PHYSIOLOGICAL EFFECT OF COPPER (II) ON SUNETTA SCRIPTA. L

THESIS SUBMITTED TO THE COCHIN UNIVERSITY OF SCIENCE AND TECHNOLOGY IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

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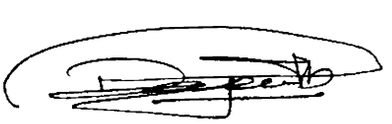
AUGUST 1986

CERTIFICATE

This is to certify that this is an authentic record of the work carried out by Latha Thampuran, M.Sc. under our supervision in the School of Marine Sciences of the Cochin University of Science & Technology and that no part thereof has been presented for amy other degree in any University.



Dr. R. Damodaran,
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Cochin - 16,
25..8..1986.

DECLARATION

I hereby declare that the thesis entitled, 'PHYSIOLOGICAL EFFECTS OF COPPER (II) ON *SUNETTA SCRIPTA* L.', is an authentic record of research carried out by me under the supervision and guidance of Dr.R.Damodaran and Dr.P.N.K.Nambisan in partial fulfilment of the requirements of the Ph.D. Degree of the Cochin University of Science & Technology and that no part of it has previously formed the basis for the award of any degree, diploma or associateship in any University.

LATHA THAMPURAN.

Cochin-16,
25.8.1986.

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L.T.

C O N T E N T S

INTRODUCTION

An organism, or a group of organisms living in a set of environmental conditions are bound to interact with the environment and modify it. These modifications often become detrimental to the organisms concerned. Man is no exception to this situation. Industrialisation, an outcome of man's attempt to increase his sphere of activity and living comforts is becoming a threat to his own survival, by polluting air, water and land. The industrial effluents being discharged mostly into Oceans, the pollution of the marine environment has become inescapably a world problem. The problem of pollution whether aquatic or terrestrial calls for rational exploitation of resources with environmental consciousness.

A pollutant is defined as any substance added to the environment which has a measurable and generally detrimental effect on the environment. The chief pollutants of the sea fall under five categories. They are (1) domestic sewage, (2) industrial effluents constituting the heavy metals, (3) halogenated hydrocarbons, (4) transuranic nuclides and (5) petroleum hydrocarbons.

The pollutants in general, react by imposing a physiological stress on the organism, bringing about various abnormalities in the concerned animal which ultimately affect populations. Elimination or even reduction in the number of a species or particular stage in the life history of an organism may upset the pattern of the ecosystem and hence the community metabolism. By the human consumption of contaminated sea foods, man may also be affected by various disorders.

The current alarm of metal pollution in sea started with the 'Minamata disaster' which occured in Japan between 1953 and 1964. Since then attempts are made throughout the world to understand the problems of heavy metal pollution. Even though many of these heavy metals do not occur in the environment in very high concentrations so as to produce any visible effects, the ability of many of the organisms to concentrate them in their body tissues and being bioaccumulated through the food chain is a problem of serious concern to man.

Like any other nation, in India too, industrial pollution has become a subject of increasing concern.

Incidents of industrial pollution have been reported from many parts of the country. Cochin, the collection site of the present study, being the industrial capital of Kerala is also a harbour, is vulnerable to pollution by trace metal contaminants.

In the recent times, pollutants of greatest concern in the aquatic environment are those which are persistent such as toxic heavy metals and the chlorinated hydrocarbons which include insecticides and pesticides. Heavy metals which form the major component of the industrial effluents attract more attention in this modern industrialised world. All metals being cumulative poisons are potentially harmful to most organisms at some level of exposure and absorption (Miettinen 1974). Mercury, copper, zinc, nickel, lead, manganese, chromium, selenium, silver, arsenic and cadmium are some of the toxic heavy metals commonly met with. Each metal react differently in different animals. Zinc is highly poisonous to fishes causing reduced growth rate and mortality. The 'Minamata' diaster, which brought to the fore the dangers of industrial pollution, was the first

reported human poisoning by mercury in sea foods (Nitta 1972). Vernberg and Vernberg (1972) had investigated the toxic effects of Mercury on the fiddler crab. In crabs the gill tissue oxygen consumption was depressed when exposed to cadmium while copper had no effect in both the green crab and the rock crab (Thurberg et al 1973). MacInnes and Thurberg (1973) observed a reduction in the oxygen consumption of mud snail Nassarius obsoletus in response to silver, copper, arsenic and zinc, while the mussel Mytilus edulis and the soft shelled clam Mya arenaria showed an increase in oxygen consumption on exposure to Silver (Thurberg et al 1974). Arsenic used in the manufacture of 'Weed-Killers' entering the aquatic systems causes the disease known as 'Arsenicism' in human beings while among fish a high rate of mortality occurs (Ganapati 1975). In Mya arenaria and Mytilus edulis reduced filtration rates and disturbed ciliary activity were observed on exposure to Chromium (Capuzzo and Sasner 1977). Raymont and Shields (1964) and Calabrese et al (1973) have determined the toxic effects of chromium to marine organisms. Toxic effects of copper on marine

organisms are well documented in several reviews. Acute toxicity of copper was determined in marine invertebrates by several workers (Marks (1938); Weiss (1947); Wisely and Blick (1967); Scott and Major (1972); Saliba & Ahsanullah (1973); Delhaye & Cornet (1975); Sunda & Guillard (1976); Abel (1976); Winner & Farrell (1976); Lakshmanan & Nambisan (1977); Kumaraguru and Ramamoorthi (1978); Carmel et al (1983); Sivadasan et al (1986). Copper induced damage to gill tissue has been reported by Baker (1969) in many marine animals. Scott & Major (1972) while studying the effect of copper on the blue mussel Mytilus edulis came to a conclusion that the cupric ions cause respiratory and cardiovascular depression in the organism. In Mytilus edulis copper exhibited a depressing effect on the oxygen uptake by a direct inhibition of the ciliary action (Brown and Nowell 1972) while zinc carried no effects; Abel (1976) observed a reduction in the rate of filtration of Mytilus edulis when exposed to copper. In Meretrix casta and Perna viridis, all the four metals ie. Ag, Cr, Zn & Pb were seen to reduce the metabolic rate at sublethal concentrations. The bivalves Perna viridis and Meretrix casta showed an increased rate

of filtration at very low concentrations and reduced filtration rates at higher concentrations of copper (Mathew & Menon, 1984).

It is well known that many of the aquatic invertebrates are capable of concentrating heavy metals in their body tissues. Boyce & Herdman reported the 'green-sick' condition in the oysters as early as 1897, which was caused due to the abnormal accumulation of copper. Nambisan *et al* (1977) showed that the bivalve *Meretrix casta* when maintained in sea water containing various concentrations of copper takes up large amounts of the metal. D'silva & Kureishy (1978) observed that copper and zinc were continuously taken up into the soft parts of *Mytilus viridis* throughout the experiment. Copper toxicity in the marine environment and the kinetics of bioaccumulation are investigated by several other workers (Atkins 1932; Raymont & Shields 1964; Brooks & Rumsby 1965; Ikuta 1967; Pringle *et al* 1968; Shuster & Pringle 1969; Eustace 1974; Betzer & Pilson 1975; Philips 1976 a & b; Wright 1976; Davenport & Manley 1978; D'Silva & Qasim 1979).

In sea surface concentration of copper is estimated to be between about 10 µg Cu/l in coastal waters (Atkins 1932) and 3 µg/l in open sea (Goldberg 1963). Currently copper is attracting widespread attention because its concentration is increasing significantly in the sea (D'Silva and Kureishy 1978). Copper refineries, pesticide and fungicide manufacturing industries are important sources which bring copper to the aquatic systems. The use of copper for various other purposes such as in the manufacture of antifouling paint, in the treatment of diseases of fishes and as an algicide increases its importance as a pollutant. Cochin, being an important harbour is vulnerable to copper pollution since copper forms an important constituent of the antifouling paints which are designed to steadily release copper in a biologically available form.

Copper is a normal constituent of many marine animals and is essential for the normal growth and development (Bryan 1971). It forms a part of hemocyanin, the oxygen carrying pigment found in almost all crustaceans and molluscs. The bivalves are an exception to this, they do not appear to have a functional oxygen carrying pigment (Morton 1958). But copper being an important constituent

of the enzymes tyrosinase and cytochrome oxidases, has a universal distribution in the Animal Kingdom. Although copper is required by living organisms in trace amounts, small increments above the required level are highly toxic (Scott and Major 1972). Hence the biotic effects of copper pollution are of particular interest.

The usefulness of biological indicators as an alternative to studies of metals in water or sediments is widely accepted. Biological effects monitoring has attracted the attention of the regulating authorities concerned with the environmental management for the assessment of the environmental quality in the recent years. An ideal indicator could be used to identify areas of pollution, to monitor the progress of counter-pollution measures, to detect changes, either deterioration or improvement in environmental quality, etc. The metal concentration present in the indicator organism may be 10^3 to 10^6 times higher than that in the ambient water, allowing direct analysis without preconcentration (Phillips 1976 a, b). Hang et al (1974) suggest that a simple correlation should exist between the metal content of the organism and the average metal concentration in the

surrounding water. An ideal indicator species exhibit characters like: the ability to accumulate high concentrations without dying; a sedentary life history; high numerical abundance; of sufficient life span to permit sampling of more than one year class throughout the monitoring period; large size so that ample tissue is available for analysis; and good adaptation to laboratory conditions (Eisler 1981).

The molluscs in general are seen to take up large amounts of metal ions from solutions (Nambisan et al 1977). Owing to the ability to reflect environmental levels of metallic pollutants both in marine and estuarine ecosystems the bivalves play a good role in the current literature. The bivalves were used in the assessment of toxic heavy metals and other contaminants of the marine environment even in the past (Boyce & Herdman 1897, Phillips 1976 a, 1976 b, Goldberg 1975). They possess several characters of ideal indicator species (Darracott & Watling 1975; Phillips 1977). Goldberg (1975) has emphasized the need of a global mussel watch for the assessment of environmental levels of certain contaminants.

The sublethal concentrations of metals are considered more deleterious and harmful than even lethal concentrations since they bring about many physiological changes in the concerned animal. Marine bivalves under physiological stress have shown many abnormalities like (1) decreased growth rates (Galstoff *et al* 1947), (2) loss of carbohydrate and protein reserves (Bayne & Thompson 1970), (3) interference with spawning time (Roberts 1972), (4) production of abnormal offspring (Bayne 1975). Sublethal effects of this kind ultimately affect the population as a whole without the danger being noticed. Physiological changes brought about by the sublethal concentrations of metals on aquatic organisms have not been sufficiently investigated so far in this country. Hence, the present study was undertaken.

The clam, *Sunetta scripta* L. chosen for the present study is distributed widely along the east and west coasts of India. The important clam bed in the Cochin area are located on the northern side of Cochin barmouth and also in Munampam, South-West coast of India.

