INVESTIGATIONS ON THE CHEMICAL CONSTITUENTS AND TRACE METAL INTERACTIONS IN SOME BIVALVE MOLLUSCS OF THE COCHIN BACKWATERS

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BY

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C E R T I F I C A T E

This is to certify that this Thesis bound herewith is an authentic record of the research carried out by Mr. P.T.Lakshmanan, M.Sc., under my supervision and guidance in the Department of Marine Sciences, in partial fulfilment of the requirements of the Ph.D. degree of the University of Cochin and no part thereof has been presented before for any other degree in any University.

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15.1.1982
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PREFACE

The coastal and estuarine waters have become zones of great economic importance in view of the aquaculture prospects. However, its potential vulnerability to the impact of industrial and urban development has aroused serious concern the world over. This Thesis is an attempt to explore the effects of heavy metal contaminants on some aquatic molluscs. The earlier chapters of the Thesis deal with the seasonal variations of the biochemical constituents and some heavy metals (viz. copper, zinc, iron and lead) in three commercially important molluscs, viz. Villorita cyprinoides var. cochinensis (Hanley), Meretrix casta (Chemnitz) and Perna viridis (Linnaeus) so as to identify the best harvesting season and also to establish baseline concentration of these metals in the molluscs. The determination of heavy metals in the organisms is important since it gives indications of the quality of the waters around and also the possible future changes in the metal content.

The subsequent chapters of the Thesis deal with the toxic effects of mercury, copper, zinc and lead on these bivalve molluscs, their accumulation and distribution among various organs of the animals and also the metal retention kinetics by the three species. Static bioassay tests have been conducted

in these studies. It was found that the concentrations of the various metals studied in these organisms are well below the permitted level given for some shellfishes (crab and shrimp) and that these molluscs are very good integrators of trace metals from their environment and may be used as an indicator organism of metal pollutants.

The present investigations emphasis the need for a clean coastal water and gives a serious warning regarding the possible route of heavy metals in to human body through marine food chain.

A part of the results of the present investigations have been published as indicated below:

1. "Toxicity of copper on the Bivalve Villorita cyprinoides var. cochinensis" - Indian Journal of Marine Sciences, 6, 83-85 (1977).

2. "On the uptake of copper by Meretrix casta (Chemnitz), an indicator species of Metal pollution" - Current Science, 46 437-440 (1977).

3. "Accumulation of mercury by the mussel, Perna viridis (Linnaeus)" - Current Science, 48, 672-674 (1979).

4. "Biochemical composition of the bivalve molluscs, Villorita cyprinoides var. cochinensis (Hanley) and Meretrix casta (Chemnitz)" - Indian Journal of Marine Sciences, 9, 65-67 (1980).

A B B R E V I A T I O N S

AAS	=	Atomic absorption spectrophotometer
BOD	=	Biochemical oxygen demand
CF	=	Concentration factor
IAPSO	=	International Association for Physical Sciences of the Oceans
IS	=	Indian standard
SD	=	Standard deviation
TCA	=	Trichloroacetic acid
Cal	=	kilocalorie
g	=	gram
mg	=	milligram
µg	=	microgram
ng	=	nanogram
km	=	kilometre
mm	=	millimetre
nm	=	nanometre
ml	=	millilitre
mA	=	milliampere
ppm	=	parts per million
rpm	=	revolutions per minute
hr	=	hour(s)

C O N T E N T S

CHAPTER 1
INTRODUCTION

I N T R O D U C T I O N

1.1. Importance of Molluscan Fisheries:-

The molluscs, comprising of different groups like mussels, oysters, clams, chanks, cowries, squids and cuttlefish form a subsistence fishery of India. Among these the clams and mussels constitute smaller fisheries of considerable local importance. The present studies are confined to the clams, Villorita cyprinoides var. cochinensis (Hanley) and Meretrix casta (Chemnitz) and the mussel, Perna viridis (Linnaeus) which form particularly good fisheries in Kerala.

The coastal seas, backwaters and estuaries form ideal habitat for the growth of many clams. An estimated quantity of 88000 tonnes of live clams of the genera Villorita and Meretrix were fished in Indian waters (George and Sebastian, 1972). A recent survey showed that the mussel was under-exploited in India and the total annual production was estimated to be 3079 tonnes(Alagarswami et al., 1980).

P. viridis enjoys a wider distribution along both the east and west coasts of India including the Andaman Islands. Alagarswami et al. (1980) had delineated three zones along the west coast, namely (a) Ratnagiri to Gangoli (b) Cannanore to Calicut and (c) Kovalam to Muttam. Among these, the Malabar

area is the zone of importance, both from abundance and exploitation. The mussel is usually found attached to intertidal and submerged rocks, concrete and wooden pilings, jetties and other firm substrata. It is fished at Cochin, Calicut, Karwar, Ratnagiri, Bombay and Madras for human consumption. The mussels marketed are in the size range of 40-120 mm in shell length.

M. casta is distributed in many estuaries of the East and West coasts and there is a good fishery in Kerala (Hornell, 1917; Silas and Alagaraswami, 1967; Parulekar et al., 1973). V. cyprinoides is mainly found in parts of the backwaters further distant from the sea where the bottom deposit consists of sand and silt (Hornell, 1917). Rich beds are found in Cochin backwaters. The fishing of M. casta is done throughout the year but mainly during summer months at Adayar estuary, Ennore estuary, Pulicat lake and in Kerala, near Azhikode and Kaliaputtam.

The importance of mussels and clams as a good source of protein food has been widely recognised. Their potentiality as human food has been emphasised by Mukundan (1968). From the nutritional point of view it has got the advantage of easy digestibility and is a good source of minerals and vitamins.

The major biochemical constituents are protein, carbohydrate and fat. In a country like India where nutritious food is a long standing problem, the molluscs could serve as a potential source of animal protein. The animal shell is industrially important as it is extensively used for the manufacture of lime.

For the exploitation and economic utilisation of the molluscan resources a good knowledge on the biochemical constituents is needed. The earlier investigations on the molluscan body constituents are reviewed under chapters 3 and 4.

1.2. Trace metals in molluscs

However, toxic materials, particularly heavy metals * and pesticide residues are likely to be present in molluscs or any other marine organisms from polluted waters which restrict their use as food and this is of global concern. The toxic metals enter the marine or estuarine ecosystem through industrial wastes and urban domestic releases as also by precipitation and river run off. Industrial discharges are however, considered to be the largest single source. As a result of the continuous input, the concentration of some of these metals in the aquatic environment gradually increases.

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*The terms 'trace metals' and 'heavy metals' are synonymously used in the text.

Benthic filter feeding organisms like the molluscs are noted for their ability to concentrate trace metals in their tissues to a very high level, from the surrounding waters. So a study on the occurrence and seasonal variation of some of the trace metals in the molluscs is most warranted. This may help to provide information on the base line concentration of the metals in the organisms and also the quality of the habitat water.

1.3. Heavy metal pollutants in Aquatic environment and their toxic effects

Among various chemicals, the heavy metals form a group of highly toxic pollutants to the aquatic organisms. The term 'heavy metal' is a loose terminology as used in aquatic sciences. Normally this group includes the transition metals Cr, Co, Ni, Cu, Zn, Cd and Hg together with Pb. These elements almost all are relatively toxic and readily concentrated by aquatic organisms in comparison with other metals. The coastal and backwaters stand a greater chance of contamination. Fish poisoning due to heavy metal pollutants is reported from many parts of the world (Halstead and Courville, 1965, 1967; Pitta, 1972 and Davis, 1972).

The incidence of fish mortality as a result of the discharge of industrial effluents containing heavy metals have been reported in many parts of the country (Ganapathi and Raman, 1976; Unnithan et al., 1977; Venugopal et al., 1980). The Hoogly estuary in West Bengal hit by the untreated industrial wastes resulted in the decimination of fish, fish eggs and larvae. The Periyar river near Alwaye has been polluted by the industrial complex at Eloor. The effluents from a rayons factory near Calicut have not only effected the fishing in the Chaliyar river but has also caused extensive damage to agriculture.

The four most toxic metal ions (viz. Hg^{2+}, Cu^{2+}, Zn^{2+} and Pb^{2+}) have been used in the present investigation for their toxic effect on the three bivalve molluscs, and the kinetics of uptake and loss by these organisms have also been studied.

Mercury and mercury compounds have been proved to be one of the most toxic substances entering the hydrosphere (Wisely and Blick, 1967). The first reported human poisoning by Hg in seafoods occurred in Japan, between 1953 and 1964, which is known as the 'Minamata disease'(Kitta, 1972). Mercury has many industrial uses such as in the manufacture of plastics, chlorine, caustic soda, paints and certain fungicides and

pesticides. The effluents coming from such factories pollute the aquatic environment.

Copper, an essential micro-nutrient and a necessary constituent of all organisms, is highly toxic even at small concentrations (Wisely and Blick, 1967; Pringle et al., 1968). Copper in ionic form is found to be very poisonous for photosynthesis and to the growth of unicellular algae (Nielson and Anderson, 1970). The phenomenon of green-sick oysters is caused by high content of copper in the environmental water. The effluents from copper refineries, pesticide and fungicide manufacturing industries bring copper to the aquatic systems.

Zinc is another heavy metal that is very toxic to fish and other aquatic organisms (Pringle et al., 1968). It has adverse effects on fish growth rate and cause mortality at higher concentrations. The main sources of Zn in the aquatic systems are the effluents from factories manufacturing zinc compounds, from zinc plating wastes, galvanising wastes, rayon wastes, etc.

Lead, like many other heavy metals is highly toxic to aquatic life. Lead enters as a pollutant in the aquatic environment in several ways-by weathering of lead deposits and as gases during volcanic activity. Other sources of lead pollution

are mainly from manufacture of acids, electrical storage battery industry and paint industry. Lead-alkyl antiknock compounds used in petroleum is another major source of lead pollution.

1.4. Bioassay studies on molluscs

Bioassay studies for detecting and evaluating the toxicity of industrial wastes in connection with their treatment and safe disposal are being widely used. Studies on the toxicity of heavy metals on local biota will provide a measure of lethal concentrations. More directly, the information will provide a basis for assessing safe levels of metals for aquatic organisms in their natural environment.

The great variety of molluscs available in unpolluted estuarine areas, the sensitivity of certain of their life stages to low concentration of pollutants, and the ease with which they are caught and maintained contribute to their usefulness as test animals in bioassay experiments of heavy metals. So the three bivalve molluscs which are abundantly found in Cochin backwaters and in various other parts of Kerala waters are made use of for the study.

1.5. Suitability of molluscs as an indicator organism of metal pollution

The determination of heavy metals in natural waters is

often difficult because of the extremely low levels involved. Organisms are being sought which, by enriching these elements in their tissues, permit a more reliable analysis. Benthic filter feeding organisms like the molluscs are noted for their ability to concentrate trace metals in their tissues to a very high level with respect to their concentration in the environment. Goldberg (1975) has urged for a global mussel watch on the concentration of certain contaminants in the tissues of different species of Mytilus to provide an integrated index of environmental condition. They are sedentary, filter feeders and are forced to experience the varying conditions prevailing in the water mass. Thus, molluscs continuously sample their environment, so that the concentrations of heavy metals in their tissues reflect the average concentration of the metals in the habitat water. Similarly, any depletion in the environmental water should also reflect in the animal body by a loss of metals. On satisfying the above requirements, the animal can be considered as an indicator organism of heavy metals. Schulz-Baldes (1974) and Phillips (1976 a,b) found that, the mussel Mytilus edulis could be used as an indicator of heavy metal pollution. Here, an attempt has been made to study whether the mussel Perna viridis and the clams Villorita cyprinoides and Meretrix casta can be used as indicator organisms of heavy metal pollution.

1.6. Objectives of the present investigation

The main objectives of the present investigation were: (i) To provide sufficient data on the biochemical constituents of the molluscan body so as to facilitate an economic exploitation of the resources. Hence, seasonal changes in various constituents were studied. (ii) To provide data on the background levels of some selected heavy metals including non-essential elements during the whole cycle of the year which will enable to assess the water quality. (iii) To study the interaction of some heavy metals with the living molluscs - lethal and sub-lethal effects, accumulation of the metals, distribution and retention kinetics which may provide information on the safety levels of these pollutants, the transfer of toxic metals from the hydrosphere to biosphere etc. and also to know whether these organisms could be used as an indicator of heavy metal pollution.

No attempt has been made to study the biology of the molluscs; so also no distinction has been made between male and female animals as the study was from the view point of human consumption.

CHAPTER 2
MATERIALS AND METHODS

CHAPTER 2

MATERIALS AND METHODS

A. MATERIALS

2.1. Animals and water samples

Monthly collections of the clams, Villorita cyprinoides var. cochinensis and Meretrix casta (Chemnitz) were made between September 1976 and August 1978. M. casta was collected using a dredge from a place about 2 km south east of Cochin barmouth in the Cochin backwaters. V. cyprinoides was also collected from the backwaters from a place about 8 km north east to the barmouth. The depths of the clam beds were about 1.75 metres (M. casta) and 2 metres (V. cyprinoides) from the surface. There was a break of 3 months (from September 1977 to November 1977) in data collection for want of facilities.

The mussel, Perna viridis was collected from a site close to the Cochin barmouth, during February 1977 to July 1977 and February 1978 to August 1978. The species are not available during the rest of the months at this particular area due to the influx of freshwater, when the salinity goes down to low values and the animals fail to survive.

The bottom water samples from the clam beds/mussel beds were collected using a water sampler designed at the Department

of Marine Sciences, University of Cochin, which was similar in operation to van Dorn sampler. The location of collection of the samples (clams/mussel) is shown in figure 2.1.

Following collections, the animals were brought to the laboratory in live condition imparting as little shock as possible. They were kept in large polythene basins containing filtered seawater of the habitat salinity of the respective animals for a period of 24 hours to remove the pseudofaecal materials which may otherwise cause interference in the biochemical analysis. 10-15 animals were taken each time for the preparation of materials used for the determination of biochemical constituents and of the trace metal content.

Particular attention was taken to collect animals of nearly the same size every month so as to avoid any possible error due to size difference. The two clams and the mussel collected were of commercial size, from the view point of exploitation. The average shell lengths of *V. cyprinoides*, *A. casta* and *P. viridis* were 35.85 mm , 32.02 mm and 61.67 mm respectively (Tables 3.1 a to c).

2.2. Reagents

a) Digestion Mixture

10 g of A.R. Potassium Sulphate was ground with 0.5 g of anhydrous Copper Sulphate and well mixed (Vogel, 1970).

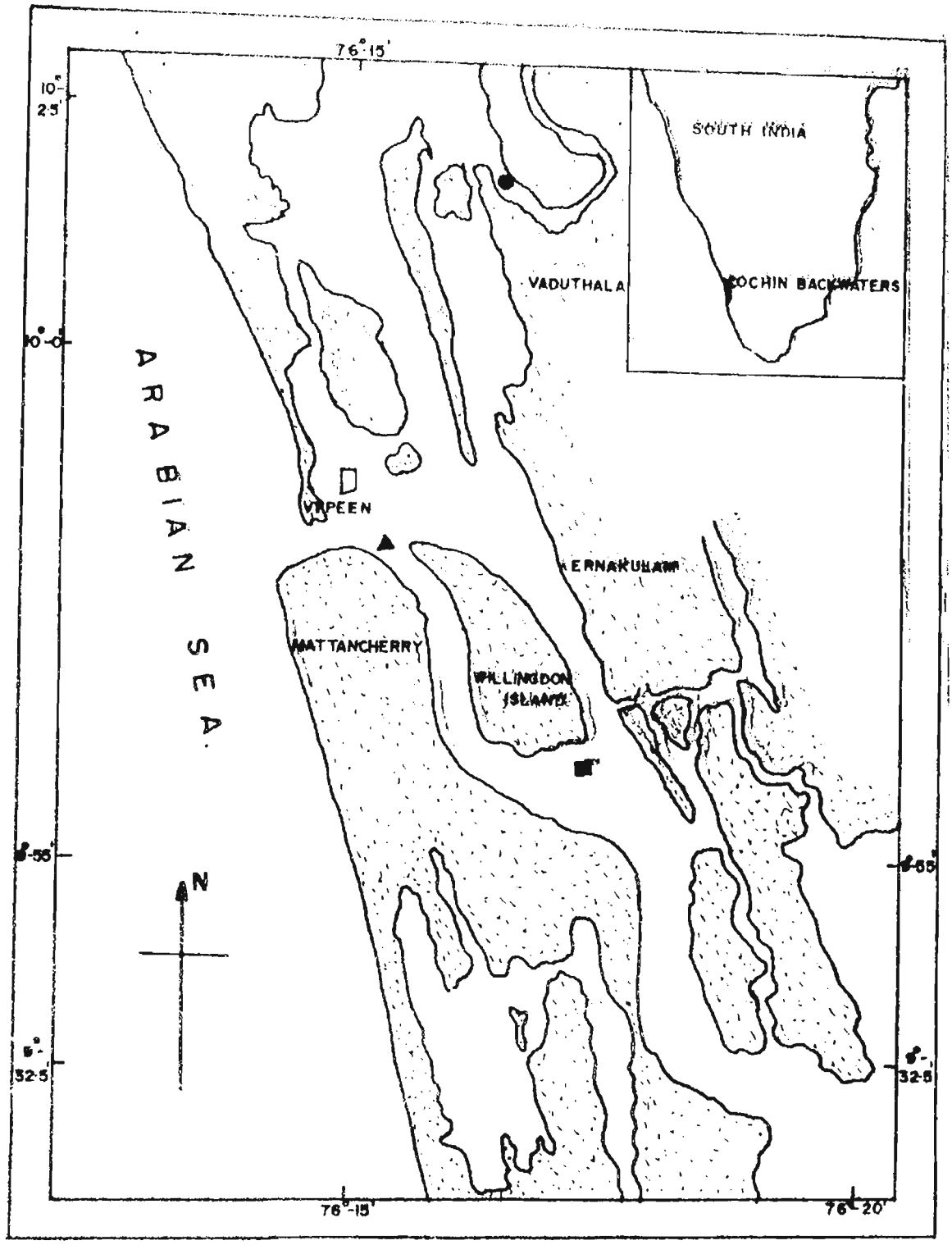

Fig 2.1 Map showing the site of collection (● Villorita Cyprinoides, ■ Meretrix casta and ▲ Perna viridis.)

b) Tarshiro's Indicator

A mixed solution containing three volumes of 0.1% methyl red and two volumes of 0.1% methylene blue, both solutions being made up in 95% ethanol was used.

c) Ammonium Molybdate solution

15 g of analytical reagent quality Ammonium Molybdate, $(NH_4)_6\ Mo_7O_{24}.4H_2O$, was dissolved in 500 ml of distilled water. The solution was stored in a polythene bottle.

d) Ascorbic Acid

27 g of good quality Ascorbic Acid was dissolved in 500 ml distilled water. The solution was stored in a plastic bottle in a freezer, when not in use.

e) Potassium Antimonyl Tartrate solution

0.34 g of Potassium Antimonyl Tartrate was dissolved in 250 ml of distilled water.

f) Mixed reagent for Phosphorous

100 ml of ammonium molybdate, 250 ml of dil. H_2SO_4 (140 ml con. H_2SO_4 added to 900 ml water), 100 ml ascorbic acid and 50 ml of potassium antimonyl tartrate were mixed just before use.

g) Manganese salt solution (Winkler solution A)

Dissolved 400 g of Manganese Chloride, $MnCl_2.4H_2O$, in distilled water and added 2 ml of con. HCl. The solution was then diluted to 1 litre.

h) Alkaline Potassium Iodide reagent (Winkler solution B)

360 g of sodium hydroxide pellets was dissolved in 500 ml distilled water and cooled. 400 g of potassium iodide was dissolved in 450 ml of distilled water. The two solutions were mixed and diluted to 1 litre.

i) Standard Silver Nitrate solution

37.11g of A.R. $AgNO_3$ crystals was dissolved in ion-free distilled water and made up to 1 litre.

j) Potassium Chromate indicator solution

5 g of K_2CrO_4 was dissolved in 100 ml of distilled water.

k) Standard sea water

A sample of Eau de Mer Normale with a stated chlorinity, ($Cl‰$) value of 19.375 was used.

l) p. Hydroxy Diphenyl reagent

Dissolved 1.5 g of p-hydroxy diphenyl in 100 ml of

0.5% sodium hydroxide solution.

m) Stannous Chloride solution (20% W/V)

20 g of $SnCl_2.2H_2O$ was dissolved in 10 ml distilled con. HCl and boiled for about a minute. The solution was then cooled and diluted to 100 ml with distilled water. 1-2 g of tin metal was added to the solution after the preparation of the solution.

n) Standard solution of $HgCl_2$ (for the determination of mercury)

0.1354 g of $HgCl_2$ was dissolved in 25 ml of 5% HNO_3. About 1 ml of 1% $K_2Cr_2O_7$ solution was added to it and made upto 100 ml with 5% HNO_3. This solution has a mercury content of 1 mg/ml.

Secondary standards were prepared by diluting the required volume using 5% HNO_3 and maintaining 0.01% $K_2Cr_2O_7$ in the solution.

All the reagents and chemicals used were of analytical reagent grade.

B. METHODS

2.3. Preparation of Tissue samples

a) Whole soft parts:- The animals were washed with

distilled water. 10-15 individual clams or mussels were opened by cutting the adductor muscle using a stainless steel scalpel. The soft portions were dried by gently pressing with in filter paper folds. The tissues were homogenised in a glass morter. The homogenised mass was dried to constant weight at 80°C. The dried material was finely powdered and stored in a decicator over silica gel until analysed. These materials were used for the estimations of biochemical constituents and of trace metals other than mercury. For determining Hg, the homogenised wet tissues were kept in deep freezer in polythene bags until analysis.

b) Component-wise analysis for trace metal content:- About 6 animals were dissected and separated into mantle (mantle+gonad), muscle (adductor muscle+foot), gills and the remaining part was taken as visceral mass.

2.4. Determination of water content

The whole soft portions were removed from 4-6 animals and were freed from the adhering fluids by pressing within filter paper folds and the total weight was determined. It was then dried at 80°C in an oven till constant weight was obtained. Knowing the wet and dry weights of the material, the moisture content was estimated (Barnes and Black Stock, 1973).

2.5. Biochemical Analysis

a) Determination of protein:- Protein was determined by a standard micro-kjeldahl method (AOAC, 1975).

About 0.5 g aliquot of the dried, homogenised tissues was digested with 10 ml of con. sulphuric acid in a kjeldahl flask after adding about 0.5 g of the digestion mixture. The heating was continued till a colourless solution was obtained. The clear solution was cooled and made up to 100 ml with distilled water. An aliquot of the sample (5 ml) was refluxed with 40% NaOH in a micro-kjeldahl apparatus and the evolving NH_3 was absorbed in 10 ml of 2% boric acid containing tarshiro 's indicator. It was then titrated against standard 0.02N H_2SO_4. The value for kjeldahl nitrogen was multiplied by 6.25 to give protein content.

b) Determination of carbohydrate:- Carbohydrate was determined by the furfural colorimetric method after treatment with con. H_2SO_4. About 30-50 mg of material was weighed out into a 20 ml centrifuge tube and heated in a boiling water bath for 30 minutes with 4 ml of 10% TCA and about 30 mg of Ag_2SO_4. After centrifuging, the clear supernatant and the subsequent washings of the residue with the TCA solution were transferred to a 25 ml graduated flask and made up to the

volume. 2 ml aliquots were taken in duplicate and carefully layered over 6 ml of con. H_2SO_4 taken in a boiling tube. The tubes were quickly agitated to mix the contents thoroughly and then heated for 6.5 minutes in a vigorously boiling waterbath; after rapid cooling to room temperature ($\simeq$28°C) the optical density was measured at 520 nm. Blanks were run with each batch of analysis. Glucose was used to obtain the standard curve (Heath and Barnes, 1970).

c) Determination of total lipids:- The total lipids were determined by the methanol-chloroform method of Heath and Barnes (1970). An accurately weighed amount of the material (ca 0.5g) was homogenised with 3 ml of chloroform-methanol mixture (2:1v/v) in a homogeniser. The homogenate was centrifuged and the supernatant transferred to a stoppered tube; the residue was washed with small amounts of chloroform-methanol mixture, centrifuged, and the washings added to the previous supernatent to give a total volume of just over 7 ml. Then 2 ml of 0.9% NaCl solution was added to the combined supernatant and the mixture gently shaken. The emulsion was allowed to stand overnight in a refrigerator to remove water soluble impurities. After transferring to a separating funnel the lower layer of organic solvent was removed. The aqueous layer was washed with 2 ml portions of chloroform-methanol

mixture and the washings again added after separation, to the organic layer. The solution of lipids was then evaporated carefully to dryness on a waterbath just below the boiling point of $CHCl_3$ and was finally dried in an oven at 105°C. The weight of the lipid was then found out.

d) Determination of total phosphorous:- The combined phosphorous was converted into orthophosphate by wet digestion with nitric acid and perchloric acid (Standard Methods, 1975).

About 100 mg of the finely powdered material was accurately weighed into a 100 ml conical flask and to this were added 10 ml demineralised water and 2 ml con. HNO_3. After a preliminary oxidation by evaporation of water and HNO_3 on a hot plate, 2 ml of con. $HClO_4$ (60%) was added and the sample was boiled until clear. After cooling, it was diluted to 100 ml in a volumetric flask and an aliquot was treated with a mixed reagent containing ammonium molybdate, ascorbic acid, sulphuric acid and potassium antimonyl tartarate as described by Murphy and Riley (1962). Blanks and standards were treated as above. K_2HPO_4 was used as standard. The optical density was measured at 880 nm against the reagent blank.

(c) Determination of calcium:- Calcium in the molluscan tissue was estimated by the Flame photometric method (Bernard and Chayen, 1965).

About one gram of the dried and finely powdered tissue sample was ashed in a silica crucible at 500-550°C in a muffle furnace for 4-6 hours. It was cooled; leached with minimum amount of hot 1:1 HCl and filtered through a NO.42 Whatmann filter paper. The crucible and filter paper were washed several times with hot water and all washings were collected in a standard flask (100 ml) and made up to the mark using distilled water.

Standard solutions of calcium were prepared by dissolving 0.25 g of calcium carbonate in the minimum volume of 1:1 HCl and then making up to 100 ml (this solution contains 1000 ppm Ca). Suitable aliquots were diluted using distilled water to give the required concentrations (viz. 125 ppm, 100 ppm, 75 ppm, 50 ppm, & 25 ppm).

A flame photometer (Systronics Ahammadabad, Type 121) was used for the analysis.

With the correct filter in position, the instrument was calibrated using distilled water and standard solutions of calcium. The galvanometer reading was adjusted to zero when aspirated with deionised water and 100 when aspirated with

the 125 ppm solution. It was repeated with other standard solutions. Then, the sample solution was atomised and the galvanometer reading was noted. Triplicate determinations were made in each cases.

f) Determination of ash content:- About 0.5 g of the dried and homogenised material was weighed in to a previously weighed silica crucible and was ashed at 500-550°C in a muffle furnace, for about 4-6 hours. The crucible and ash were cooled in a desiccator and then weighed. The residual weight would give the ash content (AOAC, 1975).

g) Determination of lactic acid:- Lactic acid in the sample material was quantitatively converted in to acetaldehyde by heating with con. H_2SO_4. The acetaldehyde was determined by measurement of the purple colour formed with p-hydroxy diphenyl reagent (Barker, 1957).

The animal was cut open using a scalpel and the soft parts were dried by pressing within filter paper folds and the wet weight determined. It was then homogenised in a glass mortar with cold 10% TCA (10 ml) and purified sea sand. The liquid was then centrifuged at 10,000 r.p.m. in a refrigerated centrifuge for 30 minutes. 1 to 2 ml of the protein free filtrate was treated with 1 ml of 20% $CuSO_4$ solution and

diluted to 10 ml. Approximately 1 g of powdered $Ca(OH)_2$ was added and shaken vigorously; it was allowed to stand at room temperature for atleast 30 minutes with occasional shaking and was then centrifuged. Duplicate aliquots of 1.0 ml of the supernatant fluid was withdrawn in to clean test tubes and one drop of 4% $CuSO_4$ added and the tubes were chilled in ice. Exactly 6 ml of con. H_2SO_4 (chilled) was added slowly and the contents were mixed. The tubes were placed in a boiling waterbath for 5 minutes, removed and cooled to below 20°C.

Two drops (0.05ml) of 1.5% p-hydroxy diphenyl reagent was added, dispersed quickly and the tubes were placed in a waterbath at 30°C and allowed to stand for 30 minutes. Excess reagent if any, was dissolved by heating the tubes for 90 seconds in a boiling waterbath. The extinction was measured at 560 nm against reagent blank. Lithium lactate was used for preparing standard curve.

h) <u>Determination of glycogen</u>:- The modified Pflüger method as given by Hassid and Abraham (1959), was used for the determination of glycogen. The muscle tissues (adductor muscle+foot) were separated from the animals and were used for the analysis.

A known quantity of the muscle (about 1 g) was dropped in to a 15 ml graduated centrifuge tube containing 3 ml of 30% KOH solution. The tissue was then digested by heating the tube in a boiling waterbath for 20 to 30 minutes. When the tissue was dissolved, 0.5 ml of saturated Na_2SO_4 solution was added and the glycogen was precipitated by the addition of 1.1 to 1.2 volume of 95% alcohol. The contents were stirred, heated to boiling, then cooled and centrifuged at 3000 r.p.m. for about 20 minutes. The mother liquor was decanted off, the tube was drained and the precipitate was dissolved in 2 ml of distilled water and reprecipitated with 2.5 ml of 95% EtOH, the alcoholic supernatent liquid decanted, and the tube drained as before.

The purified glycogen was hydrolysed to glucose by refluxing with 6 ml of 0.6N HCl in a test tube provided with an air condenser over a boiling water bath for about 3 hrs. The solution was cooled, neutralised with 0.5N NaOH and made up to 50 ml. 1 to 2 ml aliquots were withdrawn from the solution and glucose was estimated as described earlier (Heath and Barnes, 1970). The glucose value was converted to glycogen using the conversion factor 0.93.

2.6. Analysis of water samples

a) Determination of salinity:- The content of dissolved salts in sea water is usually expressed as salinity (S‰) a convention which approximates to the weight in grams of the inorganic salts (in vacuo) contained in 1 kg of sea water (weighed in vacuo), when the solids have been dried to constant weight at 480°C, the organic matter completely oxidised, the bromide and iodide replaced by an equivalent amount of chloride, and all carbonates have been replaced by an equivalent amount of oxides.

In practice, the salinity is defined in terms of chlorinity by the Knudsen equation:

$$S‰ = 0.030+1.8050\ Cl‰$$

Chlorinity - Cl‰ of seawater is defined as 0.3285234 times the weight of silver precipitated as silver halides from 1 kg of sea water, all weighings being in vacuo.

The original Knudsen equation suffered from certain draw backs. Thus if two sea water samples of different salinities were mixed, instead of the mean salinity of the two, a slightly altered value was obtained using Knudsen's equation. Again, he assumed that a definite chloride: salinity ratio

existed in heavily diluted water. The generally accepted and universally used (IAPSO) salinity chlorinity relation is

$$S‰ = 1.80655\ Cl‰$$

The determination of salinity was made by the modified Mohr's method developed by Knudsen, using ordinary burette and pipettes (Strickland and Parson, 1968).

10 ml of the water sample was pipetted out in to a conical flask and diluted to about 25 ml with distilled water. 6 drops of 8% K_2CrO_4 solution (indicator) was added to this and was titrated with standard $AgNO_3$ solution with vigorous shaking. The end point was the appearance of reddish brown colour to the precipitate, persistent for 30 seconds.

The silver nitrate solution (37.11 g in 1000 ml deionised water) was standardised using standard sea water of known chlorinity ($Cl‰ = 19.375$), "Eau de Mer Normale", obtained from the Depot d'Eau Normale, Laboratoric Hydrographique, Charlottenland Slot, Copenhagen, Denmark.and the αC value was determined. Then, the chlorinity of the sample was calculated and the salinity corresponding to the corrected chlorinity was read from the Knudsen's hydrographic table.

b) Determination of dissolved oxygen:- The dissolved oxygen in the water sample was estimated by the classical Winkler method as given by Anderson and Poyn Jr. (1969).

The principle of this method is based on a set of chemical reactions in which the dissolved O_2 in a sample of water is converted to a chemically equivalent quantity of I_2 followed by determining the I_2 produced iodometrically.

The water sample was taken in a B.O.D. bottle using a rubber tube with maximum care so as to exclude air bubbles. 1 ml of $MnCl_2$ solution was added using a pipette, by dipping the tip at the bottom of the bottle. This was followed by the addition of 1 ml of alkaline KI solution. The bottle was stoppered and the contents were thoroughly mixed by vigorous shaking and the bottle kept in a wooden box, protected from light. It was brought to the laboratory, acidified with 2 ml of 50% H_2SO_4 and shaken vigorously to dissolve the precipitate. 50 ml of the above solution was pipetted out into a conical flask and titrated with standard (0.02N)$Na_2S_2O_3$ solution to the starch end point.

c) Measurement of temperature:- The temperature of water sample was measured immediately after the sample was taken using an ordinary mercury-in-glass thermometer, calibrated

to 1/10°C. Since the depths at the three stations (from where the animals were collected) were small,(<2 metres) Nansen bottle or reversing type thermometer was not used.

d) Measurement of pH:- The pH of water sample was measured at the laboratory temperature and pressure using a pH meter(Elico model - EL 101) immediately after it was brought to the laboratory. A glass indicator electrode and saturated colomal reference electrode were used. The instrument was standardised using buffer solutions of pH 4.2 and 9.0.

2.7. Trace metal analysis

a) Digestion procedure for the determination of Copper, Zinc, Iron and Lead:- The sample material for the determination of Cu, Zn and Fe was dried at 105°C overnight. About 1-2g of the sample was taken in a Kjeldahl flask and added 10 ml of con. HNO_3 and 5 ml of con. H_2SO_4. It was heated gently first and then strongly, but cautiously. Oxidising condition was maintained in the mixture by adding small amounts of con. HNO_3. The digestion was continued until all the organic matter was destroyed and SO_3 fumes copiously evolved. Excess HNO_3 was destroyed by adding saturated ammonium oxalate solution to the cooled, digested

solution. It was evaporated again to get SO_3 fumes. The solution was cooled and diluted to 50 ml in a standard flask using redistilled water (AOAC, 1975).

The material used for the determination of lead was weighed (1-2 g of dry tissue) in a silica crucible and placed in a furnace at 250°C. The temperature was slowly raised to 350°C (in 50°C increments) and held at this temperature till smoking ceased. The temperature was increased to 500°C and the sample was ashed at this temperature for 16 hr (overnight). The carbon free ash was cooled and dissolved in 5 ml of 1N HNO_3. It was warmed on a hot plate to aid dissolution and was made up to 50 ml in a standard flask using doubly distilled water (AOAC, 1975).

b) Determination of Cu, Zn, Fe and Pb by Atomic Absorption Spectrophotometry:- These metals were estimated by atomic absorption spectrophotometric method. The analysis was done directly using a Varian Techtron AAS, Model 1100. The samples were aspirated directly into the flame and the corresponding readings were noted. Standard graphs were prepared using standard solutions of copper sulphate, $CuSO_4.5H_2O$; zinc sulphate, $ZnSO_4.7H_2O$; ferrous ammonium sulphate, $(NH_4)_2SO_4FeSO_4.6H_2O$ and lead nitrate, $Pb(NO_3)_2$.

The concentration range, wave lengths, slit width lamp current and fuel used in the estimations of various metals are given below:

Element	Wave length (nm)	Lamp current	Spectral band width (nm)	Concentration range (µg/ml)	Fuel
1. Copper	324.7	3mA	0.5	2-8	Air-Acetylene
2. Zinc	213.9	5mA	0.5	0.4-1.6	"
3. Iron	248.3	5mA	0.2	2.5-10	"
4. Lead	217.0	5mA	1.0	5-20	"

Standard addition technique was employed in a number of samples at random; good agreement in values from the two different methods was observed.

c) Digestion procedure for the determination of mercury:- Wet sample was used for the determination of mercury. The digestion was carried out by the method recommended by the Analytical Methods Committee (1965) using the modified Bethge Apparatus (Fig 2.2). This digestion procedure would prevent the loss of mercury by volatilisation.

About 5-10 g of the wet material was accurately weighed and transferred to the oxidation flask followed by the addition of a cold mixture of con. HNO_3 and con. H_2SO_4 in the ratio

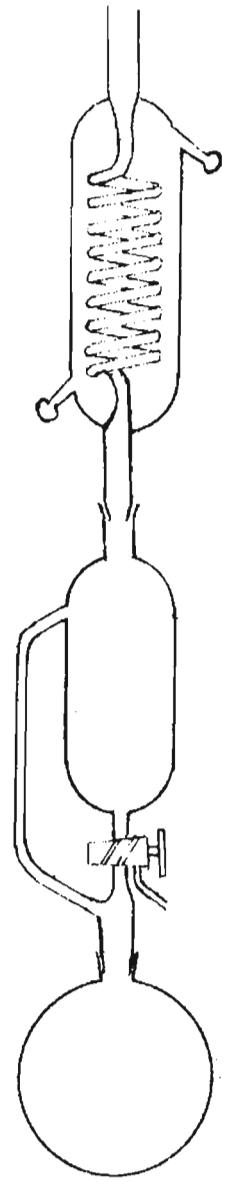

Fig.2.2 BETHGE APPARATUS

4:1 ($v/_v$). It was heated, cautiously at first, collecting the distillate in the reservoir. When the mixture started to darken, a little of the distillate was run from the reservoir to the flask. This procedure was continued maintaining a slight excess of HNO_3 in the oxidation flask, until the solution ceased to darken and fumes of H_2SO_4 were evolved. The solution was allowed to cool and the contents and the distillate in the reservoir were transferred into a volumetric flask (50 ml) and made up to the mark with redistilled water.

d) Determination of Mercury using Mercury Analyser:- Mercury was analysed using a Mercury Analyser (Model MA-77 ser. No. 005, designed by Analytical Chemistry Division, BARC, Bombay) by cold vapour atomic absorption technique.

A suitable aliquot of the sample (5-10 ml) was pipetted out in to the reaction vessel of the apparatus and the required amount of 10% HNO_3 solution was added in order to maintain total volume of 10 or 20 ml. 2 ml of 20% $SnCl_2$ solution was then added and the stopper was replaced as quickly as possible. The magnetic stirrer was switched on and the contents were stirred vigorously for 5 minutes. Holding the shutter in the open position, the instrument was switched to 'Peakhold' mode of operation. The absorbence reading was noted on the meter as early as possible. The same procedure was adopted with a

reagent blank and series of standards. The reaction vessel was thoroughly cleaned before each measurement. Standard curve was prepared by using the $HgCl_2$ solutions.

2.8. Studies on Heavy Metal Toxicity, Accumulation and Retention Kinetics

a) Static Bioassay Tests:- Freshly collected and acclimatised organisms, viz. *V. cyprinoides*, *M. costa* and *P. viridis* were used in the toxicity experiments. Following collections, the animals were brought to the laboratory and maintained in filtered seawater of habitat salinity. The desired salinity was obtained by suitable dilution of a seawater sample of known salinity using deionised water. The animals were acclimated to the test conditions of salinity, pH, temperature etc. for at least a week. Whenever a study in different salinity regime of a species was desired, the animals were conditioned to that particular set of environmental parameters via a stepwise gradation process. For instance, if the change was from a holding salinity of 25‰ to an experimental value of 10‰, the animals were first held in 20‰ for 2-3 days, then in 15‰ for a similar period and so on.

Static bioassay tests were conducted to determine the LC_{50} values, the kinetics of uptake and loss and the distribution

of the test metals among the various organs of the animals in the three bivalve molluscs. The tests were done in large glass troughs, which were thoroughly cleaned. All the glassware used in the trace metal studies were kept soaked in 6N nitric acid overnight and then washed with copious amounts of deionised water. Each trough was then filled with 5 litres of sea water of desired salinity. The water was aerated (with minimum agitation) to give oxygen saturation.

A series of preliminary studies were undertaken to determine the approximate lethal and sub-lethal concentrations of each metal to the three species of molluscs.

In acute toxicity studies the animals were exposed to different concentrations of the metal ions under consideration for a specified time. The mortality of the animals were noted at every 24 hr. An animal was considered dead, if its shell was wide-opened and failed to close on touching with the tip of a glass rod. The dead animals were removed and the water was changed daily. (A stock volume of seawater of desired salinity was prepared and kept in large polythene tanks for ready use). Aeration of the experimental trough was done twice a day for about half an hour each time and the O_2 concentration of the water was measured morning and evening.

Stock solutions of the various heavy metals studied, were prepared from their normal salts (A.R.grades) by dissolving in redistilled water (Sec. 2.2).

The metal ions were provided in the experimental tank as known concentrations of metallic salt solutions. The concentrations of metals given in the text were the calculated values and not the effective concentrations.

b) Determination of LC_{50} values:- The concentration which kills 50% of the test organisms in a stipulated time is called LC_{50} value. The LC_{50} value was determined by the straight-line graphical interpolation method (Standard Methods, 1975). The method involves plotting the data on semilogarithmic coordinate paper with concentration on the logarithmic and percentage mortality on the arithmatic scale. A straight line was drawn between two points representing the percentage dead at the two successive concentrations one of which was lethal to more than half and the other was lethal to less than half of the test organisms. From the graph, the concentration required to kill 50% of the animals (LC_{50}) in a given time was found out.

The 96 hr LC_{50} value is the concentration of a material that is lethal to 50% of the test organisms in 96 hr period.

c) Kinetics of Heavy Metal Accumulation

(i) Metal in whole soft parts:- Metal accumulation tests were conducted in glass troughs containing filtered sea water of desired salinity. The test water was collected from open sea and diluted to the specified salinity using deionised water. Usually 10 individual animals were kept in each trough containing sea water. The water was aerated 2 or 3 times a day. The animals used were not fed during the experimental period. Animals of the same age group were selected from each class for the experiment. Metals were administered as their soluble salts (prepared in distilled water) into the medium to obtain various concentrations of the metal.

The water characteristics, the experimental concentrations of the metals in various troughs for each set of accumulation study, total number of animals used etc. are given in table (7.1). Only one metal salt solution was given in the medium at a time. The concentrations selected include both lethal and sublethal levels to the test animals. The salinity and temperature selected were those of their habitat waters. The water was changed daily and metal salt solutions were added to give the same experimental concentrations.

The rates of uptake of the metals by the molluscs were determined by analysing the metal content in the soft parts of the animals at fixed intervals of time. Control animals were always analysed for the background level of each metal.

(ii) Metals in different organs:- The molluscs were maintained in sea water of the specified salinity and the metal was introduced as their salt solution. Only one concentration of the metal was selected for each class of mollusc in all cases. However, the selected concentration of each metal was different for the three class of organisms. The details of the experimental parameters selected in each case are given along with the results (Tables 7.2b to 7.13b).

The molluscs were cut open and then dissected into different organs and treated as muscle (adductor muscle+foot), mantle (mantle+gonad), gills and the remaining part was treated as visceral mass. 6 to 8 animals were dissected at definite intervals of time and metal content in each organ was determined. Stainless steel scissors, forceps and scalpel were used for dissecting the animals. Metals were determined as described under (sec. 2.7).

d) Kinetics of Heavy Metal Loss:- The molluscs Perna viridis, Villorita cyprinoides and Meretrix casta were exposed to seawater

containing heavy metal ions viz. Hg^{2+}, Cu^{2+}, Zn^{2+} or Pb^{2+} (only one metal salt was introduced per tank) for a period of 96 hr. The water salinity and the concentration of metal used in each case are given along with the results (Tables 8.1 to 8.3).

After 4 days of exposure, 4 animals were removed from each tank and subjected to metal analysis. Control animals in all cases were also analysed for the metal to know the background level of each metal.

The remaining animals were then transferred in to large glass aquaria containing seawater of their respective experimental salinities. This water contained no detectable amount of any of the test metals.

The depuration study was continued for 24 days in the case of Perna viridis and Villorita cyprinoides and for 20 days in the case of Meretrix casta.

The kinetics of metal loss in various organisms was determined by analysing the metal content in the whole soft parts of the molluscs at definite intervals of time. (Kinetics of mercury loss in the mussel , Perna viridis could not be conducted)

CHAPTER 3

SEASONAL VARIATIONS OF BIOCHEMICAL CONSTITUENTS

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SEASONAL VARIATIONS OF BIOCHEMICAL CONSTITUENTS

A study of biochemical constituents could be of considerable use in the economic utilisation of the molluscan fishery resources and to gather information on their food value. In view of the increasing importance of molluscan fisheries, a detailed study on their biochemical composition is called for.

Vinogradov (1953) in "The Elementary Chemical Composition of Marine Organisms" had compiled the results of the investigations carried out in molluscs till the earlier part of this century. Giese (1969) had presented a good account of the biochemical constituents of certain body components in molluscs. Seasonal changes in the biochemical composition of certain bivalves from Clyde sea area had been elucidated by Ansell (1974a, b,c,d). Other works include that of Giese and Hart (1967), Ansell (1972), Taylor and Venn (1979) etc.

A literature survey showed that the studies carried out on the chemical constituents of molluscs from Indian waters are limited. The earlier investigations are summed up below. Venkataraman and Chari (1951) had given a brief account of the biochemical composition of Meretrix casta from Ennore

backwaters. Durve and Bal (1961) had studied the chemical composition of the oyster, Crassostrea gryphoides (Schlotheim). Suryanarayanan and Alexander (1972), and Suryanarayanan et al. (1973) had evaluated the nutritional status of some gastropodes and cephalopodes. Ansell et al. (1973), had determined the biochemical constituents of four invertebrates including two bivalves, Donax incarnatus and D. spiculum, sampled from Thevara and Cochin area. Sivankutty Nair and Shynamma (1975) studied the seasonal changes in the lipid content and calorific values in V. cyprinoides. Sarvaiya (1977) had determined the chemical composition of some molluscs of Saurastra Coast. Mohammed Salih (1977) had studied some aspects of the biochemical constituents of M. casta, off Cochin harmouth. Suryanarayanan and Balakrishnan Nair (1976) had presented the variations in moisture, glycogen, protein, total lipids and ash content in the tropical intertidal limpet Cellana radiata. It clearly reveals that the chemical compositions of a large number of commercially important molluscs are lacking. Information on these aspects of the bivalves P. viridis, V. cyprinoides and M. casta is either very scanty or fragmentary. It would be interesting to note the changes in the chemical constituents of the body of the molluscs due to the drastic changes in environmental water quality brought

about by the monsoon floods. To a study of the seasonal changes of the biochemical composition in these organisms were undertaken to determine the most favourable season for harvest, from the point of view of both economy and nutrition.

3.1. Materials and methods:- The samples collected at monthly intervals during 1976-77 and 1977-78 were used for the study of seasonal variations of biochemical constituents. The details regarding the collections of the animals including sample sites etc. are given in section 2.1. The shell length and depth of the animals and the characteristics of the habitat water (bottom) from the site of collection are given in Tables 3.1a to 3.1c. Standard methods as described in Chapter 2 were followed for the determination of various biochemical constituents.

3.2. Results:- The biochemical constituents determined in the three species of molluscs are detailed in tables 3.2a to 3.5c. Figs 3.1a to 3.4c indicate the seasonal changes in the different parameters studied. The biochemical data are given in percentage of dry weight.

a) Water content:- The variation of water content in the molluscs was relatively low. The fluctuations were from 75.46% to 83.10% in V. cyprinoides, 77.11% to 85.45% in V. casta and 78.28% to 83.74% in P. viridis. In general, high values for water content were found during June to August in all the

three species. Another period of high water content was found during September to December in V. cyprinoides and during November to December in M. casta. The rise in water content approximately synchronised with the monsoon rains suggesting that the seasonal changes in water content of the molluscan body is associated with the change in salinity of the ambient water (the period of low salinities). The tissue water content in these animals was comparatively low during the period of high salinity (Table 3.2a to 3.2c). The body water level showed strong negative correlation with the environmental salinity. The correlation coefficients (r) and probability factor (P) in each case were found to be

$r = -0.8292$ ($P < 0.001$) in V. cyprinoides

$r = -0.7548$ ($P < 0.001$) in M. casta

and $r = -0.7897$ ($P < 0.01$) in P. viridis

the corresponding regression equations being

$$Y = 81.9830 - 0.2155X \quad \ldots \quad 3.1$$

$$Y = 84.6674 - 0.1621X \quad \ldots \quad 3.2$$

and $$Y = 91.7188 - 0.3440X \quad \ldots \quad 3.3$$

respectively, where X = salinity (‰) of habitat water and Y = body water content (%). Figs 3.2a to 3.2c represent the above equations.

Table 3.2 a

Variation of water and ash contents in the clam, Villorita cyprinoides var. cochinensis collected during 1976-77 and 1977-78.

Date of collection	Salinity of water (‰)	Water content (%)	Dry wt. (%)	Ash (%) (dry wt. basis)
2.9.1976	0.00	80.73	19.27	3.38
1.10.'76	1.53	82.33	17.67	4.51
1.11.'76	1.00	82.56	17.44	4.85
1.12.'76	0.50	82.75	17.25	4.92
2.1.1977	15.21	78.06	21.94	6.64
1.2.'77	22.81	77.10	22.90	0.94
6.4.'77	20.30	76.46	23.54	6.11
2.5.'77	8.35	80.38	19.62	4.74
1.6.'77	4.34	80.17	19.83	5.85
13.7.'77	0.00	78.69	21.31	4.90
2.8.'77	0.00	81.83	18.17	4.26
2.12.'77	1.93	82.93	17.07	5.09
5.1.1978	11.67	80.75	19.25	6.30
9.2.'78	14.52	80.14	19.86	6.67
6.3.'78	17.63	77.21	22.79	6.02
10.4.'78	16.00	78.22	21.78	6.28
10.5.'78	11.13	80.42	19.58	5.23
8.6.'78	0.54	83.10	16.90	3.84
7.7.'78	0.00	81.21	18.79	5.16
4.8.'78	0.00	82.30	17.70	4.93
Mean value =		80.36	19.63	5.44
Standard deviation =		±2.05	±2.05	±1.21

Table 3.2 b

Variation of water and ash contents in the clam, Meretrix casta collected during 1976-77 and 1977-78.

Date of collection	Salinity of water (‰)	Water content (%)	Dry wt. (%)	Ash (%) (dry wt. basis)
2.9.1976	27.12	79.66	20.34	5.72
1.10.'76	31.73	77.11	22.89	6.03
1.11.'76	28.46	81.80	18.20	5.93
2.12.'76	30.70	79.85	20.15	8.73
2.1.1977	31.92	80.48	19.52	10.59
1.2.'77	32.40	79.73	20.27	11.11
6.4.'77	24.00	78.73	21.27	9.83
2.5.'77	24.16	78.18	21.82	7.74
1.6.'77	17.25	84.87	15.13	6.27
13.7.'77	10.50	85.45	14.55	6.99
2.8.'77	5.72	85.31	14.69	5.98
2.12.'77	24.54	81.30	18.70	9.83
5.1.1978	30.62	79.76	20.24	11.12
9.2.'78	27.65	80.03	19.97	9.62
6.3.'78	30.50	79.42	20.58	8.11
10.4.'78	23.19	81.68	18.32	8.28
10.5.'78	22.11	81.40	18.60	8.58
8.6.'78	1.76	83.07	16.93	7.67
7.7.'78	0.95	82.77	17.23	8.59
4.8.'78	2.34	83.45	16.55	8.76
Mean value =		81.20	18.80	8.27
Standard deviation =		±2.30	±2.30	±1.69

Table 3.2 c

Variation of water and ash contents in the mussel, Perna viridis collected during 1977 and 1978.

