddler crab, *Uca pugilator*.

Effects of sublethal concentrations of cadmium on adult Palaemonetes pugio under static and flow through conditions.

A plea for the study of temperature influences on osmoregulation.

'Copper' granules in the barnacle Balanus balanoides.

Barnacles: Possible indicators of zinc pollution?

WARNER, R.E., (1967).
Bioassays for microchemical environmental contaminants with special reference to water supplies.

On the influence of temperature on the osmoregulation of Crangon crangon and it's significance under estuarine conditions.

Heavy metals in macroinvertebrates and fish from the lower Medway estuary, Kent.

Control of the biological availability of trace metals to a Calanoid copepod in a coastal fjord.

Acute and chronic toxicity of copper to four species of Daphnia.
Zinc enzymes in *Crassostrea virginica.*

Heavy metals in animals from the North East coast.

The effect of salinity on cadmium uptake by the tissues of the shore crab *Carcinus maenas.*

The effect of calcium on the cadmium uptake by the shore crab *Carcinus maenas.*

The uptake of cadmium into the haemolymph of the shore crab *Carcinus maenas* : the relationship with copper and other divalent cations.

The transfer of $^{65}$Zn and $^{59}$Fe along two marine food chains.

Biostatistical Analysis.

Hémocyanine et cuivre chez un crustacé décapode, dans leurs rapports avec le cycle d'intermée.