The clams belonging to the genus *Sunetta*, have a wide distribution. Eleven species of the genus are known chiefly from Senegal, India, Japan and Australia.

Sunetta meroe is another common South Indian species recorded from Madras.

The animal is economically important being exploited both for flesh and shell. Along the Cochin coast it is used by poor people as it forms a cheap source of protein food. The clam meat is also used as a good poultry feed. The thickness of the shell makes it highly suitable as raw material for lime and cement industry.

Sunetta scripta is a sedentary filter feeder inhabiting the coastal marine realms. The animal is easily available almost throughout the year and can be reared in the laboratory with ease. The animal is highly tolerant to a wide range of environmental conditions. Pilot experiments have shown that the animal can withstand very high concentrations of copper without any visible effects and that it is also capable of concentrating the heavy metal in its body tissues from solutions. Hence, its suitability as a bio-assay organism for copper in sea water.

The animals collected from the clam bed situated on the northern side of Cochin barmouth are subject to wide fluctuations in salinity both seasonal and tidal. Also, salinity is considered as an important parameter influencing the physiological functioning of an organism. Hence, the salinity tolerance of the animal is worked out. Considering the potential vulnerability of Cochin backwaters to heavy metal pollution, the impact of heavy metal copper (II) on the bivalve Sunetta scripta was conceived. Static bioassays were conducted for the determination of the sublethal concentrations of the metal as a preliminary step towards the toxicity studies. Oxygen consumption and filtration rate which are considered as reliable sublethal toxicity indices were employed for investigating the toxic effects of the metal. Bioaccumulation, a physiological phenomenon which can be of importance from the public health point of view, and also in the assessment of environmental quality is also dealt with.

SALINITY TOLERANCE

Salinity is considered as one of the important environmental parameters initiating structural and functional responses in marine invertebrates. Salinity affects the organisms through changes in the total osmo-concentration, relative proportion of solutes, co-efficients of absorption and saturation of dissolved gases, density and viscosity of the medium (Kinne 1971). It is well known that the estuaries and the adjoining marine realms in general are subjected to wide variations in salinity under the impact of seasonal changes. In addition to seasonal variations, there is also diurnal variation in salinity due to tides. Animals inhabiting such habitat adopt different mechanisms for their survival. The clam Sunetta scripta like any other bivalve closes its shell valves and isolates its tissues during such unfavourable conditions. This is the first response of many bivalves to an environmental stressor (Bayne 1973). The long term effects of salinity are counteracted by other physiological mechanisms.

The clam bed situated on the northern side of Cochin barmouth, chosen as the collection site for the present study is subject to wide variations in salinity (Table 1).

In spite of these salinity variations, different age groups of the clam Sunetta scripta is found abundantly in this area almost throughout the year. Hence, with a view to determine the tolerance capacity of the animals to different salinities, studies were conducted in the different salinity media ranging from 5 x 10^{-3}S to 40 x 10^{-3}S at 5 x 10^{-3}S interval.

Table 1: Monthly Distribution of average bottom salinity of the water over the clam bed (Salih, 1978).

Months	Salinity x 10^{-3}	Months	Salinity x 10^{-3}
March (1972)	32.39	March (1973)	33.28
April	34.33	April	33.71
May	23.30	May	35.28
June	13.29	June	26.10
July	6.82	July	10.11
August	2.30	August	5.28
September	23.24	September	7.28
October	28.30	October	28.00
November	30.46	November	28.25
December	29.25	December	28.10
January (1973)	29.38	January (1974)	30.25
February	33.80	February	32.18

The effect of salinity variations on bivalves has been investigated by many workers. Abraham (1953) studied the influence of salinity on the survival of the clam, Meretrix casta. The range of tolerance of salinity was determined by Motwani (1955) in Mytilus edulis. The capacity to tolerate different salinities was worked out by Nagabhushanam (1955) on Martesia striata. Loosanoff (1950) studied the effect of transference of the oyster, Crassostrea virginica, from low to high salinities. Cheriyan (1966) has worked on the tolerance of Nausitora hedleyi to different salinities and Pierce (1970) on Modiolus demissus. The range of tolerance in Rangia cuneata was determined by Bedford and Anderson (1972). The mode of tolerance of Katelysia opima to salinity fluctuations was worked out by Mane (1974). Studies were conducted on different bivalves namely Crassostrea madrasensis, Meretrix meretrix and Mytilus viridis by Sundaram and Shafee (1975) and Crassostrea cucullata by Nagabhushanam and Bidarkar (1975) in view of determining their tolerance to different salinities. The lethal salinity on the basis of 50% survival was determined in Donax cuneatus by Talikhedkar and Mane (1976). The rate of mortality in the different salinity media was determined by Alagarswami & Victor (1976)

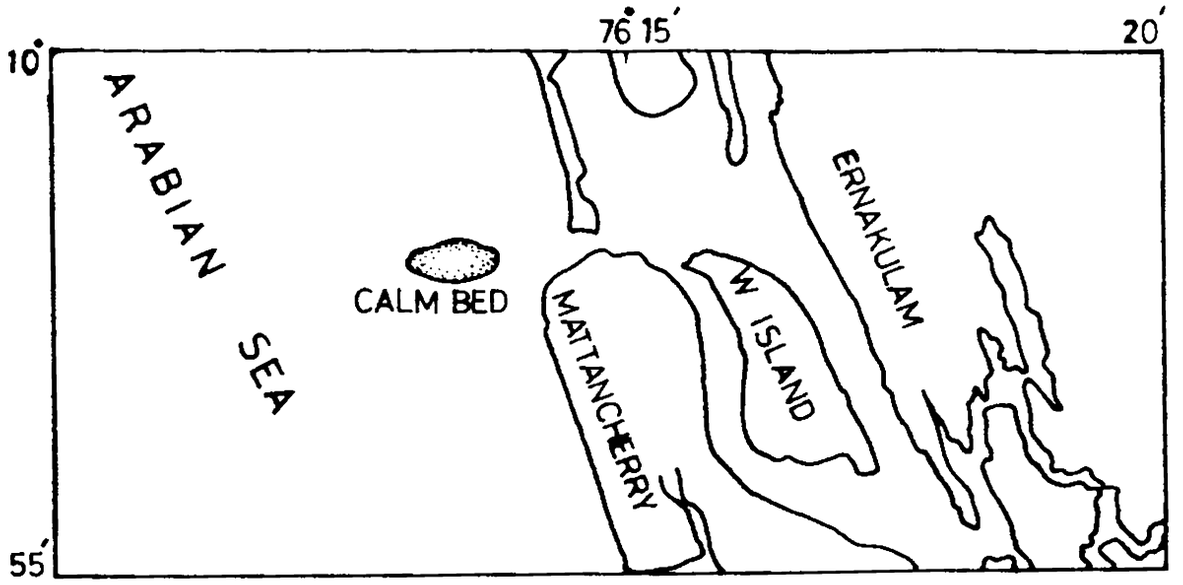
in the pearl oyster, Pinctada fucata. The tolerance range in the different size groups was determined by Sivankutty Nair & Shynama (1975) on Villorita cyprinoides and Salih (1978) on Meretrix casta. Mohan (1979) has worked on the salinity tolerance of Nausitora hedleyi to sub and supra normal salinities. It has been already mentioned that the bivalves in general rely on behavioural mechanisms like valve closure for combating salinity changes. This aspect has been discussed by various authors [Maloeuf (1937), Mane (1974), Talikhedkar & Mane (1976), Alagarswami & Victor (1976), Shummway (1977), and Davenport (1979)].

In the natural habitat the effects of salinity may be modified by the influence of other environmental parameters. Hence laboratory experiments under controlled conditions will give more reliable information as far as effects of salinity are concerned.

2.2 MATERIALS AND METHODS

The clams, Sunetta scripta were collected from the clam bed situated on the northern side of the Cochin barmouth which lies parallel to the sand bar, which is perpendicular to the southern extremity of Vypeen island (Fig.1). The total area of the clam bed approximately covers about ½ sq. km. The bottom sediment is composed

FIG. 1



MAP SHOWING THE LOCATION OF THE CALM BED.

predominantly of sand with clay and silt forming a small percentage. Salinity varies widely during the monsoon and premonsoon period ranging from about 5×10^{-3} S to 35×10^{-3} S. The depth of the water ranges from 1 m to 4 m. The clams Sunetta scripta and Meretrix casta occur conjointly in this area.

Specimens for the present study were collected only during the pre-monsoon and post-monsoon period when the salinity was around 30×10^{-3} S. They were brought to the laboratory in polythene bags unexposed to the sun. In the laboratory the animals were thoroughly cleaned of the lingering algae and dirt and the barnacles attached to the shells. They were then put in large plastic basins and allowed to acclimatise in the habitat salinity and were fed on the blue green algae, Synnechocystis sp. grown in a metal free medium during the acclimatisation period. The water was changed once in two days besides giving artificial aeration daily.

The water of different salinities used in the experiments was prepared either by evaporation or by dilution of the sea water with deionised water. The salinity of the water samples was determined by modified Mohr Knudson method.

Sea water was filtered through 41 Whatman filter paper for use in the experiments. Animals of three size groups, ie. 2.0 ± 0.5 cm. in length, considered as small, 3.0 ± 0.5 cm. as medium sized and 4.0 ± 0.5 cm. as large sized, were selected for the study. Size of the animals was measured using vernier calipers (antero-posterior axis). The test vessels and all the glasswares were washed with 2 N HNO_3 and then thoroughly rinsed in deionized water before use. The animals were not fed while running the experiments.

The three size groups of Sunetta scripta after acclimating in the habitat salinity were introduced in the different salinities ranging from 5×10^{-3} S to 40×10^{-3} S at 5×10^{-3} S interval for studying their tolerance. Batches of animals, ten in each, were introduced carefully into the glass troughs containing 4 litres of water having the experimental salinities. The water in the trough was changed every two days so as to reduce interference of mucus secreted by the animals and also to avoid any perceptible increase in salinity due to evaporation. The troughs were also aerated twice a day giving least disturbance to the test organism. The experiments were run for a period of 15 days. Temperature of the medium ranged

from 27°C to 29°C. The rate of mortality was taken as the criterion of tolerance. The troughs were checked daily for survivors and the activity of the animals was observed continuously. Animals which failed to close their shells even after prodding was considered as dead. Lack of response of siphons to tactile stimuli is another factor by which mortality was decided. Average value from repeated experiments was taken into consideration. Percentage mortality was calculated in each salinity for the determination of the tolerance capacity of the animal.