Date of collection	Salinity of habitat water (‰)	Water content (%)	Dry Wt. (%)	Ash (%) (dry wt. basis)
1.2.1977	34.47	79.58	20.42	13.29
2.3.'77	34.40	78.77	21.33	13.77
5.4.'77	26.62	83.59	16.41	8.57
2.5.'77	29.86	82.52	17.48	8.07
1.6.'77	24.75	83.74	16.26	10.22
13.7.'77	20.90	82.66	17.34	8.83
9.2.'78	32.82	80.60	19.39	8.50
13.3.'78	33.60	79.32	20.68	8.04
10.4.'78	33.21	80.52	19.48	6.99
8.5.'78	34.42	81.39	18.61	7.48
6.6.'78	25.90	83.27	16.73	8.35
7.7.'78	32.79	78.28	21.72	9.97
7.8.'78	25.75	83.44	16.56	9.50
Mean value =		81.36	18.65	9.35
Standard deviation =		± 1.89	± 1.90	±1.98

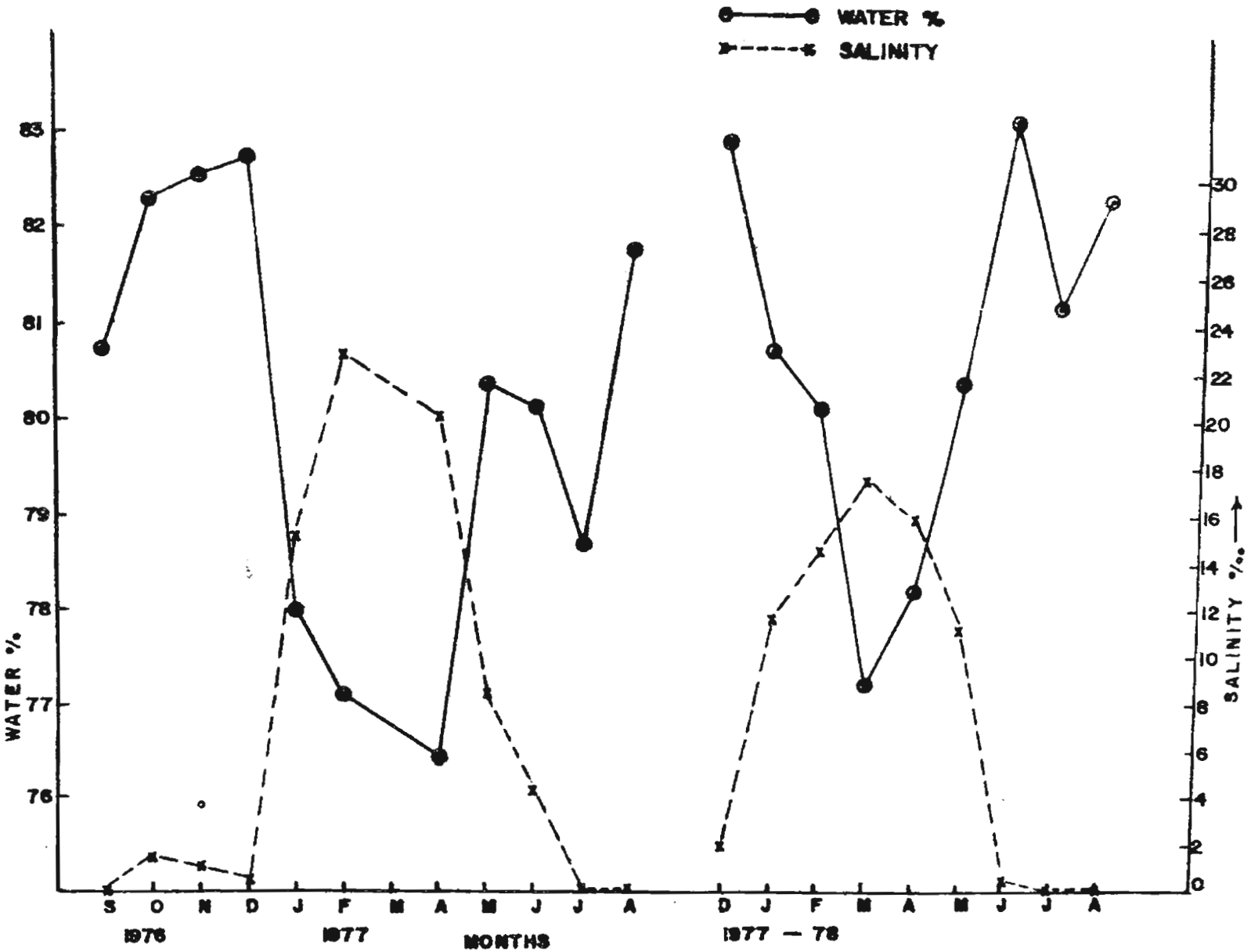


Fig. 3.1a Seasonal variation of water content in the whole soft parts of Villorita cyprinoides in relation to environmental water salinity

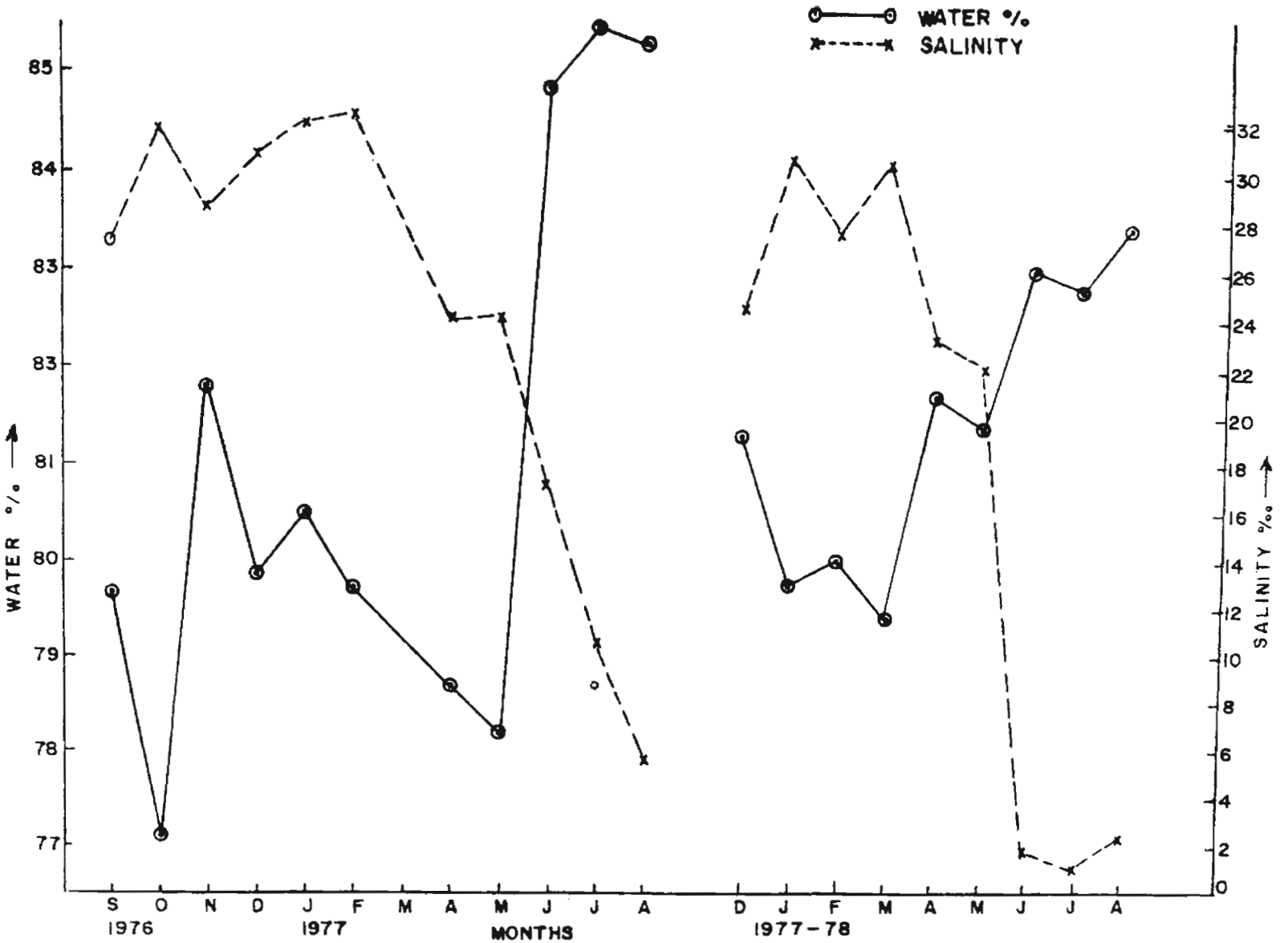


Fig. 3. 1b. Seasonal variation of water content in the whole soft parts of Meretrix casta in relation to environmental water salinity.

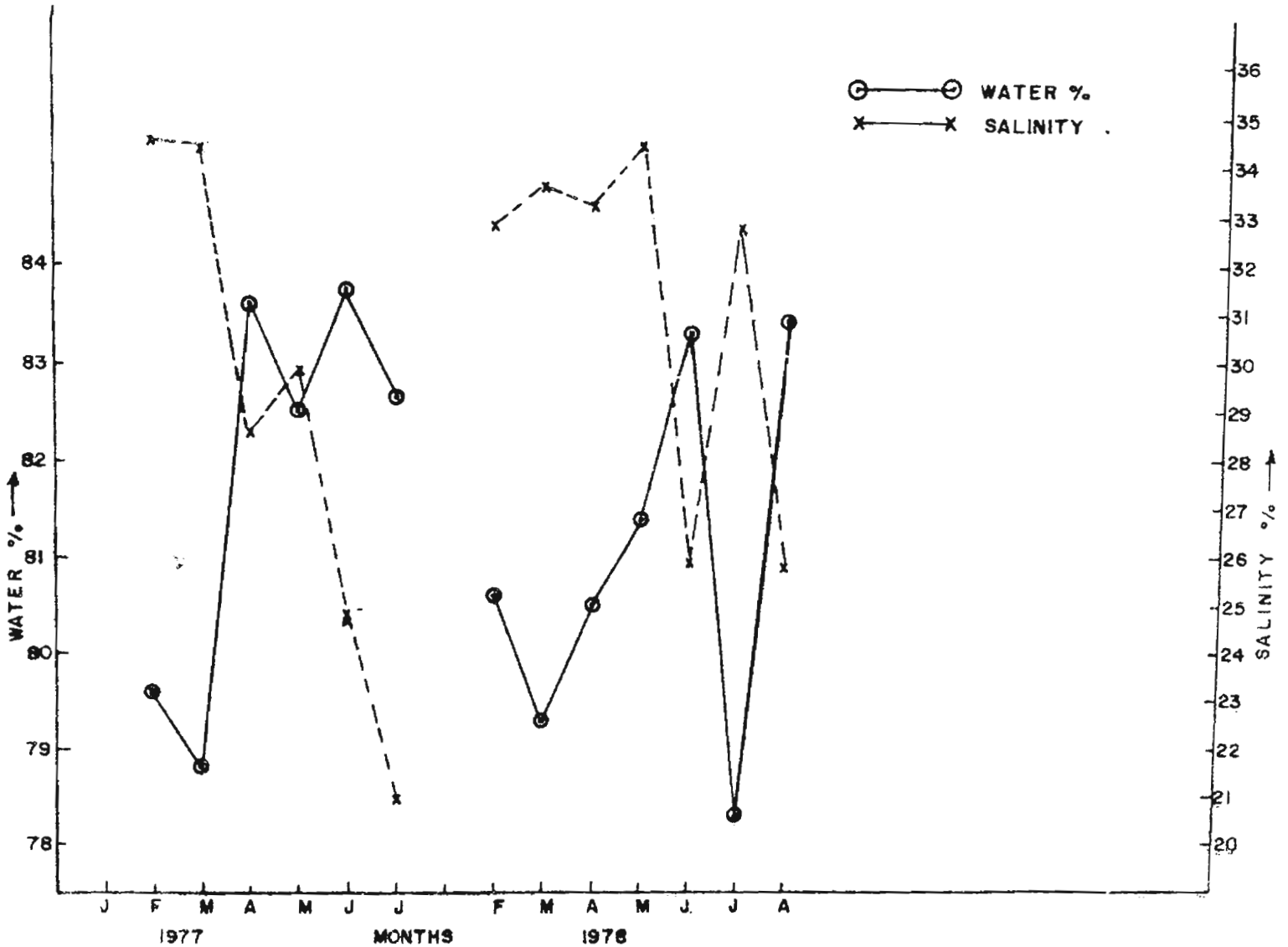


Fig. 3. Ic. Seasonal variation of water content in the whole soft parts of Perna viridis in relation to environmental water salinity.

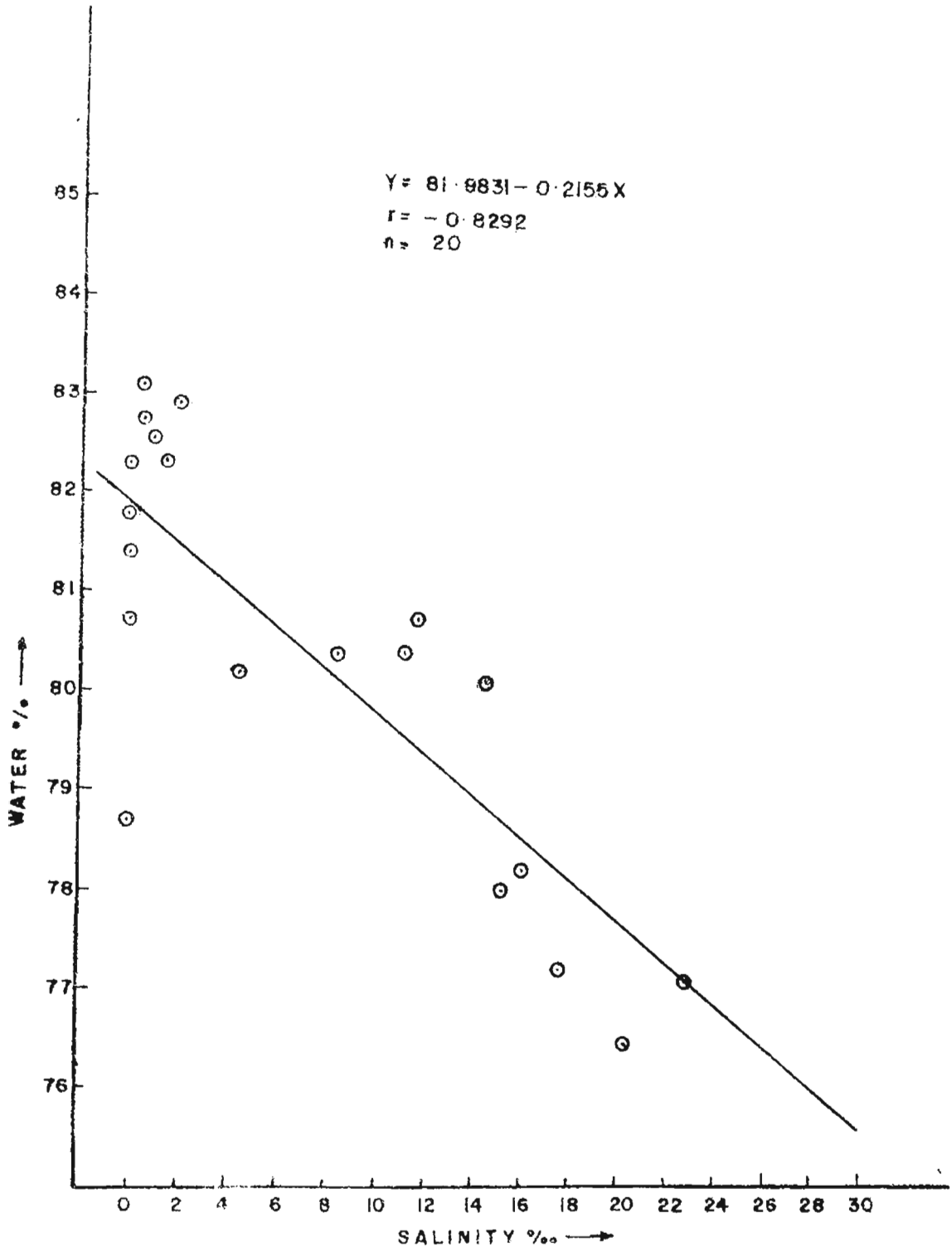


Fig. 3.2a. Relationship between body water content of Villorita cyprinoides and habitat water salinity.

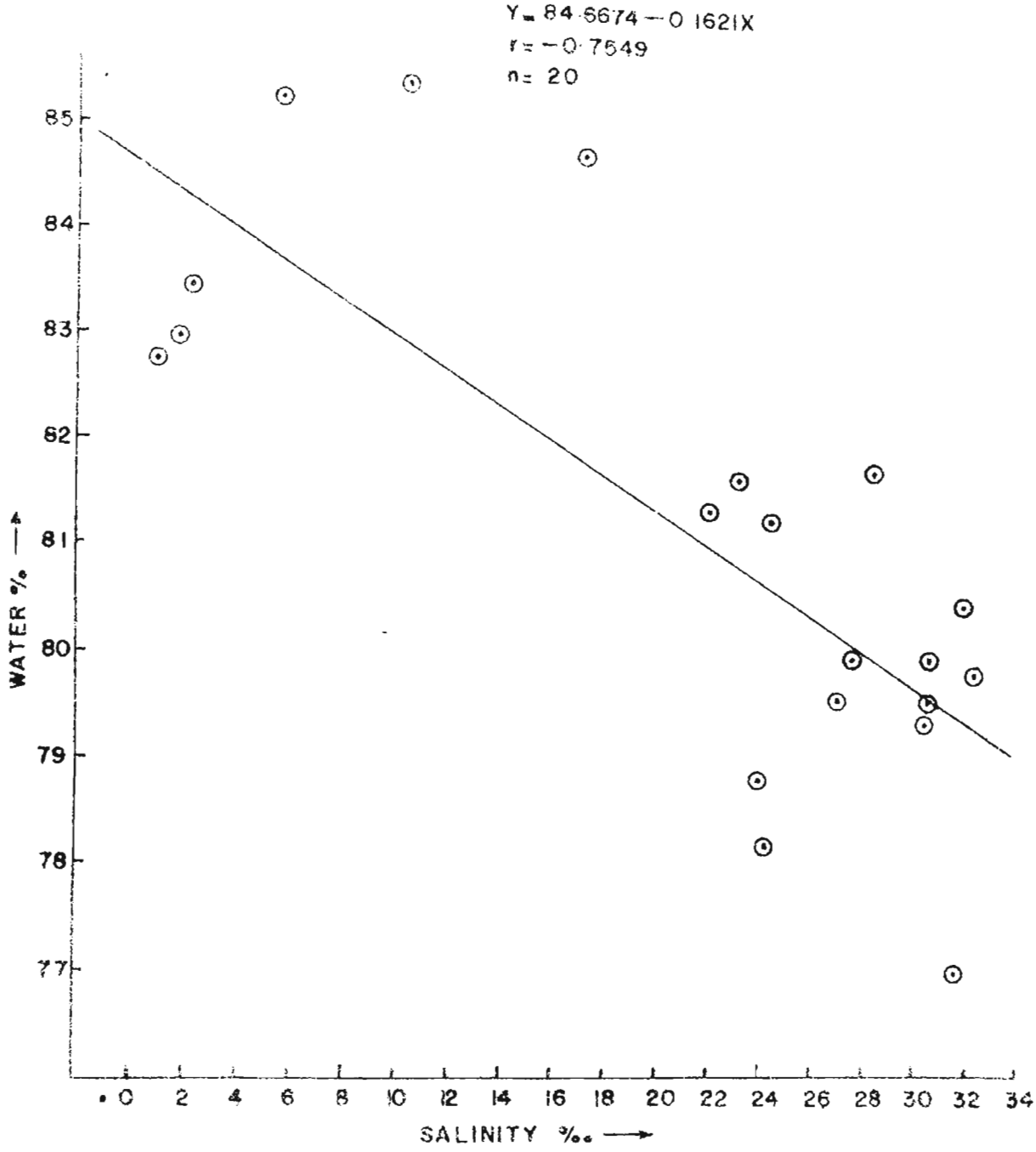


Fig. 3.2 b Relationship between body water content of Meretrix casta and habitat water salinity.

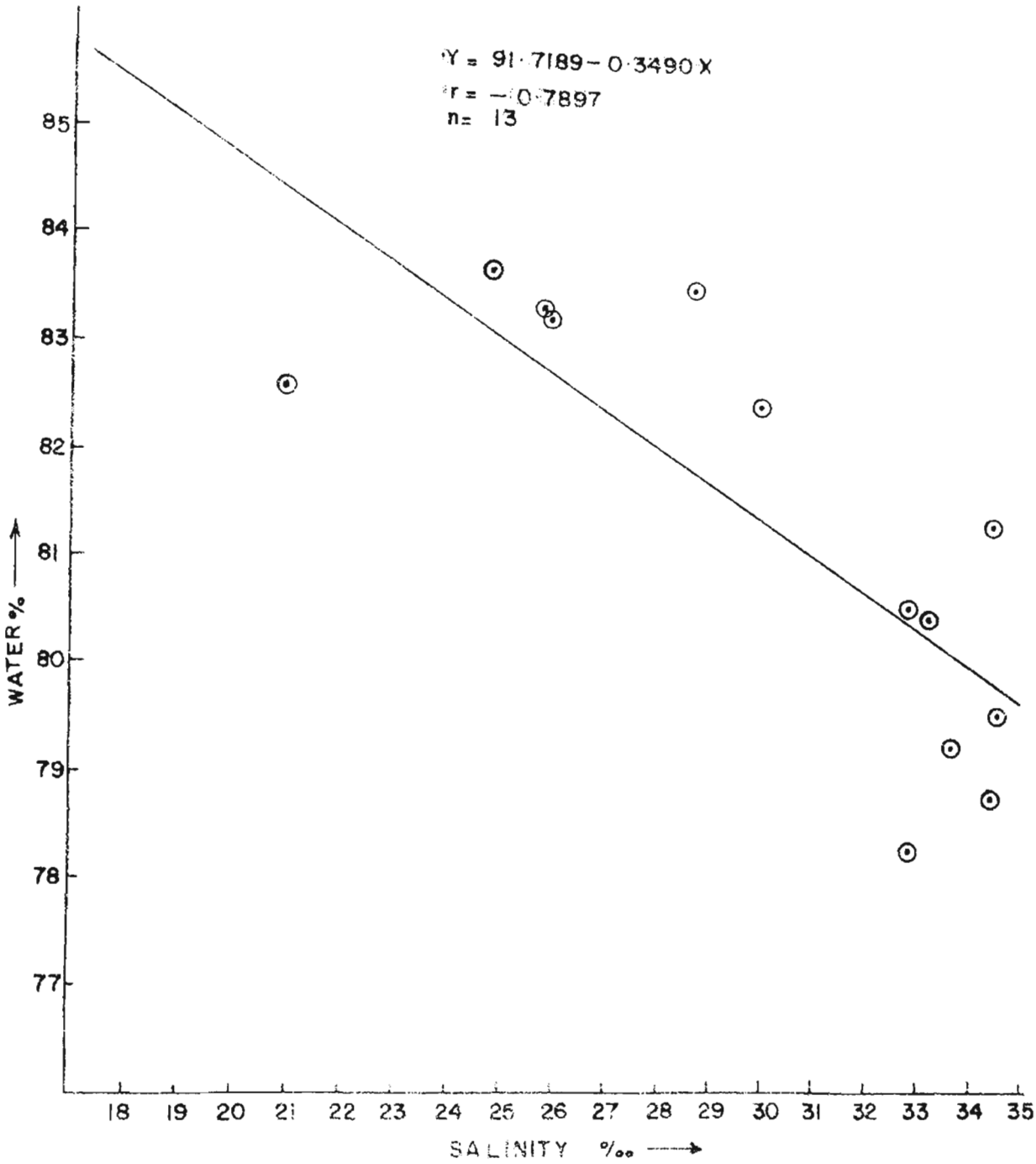


Fig. 3.2c Relationship between body water content of Perna viridis and habitat water salinity.

b) Protein:- Protein, the major organic constituent in the body components of the bivalves, varied markedly with season and species. High values for protein were observed during November to January (with a peak value in January each year) in the black clam, V. cyprinoides. The values ranged from 41.34% to 73.66%. Although protein content was generally low and remained rather steady during the rest of the months, they however, declined to give the lowest value either in May or June (Table 3.3a). Protein level was generally found to be high during December to April in M. casta (Table 3.3b). Highest value for protein was found in February, 1977 (67.01%) and January, 1978 (69.43%). The months of January and February can be considered as the peak period for protein as far as this species is concerned. Protein content was relatively low, in the species, during the rest of the year. The lowest values were obtained in September, 1976 (43.20%) and also in June, 1978 (42.54%). The seasonal variation of protein content in the mussel, P. viridis showed marked deviation from the other two species. In this case, higher values for protein were noticed during June-July (Table 3.3c). The tissue protein content registered low values during April to May. The values were in the range of 46.55% to 69.95%. The slight shift observed in the period

Table 3.3 a

Seasonal variation of protein and carbohydrate contents in the clam, Villorita cyprinoides collected during 1976-77 and 1977-78.

Date of collection	% protein		% carbohydrate	
	Mean value	± S.D.	Mean value	± S.D.
2.9.1976	44.96	0.806	39.13	1.535
1.10.'76	52.98	0.933	27.39	1.092
1.11.'76	58.04	1.396	23.44	1.113
1.12.'76	57.65	1.619	22.32	1.108
2.1.1977	73.66	1.526	4.06	0.187
1.2.'77	53.09	1.211	27.92	1.085
6.4.'77	47.38	1.749	31.57	1.035
2.5.'77	41.34	1.003	34.04	1.351
1.6.'77	55.55	1.605	29.49	1.083
13.7.'77	50.36	1.256	32.97	1.002
2.8.'77	49.96	1.206	32.74	0.980
2.12.'77	60.06	1.074	15.37	1.037
5.1.1978	68.39	1.152	5.74	0.298
9.2.'78	56.92	1.334	18.19	1.278
6.3.'78	50.75	1.189	27.67	1.050
10.4.'78	48.60	1.116	23.39	0.943
10.5.'78	54.79	1.168	20.58	1.046
8.6.'78	43.15	1.292	37.83	0.862
7.7.'78	46.62	1.104	34.56	1.120
4.8.'78	51.25	1.697	30.24	1.152
Mean value =	53.275 ± 7.739		25.862 ±9.333	

Table 3.3 b

Seasonal variation of protein and carbohydrate contents in the clam, *Meretrix casta* collected during 1976-77 and 1977-78.

Date of collection	% protein		% carbohydrate	
	Mean value	± S.D.	Mean value	± S.D.
2.9.1976	43.20	1.033	45.97	1.183
1.10.'76	47.83	1.066	39.13	0.803
1.11.'76	49.06	1.409	37.00	0.814
2.12.'76	53.61	1.166	28.56	0.633
2.1.1977	64.57	1.477	6.49	0.333
1.2.'77	67.01	1.118	6.36	0.219
6.4.'77	57.84	1.319	20.67	0.641
2.5.'77	52.27	1.005	28.81	0.706
1.6.'77	50.30	1.088	32.60	0.854
13.7.'77	55.91	1.245	28.24	0.951
2.8.'77	57.24	1.120	27.18	0.763
2.12.'77	67.20	1.045	5.80	0.136
5.1.1978	69.43	1.043	4.15	0.115
9.2.'78	66.77	1.064	7.92	0.302
6.3.'78	67.66	1.606	7.04	0.300
10.4.'78	61.66	1.096	14.61	0.348
10.5.'78	56.41	0.997	22.69	0.621
8.6.'78	42.54	0.960	35.63	0.783
7.7.'78	49.36	1.037	26.83	0.575
4.8.'78	55.21	1.083	23.58	0.605
Mean value =	56.764 ±8.1661		22.460 ±12.476	

Table 3.3 c

Seasonal variation of protein and carbohydrate contents in the mussel, Perna viridis, collected during 1977 and 1978.

Date of collection	% protein		% carbohydrate	
	Mean value	± S.D.	Mean value	± S.D.
1.3.1977	61.03	0.937	13.28	0.333
2.3.'77	62.05	1.024	11.45	0.310
5.4.'77	56.50	0.944	20.08	0.463
2.5.'77	46.55	1.108	30.04	0.738
1.6.'77	58.32	1.100	11.29	0.255
13.7.'77	69.93	1.081	3.16	0.170
9.2.1978	57.07	0.919	14.78	0.484
13.3.'78	54.27	0.907	18.88	0.530
10.4.'78	54.86	0.918	21.30	0.646
8.5.'78	53.80	1.014	25.80	0.759
8.6.'78	60.16	1.091	15.84	0.494
7.7.'78	68.15	1.125	6.23	0.151
7.8.'78	59.06	0.903	16.12	0.563
Mean value =	58.596		16.02	
	±5.869		±7.115	

of higher or lower protein values from year to year can be due to variations in environmental factors and physiological condition of the animal.

c) Carbohydrate:- Carbohydrate content in the molluscs showed marked seasonal variations. Striking changes in carbohyarate content was seen in all the three species. In V. cyprinoides it varied from 4.06%to 39.13%. There was a steady fall in carbohydrate content from September, 1976 to January 1977. The trend was maintained in 1978 also (Table 3.3a). The carbohydrate minima coincided with protein maxima at all times. In M. costa as well, carbohydrate level steadily declined from September 1976 to January 1977 (Table 3.3b). In 1978 also, the lowest value was found in January (4.15%). The highest value observed in this species was 45.97%, during September 1976.

In P. viridis also carbohydrate fluctuated widely (Table 3.3c). Carbohydrate content gradually increased from February and recorded the maximum value in May during both the years. Thereafter, a perceivable fall in carbohydrate level was observed and reached a minimum value in July each year. The values ranged from 3.16% to 30.04%.

In all the three species, a decrease in carbohydrate content was invariably followed by an increase in protein level.

The carbohydrate minima coincided with protein maxima at all times (Figs 3.3a to 3.3c).

Significant negative correlations were found between protein and carbohydrate in all the three species. The correlation coefficients (r) and probability factor (P) are given below:

$r = -0.9254$ ($P < 0.001$) in *V. cyprinoides*

$r = -0.9544$ ($P < 0.001$) in *V. casta*

and $r = -0.9488$ ($P < 0.001$) in *P. viridis*

the corresponding regression equations are

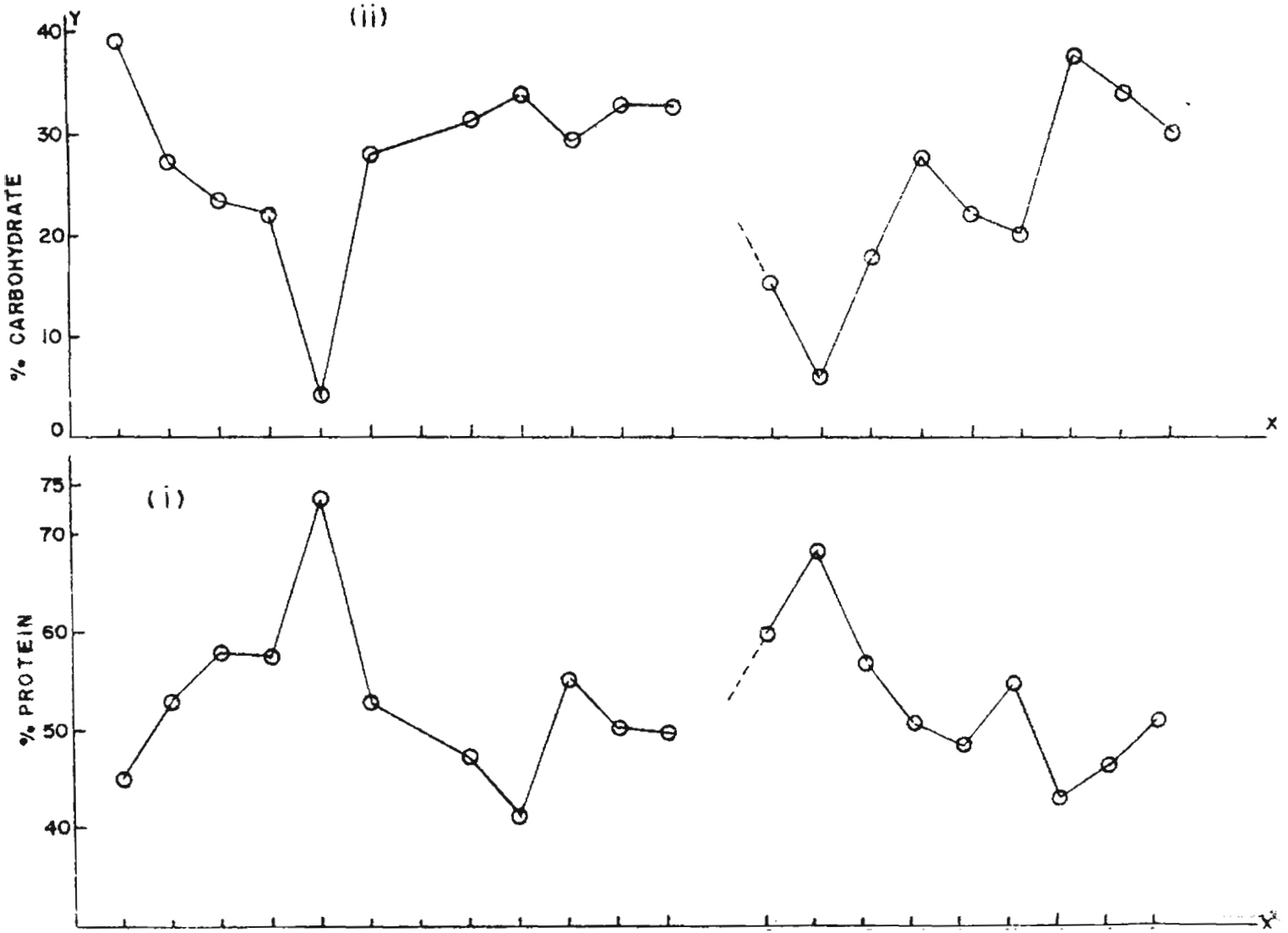
$$Y = 85.3344 - 1.1160X \quad \ldots \quad 3.4$$

$$Y = 107.2685 - 1.4852X \quad \ldots \quad 3.5$$

and $$Y = 83.4272 - 1.1504X \quad \ldots \quad 3.6$$

respectively, where X represents protein (%) and Y represents carbohydrate (%). Figs 3.4a to 3.4c represent the above equations.

d) Total lipids:- There was also a distinct seasonal variation in lipid content, at least in the two clams. Figs (3.3a to 3.3c) represent the general trend in the change. In *V. cyprinoides*, lipid level varied from 9.21% to 18.12%. In general, higher levels of lipid were found from October to December 1976, April to May 1977 and January to May 1978. In *V. casta*, the variation in lipid content was more marked (4.53% to 13.40%). The lipid level was usually high during


(ii)
Y
40
30
20
10
0
% CARBOHYDRATE
X
(i)
75
70
60
50
40
% PROTEIN
X'

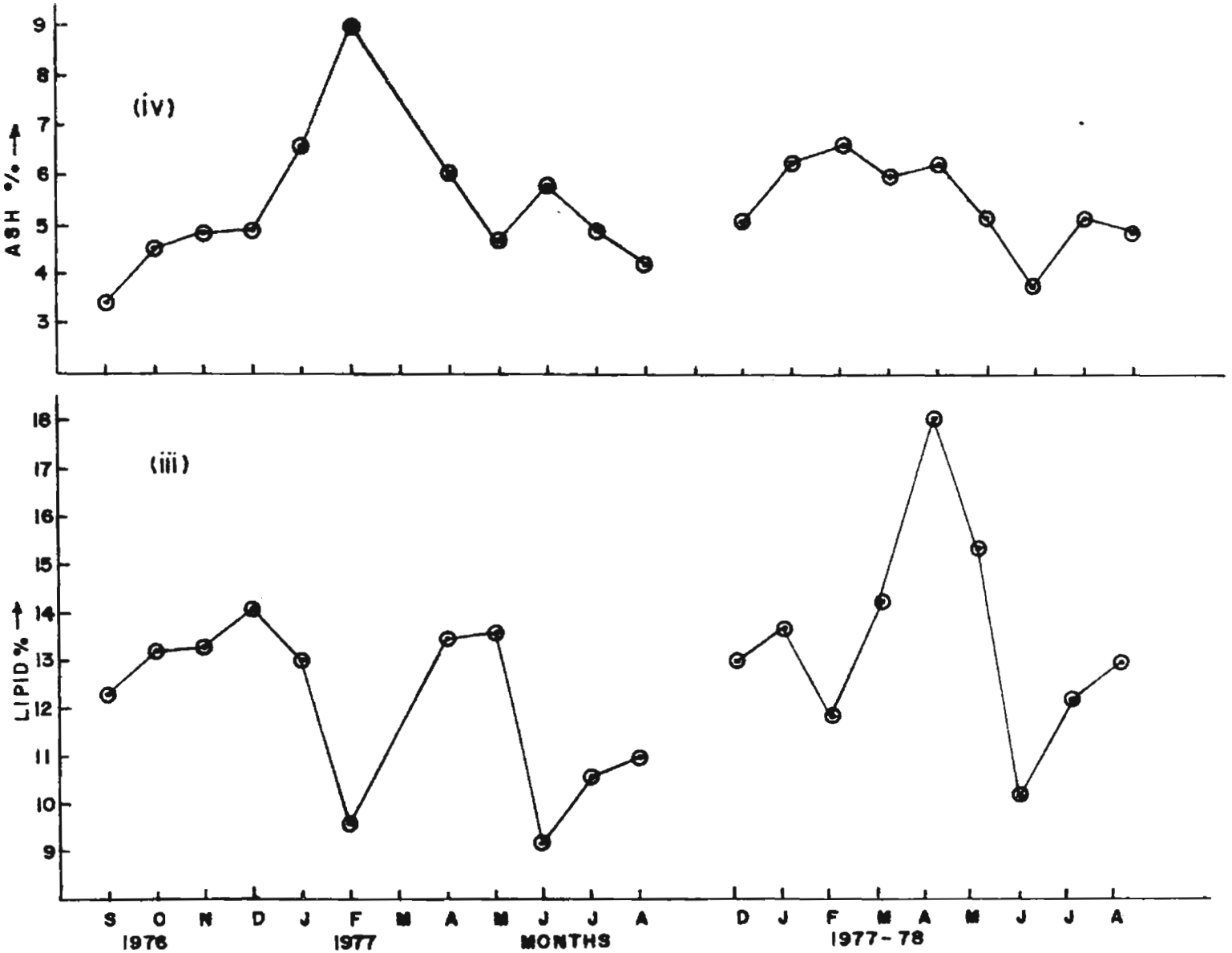


Fig 3.3a (contd.) Seasonal variation of (iii) Total lipids and (iv) Ash content in Villorita cyprinoides

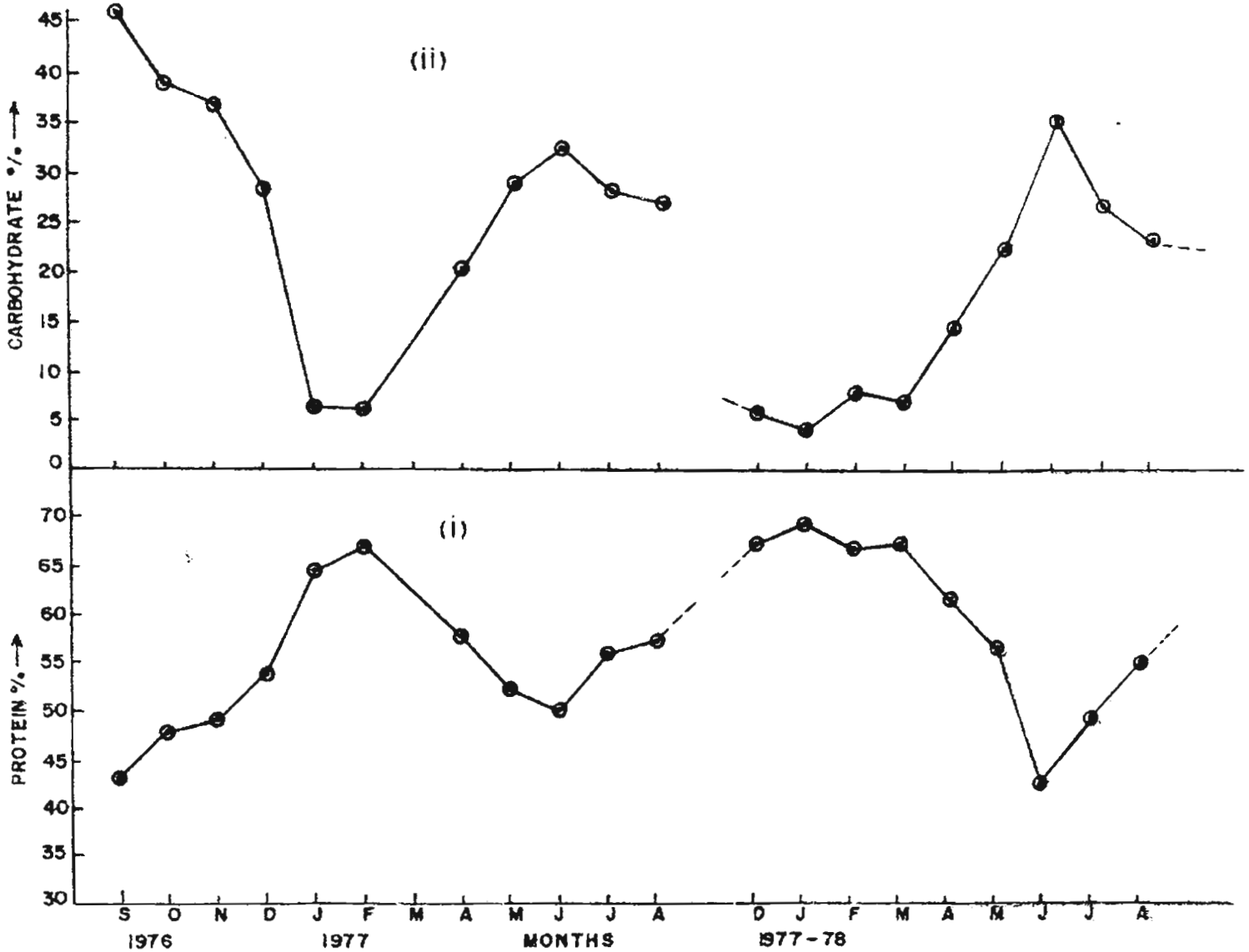


Fig. 3. 3 b. Seasonal variations of (i) Protein and (ii) Carbohydrate in Merstrix casta

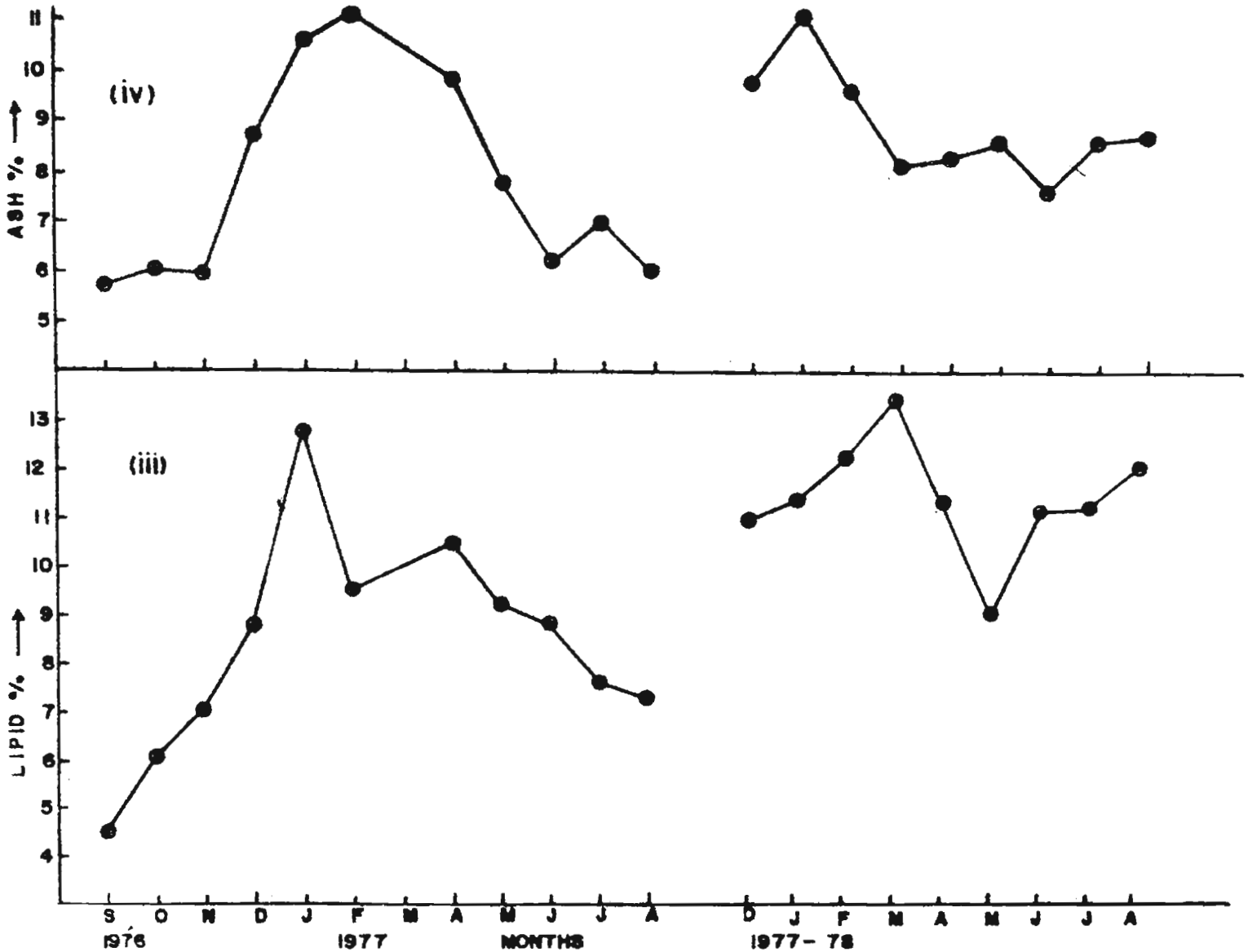


Fig. 3.3 b (contd.) Seasonal variations of (iii) Total lipids and (iv) Ash content in Meretrix casta

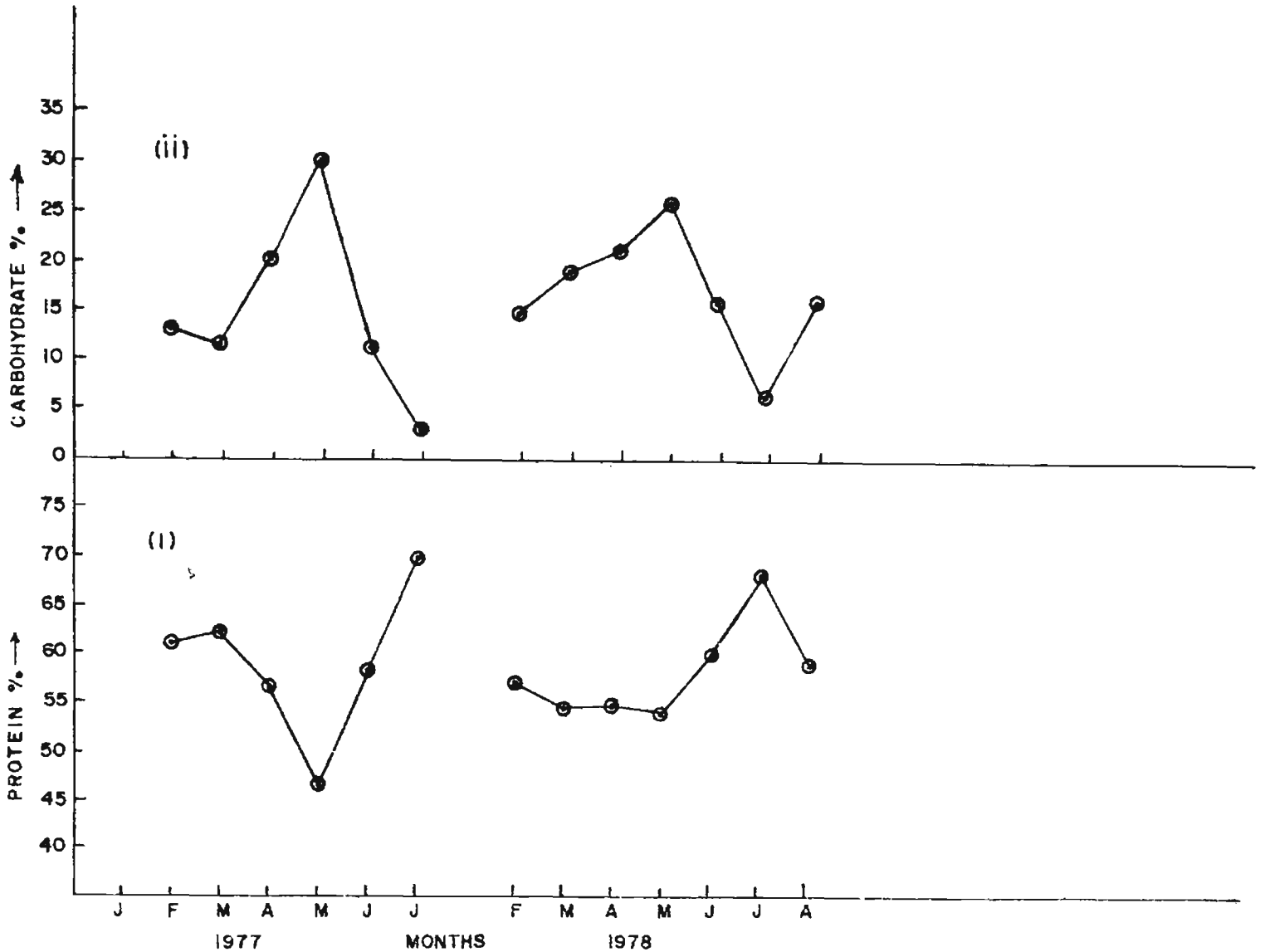


Fig. 3.3c. Seasonal variations of (i) Protein and (ii) Carbohydrate in Perna viridis.

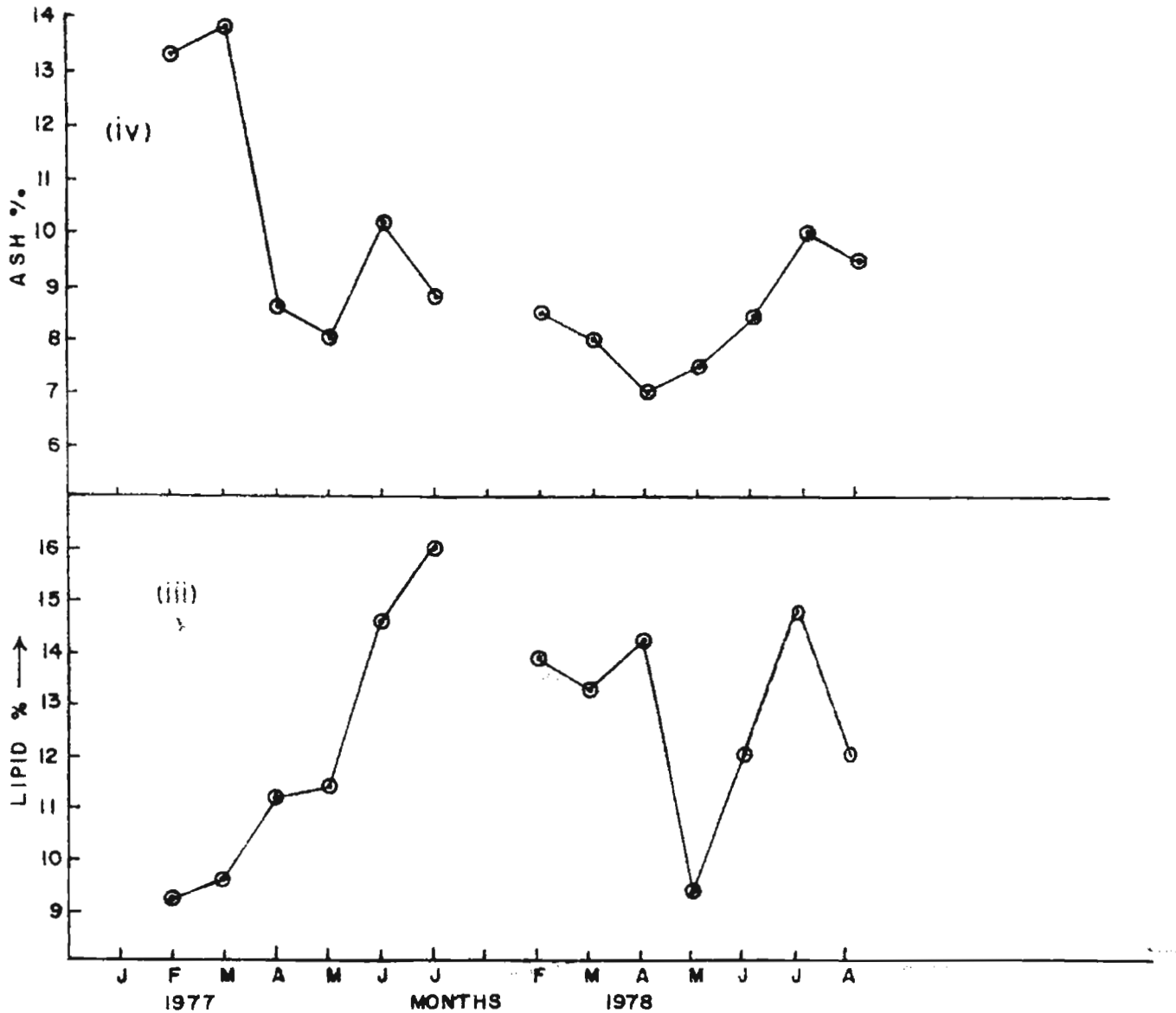


Fig. 3.3c. (contd.) Seasonal variations of (iii) Total lipids and (iv) Ash contents in Perna viridis.