2.3 RESULTS

Salinity tolerance experiments run for a period of 15 days indicate that *Sunetta scripta* belonging to the large size group (4 cm to 4.5 cms) exhibit 100% survival in salinities ranging from 25×10^{-3} S to 35×10^{-3} S. The medium sized clams also have the same range of tolerance but it appears that the medium sized ones tolerate slightly lower salinities. Small clams were all alive in salinities ranging from 15×10^{-3} S to 40×10^{-3} S during the course of 15 days. Highest percentage of mortality was recorded in the lowest salinity tested ie. at 5×10^{-3} salinity and it was noted that the mortality rate increased as the age of

the clams advanced. There was a total mortality percentage of 33.31%, 19.99% and 13.32% in large, medium and small clams respectively in this salinity over a 15 days period. In 10 x 10^{-3} S mortality was noticed but in a lesser degree than in 5 x 10^{-3} S. Here also large and medium sized clams showed higher mortality rate than small ones. Large, medium and small clams suffered a mortality of 23.31%, 16.65% and 9.99% respectively.

The small clams were all alive throughout the experimental period in 15 x 10^{-3} S while in medium sized and large clams there was a mortality of 9.99% and 20%. Only large clams showed a negligible mortality of 3.33% in 20 x 10^{-3} salinity. Next to 5 x 10^{-3} salinity 40 x 10^{-3} salinity showed a higher percentage of mortality in the case of large clams. Small clams showed 100% survival in 40 x 10^{-3} S. In large and medium sized clams mortality rate in this salinity was 23.32% and 6.66% respectively.

The relative salinity tolerance capacity of large, medium and small clams is evident from Fig.2.

Commencement of mortality in the different salinity media followed the same trend as in the percentage mortality.

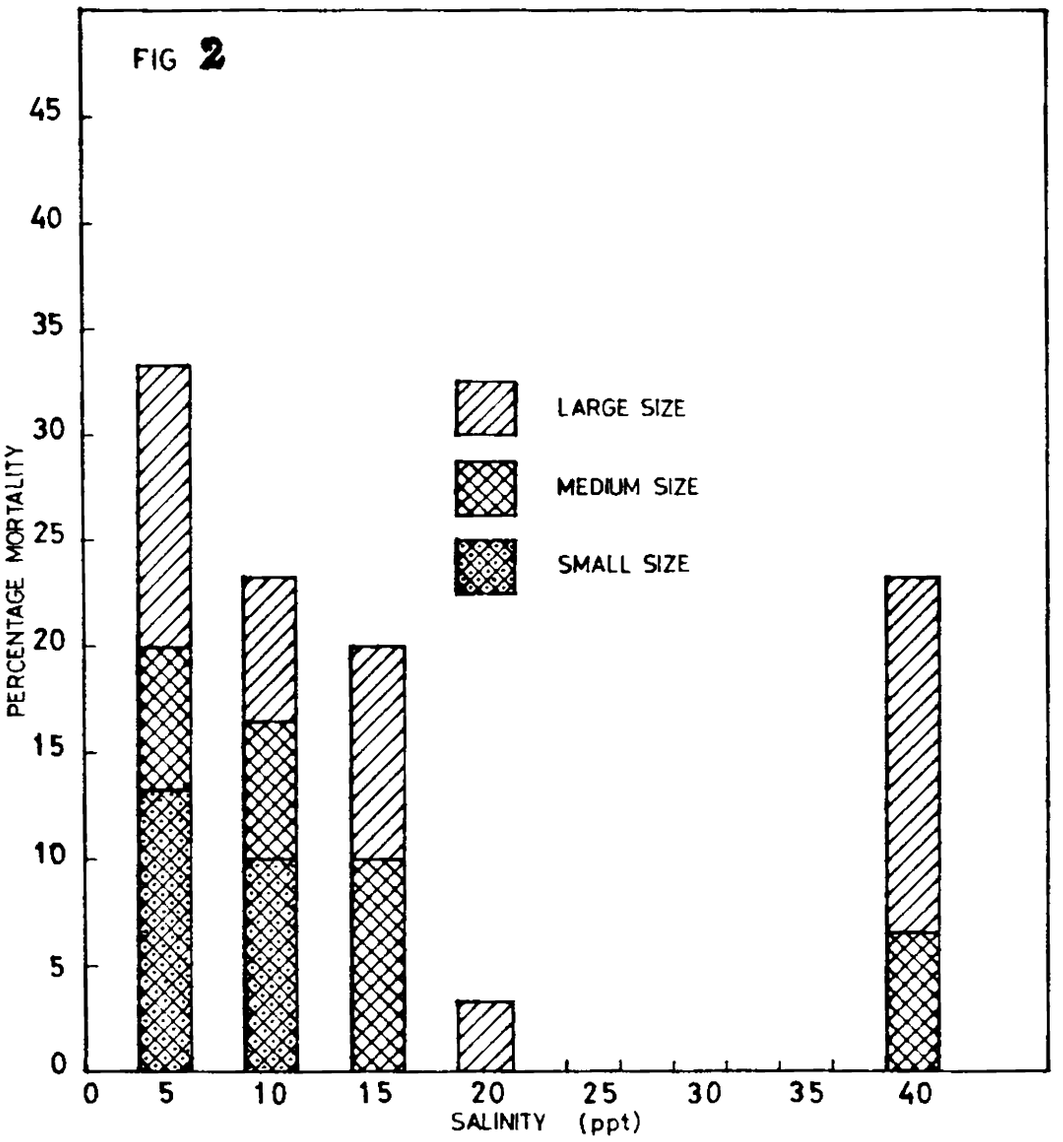


Fig. 2 :- Relative salinity tolerance capacity of Sunetta scripta belonging to small (20-25 mm), medium (30-35 mm) and large (40-45 mm) size groups.

In 5 x 10^{-3} S the mortality in large, medium and small clams commenced on the 7th, 9th and 10th day respectively over a 15 day period. Initial mortality was observed on the 8th day in large clams, on the 10th day in medium sized and on the 13th day in the small clams in 10 x 10^{-3} S. The trend was maintained in 15 x 10^{-3} salinity also and mortality started on the 10th and 12th day in large and medium sized clams. Only the larger size group showed mortality at 20 x 10^{-3} S and began on the 13th day. In 40 x 10^{-3} S even though the mortality percentage was high the initial mortality occurred late, when compared to the lower salinities. It had first occurred only on the 11th day in the larger size group and 14th day in the medium sized ones. It wasobserved during the course of the experiment that in salinities where comparatively higher mortality occurred the animals showed increased mucus secretion. The clams were active only in favourable salinities while they tightly closed their valves in all other media.

Table 2 gives a detailed picture regarding the effect of different salinity media on the survival of the clam Sunetta scripta belonging to three size groups.

Table - 2 Percentage mortality in the different salinities in three size groups.

Nb. of days	$5x10^{-3}$s			$10x10^{-3}$s			$15x10^{-3}$s			$20x10^{-3}$s			$25x10^{-3}$s			$30x10^{-3}$s			$35x10^{-3}$s			$40x10^{-3}$s		
	L	M	S	L	M	S	L	M	S	L	M	S	L	M	S	L	M	S	L	M	S	L	M	S
1		-	-	-	-		-	-	-	-	-		-	-	-		-	-	-	-	-	-		-
2	-	-	-		-	-	-	-	-	-	-		-		-	-								--
3		—	-		-		-	-			-	-	-		-	-		-		--				-
4	-	-	-	-	-	-	-	-		-	-				-	-								
5						-	-						-	-	-	-	-							
6					-	-	-			-						-							-	
7	6.66	-	-	-	-					-			--	-	-		-							
8	6.66	-	-	6.66	-	-		-	-	-	-	-	-	-	-		-			-			-	-
9	3.33	10.00	-	3.33	-	-	-	-	-	-	-	-	-		-									-
10	6.66	-	3.33	6.66	6.66	-	10.00			-								-		-	-	-	-	-
11	-	-	6.66	3.33	-		-	-	-	-			-		-								3.33	
12	-	6.66	-	3.33	6.66	-	10.00	3.33	-							-	-	-	-		-		10.00	-
13	10.00	-	3.33	-	-	6.66	-	6.66	-	3.33	-	-		-	-		-	-					3.33	
14		3.33	-		3.33	-					-			-	-			-		-				3.33
15			-		-	3.33	-	-		-	-	-	-										6.66	3.33	
Total % Mortality	33.31	19.99	13.32	23.31	16.65	9.99	20.00	9.99	-	3.33	-	-		-				-			-		23.32	6.66	-

L = large size (40-45 mm) M = medium size (30-35 mm) S = small size (20-25 mm)

2.4 DISCUSSION

Molluscs in general are capable of withstanding wide range of salinities. This euryhalinity of aquatic molluscs is attributed to their capacity to regulate the ionic and osmotic concentrations of both their blood and cells. Salinity tolerance experiments on Sunetta scripta under the laboratory conditions indicate a wide range of tolerance.

By reviewing the works of various authors on this aspect it is evident that the bivalves are able to withstand wide and rapid changes in the osmotic pressure of their environmental medium and that they react differently to different salinities. They also show wide variations in their range of tolerance and lethal limits of salinity.

It has been already mentioned that the clam bed selected for the present study exhibit wide variations in salinity and that the clams thrive well throughout the year irrespective of the very low saline conditions observed during the monsoon period. Even though the clam, Sunetta scripta is seen to tolerate lower salinities, the range from 25×10^{-3} S to 35×10^{-3} S can be considered as the zone of tolerance. Below and beyond this range they are in their resistance zone (Fig.2.).

In general, in bivalves behavioural responses play an important role in succeeding various adverse conditions. Many aquatic molluscs respond to changes in salinity of their medium or even resist dessication for relatively long periods merely by closing their shell valves tightly. Thus they escape themselves from the osmotic changes in the external medium by isolating their tissues from it and exposing it to the action of the fluid trapped in the mantle cavity. This "Shell-closing mechanism" adopted by bivalves to resist sudden changes in salinity was first reported by Bendant in 1816 as quoted by Maloeuf (1937). Nagabhushanam & Badarkar (1975) while investigating the effect of low salinities on the survival and behaviour of Crassostrea cucullata, suggested that salinity plays an important role in opening and closing of the shell valves. Motwani (1955) and Mane (1974) obtained identical results. Pierce (1971 a) while studying the valve movements in different species of Modiolus concluded that the animals respond to a salinity change by closing their shell valves immediately. Osmo-regulation by valve closing process was also reported by Gilles (1972) in Mytilus edulis, Glycymeris glycymeris and Acanthochitona diserepaus. Talikhedkar and Mane (1976) observed in Donax cuneatus that the clams subjected to higher

concentrations took more time to open their valves than those subjected to dilutions. But in the pearl oyster Pinctada fucata a reverse response was noticed (Alagarswami & Victor 1976). It was suggested by Shummway (1977) that Mytilus is an osmoconformer which depends on its behavioural mechanism for protection from low saline conditions for short durations by valve closure. Davenport (1979) recorded the lowest salinity of the contents of the mantle cavity in Mytilus edulis as 19.5×10^{-3} S irrespective of the external low saline conditions.