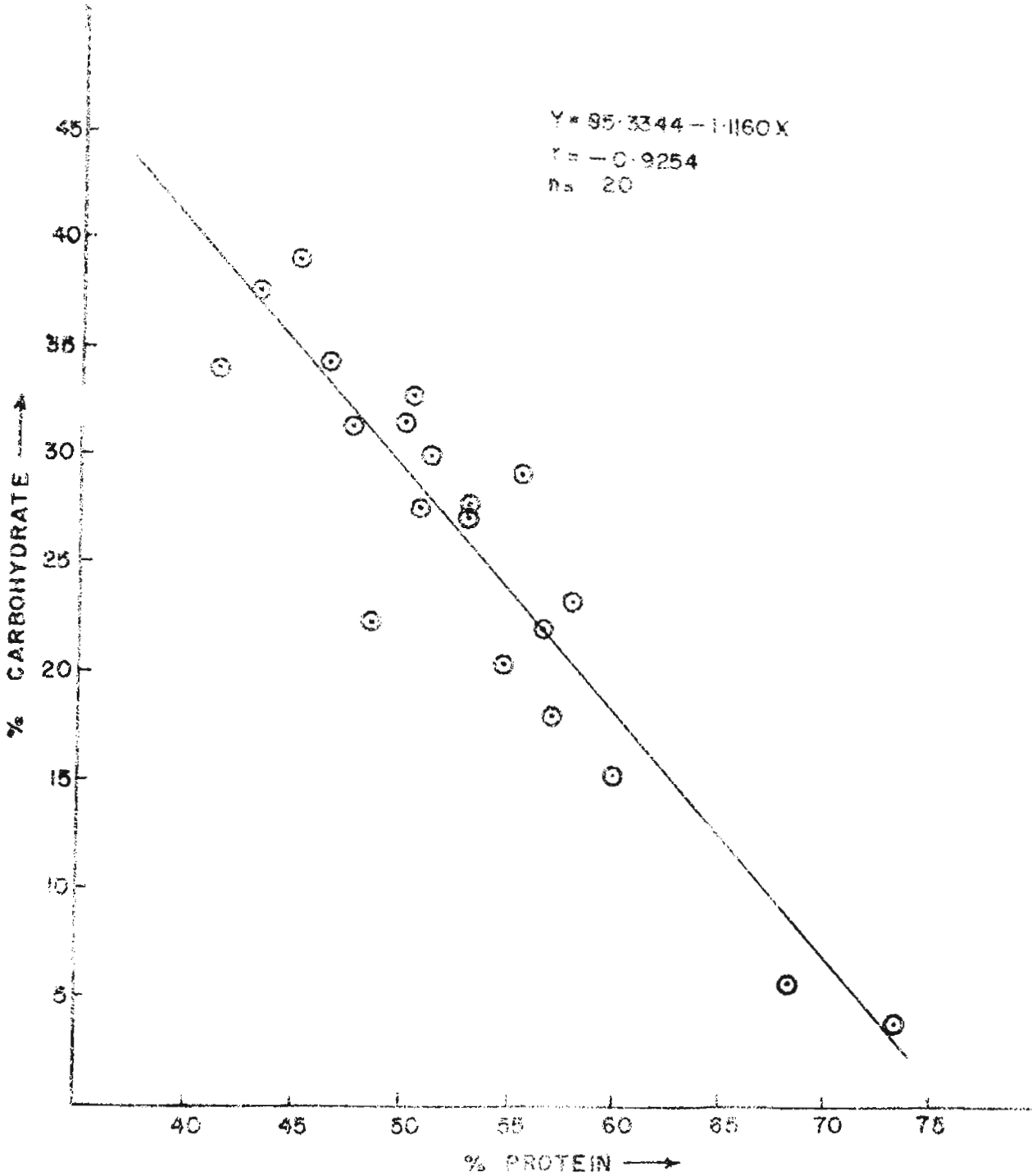


Fig 3.4a Relationship between Protein and Carbohydrate in Villorita cyprinoides

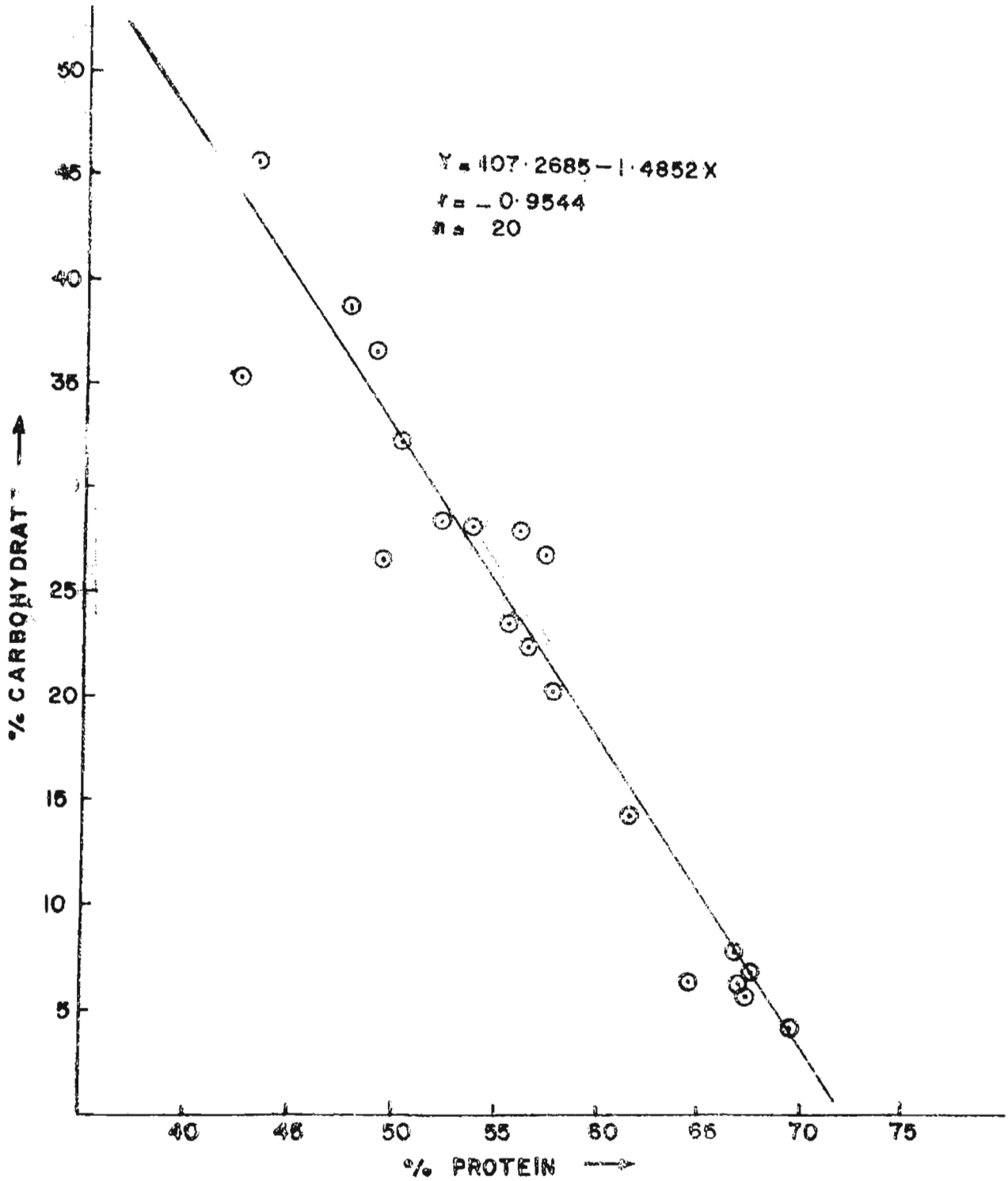


Fig 3.4b Relationship between Protein and Carbohydrate in Meverrix casta

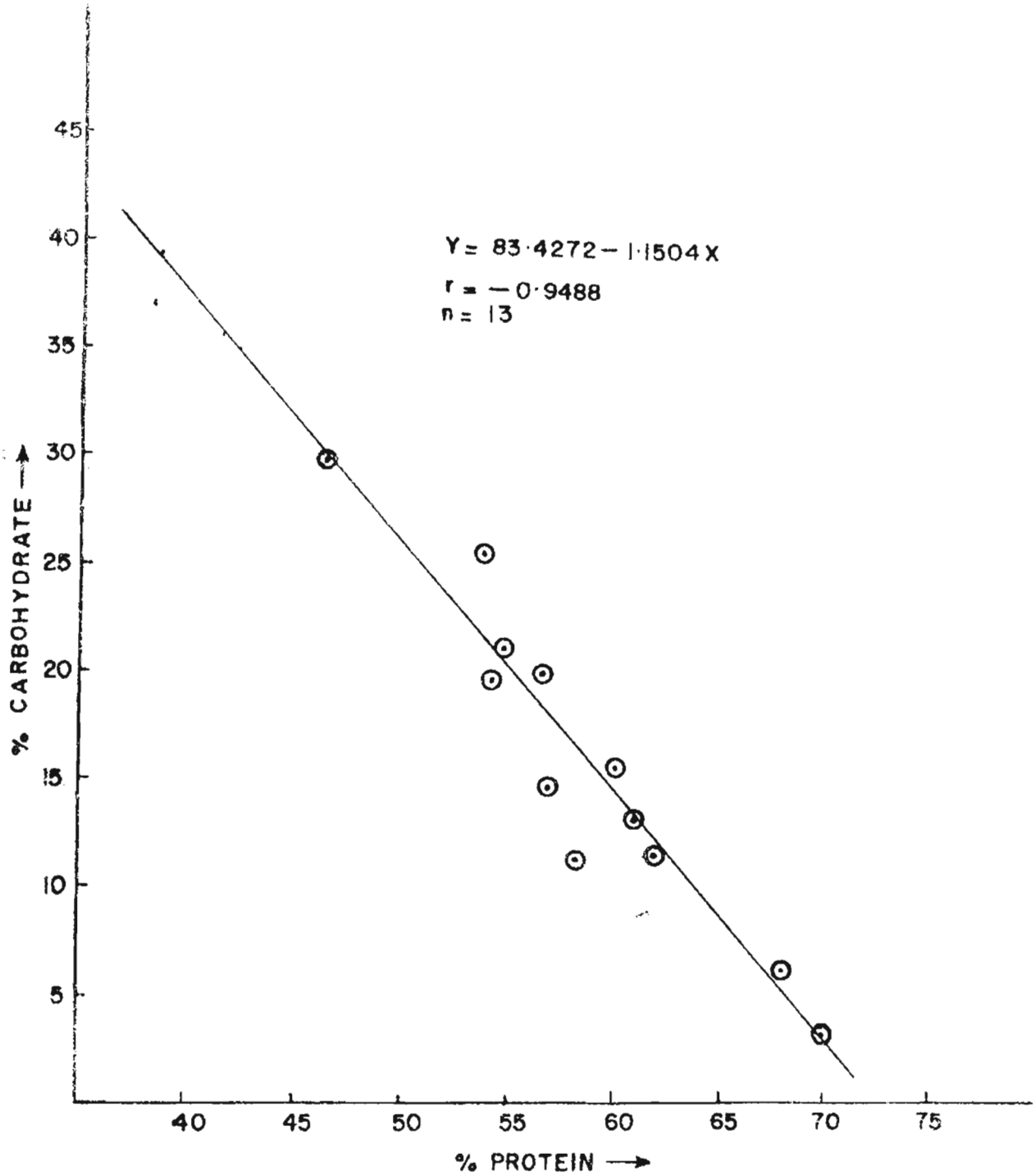


Fig. 3.4c Relationship between Protein and Carbohydrate in Perna viridis.

January to April. Higher values for lipid coincided with the high saline summer months in both the species (Tables 3.4a to 3.4b).

In P. viridis, lipid level ranged from 9.18% to 16.03%. However, the variation was not so regular as noticed in the other two species (Table 3.4c). Highest value was attained in early July each year.

c) Ash content:- Ash content also showed a seasonal cycle of change in all the species (Tables 3.2a to 3.2c). Ash conten was relatively low (mean value 5.44%) in V. cyprinoides and it varied from 3.38% to 9.04%. The ash content was high during January-April in each year (1977 and 1978). In P. canta, the percentage of ash ranged from 5.72% to 11.12% with an average value of 8.27%. It could be seen that, in both the species, the ash content went up when the environmental water salinity was high and declined with the lowering of salinity, as a consequence of the influx of monsoon. In P. viridis ash conten varied between 6.99% and 13.77% with a mean value of 9.35%. However, in this species, the variation in ash content did not maintain any trend as in the other two species, eventhough higher values were found during high saline months.

f) Phosphorous:- Phosphorous content of all the three ani mals varied with season (Tables 3.5a to 3.5c). In V. cyprinoide

Table 3.4 a

Seasonal variation of lipid and calorific value in the clam, Villorita cyprinoides collected during 1976-77 and 1977-78.

Date of collection	% lipid		Calorific value Cals/g dry wt.
	Mean value	± S.D.	
2.9.1976	12.30	0.349	5.346
1.10.'76	13.19	0.348	5.390
1.11.'76	13.27	0.396	5.518
1.12.'76	14.11	0.235	5.528
2.1.'77	12.97	0.306	5.558
1.2.'77	9.58	0.263	5.077
6.4.'77	13.47	0.209	5.276
2.5.'77	13.56	0.296	5.047
1.6.'77	9.21	0.238	5.248
13.7.'77	10.60	0.233	5.232
2.8.'77	11.01	0.297	5.238
2.12.'77	13.05	0.246	5.272
5.1.1978	13.74	0.236	5.404
9.2.'78	11.87	0.238	5.102
6.3.'78	14.27	0.281	5.378
10.4.'78	18.12	0.502	5.399
10.5.'78	15.44	0.366	5.419
8.6.'78	10.24	0.343	4.995
7.7.'78	12.23	0.235	5.241
4.8.'78	13.02	0.299	5.396
Mean value	12.763		5.3032
	±2.01		0.156

Table 3.4 b

Seasonal variation of lipid and calorific value in the clam, Meretrix casta collected during 1976-77 and 1977-78.

Date of collection	% lipid		Calorific value Cal/g dry wt.
	Mean value	± S.D.	
2.9.1976	4.53	0.089	4.800
1.10.'76	6.01	0.151	4.914
1.11.'76	7.02	0.152	4.989
2.12.'76	8.75	0.176	5.067
2.1.1977	12.76	0.428	5.127
1.2.'77	9.54	0.224	4.955
6.4.'77	10.44	0.382	5.123
2.5.'77	9.17	0.131	5.030
1.6.'77	8.84	0.149	5.047
13.7.'77	7.61	0.143	5.064
2.8.'77	7.31	0.134	5.066
2.12.'77	10.94	0.230	5.074
5.1.1978	11.35	0.208	5.170
9.2.'78	12.22	0.208	5.260
6.3.'78	13.39	0.212	5.384
10.4.'78	11.31	0.247	5.166
10.5.'78	9.02	0.214	4.993
8.6.'78	11.17	0.210	4.956
7.7.'78	11.21	0.235	4.975
4.8.'78	12.03	0.222	5.246
Mean value =	9.7313		5.070
	2.3093		±0.130

Table 3.4 c

Seasonal variation of lipid and calorific value in the mussel, Perna viridis collected during 1977 and 1978.

Date of collection	% lipid		Calorific value Cals/g dry wt.
	Mean value	± S.D.	
1.2.1977	9.18	0.252	4.874
2.3.'77	9.56	0.224	4.890
5.4.'77	11.22	0.263	5.096
2.5.'77	11.38	0.286	4.967
1.6.'77	14.59	0.286	5.148
13.7.'77	16.03	0.440	5.599
9.2.1978	13.90	0.249	5.159
13.3.'78	13.77	0.186	5.160
10.4.'78	14.23	0.187	5.339
8.5.'78	9.34	0.143	5.006
8.6.'78	12.06	0.181	5.204
7.7.'78	14.71	0.184	5.502
7.8.'78	11.89	0.192	5.138
Mean value =	12.4504		5.160
	±2.1726		±0.208

Table 3.5 a

Seasonal variations of phosphorous and calcium content in the clam, Villorita cyprinoides var. cochinensis, collected during 1976-77 and 1977-78.

Date of collection	% phosphorous	± S.D.	% calcium	± S.D.
2.9.1976	0.733	0.016	0.1967	0.007
1.10.'76	0.994	0.018	0.1897	0.005
1.11.'76	1.240	0.021	0.1685	0.004
1.12.'76	1.554	0.030	0.1797	0.004
2.1.1977	1.681	0.032	0.2510	0.005
1.2.'77	0.939	0.024	0.3492	0.005
6.4.'77	1.055	0.035	0.2803	0.005
2.5.'77	0.715	0.023	0.1414	0.002
1.6.'77	0.802	0.025	0.2336	0.004
13.7.'77	0.810	0.024	0.1723	0.004
2.8.'77	0.833	0.023	0.1147	0.004
2.12.'77	1.087	0.030	0.1730	0.004
5.1.1978	1.064	0.026	0.2471	0.005
9.2.'78	0.973	0.026	0.3455	0.006
6.3.'78	0.714	0.020	0.2923	0.004
10.4.'78	0.730	0.021	0.2600	0.007
10.5.'78	0.731	0.023	0.3131	0.005
5.6.'78	0.603	0.021	0.1433	0.004
7.7.'78	N.D.	N.A.	0.1729	0.005
4.8.'78	N.D.	N.A.	N.D.	N.A.
Mean value =	0.9693		0.2223	
	±0.2745		±0.0684	

Table 3.5 b

Seasonal variations of phosphorous and calcium contents in the clam, Meretrix casta (Chemnitz) collected during 1976-77 and 1977-78.

Date of collection	% phosphorous	± S.D.	% calcium	± S.D.
2.9.1976	0.659	0.012	0.3730	0.004
1.10.'76	0.698	0.017	0.3885	0.005
1.11.'76	0.783	0.016	0.3950	0.005
2.12.'76	0.797	0.016	0.3740	0.004
2.1.1977	1.264	0.019	0.4641	0.004
1.2.'77	1.170	0.021	0.5175	0.005
6.4.'77	0.864	0.013	0.3720	0.008
2.5.'77	0.864	0.017	0.2892	0.004
1.6.'77	0.794	0.016	N.D.	-
13.7.'77	0.758	0.020	0.2491	0.004
2.8.'77	0.697	0.020	0.1086	0.004
2.12.'77	0.669	0.014	0.4036	0.004
5.1.1978	0.656	0.015	0.4130	0.006
9.2.'78	1.011	0.020	0.4929	0.005
6.3.'78	0.878	0.021	0.4173	0.004
10.4.'78	0.869	0.021	0.3777	0.005
10.5.'78	0.592	0.016	0.4149	0.004
8.6.'78	0.762	0.020	0.4269	0.005
7.7.'78	0.934	0.021	0.5725	0.006
4.8.'78	0.882	0.019	N.D.	N.A.
Mean value =	0.8301 ±0.1655		0.4011 ±0.1051	

Table 3.5 c

Seasonal variations of phosphorous and calcium contents in the mussel, Perna viridis, collected during 1977 and 1978.

Date of collection	% phosphorous		% calcium	
	Mean value	± S.D.	Mean value	± S.D.
1.2.1977	1.016	0.028	0.4523	0.004
2.3.'77	0.886	0.033	0.4726	0.004
5.4.'77	0.662	0.018	0.3130	0.002
2.5.'77	0.583	0.017	0.2170	0.002
1.6.'77	0.861	0.018	0.2590	0.004
13.7.'77	0.932	0.031	0.2450	0.003
9.2.'78	0.753	0.023	0.3090	0.003
13.3.'78	0.646	0.014	0.3767	0.004
10.4.'78	0.696	0.016	0.3697	0.004
8.5.'78	0.487	0.014	0.3745	0.004
8.6.'78	0.685	0.018	0.3940	0.004
7.7.'78	0.598	0.014	0.3263	0.004
7.8.'78	0.713	0.017	N.D.	-
Mean value =	0.7320		0.3424	
	±0.1460		±0.0759	

the level of phosphorous was greater and lay in the range of 0.714% to 1.681% on a dry weight basis. In M. casta, the amount of phosphorous varied from 0.592% to 1.264% whereas in the mussel, P. viridis the distribution was from 0.487% to 1.016%. In general a higher percentage of phosphorous was accompanied by a higher percentage of lipid (Tables 3.4a to 3.5c).

g) Calcium:- The seasonal variations of calcium content in the bivalve molluscs are given in Tables 3.5a to 3.5c. Like most other shellfish, the three species studied were also rich in calcium. Higher values for Ca were found during January to April or May, the pre-monsoon months when the ambient water salinities were high. This was true of all the species. The amount of tissue Ca content decreased during monsoon periods (June to August), when the water salinity was at a minimum. The variations of Ca content in the three bivalves, V. cyprinoides, M. casta and P. viridis were from 0.1147% to 0.3492%, 0.1086% to 0.5175% and 0.2170% to 0.4726% respectively. The fluctuations in Ca content was at a minimum in P. viridis since the variation in salinity was also small in magnitude.

h) Calorific values:- Energy values in terms of calories were calculated using the appropriate calorific equivalents of 5.65 for protein, 9.45 for lipid and 4.2 for carbohydrate

(Ansell, 1974a). The total calorific values for the whole tissue (K. Cals/g. dry wt.) of the three species are given in Tables 3.4a to 3.4c. The calorific content of the tissue varied between 4.995 Cals to 5.558 Cals/g dry tissue weight in V. cyprinoides, between 4.800 Cals to 5.384 Cals/g in M. casta and between 4.874 Cals to 5.599 Cals/g dry tissue weight in P. viridis.

3.3. Discussion

The results indicated that the biochemical constituents studied, viz. protein, carbohydrate, lipid, ash etc. showed distinct seasonal variations in all the three bivalves. The changes in the biochemical constituents may be attributed, in general, to factors like availability of food, spawning and maturity and environmental parameters like salinity, temperature etc.

The seasonal changes in the body water content of the bivalves showed a direct relation to environmental water salinity. This component increased steadily during the monsoon period, when the ambient water salinities were low and decreased during the high saline periods (Tables 3.2a to 3.2c). This was true of all the three species studied and the same trend was followed during both the years. Thus, the variations in the water content of the soft parts of these three bivalve molluscs is associated

with the change in salinity of the ambient water. This may be explained as due to the hyposmotic condition of the environment. The increase in water content during low saline periods can be due to the osmotic flooding of water. In summer months, when the ambient water salinity was high, water may be abstracted by the hyper osmotic ambient media. This must be true of these bivalves which are euryhaline in nature, withstanding a broad range of external salinities.

Robertson (1964) observed that in concentrated seawater, Aplysia fasciata loses weight as water is abstracted by the hyper osmotic solution and noted that there was outward diffusion of ions, particularly Na^+ and Cl^-. When Mytilus edulis was kept in 50% seawater, the water content of the muscle increased from 75% to 78%. Again, the brackish-water clam Rangia cuneata showed a decrease in the water content of the body (minus shell) with increased salinity of the medium, from 81% (S=3‰) to 74% water content (S=25‰). Similar observation in the seasonal changes in water content has been reported by Suryanarayanan and Balakrishnan Nair (1976) in the mollusc, Cellana radiata.

The protein content showed a systematic increase from September onwards and touched peak values in January in the clam V. cyprinoides. Thereafter, the protein content started decreasing and reached a minimum value in May or June(Table 3.3a).

Carbohydrate, on the other hand showed an opposite trend in its variation. The lipid content was found generally high during the summer months, the period of high salinity (Table 3.4a) in V. cyprinoides. The seasonal variations may be attributed to food abundance and also, to spawning. The sedimentation of detritus and other dead organisms reaching the bottom may also affect the biochemical composition.

The increase in protein and lipid levels coincided closely with the periods of maximum phytoplankton abundance in the water (K.J.Joseph, 1978; personal communication). The steady fall in carbohydrate content during September to December 1977 and in April-May 1978 (Table 3.3a) may be attributed to the utilisation of this component for biochemical processes such as growth and maintenance and gonad development. The sudden change over from a minimum value of carbohydrate (in January) to a high value (in February) and the sharp declin in protein value during these periods may partly be attributed spawning occurred in January. Two breeding periods were observ for this clam in the area: one from January to February and the other from June to August (Sivankutty Nair and Shynamma, 1975). However, such a drastic change in the levels of the biochemical components was not observed during the latter period. The usua decrease in lipid content during monsoon periods can be considered as a consequence of high water content in the tissue. Su inverse relationship between tissue water content and lipid lev

in fish had been observed by many workers (Love, 1970). It is difficult to interpret whether the low lipid level observed during June to August is due to salinity effect or spawning or both.

In M. costa also, the variations in protein and carbohydrate followed almost a similar trend as observed in V. cyprinoides. Carbohydrate and protein showed reciprocal relations in their variations. The comparatively low values of protein from May to August and again from September to November (Table 3.3b) can be due to the low concentrations of planktors in the ambient water and may also be due to the abrupt and drastic changes in the ambient water salinity and temperature. Higher percentage of protein observed during December to April (Table 3.3b) in these cases also, corresponded to an increase in phytoplankton population in the habitat water (K.J.Joseph, 1978; personal communication). Lipid content was also at a higher level during the period (Table 3.4b). The increase in protein value was always followed by an abrupt fall in carbohydrate content indicating that this component might have been utilised for metabolic needs. The slight difference in seasonal variation of these two components from year to year may be related to environmental factors. It could be seen that there was an abrupt fall in environmental salinity from February (32.4‰) to April (24.0‰) in 1977 and from March

(30.5‰) to April (23.19‰) in 1978 due to pre-monsoon floods. And these periods coincided with low protein content of the tissue. The very low values for carbohydrate observed during January to March may be due to the intense pre-breeding activity in these months. The subsequent decrease in protein content can be attributed to the increase in carbohydrate level which might have occurred at the end of spawning. Mohammed Sal (1973) had reported two spawning periods in M. casta in the area viz. January and October. However, such a distinct variation in these components was not seen during October. Panikkar and Aiyar (1937) hold that the breeding of M. casta is a discontinuous one during the year and is often interrupted.

The pattern followed in the seasonal cycle of change in the biochemical constituents of the mussel was different from the two clams. Here, the highest protein content was noted in early July each year (Table 3.3c). The protein maxima synchronised with carbohydrate minima. Lipid level was also high in July. The variations in these components may be related to spawning in addition to food abundance. Virabhadra Rao et al. (1975) stated that spawning in the mussel (shell length ≥60 mm) commences from about July and lasts upto December.

Ansell (1972) found that there was a net increase in body weight and metabolic reserves in the bivalves Donax vittat

and Tellina tenuis of the Clyde Sea area during the periods of maximum phytoplankton abundance. He regarded the availability of food as a major factor determining the seasonal cycle of biochemical constituents. A similar increase in tissue weight was observed in both Abra alba and Chlamys septemradiata during the spring increase in phytoplankton population in the ambient water (Ansell, 1974a,b). These reserves are later utilised for maintenance during periods when food is available in insufficient quantity to support the animal's metabolic requirements. Gabbott and Bayne (1973) from an experimental study observed that nutritional stress affects the biochemical composition of Mytilus edulis L. Virabhadra Rao et al. (1975) also found that the frequencies of planktonic larvae in the water effects the biochemical composition of Perna viridis. The present observations are in good agreement with the above findings.

Giese (1969) observed that protein value reached a peak during the height of the reproductive season (in Amphineura) and declined to the minimum after spawning. The decrease in protein value was attributed to the increase in carbohydrate level. Suryanarayanan and Balakrishnan Nair (197 noted that there was a perceivable fall in glycogen content with the onset of breeding season. Ansell (1974a,b,c) had reported that spawning in the bivalves Abra alba, Chlamys

septemradiata and Nucula sulcata were accompanied by a rapid decline in carbohydrate content. However, in the present study no attempt had been made to relate the variations in biochemical constituents with spawning in the species since the prime objective was to establish the best harvesting season for these species.

Galtsoff (1964) recorded monthly variations in protein level in the oyster, Crassostrea virginica and found no relation to the reproductive season. He attributed the variations to the differences in nutrient conditions or other variables in the environment.

The inverse relationship between protein and carbohydrate observed here in the three bivalves has been supported by the earlier works of Giese (1969) in the mollusc Katharina tunicata, Durve and Bal (1961) in the oyster Crassostrea gryphoides and of Suryanarayanan and Balakrishnan Nair (1976) in the limpet, Cellana radiata.

The results indicated that higher values of calorific equivalents were associated with higher percentage of lipid content (Table 3.4a to c) in all the three bivalves.

Ash content in all the three species were higher when the salinity of their habitat water was high. The decrease in ash content during monsoon periods can be due to the

increased tissue water content. When the environmental water salinity goes up, there must be a corresponding increase in the uptake of major ions which is quite essential for the ionic regulation in these organisms. The high value of calcium content observed during these periods in these bivalves clearly supports the above argument.

Since it is highly essential in the building up of shells in all the three organisms, a greater rate of uptake of calcium can be expected during high saline periods when the calcium content in the environment is also high. Love (1970) found that calcium was absorbed directly from the surrounding water by fish also and stated that the Ca content of various tissues increases when euryhaline fish go from fresh water to the sea.

The phosphorous content also showed a similar trend to that of calcium (Table 3.5a to 3.5c)The increase in phosphorous content, in general was always accompanied by an increase in lipid level, in all the three species. This may probably be due to the presence of phosphorous as phospholipids in the muscle as observed by other workers (Jafri et al., 1964).

The Ideal Harvesting Periods for the Bivalves

From the nutritional point of view molluscan flesh forms an ideal source of animal protein. Hence the fishing

of these species should be made when the protein content is the highest. For Villorita cyprinoides, the period from December to February (with January the peak season) seemed to be ideal for harvest. This is the period of high protein content, high lipid content and hence of generally high nutritive value. The water content was only medium. In the case of Meretrix casta protein value was generally high during December to early April. The lipid content was also high during the period. So, M. casta may best be harvested during December to April. Perna viridis, on the other hand attains maximum nutritive value in June-July. The highest value for protein and lipid was found in July for the species. Therefore, the fishing may be carried out mainly during this period.

CHAPTER 4

SEASONAL VARIATION OF TRACE METAL CONTENT

CHAPTER 4

SEASONAL VARIATION OF TRACE METAL CONTENT

In recent years, monitoring the concentration of heavy metals in marine and fresh water organisms has received much attention. A broad understanding of metal levels in a large variety of fish/shell fish would help provide in establishing a baseline for some of the toxic metals. However, to draw a baseline for the metal levels in these organisms, the seasonal changes of the various trace metals in these animals must be considered. The availability of metal ions in the ambient water would influence the tissue metal levels. It is generally agreed that metal ions in water are not present as free hydrated ions but are multi complexed with inorganic and organic ligands (Zirino and Yamamoto, 1972; Blustein and Smith, 1978). The situation in fresh water is less complex compared to seawater. Changes in salinity, therefore will have the potential to change metal uptake. A typical system is an estuarine environment, where the salinity fluctuates from an almost zero value to an average seawater salinity of 35‰. This must be largely influencing the trace metal distribution in an organism. This fact emphasises the need for the study of seasonal variations of trace metals in aquatic organisms.

Other environmental parameters may exert physico-chemical influences on metals in the water, rendering them less available for uptake. Also, stresses such as temperature, salinity or turbidity and physiological condition of the animal may affect metabolic rates, resulting in altered uptake of metals.

The investigations carried out mainly in the industrialis affluent countries are briefly reviewed below. The results of t earlier investigations had been compiled by Vinogradov (1953).

Brookes and Rumsby (1965) made a quantitative study of certain trace metals in some bivalve molluscs of Newzealand. Segar et al. (1971) gave the distribution of six major and thirteen minor elements in the shells and entire soft parts of eleven species of molluscs from the Irish Sea including those of one freshwater species. Bertine and Goldberg (1972) made a detailed study on the trace metals in clams, mussels and shrimp. Another important piece of work worth mentioning is by Bryan (1973) on the occurance and seasonal variations of Cu, Fe, Zn, Mn, Pb, Ni, Co, Cr, Cd and Al in the scallops Pecten maximus (L) and Clamys opercularis (L). Holden (1973) had reviewed the work on mercury in fish and shellfish. Hall (1974) had estimated the Hg content in several brands of different commercial canned seafood products including certain clams and oysters. Eustace (1974) estimated the concentrations

of Zn, Cd, Cu and Mn in certain species of finfish and shellfish caught from Derwent estuary in Tasmania. Other important investigations on the occurance of trace metals in molluscs were those of Preston et al. (1972), Topping (1973), Martin (1974), Ratkowsky et al. (1974), Boyden (1974), Keeney et al. (1976), Bryan et al. (1977), Bryan and Hummerstone (1977), Karbe et al. (1977), Posch et al. (1977), Wharfe and Van den Broeke (1977), Bryan and Hystal (1978) and Davies and Pirie (1978). However, work carried out in India, in these lines is scant. Some of the attempts made are mentioned below.

Somayajulu and Rama (1972) had estimated mercury in seafoods collected from the coastal waters off Bombay (in lobster, pomfrets, Bombay duck, salmon and red prawns), Jagadeson and Venugopal (1973) determined certain trace elements in the particulate matter of Porto-Novo waters. Zingade et al. (1976) had analysed As, Cu, Zn and Mn in certain fish and shellfish caught from the estuaries around Goa. Sankaranarayanan et al. (1978) had determined certain heavy metals in the oyster, Crassostrea virginica.

Thus, literature pertaining to the occurance and distribution of trace metals in fish/shellfish from Indian waters is very much limited. So, it was found desirable to undertake a study on the occurance, distribution and seasonal variations

of certain trace metals in the bivalve molluscs, V. cyprinoides, M. casta and P. viridis of the Cochin backwaters and hence to establish a base line for these metals which may be useful for comparison with possible future changes.

4.1. Materials and methods:- The samples collected at monthly intervals during 1976-77 and 1977-78 were used for the estimation (See chapter 2). The estimations of copper, zinc, iron and lead were carried out using Atomic Absorption Spectrophotometer. The details of the analytical procedure are given in 2.7a & b.

4.2. Results:- The results of the various analysis are presented in tables (4.1a to 4.4c) and their seasonal trend can be seen from the figures (4.1a to 4.4c). Metal concentrations are given on a dry weight basis.

Among the metals studied concentration of lead was found to be the lowest in all the three species and the highest values were observed for iron. The levels of Zn and Cu were comparable in magnitude in the three species.

a) Copper:- The distribution of copper in the three bivalve molluscs seemed to be well influenced by season, especially by the two monsoons: the southwest monsoon (June to August) and the northeast monsoon (October to December). The

levels of copper in the whole soft tissues ranged from 18.13 to 44.46 µg/g in V. cyprinoides, 17.35 µg/g to 46.89 µg/g in M. casta and 15.20 µg/g to 30.41 µg/g in P. viridis. The con trations of copper seemed to be comparable in V. cyprinoides M. casta. It can be seen that higher values were often found during low saline periods (Table 4.1a to c) with a few discre pencies found here and there. In V. cyprinoides, higher conc trations were found between June to August and September to December. Low values, in general, were observed between Janu to May, with one exception in January 1978. Higher values bo in _. casta and P. viridis were found between June to August comparatively low values during the rest of the year. The co trations of copper in P. viridis were rather low compared to other two species. The fluctuations in copper content in P. viridis with season were also a minimum (11.40 µg/g to 30.41 µg/g). Copper content in the molluscs showed a signifi negative correlation with the environmental water salinity. correlation coefficients (r) and probability factor (P) were found to be

$r = -0.8744$ $(P < 0.001)$ in V. cyprinoides

$r = -0.9063$ $(P < 0.001)$ in M. casta

and $r = -0.8548$ $(P < 0.001)$ in P. viridis

the corresponding equations being

Table 4.1 a

Seasonal variation of copper content in the clam, Villorita cyprinoides var cochinensis (Mean concentrations of the metal in the whole soft parts and standard deviation, on a dry wt. basis)

Date of collection	Habitat salinity (‰)	pH of water	Copper µg/g dry wt.	± S.D.
2.9.1976	0.00	6.30	39.02	1.15
1.10.'76	1.53	6.60	37.46	1.48
1.11.'76	1.00	6.40	41.27	1.65
1.12.'76	0.50	6.80	44.46	1.98
2.1.1977	15.21	7.80	23.93	1.58
1.2.'77	22.81	8.00	20.26	1.15
6.4.'77	20.30	7.60	24.93	1.37
2.5.'77	8.35	7.00	32.62	1.41
1.6.'77	4.34	6.80	37.70	1.07
13.7.'77	0.00	6.90	40.25	2.21
2.8.'77	0.00	6.90	34.91	1.36
2.12.'77	1.93	6.20	40.56	1.38
5.1.1978	11.67	6.80	38.20	1.77
9.2.'78	14.52	7.05	18.13	0.94
6.3.'78	17.63	8.10	19.71	1.25
10.4.'78	16.00	7.25	24.18	1.18
10.5.'78	11.13	8.65	26.61	1.64
8.6.'78	0.54	6.20	33.97	2.19
7.7.'78	0.00	6.20	37.65	1.55
Mean value =			34.41	
			±8.15	

Table 4.1 b

Seasonal variation of copper content in the clam, Meretrix casta (Chemnitz). (Mean concentrations of the metal in the whole soft parts and standard deviations, on a dry weight basis)

Date of collection	Habitat salinity (‰)	pH of water	Copper µg/g dry wt.	± S.D.
2.9.1976	27.12	7.80	26.95	2.02
1.10.'76	31.73	8.15	23.90	1.54
1.11.'76	28.46	8.00	19.27	1.37
2.12.'76	30.70	8.10	20.63	1.61
2.1.1977	31.92	8.20	25.49	2.10
1.2.'77	32.40	8.10	17.35	0.97
6.4.'77	24.00	7.80	31.31	1.96
2.5.'77	24.16	8.00	26.43	1.85
1.6.'77	17.25	7.80	28.74	1.54
13.7.'77	10.50	7.20	34.25	1.36
2.8.'77	5.72	7.00	38.89	1.59
2.12.'77	24.54	7.50	25.98	1.39
5.1.1978	30.62	7.80	21.23	1.34
9.2.'78	27.65	7.80	22.33	1.72
6.3.'78	30.50	8.25	23.23	1.03
10.4.'78	23.19	7.60	25.42	2.08
10.5.'78	22.11	8.00	27.97	0.92
8.6.'78	1.76	6.10	39.79	0.83
7.7.'78	1.00	6.35	46.89	1.67
4.8.'78	2.34	6.40	38.81	1.81
Mean value =			28.24	
			±7.62	

Table 4.1 c

seasonal variation of copper content in the mussel, Perna viridis (Linnaeus) (Mean concentrations of the metal in the whole soft parts and standard deviations on a dry weight basis).

Date of collection	Habitat salinity (‰)	pH of water	Copper µg/g dry wt.	± S.D.
1.2.1977	34.47	8.20	22.79	0.76
1.3.'77	34.40	8.15	17.09	0.85
2.4.'77	28.62	7.82	23.02	1.28
2.5.'77	29.86	7.80	24.64	1.52
1.6.'77	24.75	7.25	28.59	1.67
13.7.'77	20.90	7.15	30.41	1.82
9.2.1978	32.82	7.80	17.72	0.89
13.3.'78	33.60	8.20	19.03	1.08
10.4.'78	33.21	8.20	11.40	1.01
8.5.'78	34.42	8.30	15.48	1.33
5.6.'78	25.90	7.20	27.68	1.11
7.7.'78	32.79	6.65	23.44	1.61
7.8.'78	25.75	6.60	28.20	1.20
Mean value =			22.26	
			±5.55	

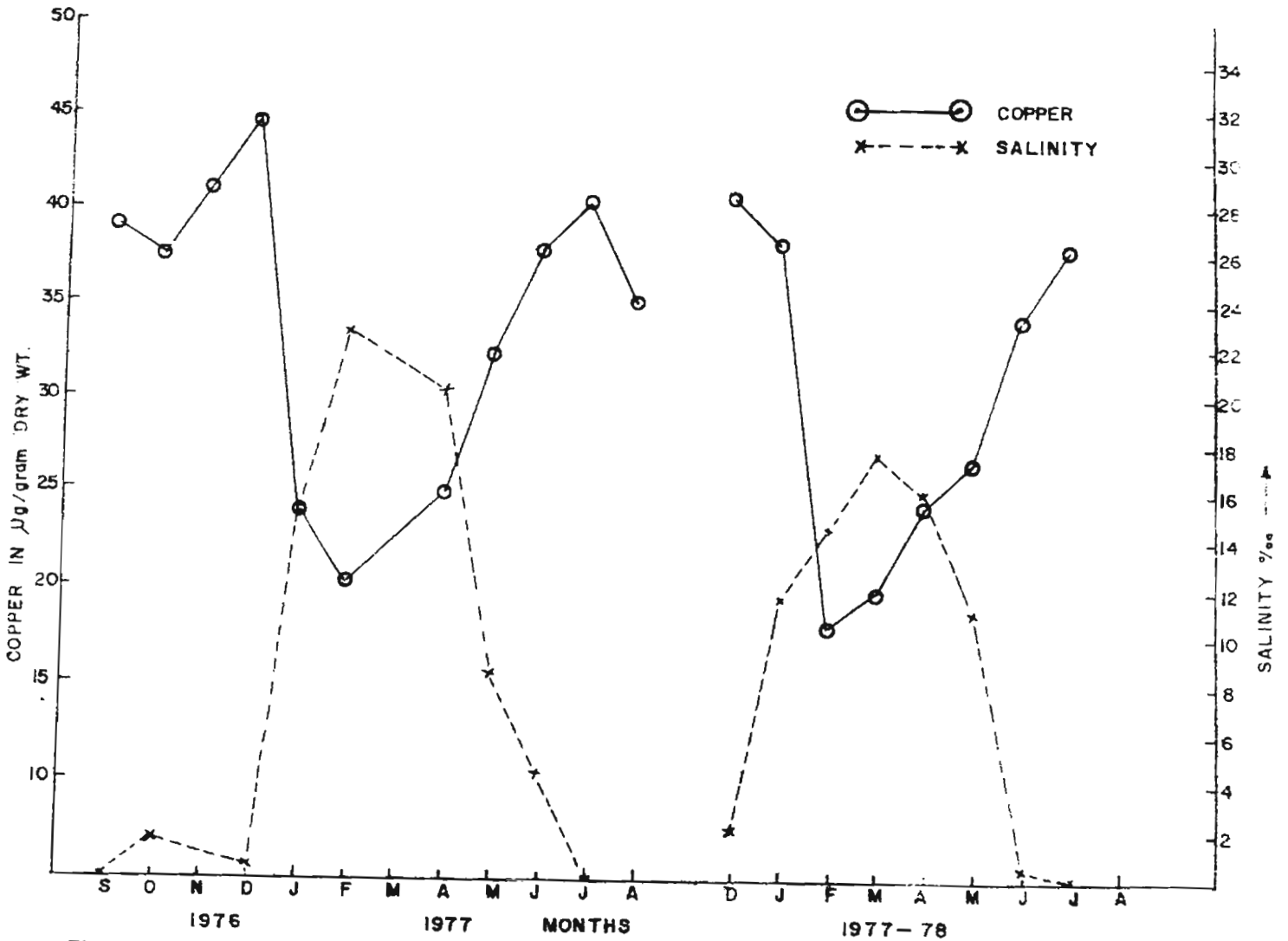


Fig. 4.1a. Seasonal variation of copper content in the whole soft parts of Villorita cyprinoides in relation environmental water salinity.

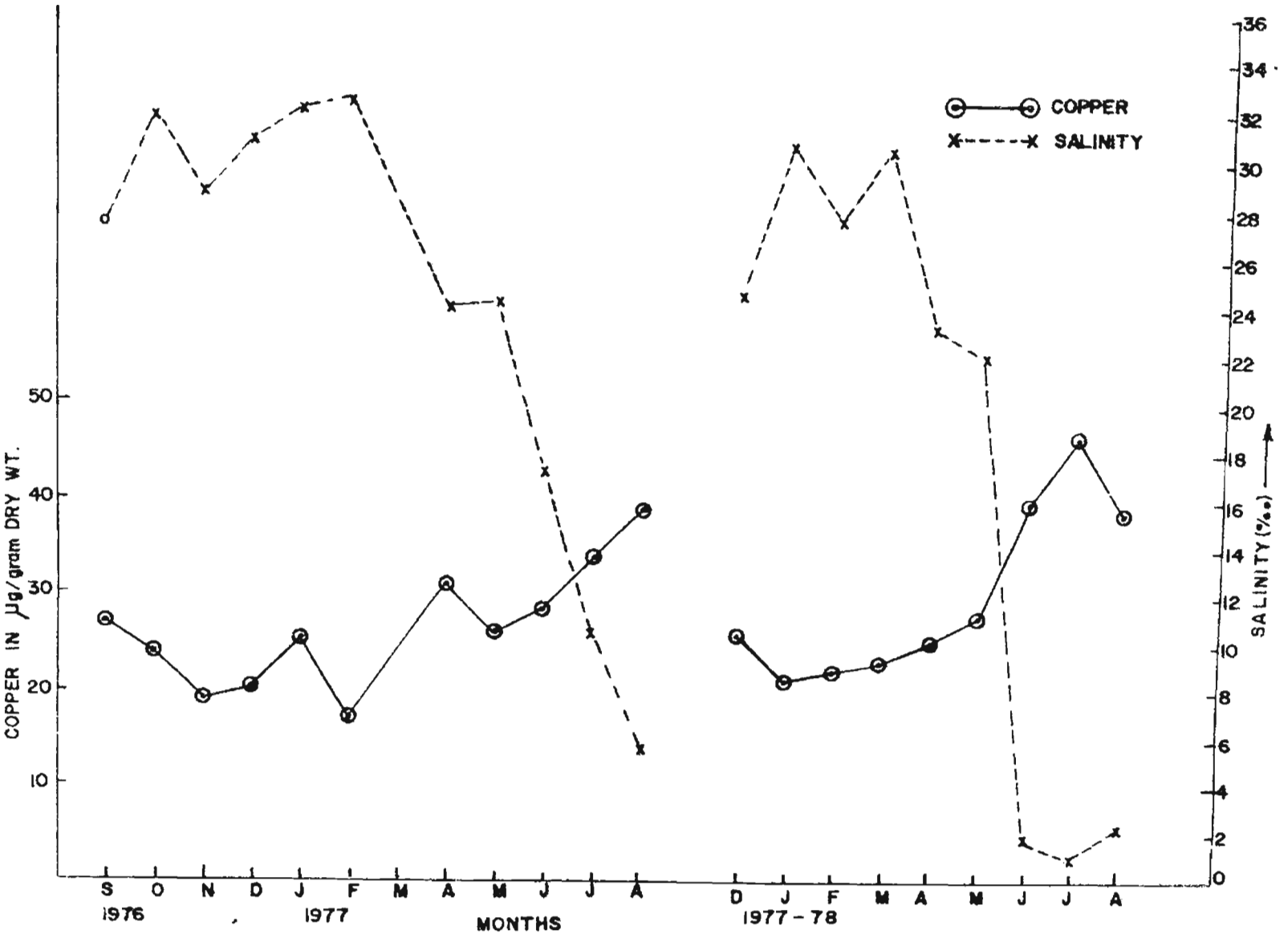


Fig. 4.1b. Seasonal variation of copper content in the whole soft parts of Meretrix casta in relation to environmental water salinity.

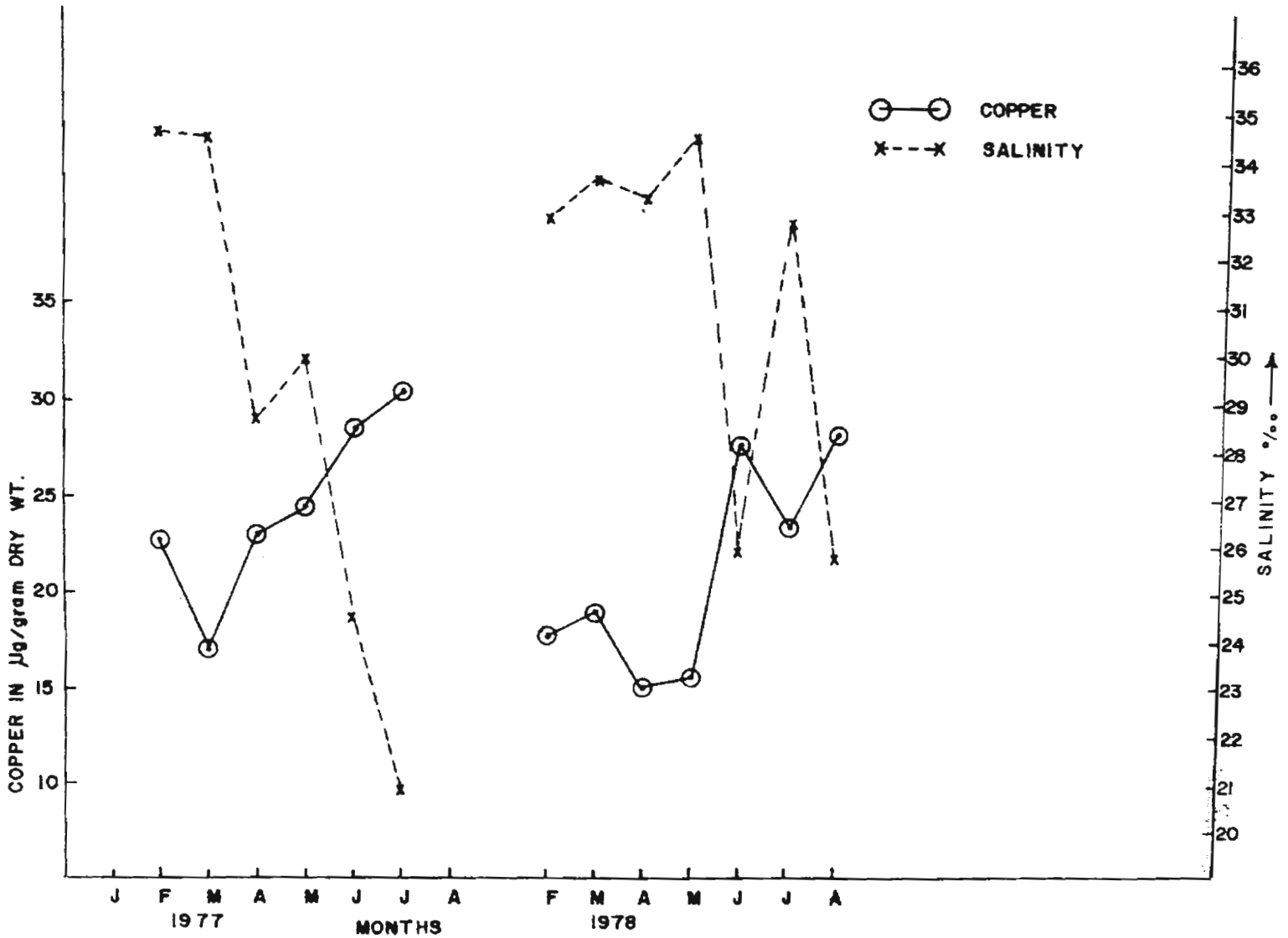
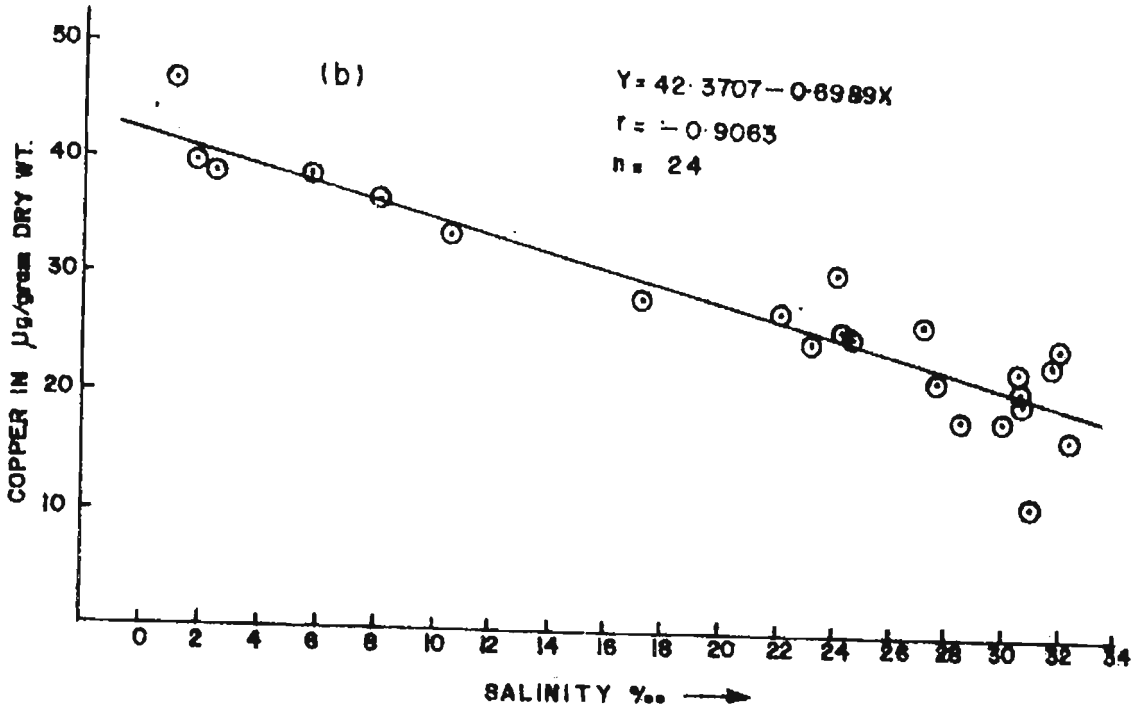


Fig. 4.1c. Seasonal variations of copper content in the whole soft parts of Perna viridis in relation to environmental water salinity.