Increased secretion of mucus observed in the experimental clams during unfavourable conditions may also help them in reducing contact with the external conditions. The shell closing mechanism, secretion of mucus etc. are only devices which help the animal for relatively short period of time. Ionic regulation plays an important role in aquatic molluscs especially in marine species. True euryhalinity met within molluscs is chiefly due to their capacity to regulate the osmotic pressure of the cells with respect to changes in the blood osmolarity. By reviewing the results of the salinity tolerance experiments on Sunetta scripta it is evident that the animals are more capable of withstanding higher concentrations than dilutions.

It may be due to the reason that the clams can resist in a better way the loss of water and a gain of ions that occur in higher concentrations than a loss of ions and gain of water happening in dilutions.

The data also indicate that the salinity tolerance capacity of Sunetta scripta decreases with increase in size. It can be noted that the animals of the larger size group exhibited the highest percentage mortality followed by the medium sized and then the small clams which showed the lowest rate of mortality in all the salinity media. Identical results were obtained in Villorita cyprinoides (Sivan Kutty Nair & Shynamma 1975). Salih (1978) also came to the conclusion that the smaller specimens of Meretrix casta are capable of withstanding greater variation in salinity when compared to the larger ones. It was observed that the clams of smaller size group were able to tolerate salinities ranging from 15×10^{-3} S to 35×10^{-3} S while the medium sized and large clams could tolerate a salinity range of 20×10^{-3} S to 35×10^{-3} S only. Experiments on the salinity tolerance of Nausitora hedleyi also showed that smaller animals have better tolerance to fluctuations in salinities than larger ones (Mohan 1979).

By closely observing the activity of Sunetta scripta during the course of the experiment, it is seen that they are most active in 30×10^{-3} salinity than in any other salinity. Hence 30×10^{-3} S may be considered as the most favourable salinity as far as Sunetta scripta is concerned. It is well known that salinity plays a paramount role in limiting the distribution of animal populations in marine and brackish water environments (Kinne 1967). The remarkable capacity of Sunetta scripta to withstand osmotic changes explains the reason for its occurrence and abundance almost throughout year near the Cochin barmouth which is subjected to diurnal and seasonal fluctuations in salinity.

TOXICITY STUDIES

It has been mentioned earlier that the estuaries and the adjoining marine realms have become centres of pollution with the promotion of trade and industry. Besides the fluctuations of environmental variables like salinity, temperature, dissolved oxygen content, pH, current, etc., a major hazard which the coastal marine animals in general have to face in recent times is the increasing amounts of various chemical-pollutants, both in the dissolved and suspended form. The heavy metals which form an important component of the industrial effluents deserve special attention in this context. Heavy metals* are introduced into the marine/aquatic environment through a number of agencies: industrial waste discharges, land (especially agricultural) run-off, mining and metallurgical operations, automobiles, etc.

In recent times, the usefulness of bioassays in pollution studies has been well recognized. Bioassay (toxicity) tests are defined as estimations of the amount

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*The term 'heavy metals' is used to refer to both light and heavy metals which are present in trace to ultra trace amounts and which are biologically active.

of biologically active substances by the level of their effect on test organisms. Bayne et al (1976) suggest that a useful index of pollution should involve a response which may be shown to have a detrimental effect on growth, reproduction or survival. Survival is the best index of stress since it is provided to be more sensitive than others and is also the least variable (Winner & Farrell, 1976).

It is true that in trace amounts most of these metals are essential for the normal metabolism of aquatic organisms. But excessive amounts of these metals have proved lethal. In recent years considerable attention is devoted to the discussions on mortality of marine animals after exposure to varying amounts of these metals. Bryan & Hummerstone (1971) had inyestigated the adaptations of the polychaete Nereis diversicolor to sediments containing high concentrations of Zn, Cd & Cu. Copper and zinc poisoning in Artemia was studied by Brown & Ahsanullah (1971). The acute toxicity of cadmium and zinc was studied in seven invertebrate species by Ahsanullah (1976). Brown (1976) has worked on the copper and lead tolerance of the isopod Asellus meridianus. Winner & Farrell (1976) conducted

experiments on the acute and chronic toxicity of copper on four species of Daphnia.

Wisely & Blick (1967) made observations on the mortality of the larvae of some species of bryozoans, tube worms, bivalve molluscs including Mytilus edulis and Crassostrea commercialis and the brine shrimp on exposure to Hg, Cu & Zn. Schulz-Baldes (1972) investigated the toxicity of copper on the mussel, Mytilus edulis. Influence of ferric hydroxide flakes on the rate of mortality of Mytilus edulis was determined by Winter (1972). Lakshmanan & Nambisan (1977) while investigating the toxic effects of copper on the bivalve Villorita cyprinoides had determined the LC_{50} for a 240 h period. Percentage mortality was determined in Meretrix casta by Nambisan et al (1977) in 0.5 ppm, 1 ppm, 5 ppm and 10 ppm copper concentrations upto a period of 20 days.

D'Silva & Kureishy (1978) evaluated the acute toxicity of copper and zinc in the green mussel Mytilus viridis (Perna viridis) and 48 h LC_{50} value was determined for each metal. Kumaraguru & Ramamoorthi (1978) determined the LC_{50} values for a 96 h period in the estuarine bivalves Anadora granosa, Meretrix casta and Crassostrea madrasensis.

Acute toxicity tests were conducted on the clam Meretrix lusoria for the metals Hg, Cu & Cd by Park and Kim (1979 a). Lakshmanan & Nambisan (1979) determined the 96 h LC_{50} value for Hg in the mussel Perna viridis.

The toxic effects of metals varies from metal to metal, among the different organisms and also depends on the various environmental parameters. It has been stated by Bryan (1971) that the severity of the effect depends on the form of the metal, presence of other metals, physiological condition of the organism and the environmental conditions. The physiological conditions include the size and age of the organism, stage in the life history and activity and acclimatization to metals (D'Silva & Kureishy 1978). It has been observed that the trace metal concentrations in the bivalve molluscs and the net uptake of metals are size dependent (Mark 1938, Boyden 1974, 1977). Effect of body size on the percentage mortality of Penaeus indicus was investigated by Carmel et al (1983) and the 120 h LC_{50} was determined for different size groups (1.5 cm to 6.5 cm). Sivadasan et al (1986) had determined the acute toxicity of Hg, Cu, & Zn to Metapenaeus dobsoni belonging to the juvenile (30 mm to 50 mm) and maturing stages (50 mm to 70 mm).

The ability of marine bivalves to accumulate copper in excess of environmental levels have been well documented (Scott & Major 1972, Nambisan *et al* 1977, D'Silva & Kureishy 1978, D'Silva & Qasim 1979, Ikuta 1967, Shuster and Pringle 1969). It has been shown that the accumulation of metals may cause death of marine organisms affecting both the larvae as well as the adults (D'Agostino and Finny 1974, Wisely & Blick 1967, Martin & Stephenson 1977). The effect of salinity on the trace metal content and accumulation in the different body tissues is dealt with in various marine species (Wolfe & Coburn 1970, O'Hara 1973, Jones 1975, Phillips 1976a, 1977, Fowler *et al* 1975). Carmel *et al* had conducted static bioassays for determining the effects of copper in different salinities on *Penaeus indicus* belonging to three size groups. Oslon & Harrel (1973) studied the salinity impact on the acute toxicity of Hg, Cu and Cr for *Rangia cuneata*.

The above reviews bring to light the fact that the toxicity of metals are influenced by different biological and environmental variables like body size and salinity. Acute toxicity tests were conducted on *Sunetta scripta* belonging to three size groups in the three selected salinities.

3.2 MATERIALS & METHODS

Collection, acclimation and preparations were carried out as described earlier (Chapter 2). Animals were allowed to acclimatise in the laboratory condition for a period of two days. They were given artificial aeration and fed on blue green algae Synechocystis sp. grown in metalfree medium during the entire period of acclimatisation. The clams acclimated in the habitat salinity were transferred to the experimental salinities, 20×10^{-3}, 25×10^{-3} and 30×10^{-3} for acclimatisation for a period of 10 days. Selection of the experimental salinities is based on the results of the salinity tolerance experiments. As described by Portman & Connor (1968), Ahsanullah (1976) and D'Silva and Kureishy (1978), static bioassays were conducted for determining the acute toxicity of copper on Sunetta scripta. Test animals of the same size and number (ten in each) were transferred to glass troughs containing four litres of the filtered sea water of the desired salinity. The clams acclimated for two days in the above conditions were exposed to a range of concentrations of the metal under test. Calculated amounts of a 1000 ppm Cu(II) (as aqueous solution of $CuSO_4.5H_2O$) was added to the filtered sea water contained

in glass troughs to obtain final concentrations of 1, 2, 3, 4, 5, and 6 mg Cu l^{-1}(ppm). A control and a duplicate were run for each set of experiment. Troughs were aerated once a day and the D.O. was maintained within 90 ± 10% saturation. In order to avoid any possible loss of copper due to precipitation or adsorption to the sides of the glass trough, the medium was changed every day. Temperature of the water was 27 ± 2°C. Mortality was recorded every 24 h for a period of 120 h. Death criteria was lack of response to mechanical stimuli and inability to close the shell valves even when pressed together.

3.3 RESULT & DISCUSSION

No mortality was observed even in the highest concentration tested (6 ppm) in all the three size group viz. 20 mm to 25 mm, 30 mm to 35 mm and 40 mm to 45 mm in any of the experimental salinities. Therefore concentrations upto 6 ppm of copper can be considered as sublethal as far as Sunetta scripta is concerned in the salinity range 20×10^{-3} to 30×10^{-3} and temp. 27 ± 2°C. It was also observed that the animals on exposure to concentrations of copper secreted mucus, the amounts of secretion increasing with increasing copper concentrations.

The animals were also observed to keep their valves closed tightly in all the test media.

The threshold concentration of metals above which they are toxic varies from metal to metal, among the different organisms; it also depends on the environmental conditions. In Meretrix casta, Nambisan et al (1977) observed no mortality in an unbuffered medium of 10 ppm Cu even upto 2 months. Lakshmanan & Nambisan (1977) determined the 240 h LC_{50} for Cu in the bivalve Villorita cyprinoides as 2 ppm and concentration upto 0.5 ppm was observed to be sublethal. The 48 h LC_{50} for copper in Mytilus viridis was determined to be 0.14 ppm (D'Silva & Kureishy 1978). Kumaraguru & Ramamoorthi (1978) observed that the LC_{50} values for a period of 96 h in the estuarine bivalves Anadora granosa, Meretrix casta and Crassostrea madrasensis, was 0.06 ppm, 0.072 ppm and 0.08 ppm respectively. In Penaeus indicus, animals of smaller size had a low LC_{50} value at higher salinities while in low salinities the trend was reversed (Carmel et al 1983). In Metapenaeus dobsoni, belonging to juvenile and maturing stages 96 h LC_{50} values for copper was determined by

Sivadasan *et al* (1986) as 0.84 µg/l and 2.25 µg/l respectively. Wisely & Blick (1967) observed that 50% of the larvae of the mussel *Mytilus edulis* died in 2 hrs at 22.3 ppm copper concentration. This high resistance as explained by them was due to the ability to withdraw into their shells thereby reducing entry of the metal into the tissues. It was observed that the animals in all the experimental solutions had their valves tightly closed thereby avoiding contact of the toxic medium which results in reduced rate of mortality. Percentage mortality in some of the bivalves exposed to different copper concentrations is summarised in table 3.

One of the foremost observation in organisms under metal toxicity test is the damage of the gill epithelial cells and copious secretion of mucus (Scott & Major 1972, Engel & Fowler 1979). Increased secretion of mucus in *Sunetta scripta* observed with increasing concentration of copper is in agreement with report of the workers mentioned above. Secretion of mucus and the valve closing mechanism exhibited by bivalves can be considered as some of the methods employed for counter acting pollution. The copper ions may get bound to the mucus and thus get eliminated as

Table - 3

Percentage mortality of some bivalves exposed to copper concentrations:

Species	Time (in h)	Size (in mm)	Concentration (in ppm)	Mortality (%)	Source
Meretrix casta	480	30.0±0.5	10	40	Nambisan *et al* (1977)
Villorita cyprinoides	240	25±1	2	50	Lakshmanan & Nambisan (1978)
Mytilus viridis	48	15.88-16.07	.14	50	D'Silva & Kureishy (1978)
Anadara granosa	96	29-44	.060	50	Kumaraguru & Ramamoorthi (1978)
Meretrix casta	96	25-42	.072	50	
Crassostrea madrasensis	96	31-115	.088	50	
Mytilus edulis	2	Larvae	22.3	50	Wisely & Blick (1967)

suggested by Scott & Major (1972). Koringa (1952) reported that cations can be adsorbed on the mucus of the gills of Crassostrea virginica. Copper may also be transferred to metabolic wastes and removed via the faeces (Scott & Major 1972). Winter (1972) noted that very high concentrations of iron is tolerated by Mytilus edulis for a short time since almost the total amount is refused and returned as pseudo-faeces and only a small amount of iron enters the digestive tract. Several other mechanisms of detoxification have been suggested by various authors. Moreover, the chemistry of copper is known to be dominated by the phenomena of hydrolysis and precipitation. It is stated by Reeve et al (1977) that measurable copper was only 50-70% of the added quantity even when measurements are made soon after the addition. It is generally believed that copper is toxic to aquatic organisms only in ionic form.

Usually metals with a physiological role would be regulated but those without a physiological role are accumulated (Piccinni & Coppellotti 1982). Copper, being a normal constituent of many marine organisms and essential, for the normal growth and development (Bryan 1971) may be regulated by different mechanisms in different animals. Again, the biological response of different organisms to

trace metal concentrations can be very different. Bryan & Hummerstone (1971) noted that specimens of Nereis diversicolor having high concentrations of copper survive in areas of copper pollution since they have acquired a tolerance to the toxic effects of the metal.

Death by suffocation is the primary mode of action of heavy metals. The primary site of action of the heavy metals being the gills, thickening of the epithelial walls occurs and finally results in death by suffocation as oxygen consumption is impeded (Brown et al 1968). Precipitation of metal around the gills also may cause suffocation and death (Portman 1972).

Even though survival is considered as the best index of a copper stress (Winner & Farrell 1977), sublethal toxicity indices need much attention since they have proved to be more sensitive than the indicators of lethal toxic stress. Physiological damage is considered just as important as mortality since it may effect the efficiency of the animal to cope up with the surroundings and ultimately reduce its chances for survival. The elimination of a marine species by such low intensity selective factors may be even more serious than instantaneous death, since

it is impossible to be observed and corrected (Thurberg *et al* 1973). According to Clarke (Waldichuk (1974)), the only observed effect of some of the metals present in high concentrations in estuaries of the United Kingdom is a tendency for the organisms to be more sluggish in response which may be a clue to some rather interesting sublethal effects on the organisms. The capacity of *Sunetta scripta* to tolerate very high concentrations of copper without any mortality shows its importance as a bioassay organism for copper and possibly other metals too in sea water.

OXYGEN CONSUMPTION

It is well known that among the invertebrates the molluscs exhibit the greatest diversity in form and physiology. As a consequence, the metabolic rates also differ widely from one group to another. Moreover it is a known fact that the rate of metabolism varies widely depending upon the intrinsic and extrinsic factors even within a single species in any animal phylum. Oxygen consumption can be considered as a convenient measure of energy transformation (Scott & Major 1972). According to Ghiretti (1966) the class Bivalvia shows the greatest variability in the respiration rate. Even under constant external conditions the oxygen consumption of a given specimen varies considerably. Since the metabolic rate of an organism is influenced both by the intrinsic and extrinsic factors, it is always advisable to estimate the rate of oxygen consumption as a function of a single parameter keeping all other factors constant (Ghiretti 1966 and Vernberg & Vernberg 1972 a).

The oxygen uptake is an exponential function of the body weight (Zeuthen 1947, 1953, Hemmingsen 1950, 1960).

This relationship is most commonly expressed in the form of an allometric equation :

$$Y = a\ x^{b}$$

where 'Y' is the rate of O_2 consumption, 'X' is the body weight, 'a' is the Y intercept and 'b' is the slope. The equation may be linearised by taking the logarithm of both sides:

$$\text{Log } Y = \text{Log } a + b \log x$$

In a double logarithmic coordinate system in which the oxygen consumption is shown on the ordinate and weight on the abscissa, a straight line rather than an experimental curve is obtained.

The oxygen uptake per unit body weight per unit time ie. the metabolic rate is obtained by dividing the above equation by weight :

$$\frac{Y}{X} = QO_2 = a\ x^{b-1}$$

In the linearized form this expression for the 'weight specific' rate of O_2 consumption should be written

$$\text{Log } QO_2 = \log a + b\text{-}1 \log x$$

Where b-1 is the slope. The 'b' value is usually less than 1 and so 'b-1' has a negative value. There can also be negative 'b' values (Newell and Northcroft 1965) and 'b' values greater than 1 (Ansell 1973).

Zeuthen (1953) found a respiration coefficient (b) of 0.67 applicable to homeotherms suggesting that respiration is dependent on the surface volume relationship. However, the value of 'b' is seen to vary from 1 to negative numbers. Hemmingsen (1960) evolved a 'b' value of 0.751 $\pm$ 0.015 for poikilotherms. He had proposed that the metabolism is proportional not to the cell surface but to vascularization and development of a respiratory system. While reviewing the problem of size related metabolism of various animal groups three metabolic types have been suggested by Von Bertalanffy (1957) ie. proportionality of metabolic rate to surface area ('b' value 0.67) to weight ('b' value -1) and intermediate between surface and weight proportionality ('b' value 0.67-1). However many more metabolic types also exist.

Variations in 'b' values occur depending upon the changes in intrinsic and extrinsic factors. The reason for these variations is not explained fully so far.

Dehnel & McCaughran (1964) have suggested that the environmental factors and inherent physiological mechanisms are responsible for the occurrence of various 'b' values found among poikilotherms. According to some authors 'b' values of 0.80, 0.95 and 0.65 characteristic of different stages in the life history of Mytilus edulis. It has been suggested by Davies & White (1966) that variations in the respiration coefficients may be considered as deviations from the phylogenetic 'b' value of 0.75. Various other authors like Read (1962 a), Pamatmat (1969) and Ansell (1973) reported that there is no significant variations of 'b' over a range of temperatures, at different levels of ration and at different times of the year.

Body size is one of the important factors affecting the respiration of many animals (Huebner 1973). In general it has been established that in most of the animals, the oxygen uptake per unit time is proportional to some exponential function of the animal body weight (Zeuthen 1953). As mentioned earlier the relationship between oxygen uptake and body weight is expressed both intra-specifically and inter-specifically by the allometric equation $Y = a \times ^{b}$. Since the mass exponent 'b' is

smaller than unity, the mass specific metabolic rate $\frac{y}{x}$ decreases with increasing body mass. Rubner has explained this phenomenon as the 'surface rule' as early as 1883 in homeotherms. Many authors have discussed the above phenomenon (Winberg 1956, Kleiber 1961). Considerable information has gathered on the effect of body size on the respiration of marine and estuarine organisms. The relationship between body weight and oxygen consumption was investigated by Von Brand et al (1948) in snails. 'b' values for oxygen consumption and body size were determined by Rotthauwe (1958) and Kruger (1960) on Mytilus edulis, Kuenzler (1961) on Modiolus demissus and Read (1962 a) on Modiolus demissus and Mytilus edulis for different size ranges and under different experimental conditions. Read (1962 b) studied the respiration of Mytilus and Branchidontus as a function of size and temperature. The influence of body weight on the oxygen consumption rate was worked out by Srinivasan (1965) in Martesia fragilis, Nagabhushanam (1966) in Martesia striata and Davies (1966) in Patella vulgata. 'b' value for Perna perna was determined by Bayne (1967) while Vahl (1972 a, 1973) had determined the oxygen consumption of Cardium edule and Mytilus edulis belonging to different

size groups respectively. Similarly Ansell (1973) had worked on Donax vittatus. Ranade (1973) had studied the oxygen consumption of the clams Meretrix meretrix and Katelysia opima in relation to size and salinity. Bayne et al (1973) had determined both 'routine' and 'standard' rates of oxygen uptake in Mytilus edulis ranging in size 0.07 - 3 gms dry weight during summer and winter. Weight specific 'b' values for oxygen consumption was determined by Mangapati Rao et al (1974) in Congeria sallei and Bayne et al (1975) in Mytilus californianus the gastropuod Turbo intercosfalis. Salih (1978) had studied oxygen consumption in the clam Meretrix casta in relation to size. The effect of seasonal changes in the oxygen consumption of the iceland scallop Chlamys islandica belonging to different sizes was determined by Vahl (1978). Mohan (1979) determined the oxygen consumption of the ship worms Nausitora hedleyi and Teredo furcifera in relation to body weight. Oxygen consumption of the clam Meretrix meretrix was studied in relation to body size by Deshmukh (1979). Famme (1980) studied the relation between oxygen consumption and body weight in starved specimens of Mytilus edulis. Rao et al (1982) investigated size related metabolism in Perna viridis. Hamburger et al (1983) had

studied the relationship between oxygen consumption and tissue dry mass in Mytilus edulis from very early veliger to the adult stage.