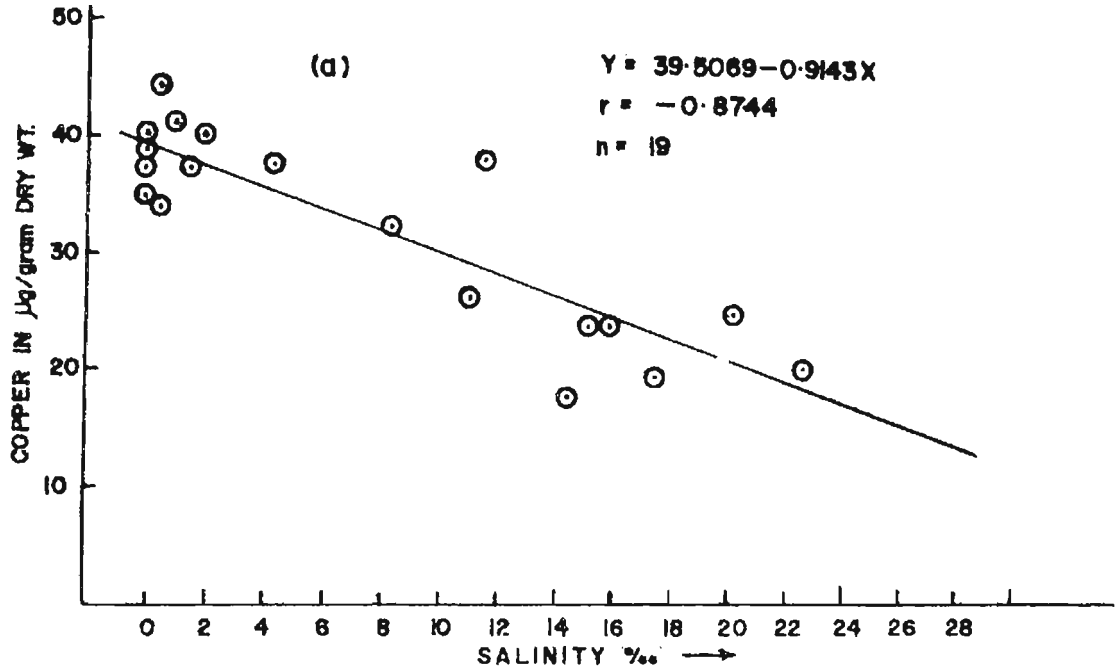


Fig. 4.5 a & b. Relationship between copper content in the whole soft parts and environmental water salinity of (a) V. cyprinoides & (b) M. casta.

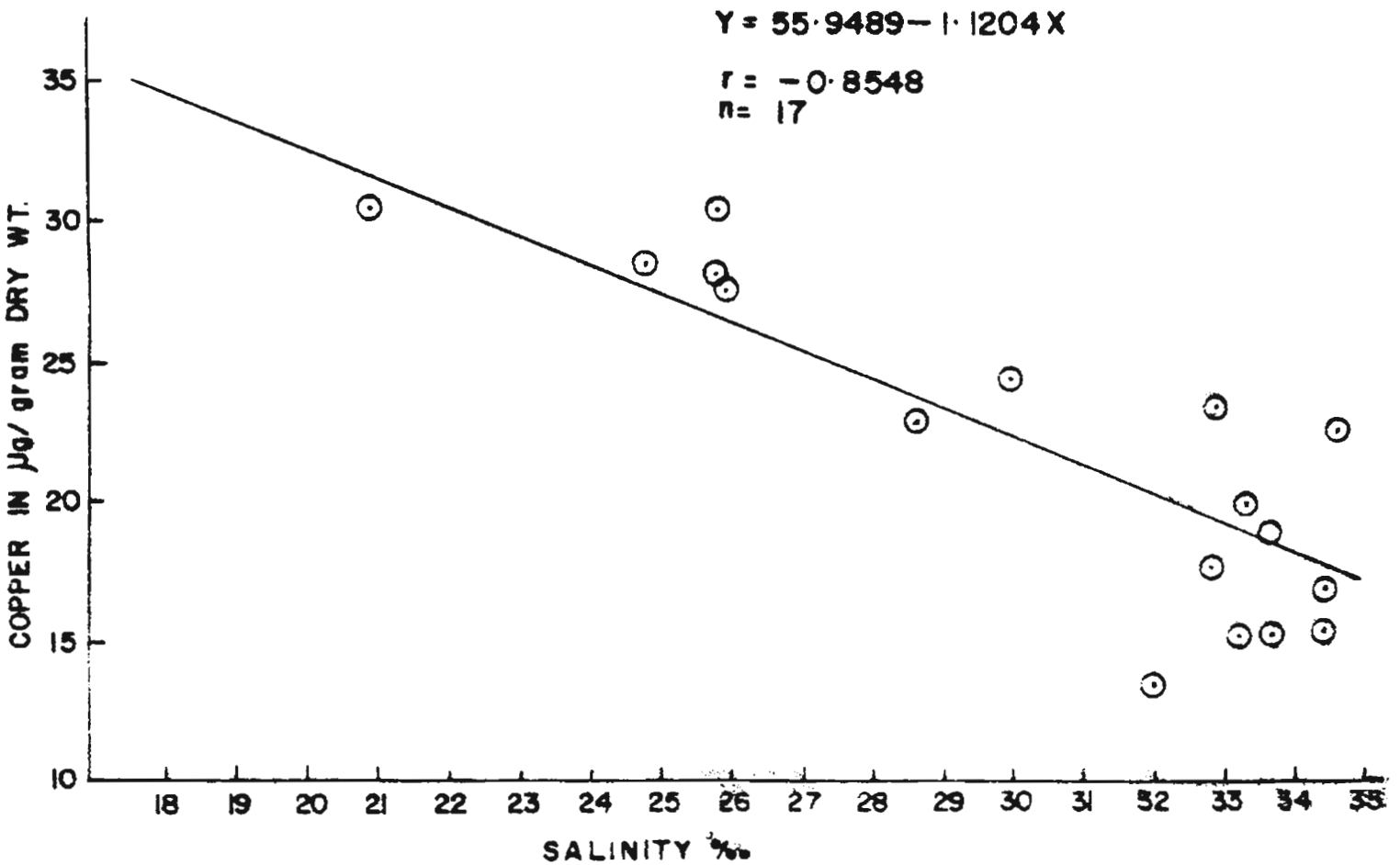


Fig. 4.5c Relationship between copper content in the whole soft parts of P. viridis and environmental water salinity.

$$Y = 39.5069 - 0.9143X \quad \ldots 4.1$$

$$Y = 42.3707 - 0.6969X \quad \ldots 4.2$$

and $$Y = 55.9489 - 1.1204X \quad \ldots 4.3$$

respectively, where Y = metal content and X = salinity of the habitat water. Figs 4.5a to 4.5c represent the above equations.

b) Zinc:- The distribution of zinc in the three species also varied with season. (Tables 4.2a to 4.2c and Figs 4.2a to 4.2c). The concentrations of the metal in all the three bivalves were apparently similar in magnitude. Zinc content was found to be highest in *V. cyprinoides* with a maximum value of 105.58 µg/g in September 1976 (S = 0‰). The concentration declined with increased salinity and the lowest value was found during April 1977 (53.07 µg/g), when the salinity was 20.30‰. It can be seen that lower values were observed during the pre-monsoon months and higher values during low saline period (monsoon months).

In *M. casta* also the distribution of zinc followed almost a similar pattern as in *Villorita*. In *M. casta* higher levels of Zn were found during and immediately after the monsoon periods and low values during the summer months (Table 4.2b). The highest concentration of zinc was found in July 1978 (83.35 µg/g when the salinity was ≈ 1‰. The lowest zinc content was found to be 49.82 µg/g in February 1977, when the salinity was 32.4‰.

Table 4.2 a

Seasonal variation of zinc content in the clam, Villorita cyprinoides var cochinensis. (Mean concentrations of the metal in the whole soft parts and standard deviations, on a dry weight basis).

Date of collection	Habitat salinity (‰)	pH of water	Zinc µg/g dry wt.	± S.D.
2.9.1976	IL	6.30	105.58	5.79
1.10.'76	1.53	6.60	75.68	3.60
1.11.'76	1.00	6.40	82.80	4.47
1.12.'76	0.50	6.80	65.62	2.19
2.1.1977	15.21	7.80	80.55	3.14
1.2.'77	22.81	8.00	54.33	1.99
6.4.'77	20.30	7.60	53.07	1.54
2.5.'77	8.35	7.00	88.84	3.46
1.6.'77	4.34	6.80	90.92	3.92
13.7.'77	0.00	6.90	94.17	3.49
2.8.'77	0.00	6.90	89.81	3.80
2.12.'77	1.93	6.20	91.99	4.56
5.1.1978	11.67	6.80	86.32	3.55
9.2.'78	14.52	7.05	77.62	3.83
6.3.'78	17.63	8.10	71.26	2.65
10.4.'78	16.00	7.25	58.00	2.83
10.5.'78	11.13	8.65	74.19	3.32
8.6.'78	0.54	6.20	84.97	3.73
7.7.'78	0.00	6.20	81.45	3.14
Mean value =			79.32 ±13.70	

Table 4.2 b

Seasonal variation of zinc content in the clam, Meretrix casta (Chemnitz). (Mean concentrations of the metal in the whole soft parts and standard deviations, on a dry weight basis).

Date of collection	Habitat salinity (‰)	pH of water	Zinc µg/g dry wt.	± S.D.
2.9.1976	27.12	7.80	64.44	2.32
1.10.'76	31.73	8.15	52.03	2.31
1.11.'76	28.46	8.00	64.92	3.55
2.12.'76	30.70	8.10	59.09	3.53
2.1.1977	31.92	8.20	63.44	3.02
1.2.'77	32.40	8.10	49.82	2.55
6.4.'77	24.00	7.80	60.01	2.95
2.5.'77	24.16	8.00	68.79	2.89
1.6.'77	17.25	7.80	66.53	3.20
13.7.'77	10.50	7.20	70.01	3.41
2.8.'77	5.72	7.00	72.34	3.41
2.12.'77	24.54	7.50	57.47	2.32
5.1.1978	30.62	7.80	54.01	2.46
9.2.'78	27.65	7.80	69.63	3.13
6.3.'78	30.50	8.25	59.28	2.88
10.4.'78	23.19	7.60	58.47	2.13
10.5.'78	22.11	8.00	57.63	3.33
8.6.'78	1.76	6.10	76.80	3.21
7.7.'78	1.00	6.35	83.35	3.34
4.8.'78	2.34	6.40	73.62	3.55
Mean value			64.11	
			±8.48	

Table 4.2 c

Seasonal variation of zinc content in the mussel, Perna viridis (Linnaeus). (Mean concentrations of the metal in the whole soft parts and standard deviations, on a dry weight basis).

Date of collection	Habitat salinity (‰)	pH of water	Zinc µg/g dry wt.	± S.D.
1.2.1977	34.47	8.20	67.48	2.95
1.3.'77	34.40	8.15	72.50	3.75
2.4.'77	28.62	7.82	80.58	3.81
2.5.'77	29.86	7.80	71.68	2.79
1.6.'77	24.75	7.25	94.90	3.60
12.7.'77	20.90	7.15	95.30	4.03
9.2.'78	32.82	7.80	75.80	3.81
13.3.'78	33.60	8.20	71.20	3.07
10.4.'78	33.21	8.20	79.49	3.65
8.5.'78	34.42	8.30	56.12	2.50
5.6.'78	25.90	7.20	91.20	3.16
7.7.'78	32.79	6.65	74.31	2.49
7.8.'78	25.75	6.60	100.88	3.74
Mean value =			79.18	
			±12.44	

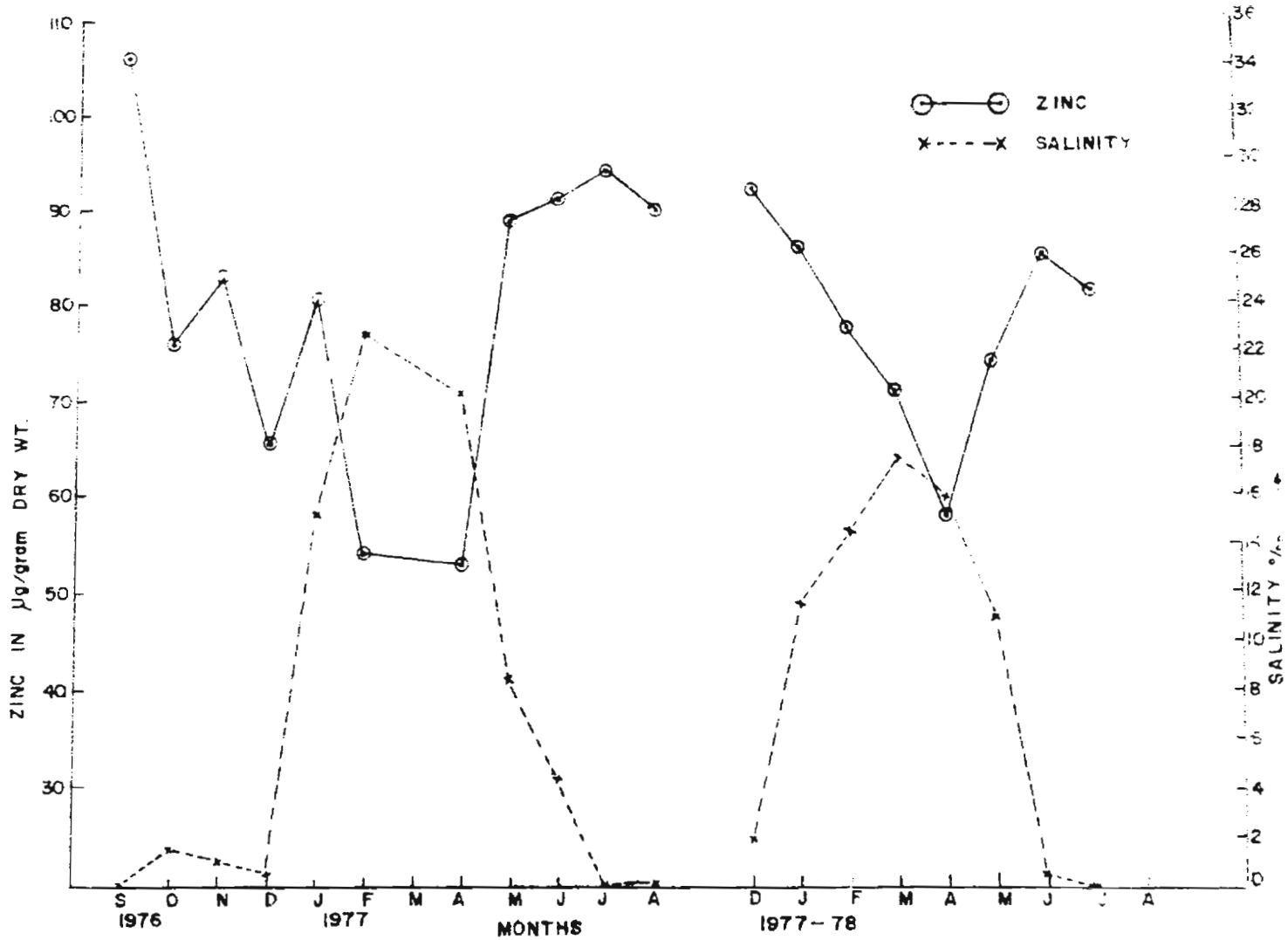


Fig. 4.2 a. Seasonal variation of zinc content in the whole soft parts of Villorita cyprinoides in relation to environmental water salinity.

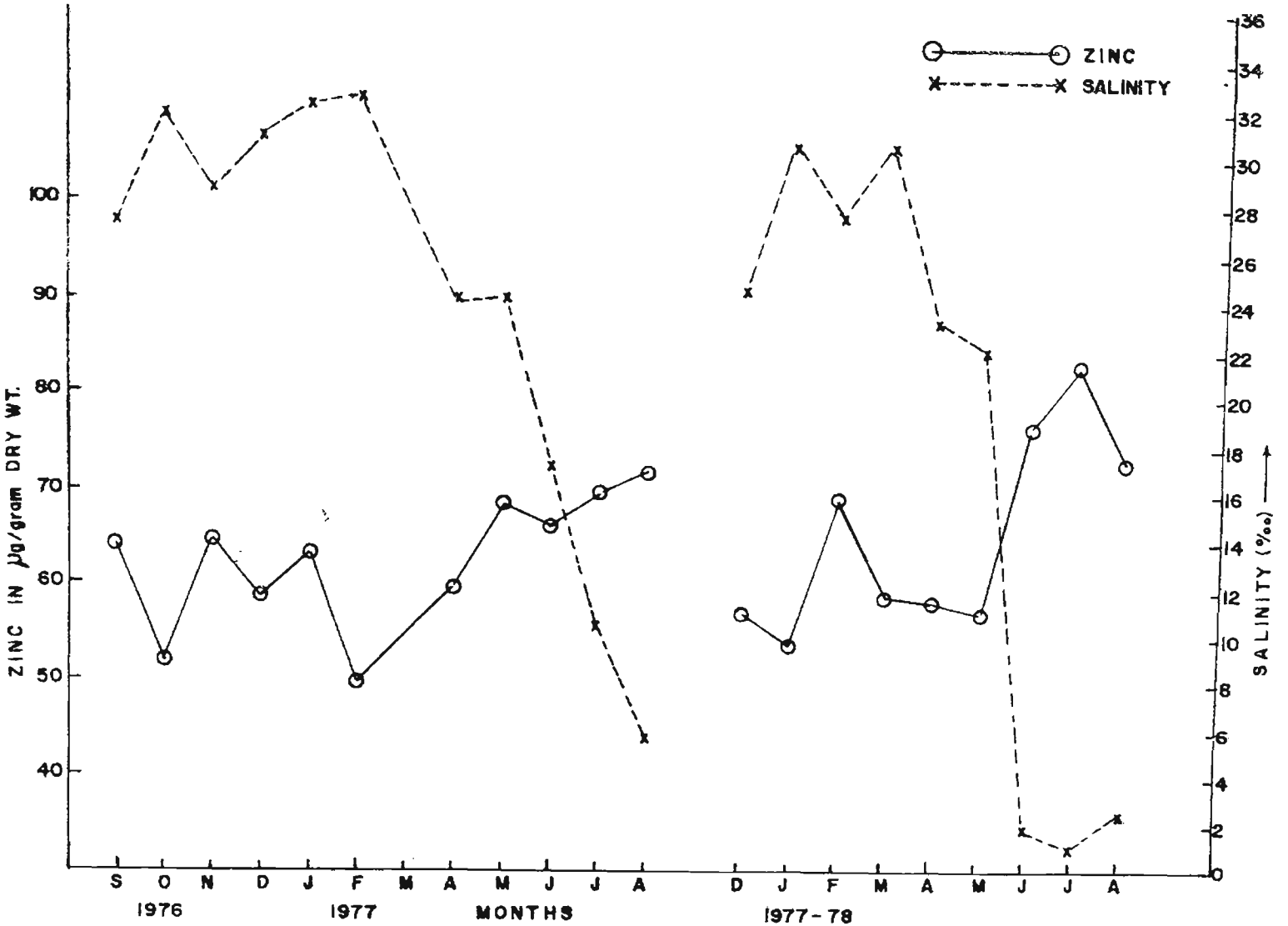


Fig. 4·2 b. Seasonal variation of zinc content in the whole soft parts of Meretrix casta in relation to environmental water salinity.

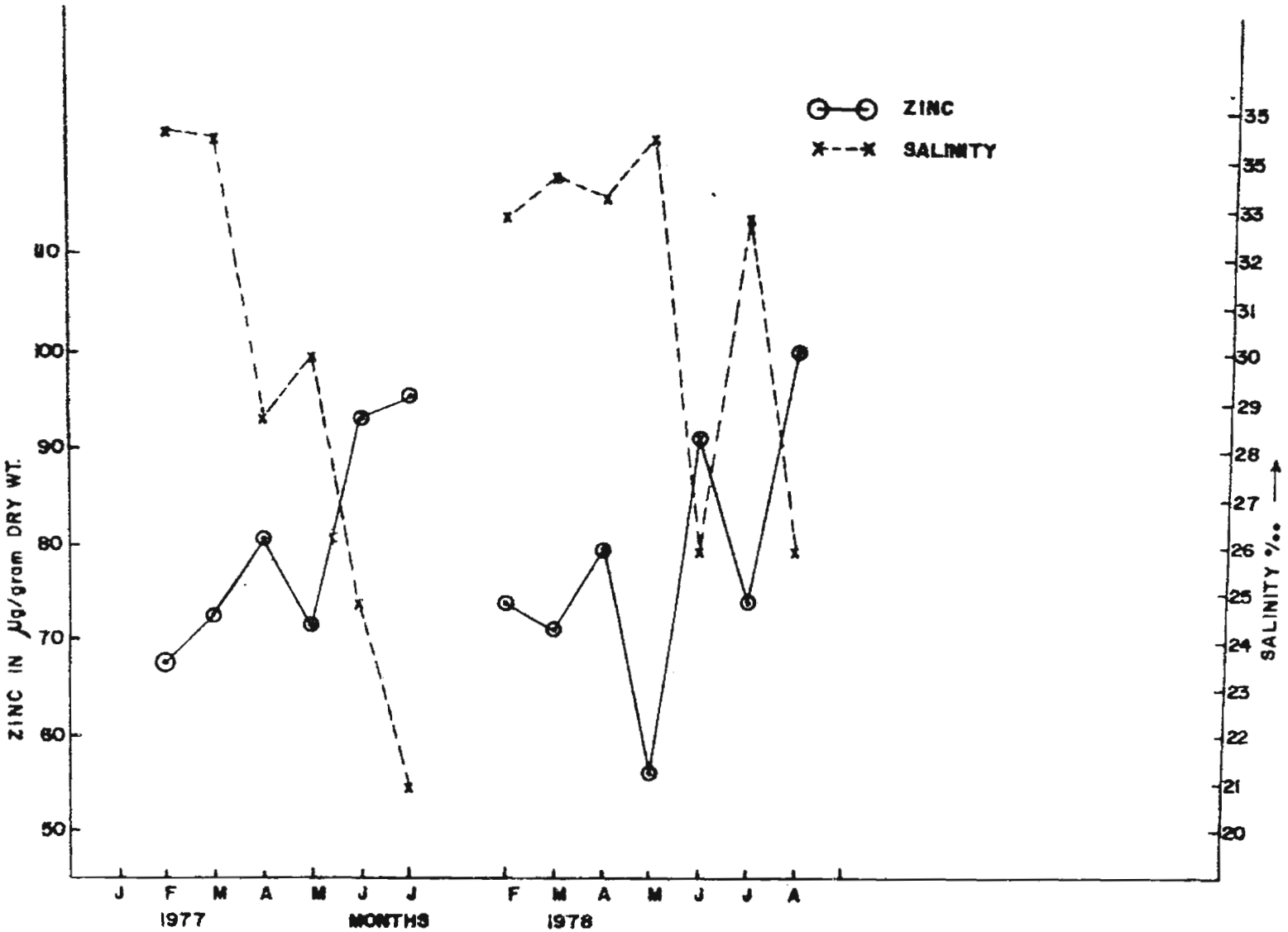


Fig. 4. 2c, Seasonal variation of zinc content in the whole soft parts of Perna viridis in relation to environmental water salinity.

Zinc content in P. viridis falls in the range of 56.12 µg/g to 100.80 µg/g. The distribution of Zn was influenced by season in this species also. It should be noted here that fluctuation in water salinity is low in magnitude (20.90‰ to 34.47‰). However, the higher values of Zn, in P. viridis always coincided with lower values of salinity. The highest value was found in August 1978 (100.80 µg/g) at a salinity of 25.78‰. The lower values were found during the pre-monsoon periods; thus, the lowest value observed was 56.12 µg/g during May 1978 (S = 34.42‰). Among the three species Zn content was in the order V. cyprinoides ≃ P. viridis > M. casta, the respective average values being 79.33, 79.19 and 64.11 µg/g dry weight respectively.

Zinc content in the three species of bivalves showed significant negative correlations with salinity of the habitat water, the correlation coefficients (r) and(P) values being

$$r = -0.5229 \quad (P < 0.05) \text{ in } V. \text{ cyprinoides}$$

$$r = -0.6671 \quad (P < 0.001) \text{ in } M. \text{ casta}$$

$$\text{and } r = -0.7035 \quad (P < 0.01) \text{ in } P. \text{ viridis}.$$

The corresponding regression lines were plotted and are given in figures (4.6a to 4.6c).The respective regression equations for V. cyprinoides, M. casta and P. viridis are given below,

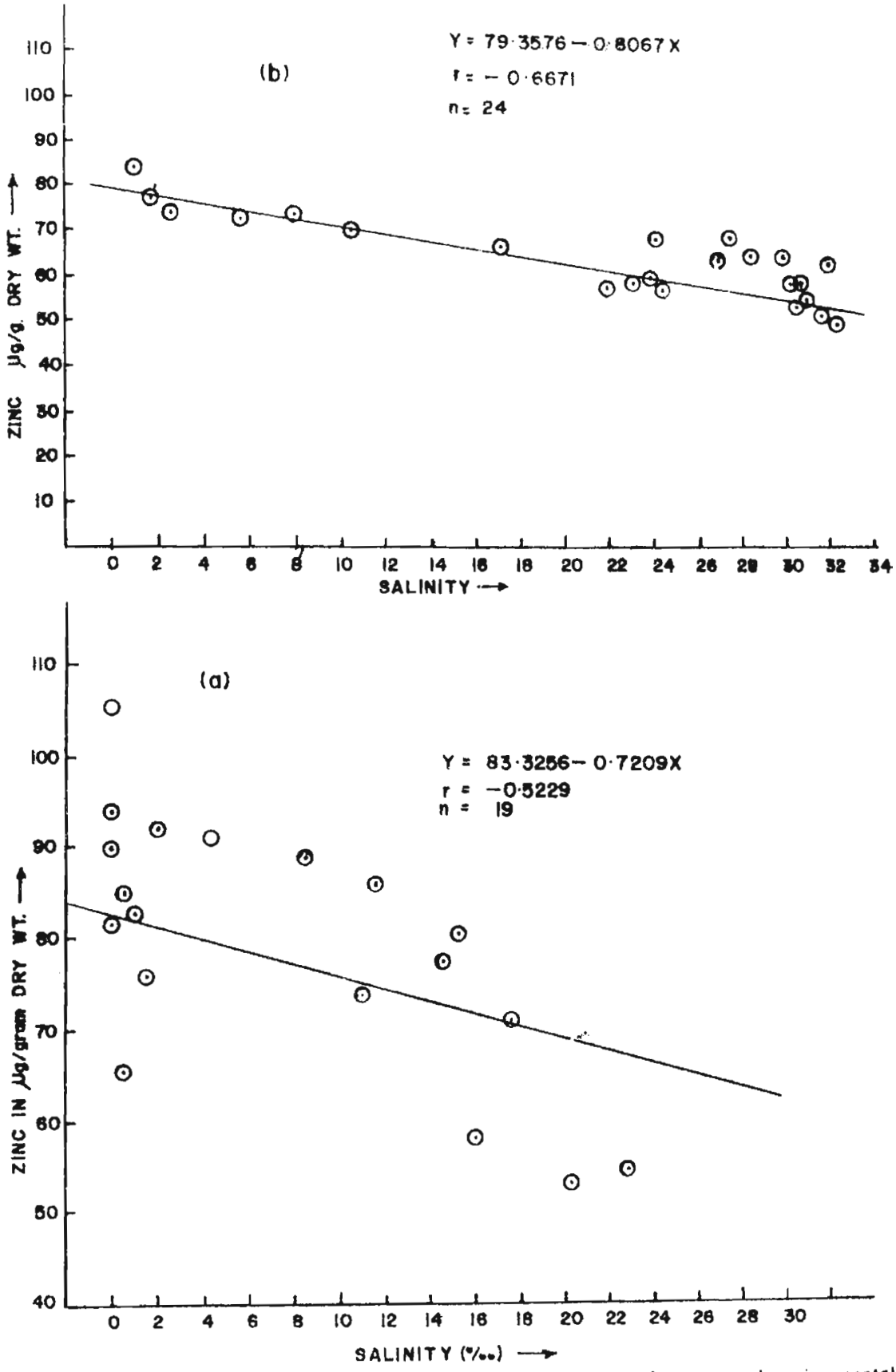


Fig. 4.6 a & b. Relation between zinc contents in the whole soft parts and environmental water salinity of (a) V. cyprinoides and (b) M. casta.

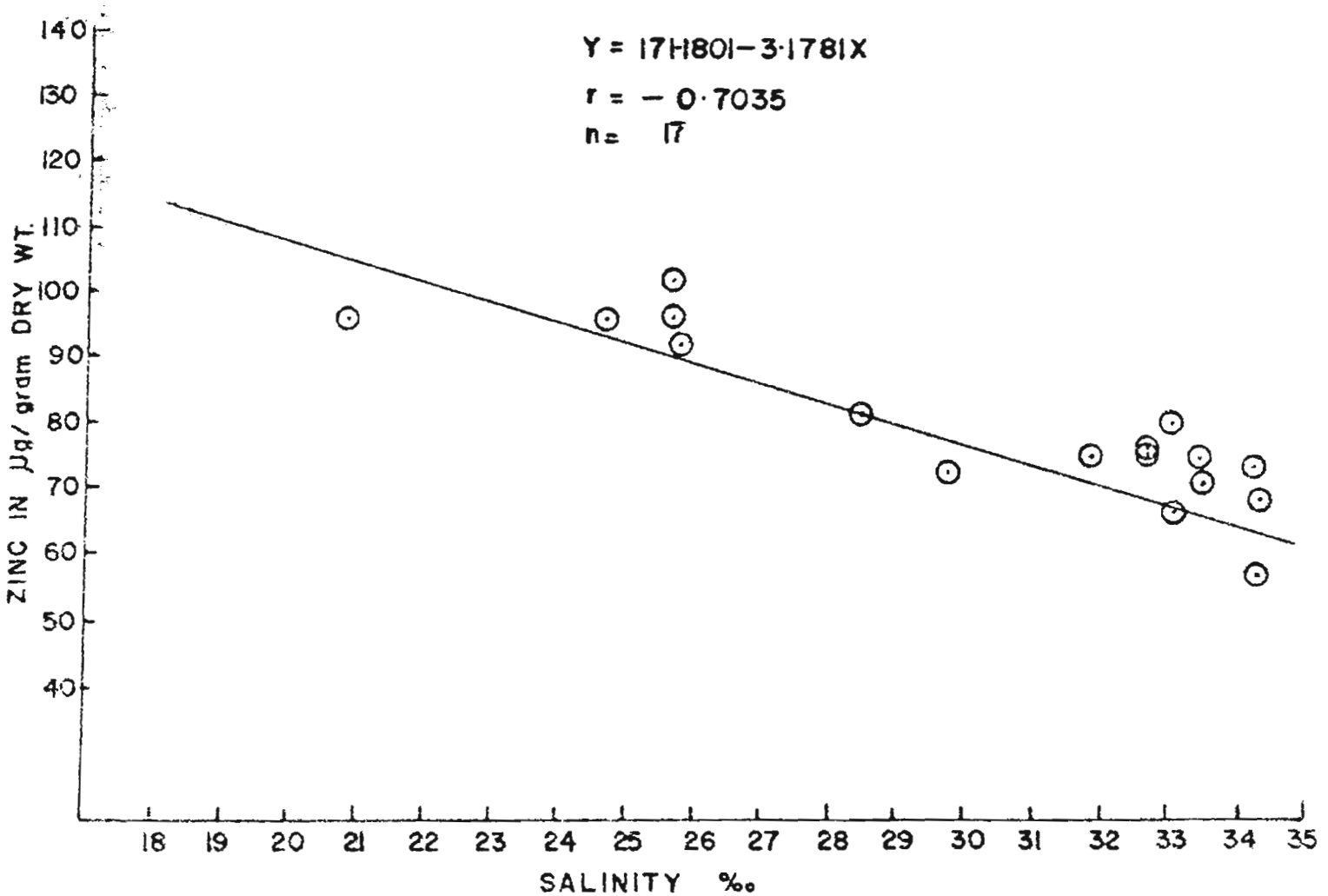


Fig. 4.6.c. Relationship between zinc content in the whole soft parts of P. viridis and environmental water salinity.

$$Y = 83.3256 - 0.7209X \quad \ldots \quad 4.4$$

$$Y = 79.3576 - 0.8067X \quad \ldots \quad 4.5$$

$$\text{and } Y = 171.1801 - 3.1781X \quad \ldots \quad 4.6$$

where Y, represents zinc content in the animals and X, represents salinity of water.

c) Iron:- Of all the trace metals iron can be considered as the major constituent in the three organisms. In V. cyprinoides it showed a marked seasonal variation, higher values being found during the monsoon season and lower values during pre-monsoon periods (Table 4.3a). The iron content in V. cyprinoides varied from 200.59 µg/g to 665.41 µg/g (dry wt.); the highest value was found during July 1978 (S = 0‰) and the lowest value was observed in March 1978 (S = 17.63‰). However, the seasonal variations of iron content in V. cyprinoides followed a similar pattern during 1976-77 and 1977-78. The Fe content in M. costa was generally low in comparison to its values in the other two species; the average value was found to be 250.19 µg/g. The distribution and the nature of seasonal variations could be seen from the table (4.3b) and figure (4.3b) The range of values falls in between 181.22 µg/g and 338.84 µg/g The highest value was recorded in July 1978 (S = 1‰) and the lowest concentration in April 1978 (S = 23.19‰). The variation of iron content in M. costa is not as regular as observed in the case of V. cyprinoides.

Table 4.3 a

Seasonal variations of iron in the clam, Villorita cyprinoides var. cochinensis. (Mean concentrations of the metal in the whole soft parts and standard deviation, on a dry weight basis).

Date of collection	Habitat salinity (‰)	pH of water	Iron µg/g dry wt.	± S.D.
2.9.1976	0.00	6.30	459.43	14.16
1.10.'76	1.53	6.60	396.26	4.92
1.11.'76	1.00	6.40	377.12	9.89
1.12.'76	0.50	6.80	307.71	3.18
2.1.1977	15.21	7.80	466.96	4.48
1.2.'77	22.81	8.00	293.97	3.20
6.4.'77	20.30	7.60	250.51	2.73
2.5.'77	8.35	7.00	376.77	14.57
1.6.'77	4.34	6.80	488.52	7.48
13.7.'77	0.00	6.90	534.19	7.50
2.8.'77	0.00	6.90	584.60	9.08
2.12.'77	1.93	6.20	401.20	8.74
5.1.'78	11.67	6.80	474.05	8.08
9.2.'78	14.52	7.05	241.19	6.22
6.3.'78	17.63	8.10	200.59	5.52
10.4.'78	16.00	7.25	359.15	4.23
10.5.'78	11.13	8.65	367.93	6.88
8.6.'78	0.54	6.20	439.50	7.89
7.7.'78	0.00	6.20	665.41	7.78
Mean value			399.16	
			±116.55	

Table 4.3 b

Seasonal variation of iron, in the clam M. Canta (Chemnitz). (Mean concentrations of the metal in the whole soft parts and standard deviation, on a dry weight basis).

Date of collection	Habitat salinity (‰)	pH of water	Iron µg/g dry wt.	± S.D.
2.9.1976	27.12	7.80	279.90	4.97
1.10.'76	31.73	8.15	239.95	4.81
1.11.'76	28.46	8.00	263.95	8.02
2.12.'76	30.70	8.10	238.91	5.81
2.1.1977	31.92	8.20	217.50	5.32
1.2.'77	32.40	8.10	250.97	4.75
6.4.'77	24.00	7.80	262.15	5.67
2.5.'77	24.16	8.00	222.48	5.38
1.6.'77	17.25	7.80	247.40	5.92
13.7.'77	10.50	7.20	252.00	6.62
2.8.'77	5.72	7.00	248.35	6.08
2.12.'77	24.54	7.50	257.49	6.60
5.1.1978	30.62	7.80	196.22	5.02
9.2.'78	27.65	7.80	266.40	5.42
6.3.'78	30.50	8.25	198.17	5.78
10.4.'78	23.19	7.60	181.22	5.20
10.5.'78	22.11	8.00	245.60	7.86
8.6.'78	1.76	6.10	272.60	6.27
7.7.'78	1.00	6.35	338.82	5.23
4.8.'78	2.34	6.40	323.64	7.79
Mean value =			250.18	
			±37.25	

Table 4.3 c.

Seasonal variation of iron, in the mussel, Perna viridis (Linnaeus). (Mean concentration of the metal in the whole of soft parts and standard deviations, on a dry weight basis).

Date of collection	Habitat salinity (‰)	pH of water	Iron µg/g dry wt.	± S.D.
1.[illegible].1977	34.47	8.20	328.35	6.78
1.3.'77	34.40	8.15	225.81	5.78
2.4.'77	28.62	7.82	281.31	7.66
2.5.'77	29.86	7.80	271.32	6.51
1.6.'77	24.75	7.25	N.D.	
12.7.'77	20.90	7.15	939.94	9.01
9.2.'78	32.82	7.80	203.33	5.23
13.3.'78	33.60	8.20	238.20	6.84
10.4.'78	33.21	8.20	220.40	8.39
8.5.'78	34.42	8.30	385.22	7.70
7.7.'78	32.79	6.65	940.16	10.95
7.8.'78	25.75	6.60	226.80	4.60
Mean value =			374.57	
			±257.65	

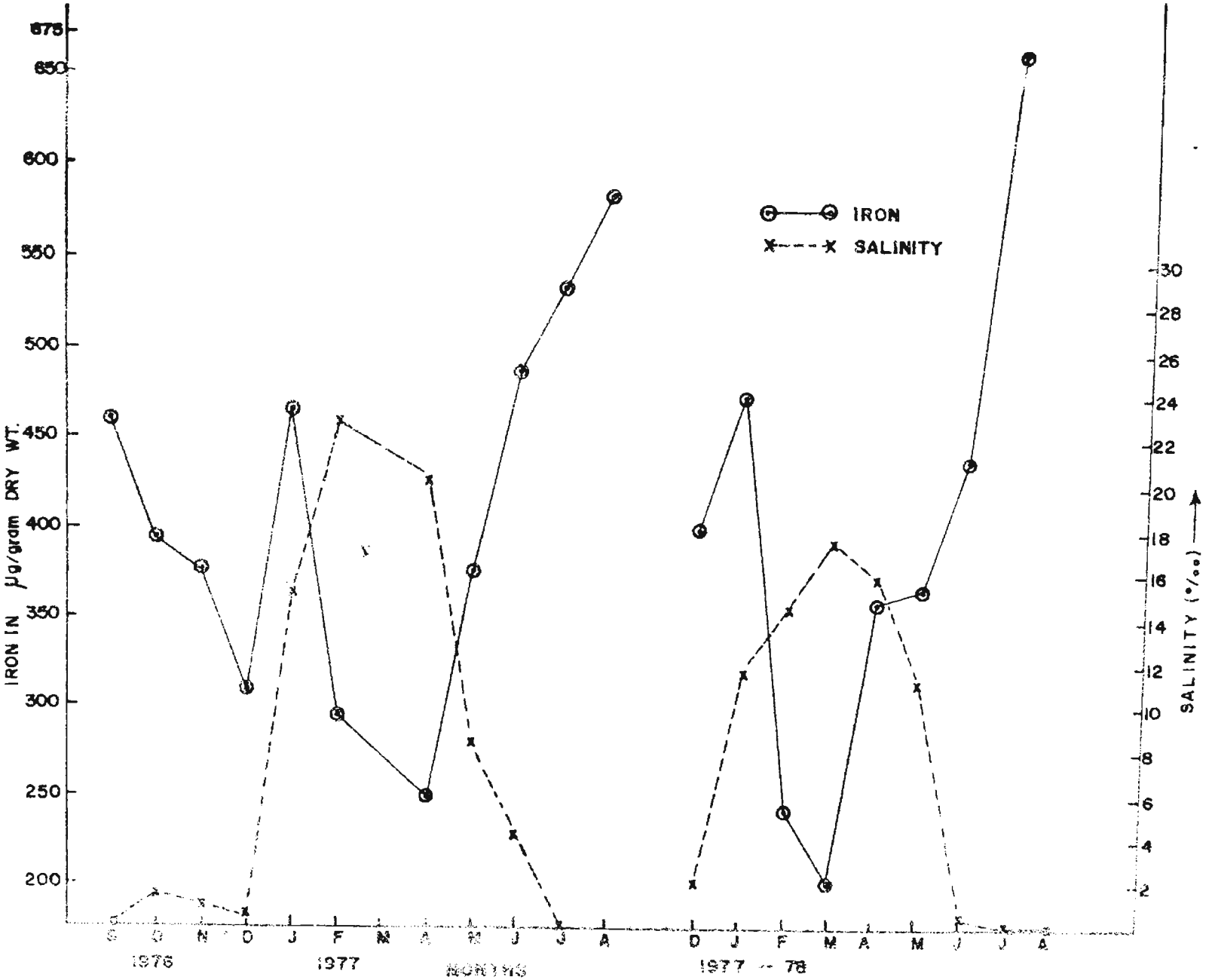


Fig. 4.3a Seasonal variation of iron content in the whole soft parts of *Villorita cyprinoides* in relation to environmental water salinity

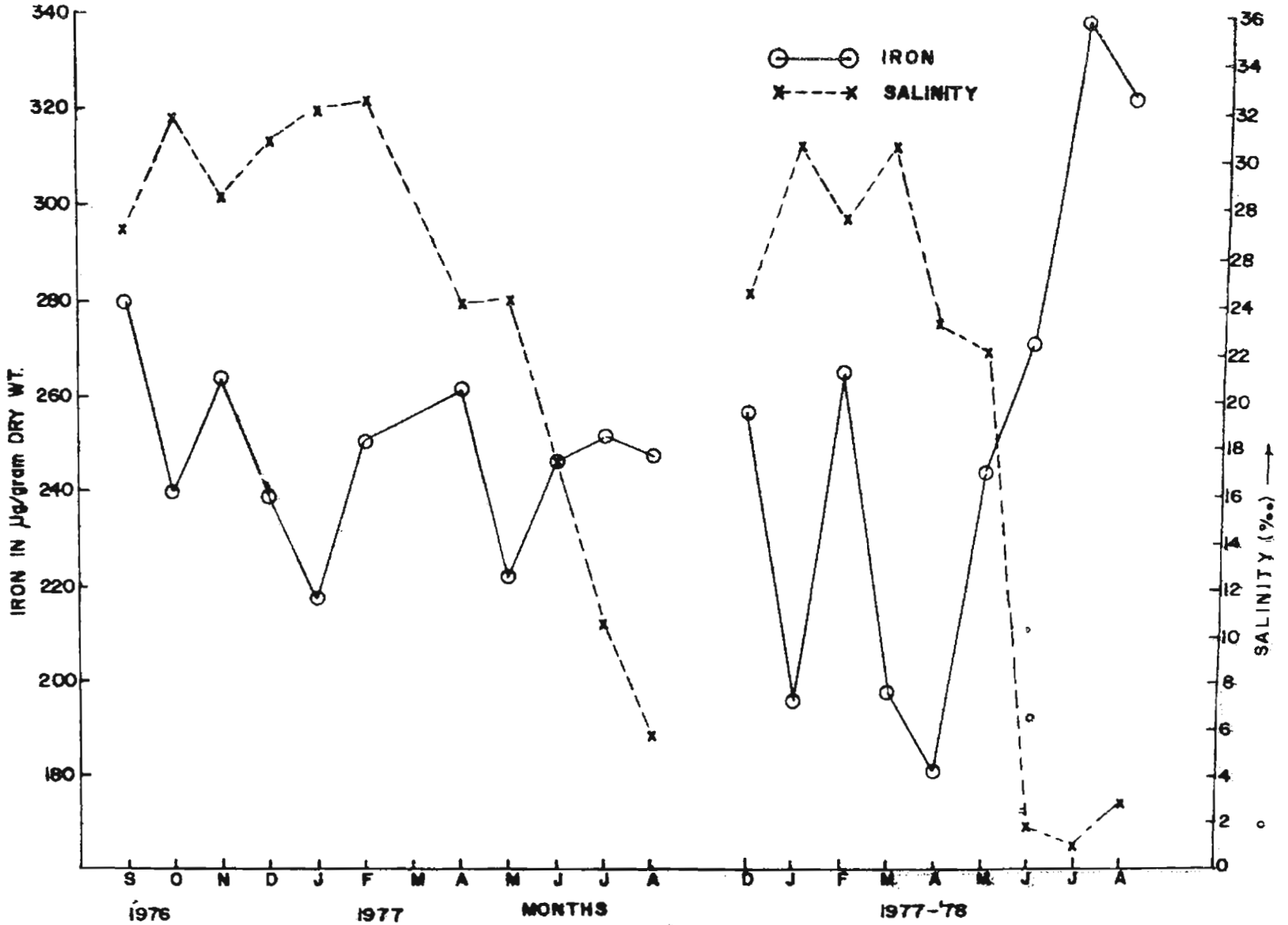


Fig. 4.3 b. Seasonal variation of iron content in the whole soft parts of Meretrix casta in relation to

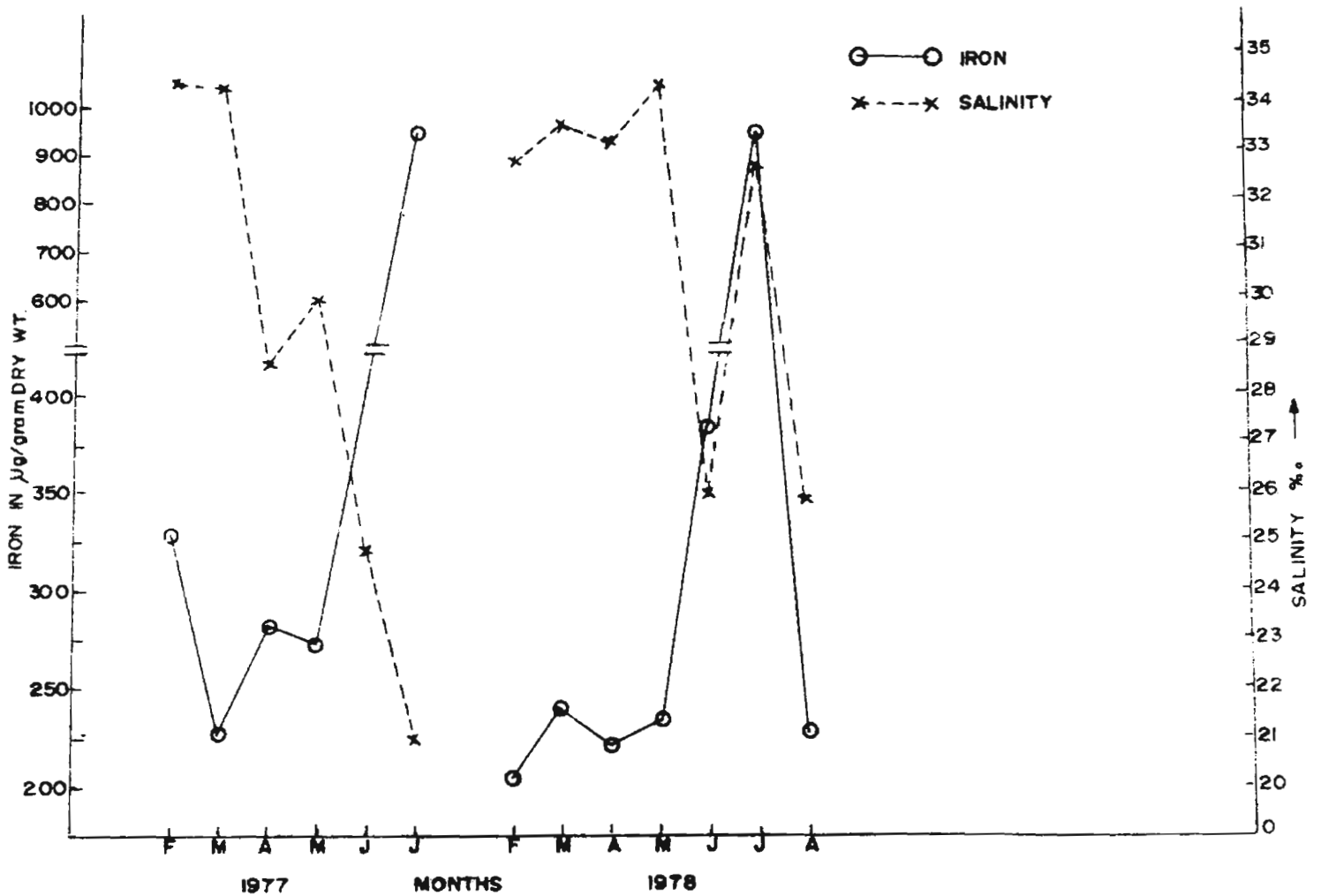


Fig. 4.3c. Seasonal variation of iron content in the whole soft parts of Perna viridis in relation to environmental water salinity.

The level of iron content was found to be rather high in P. viridis with a maximum value of 940.16 µg/g in July 1978. The higher values were found during the monsoon periods. However, it was observed that the seasonal variation of Fe in P. viridis was rather irregular. The lowest concentration of iron (203.30 µg/g) was found in February 1978.

There exists significant negative correlation between salinity and Fe content in the whole soft tissues of V. cyprinoides and M. casta, the correlation coefficient (r) and (P) values being

$$r = -0.6359 \quad (P < 0.01) \text{ in } V. \text{ cyprinoides}$$

and $r = -0.5796$ $(P < 0.01)$ in M. casta.

However, in P. viridis no significant correlation was found between these two parameters ($r = -0.3659$, P - not significant). The respective regression equations for V. cyprinoides and M. casta are given below: (See also Fig. 4.7a to 4.7b).

$$Y = 477.94 - 9.4728X \quad \ldots \quad 4.7$$

$$Y = 290.91 - 1.9427X \quad \ldots \quad 4.8$$

where Y represents Fe content and X represents the habitat salinity.

d) Lead:- The seasonal variations and the concentration ranges of lead content in the three bivalve molluscs are given in Tables (4.4a to 4.4c)and figures (4.4a to 4.4c).The lead conten

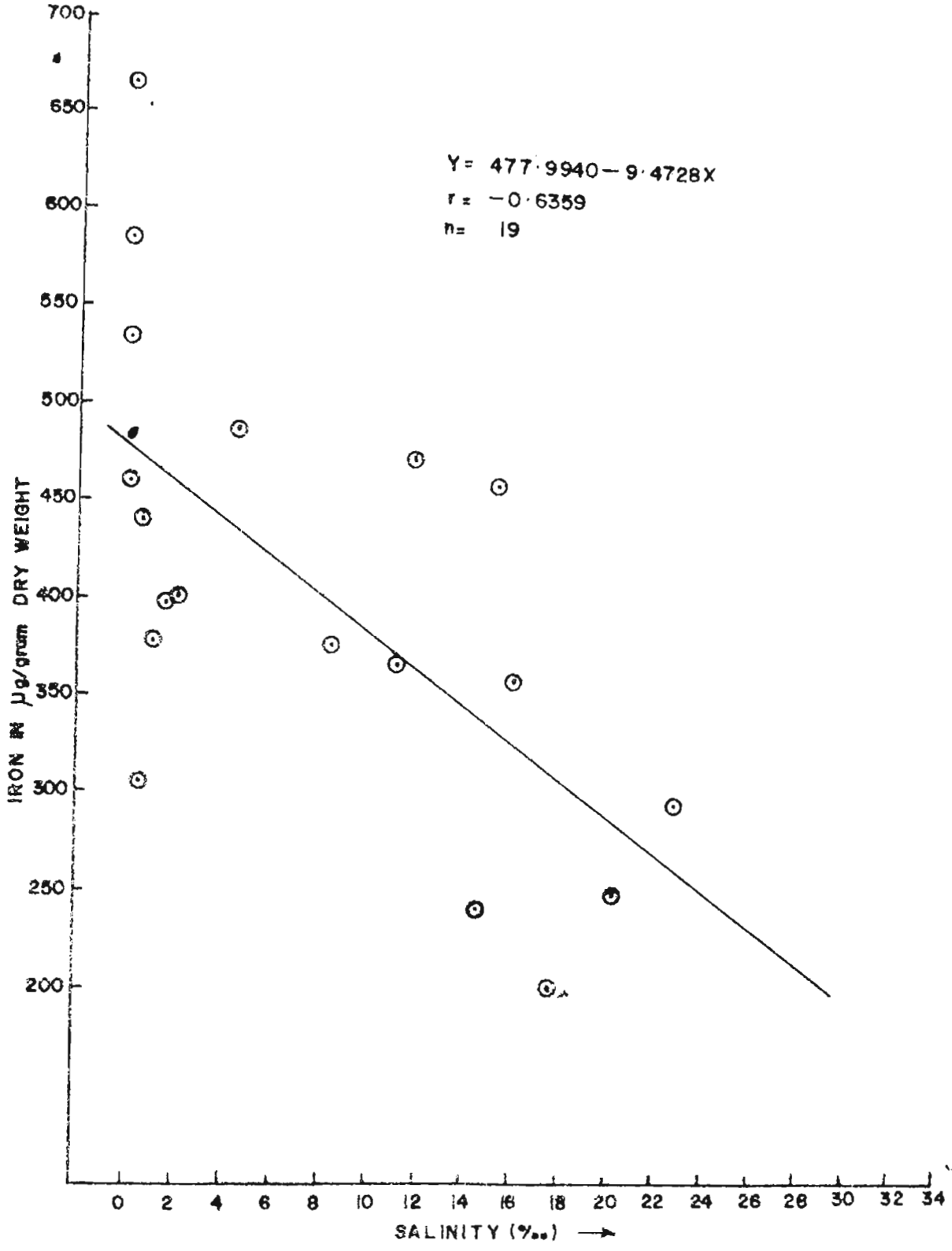


Fig. 4.7a. Relationship between iron content in the whole soft parts of Villorita cyprinoids and environmental water salinity.

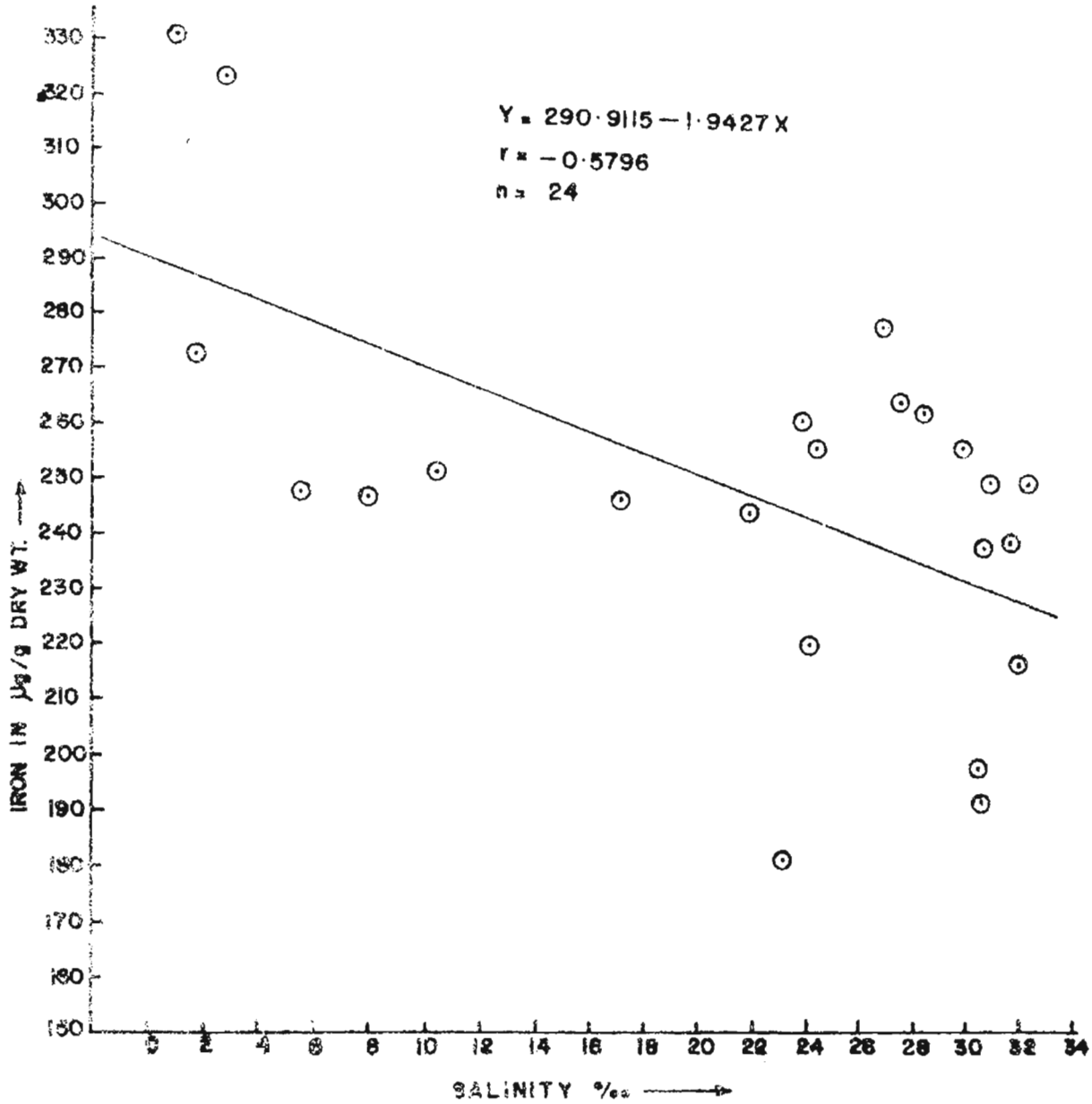


Fig. 4.7b. Relationship between iron content in the whole soft parts of **Meretrix casta** and environmental water salinity.

was quite low in all the species. In V. cyprinoides, the distribution and seasonal variation of Pb followed almost an identical pattern with that observed for Zn. Higher values were observed when the salinity was low (eg. 10.56 µg/g in July 1978, S = 0‰), while lower values corresponded with higher salinity (eg. 6.24 µg/g in February 1977, S = 22.81‰).

Lead was found in higher amounts in M. casta, the maximum being 23.46 µg/g in July 1978 (S = 1‰). The lowest value recorded 6.73 µg/g) was in the month of January, 1977 (S = 31.92‰).

In P. viridis, the lead content was rather low. The values ranged from 5.23 µg/g to 9.85 µg/g (dry wt.). The pattern followed in its seasonal variation could be seen from the table (4.4c) and figure (4.4c). Lead content in the two clams, V. cyprinoides and M. casta was found to be significant negatively correlated with salinity of the habitat water. However, there was no significant correlation between these tw parameters in the case of P. viridis.

The correlation coefficient (r) and (P) values for V. cyprinoides and M. casta are

$$r = -0.7452 \quad (P < 0.001)$$

and $r = -0.9533$ ($P < 0.001$) respectively. The corresponding regression lines are given below:

Table 4.4 a

Seasonal variation of lead content in the clam, Villorita cyprinoides var. cochinensis. (Mean concentrations of the metal in the whole soft parts and standard deviations, on a dry weight basis).

Date of collection	Habitat salinity (‰)	pH of water	Lead µg/g dry wt.	± S.D.
2.9.1976	0.00	6.30	9.23	0.35
1.10.'76	1.53	6.60	8.67	0.26
1.11.'76	1.00	6.40	7.60	0.31
1.12.'76	0.50	6.80	8.82	0.47
2.1.1977	15.21	7.80	8.52	0.34
1.2.'77	22.81	8.00	6.24	0.21
6.4.'77	20.30	7.60	6.59	0.34
2.5.'77	8.35	7.00	8.32	0.30
1.6.'77	4.34	6.80	N.D.	-
13.7.'77	0.00	6.90	9.42	0.33
2.8.'77	0.00	6.90	N.D.	-
2.12.'77	1.93	6.20	9.58	0.35
5.1.1978	11.67	6.80	8.44	0.24
9.2.'78	14.52	7.05	8.68	0.29
6.3.'78	17.63	8.10	7.76	0.31
10.4.'78	16.00	7.25	6.70	0.30
10.5.'78	11.13	8.65	9.00	0.38
8.6.'78	0.54	6.20	8.61	0.34
7.7.'78	0.00	6.20	10.06	0.49
Mean value			8.37	
			±1.04	

Table 4.4. b

Seasonal variation of lead content in the clam, Meretrix casta (Chemnitz). (Mean concentrations of the metal in the whole soft parts and standard deviations, on a dry weight basis).

Date of collection	Habitat salinity (‰)	pH of water	Lead µg/g dry wt.	± S.D.
2.9.1976	27.12	7.80	11.20	0.51
1.10.'76	31.73	8.15	10.08	0.49
1.11.'76	28.46	8.00	10.94	0.37
2.12.'76	30.70	8.10	6.83	0.31
2.1.1977	31.92	8.20	6.73	0.34
1.2.'77	32.40	8.10	N.D.	-
6.4.'77	24.00	7.80	10.67	0.45
2.5.'77	24.16	8.00	11.05	0.47
1.6.'77	17.25	7.80	11.70	0.51
13.7.'77	10.50	7.20	16.49	0.89
2.8.'77	5.72	7.00	17.36	0.95
2.12.'77	24.54	7.50	12.57	0.75
5.1.1978	30.62	7.80	9.41	0.35
9.2.'78	27.65	7.80	10.33	0.60
6.3.'78	30.50	8.25	9.21	0.39
10.4.'78	23.19	7.60	11.78	0.61
10.5.'78	22.11	8.00	N.D.	-
8.6.'78	1.76	6.10	22.56	1.28
7.7.'78	1.00	6.35	23.46	1.10
4.8.'78	2.34	6.40	N.D.	-
Mean value =			12.49	
			±4.65	

Table 4.4 c

Seasonal variation of lead content in the mussel, Perna viridis (Linnaeus). (Mean concentrations of the metal in the whole soft parts and standard deviation, on a dry weight basis).

Date of collection	Habitat salinity (°)	pH of water	Lead µg/g dry wt.	± S.D.
1.2.1977	34.47	8.20	7.60	0.31
1.3.'77	34.40	8.15	7.31	0.30
2.4.'77	28.62	7.82	8.03	0.40
2.5.'77	29.86	7.80	7.78	0.39
1.6.'77	24.75	7.25	N.D.	-
12.7.'77	20.90	7.15	7.91	0.41
9.2.1978	32.82	7.80	7.85	0.44
13.3.'78	33.60	8.20	5.26	0.18
10.4.'78	33.21	8.20	N.D.	-
8.5.'78	34.42	8.30	5.23	0.22
8.6.'78	25.90	7.20	8.05	0.36
7.7.'78	32.79	6.65	8.61	0.42
7.8.'78	25.75	6.60	9.85	0.48
Mean value =			7.59	
			±1.27	

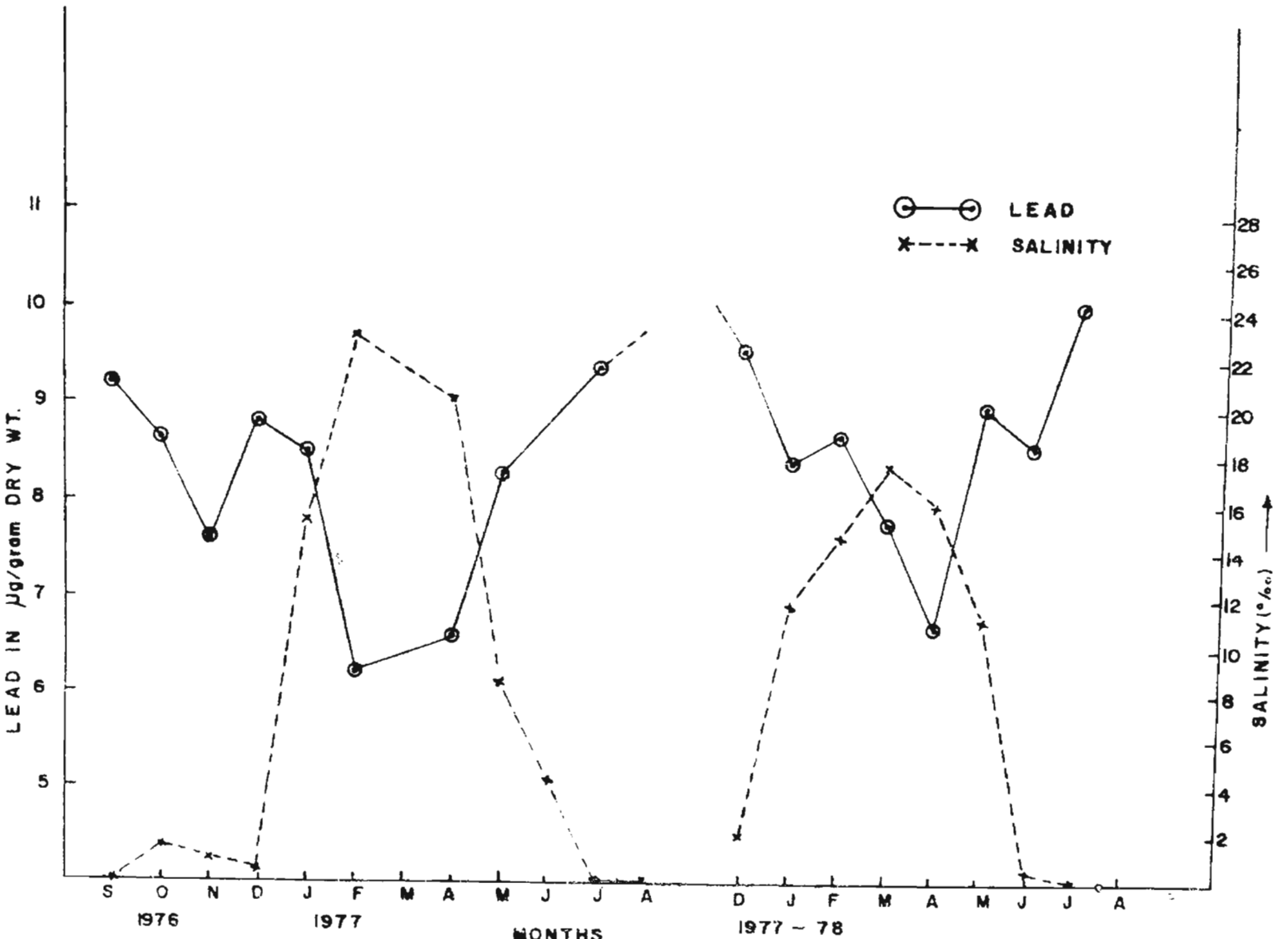


Fig. 4. 4a. Seasonal variation of lead content in the whole soft parts of Villorita cyprinoides in relation to environmental water salinity.

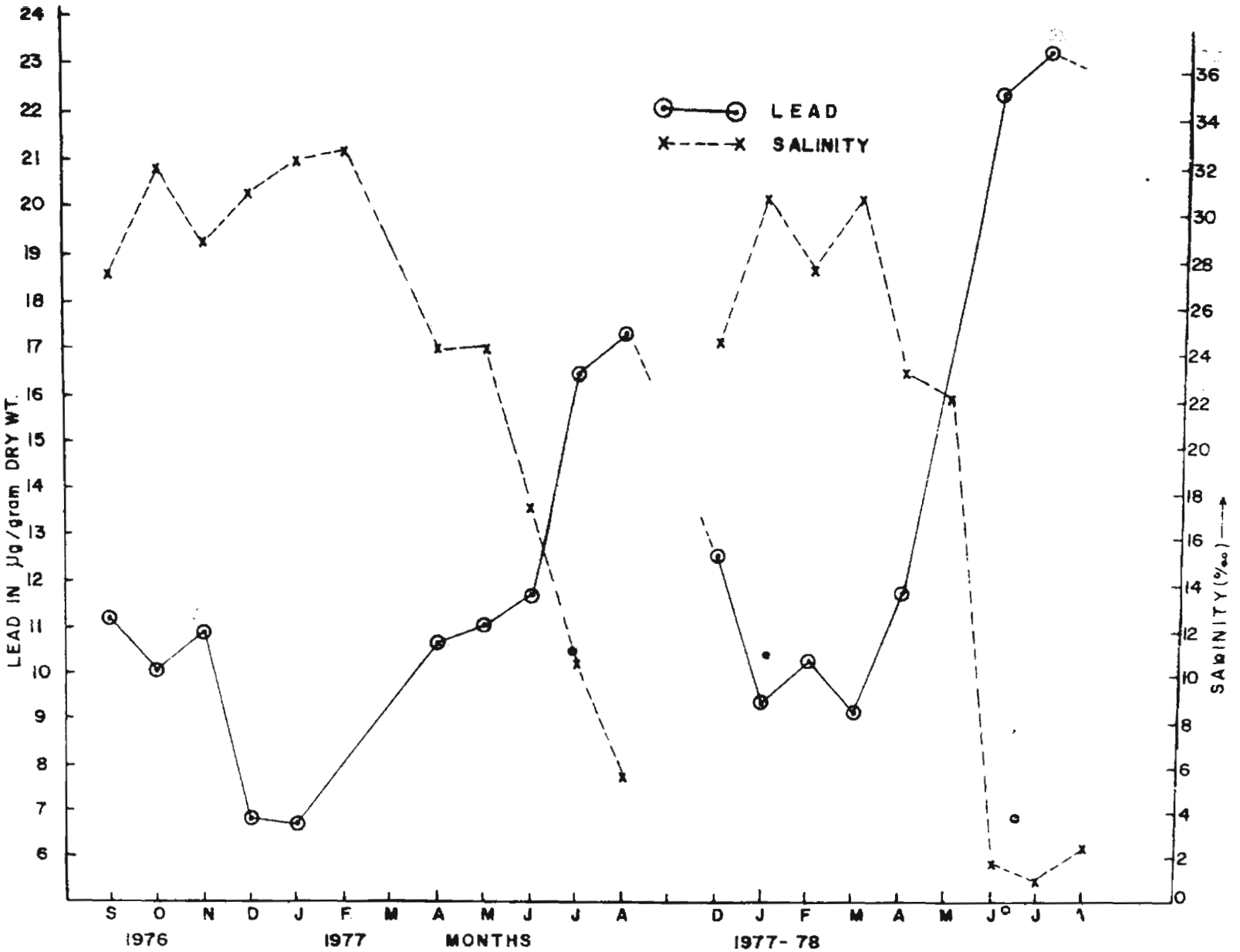


Fig. 4. 4b. Seasonal variation of lead content in the whole soft parts of Meretrix casta in relation to environmental water salinity.

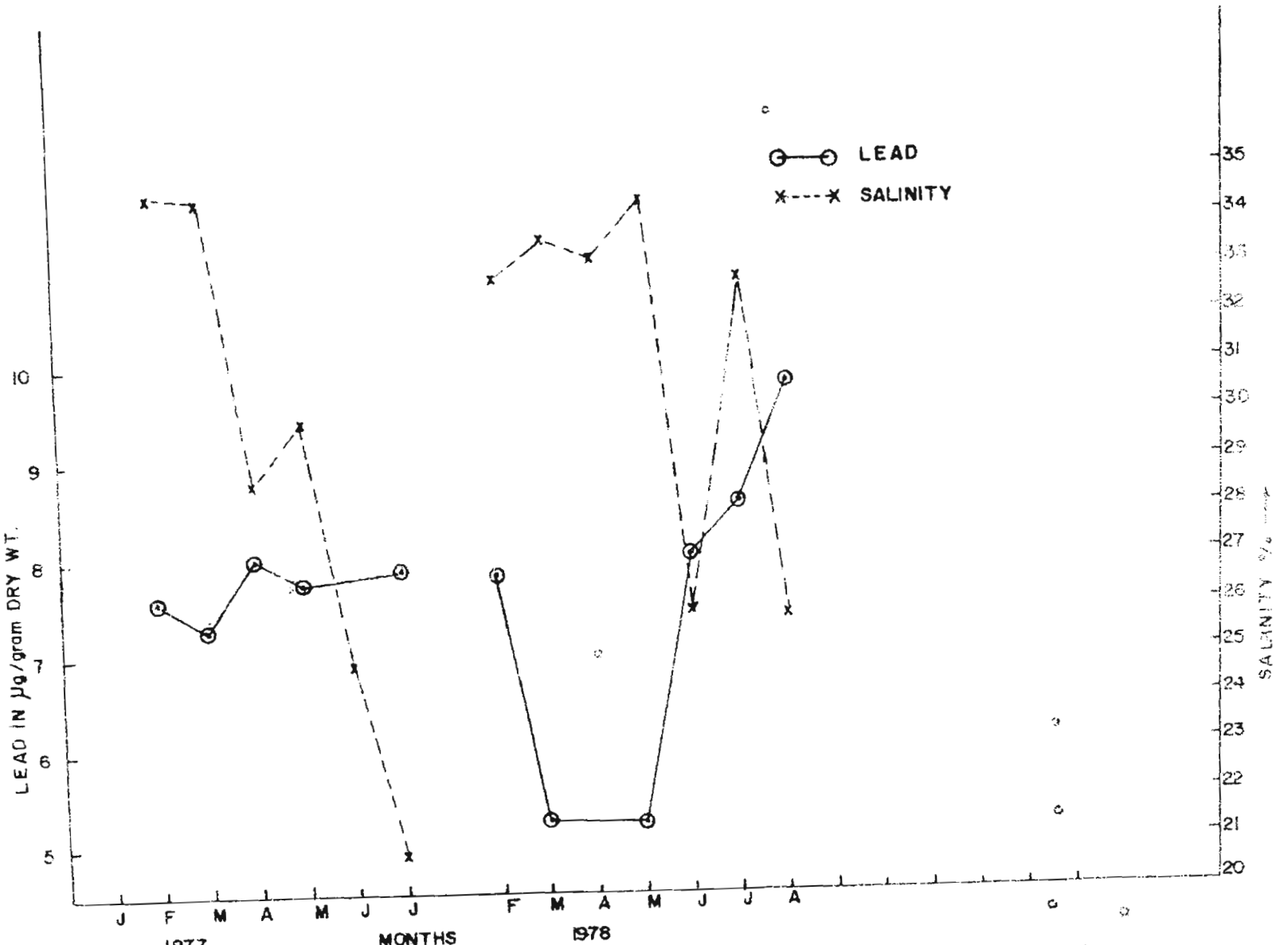


Fig. 4.4c Seasonal variation of lead content in the whole soft parts of Perna viridis in relation to environmental water salinity.

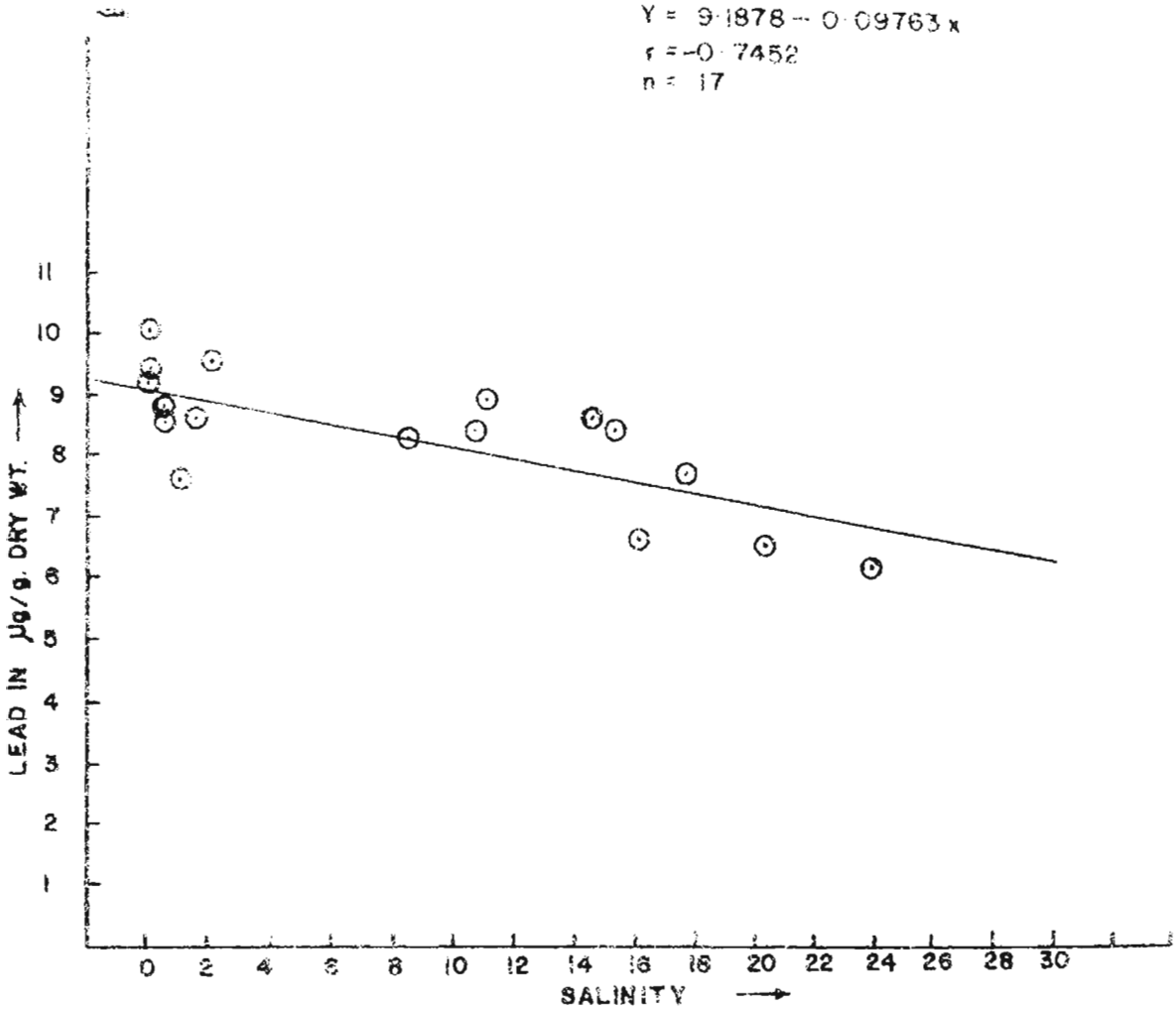


Fig. 4.8 a. Relationship between lead content in the whole soft parts of *V. cyprinoides* and environmental water salinity.

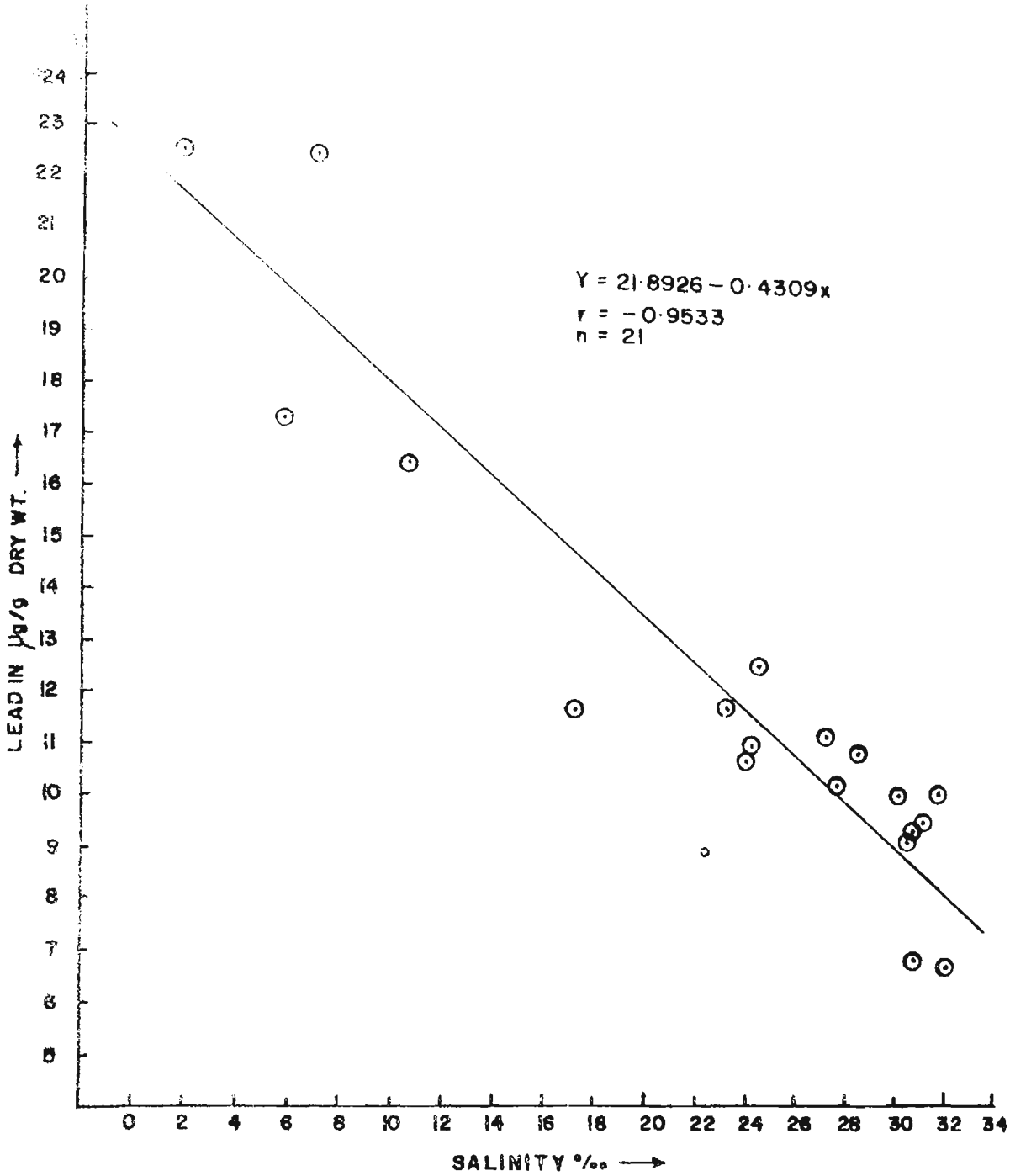


Fig 4.8 b Relationship between lead content in the whole soft parts of M. casta and environmental water salinity.

$$Y = 9.1878 - 0.09763X \quad \ldots \quad 4.9$$

$$\text{and } Y = 21.8926 - 0.4309X \quad \ldots \quad 4.10$$

where Y = lead content and X = salinity.

Although the highest and lowest values of the three metals in the three molluscs were not observed at the same time of the year, it is generally seen that the higher value were found during monsoon and post monsoon periods (periods of low salinity) whereas low values were found in summer months when the salinity and pH of the water went up.

Nearly all the four metals were found to be positive inter correlated in the two clams, V. cyprinoides and M. cas ($P < 0.001$ to $P < 0.01$) in most cases), the only exception b Cu/Pb combination in V. cyprinoides. The correlation coeffi cents were calculated in each case and are given in Table 4.5a to c). However, in P. viridis only a few combinations (Cu/Z Cu/Pb and Zn/Pb) were found to be significantly correlated ($P < 0.05$).

4.3. Discussion

The concentrations of the four trace metals studied, viz. Cu, Zn, Fe and Pb varied markedly with season in all th three species of mol uscs. The variation in concentrations showed a definite pattern. In general, high concentrations metals were confined to low saline periods, i.e. from June t

Table 4.5 a

Correlation coefficients(r) of salinity and trace metal concentrations in *V. cyprinoides*.

	S‰	Cu	Zn	Pb	Fe
Cu	-0.8744	-	-	-	-
Zn	-0.5229	0.5716	-	-	-
Pb	-0.6873	0.4104	0.7513	-	-
Fe	-0.6359	0.5618	0.6084	0.6298	-

Table 4.5 b

Correlation coefficients(r) of salinity and trace metal concentrations in *M. costa*.

	S‰	Cu	Zn	Pb	Fe
Cu	-0.9063	-	-	-	-
Zn	-0.6671	0.8130	-	-	-
Pb	-0.9465	0.9062	0.7990	-	-
Fe	-0.5796	0.6225	0.6319	0.6264	-

Table 4.5 c

Correlation coefficients(r) of salinity and trace metal concentrations in *P. viridis*.

	S‰	Cu	Zn	Pb	Fe
Cu	-0.8548	-	-	-	-
Zn	-0.7035	0.7068	-	-	-
Pb	-0.4290	0.6771	0.6387	-	-
Fe	-0.3859	0.5175	0.2864	0.2744	-

August (Tables 4.1a to 4.4c). This is the period of southwe monsoon, when the salinity and pH of the habitat water recor low values. In V. cyprinoides, yet another period of hi h metal content was found durin September to December and in January (Table 4.1a, 4.2a, 4.3a and 4.4a) the period of less fierce winter monsoon (northeast). On the otherhand metal concentration decreased in all the three species durin summ months, the period of highest salinity and pH values for the ambient water. The significant negative correlation between metal concentrations in the whole soft parts of the molluscs and salinity of the habitat water (except for Pb in P. virid suggests that salinity does play an important role.

The monsoon floods cause drastic seasonal changes in the salinity and pH of the Cochin backwaters. This must hav definite effect on the distribution, species variation etc. the heavy metal ions present in the water or sediment system This process influences the availabi ity of metal ions to th biological species. So to interpret the experimental result the chemical species of the metals in the aquatic environmen should be considered in detail. The uptake of metals by mar organisms is highly dependent on the chemical species presen in the ambient water. The variation in salinity may also al the filtration rates in these molluscs.

tated in the estuary due to sudden increase in salinity of the water. Similar iron precipitation has been reported by Subramanian et al. (1979) in Vellar estuary.

The effect of pH upon the stability, solubility and absorption of 15 metal ions at low concentrations in aquous solutions has been studied by Smith (1973) under laboratory conditions; it is shown that pH of 1.5 or less was necessary to ensure that all of the metal ions remained in solution. There is considerable change in metal species in the pH range of 6.5 to 8.0.

The seasonal variation of the levels of Cu, Zn, Fe and Pb in the three bivalve molluscs could be explained in the light of the above facts.

Thus, high concentrations of the metals observed during June to August in all the three species of the bivalves may also be due to an increase in the filtration rate and bio-availability of the metals in their habitat water. As a result of the monsoon rains, the backwater gets flushed with fresh water discharge which brings down the salinity and pH of the water. In the case of V. cyprinoides, the values for salinity and pH of the environmental water at the two periods of high metal content in the body of the mollusc were in the range of; S = 0‰ to 4.34‰ and pH = 6.2 to 6.8. In M. casta the lowest values for these two parameters were, S = 100‰ and pH = 6.1 and in P. viridis the

corresponding lowest values were, S = 20.9‰ and pH = 6.6 respectively, when the metal content was highest in their body. These low pH values (a consequence of low salinity) may facilitate the dissolution of precipitated form of the metal (hydroxide, carbonate or chloro complexes) and increase the amount of ionic species in solution. This coupled with the increased rate of filtration would naturally increase the level of metals in the organisms.

It is also possible that the freshwater discharge might bring effluents and other waste material in to the estuary. The main flow of fresh water in this estuary is through Periyar river. Since the river gets effluents and other waste materials from an industrial area near Eloor where a number of chemical factories including one copper sulphate factory and zinc factory are located, it also may contribute to the heavy metals in the water. This may be another reason for the increase in concentration of the heavy metals in the molluscs during monsoon periods.

During October to December (winter monsoon) metal levels were high in Villorita cyprinoides, but there was only little increase in the other two species. Since, the winter monsoon is less severe, it did not influence much the water salinity or pH in the latter two cases (Tables 3.1b to 3.1c).

owing to their proximity to the sea. On the otherhand the Villorita bed was very much affected by the fresh water discharge during the winter monsoon also. And hence, the variation of the metals in this species.

Generally low concentration of the metals in the animals during summer months may be due to the low bioavailability of the metal ions in the water owing to the high salinity and pH.

Secondly the incorporation of metals by phytoplankton or detritus and chelation by other extracellular products might reduce the metal availability to these organisms. The significant negative correlation between metal content in the animal soft parts and salinity of the environmental water (in most cases) is consistent with the above reasoning.

Bryan and Hystal (1978) suggested that salinity was an important factor governing the availability of metals to the organisms. Thus they found higher levels of Fe and Zn in the bivalve, Sacrobicularia plana at upstreams of the Tamar Estuary than at downstreams. The levels were highest during early part of the year when less saline conditions existed. Mohammed Salih (1977) has also observed high values for Fe and Cu in M. casta during monsoon periods and low values in the summer months.

Sankaranarayanan and Reddy (1973) observed high values for Cu, in estuarine water during the monsoon periods when the fresh water discharge was maximum. A significant negative correlation was obtained between Cu concentration and salinity in Porto Novo waters (Sunderraj and Krishnamoorthy, 1972). Subramanian et al. (1979) found that reactive iron concentration was maximum in low saline waters and decreased with increasing salinity.

Bryan (1973) had observed low metal concentrations in the scallop during the period of highest productivity. He explained that the very much greater availability of food probably caused increased metabolic rates of the animals and the excretion of waste products. He also remarked that the incorporation of metals by the phytoplankton would reduce the amount of dissolved ions in the water and also that the extra cellular products might chelate metals in the water leaving them unavailable for absorption by other organisms. ~~Frank~~ Slowey et al. (1972) also got evidence for copper-complexation with organic compounds in seawater. The above observations of the various workers are in agreement with the present findings.

The levels of copper and zinc in the three bivalve molluscs observed in the present study are comparable in magnitude to the concentrations reported by Zingde et al. (1976) in some

bivalve molluscs from the estuaries of Goa. However, high concentrations of Zn, Cu and Fe were observed in the oyster, Crassostrea madrasensis collected from the Cochin backwaters (Sankaranarayanan et al., 1978). In the present study no such high value was observed in the molluscs.

Iron was by far the most abundant of the trace metals in all the three species. High levels of Fe content in molluscan body had been reported by many workers (Brookes and Rumsby, 1965; Bryan, 1973).

The concentration levels of the various metals studied (including that of mercury - control values from Chapter 7), in the three bivalve molluscs may be compared with the permissible levels in some of the marine products* (IS: 7582-1975, IS:2236-1968 and Connell, 1980). The average values as well as

Element	Permitted level (ppm)	Metal levels in the molluscs (ppm) Highest concentration and mean value given in brackets. V. cyprinoides	M. costa	P. viridis
†Mercury	0.50-1.00	(0.232)	(0.195)	(0.086)
Lead	5.00	2.327 (2.045)	4.912 (2.892)	1.955 (1.739)
Copper	10.00	9.268 (7.921)	9.761 (6.538)	6.379 (5.101)
Zinc	50.00	25.200 (9.387)	17.450 (14.840)	20.021 (18.142)

*No IS specifications for metals in molluscs are available.

†The food regulatory and health authorities of some countries

CHAPTER 5

STUDIES ON THE TOXIC EFFECTS OF HEAVY METALS

PART I. DETERMINATION OF LC_{50} VALUES.

CHAPTER 5

STUDIES ON THE TOXIC EFFECTS OF HEAVY METALS

PART I DETERMINATION OF LC_{50} VALUES

Coastal aquaculture has been realised as a potential means of increasing the country's fish/shellfish production. A clean coastal water free from all kinds of pollutants is highly essential for culture activities. Heavy metals have been recognised as serious pollutants of the aquatic environment with deleterious effects on associated organisms. It may cause (i) high mortality (ii) reduced growth rate (iii) reduced quality and export value of the product. So broad suggestions have been made for world wide monitoring of the coastal marine environment for heavy metals using sedimentary organisms like the molluscs.

As mentioned earlier, bioassay studies play an important role in water quality studies. Lethal concentration (LC) is used to express the results of bioassays having lethality as the criterion of toxicity. A number is used with it to indicate the percentage of the test animals killed at a given time of the test material. The 96 hr LC_{50} is the concentration of a material that is lethal to 50% of the test organisms in 96 hours.

Wisely and Blick (1967) had studied the mortality of the larvae of the mussel, Mytilus edulis and the oyster, Crassostrea commercialis exposed to solutions of mercury, copper and zinc. Pringle et al. (1968) had studied the toxic effect of heavy metal ions on some estuarine molluscs. Portmann (1970) had also conducted acute toxicity tests with the oyster, Ostrea edulis using heavy metals. Arthur and Leonard (1970) had investigated the effects of copper on three invertebrate species in soft water. The toxic effects of 21 metal salts on Daphnia magna were studied by Biesinger and Christensen (1972). The acute toxicity of 11 heavy metals to embryos of the American oyster, Crassostrea virginica was studied by Calabrese et al. (1973). Other works in this line of research are: Lloyd (1960), Skidmore (1964), Portmann (1968), Brown et al (1968), Nielson and Anderson (1970), Bryan (1971), Mackin and Benoit (1971), Brown and Newell (1972), Burton et al. (1972), Scott and Major (1972), Martin (1974), Davies (1976), Reish et a (1976), Davenport (1977), Fisler (1977), Davenport and Wasely (1978), Miller and Mackey (1980) etc.

However, the investigations carried out on these and related aspects in India are scant (Lakshmanan and Krishnan Nambisan, 1977 and 1979; Krishnan Nambisan et al., 1977; Classy D'silva and Kureishy, 1978). The paucity of information in the field prompted to carry out a study on the toxicity of some heav metals viz. mercury, copper, zinc and lead to the bivalve mollus V. cyprinoides, M. casta and P. viridis which are abundantly foun in Cochin backwaters and in various parts of Kerala waters.

5.1. Materials and methods

The general procedure followed in the bioassay tests, the description of the animals and water used in the experiment are given in Chapter 2 (2.8a,b). Preliminary experiments were conducted to select the appropriate test concentrations of various metal salts Hg(II), Cu(II), Zn(II) and Pb(II) to the three animals. Toxicity of heavy metals to the organisms were conducted at their habitat salinity and temperature conditions. The concentrations mentioned are those obtained on addition of calculated amounts of the metal salt solutions to the medium (the background concentration not considered). 10 animals were used in each trough containing 5 litres of filtered sea water. Duplicate troughs were kept at all concentrations including control animals. The troughs were aerated three times a day and dissolved O_2 concentrations were measured both morning and evening. Mortality of the animals were noted at every 24 hr . The results of successive tests were then averaged and the LC_{50} value was determined by the straight line graphical interpolation method. Water was changed daily. The average shell lengths of the various bivalve molluscs used namely, *V. cyprinoides*, *M. casta* and *P. viridis* were in the range of 34-38, 30-32 and 55-60 mm respectively. The water characteristics and the concentrations of the metal used in each case are presented in the

table 5.1. The metals were administered as solutions of their salts viz. $HgCl_2$, $CuSO_4.5H_2O$, $ZnSO_4.7H_2O$, and $Pb(NO_3)_2$ in the experimental tank; all solutions were prepared in doubly distilled water.

5.2. Results

The acute toxicity of four heavy metals, as inorganic salts, to the three bivalve molluscs are shown in Tables 5.2a to 5.4c and the LC_{50} values of these metals to these organisms are presented in Table 5.5. The toxic effects of these metals* to individual organisms are described below.

a) Perna viridis:- Of the four metals tested, mercury and copper were found to be extremely toxic to the mussel, P. viridis at very low concentrations. Mercury above 0.1 ppm in the medium proved to be very toxic to the mussel. Mortality occurred in all the experimental tanks having Hg concentrations of 0.2 ppm and above, during a 96 hr period. 20% of the animals died in 0.2 ppm solution on the fourth day (96hr) of the experiment; other animals in the same tank had become quite inactive. Mercury was 100% lethal to P. viridis at 0.5 ppm in 96 hr period. The rates of mortality can be seen from the Table 5.2a. The following effects were observed within 30 minutes of addition of metal salt solution:-

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*The term 'metal' refers to metal ions, viz. M^{2+} in the text.

Table 5.1

Table showing the number of animals used in each tank, the concentrations of various metals tested and characteristics of water used in LC_{50} value determinations.

Metal ions tested	Name of the animal and number used per tank		Metal concentrations in ppm						Water characteristics: Salinity (S‰)	Oxygen Saturation	Temp. °C	pH
Hg^{2+}	P. viridis	(10)	0.05	0.10	0.20	0.30	0.40	0.50	25.0	95±5	29-30.5	7.5±0.2
	V. cyprinoides	(10)	0.10	0.20	0.50	0.75	1.00	2.00	3.50	95±5	28-30	6.8±0.1
	M. casta	(10)	1.00	2.00	3.00	5.00	-	-	17.0	95±5	29-31	7.4±0.2
Cu^{2+}	P. viridis	(10)	0.05	0.10	0.20	0.50	0.75	-	25.0	95±5	28-31	7.5±0.2
	V. cyprinoides	(10)	0.10	0.50	1.00	2.00	5.00	-	10.0	95±5	28-30	7.1±0.1
	M. casta	(10)	0.25	0.50	1.00	5.00	10.00	-	25.0	95±5	27-30	7.2±0.1
Zn^{2+}	P. viridis	(10)	0.50	1.00	2.00	3.00	5.00	-	25.0	95±5	30-32	7.4±0.1
	V. cyprinoides	(10)	1.00	2.00	5.00	10.0	-	-	3.5	95±5	28-30	6.8±0.1
	M. casta	(10)	1.00	2.00	3.00	5.00	10.0	-	25.0	95±5	29-31	7.2±0.1
Pb^{2+}	P. viridis	(10)	1.00	2.00	3.00	5.00	10.0	-	25.0	95±5	29-31	7.4±0.1
	V. cyprinoides	(10)	1.00	2.00	3.00	5.00	10.0	-	8.0	95±5	29-31	6.8±0.1
	M. casta	10)	1.00	2.00	3.00	5.00	10.0	-	15.0	95±5	28-30	6.9±0.1

(i) In 0.05 and 0.1 ppm Hg solutions there was no mucus secretion and the valve gape was normal.

(ii) At 0.2 ppm there was slight mucus secretion but normal valve gape was maintained.

(iii) Above 0.2 ppm and upto 0.5 ppm, there was considerable amount of mucus secretion and the animals showed a tendency to close the shells.

(iv) Above 0.5 ppm the animals closed their valves completely and a lot of mucus secretion was observed.

It seemed that the amount of mucus exuded by the animal was dependent on the extend of irritation. The LC_{50} value for a 96 hr period was determined by the graphical interpolation method and was found to be 0.34 ppm of Hg at the particular salinity and temperature.

Copper was found to be more toxic to the mussel when compared to mercury. Copper concentrations of 0.1 ppm and above were found to be lethal to the animal. At lower concentrations ($<$ 0.10 ppm) all the animals were found to be quite active, showing normal gape width and did not secrete any

mucus. At concentrations of 0.1 ppm and above all the mussels became very passive and closed their shell valves. The gape width decreased with increased concentrations of copper. There was increased mucus secretion at higher concentrations. The water in the experimental trough became turbid and the turbidity also increased with increased metal concentrations.

Mortality started after an elapse of 24 hr in 0.5 ppm and 0.75 ppm of Copper and after 48 hours in 0.2 ppm solution. 100% mortality occurred at 0.5 ppm in 72 hr. The 96 hr LC_{50} value was determined as described before and was found to be 0.174 ppm.

Compared to mercury and copper, zinc was found to be less toxic to P. viridis. There was no lethality in concentrations of 1.00 ppm and below of Zn during the 7 days period. Even at higher concentrations of Zn mortality started late. At 2 ppm level, 10% of the animals died on the 4th day. At 3 ppm and 5 ppm Zn solutions mortality occurred on the third day. However, once the mortality started, it continued at a greater speed in all the tanks. Thus 100% mortality occurred at 5 ppm and 3 ppm level Zn in 4 days and 6 days respectively. The rate of mortality can be seen from the Table 5.4a. The 96 hr LC_{50} value was determined to be 3.00 ppm.

Table 5.2a

*Cumulative mortality (%) of *E. viridis* exposed to different concentrations of $HgCl_2$ solutions for varying lengths of time.

Con. of Mercury in ppm	Mortality (%)					
	Exposure time (in hrs)					
	24	48	60	72	84	96
0.05	-	-	-	-	-	-
0.10	-	-	-	-	-	-
0.20	-	-	-	-	-	20
0.30	-	-	-	10	20	40
0.40	-	-	10	30	35	60
0.50	-	20	25	50	50	100
Control	-	-	-	-	-	-

Table 5.3a

*Cumulative mortality (%) of *E. viridis* exposed to different concentrations of $CuSO_4$ solutions for varying lengths of time.

Con. of Copper in ppm	Mortality (%)						
	Exposure time (in hrs)						
	24	36	48	60	72	84	96
0.05	-	-	-	-	-	-	-
0.10	-	-	-	-	10	10	25
0.20	-	-	25	35	50	50	60
0.50	10	25	50	75	100		
0.75	15	25	50	75	100		
Control	-	-	-	-	-	-	-

*The results from duplicate experiments were averaged and given.

Table 5.4a

*Cumulative mortality ( ) of P. viridis exposed to different concentrations of $ZnSO_4$ solutions in sea water for varying lengths of time.

Con. of Zinc in ppm	Mortality (%)						
	Exposure time (in hrs)						
	24	48	72	96	120	144	168
0.50	-	-	-	-	-	-	-
1.00	-	-	-	-	-	-	-
2.00	-	-	-	10	25	50	50
3.00	-	-	25	50	85	100	
5.00	-	-	45	100			
Control	-	-	-	-	-	-	-

*The results from duplicate experiments were averaged and given.

At lethal as well as at sublethal levels the animals secreted a lot of mucus and the amount increased with increasing concentration of zinc. Similarly the gape width also decreased with increasing levels of zinc.

Lead was found to be non-lethal to the mussel, P. viridis up to 10 ppm level of Pb (highest concentration tested) during an experimental period of 10 days. No mortalit or any other symptoms of toxicity was visible in any of the test tanks. All the animals were found to be quite active as evidenced by normal gape width similar to the control mussels.

b) Villorita cyprinoides:- The general trend in toxicity of the four metals to the clam, V. cyprinoides was similar to that observed in the case of P. viridis. Among the four metals, copper and mercury were again found to be more toxic than zinc and lead.

Mercury from 0.5 ppm and above were found to be lethal to the clam during a 7 days period. The onset of mortality of the animals were delayed in 0.5 ppm and 0.75 ppm solutions. 100% mortality occurred at 2.00 ppm Hg media in 6 days. The LC_{50} value was found to be 1.57 ppm for 96 hr period.

Visible symptoms of toxicity was seen in animals maintained at 1.00 and 2.00 ppm environmental mercury. Moribund symptoms were often observed: the animals kept

opened their shell valves as if dead. And, they were not at all sensitive to touch, took long time to close the valve and retreat the foot.

It was seen that in experiments having mercury concentrations 0.5 ppm, a milkiness or turbidity appeared which increased with increased levels of the metal salt in the medium.

The LC_{50} value (96 hr) for copper, (Table 5.1) showed that it was only slightly ~~little~~ more toxic than Hg to the clam, V. cyprinoides. However, a 100% mortality occurred only at 5 ppm level of copper in 6 days. 2 ppm level of copper could not produce 100% mortality in 7 days period, as in the case of mercury. The trends followed in the mortality rate from copper and mercury can be compared from the Tables (5.2b & 5.3b).

Zinc was found to be less toxic to V. cyprinoides, when compared to Hg and Cu. In the concentration range chosen, 1.00 to 10.00 ppm, 96 hr LC_{50} value could not be estimated; instead the LC_{50} value was determined for a 10 day period. The results clearly showed that zinc markedly differed in toxic effect to the clam (Table 5.4b). Mortality of the clams started very late, after a lag period of about a week in test concentrations up to 5 ppm of Zn. Even at higher concentrations

Table 5.2b

*Cumulative mortality (%) of *V. cyprinoides* exposed to different concentrations of $HgCl_2$ solution in sea water for varying lengths of time.

Con. of Mercury in ppm	Mortality (%) Exposure time (in days) 1	2	3	4	5	6	7
0.10	-	-	-	-	-	-	-
0.20	-	-	-	-	-	-	-
0.50	-	-	-	-	10	20	25
0.75	-	-	10	20	35	50	60
1.00	10	10	35	60	75	85	85
2.00	10	20	35	60	75	100	
Control	-	-	-	-	-	-	-

Table 5.3b

*Cumulative mortality (%) of *V. cyprinoides* exposed to different concentrations of $CuSO_4$ solution in sea water for varying lengths of time.

Con. of Copper in ppm	Mortality (%) Exposure time (in days) 1	2	3	4	5	6	7
0.25	-	-	-	-	-	-	-
0.50	-	-	10	10	20	20	20
0.75	-	-	20	25	35	40	40
1.00	10	10	30	40	50	60	65
2.00	10	20	45	65	65	70	80
5.00	20	30	50	75	85	100	
Control	-	-	-	-	-	-	-

*The results from duplicate experiments were averaged and given.