Changes in 'b' values due to variations in salinity were observed by many authors. Kennedy & Mihursky (1972) on Mya arenaria, Macoma balthica & Mulinia laternalis, Shafee (1976) on Mytilus viridis, Salih (1978) on Meretrix casta and Mohan (1979) on Nausitora hedleyi. Thus estuarine and marine organisms subjected to variations in salinity exhibit various metabolic types deviating from the basic pattern. According to Kinne (1971) marine and brackish water invertebrates exhibit 4 different types of respiratory behaviour when salinity varies. Within the tolerance range, it can be (1) an increase in sub-normal salinities and/or decrease in supra-normal salinities (2) an increase in sub and supra normal salinities (3) decrease in sub and supra normal salinities (4) essentially unaffected. The first two types of metabolic responses are represented by euryhaline invertebrates while the third and fourth types are shown by stenohaline and extremely euryhaline animals respectively (Kinne 1971, Vernberg & Vernberg 1972).

The effect of temperature on the variations in 'b' values for bivalves was observed by many authors like Kuenzler (1961), Hughes (1970) and Ansell (1973). Different 'b' values with changes in oxygen tension were determined by Cherian (1978 a), Mohan (1979), Mohan & Cheriyan (1980) and Famme (1980 a & 1980 b). Influence of season on the value of 'b' was determined by Bayne et al (1973) in Mytilus edulis.

It is well known that salinity has considerable influence on the metabolic activities of animals. The metabolic response of aquatic poikilotherms to osmotic stress has been investigated by many workers. But the information available on the effect of salinity on the oxygen consumption of bivalve molluscs is meagre. The effect of salinity variations on the oxygen consumption rate of Mytilus galloprovincialis was investigated by Bouxin (1931). Hiscock (1953) measured the oxygen consumption rate of the Australian freshwater mussel Hyridella australis in relation to osmoregulation. Rao (1958) studied the influence of salinity on the oxygen consumption of Metapenaeus monoceros from marine and brackish water environments. Changes in metabolic rates

due to osmotic stress was reviewed by Remane & Schlieper (1971) in various poikilotherms. Lagerspetz & Sirkka (1959) had investigated the effect of salinity variations on the oxygen consumption rate of Mytilus edulis. Nagabhushanam (1962) has studied the respiration of Martesia striata in different salinities. Potts & Parry (1964) had reviewed the changes in the metabolic rates due to osmotic stress in various poikilotherms. Influence of salinity on the respiration of marine invertebrates was reviewed in detail by Kinne (1964 a, 1964 b). Kinne (1964 a) has cited the response of Mytilus viridis acclimated in low salinity and transferred to high salinity and vice versa. The effect of salinity on the oxygen consumption rate was studied by King (1965) in Maja verrucosa, Libinia emarginata, Carcinus mediterraneus and Calinectes sepidus. Ghiretti (1966) has made an overall review of the work on the oxygen consumption of molluscs in relation to different salinities. Salinity impact on the oxygen consumption rate of Mytilus perna was studied by Bayne (1967). Remane & Schlieper (1971) have also made a detailed review regarding the effect of salinity on the respiration of marine invertebrates. Influence of salinity on the oxygen consumption of the

fresh water mussel Parreysia corrugata was worked out by Lomte & Nagabhushanam (1971). Bayne (1973) had conducted similar studies on the bivalves Geolina ceylonica, Anadora granosa and Mytilus edulis. The metabolism of Katelysia opima and Meretrix meretrix was investigated by Ranade (1973) in different salinities. The changes in the oxygen consumption in varying salinities was studied by Shafee (1976) in Mytilus viridis and Salih (1978) in Meretrix casta. Impact of salinity on the oxygen consumption rate was studied by Newell (1979) on many marine invertebrates. Mohan (1979) determined the effect of varying salinity on the oxygen consumption of the shipworm Nausitora hedleyi acclimated in 5 x 10^{-3} and 20 x 10^{-3} salinities. The respiration rate of Mytilus edulis and Katherina tunicata was determined under varied salinity conditions by Stickle & Sabourin (1979). Deshmukh (1979) studied the oxygen consumption of the clam Meretrix meretrix in relation to low salinity. Considerable information is also available regarding the effect of salinity on isolated gill preparations (Lagerspetz & Sirkka 1959; Lange 1968 and Van Whinkle 1968).

Currently when pollution has been the focus of attention of several reviews the biological impact of the pollution load can be best understood by the biochemical and physiological responses of organisms through the various known indices. Among such potential indicators of sublethal toxic stress, oxygen consumption has received much attention. The heavy metals in general are known to interfere with the various important physiological functions and hence they bring about changes in the metabolic rate. This change can be well understood through oxygen consumption studies since utilization of oxygen is a direct measure of degree of activity, food conversion and heat production in animals.

There is only scant information regarding the effect of heavy metals on the oxygen consumption of aquatic invertebrates. Effect of various heavy metals like Cr, Ag, Cd, Hg, and Zn were worked out by Fromm & Stokes (1962), Thurberg et al (1974), and Tort et al (1982) respectively. Capuzzo & Sasner (1977) investigated the effect of Cr on the oxygen consumption rates of excised gill tissue of Mytilus edulis. Very few authors have worked on the impact of copper (II) on the respiratory

rate of the animals. Shapiro (1964) studied the oxygen consumption in Mytilus galloprovincialis maintained in sea water containing magnesium, copper, nickel and molybdenum ions. Baker (1969) had observed the toxic effects of copper on the gill tissue of many marine animals. Scott & Major (1972) had studied the effect of copper (II) on the oxygen consumption of the blue mussel, Mytilus edulis. The impact of copper and zinc on the oxygen uptake was investigated by Brown & Newell (1972) on Mytilus edulis. Thurberg et al (1973) had determined the effect of cadmium and copper on the gill tissue oxygen consumption of the crabs Carcinus maenas and Cancer irroratus. The effect of heavy metals, copper, silver, arsenic, zinc and cadmium on the oxygen consumption of the mud snail Nassarius obsoletus was worked out by MacInnes & Thurberg (1973). Delhaye & Cornet (1975) determined the effect of copper on the oxygen consumption of the bivalve Mytilus edulis. Effect of heavy metals Ag, Cu, Zn & Pb on the oxygen consumption of the bivalves Perna viridis and Meretrix casta was studied by Mathew & Menon (1983).

From the foregoing account, the influence of body size and salinity on the respiration of marine organisms is

quite evident. The impairement of the respiratory process by the heavy metal copper in marine invertebrates is also brought to light from the above reviews. Taking into consideration the fact that the natural habitat of the clam Sunetta scripta is subjected to wide variations in salinity, animals of different size groups were experimented in the different salinity media for a clear understanding of the impact of the heavy metal copper (II) on the oxygen consumption of the animal.

4.2 MATERIALS & METHODS

Collection of specimens, acclimation, preparations, etc. were done as described in the Chapter 2 salinity tolerance. The oxygen consumption rates were studied in the same salinity regimes as in the toxicity studies (20×10^{-3}, 25×10^{-3} and 30×10^{-3} salinities) because the animals were found to be less active below and beyond this range. The clams acclimated in the habitat salinity were kept in the experimental salinities for an acclimatisation period of 2 days.

Animals of different sizes (21 mg - 1096 mg dry wt.) were experimented in the three salinity regimes with 1 ppm and 2 ppm copper concentrations for studying the effect of

the metal on the oxygen consumption rate of the animal. Choice of the above two concentrations of copper is on the ground that preliminary experiments with lower concentrations of the metal did not show any notable difference when compared to the control and that copper concentrations above 2 ppm is an unusual condition as far as copper contamination in nature is concerned.

The animals were pre-exposed to the respective copper concentrations in the desired salinity regimes for 24 hrs before experimenting. In order to prevent closure of valves, small glass pieces were introduced in between the valves carefully without injuring the animal. A control identical in all respects excepting the presence of the pollutant was run for each set of the experiment.

The respirometer designed by Mohan & Cheriyan (1980) was employed for the oxygen consumption rate studies (Fig 3). It consists of a respiratory chamber with an air tight lid carrying a 30 ml syringe, two inlets and an outlet. One of the inlets, 'A' which extends to the bottom of the respiratory chamber is connected to a constant level overflow tank 'R' and the other inlet, 'C' is connected to a burette, 'B' of 25 ml capacity. Samples for the estimation

FIG. 3

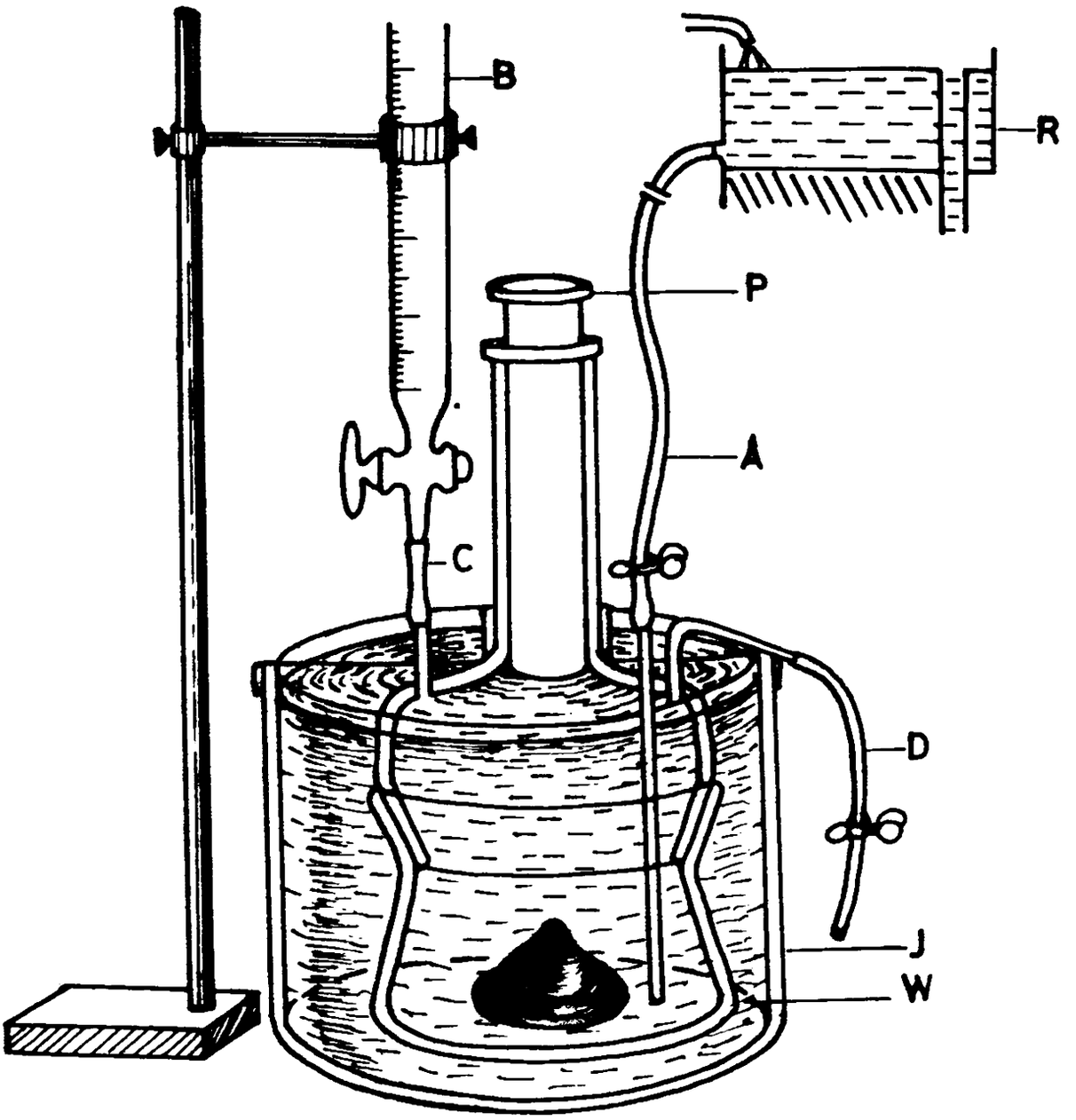


Fig. 3 :- Apparatus used for the determination of the oxygen consumption rate.

of oxygen are drawn out through the outlet, 'D'. The internal volume of the rubber tube connections is found to be photo-sensitive the respiratory chamber is covered with a black paper jacket, 'J' provided with a small window for observing the activity of the animal.