Table 5.4b

*Cumulative mortality (%) of *V. cyprinoides* exposed to different concentrations of $ZnSO_4$ solutions in sea water for varying lengths of time.

Con. of Zinc in ppm	Mortality (%)									
	Exposure time (in days)									
	1	2	3	4	5	6	7	8	9	10
1.00	-	-	-	-	-	-	-	-	-	-
2.00	-	-	-	-	-	-	-	-	10	20
5.00	-	-	-	-	-	-	10	25	35	45
7.50	-	-	5	10	10	15	25	35	50	70
10.00	-	-	10	10	15	20	30	40	60	85
Control	-	-	-	-	-	-	-	-	-	-

*The results from duplicate experiments were averaged and given.

viz. 7.5 and 10 ppm mortality occurred only on the third day. In 7.5 and 10.0 ppm solutions, the mortality rates were almost similar (Table 5.4b). The LC_{50} value was estimated to be 5.47 ppm.

Eventhough there was a lag period of about a week in which mortality set in, the animals showed moribund symptoms at higher concentrations of zinc. Often the animals secreted a waite precipitate which was identified to be due to spawning. This happened before the onset of mortality. In the moribund state, they gaped, extended their foot outside and did not close and took long time to close even after touching with a glass rod. Some animals survived in this state for 3-4 days and finally died.

Lead was found to be non-lethal to the clam even up to 10 ppm (highest concentration tested) during a 10 day period. The animals seemed to be quite active as revealed by their gape and extension of the siphon. However, at 2.00 ppm and above concentrations of the metal, spawning could be observed in the animals.

c) *Meretrix casta*:- Compared to the other two organisms, *M. casta* seemed to be more resistant to metal toxicity. However, the response of *M. casta* to the various metal ions,

viz. Hg, Cu, Zn and Pb was quite different. Copper was found to be the most toxic metal among the four metals tested. The effects of each metal to the clam is described below.

The rate of mortality of the clam in various mercury solutions can be seen from the Table 5.2c. Mortality of the clam started late in all the experimental tanks having Hg concentrations up to 3.00 ppm. At higher concentration (5 ppm), mortality occured on the second day. After an initial lag period there were high rates of mortality at these concentrations of mercury. 5 ppm Hg was found to be 100% lethal to the clam during an experimental period of 6 days (Table 5.2c). Mercury < 0.5 ppm was not lethal to the animal in 7 days. The 96 hr LC_{50} was found to be 3.25 ppm.

Eventhough mortality of the clam started late after exposure to Hg solutions, the animals showed visible symptoms of undesirable environmental conditions even from the very beginning. Mostly, they closed their shell valves and secreted mucus. The water was found to be very much turbid (colloidal solution) in 2.00 ppm and above concentrations of Hg. In fact, turbidity increased with increased concentrations of mercury.

The response of the animals to copper ions was immediate. At 1.0 ppm and above, the animals closed their

shells and did not extend their siphon out. The gape width was very little and it decreased with increasing concentrations of copper in the medium. Even before the onset of mortality, the water in the experimental tank became turbid.

Mortality was observed on the third day of exposure to copper salt solution up to 2.00 ppm and on the second day in 3.00 and 5.00 ppm. After the initial lag period, mortality was rather continuous in all the test tanks above 0.5 ppm level of copper. 5.00 ppm copper was found to be lethal to 100% of the animals in 6 days. There was no mortality at 0.5 ppm and lower concentrations during the experimental period. The general trend followed in the mortality of the clam can be seen from the table 5.3c. The 96 hr LC_{50} value was determined to be 2.188 ppm.

M. casta showed a similar response towards environmental zinc ions as V. cyprinoides. Moribund symptoms were very much pronounced, in the case of M. casta exposed to zinc solutions. At 5.00 ppm and 7.50 ppm Zn solution 2 or 3 animals were found moribund at the end of 24 hours. In 3.00 ppm, 2 animals each developed toxic symptoms at the end of 48 hours. The mortality rate can be seen from the table 5.4c. 100% of the animals died at 7.50 ppm Zn in 6 days. Zinc concentration below 2.00 ppm

Table 5.2c

*Cumulative mortality (%) of *M. casta* exposed to different concentrations of $HgCl_2$ solutions in sea water for varying lengths of time.

Con. of Mercury in ppm	Mortality (%)						
	Exposure time (in days)						
	1	2	3	4	5	6	7
0.50	-	-	-	-	-	-	-
1.00	-	-	-	10	10	10	15
1.50	-	-	-	15	15	20	30
2.00	-	-	10	25	40	50	60
3.00	-	-	25	45	60	70	90
5.00	-	15	20	60	75	100	
Control	-	-	-	-	-	-	-

Table 5.3c

*Cumulative mortality (%) of *M. casta* exposed to different concentrations of $CuSO_4$ solutions in sea water for varying lengths of time.

Con. of Copper in ppm	Mortality (%)						
	Exposure time (in days)						
	1	2	3	4	5	6	7
0.50	-	-	-	-	-	-	-
1.00	-	-	10	20	25	30	30
1.50	-	-	15	30	40	45	50
2.00	-	-	20	40	50	60	65
3.00	-	10	35	60	70	70	80
5.00	-	15	50	75	90	100	
Control	-	-	-	-	-	-	-

*The results from duplicate experiments were averaged and given.

Table 5.4c

*Cumulative mortality (%) of *M. casta* exposed to different concentrations of $ZnSO_4$ solutions in sea water for varying lengths of time.

Con. of Zinc in ppm	Mortality (%)						
	Exposure time (in days)						
	1	2	3	4	5	6	7
0.50	-	-	-	-	-	-	-
1.00	-	-	-	-	-	-	-
2.00	-	-	-	5	10	20	25
3.00	-	-	-	10	20	60	70
5.00	-	-	10	30	70	90	90
7.5	-	-	10	60	80	100	
Control	-	-	-	-	-	-	-

*The results from duplicate experiments were averaged and given.

was non-lethal to the clam in the experimental period of one week.

It would be interesting to narrate the moribund symptoms of the clam due to the toxic effect of zinc. At concentrations of 3 ppm and above, the animals wide opened their shells, extended their foot outside and seemed to be very passive. It took long time to close the valve when touched with a glass rod. In the moribund state, the animals could survive for few more days and then died. The LC_{50} value was estimated to be 6.67 ppm during a 96 hr period.

Lead ions could not produce any lethality to the clam up to a concentration of 10 ppm during an exposure period of 10 days. No symptoms of acute toxicity could be seen in *V. costa* exposed to various concentrations of Pb ions. The animals were found to be quite active as in the control tanks as evidenced by gape and extension of siphon.

5.3. Discussion

The results of the present experiments clearly reveal that even short term exposure to heavy metal ions, viz. Hg^{2+}, Cu^{2+}, Zn^{2+} and Pb^{2+} have detrimental effect on all the three species of bivalve molluscs studied. However, the toxic effect as symbolised by LC_{50} values varied markedly with species. The mussel, *P. viridis* seemed to be very

susceptible to heavy metal ions when compared with other two species. The rate of mortality and LC_{50} values obtained for each animal would clearly confirm this (Tables 5.2a to 5.4c and 5.5). M. costa was less sensitive (except for Zn) than the other two species to heavy metal ions.

Of the four metals tested in this study, Cu was found to be the most toxic metal to all the species. There were considerable differences in the toxicity of Cu, Hg, Zn and Pb. The order of toxic effect was Cu > Hg > Zn ≫ Pb and was true for the three organisms. The no effect level of the metal ions copper and mercury to the various species was very low when compared to the other two metal ions. Lead salt could not produce any mortality in any of the species even up to 10 ppm level. Copper and mercury ions produced quicker toxic effects on the animals as described earlier (visible symptoms of toxic effect was noticed in these cases). It must be pointed out that zinc ions differ from copper or mercury ions in that it seemed to affect more slowly than the latter ions. Thus, no lethal effect could be observed in the first 48 hr of exposure, on any of the species. However, once mortality was started, there was high rate of death (Tables 5.4a to 5.4c).

The toxic effect of the metal would be influenced by so many factors. The physiological condition of the organisms,

the form of the metal present, presence of other metal ions and environmental conditions like the physical and chemical properties of water etc would include this (Nielsen and Anderson, 1970; Bryan, 1971). The LC_{50} values would also be influenced by the length of time that a particular test species could remain closed (Calabrese et al., 1973). In the present study no attempt was made to account for any metal loss by way of adsorption or uptake and precipitation. The concentration reported was the added concentration of the metal ion to the sea water medium. This was with an objective to study the effect in a nearly natural condition. Some losses were overcome by daily changing the medium. However, visible precipitates of some of the metals, especially in the case of copper, was seen in the test tanks at higher concentrations.

The high resistance of the two clams, particularly M. casta, as revealed by high LC_{50} values might be due to the ability of these organisms to close their shell valves, thereby reducing penetration of toxic metals into the soft parts of the animals. This was confirmed by the fact that some variability in LC_{50} values were found in the two clams conducted at different times. Similar problems were encountered by Calabrese et al. (1973) while working with the American oyster, Crassostrea virginica.

Wisely and Blick (1967) exposed larvae of the mussel, Mytilus edulis and the oyster, Crassostrea commercialis to copper and mercury salts. 50% of the mussel larvae died in 2 hr at concentration of 32.3 ppm Cu and 13.0 ppm Hg (separately) and oyster larvae at 180.5 ppm Hg. They concluded that this comparatively high resistance was due to the ability of these organism to remain closed their shell valves and thereby reducing the penetration of the toxic material.

Pringle et al. (1968) included Cu, Hg, Zn and Pb in the group of very high potential pollutants. These authors found that an experimental level of 0.02 ppm Cu was extremely toxic to molluscs and that the toxicity of zinc and lead salts was generally reduced in presence of soluble calcium salts. Wisely and Blick (1967) also found Hg and Cu very toxic to the larvae of the mussel, M. edulis and the oyster, Crassostrea commercialis. Biesinger and Christensen (1972) found that only 3 of 21 metals tested, Co, Hg and Cd were more toxic than Cu to Daphnia magna. Other authors have also indicated that copper is among the more toxic metals to aquatic animals or at least Cu is more toxic than Zn (Portmann, 1968; Reish et al., 1976). Davenport and Wasely (1978) observed that 0.09-0.1 ppm added Cu was the toxicity threshold for M. edulis. The toxicity of metal ions to embroys of the American oyster,

Crassostrea virginica was in the order $Hg^{2+} \gg Cu^{2+} > Zn^{2+} \gg Pb^{2+}$ (Calabrese et al., 1973). The present findings differ slightly, the order being $Cu^{2+} > Hg^{2+} > Zn^{2+} \gg Pb^{2+}$.

The toxic effect of heavy metals is due to poisoning the enzyme systems. The more electronegative elements (eg. Cu, Ag and Hg) have a greater affinity for amino, imino and sulfhyaral groups and are readily chelated by organic molecules (Pringle et al., 1968). Bryan (1971) pointed out that a metal would only be required in a [illegible]ly finite concentration for normal metabolic activity and, if this were exceeded, the metal might inhibit either the enzymes it was activating or other enzymes by blocking the reaction sites.

Danielli and Davis (1951) suggested that the metal ions exert their toxic influences by covalent binding at cell surfaces, and that the difference in electronegativity of the various ions is a toxicity determining factor. The site of action between the metal and the organic ligand appeared to involve =S or other electronegative functional groups, Shaw and Gruskin (1957) and Shaw (1961) related the toxicity of many organisms and metal sulfide solubility and found a positive correlation.

Biesinger and Christensen (1972) studied the effects of 21 metals on survival, growth, reproduction and metabolism

of Daphnia magna. These workers observed that the correlation between toxicity and solubility of metal sulfides suggests the possibility that the metals may combine in vivo with -SH groups of enzymes, which affects their solubility and catalytic activity.

The electronegativity data, pKs value of the metal sulphide and ionic radii of the four metal ions viz. Cu^{2+}, Hg^{2+}, Zn^{2+} and Pb^{2+} are given in the table (5.6). Based on these data it may be argued that Hg must be more toxic than Cu or that Pb must be more toxic than Zn, in conflict with the present results. However, the reactivity of an ion must be influenced by its ionic radius. Here it appears that higher the electronegativity and pKs values of the metal sulphide and lower the ionic radiusof the metal ion, the more toxic the metal would be. Hence, though Cu and Hg are equivalent in electronegativity values, the small ionic radius of Cu^{2+}, might have made it more reactive. A similar argument holds true for Zn and Pb. Eventhough, the very low or no toxic effect of Pb could not be explained it can be argued that the high level of soluble calcium salts in the culture water probably reduced the toxic effect of Zn and Pb.

Table 5.5

The 96 hr LC_{50} values for the heavy metals, mercury, copper, and zinc determined by the straight line graphical interpolation method tested against the three bivalve molluscs.

Organism	Hg (ppm)	Cu (ppm)	Zn (ppm)
P. viridis	0.34	0.174	3.00
V. cyprinoides	1.57	1.214	5.47*
M. costa	3.25	2.188	6.67

*10 day LC_{50} value.

Table 5.6

Some physico-chemical properties of the metal ions and metal sulphide (After Pauling*, 1975 and Meites†, 1963)

Property	Name of the element			
	Cu	Hg	Zn	Pb
1. *Electronegativity	1.9	1.9	1.6	1.8
2. *Ionic radii (M^{2+} ion) °A	0.72	1.10	0.83	1.20
3. †pKs value of the metal sulphide	35.2	52.4	23.8	27.9

CHAPTER 6

STUDIES ON THE TOXIC EFFECTS OF HEAVY METALS

PART II. PHYSIOLOGICAL EFFECTS

CHAPTER 6

STUDIES ON THE TOXIC EFFECTS OF HEAVY METALS

PART II PHYSIOLOGICAL EFFECTS

Studies on the poisonous effects of heavy metals on aquatic organisms were mainly concentrated on the acute toxicity and uptake kinetics (Chapters 5 and 7). Little information is available on the effects of the metals on metabolic processes of the affected animals. The physiological and biochemical changes and causes of death on exposure of fish/shellfish to heavy metals are not yet fully investigated. Skidmore (1970) had studied the changes in several respiratory parameters of rainbow trout (Salmo gairdneri) due to zinc sulfate toxicity. Carpenter (1927, 1930) had studied the lethal action of metalic salts on some fishes and established that fish died from asphyxiation. Burton et al. (1972) had also conducted toxicity tests using zinc compounds to the Rainbow trout (Salmo gairdneri) and confirmed the hypothesis that death due to metal toxicity was related to tissue hypoxia. Shaffi (1978a,b) had studied the effects of cadmium on tissue glycogen content and the variations in tissue glycogen content, serum lactate and glucose levels due to copper intoxication in three freshwater teleosts.

To test the hypothesis that death from acute metal toxicity is related to tissue hypoxia, the variations in tissue lactic acid and glycogen content were measured after exposure of the molluscs to metal ions (tests were conducted using Cu^{2+} and Hg^{2+} only). In animal tissues, one of the breakdown products that may be formed due to glycolysis is lactic acid.

6.1. Materials and methods

P. viridis, V. cyprinoides and M. casta were exposed to various concentrations of copper and mercury in seawater of their respective habitat salinity for varying lengths of time. The concentrations of the metal ions selected and the salinities of the waters used in each case are given along wit the results in tables (6.1a to 6.2c).

Individual animals were killed at definite intervals of time (Table 6.1a to 6.2c) and the soft parts were used for determining lactic acid. For the estimation of glycogen, 3-4 animals were dissected for muscle and liver and each component was pooled for further analysis. The analytical procedure for the estimation of lactic acid and glycogen are given under 2.5g,h. (In V. cyprinoides and M. casta, liver portion could not be dissected out)

6.2. Results:

The results of the experiments are presented in tables (6.1a to 6.2c). The salient features of the observations are given below.

a) P. viridis:- The tissue lactic acid content increased in the mussel exposed to both Cu and Hg salt solutions in the medium when compared to the control animals. The lactic acid content increased with increased metal ion concentration and exposure time. Thus, the level of lactic acid in the whole soft parts increased to 621.44 µg/g and 581.79 µg/g (wet wt.) in 2 ppm environmental levels of Cu^{2+} and Hg^{2+} respectively, at the end of 24 hr exposure. These concentrations may be compared to the lactic acid content in the control animals (46.61 µg/g and 50.04 µg/g wet wt. respectively

On the otherhand, the muscle and liver glycogen content declined in the mussel exposed to Cu^{2+} or Hg^{2+} solutions. At 0.5 ppm concentration of either of the metal ions, the muscle glycogen content was reduced to a non-detectable level in 24 hr of exposure. The fall in glycogen content in the metal intoxicated animals, with respect to the control animals can be better appreciated from tables (6.1a & 6.2a).

Thus, an inverse relationship between lactic acid in the tissue and glycogen content in the liver or muscle of

Table 6.1a

Changes in the tissue lactic acid content of the mussel, Perna viridis exposed to different concentrations of copper and mercury ions in sea water of salinity, 23‰ for varying lengths of time.

Con. of metal ions (ppm)	Lactic acid, µg/g wet wt. ± S.D.		
	Exposure time		
	2 hrs	4 hrs	24 hrs
Cu			
0.50	163.56 ± 15.39	190.78 ± 11.07	392.18 ± 33.43
1.00	204.12 ± 21.46	231.95 ± 21.02	445.74 ± 48.82
2.00	297.50 ± 26.08	327.44 ± 18.50	621.44 ± 58.06
Control	48.25 ± 4.13	43.80 ± 3.52	46.61 ± 3.76
Hg			
0.50	127.26 ± 9.67	142.17 ± 9.46	344.17 ± 26.38
1.00	160.52 ± 18.30	176.56 ± 19.28	439.84 ± 37.15
Control	48.25 ± 4.13	46.55 ± 3.82	50.04 ± 4.16

Table 6.2a

Changes in the muscle and liver glycogen content of the mussel, Perna viridis exposed to different concentrations of copper and mercury in sea water of salinity, 25‰ for varying lengths of time.

Con. of metal ions (ppm)	Organ	Glycogen, µg/g wet wt. ± S.D.					
		Exposure time					
		4 hrs		12 hrs		24 hrs	
Cu							
0.20	Muscle	632.37 ±	20.21	478.50 ±	14.64	380.53 ±	11.63
	Liver	1908.85	42.64	1651.44	31.74	1379.37	25.01
0.50	Muscle	586.90	17.58	372.67	11.31	N.D.	
	Liver	1748.59	35.93	1367.36	32.69	811.06	13.48
Control	Muscle	696.69	13.33				
	Liver	2020.29	43.70				
Hg							
0.20	Muscle	657.23 ±	19.62	571.90 ±	14.50	429.70 ±	12.64
	Liver	1943.64	33.98	1638.55	24.49	1405.35	26.87
0.50	Muscle	618.30	11.81	385.63	11.94	N.D.	
	Liver	1830.45	41.05	1409.78	25.77	792.68	12.80
Control	Muscle	696.69	13.33				
	Liver	2020.29	43.70				

P. viridis was observed. Since different animals were used in these two experiments, individual variation is not ruled out. However, the major objective was to focus on any change in glycogen content and consequent lactic acid accumulation in the tissues as a result of metal toxicity.

b) V. cyprinoides:- It could be seen that the lactic acid content in the tissue of the clam increased significantly with increasing concentrations of metal ions. Thus greatest amount of lactic acid was found in clams exposed to highest concentrations of copper or mercury. The tissue lactic acid increased to 423.54 µg/g and 372.65 µg/g wet wt. after 24 hr in 1 ppm solutions of copper and mercury respectively; the value for the control animals was in the range of 24.73 µg/g to 27.79 µg/g (wet wt.).

The muscle glycogen content showed a steady decrease in the animals exposed to heavy metal ions at the toxic levels. Thus, the muscle glycogen content was reduced to 900.94 µg/g and 974.10 µg/g at 1.00 ppm levels of copper and mercury in 24 hr from the control value of 2350.53 µg/g. It decreased further with elapse of time in the two test cases (Tables 6.1b & 6.2b).

c) M. casta:- The muscle glycogen content in the clam at different intervals of exposure time to various concentra-

Table 6.1b

Changes in the tissue lactic acid content of the clam, Villorita cyprinoides exposed to different concentrations of copper and mercury in sea water of salinity, 10‰ for varying lengths of time.

Con. of metal ions (ppm)	Lactic acid, µg/g wet wt. ± S.D.		
	Exposure time		
	2 hrs	4 hrs	24 hrs
Cu			
0.50	50.22 ± 3.73	74.80 ± 4.84	289.60 ± 33.50
1.00	85.65 ± 5.10	116.78 ± 8.52	423.54 ± 48.05
Control	26.50 ± 1.62	28.82 ± 1.90	27.48 ± 1.77
Hg			
0.50	49.25 ± 3.95	68.12 ± 5.07	254.84 ± 19.78
1.00	73.85 ± 4.68	110.34 ± 7.11	372.65 ± 46.68
Control	25.40 ± 1.44	23.65 ± 1.38	24.73 ± 1.56

Table 6.2b

Changes in the muscle glycogen content of the clam, Villorita cyprinoides exposed to different concentrations of copper and mercury ions in sea water of salinity, 10‰ for varying lengths of time.

Con. of metal ions (ppm)	Glycogen, µg/g wet wt. ± S.D.		
	Exposure time		
	12 hrs	24 hrs	48 hrs
Cu			
0.50	1875.74±22.40	1373.16±44.75	704.54±45.67
1.00	1643.50±24.30	900.94±55.01	574.73±29.78
Control	2350.53±39.70	2342.60±35.90	2356.24±40.35
Hg			
0.50	2016.49±36.21	1487.83±34.70	824.50±30.60
1.00	1809.64±33.33	974.10±42.26	486.14±29.56
Control	2350.53±39.70	2342.60±35.90	2356.24±40.35

Table 6.2c

Changes in the muscle glycogen content of the clam, Meretrix casta exposed to different concentrations of copper and mercury ions in sea water of salinity, 17‰ for varying lengths of time.

Con. of metal ions (ppm)	Glycogen, µg/g wet.wt. ± S.D.		
	Exposure time		
	12 hrs	24 hrs	48 hrs
Cu			
0.50	1270.89±45.51	1140.23±45.56	774.86±26.13
1.00	1231.47±46.49	1012.34±37.20	575.20±19.25
Control	1484.37±50.11	1490.25±51.60	1471.82±48.44
Hg			
0.50	1319.63±55.75	1036.52±45.43	716.42±26.53
1.00	1302.76±49.89	977.43±33.12	486.35±16.07
Control	1484.37±50.11	1490.25±51.60	1471.82±48.44

tions of copper and mercury are given in table (6.2c). (Lactic acid was not estimated in the animal)

The muscle glycogen content steadily decreased in the clam, with increasing metal concentrations in the medium. The lowest values were found in animals maintained at 1.00 ppm levels of copper and mercury, which was the highest concentration tested. At the end of 48 hrs the glycogen content was reduced to 575.20 µg/g and 486.35 µg/g (wet wt.) in 1 ppm concentrations of copper and mercury respectively. The muscle glycogen value in the control animals was 1484.37 µg/g.

6.3. Discussion

The results of the experiment clearly showed that heavy metal (viz. copper and mercury) intoxication had caused lactic acid accumulation in the tissues and glycogen depletion in the muscle (also in the liver of *P. viridis*) of the three bivalve molluscs. The metal intoxication might have caused severe anaerobic stress resulting in the breakdown of tissue glycogen possibly to meet the energy demands in the muscle. The end product of the anaerobic degradation of glycogen being lactic acid, the increase in level of this component in the tissue may be attributed to this. The increased level of lactic acid (or a corresponding decrease of glycogen) with increasing concentration of metal ions must be due to the

relative stress produced on the animals at various levels of metal ions in the habitat water.

In the present study, it was observed that all the three bivalves produced a lot of mucus (which increased with increasing metal concentration in the environmental water and was maximum in the case of P. viridis) on exposure to metal ions. The deposition of mucus on the gills might have prevented the capacity of blood to transfer O_2 to various internal organs. This in turn must have led to severe hypoxic stress which resulted in the breakdown of tissue glycogen. The lactic acid formation can account for the decline of the glycogen content. So the mortality caused by acute heavy metal poisoning can partly be attributed to tissue hypoxia.

Burton et al. (1972) attributed the acute zinc toxicity to Rainbow trout (Salmo gairdneri) to the coagulation or precipitation of mucus on the gills and cytological damage to the gills. The physiological mechanism of death by either of the above cause is related to a breakdown in gas exchange at the gills, which results in hypoxia at the tissue level. It confirms Skidmore's (1970) hypothesis that gill damage modified gas exchange and created hypoxia at the tissue level. Similar observations were made by Khaffi (1976a,b in three freshwater teleosts due to cadmium and copper intoxications;

in liver and muscle, an inverse relationship between the concentration of cadmium or copper and fall in glycogen content was observed. He stated that the coagulated mucus on the gills of the fishes might have reduced the O_2 transfer to various internal organs which in turn might have caused the process of tissue acidosis. The accumulation of lactic acid in serum was related to the above processes.

The results of the present investigations are in good agreement with the above observations, though the comparison made is between fishes and molluscs.

CHAPTER 7

KINETICS OF HEAVY METAL UPTAKE AND LOSS

PART I. KINETICS OF HEAVY METAL UPTAKE

CHAPTER 7

KINETICS OF HEAVY METAL UPTAKE AND LOSS

PART I KINETICS OF HEAVY METAL UPTAKE

As mentioned earlier, certain bivalve molluscs are important in further concentrating trace metals from their environment to a very high level. Since, these organisms invariably reflect the metal levels of the ambient water in their tissues, they are widely recognised as environmental indicator organisms for trace metal pollutants. So a study on the kinetics of heavy metal accumulation by the three bivalve molluscs and the distribution of the metals among the various organs is all important in understanding whether these benthic organisms if exposed to metallic pollutants would refl elevated concentration in their tissues. The great variety of bivalve molluscs available in unpolluted estuarine areas and the ease with which they are caught and maintained contribute to their usefulness as test animals in this regard.

The trace metal accumulation of estuarine molluscs had been studied by various workers (Irukayama, 1967; Skidmore, 1964; Brookes and Rumsby, 1965; Wisely and Blick, 1967; Pringle et al., 1968; Arthur and Leonard, 1970; Jernelov and Lann, 1971; Nitta, 1972; Cunningham and Tripp, 1973, 1975; Ayling, 1974; Eustace, 1974; Schulz-Baldes, 1974, 1978; Ratkowsky et al., 1974; Smith et al., 1975; Phillips, 1976a, b,

1978; ~~Srungs et al., 1976~~; Davies, 1976; Davies and Virie, 1978; Eganhouse and Young, 1978; Isao Aoyama et al., 1978 and Simpson, 1979).

The literature survey showed that very little attention has been bestowed on the trace metal accumulation or loss studies in species from Indian waters (Krishnan Nambisan et al., 1977; Lakshmanan and Krishnan Nambisan, 1979; D'Silva and Kureishy, 1978). Hence an attempt was made to study the kinetics of accumulation of some heavy metals viz. Hg, Cu, Zn and Pb by the bivalve molluscs, P. viridis, V. cyprinoides and M. casta.

7.1. Materials and methods

The kinetics of heavy metal uptake was studied by exposing a fixed number of organisms to various environmental levels of the metal, added as metal salt solutions to seawater and estimating the metal content in the soft parts of the animals at fixed intervals of time (given in the tables 7.2a to 7.13b). The details of the methods adopted are described in section 2.8c. The concentrations of the metal ions tested for each species and the characteristics of water used etc. are presented in table 7.1.

Table 7.1

Showing the concentrations of various metal salts in sea water used in metal uptake studies by the three bivalve molluscs and the water characteristics used in each study.

Metal ions tested	Name of the animal	Metal concentrations in ppm				Water characteristics			
						Salinity (S‰)	Oxygen saturation	Temp. °C	pH
Hg^{2+}	P. viridis	0.05	0.10	0.20	-	25.0	95±5	29-30.5	7.5±0.2
	V. cyprinoides	0.25	0.50	1.00	2.00	5.0	95±5	28-30	6.7±0.1
	M. casta	1.00	2.00	3.00	5.00	10.0	95±5	28-30.5	6.9±0.1
Cu^{2+}	P. viridis	0.025	0.05	0.10	-	25.0	95±5	28-31	7.5±0.2
	V. cyprinoides	0.25	0.50	1.00	2.00	5.0	95±5	28-30	6.8±0.1
	M. casta	-	0.50	1.00	2.00	10.0	95±5	29-31	7.0±0.1
Zn^{2+}	P. viridis	-	0.50	1.00	2.00	25.0	95±5	30-32	7.4±0.1
	V. cyprinoides	0.25	0.50	1.00	2.00	8.0	95±5	29-31	6.9±0.1
	M. casta	-	0.50	1.00	2.00	25.0	95±5	30-32	7.3±0.1
Pb^{2+}	P. viridis	0.25	0.50	1.00	2.00	25.0	95±5	29-31	7.4±0.1
	V. cyprinoides	0.25	0.50	1.00	2.00	8.0	95±5	29-31	6.8±0.1
	M. casta	-	0.50	1.00	2.00	11.5	95±5	30-32	6.9±0.1

7.2. Results

The results of the investigation are presented metalwi in the following subsections. The term concentration factor* is extensively used to describe the toxicant accumulation dat

A. Accumulation of mercury

a) By Perna viridis:- The mercury content in the whole soft tissues analysed at definite intervals of time are given in table 7.2a and Fig 7.1a .

Mercury content of the whole soft parts of the mussel in all the experimental tanks increased considerably, when compared to the background level of Hg in the body. It increased from a background level of 0.1 µg/g to 25.18 µg/g (wet wt.) in 0.2 ppm Hg solution within 5 days. It is seen that the uptake was proportional to the concentration of Hg^{2+} in the medium.

At higher concentrations there was rapid initial uptak The rate decreased subsequently. The concentration factor wa maximum in 0.05 ppm (403.6 after exposure for 6 days) and the was a gradation in the concentration factor with increasing concentration of mercury(Table 7.2a).

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*'Concentration factor' (CF) is defined as the ratio of the tissue concentration of the metal (in ppm) to the concentration in water (in ppm)

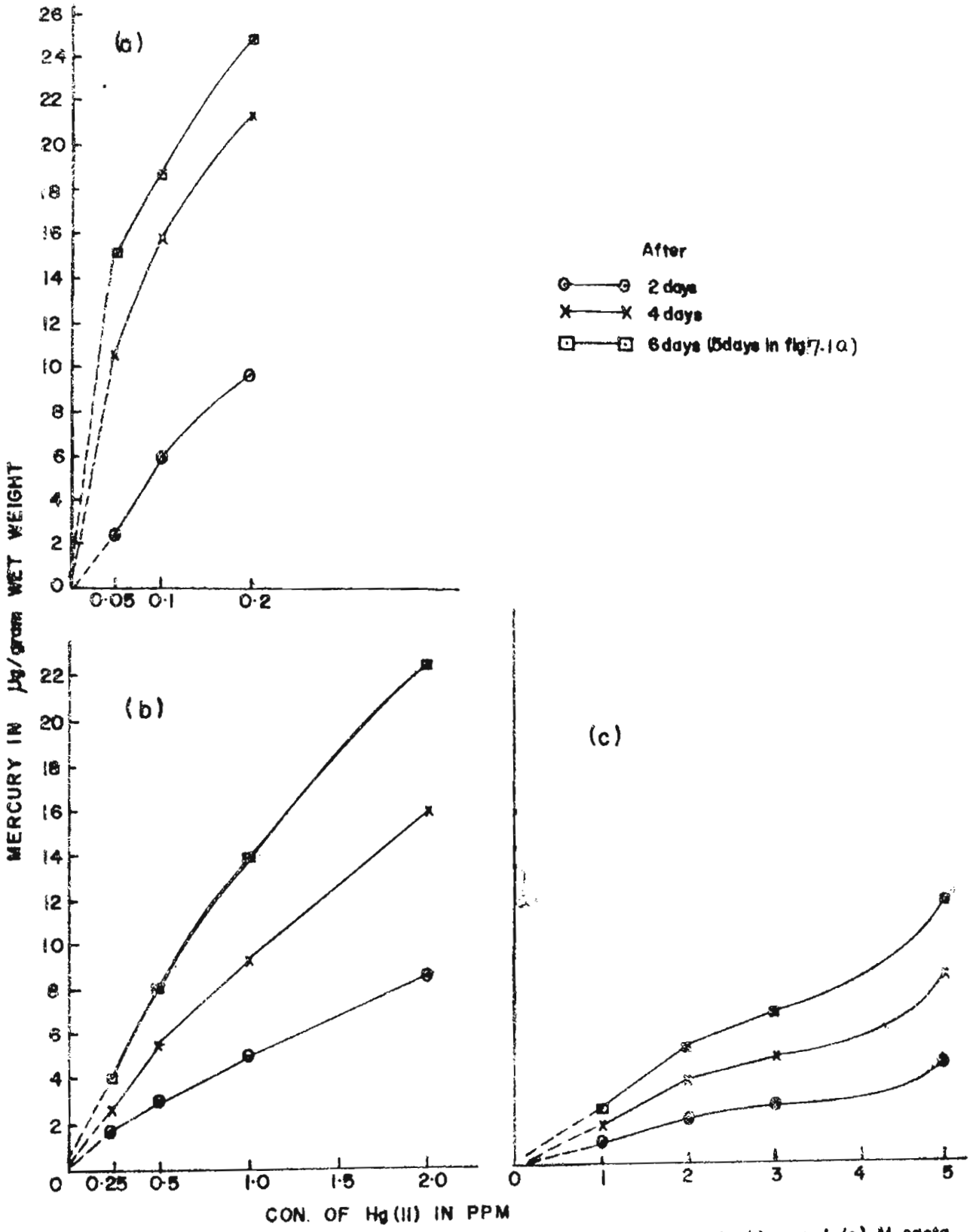

Fig 7. 1 Accumulation of mercury by (a) P. viridis, (b) V. cyprinoides and (c) M. casta in relation to mercury concentration in the medium at different periods of time.

Distribution of mercury in organs:- The distribution pattern of Hg among different organs on exposure of the animal to 0.1 ppm Hg^{2+} and their concentration factors are given in Fig 7.5a and table 7.2b respectively. Mercury was rapidly accumulated in all the organs, but gills had the highest concentration of mercury at all times. It was distributed rather uniformly in all other organs. The order of accumulation in different organs are Gills > Viscera > Muscle > Mantle.

Table 7.2a

Accumulation of Hg in the tissues of the mussel, Perna viridis (Linnaeus) exposed to various concentrations of $HgCl_2$ solution in sea water of salinity, 25‰ for varying lengths of time.

Test con. of mercury in ppm	Tissue con. of Hg, µg/g wet wt. Mean ± S.D.				Con. factor at 5 days
	2 days	4 days	5 days	6 days	
0.05	2.5005 0.24	10.560 0.81	15.200 0.96	20.180 0.97	403.60
0.10	6.004 0.32	15.852 0.89	18.650 1.09	21.286 1.05	212.86
0.20	9.756 0.72	21.634 1.22	25.180 1.31	-	125.90 (at 5 days
Control	0.0861				

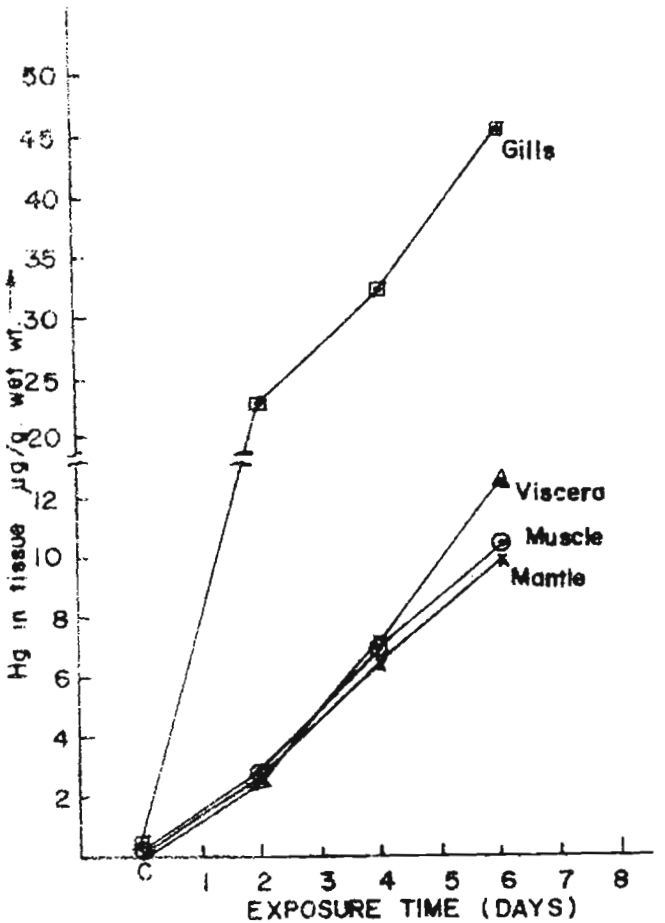

Fig. 7.5a. Accumulation of mercury among various organs of P. viridis at 0·1ppm Hg^{2+} solution.

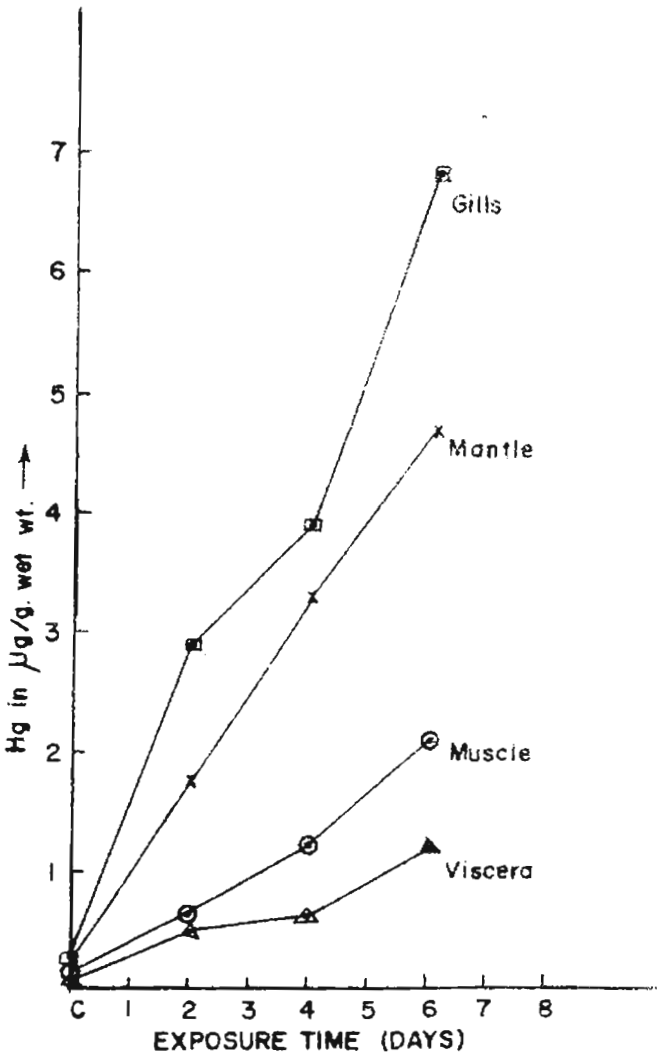

Fig. 7.5b Accumulation of mercury among various organs of M. casta, at 1·00 ppm Hg^{2+} solution.

Table 7.2b

Accumulation of Hg among various organs of the mussel, Perna viridis exposed to 0.1 ppm mercury solution in sea water of salinity, 25‰.

Organ	% of body weight	Hg, µg/g wet wt. (control)	Con. of Hg, µg/g wet wt. Mean ± S.E. Exposure time 2 days	4 days	6 days	Con. fact at 6
Muscle	26.71	0.075	2.800	6.925	10.493	104.9
		0.004	0.102	0.186	0.180	
Mantle	32.47	0.077	2.760	6.730	10.095	100.9
		0.005	0.108	0.114	0.370	
Gills	16.79	0.195	23.050	32.306	45.697	456.9
		0.011	1.05	1.814	0.850	
Viscera	24.04	0.082	2.640	7.220	12.522	125.2
		0.004	0.079	0.211	0.330	

b) By Villorita cyprinoides:- V. cyprinoides acumulated Hg from the medium and it was found that the uptake was linear (Table 7.3a). There was about 100 fold increase in the tissue concentration of Hg in the clam kept in 2 ppm Hg solution at t end of 6 days. The concentration factor was the highest at 0.25 ppm (the lowest concentration of Hg tested) and it decrea with increasing concentrations.

It can be seen that (Table 7.3a) the rate of uptake decreases with time in all the concentrations tested. After a

exposure time of 2 days the mercury concentrations in the tissue of the clams were 1.820, 2.950, 5.029 and 8.682 µg/g (wet wt.) in 0.25, 0.50, 1.00 and 2.00 ppm of Hg respectively and at the end of 6 days the corresponding values were only 4.094, 8.165, 13.938 and 22.646 µg/g (wet wt.).

Table 7.3a

Accumulation of Hg in the tissues of Villorita cyprinoides var. cochinensis exposed to various concentrations of $HgCl_2$ solution in sea water of salinity, 5‰ for varying lengths of time.

Con. of mercury in ppm	Tissue con. of Hg, (µg/g wet wt.) Mean ± S.D.			Con. factor at 6 days
	Exposure time			
	2 days	4 days	6 days	
0.25	1.820	2.686	4.094	16.38
	0.056	0.088	0.069	
0.50	2.950	5.568	8.117	16.23
	0.077	0.105	0.152	
1.00	5.029	9.540	13.938	13.94
	0.067	0.092	0.196	
2.00	8.682	16.209	22.646	11.32
	0.135	0.142	0.337	
Control	0.232			

(Distribution of Hg among various organs of the clam, was not studied)

c) By Meretrix casta:- The rate of Hg uptake and the tissue level of Hg in the clam at various concentrations of mercury are given in the table (7.4a). The nature of uptake can be seen from Fig 7.1c . The metal uptake was proportional to the mercury ion concentration in the medium. However, the rate of accumulation of Hg by M. casta seemed to be a slow process compared to the other two species, as indicated by the concentration factors. Here the C.F. appeared to be more or less same at all concentrations of Hg (Table 7.4a). The tissue concentration showed a ≈ 12 fold increase of mercury level in 1.00 ppm solution and 60 fold increase in 5.00 ppm solution. The highest level of tissue Hg content was 12.12 µg/g(wet wt.) in 5 ppm solution during 6 days period.

Table 7.4a

Accumulation of Hg in the tissues of Meretrix casta exposed to various concentrations of $HgCl_2$ solution in sea water of salinity, 10‰ for varying lengths of time

Con. of mercury in ppm	Tissue con. of Hg, (µg/g wet wt.) Mean ± S.D.			Con. factor at 6 days
	Exposure time			
	2 days	4 days	6 days	
1.00	1.022 0.050	1.845 0.049	2.500 0.051	2.50
2.00	2.147 0.066	3.894 0.087	5.450 0.175	2.73
3.00	2.675 0.082	4.942 0.090	7.004 0.080	2.34
5.00	4.714 0.080	8.503 0.090	12.120 0.195	2.42
Control	0.195			

Distribution of mercury in organs:- Mercury content increased in all the body components studied. However, the rate of uptake and distribution pattern varied with organs (Table 7.4b and Fig 7.5c). Highest tissue burden of Hg was found in the gills (6.815 µg/g wet wt. after 6 days) which was about 3 times of the whole soft part concentration. The order of accumulation among various components were Gills > Mantle > Muscle > Viscera.

Table 7.4b

Accumulation of Hg among various organs of Meretrix casta exposed to 1.00 ppm of mercury solution in sea water of salinity, 10‰.

Organ	% of body weight	Hg, µg/g wet wt. (control)	Con. of Hg, µg/g wet wt. Mean ± S.D. Exposure time 2 days	4 days	6 days	Con. factor at 6 days
Muscle	27.58	0.135	0.625	1.190	2.085	2.08
		0.01	0.058	0.127	0.104	
Mantle	23.37	0.146	1.768	3.334	4.701	4.70
		0.012	0.071	0.215	0.161	
Gills	12.78	0.203	2.935	3.873	6.815	6.82
		0.012	0.110	0.167	0.195	
Viscera	36.27	0.078	0.456	0.612	1.182	1.18
		0.005	0.036	0.08	0.059	

B. Accumulation of copper

a) By Perna viridis:- The results of the investigations on the accumulation of copper by P. viridis is shown in table 7.5a and Fig 7.2a .

The tissue concentration of copper in the mussel increased in all the concentration studied. The highest level of copper (120.70 µg/g dry wt.) was found in animals from 0.1 ppm solution after an exposure period of 6 days. The efficiency of accumulation can be assessed from the concentration factors.

Table 7.5a

Accumulation of Cu in the tissues of the mussel, P. viridis exposed to various concentrations of $CuSO_4$ solution in sea water of salinity, 25‰ for varying lengths of time.

Con. of Copper in ppm	Tissue con. of Cu, µg/g dry wt. Mean ± S.D.			Con. factor at 6 days
	Exposure time			
	2 days	4 days	6 days	
0.025	22.80	43.79	61.25	2450.00
	2.08	2.50	2.86	
0.050	28.67	58.20	80.34	1606.80
	2.49	1.64	2.83	
0.100	37.20	81.23	120.70	1207.00
	3.17	4.98	4.06	
Control	17.72			
	0.93			

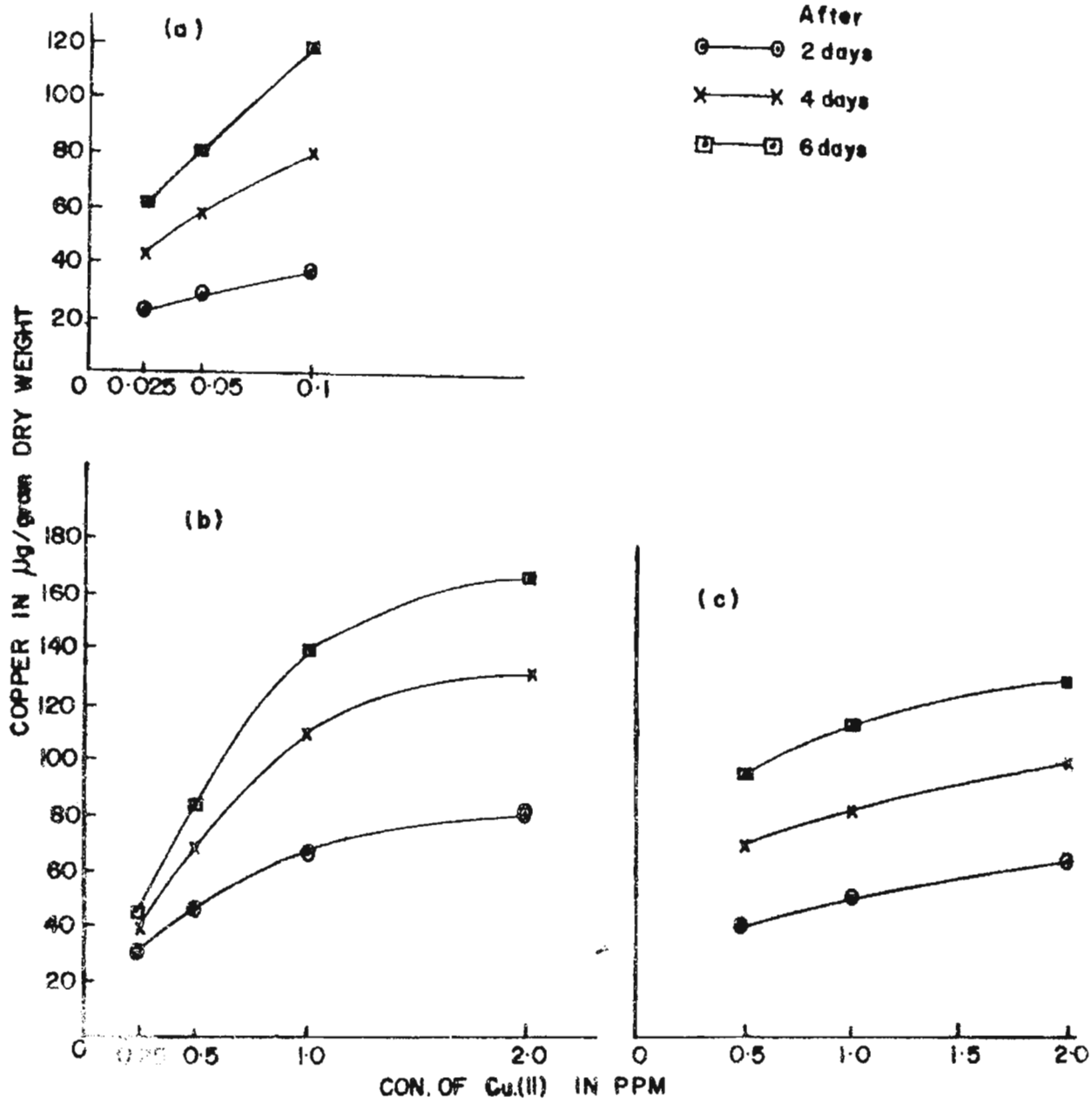

Fig. 7·2. Accumulation of copper by (a) P. viridis (b) V. cyprinoides and (c) M. casta in relation to copper concentration in the medium at different periods of time.

It was found that the C.F. decreases with increasing concentration, the highest value being 2450 at 0.025 ppm and the lowest value (1207) at 0.1 ppm solution. This again shows that the efficiency of uptake is maximum at lower concentrations. An examination of the uptake data at different intervals showed that the rate decreased with time. However, the rate of uptake was found to be related to the metal concentration in the environmental water.

Distribution of copper among various organs:- The distribution pattern of copper in various organs was studied at 0.1 ppm copper solution; the results are given in Table 7.5b and Fig 7.6a .

Table 7.5b

Accumulation of Cu among various organs of *P. viridis* exposed to 0.1 ppm copper solution in sea water of salinity, 25‰.

Organ	% of body weight	Cu, µg/g dry wt. (control)	Con. of Cu, µg/g dry wt. Mean ± S.D. Exposure time 2 days	4 days	6 days	Con. factor at 6 days
Muscle	22.84	26.71	36.99	29.29	58.38	583.75
		1.07	1.79	1.56	1.80	
Mantle	37.73	21.27	32.60	58.44	76.87	768.70
		1.03	1.68	2.32	2.71	
Gills	13.72	45.71	78.93	119.54	166.18	1661.80
		1.64	3.12	3.35	4.30	
Viscera	25.69	27.17	56.28	88.18	119.56	1195.60
		1.11	1.94	3.46	3.98	

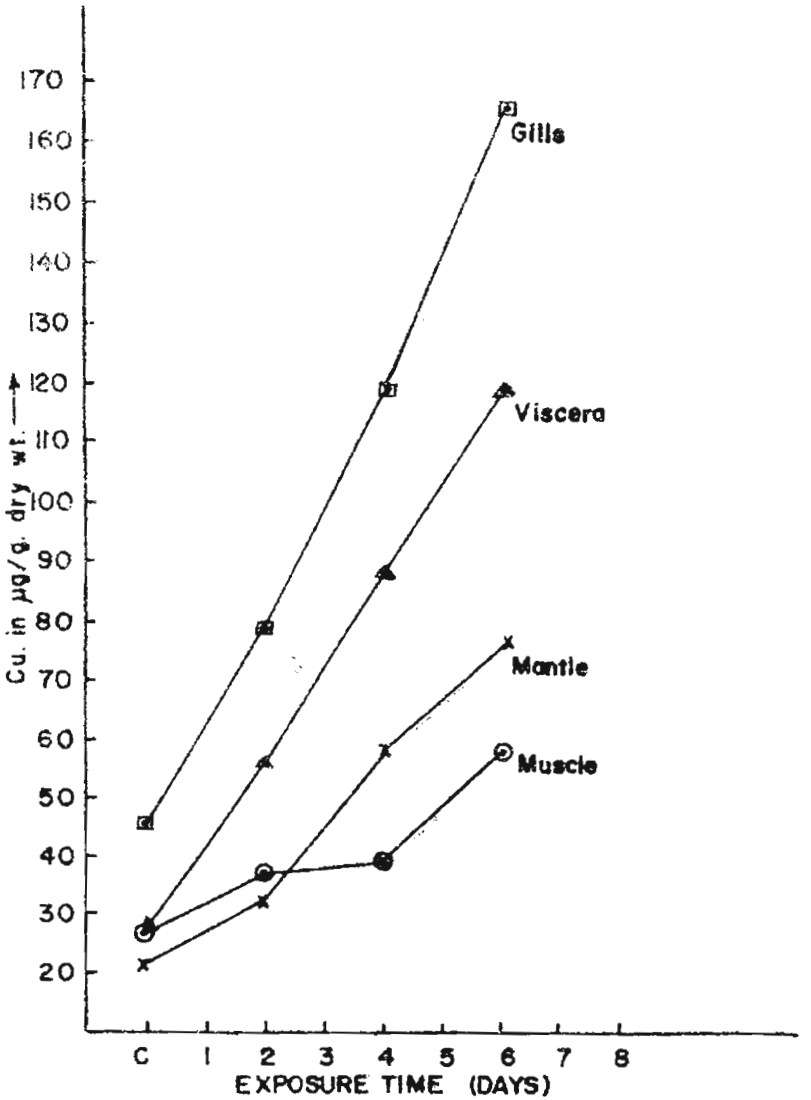

Fig. 7.6a. Accumulation of copper among various organs of P. viridis at 0·1 ppm Cu^{2+} solution.

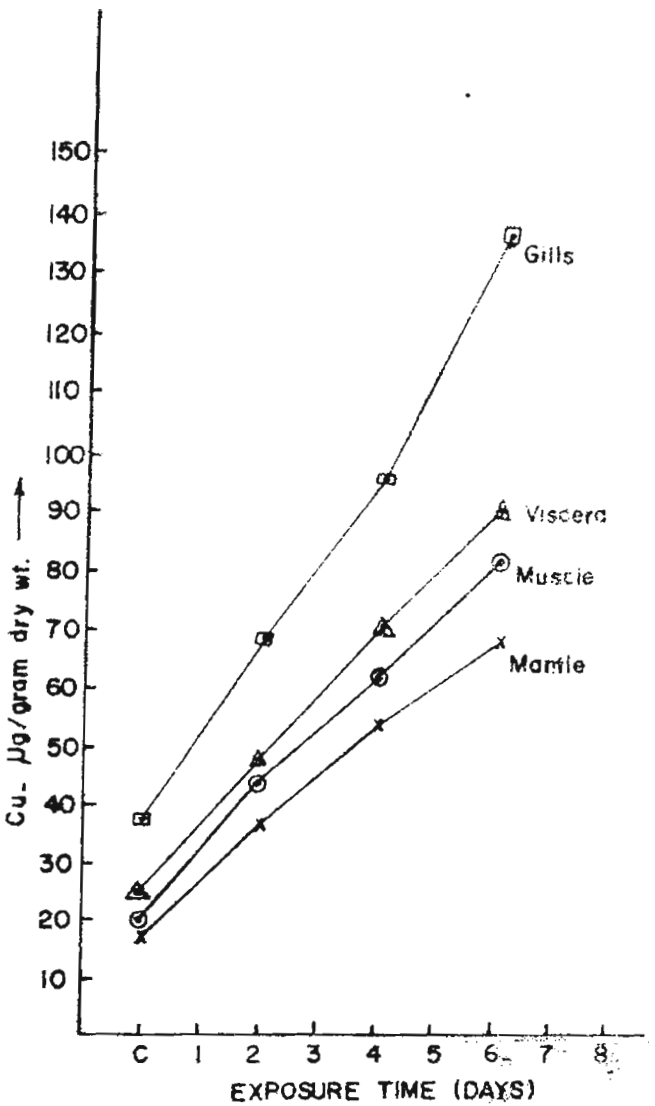

Fig. 7.6b. Accumulation of copper among various organs of V. cyprinoids at 0·5 ppm Cu^{2+} solution.

Copper was found to be accumulated by all the components studied. In all the experiments the highest concentration of copper was found in the gills. The order of distribution was Gills > Viscera > Mantle > Muscle.

b) By Villorita cyprinoides:- The results of the accumulation studies are given in Table 7.6a and Fig 7.2b .

The accumulation of copper was found to be dependent on the environmental level of the metal. The highest tissue concentration of copper was found at 2.00 ppm Cu solution

Table 7.6a

Accumulation of Cu in the tissues of the clam, V. cyprinoides exposed to various concentrations of $CuSO_4$ solution in sea water of salinity, 5‰, for varying lengths of time.

Con. of Copper in ppm	Tissue con. of Cu, µg/g dry wt. Mean ± S.D.			Con. factor at 6 days
	Exposure time			
	2 days	4 days	6 days	
0.25	31.68	37.62	44.29	177.16
	0.81	1.35	1.87	
0.50	45.12	66.81	82.70	165.40
	1.25	2.16	2.44	
1.00	67.38	110.56	141.67	141.67
	1.70	2.85	4.37	
2.00	80.63	132.77	168.68	84.34
	1.61	2.93	2.66	
Control	26.32			
	0.65			

(168.68 µg/g dry wt.) at the end of 6 days. At the lowest concentration tested (0.25 ppm), tissue copper content increased by 1.68 times that in the control animals.

The concentration factor was highest (177.16) in 0.25 ppm and the C.F. value decreased with increasing metal concentration. Thus at 2.00 ppm copper, it was only 84.34.

Distribution of copper among various organs:- The nature of uptake and distribution pattern of copper among the various organs of the clam, V. cyprinoides are presented in Table 7.6b and Fig 7.6b .

Table 7.6b

Accumulation of Cu among various organs of V. cyprinoides exposed to 0.5 ppm copper solution in sea water of salinity, 5‰.

Organ	% of body weight	Cu, µg/g dry wt. (control)	Con. of Cu, µg/g dry wt. Mean ± S.D. Exposure time 2 days	4 days	6 days	Con. factor at 6 days
Muscle	22.43	19.81	43.73	62.40	81.98	163.96
		1.54	2.46	2.22	2.90	
Mantle	16.04	15.99	36.07	53.36	68.47	136.94
		0.85	2.16	2.09	2.02	
Gills	11.01	37.39	68.28	95.75	136.58	273.16
		1.67	2.20	2.41	3.11	
Viscera	50.53	24.25	46.92	69.84	90.68	181.36
		1.32	1.42	2.45	2.32	

Gill tissue of the clam was found to be the major site of copper deposition . The copper content in the gills increased from a background level of 37.39 µg/g (dry wt.) to the highest value of 136.58 µg/g (dry wt.) after an exposure time of 6 days. The next major site of accumulation was viscera (90.68 µg/g) followed by the muscle and then the mantle. The highest concentration factor was found with the gills. The C.F. decreased in the order: Gills > Viscera > Muscle > Mantle (the ratio being 20:13:12:10).

c) By *Meretrix casta*:- The accumulation of copper from various environmental levels of the metal ion and the concentration factor determined at 6 days are shown in the Table 7.7a

Table 7.7a

Accumulation of copper in the tissues of *M. casta* exposed to various concentrations of $CuSO_4$ solution in sea water of salinity, 10‰ for varying lengths of time.

Con. of Copper in ppm	Tissue con. of Cu, µg/g dry wt. Mean ± S.D.			Con. factor at 6 day
	Exposure time			
	2 days	4 days	6 days	
0.50	40.85 1.25	72.02 2.35	96.81 2.21	193.61
1.00	51.10 2.17	85.32 2.52	116.52 2.63	116.52
2.00	63.95 2.33	101.53 2.78	132.65 3.17	66.33
Control	19.64 0.87			

Copper was taken up in the tissues of M. casta at all environmental concentrations. The uptake was found to be dependent on the metal ion concentration in the medium (Fig 7.2c). At the end of 6 days, the tissue level of copper increased from 19.64 µg/g to a maximum value of 132.65 µg/g (dr. wt.) in 2.00 ppm solution. However, the concentration factors decreased with increasing concentration of metal.

Distribution of copper among various organs:- The results are given in Table 7.7b and Fig 7.6c .

Table 7.7b

Accumulation of copper among various organs of M. casta exposed to 1.00 ppm copper solution in sea water salinity, 10‰.

Organ	% of body weight	Cu, µg/g dry wt. (control)	Con. of Cu, µg/g dry wt. Mean ± S.D. Exposure time 2 days	4 days	6 days	Con. factor at 6 days
Muscle	30.86	30.59	59.70	102.69	128.60	128.60
		2.23	2.59	4.16	5.36	
Mantle	20.11	19.53	38.92	46.05	57.14	57.14
		1.80	3.08	5.44	2.54	
Gills	9.59	68.34	98.37	145.10	179.76	179.76
		6.11	5.97	6.96	5.24	
Viscera	39.44	25.79	37.76	50.85	58.39	58.39
		1.11	2.76	3.64	2.96	

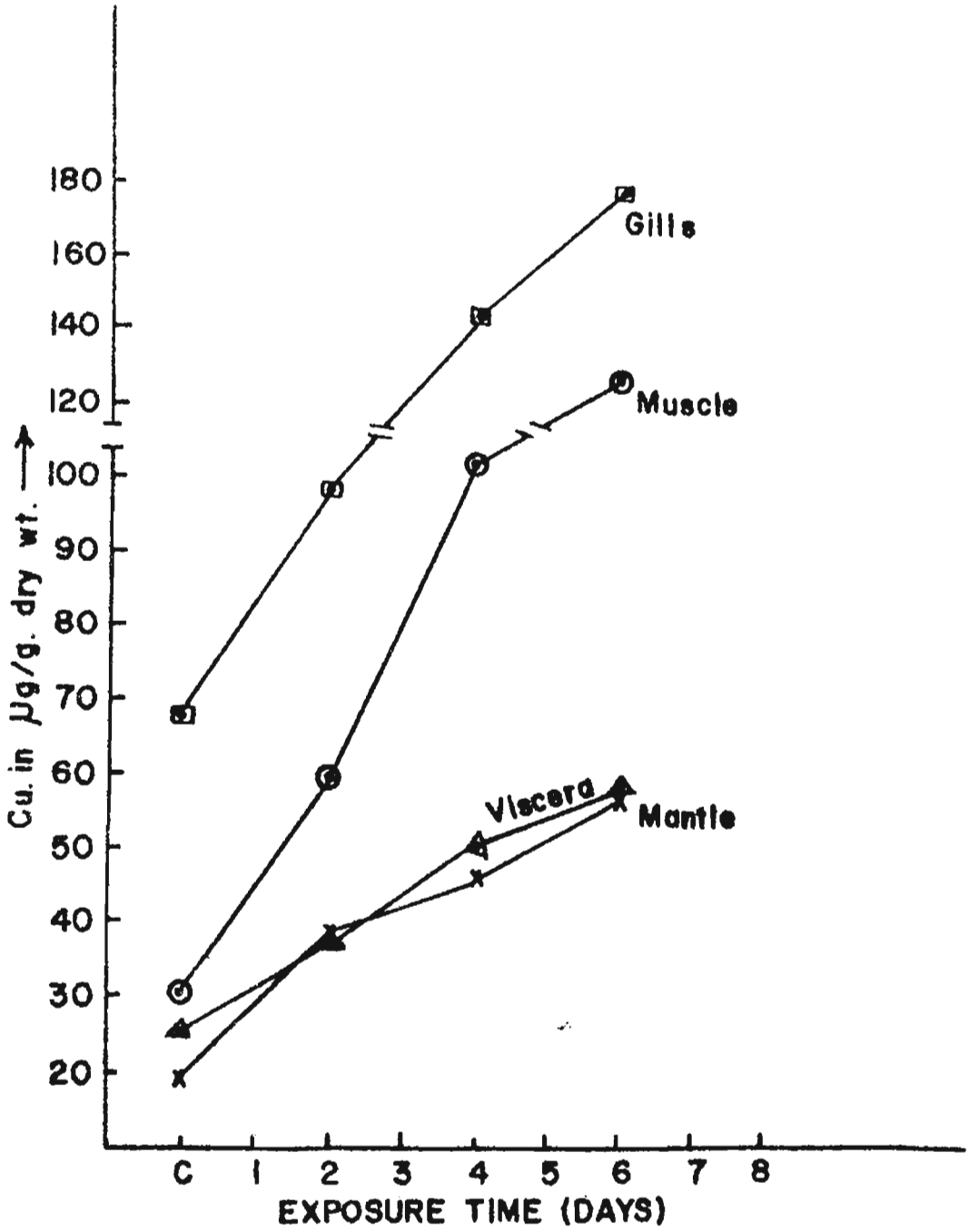

Fig. 7.6c. Accumulation of copper among various organs of M. casta, at 1·00ppm Cu^{2+} solution

The copper content registered a significant increase in all the components studied. Gills of the animal was again found to serve as the major site of metal accumulation. The copper content in the gills increased from 68.34 µg/g (dry wt.) (of control animals) to a value of 179.75 µg/g during a 6 day period. The muscle tissue was the next major site of copper accumulation (128.60 µg/g) in 6 days period. The levels of copper in mantle and viscera followed almost a similar pattern during the experimental period. The order of copper distribution in P. costa was: Gills > Muscle > Viscera > Mantle.

C. Accumulation of zinc

a) By Perna viridis:- P. viridis, when exposed to various concentrations of zinc ions (in the medium), took up the metal in its tissues. The results are given in Table 7.8a . The uptake pattern was almost linear with time (Fig 7.3a). The zinc content in the tissue showed a 4 fold increase in 2.00 ppm solution at the end of 6 days. At 0.5 ppm and 1.00 ppm solutions, an increase of 1.5 and about 3 fold were recorded during the same length of time. The concentration factor on the other hand decreased from 0.5 ppm to 2.00 ppm solutions and the values ranged from 219.04 to 144.25.

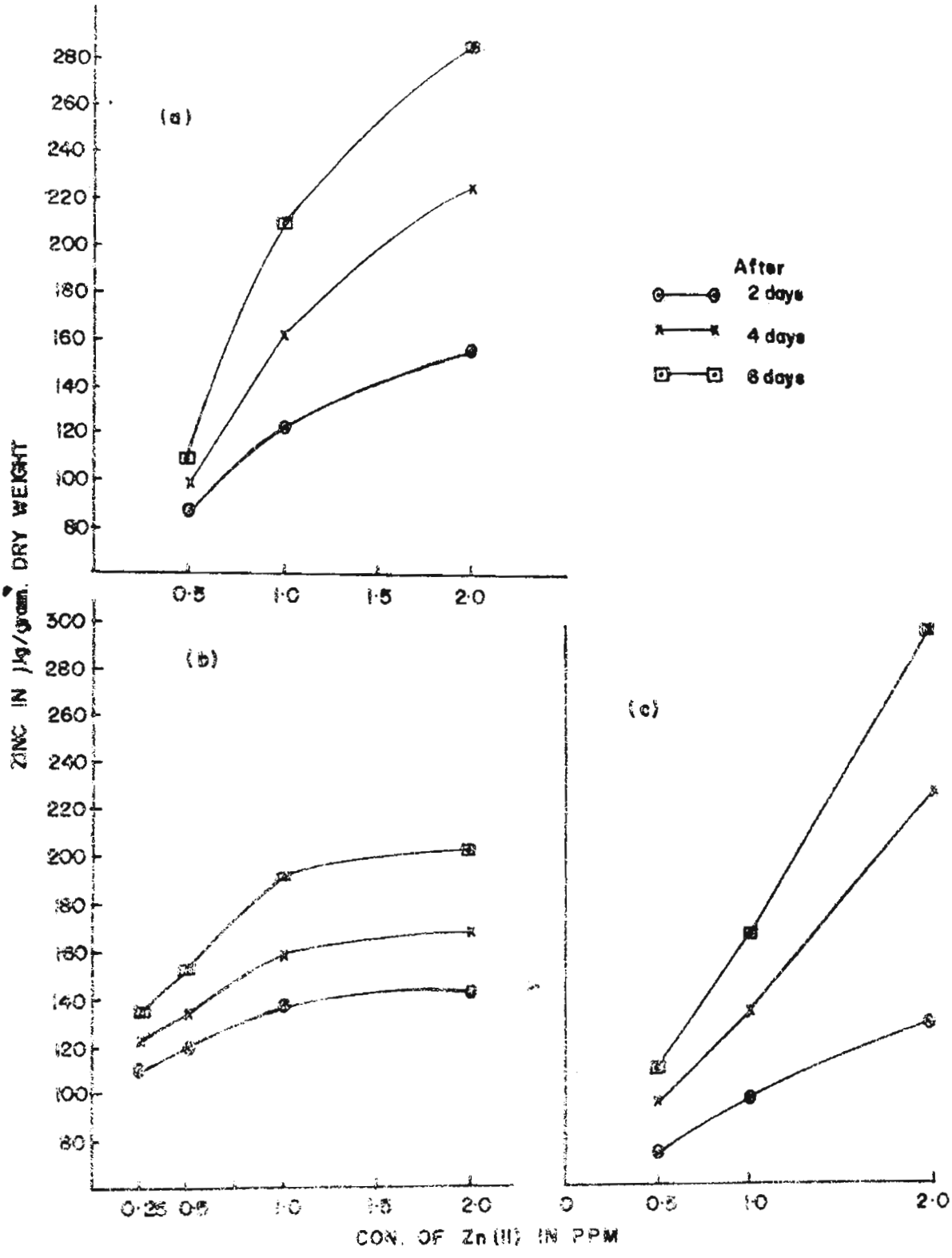

Fig. 7.3 Accumulation of zinc by (a) P. viridis, (b) V. cyprinoides and (c) M. casta in relation to zinc concentration in the medium at different periods of time

Table 7.8a

Accumulation of Zn in the tissues of P. viridis exposed to various concentrations of $ZnSO_4$ solution in sea water of salinity, 25‰ for varying lengths of time.

Con. of Zinc in ppm	Tissue con. of Zn, µg/g dry wt. Mean ± S.D. Exposure time 2 days	4 days	6 days	Con. factor at 6 days
0.50	86.04	97.85	109.82	219.64
	2.25	2.60	3.19	
1.00	123.40	162.18	211.09	211.09
	3.64	4.64	4.90	
2.00	155.37	226.36	288.50	144.25
	4.75	5.61	7.86	
Control	73.20			
	2.34			

Distribution of zinc among various organs:- The results of the analysis of various tissue components are given in Table 7.8b and Fig 7.7a..

Zinc was accumulated in all the body components tested, however, differed in magnitude. Gills and viscera were the major site of Zn accumulation and gills showed the highest value (247.50 µg/g dry wt.) at the end of 6 days. The ranking was in the order: Gills > Viscera > Muscle > Mantle, based on the concentration factors.

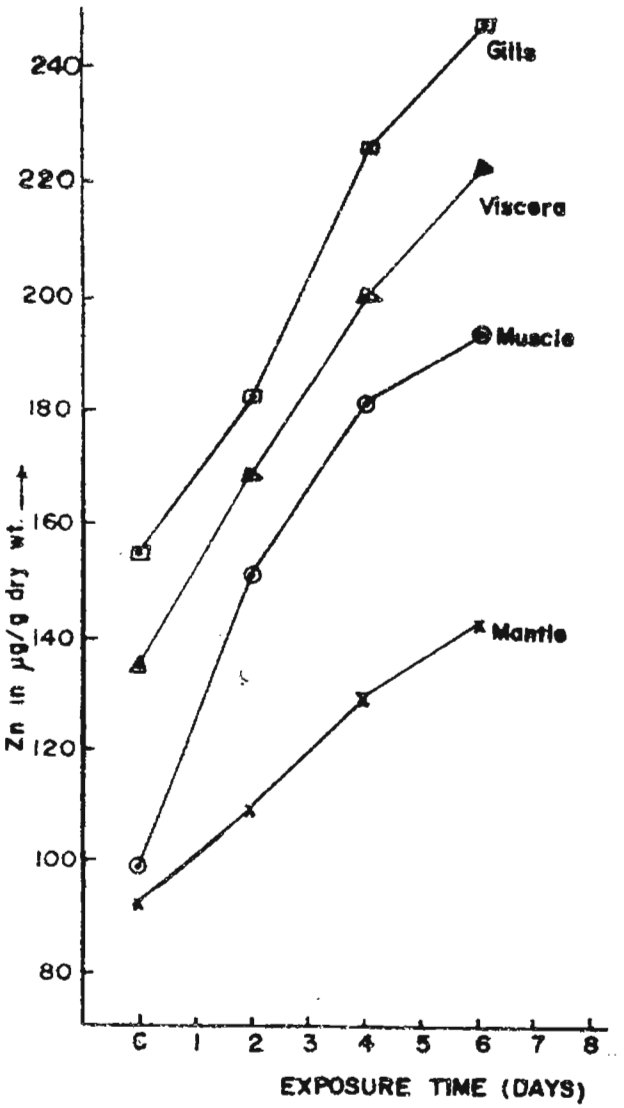

Fig. 7.7a. Accumulation of zinc among various organs of P viridis at 1 ppm Zn^{2+} solution.

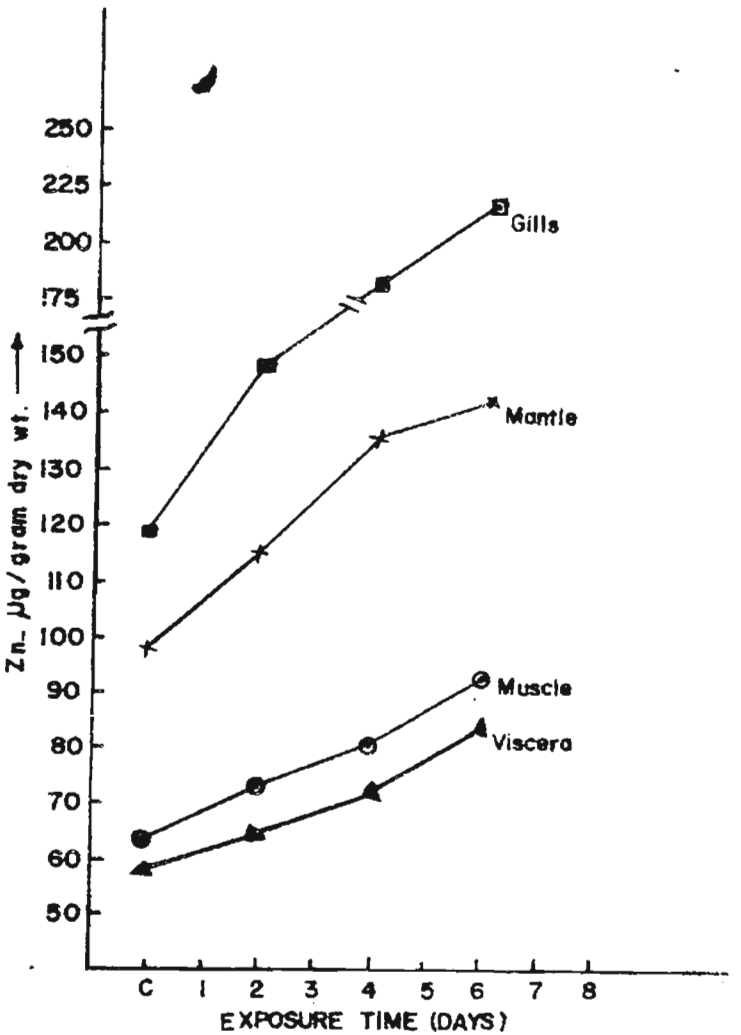

Fig. 7.7b. Accumulation of zinc among various organs of V. cyprinoides at 1 ppm Zn^{2+} solution.

Table 7.8b

Accumulation of Zn among various organs of P. viridis exposed to 1.00 ppm zinc solution in sea water of salinity, 25‰.

Organ	% of body weight	Zn, µg/g dry wt. (control)	Con. of Zn, µg/g dry wt. Mean ± S.D. Exposure time 2 days	4 days	6 days	Con. factor at 6 days
Muscle	20.62	98.73	151.36	182.36	194.34	194.34
		2.52	5.57	4.23	5.64	
Mantle	39.59	91.88	108.81	129.35	142.78	142.78
		2.51	2.94	4.76	4.63	
Gills	14.11	154.26	182.58	226.19	247.50	247.50
		3.92	4.65	5.96	9.32	
Viscera	25.68	134.65	169.22	200.48	223.63	223.63
		2.75	3.99	6.35	6.19	

b) By Villorita cyprinoides:- The tissue concentration of zinc increased at all levels of environmental zinc ions and the values were dependent on the concentration of Zn in the medium. The results are shown in Table 7.9a and Fig 7.3b .

There was a 2 fold increase in the level of tissue concentration of Zn in 2.00 ppm solution after 6 days. The uptake of Zn showed little difference at higher concentrations (e.g. 1.00 and 2.00 ppm). The concentration factor was the highest at 0.25 ppm of Zn (534.28) and lowest at 2.00 ppm (101.17).

Table 7.9a

Accumulation of Zn in the tissues of *V. cyprinoides* exposed to various concentrations of $ZnSO_4$ solution in sea water of salinity, 8‰ for varying lengths of time.

Con. of Zinc in ppm	Tissue con. of Zn, µg/g dry wt. Mean ± S.D. Exposure time 2 days	4 days	6 days	Con. factor at 6 days
0.25	109.09	121.07	133.57	534.28
	2.33	2.51	4.97	
0.50	118.96	133.17	152.30	304.60
	2.06	3.67	4.92	
1.00	135.80	159.52	191.48	191.48
	2.71	6.52	5.67	
2.00	141.80	168.32	202.33	101.17
	3.73	5.04	5.76	
Control	98.72			
	3.50			

Distribution of zinc among various organs:- The rate of uptake and distribution pattern of Zn among the various tissue components are presented in Table 7.9b and Fig 7.7b .

The highest concentration was found in the gill tissues (218.34 µg/g). Muscle and viscera followed almost a similar type of uptake. The order being: Gills > Mantle > Muscle > Viscera.

Table 7.9b

Accumulation of Zn among various organs of V. cyprinoides exposed to 1.00 ppm zinc solution in sea water of salinity, 16‰.

Organ	% of body weight	Zn, µg/g dry wt. (control)	Con. of Zn, µg/g dry wt. Mean ± S.D. Exposure time 2 days	4 days	6 days	Con. factor at 6 days
Muscle	28.87	63.60	72.87	80.98	93.52	93.52
		2.34	3.18	3.11	2.72	
Mantle	11.31	98.37	115.63	136.46	143.25	143.25
		2.42	3.65	3.30	4.70	
Gills	9.54	119.40	148.69	183.75	218.34	218.34
		2.86	3.76	4.21	10.00	
Viscera	50.28	58.17	64.23	72.19	83.70	83.70
		1.01	2.21	2.51	2.63	

c) By Meretrix casta:- The rate of uptake of Zn by the clam from different environmental levels of the metal are given in Table 7.10a .

The tissue level of Zn was highest (298.25 µg/g) at 2.00 ppm concentration and lowest (109.56 µg/g) at 0.5 ppm after an exposure time of 6 days. The uptake of Zn was found to be proportional to the environmental concentration of the metal. The uptake behaviour can be seen from the Fig 7.3c .

The concentration factor decreased with increasing concentration of Zn. The values ranged from 149.13 to 219.12.

Table 7.10a

Accumulation of Zn in the tissues of M. costa exposed to various concentrations of $ZnSO_4$ solution in sea water of salinity, 25‰ for varying lengths of time.

Con. of Zinc in ppm	Tissue con. of Zn, µg/g dry wt. Mean ± S.D. Exposure time 2 days	4 days	6 days	Con. factor at 6 days
0.50	72.85	93.70	109.56	219.12
	2.77	2.57	3.01	
1.00	97.34	134.82	166.79	166.79
	3.11	3.51	6.52	
2.00	129.70	228.60	298.25	149.13
	3.96	7.31	8.89	
Control	54.10			
	1.36			

Distribution of zinc among various organs:- Zinc was distributed in all the body components studied, but differed in magnitude. Highest levels were found in the gill tissues (310.22 µg/g) and muscle tissue (283.91 µg/g) during 6 days period. Viscera and mantle accumulated comparatively lesser amounts of the metal. The order of distribution being: Gills > Muscle > Viscera > Mantle, with respect to the C.F. The details of the results are presented in Table 7.10b and Fig 7.7c would indicate the general trend in the uptake pattern.

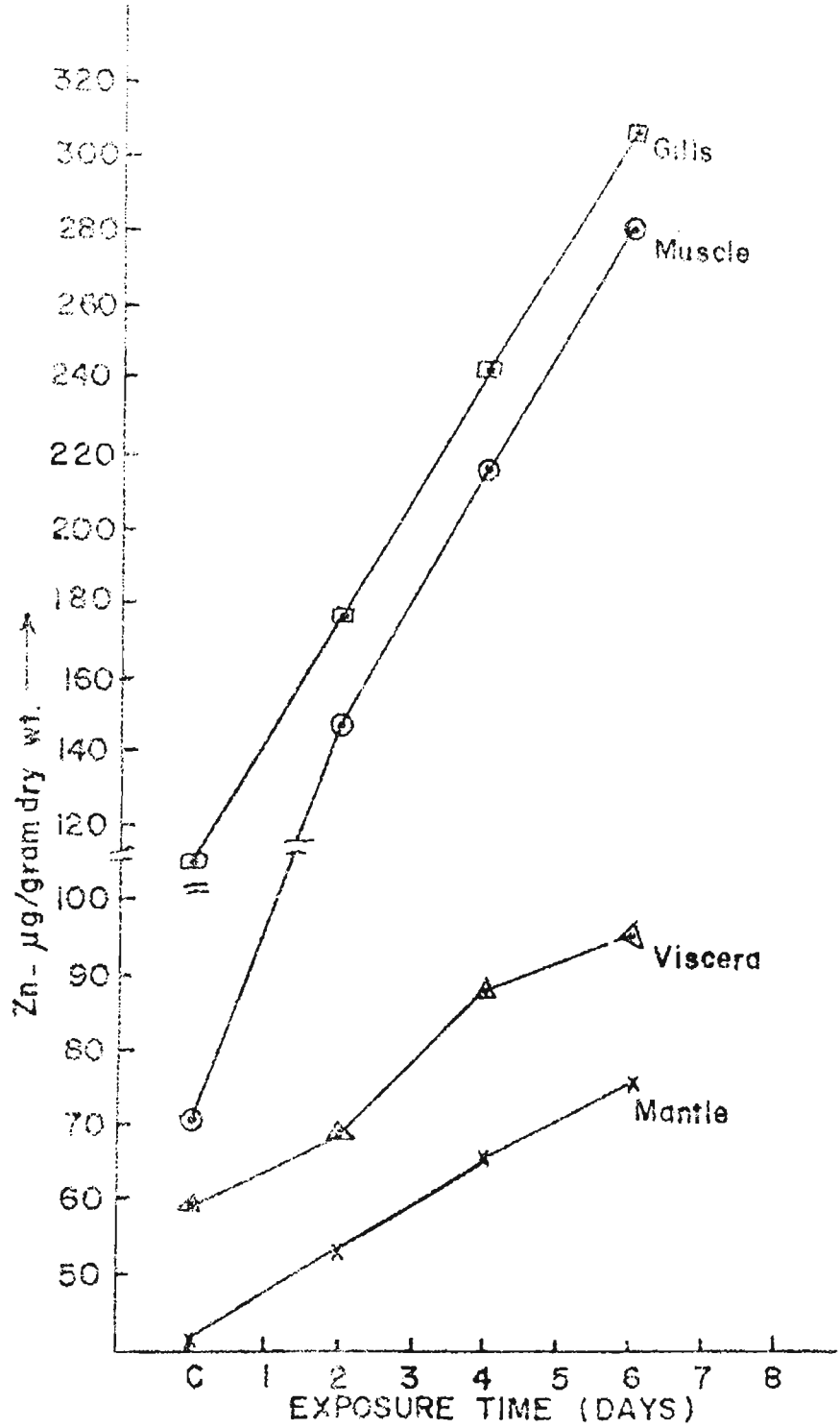

Fig 7.7c. Accumulation of zinc among various organs of M. casta, at 2·00 ppm Zn^{2+} solution.

Table 7.10b

Accumulation of Zn among various organs of M. costa exposed to 2.00 ppm zinc solution in sea water of salinity, 10‰.

Organ	% of body weight	Zn, µg/g dry wt. (control)	Con. of Zn, µg/g dry wt. Mean ± S.D. Exposure time 2 days	4 days	6 days	Con. factor at 6 days
Muscle	29.44	70.79	147.98	217.62	283.91	141.96
		2.27	4.46	8.83	9.58	
Mantle	22.62	41.42	53.29	65.61	76.09	38.04
		1.78	2.56	3.04	3.30	
Gills	10.38	109.95	177.76	245.05	310.22	155.11
		3.79	5.05	7.45	9.85	
Viscera	37.57	59.09	68.78	87.46	95.89	47.95
		2.05	1.98	2.34	2.70	

D. Accumulation of lead

a) By Perna viridis:- Lead was found to be concentrated to a very high level in the mussel tissue. The metal uptake was found to be directly proportional to the concentration of lead in the medium. The results are given in the Table 7.11a and Fig 7.4a .

The levels of lead in the tissue increased from a background value of 10.03 µg/g to 429.18 µg/g (dry wt.) in 0.25 ppm and to 4938.01 µg/g (dry wt.) in 2.00 ppm environments

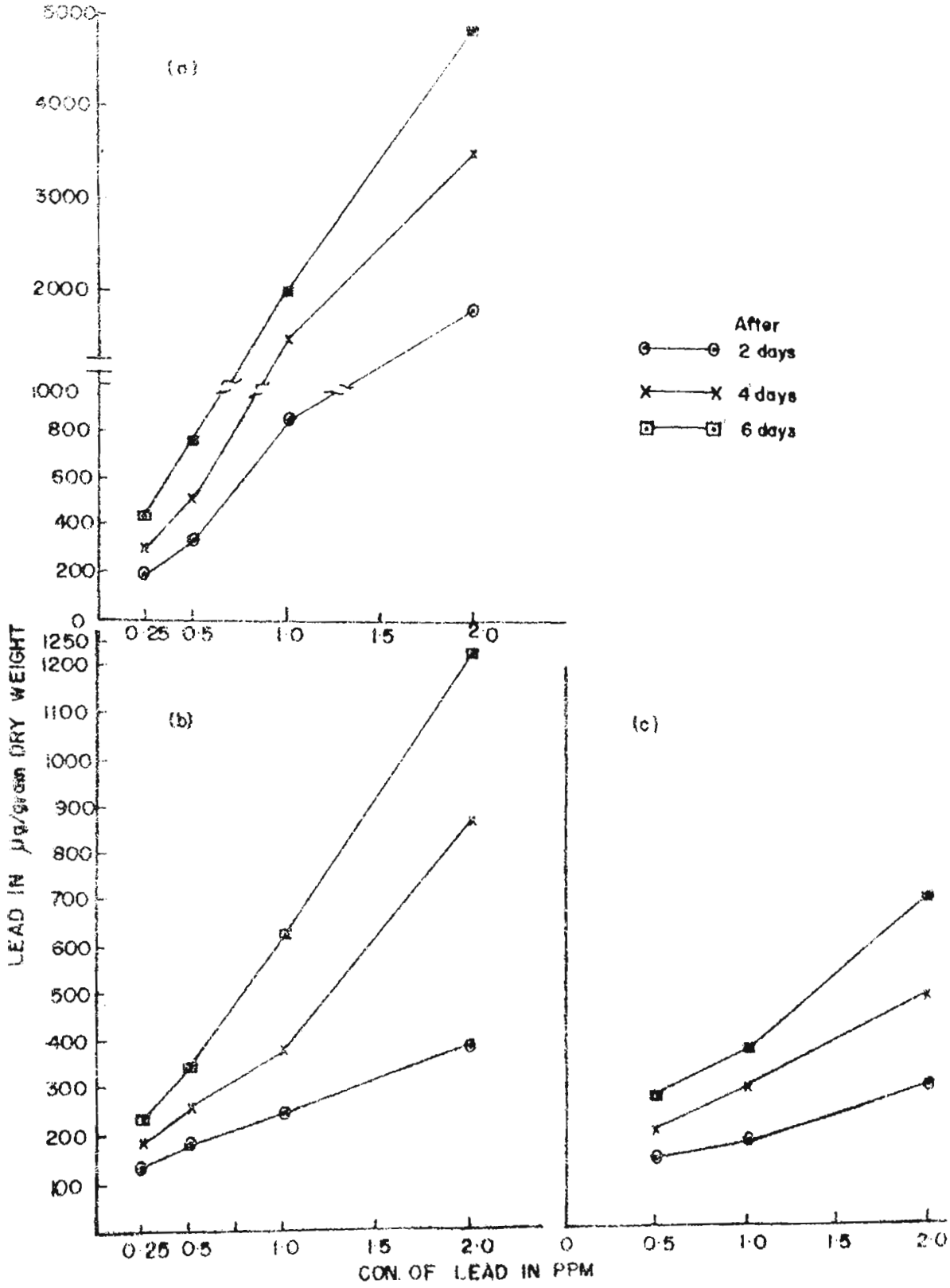

Fig. 7.4. Accumulation of lead by (a) P. viridis, (b) V. cyprinoides and (c) M. casta in relation to lead concentration in the medium at different periods of time.

lead. Thus at 2.00 ppm, there was a magnification of about 492 times during 6 days. At lower concentrations the increase were 42.8, 75.5 and 213.5 fold in 0.25, 0.5 and 1.00 ppm solutions respectively. In the case of lead uptake, the concentration factor increased with increasing concentration of lead in the medium (Table 7.11a).

Table 7.11a

Accumulation of Pb in the tissues of P. viridis exposed to various concentrations of $Pb(NO_3)_2$ solution in sea water of salinity, 25‰ for varying lengths of time.

Con. of Lead in ppm	Tissue con. of Pb, µg/g dry wt. Mean ± S.D.			Con. factor at 6 days
	Exposure time			
	2 days	4 days	6 days	
0.25	188.74	296.45	429.18	1716.72
	6.57	5.38	7.05	
0.50	329.64	561.30	757.18	1514.36
	8.99	20.43	22.38	
1.00	851.03	1505.63	2141.69	2141.69
	15.19	21.27	25.53	
2.00	1821.18	3571.19	4936.21	2469.01
	23.55	34.41	38.40	
Control	10.03			
	0.44			

Distribution of lead among various organs:- The rate of uptake of lead was high for all the organs. However, it was distributed rather unevenly among these various tissue components. Gills of the mussel was by far the major site of lead accumulation. The tissue burden of lead in the gills was as high as 5330.74 µg/g at the end of 6 days. The next major site of lead accumulation was the visceral part(2995.61 µg/g in 6 days). The distribution pattern is, Gills > Viscera > Muscle > Mantle. The results along with the C.F. values are presented in Table 7.11b . The nature of uptake can be seen from the Fig 7.8a .

Table 7.11b

Accumulation of Pb among various organs of P. viridis exposed to 2.00 ppm of lead solution in sea water of salinity, 25 °.

Organ	% of body weight	Pb, µg/g dry wt. (control)	Con. of Pb, µg/g dry wt. Mean ± S.D. Exposure time 2 days	4 days	6 days	Con. factor at 6 days
Muscle	30.30	9.72	881.77	1530.29	2240.17	1120.08
		0.23	25.36	35.77	44.48	
Mantle	42.77	6.35	440.41	835.31	1083.26	541.63
		0.18	28.37	28.98	26.88	
Gills	10.46	13.45	1988.72	3801.67	5330.74	2665.37
		0.45	22.70	47.23	68.95	
Viscera	16.47	8.84	1046.35	2118.95	2995.61	1497.81
		0.22	12.48	33.10	34.44	

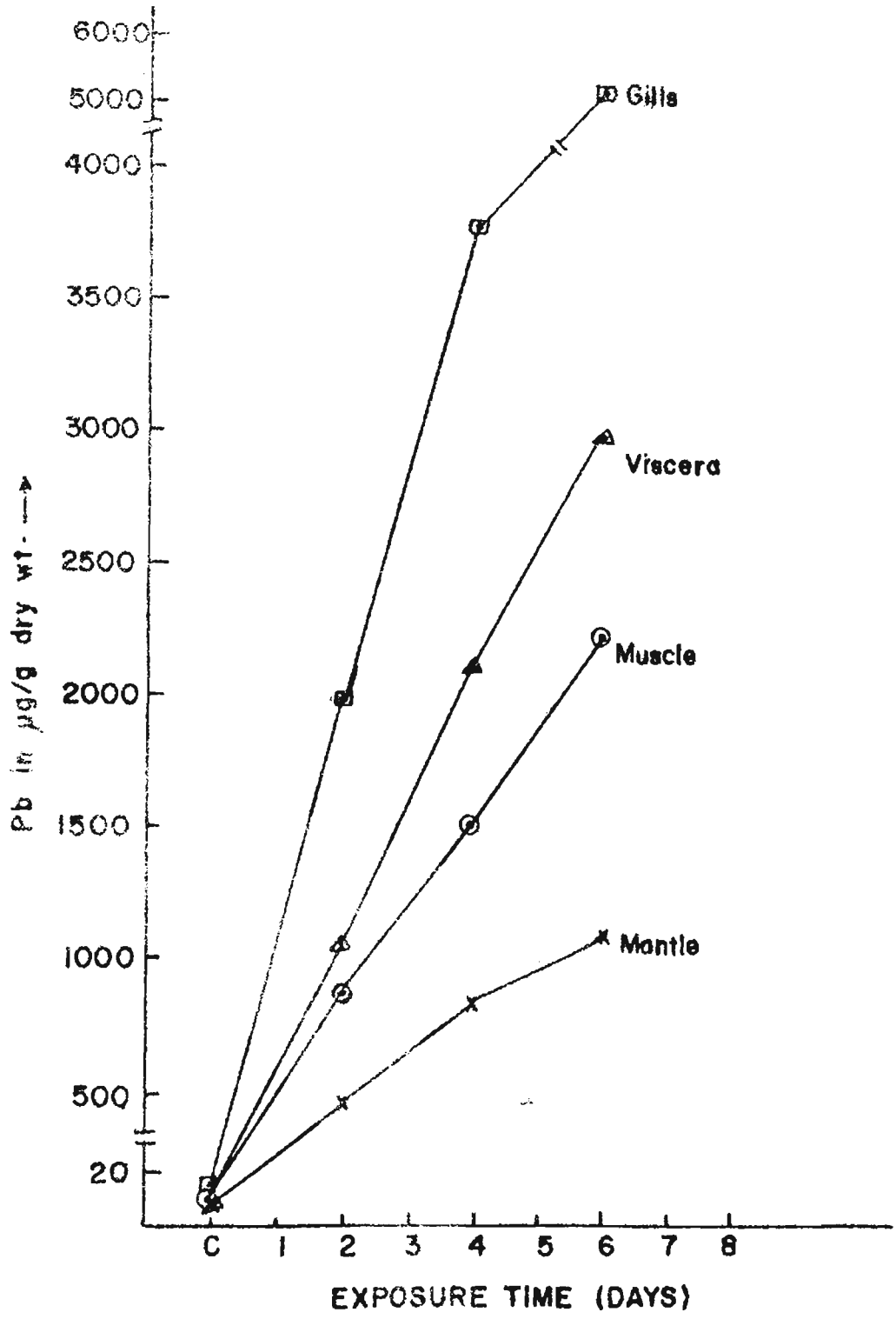

Fig. 7. 8a. Accumulation of lead among various organs of P. viridis at 2 ppm Pb^{2+} solution.

b) By Villorita cyprinoides:- There was a greater rate of uptake of lead in the tissues of the clam. The lead content in the soft parts went up to 1240.78 µg/g (dry wt.) in 2.00 ppm solution at the end of 6 days from a background value of 10.05 µg/g. This was equivalent to a 123.5 fold increase. In 1.00 ppm solution the lead content in the tissue increased by 63 fold compared to the control animals. The C.F. was highest (952.8) at 0.25 ppm solution and decreased with increasing concentration of the metal. Results are given in Table 7.12a . The uptake behaviour can be seen from the Fig 7.4b .

Table 7.12a

Accumulation of Pb in the tissues of V. cyprinoides exposed to various concentrations of $Pb(NO_3)_2$ solution in sea water of salinity, 8‰ for varying lengths of time.

Con. of Lead in ppm	Tissue con. of Pb, µg/g dry wt. Mean ± S.D. Exposure time 2 days	4 days	6 days	Con. factor at 6 days
0.25	139.17 5.26	188.21 6.72	238.20 9.04	952.80
0.50	184.03 6.79	264.75 6.58	352.25 9.90	704.50
1.00	247.81 8.74	386.82 9.08	633.48 15.06	633.48
2.00	395.14 14.62	876.39 23.14	1240.78 25.69	620.39
Control	10.05 0.39			

Distribution of lead among various organs:- Table 7.12b gives the lead content in the various organs of the animal on exposure to 2.00 ppm solutions of lead. The rate of uptake of lead was highest with the gills. The gill tissue content of lead increased to 3208.74 µg/g (dry wt.) from a control value of 26.47 µg/g. The order of distribution being: Gills ≫ Mantle > Viscera > Muscle. The uptake pattern was almost linear with time (Fig 7.8b). The concentration factors ranged from 316.20 to 1604.37.

Table 7.12b

Accumulation of Pb among various organs of *V. cyprinoides* exposed to 2.00 ppm of lead solution in sea water of salinity, 8‰.

Organ	% of body weight	Pb, µg/g dry wt. (control)	Con. of Pb, µg/g dry wt. Mean ± S.D. Exposure time 2 days	4 days	6 days	Con. factor at 6 days
Muscle	29.97	9.46	286.45	463.57	632.39	316.20
		0.18	9.85	12.46	14.24	
Mantle	9.46	14.58	662.73	1169.63	1636.44	818.22
		0.37	15.45	27.32	38.80	
Gills	12.70	26.47	1278.52	2280.49	3208.74	1604.37
		1.03	29.94	37.51	47.94	
Viscera	47.88	7.84	312.50	534.30	744.86	372.43
		0.18	9.37	11.80	17.50	

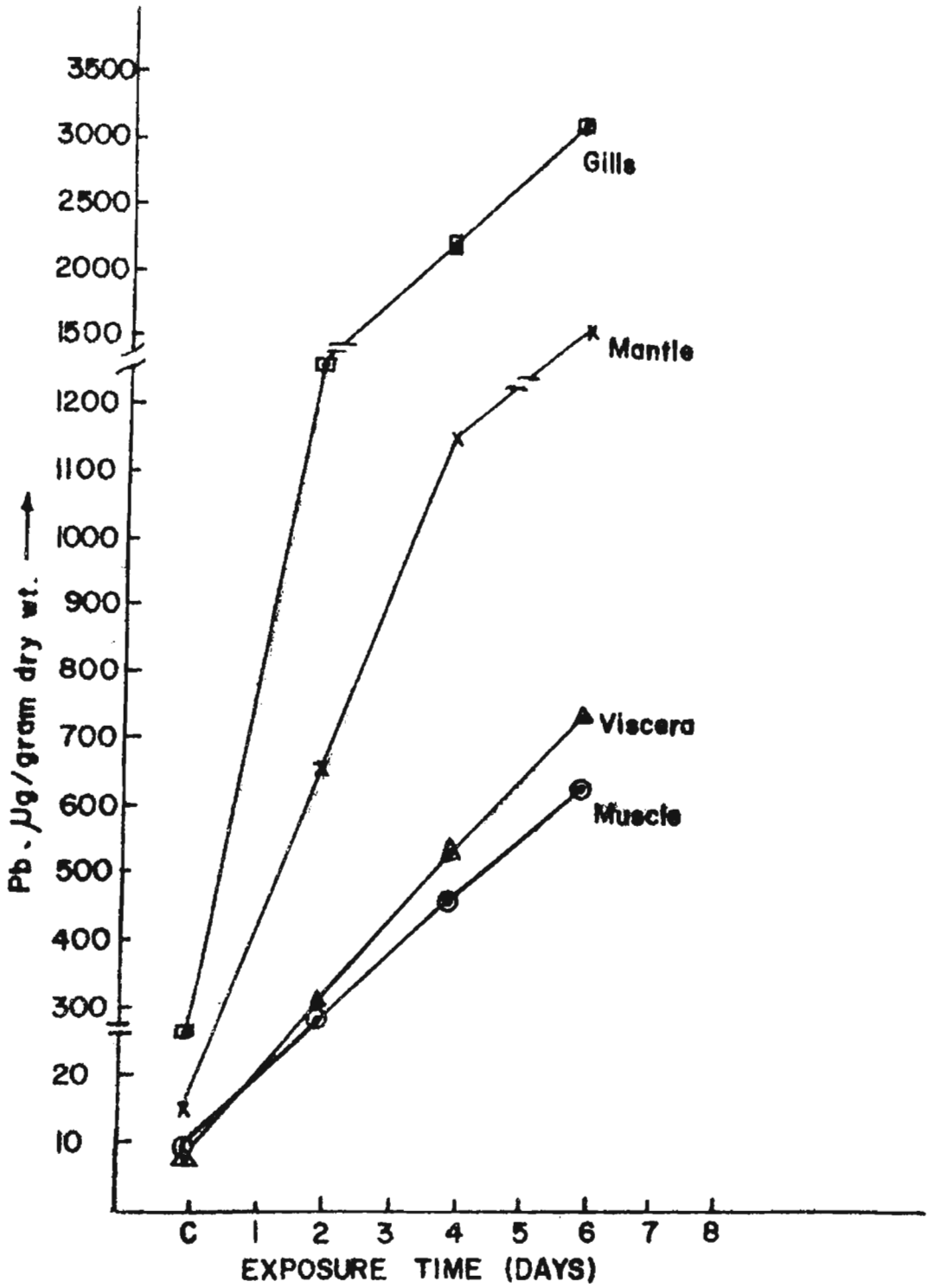

Fig .7.8b. Accumulation of lead among various organs of V. cyprinoides at 2·00 ppm. Pb^{2+} solution.

c) By *Meretrix casta*:- Lead was found to be accumulated to a very high level in the tissues of the clam from environmental water. The highest value was found in 2.00 ppm Pb (718.32 µg/g dry wt.) and lowest in 0.5 ppm solution (287.18 µg/g) during 6 days period. The highest concentration factor was obtained at 0.50 ppm (574.36) and it decreased with increasing concentration of the metal. The results are given in Table 7.13a .

It can be seen that the magnifications were 57.6, 37 and 27 folds in 2.00 ppm, 1.00 ppm and 0.5 ppm solutions respectively.

Table 7.13a

Accumulation of Pb in the tissues of *M. casta* exposed to various concentrations of $Pb(NO_3)_2$ solution in sea water of salinity, 11.5‰ for varying lengths of time.

Con. of Lead in ppm	Tissue con. of Pb, µg/g dry wt. Mean ± S.D.			Con. factor at 6 days
	Exposure time			
	2 days	4 days	6 days	
0.50	149.88	212.64	287.18	574.36
	5.48	10.54	9.56	
1.00	182.90	303.55	392.33	392.33
	5.69	14.06	10.34	
2.00	311.66	502.24	718.32	359.16
	12.90	13.17	17.57	
Control	10.63			
	0.46			

Distribution of lead among various organs:- The distribution of lead among the various body components of the clam are presented in Table 7.13b . The general trend followed in the uptake behaviour can be appreciated from the Fig 7.8c .

Rate of lead accumulation was the highest in the gills, the metal content was 924.38 µg/g(dry wt.)at 6 days. The ranking followed the order: Gills > Muscle > Viscera > Mantle.

Table 7.13b

Accumulation of Pb, among various organs of *M. casta* exposed to 2.00 ppm lead solution in sea water of salinity, 11.5‰.