To set the experiment, the experimental animal was carefully placed in the respiratory chamber filled with filtered sea water of the desired salinity. The chamber was closed with the air tight lid without the plunger. Sea water was then let out from the overflow tank while keeping the outlet closed. The column of air in the rubber tube connections between the apparatus and the burette was removed by running a few ml. of water from the latter. When the apparatus was completely filled and the water began to overflow, the inlet from the overflow tank was closed and the outlet was opened. The plunger was then replaced taking care not to trap any air bubbles. It was then slowly pressed down completely to expel all the air trapped in the outlet. When the apparatus was completely devoid of air bubbles, the inlet from the overflow tank was opened to have a continuous flow of sea water at a constant rate for about 30 min. for acclimatizing the animal to the experimental conditions. The

outlet was then closed and the continuous flow was cut off. Exactly 10 ml of the sea water was let into the apparatus from the burette which kept the plunger of the syringe in a raised position. The duration of the experiment was fixed from pilot experiments. Each time the sample is drawn out from the outlet the capacity of the respiratory chamber was restored to the original volume by the water drawn from the burette. The oxygen bottles of 9 ml and respiratory chambers of 250 ml capacity were used. A control was run under identical conditions without the animal. Samples were taken every one hour for a period of 3 hrs and the average value was considered. The experiments were run within a temperature range of 28 $\pm$ 1°C. No significant variation of pH in the experimental medium was noticed before and after the experiment. The test solution was not stirred during the course of the experiment for making it homogenous since the animal itself was capable of setting in a circulation of water through its siphonal activity and also even the least disturbance to the animal will bring about changes in the oxygen consumption rate.

Winkler's micromethod (Welsh & Smith, 1953) was used for the determination of the dissolved oxygen content of the water samples. Titrations were done using a 1 ml tuberculine syringe which can be read upto 5 ml. Normality of $Na_2S_2O_3$ used for titrations ranged from 0.002 ml and 0.005 ml and it was verified every time before use. The volume of the experimental animal was found out with the shells open for the determination of the actual volume of the test medium. The soft parts of the clam were separated from the shell, dried to constant weight in an air oven at 60°C. and the dry weight was determined.

To study the interdependency of the variations under reference, correlation and regression analysis were employed. The coefficient of correlation was worked out by the formula suggested by Carson. The coefficient of correlation :

$$r = \frac{1}{n}\sum\left[\frac{x-\bar{x}}{s_x}\right]\left[\frac{y-\bar{y}}{s_y}\right] \quad \text{where}$$

$$\bar{x} = \frac{\sum x}{n}, \quad \bar{y} = \frac{\sum y}{n}$$

s_x = S.D. of x $= \left[\frac{1}{n}\sum(x-\bar{x})^2\right]^{1/2}$

s_y = S.D. of y $= \left[\frac{1}{n}\sum(y-\bar{y})^2\right]^{1/2}$

and n = no. of pairs of values.

The significance of the correlation was tested by using 't' statistic.

$$t = r\left[\frac{n-2}{1-r^2}\right]^{1/2}$$

This 't' is having degrees of freedom n - 2.

Wherever the 'r' is found to be significant regression equations connecting x and y are worked out by the method of least squares. When the sum of squares of errors are minimized for the regression line of y or x, it will reduce to two normal equations $b\Sigma x^2 + a\Sigma x = \Sigma xy$ and $b\Sigma x + n\,a = \Sigma y$. From the data Σx, Σx^2, Σxy and Σy were calculated and substituted in the normal equations. Solving the normal equations the values of 'a' and 'b' are obtained. The goodness of fit of the regression equations worked out are shown in the graph.

To compare the regression coefficients in the various levels of salinity as well as concentrations of copper the analysis of covariance was employed.

4.3 RESULTS

1. Experiments on the effect of copper on the oxygen consumption of *Sunetta scripta* in relation to body weight in 30 x 10^{-3} salinity.

1.a Oxygen consumption of the animals in the control experiments in 30 x 10^{-3} salinity.

In animals of the size range 37 mg - 1072 mg dry weight the rate of oxygen consumption varied from 23.4 to 251.2 $\mu l O_2/h$. It can be noted that the rate of oxygen consumption increases with the increase in body size. A double logarithmic plot of oxygen uptake rate and body weight is shown in Fig.4. The estimated values of the regression coefficient, 'b' and log 'a' are 0.5910 and 0.5716 respectively. The oxygen uptake rate and metabolic rate for different weights of the above size range of *Sunetta scripta* under the control experiments in 30 x 10^{-3} salinity are given in Table 4.

The metabolic rate is the oxygen consumption per unit body weight in unit time ranged between 928.26 and 173.69 $\mu l O_2/g/h$. It is seen that the metabolic rate decreases with increasing body weight. In Fig.7 is shown the double logarithmic plot of metabolic rate against body

weight which showed a negative linear relationship with b-1, -0.4090.

1.b Oxygen consumption of the animals exposed to 1 ppm of Cu(II) in 30 x 10^{-3} salinity.

The oxygen uptake rate and metabolic rate of the clams of different sizes exposed to 1 ppm of copper (II) in 30 x 10^{-3} salinity is presented in Table 5. Body weight of the animals ranged between 42 mg - 899 mg dry weight while the oxygen uptake and metabolic rate varied from 14.4 to 128.2 $\mu l O_2/h$ and 548 to 73.94 $\mu l O_2/g/h$ respectively. A double logarithmic plot of the oxygen consumption and body weight is given in Fig.5 and metabolic rate and body weight in Fig.8 values of 'b' and log 'a' are 0.5129 and 0.4843 respectively while 'b-1' is estimated to be -0.4871.

1.c Oxygen consumption of the animals exposed to 2 ppm of Cu(II) in 30 x 10^{-3} salinity.

Animals ranging in size 50 mg - 877 mg dry weight consumed oxygen ranging from 12.6 to 72.2 $\mu l O_2/h$ while the rate of metabolism is seen to vary from 438 to 46.52 $\mu l O_2/g/h$. The rate of oxygen uptake and metabolic rate for animals of different weights of the above size range exposed to

2 ppm of copper (II) in 30 x 10^{-3} salinity is presented in Table 6. In Fig.6 logarithm of oxygen uptake rate is plotted against logarithm of body weight and in Fig.9 logarithm of metabolic rate is plotted against logarithm of body weight. The estimated values of b and log 'a' are 0.4327 and 0.5834 respectively. The metabolic rate and body weight showed a negative linear relationship with 'b-1' having a value of -0.5684.

2. Experiments on the effects of copper on the oxygen consumption of Sunetta scripta in relation to body weight in 25 x 10^{-3} salinity.

2.a Oxygen consumption of the animals under the control experiments in 25 x 10^{-3} salinity.

The rate of oxygen uptake and metabolic rate for control animals of different weights in 25 x 10^{-3} salinity is presented in Table 7. The oxygen uptake rate varied from 14.5 to 111.6 $\mu l O_2/h$ and the metabolic rate 627.5 to 76.27 $\mu l O_2/g/h$ in animals ranging in size 36 mg to 932 mg dry weight. In Fig.10 the double logarithmic plot of oxygen uptake rate against body weight is shown. The estimated values of 'b' and log 'a' are 0.4847 and 0.6874 respectively. In Fig.13 a double logarithmic plot of the

metabolic rate against body weight is shown. The weight specific regression coefficient is -0.5083.

2.b Oxygen consumption of the animals exposed to 1 ppm of Cu(II) in 25 x 10^{-3} salinity.

The oxygen uptake rate and the metabolic rate of the animals ranging in size 32 mg to 468 mg dry weight varied from 11 to 63.8 $\mu l O_2/h$ and 627.5 to 69.21 $\mu l O_2/g/h$ respectively (Table 8). A double logarithmic plot of oxygen uptake rate and body weight is shown in Fig.11. The 'b' and log 'a' values are estimated to be 0.4742 and 0.4691 respectively. In Fig.14 logarithm of metabolic rate is plotted against logarithm of body weight. The value of 'b-1' is estimated to be 0.5019.

2.c Oxygen consumption of the animals exposed to 2 ppm of Cu(II) in 25 x 10^{-3} salinity.

In animals of the size range 40 mg to 683 mg dry weight the rate of oxygen consumption and the rate of metabolism varied from 14.5 to 79.1 $\mu l O_2/h$ and 572.5 to 47.30 $\mu l O_2/g/h$ respectively. The rate of oxygen consumption and metabolic rate of animals of different weights in the above size range are presented in Table 9. In

Fig.12 logarithm of oxygen uptake rate is plotted against logarithm of body weight while in Fig.15 logarithm of metabolic rate is plotted against logarithm of body weight. Estimated values of 'b', log 'a' and 'b-1' are 0.4714, 0.3870 and -0.5295 respectively.

3. Experiments on the effect of copper (II) on the oxygen consumption of Sunetta scripta in relation to body weight in 20 x 10^{-3} salinity.

3.a Oxygen consumption of Sunetta scripta in the control experiments in 20 x 10^{-3} salinity.