Organ	% of body weight	Pb, µg/g dry wt. (control)	Con. of Pb, µg/g dry wt. Mean ± S.D. Exposure time 2 days	4 days	6 days	Con. factor at 6 days
Muscle	29.46	10.56	298.90	492.65	690.81	335.41
		0.37	10.92	16.74	22.21	
Mantle	18.60	7.01	216.45	306.13	484.23	242.12
		0.18	9.92	10.23	14.23	
Gills	7.94	13.23	389.76	640.67	924.38	462.19
		0.51	16.27	18.47	26.92	
Viscera	44.00	7.39	252.84	420.59	589.27	294.64
		0.21	9.55	11.71	15.90	

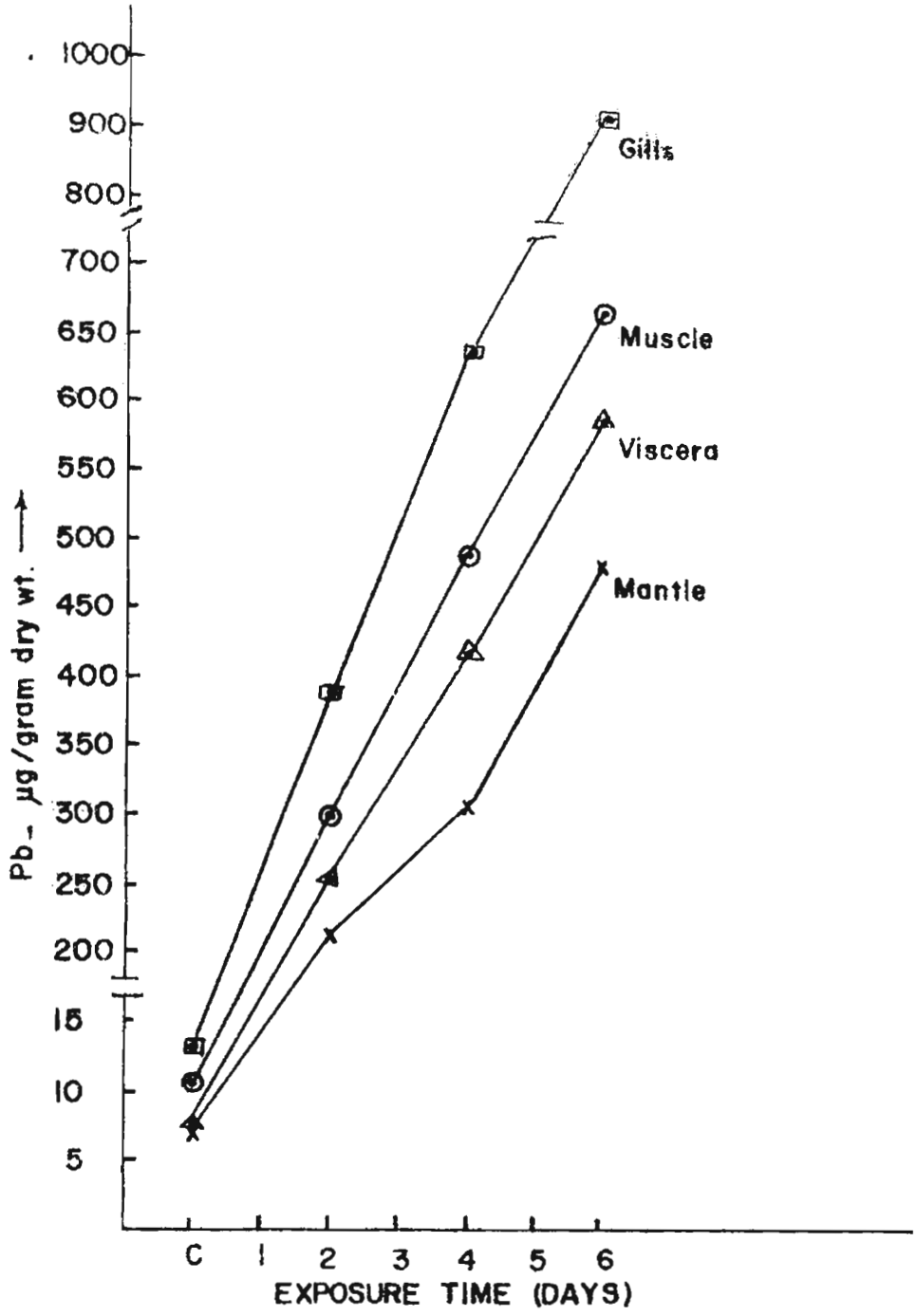

Fig 7.8c. Accumulation of lead among various organs of M.casta, at 2·00 Pb^{2+} solution.

7.3. Discussion

Tables 7.2a to 7.13b would clearly show the ability of the three bivalve molluscs to accumulate heavy metals from the environmental water much above the ambient levels. However, they also indicate dissimilarities in the response pattern followed by the three species. The important observation was that the amount of metals found in the tissue of the animals was very much dependent on the concentration of the metal in the medium. The tissue concentration of all the four metals tested (viz. Hg, Cu, Zn and Pb) showed an increase with increasing time of exposure and also with increasing concentration of metal ions (Tables 7.2a to 7.13b and Figs 7.1a to 7.dc).

In the case of mercury, highest concentration factor was obtained for P. viridis at all concentrations. In V. cyprinoides and M. casta the values were quite low (Tables 7.3a & 7.4a). The bioaccumulation factor for copper was also highest in P. viridis. In the other two species the C.F. was approximately 10 and 20 times lower than that in P. viridis. However, the rate of uptake of copper was generally high in all the three animals and the high rate of accumulation of the metal indicate that there is little regulation of this ion by these molluscs.

Zinc uptake seemed to be rather restricted in all the species (Tables 7.8a, 7.9a & 7.10a). There was only about 2 fold increase in the tissue concentration of Zn in V.cyprinoides at the highest concentration tested (2.00 ppm) after 6 days of exposure. In P. viridis there was 4 fold increase and in M.casta about 5.5 times increase in Zn content under the same conditions of metal concentration and time.

It would be seen that the concentration factor for the above three metal ions (Hg, Cu and Zn) decreased with increasing concentration of the metal in the medium showing greater uptake efficiency at lower concentrations. This may probably be due to the deactivating effect of the metal ions at higher concentrations on the organisms. Another factor would be the lower bioavailability of the metal at higher concentrations. A visible precipitate was seen at the surface of the medium as well as on the sides of the experimental trough at higher concentrations of copper. The precipitated particles are beyond the size range of the filter feeders and therefore, would not be assimilated.

However, the uptake pattern for lead was different from the other three metals. It was taken up to a very high level, without any restriction by the bivalves. The mussel, P. viridis ranks first in Pb accumulation. Thus a concentration factor

as high as 2469 was obtained at the end of 6 days. In Villorita and Paretrix also, the rate of uptake and the amount of lead incorporated was the highest compared to other metals. The maximum C.F. attained by these species were 953 and 574 respectively. An important feature of lead accumulation by the mussel was that the C.F. continued to increase with increasing concentration of the metal, showing that the efficiency of uptake was not at all impaired by higher concentrations. It may be remembered that the metal was non-lethal to the animals at these concentrations (Sec 5.2).

The results obtained from the study again indicated that accumulation pattern of tracemetals in molluscs can vary as a function of the species of molluscs and the metal ions studied. Thus, the accumulation of metals by the animals follow the order

P. viridis:	Pb > Cu > Hg > Zn
V. cyprinoides:	Pb > Zn > Cu > Hg
M. casta:	Pb > Zn > Cu > Hg

The ability of the bivalves to concentrate these metals also differed as follows

Hg: P. viridis > V. cyprinoides > M. casta

Cu: P. viridis > M. casta > V. cyprinoides

Zn: V. cyprinoides > P. viridis > M. casta

Pb: P. viridis > V. cyprinoides > M. casta

Thus, it can be seen that the mussel P. viridis has the highest biomagnifying potential (except for Zn) than the two clams. M. casta seemed to possess the least ability for metal integration.

The difference in the response pattern towards heavy metal accumulation may partially be attributed to the difference in the physiological functions of these animals.

Brookes and Rumsby (1965) studied the uptake of trace metals by some Newzealand bivalves. They found high rate of accumulation of certain heavy metals including Cu, Cr, Pb, Zn etc. by these organisms and the levels varying in scallops, mussels and oysters. Trace metal (Cu, Pb, Zn, Fe, Cd and Cr) accumulation by certain estuarine molluscs had been studied by Pringle et al. (1968) who showed that the uptake of metals by mollusc was directly proportional to the external concentration. The rate of uptake would also depend upon

(i) Species differences.

(ii) Environmental concentration level to which the species may be subjected and duration of exposure.

(iii) Temperature, salinity, dissolved O_2 and physiological conditions of the animal.

The present findings are in good agreement with the above results.

Davies and Pirie(1978) also found that, the mussel, Mytilus edulis, accurately reflected the mean total mercury concentration

in the surrounding water. Smith and Green (1975) found in three species of clams (family Unionidae) that the rate of Hg uptake increased with increased concentration in water.

Schulz-Baldes (1974) who had studied extensively on lead uptake and lead loss in the common mussel M. edulis found that a constant rate of Pb uptake, linearly dependent on the Pb concentration of the medium was taking place. These observations again give support to the present data.

Distribution among the organs:- All the experiments indicated gills as the major site of Hg accumulation in the molluscs, regardless of the concentration of the metal in solution. This is expected from filter feeders like the molluscs in which uptake occurs across this organ. Smith et al. (1975) pointed out this fact and remarked that the positive polyvalent ions adhere to the mucus feeding sheets of oysters. Since the mucus sheets pass across the gills some of the mercury measured may be due to contaminated mucus. Other organs contained less amounts of Hg.

The ranking order of different organs in the molluscs against rates of Hg uptake observed in the present studies is

P. viridis:	Gills > Viscera > Muscle > Mantle
V. cyprinoides:	N.D.
M. costa:	Gills > Mantle > Muscle > Viscera

Brookes and Rumsby (1965) had observed rapid accumulation and large concentration of Hg and other trace metals in gill tissues of some Newzealand bivalves (Scallops, mussels and oysters). The favoured sites were gills, visceral mass and intestines and considered that it was due to ingestion of sedimentary material of small particle size. Cunningham and Trip, 1975) found that on exposure to Hg^{203} in solution, the American oyster, Crassostrea virginica concentrated the metal in the following order: Gills digestive system mantle gonad muscle. Pentreath (1976) had also observed high concentration of Hg^{203} in the gills of the plaice, Pleuronectes platessa when exposed to $Hg^{203}Cl_2$ in sea water; the high value for Hg in the gills was related to the amount of particulate matter in the tank water and the amount of adherent mucus.

Copper also was found significantly in higher amounts in the gill tissues in all the animals. The distribution of copper among various organs followed the order

P. viridis:	Gills > Viscera > Mantle > Muscle
V. cyprinoides:	Gills > Viscera > Muscle > Mantle
M. costa:	Gills > Muscle > Viscera > Mantle

The high concentration of the metal in the gills may again be attributed to the filter feeding nature of the animals, absorption to the mucus sheets etc. The relatively high content of

copper found in the viscera of P. viridis and V. cyprinoidoe may be due to the high rate of uptake and subsequent loss of some Cu in the fecal matter. Similar observations were made by Brookes and Rumsby (1965) in the case of bivalves from Newzealand.

On the other hand, in M. costa, muscle contained more Cu than in viscera, probably due to the permanent fixing of the metal to the tissue and less in the visceral part.

For the other two less toxic metals, Zn and Pb, gill tissues were again found to contain the highest concentration of the metals in all the species. Based on the concentration factor attained by each body component, the order of distribution of Zn in the three organisms is

P. viridis:	Gills > Viscera > Muscle > Mantle
V. cyprinoides:	Gills > Mantle > Muscle > Viscera
M. costa:	Gills > Muscle > Viscera > Mantle

The high Zn content in the visceral part of the mussel (Table 7.5.b) probably indicates Zn excretion through fecal matter. However, no such explanation holds good in the two clams where visceral Zn level is comparatively low.

The ranking in the distribution of Pb among various organs of the molluscs is given below

P. viridis:	Gills > Viscera > Muscle > Mantle
V. cyprinoides:	Gills > Mantle > Viscera > Muscle
M. costa:	Gills > Muscle > Viscera > Mantle

It should be noted that the second major site of Pb accumulation in the mussel was viscera. However, in the other two animals this organ occupied third position in accumulation.

Schulz-Baldes (1974) found that lead was continuously taken up in the soft parts of the mussel, M. edulis. The uptake sequence obtained was kidney gills adductor muscle digestive gland foot mantle. This is in close agreement with the present findings. The level of Pb concentrated by the mussel was also comparable.

Suggested pathways of heavy metal uptake

The actual mechanism whereby the trace metals are concentrated in the marine biosphere is still not well understood. The suggested pathways, according to Pringle et al. (1968) are

(i) Particulate ingestion of suspended material from sea water.

(ii) Ingestion of elements via their pre-concentration in food material.

(iii) Complexing of metals by coordinate linkages with appropriate organic molecules.

(iv) The incorporation of metal ions in the physiologically important systems.

(v) Uptake by exchange (eg.) in to mucus sheets of the oyster.

A special characteristic of heavy metal chemicals is their strong attraction to biological tissues and in general, the slow elimination of these materials from biological systems. Once absorbed in the body, these metals are capable of reacting with a variety of binding sites. Metal complexes (coordinate compounds) are formed with -SH groups and to a lesser degree with amino, phosphate, carbodylate, imidazol and hydroxyl groups of enzymes and other essential biological proteins (Ochsme, 1976). Pringle et al. (1968) had also suggested coordination of the metals through organic molecules presumably using the -NH and or -SH groupings of the protein molecules. Davies (1976) had suggested the possibility of an -S-M-S-linkage in biological systems. Coombs (1972) found that free Zn and Cu were not present in the oysters, and suggested that large fractions of these elements were bound to membrane amino groups. Wolf (1970) demonstrated that 98% of the Zn in Crassostrea virginica was associated with protein, perhaps as metallothioneins. However, nothing much is known at present about the nature or composition of such complexes.

The electron microprobe X-ray analysis of tissues of the 'greensick' oysters along with unpolluted oysters gives direct evidence for the structural compartmentation of Cu and Zn in separate, specific granular amoebocytes, where it is further immobilised in membrane limited vesicles as different chemical compounds. This may be one possible mechanism of detoxication and storage of Cu and Zn by the oyster, Ostrea edulis (L) (George et al., 1978).

Molluscs as indicator organisms:- The results of the investigation clearly indicate that all the three bivalve molluscs are able to reflect the environmental concentration of the metals in their tissue. The metal content in the body of the molluscs increased with increased concentration of the metal ions in the experimental tank.

There was metal loss from the tissues of the molluscs (although complete clearance could not be achieved) when transferred to pollution-free media (see chapter 8). Thus, the three organisms P. viridis, V. cyprinoides and M. casta satisfy criteria required for indicator organisms of heavy metals. They may be ranked as efficient metal integrator in the order: P. viridis > V. cyprinoides > M. casta (except for Zn). However, the response pattern were different. Based on the concentration factor for the four metals, it was realised that P. viridis is the most sensitive indicator organism of Hg, Cu, Zn and Pb.

CHAPTER 8

KINETICS OF HEAVY METAL UPTAKE AND LOSS

PART II. KINETICS OF HEAVY METAL LOSS

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PART II KINETICS OF HEAVY METAL LOSS

The kinetics of metal loss from the animal body is as important as the toxicant accumulation by the animals. However, very little attention has been bestowed on the depuration of these metals. To better understand the transfer of toxicants through an estuarine trophic level, detailed retention tests should be conducted as an integral part of accumulation experiments. The contributions already made in this field were by Lockhart et al., (1972), Schulz-Baldes (1974, 1976), Pentreath (1976) and Wyne (1977).

Here, an attempt was made to study the kinetics of heavy metal depuration by the three bivalves, viz. P. viridis. V. cyprinoides and M. casta using mercury, copper, zinc and lead ions.

8.1. Materials and methods:-

The pre-treated animals were maintained in metal-free media and the tissue metal content at various intervals of time was estimated. The details of the experimental methods are given in section 2.8d.

8.2. Results

a) Rate of loss of mercury:- The rate of loss of mercury and the amount of the metal retained in the tissues of the two

clams can be seen from tables 8.2 & 8.3 and Fig 8.1.

Tissue concentration of mercury declined in both the clams when they were maintained in a metal free medium. However, only 30% of the accumulated Hg was lost in V. cyprinoides in a 24 day period. The clearance of Hg seemed to be a very slow process when compared to rate of uptake. In M. casta on the other hand about 48% of Hg was depurated in 20 days' period. The rate of loss of Hg was rapid at the initial stage; thus about 34.6% of the accumulated Hg was depurated in the first 10 days and only 13.4% was lost during the next 10 days. Total self purification could not be achieved by both the clams during the experimental period.

b) Rate of loss of copper:- The results of the depuration experiments are given in tables 8.1 to 8.3 and Fig 8.2 for the three species of molluscs.

Copper was found to be depurated faster in all the species. Thus tissue concentration of copper in P. viridis declined from 71.78 µg/g (of the copper treated animals) to 37.42 µg/g (dry wt.) in 24 days. About 33.7% of the accumulated copper was retained in the body after this period. In V. cyprinoides 90% and in M. casta about 93% of the accumulated copper was lost during a depuration period of 24 and 20 days respectively.

Table 8.1

Studies on metal retention by the mussel Perna viridis (Linnaeus) exposed to 0.1 ppm Cu^{2+}, 0.5 ppm Zn^{2+} and 1.0 ppm Pb^{2+} solutions in sea water of salinity, 25‰ and 31‰ (in the case of Zn for 4 days.

Metal con. in ppm (in the medium)	Metal µg/g dry wt control ± S.D.	Metal µg/g dry wt. after 4 days exposure ± S.D.	Metal retained by tissue, µg/g dry wt.* Mean ± S.D. No. of days Retention after 4 days	8 days	16 days	24 days
0.1 ppm	19.948	71.78	68.40	63.70	49.14	37.42
Cu	0.80	2.57	2.20	1.46	1.11	0.68
0.5 ppm	60.53	96.47	92.58	88.04	83.58	82.16
Zn	4.03	5.80	4.40	4.20	3.80	2.70
1.00 ppm	100.29	1542.05	1482.68	1435.26	1378.65	1275.31
Pb	4.35	25.57	18.57	22.80	20.24	9.55

*µg/g wet weight in the case of Hg.

Table 8.2

Studies on metal retention by the clam, Villorita cyprinoides var.cochinensis exposed to 0.5 ppm Cu^{2+}, 0.5 ppm Hg^{2+}, 1 ppm Zn^{2+} and 2 ppm Pb^{2+} solutions in sea water of salinity, 10‰ for 4 days.

Metal con. in ppm (in the medium)	Metal µg/g dry wt. control ± S.D.	Metal µg/g dry wt.after 4 days exposure ± S.D.	Metal retained by tissue, µg/g dry wt.* Mean ± S.D.			
			No. of days			
			4 days	8 days	16 days	24 days
0.5 ppm Cu	16.32 0.65	65.02 2.10	52.98 2.16	45.10 1.18	31.76 1.44	20.93 2.06
0.5 ppm Hg	0.232 0.05	5.568 0.21	5.158 0.18	4.784 0.24	4.226 0.26	3.885 0.22
0.5 ppm Zn	74.49 2.34	105.64 8.85	98.45 6.74	92.94 5.86	83.58 6.57	79.83 4.56
2.0 ppm Pb	85.40 3.68	915.30 29.44	832.41 23.79	768.59 20.60	643.80 16.34	498.13 19.98

*µg/g wet weight in the case of Hg.

Table 8.3

Studies on metal retention by the clam, Meretrix casta (Chemnitz) exposed to 0.5 ppm Cu^{2+}, 1.00 ppm Hg^{2+}, 2.00 ppm Zn^{2+} and 2.00 ppm Pb^{2+} solutions in sea water of salinity, 25‰ for 4 days.

Metal conc. in ppm (in the medium)	Metal µg/g dry wt. control ± S.D.	Metal µg/g dry wt. after 4 days exposure ± S.D.	Metal retained by tissues in µg/g dry wt* Mean ± S.D.		
			No. of days 5 days	10 days	20 days
0.5 ppm	19.812	71.748	52.019	41.168	23.62
Cu	0.85	2.37	1.49	2.01	2.24
1.00 ppm	0.1947	1.8513	1.5926	1.2779	1.0561
Hg	0.045	0.054	0.050	0.032	0.050
2.00 ppm	61.886	220.68	197.72	189.18	165.69
Zn	3.10	14.28	8.81	11.45	10.18
2.00 ppm	106.26	504.64	442.40	391.29	358.8
Pb	4.26	12.52	7.15	5.83	11.89

*µg/g wet weight in the case of Hg.

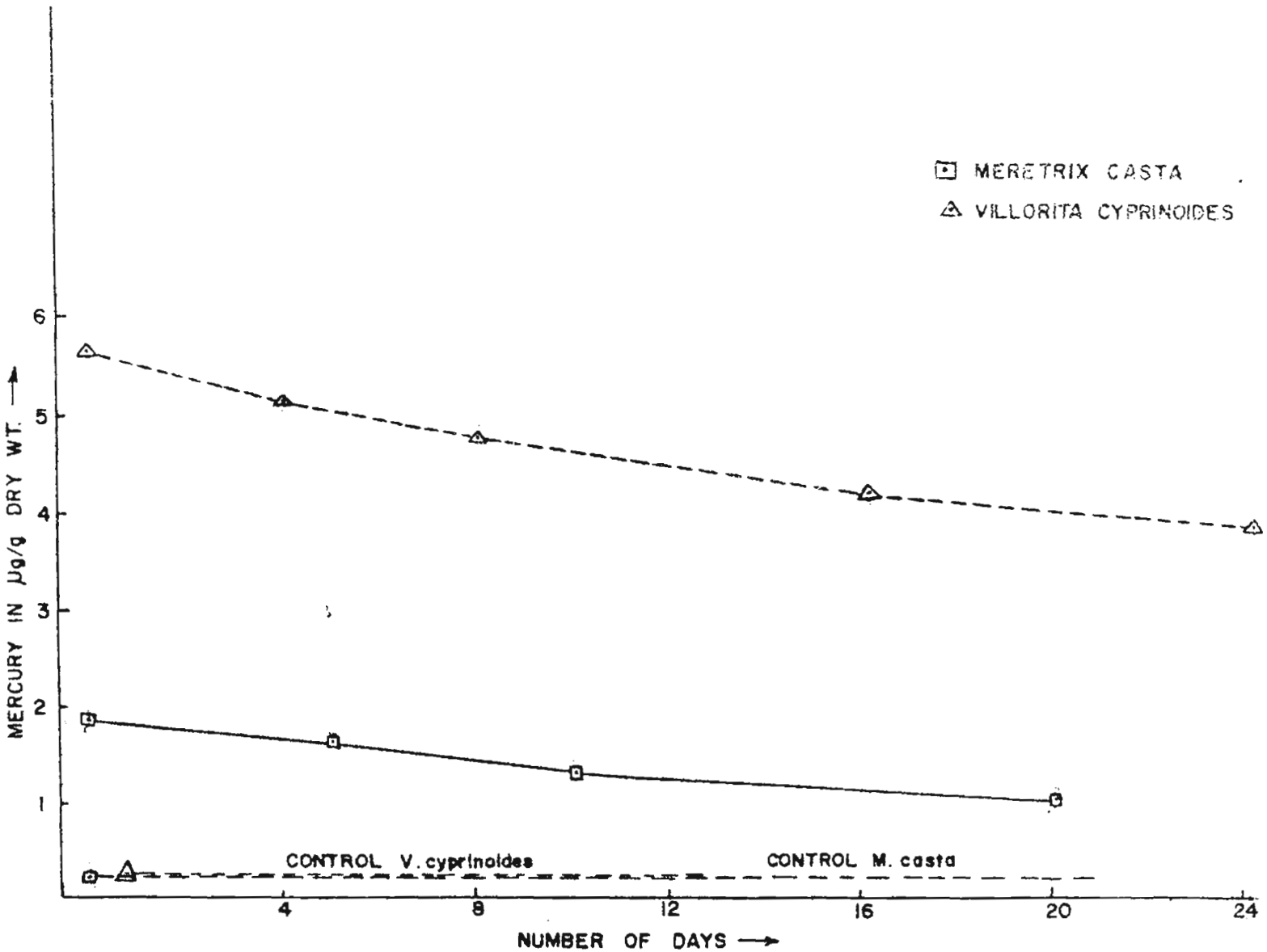

Fig. 8·1 Elimination of mercury from the body of molluscs Villorita cyprinoides (△—△) and Meretrix casta (⊡—⊡) as a function of time.

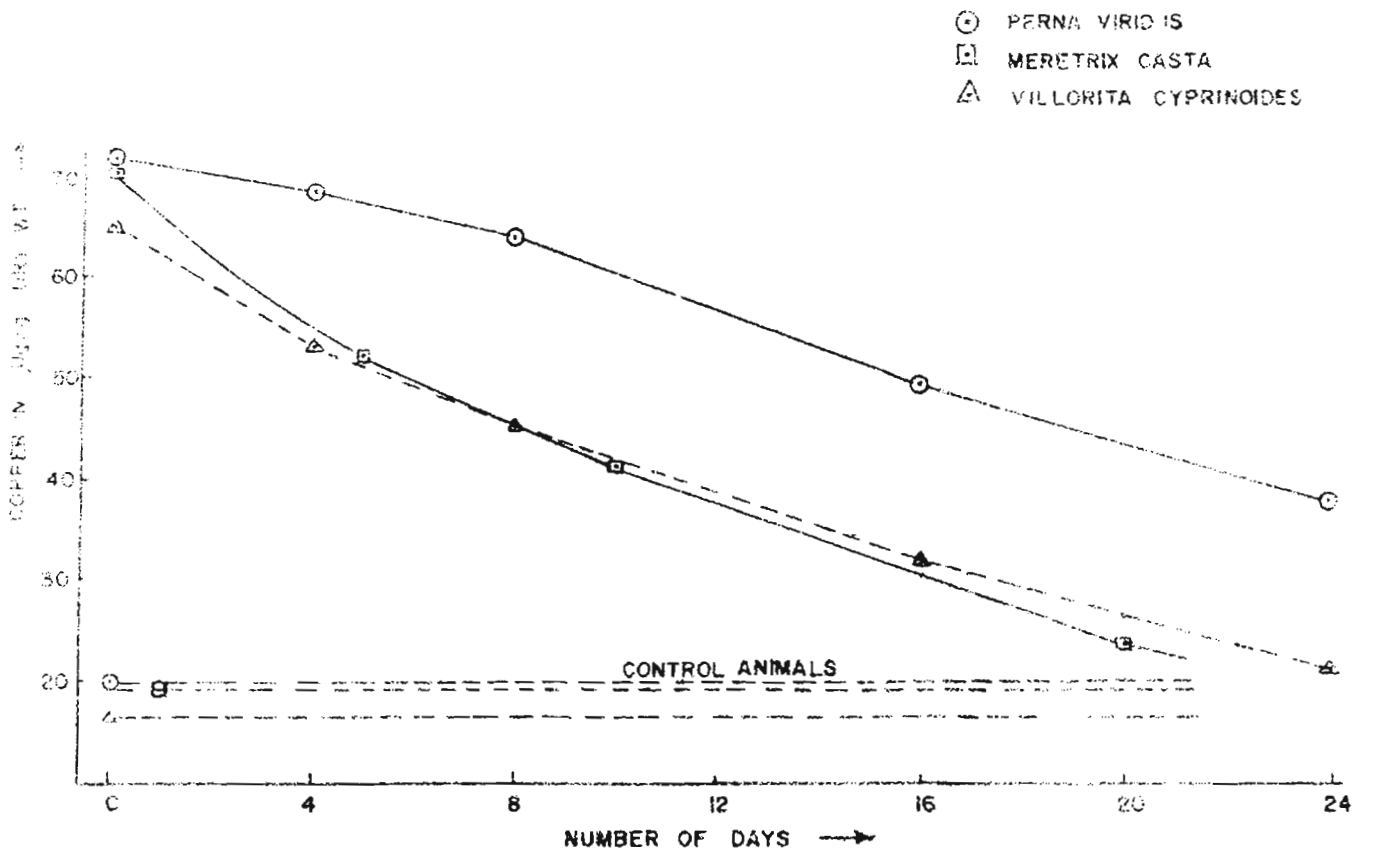

Fig. 8·2. Elimination of copper from the body of molluscs Perna viridis (⊙—⊙), Villorita cyprinoides (△—△) and Meretrix casta (□—□) as a function of time

c) Rate of loss of zinc:- The results for the three species are presented in Tables 8.1 to 8.3 and Fig 8.3 .

Zinc loss in P. viridis showed rather an exponential decrease. Thus at the end of 8 days, 23.45% of the accumulated metal was lost, after 16 days another 12.41% was released and during the last 8 days 3.95% of the metal was lost making a total of ca 40% loss at the end of 24 days.

In V. cyprinoides the rate of zinc loss was rather faster and at the end of the experimental period (24 days) 83 of the accumulated Zn was found to be depurated. The rate of loss of Zn was faster at the beginning and slowed down with time.

In M. costa, the release of the accumulated zinc seemed to be rather a slow process. Thus, 65% of the accumulated zinc was retained in the soft parts of the clam even after keeping in a metal free environment for 20 days. At the end of 10 days 19.63% of zinc was lost and during the second half time only 14.79% of the metal was lost.

d) Rate of loss of lead:- The experimental results for the three organisms are put in the Tables 8.1 to 8.3 and Fig 8.4 .

During 96 hr exposure time in sea water containing 1.00 ppm lead, the mussel, P. viridis accumulated a very high amount of lead, showing a faster uptake rate. However, the rate of lead loss in the mussel was found to be a slow process. Thus,

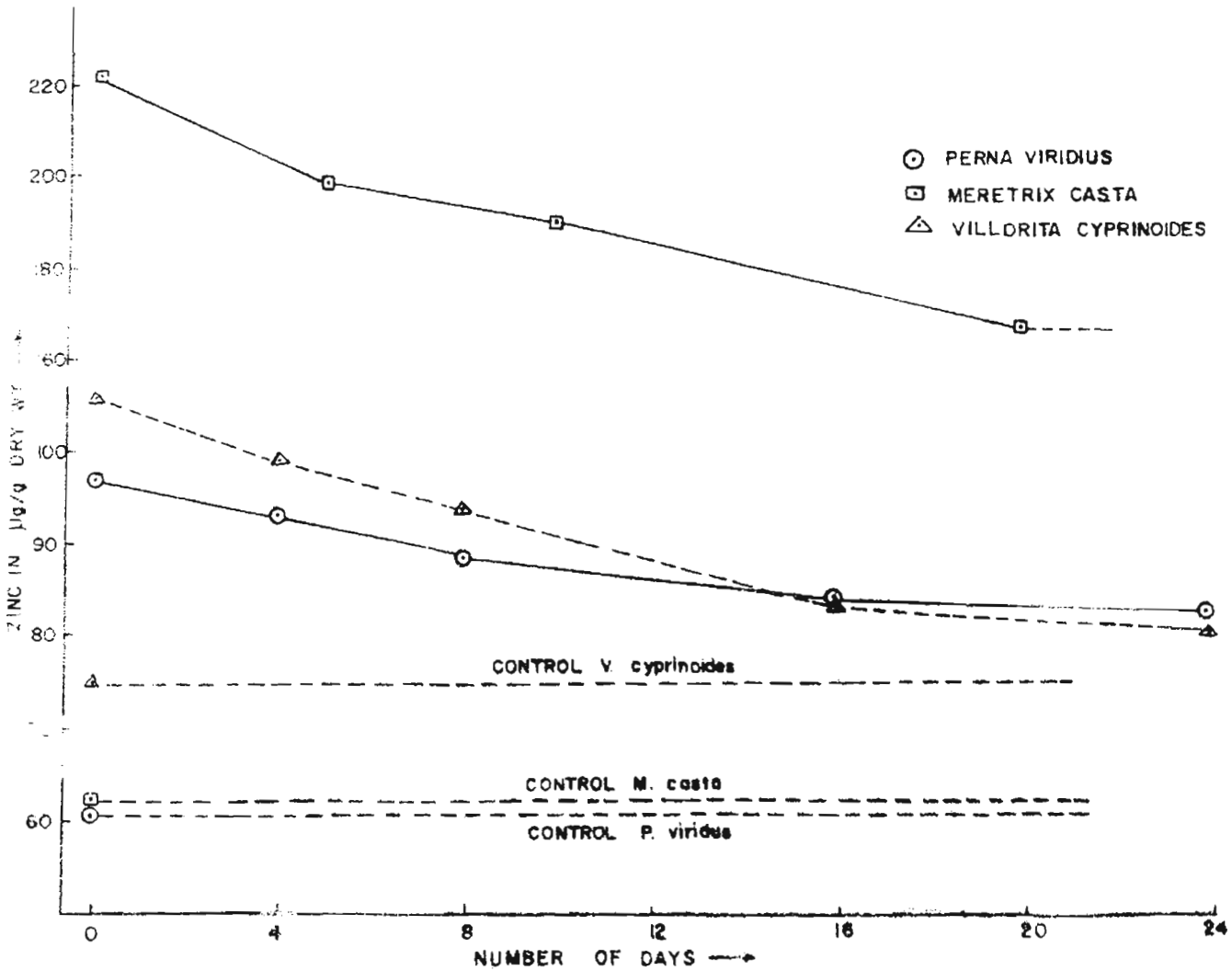

Fig. 8·3 Elimination of zinc from the body of molluscs, Perna viridis (●—●) Villorita cyprinoides (△—△) and Meretrix casta (□—□) as a function of time

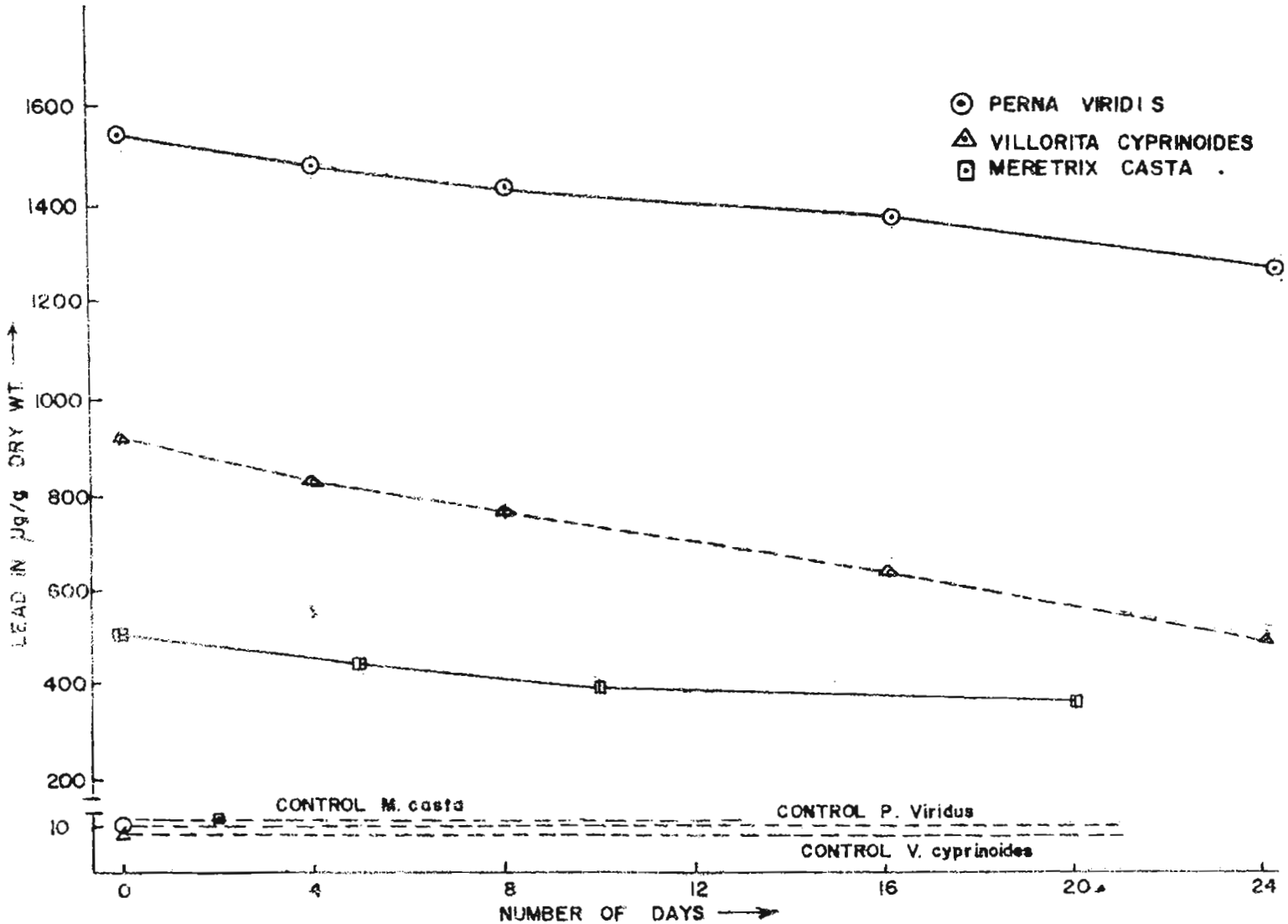

Fig 8·4 Elimination of lead from the body of the molluscs, Perna viridis (o——o), Villorita cyprinoides (△——△) and Meretrix casta (□——□) as a function of time.

as much as 81.5% of the accumulated metal was retained in the tissues at the end of a 24 day depuration period. The nature of the graph (Fig 8.4) indicates that the lead loss in the mussel is linear.

There were faster rates of release of lead in both the clams, V. cyprinoides and M. casta. Thus about 50% of the accumulated lead was lost in Villorita during 24 days depuration time. The rate of loss was found to be approximately linear (Fig 8.4). Thus, 17.68% was released at the end of 8 days, 32.71% after 16 days and 50.26% lead loss at the end of 24 days was observed.

In M. casta, 36.58% of the accumulated lead was released at the end of 20 days of which 28.45% metal was lost during the first 10 days period. The rate of release of lead was much faster at the initial stage and a slower rate with lapse of time.

8.3. Discussion

The results of the experiments clearly indicated a rapid loss of major fraction of Hg at the initial stage in all the three bivalves. This rapidly lost mercury may be due to a more labile fraction of the accumulated metal. The Hg, that was associated with mucus in the gills might be eliminated before being permanently absorbed to the major tissues. The other fraction of Hg must be involved in strong complex formation with the enzyme or protein system.

Cunningham and Tripp (1975) monitored Hg loss in the oyster, Crassostrea virginica and observed a significant depuration in the initial stage. Thus in 10 ppb Hg group, the tissue concentration declined by 21% during the first 18 days. In 100 ppb, a significant decrease of 43% in the residue concentration of Hg occurred in the same period. These authors suggested that the biological half life value ($B\frac{1}{2}$)* decreases as internal concentration of Hg is increased and remarked that this pattern of loss would have occurred if Hg were associated primarily with wandering amoebocytes and mucus which would be rapidly eliminated from the body and not associated permanently with major tissues. Total clearance of Hg would not be achieved. Miettinen et al. (1970) gave 481±40 days as the biological half-time ($B\frac{1}{2}$) for the elimination of Hg injected into the foot muscle of Tapes decusatus(L) (F) as $CH_3Hg^{203}NO_3$. They also distinguished a rapid initial loss of a small amount of Hg^{203}(fast component) in all organisms studied - a fish, a crab and two molluscs. In Tapes decussatus (L) and Mytilus galloprovincialis, it was about 20% of the administered amount. The present findings are in agreement with these results. Lockhart et al. (1972) had studied the elimination pattern of CH_3Hg^+ in the Northern pike (Esox lucius) and found that only 30% was eliminated in one

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*The biological half-life ($B\frac{1}{2}$) is the time required for a given concentration (in the animal body) to be reduced to half its original value.

year period. Pentreath (1976) also noticed a slow release of Hg in the plaice, Pleuronectes plastessa L.

As observed in the case of mercury, copper was also rapidly released by the two clams at the initial stage and after that the rate slowed down (Tables 8.2 & 8.3). This phenomenon may be attributed to the same factors as given under Hg release. The slow rate of release of Cu in P. viridis even at the initial stage indicates the possibility of forming strong Cu-complexes with the tissue components.

Scott and Major (1972) reported that Mytilus edulis accumulated copper rapidly from solution and excreted the metal in a metal free medium after several days. The nearly complete elimination of copper in the two clams is in good agreement with the above observations.

The depuration pattern for zinc was different in the three species (Tables 8.1 to 8.3 and Fig 8.3). Thus in Villorita as much as 83% of Zn was released in 24 days period. However, in the other two species more Zn was retained (Tables 8.1 & 8.3).

The difference in release pattern of the metal in the animals may be due to their differences in physiological processes. The faster rate of Zn release at the beginning can

again be attributed to the mucus absorbed Zn or the more labile fraction in the animals. It is not clear whether the rate of release is dependent on the concentration of the metal in the body.

Coombs (1972) found that most of the Zn was easily removed (dialysed) from the oyster, Ostrea edulis and suggested that either it was present as free ion, or it is bound to some smaller molecular weight compounds or it was very weakly bound and readily dissociated from a protein moiety. This observation holds good for V. cyprinoides, where most of the accumulated Zn was lost (83%) during 24 days period. In P. viridis and M. costa, the slow release of Zn may be due to the formation of strongly bound complexes with the protein system. Much of the accumulated lead was retained in P. viridis (81.5%) and M. costa (63.42%) (Tables 8.1 & 8.3). In V. cyprinoides about 50% lead was lost during 24 days depuration period (Table 8.2). The slow rate of release of lead may be due to its permanent fixation to the organic matrix. The linear release of lead in V.cyprinoides and P. viridis (Fig 8.4) indicated a steady clearance of Pb from these organisms. In M. costa a rapid initial loss of lead was observed.

Pringle et al. (1968) found that in Crassostrea virginica, lead loss was characterised by an increase in the $B_{\frac{1}{2}}$ value with

an increase in body burden of lead. When tissue residues were high little lead was lost. And they suggested permanent deposition of the metals. With the limited data available here, it is not possible to say whether the above theory holds good or not. However, it can be seen that, in P. viridis, which had the highest concentration of lead in the tissue released only 18.5% of the accumulated metal during the experimental period.

Schulz-Baldes (1974) established that the rate of loss of lead in the mussel, Mytilus edulis was very much depended on the internal lead concentration in the animal. He also observed a linear release of lead in the animal which is consistent with the present result.

SUMMARY

SUMMARY

An investigation on the seasonal variations of biochemical constituents and some of the trace metals like copper, zinc, iron and lead in three commercially important molluscs viz. Villorita cyprinoides var cochinensis, Meretrix casta (Chemnitz) and Perna viridis (Linnaeus) collected from the Cochin backwaters, at monthly intervals during 1976-'77 and 1977-'78, have been carried out. The toxic effects of some heavy metal ions viz. Hg^{2+}, Cu^{2+}, Zn^{2+} and Pb^{2+} on these organisms and the 96 hr LC_{50} values for these metals have been determined using the static bioassay procedure. The studies were conducted at the habitat salinity and temperature conditions. The physiological effects of metal pollutants on these bivalves have also been carried out. The kinetics of accumulation of these metals by the above organisms have been undertaken by exposing the test animals to media containing the metal ions at different concentrations for varying lengths of time and analysing the soft parts for the metal content. In the metal loss study the treated animals were transferred to metal free medium and the metal concentration in the tissue at different intervals of time were analysed. The localisation of the metals in various organs of the molluscs has been investigated by dissecting out the animals for muscle, mantle,

gills and viscera and determining the metal content in these tissues. The metal content has been determined using Atomic absorption spectrophotometer or Mercury analyser (in the case of mercury).

The results indicated that the tissue water content and other biochemical constituents studied viz. protein, carbohydrate, lipid, ash content, calcium and phosphorous showed a distinct seasonal cycle of change in all the three bivalve molluscs. The tissue water content increased steadily during the monsoon periods when the environmental water salinity was low. The water content varied from 74.46% to 83.10% in *V. cyprinoides*, 77.11% to 85.45% in *M. casta* and from 78.28% to 83.74% in *P. viridis*. In general high values for water content was found during June to August in all the species. The body water content showed strong negative correlation with environmental water salinity and the corresponding regression equations are given:

V. cyprinoides: $Y = 81.9830 - 0.2155\ X$

M. casta: $Y = 84.6674 - 0.1621\ X$

and *P. viridis*: $Y = 91.7188 - 0.3440\ X$

where Y = body water content (%) and X = salinity (‰) of habitat water.

Protein and carbohydrate showed reciprocal relations in their variations in all the three bivalves. The protein maxima synchronised with carbohydrate minima in these species. In *V. cyprinoides*, higher values for protein was found during November to January and in *M. casta* this period corresponded to December to April. On the other hand, in *P. viridis* higher values for protein were observed during June-July. Carbohydrate fluctuated widely in all the organisms. The lipid was found generally high during the summer months (the period of high salinity) in the two clams, *V. cyprinoides* and *M. casta*. It was generally low during June to August. In *P. viridis*, lipid content was the highest in July. The seasonal variations in the biochemical parameters were attributed in general, to factors like food availability, salinity of ambient water and spawning. The higher values for protein and lipid closely coincided with the periods of maximum phytoplankton abundance. Significant negative correlations existed between protein and carbohydrate in the molluscs and the corresponding regression equations for *V. cyprinoides*, *M. casta* and *P. viridis* are

$$Y = 85.3344 - 1.1160\ X$$

$$Y = 107.2685 - 1.4652\ X$$

$$\text{and } Y = 83.4272 - 1.1504\ X$$

respectively. (where X = protein % and Y = carbohydrate %) Higher values of ash content were found during the periods of

high salinity in all the species. Like most other shellfish, the three species studied are rich in calcium content. Higher values for tissue calcium was found, invariably during pre-monsoon and post-monsoon periods, when the salinity of the ambient water was high.

The seasonal variation in phosphorous content seemed to bear good relation to lipid content. A higher percentage of phosphorous was always accompanied by a higher percentage of lipid in all the species. The calorific content of the whole tissue varied between 4.995 to 5.558 in V. cyprinoides, between 4.800 to 5.384 in M. casta and between 4.874 to 5.599 in P. viridis (in Cals/g dry weight). Higher values for calorific content was observed during the time of high lipid and protein content. It is suggested that the fishing of the species should be made when the protein content is at the highest, viz. December to February for V. cyprinoides, December to April for M. casta and June-July, in the case of P. viridis.

The concentrations of the four trace metals studied viz. copper, zinc, iron and lead in the three bivalve molluscs are well influenced by season. The highest concentrations of all these metals, in general, were found during the period of low salinity and low pH of the habitat water (monsoon periods). Metal concentrations decreased in these species during summer

months - the period of highest salinity and pH values. Iron was by far the most abundant of the trace metals in all the three species. It varied from 200.59 to 665.41 (µg/g) in V. cyprinoides, 181.22 to 338.82 (µg/g) in M. casta and from 203.33 to 940.16 (µg/g) (on a dry weight basis) in P. viridis, copper content was in the range of 18.35 to 44.46 µg/g in V. cyprinoides, 17.35 to 46.89 µg/g in M. casta and 11.40 to 30.41 µg/g in P. viridis (dry weight basis). The level of zinc in these bivalves were apparently similar in magnitude. The variations were from 53.07 to 105.58 µg/g in V. cyprinoides, 49.62 to 83.35 µg/g in M. casta and from 56.12 to 100.88 µg/g in P. viridis. The concentrations of lead in these organisms were rather low in comparison to other metals. The distribution of the metal in the various species were from 6.24 to 10.06 µg/g in V. cyprinoides, 6.73 to 23.46 µg/g in M. casta and from 5.23 to 9.80 µg/g in P. viridis. The high concentrations of metals observed during June to August in the three species of bivalves has been attributed to an increase in the bioavailability of the metals in their habitat water, due to a decrease in salinity and pH of the medium. The generally, low concentrations of the metals in these species during summer months have been explained due to low bioavailability of the metal ions in water since the salinity and pH were high and hence, the possibility of forming inorganic complexes. The

incorporation of metals by phytoplankton or detritus and chelation by other extracellular products might also have reduced free metal ions in water.

Significant negative correlations were found between trace metal content in the tissue of the molluscs and environmental water salinity. Thus, the regression equations between copper and salinity in the three species were

V. cyprinoides: $Y = 39.5060-0.9143\ X$

M. casta: $Y = 42.3707-0.6989\ X$

and *P. viridis*: $Y = 55.9489-1.1204\ X$

The corresponding equations for zinc are

$Y = 83.3256-0.7209\ X$

$Y = 79.3576-0.8067\ X$

and $Y = 171.1801-3.1781\ X$

Iron content also bears a similar relation to environmental water salinity and the respective regression equations were

V. cyprinoides: $Y = 477.94-9.4728\ X$

and *M. casta*: $Y = 290.91-1.9427\ X$

However in *P. viridis* no significant correlation was found between salinity and concentrations of the metals iron or lead. For the other two species the regression equations for lead were

V. cyprinoides: Y = 9.1878-0.0976X

and *M. casta* Y =21.8926-0.4309X

(where X = salinity (‰) and Y = metal content (µg/g) in all cases)

Nearly all the four metals were found to be positively inter-correlated in the two clams, *V. cyprinoides* and *M. casta* (exception being Cu/Pb combination in *V. cyprinoides*). In *P. viridis* only few pairs (Cu/Zn, Cu/Pb and Zn/Pb) were found to be significantly correlated.

The important observation of the present investigation is that the concentration level of the various metals studied (including that for mercury), in the three bivalve molluscs are well below the permitted limits recommended for these metals in some marine products.

The toxicity studies using the heavy metal ions, viz. Hg^{2+}, Cu^{2+}, Zn^{2+} and Pb^{2+} on the three bivalve molluscs indicated that they are detrimental to all the species. Among the four metals studied, copper was found to be the most toxic metal ion to all the species. The order of toxic effect was $Cu^{2+} > Hg^{2+} > Zn^{2+} \gg Pb^{2+}$. Up to 10 ppm, Pb^{2+} could not produce any lethal effect to these species in 10 days period. *P. viridis* was found to be more sensitive to heavy metals than the two clams. The 96 hr LC_{50} value was found to be 0.174 ppm Cu, 0.34 ppm Hg and 3.0 ppm Zn for the mussel, *P. viridis*,

whereas for the clam, V. cyprinoides the 96 hr LC_{50} values were 1.214 ppm Cu, 1.57 ppm Hg, and a 10 day LC_{50} value of 5.47 ppm Zn. The 96 hr LC_{50} values for M. casta were 3.25 ppm Hg, 2.188 ppm Cu and 6.67 ppm Zn. M. casta was comparatively more resistant to heavy metal pollutants.(except for Zn) which can be seen from the high LC_{50} values.

The mussel, P. viridis exposed to lethal levels of Cu^{2+}, Hg^{2+} or Zn^{2+} always secreted mucus and remained passive with reduced gape width. On the other hand, on exposure to these metal ions V. cyprinoides showed moribund symptoms in addition to the secretion of mucus. M. casta responded to the presence of Hg^{2+} or Cu^{2+} in the media by secreting mucus and also by closing their shells. When exposed to Zn^{2+} moribund symptoms were developed in the animals. Lead in the environment (upto 10 ppm) could not produce any visible toxic effect on these organisms.

The toxic effect of heavy metals was explained to be due to poisoning the enzyme system. The difference in the toxic effect of the four metals to the molluscs is discussed on the basis of their electronegativity data, the ionic radii and the pKs values of the metal sulphides.

To test the hypothesis that death from acute metal toxicity of aquatic organisms is partly related to tissue

hypoxia, lactic acid and glycogen content were measured in the molluscs exposed to Hg^{2+} and Cu^{2+}. The results indicated lactic acid accumulation in the tissues and glycogen depletion in the muscle or liver of these organisms. Since the end product of the anaerobic degradation of glycogen being lactic acid, the increased level of this component in the tissue was explained to be due to severe hypoxic stress caused from heavy metals.

Bioaccumulation of the trace metals (Hg, Cu, Zn and Pb) from water occurred at all concentrations studied. The important observation is that the amount of metals found in the tissue of the molluscs is very much dependent on the concentration of the metals in the medium. In all cases (except for Zn) highest concentration factor was obtained by P. viridis. The uptake of zinc seemed to be rather restricted in all the species. V. cyprinoides showed higher C.F. for copper, mercury and lead than M. casta. The highest C.F. for Zn was obtained by M. casta. The accumulation of metals by the animals followed the order (based on their concentration factor)

P. viridis:	Pb > Cu > Hg > Zn
V. cyprinoides:	Pb > Zn > Cu > Hg
M. casta:	Pb > Zn > Cu > Hg

The concentration factor decreased with increasing concentration of the metals in the medium (except for Pb) showing

greater uptake efficiency at lower concentrations. The experiments indicated gills as the major site of metal accumulation in the molluscs. The ranking order of different organs against rates of metal uptake for P. viridis:

Hg: Gills > Viscera > Muscle > Mantle

Cu: Gills > Viscera > Mantle > Muscle

Zn: Gills > Viscera > Muscle > Mantle

Pb: Gills > Viscera > Muscle > Mantle

In the case of V. cyprinoides the following order of of uptake rates was observed:

Hg: N.D.

Cu: Gills > Viscera > Muscle > Mantle

Zn: Gills > Mantle > Muscle > Viscera

Pb: Gills > Mantle > Viscera > Muscle

and for P. costa, the ranking was in the order:

Hg: Gills > Mantle > Muscle > Viscera

Cu: Gills > Muscle > Viscera > Mantle

Zn: Gills > Muscle > Viscera > Mantle

Pb: Gills > Muscle > Viscera > Mantle

The depuration of metals in the molluscs was generally slow. Total purification could not be achieved in any case during the period of investigations.

Now certain conclusions can be drawn from the above results. The molluscs would provide a promising source of animal protein for human consumption and hence the aquaculture of the species may be popularised in the coastal waters. The heavy metal ions, viz. Hg^{2+}, Cu^{2+} and Zn^{2+} are extremely toxic and are detrimental to the molluscs if present in the environmental water above the normal level*. Lead, though not lethal to these organism, was taken up to a high degree. The accumulation and storage of the heavy metals by the organisms at sub-lethal levels pose a greater threat than affecting the organisms themselves as these metals may find way to the human body through the marine food chain. So the need for a clean coastal water is emphasised. The present investigations also establish the usefulness of benthic organisms like clams and mussels as indicator of metallic pollution. These species are extremely sensitive to heavy metal pollutants and respond quickly to it. The increase in the concentration of the metal ions in the ambient water was clearly reflected in the tissue of the organisms. Hence, it is suggested that in view of the above properties which have been highlighted in the present investigation, these bivalve molluscs can serve as a natural monitor of water quality.

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*Normal levels in seawater: Mercury = 0.00003 ppm, Copper = 0.003 ppm, Zinc = 0.01 ppm and Lead = 0.00003 ppm - values reproduced from Goldberg (1963).

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