The rate of oxygen consumption and metabolic rate of Sunetta scripta of different sizes under the control experiments in 20 x 10^{-3} salinity is presented in Table 10. The oxygen uptake rate varied from 18.6 to 162 $\mu l O_2/h$ and the metabolic rate 1143.48 to 109.16 $\mu l O_2/g/h$ in animals whose dry weight ranged between 21 mg and 682 mg. The double logarithmic plot of oxygen uptake rate against body weight is shown in Fig.16. The 'b' and log 'a' values estimated are 0.4158 and 0.6884 respectively. In Fig.19 a double logarithmic plot of metabolic rate against body weight is shown. The weight specific regression coefficient is -0.5859.

3.b Oxygen consumption of the animals exposed to 1 ppm of Cu(II) in 20 x 10^{-3} salinity.

The oxygen uptake rate and metabolic rate of the animals ranging in size 40 mg to 692 mg dry weight varied from 15.9 to 104.7 $\mu l O_2/h$ and 790 to 63.58 $\mu l O_2/g/h$ respectively (Table 14). A double logarithmic plot of oxygen consumption against body weight is shown in Fig.17. Values of 'b' and log 'a' are estimated to be 0.4096 and 0.5442 respectively. In Fig.20 double logarithmic plot of metabolic rate against body weight is shown. The estimated value of 'b-1' is -0.6039.

3.c Oxygen consumption of the animals exposed to 2 ppm of Cu(II) in 20 x 10^{-3} salinity.

In animals of the size range 22 mg - 990 mg dry weight the rate of oxygen consumption varied from 12.8 to 86.5 $\mu l O_2/h$ while the rate of metabolism ranged between 763.64 and 42.70 $\mu l O_2/g/h$. The oxygen uptake and metabolic rate of the animals of the above size range are presented in Table 12. In Fig.18 logarithm of oxygen uptake and in Fig.21 the logarithm of metabolic rate are plotted against logarithm of body weight. The estimated values of 'b', log 'a' and 'b-1' are 0.4172, 0.4701 and -0.5843 respectively.

Table - 4 Oxygen uptake rate and metabolic rate of Sunetta scripta in the control experiments in 30 x 10^{-3} salinity.

Sl. No.	Weight (mgs)	Oxygen consumption rate ($\mu lo_2/h$)	Metabolic rate ($\mu lo_2/g/h$)	Sl. No.	Weight (mgs)	Oxygen consumption rate ($\mu lo_2/h$)	Metabolic rate ($\mu lo_2/g/h$)
1.	37	23.4	632.43	21.	302	104.7	346.69
2.	38	33.1	871.05	22.	324	151.4	467.28
3.	48	42.7	889.58	23.	331	128.8	389.12
4.	46	42.7	928.26	24.	331	151.4	457.40
5.	47	42.7	908.51	25.	331	85.1	257.10
6.	59	42.7	723.73	26.	513	147.9	288.30
7.	63	30.2	479.37	27.	575	190.6	331.48
8.	76	42.7	561.84	28.	468	128.8	275.21
9.	91	79.4	872.53	29.	407	125.9	309.34
10.	105	45.7	435.24	30.	468	151.4	323.50
11.	132	64.6	489.39	31.	457	128.8	281.84
12.	145	50.1	345.52	32.	525	147.9	281.71
13.	151	87.1	576.82	33.	347	128.8	371.18
14.	162	95.5	589.51	34.	380	104.7	275.53
15.	178	95.5	536.52	35.	813	251.2	308.98
16.	195	64.6	331.28	36.	794	204.2	257.18
17.	200	100	500	37.	1096	204.2	186.31
18.	234	95.5	408.12	38.	1072	186.2	173.69
19.	263	95.5	363.12				
20.	288	85.1	295.49				

Table - 5 Oxygen uptake rate and metabolic rate of Sunetta scripta exposed to 1 ppm of copper in 30 x 10^{-3} salinity.

Sl. No.	Weight (mgs)	Oxygen consumption rate ($\mu lo_2/h$)	Metabolic rate	Sl. No.	Weight (mgs)	Oxygen consumption rate ($\mu lo_2/h$)	Metabolic rate	Sl. No.	Weight (mgs)	Oxygen consumption rate ($\mu lo_2/h$)	Metabolic rate
1.	36	14.5	402.78	21.	196	68.2	347.96	41.	394	83.8	212.69
2.	40	25.1	627.5	22.	217	63.2	291.24	42.	395	43	108.86
3.	50	19.1	381	23.	226	37.2	164.60	43.	403	84	208.44
4.	58	27.5	474.14	24.	242	66.3	273.97	44.	410	41.7	101.71
5.	63	15.8	250.79	25.	272	46	169.12	45.	423	83.8	212.69
6.	63	31.6	501.59	26.	276	52.9	191.67	46.	426	98	230.05
7.	74	32.6	440.54	27.	278	74.3	267.27	47.	438	52.2	119.18
8.	79	20	253.17	28.	291	65.2	224.06	48.	438	77.9	177.85
9.	86	26.3	305.81	29.	303	82.3	271.62	49.	440	65.5	148.86
10.	100	36.3	363	30.	309	50.6	163,75	50.	445	97.7	219.55
11.	113	47.7	422.12	31.	310	89.5	288.71	51.	465	78.3	168.39
12.	116	32.8	282.35	32.	317	84.3	265.93	52.	516	58.8	170.93
13.	121	22.4	185.12	33.	319	36.5	114.42	53.	555	58.4	105.23
14.	131	47.7	364.12	34.	321	45.6	142.06	54.	680	56	82.35
15.	136	52	382.35	35.	337	36.4	108.01	55.	740	111.6	150.81
16.	145	28.8	198.62	36.	337	47.7	141.54	56.	932	86.9	93.24
17.	154	48	311.69	37.	338	102	301.78				
18.	158	44.7	282.91	38.	344	58.8	170.93				
19.	166	50.5	304.22	39.	354	36.8	103.96				
20.	183	34.5	188.53	40.	367	98.8	269.21				

Table - 6 Oxygen uptake rate and metabolic rate of Sunetta scripta exposed to 2 ppm of copper in 30 x 10^{-3} salinity.

Sl. No.	Weight (mgs)	Oxygen consumption rate ($\mu l o_2/h$)	Metabolic rate	Sl. No.	Weight (mgs)	Oxygen consumption rate ($\mu l o_2/h$)	Metabolic rate	Sl. No.	Weight (mgs)	Oxygen consumption rate ($\mu l o_2/h$)	Metabolic rate
1.	40	22.9	572.5	21.	175	59.5	340	41.	339	39.4	116.22
2.	44	14.5	329.55	22.	180	64.2	356.67	42.	371	63	169.81
3.	49	14.5	295.92	23.	189	32.1	169.84	43.	388	63.2	162.89
4.	53	27.4	516.98	24.	190	32	168.42	44.	407	29.2	71.75
5.	55	20	363.64	25.	195	59.3	304.10	45.	409	47.4	115.89
6.	69	17.4	252.17	26.	202	46.2	228.71	46.	549	64.4	117.30
7.	69	24	347.83	27.	204	29.7	145.59	47.	561	57.7	102.85
8.	79	28.8	364.56	28.	205	64	312.20	48.	590	77.6	131.53
9.	91	17.4	191.21	29.	222	36	162.16	49.	596	46.6	78.19
10.	93	34.7	373.12	30.	227	59.5	262.12	50.	611	28.9	47.3
11.	117	32.8	280.34	31.	227	50.8	223.79	51.	651	68.3	104.92
12.	118	20.1	170.34	32.	236	25.4	107.63	52.	665	55.6	83.61
13.	119	32.2	270.59	33.	238	76.9	323.11	53.	683	77.4	113.32
14.	120	38.2	318.33	34.	246	39.4	160.16				
15.	120	34.6	288.33	35.	295	79.1	268.14				
16.	129	38.2	296.12	36.	295	31.6	107.12				
17.	134	20.2	150.75	37.	297	29.7	100				
18.	162	29.8	183.95	38.	315	47.9	152.06				
19.	164	24.1	146.95	39.	326	46.3	142.03				
20.	169	19.1	113.02	40.	327	61.7	188.69				

Table - 7 Oxygen uptake rate and metabolic rate of Sunetta scripta in the control experiments in 25 x 10^{-3} salinity.

Sl. No.	Weight (mgs)	Oxygen consumption rate (Ulo_2/h)	Metabolic rate ($ulo_2/g/h$)	Sl. No	Weight (mgs)	Oxygen consumption rate (Ulo_2/h)	Metabolic rate ($ulo_2/g/h$)	Sl. No.	Weight (mgs)	Oxygen consumption rate (ulo_2/h)	Metabolic rate ($ulo_2/g/h$)
1.	252	42.1	167.0635	24.	170	73.9	434.7059	47.	183	30.7	167.7596
2.	390	63.8	163.5897	25.	590	162	274.5763	48.	253	61.3	242.2925
3.	350	65.4	186.8571	26.	598	108	180.6020	49.	191	65	340.3141
4.	480	137.8	287.0833	27.	467	62.5	133.8330	50.	151	28	185.4305
5.	430	121.7	283.0233	28.	174	55.7	320.1149	51.	177	29.6	167.2316
6.	538	98.9	183.8290	29.	279	110	394.2652	52.	132	56	424.2424
7.	390	55.4	142.0513	30.	197	55.5	281.7259	53.	198	59	297.9798
8.	295	45	152.5424	31.	181	74	408.8398	54.	79	31.6	400
9.	221	52.3	236.6516	32.	231	55.5	240.2597	55.	74	51.3	693.2432
10.	467	59.3	126.9807	33.	395	128.3	324.8101	56.	23	26.3	1143.4783
11.	460	93	202.1739	34.	140	37.1	265	57.	50	28.8	576
12.	633	69.1	109.1627	35.	389	95.5	245.5013	58.	72	27.5	381.9444
13.	508	80.8	159.0551	36.	532	95.6	179.6992	59.	28	22.9	817.8571
14.	360	70.7	196.3889	37.	521	131	251.4395	60.	32	20	625
15.	44	37.2	845.4545	38.	484	95.6	197.5207	61.	69	22.9	331.8841
16.	29	27.9	962.0690	39.	424	95.8	225.9434	62.	50	20.9	418
17.	33	27.9	845.545	40.	604	78.2	129.4702	63.	63	36.3	576.1905
18.	35	27.9	797.1429	41.	404	78.2	193.5644	64.	79	57.5	727.8481
19.	36	18.6	516.6667	42.	541	156.3	288.9094	65.	100	33.1	331
20.	36	37.2	1033.3333	43.	367	78.2	213.0790	66.	110	28.8	261.8182
21.	21	18.6	885.7143	44.	540	136.7	253.1481	67.	120	36.3	302.5000
22.	25	18.6	744.00	45.	549	85.6	155.9199				
23.	43	24.8	576.7442	46.	682	93	136.3636				

Table - 8 Oxygen uptake rate and metabolic rate of Sunetta scripta exposed to 1 ppm of copper in 25 x 10^{-3} salinity.

Sl. No.	Weight (mgs)	Oxygen consumption rate (μlo_2/hr)	Metabolic rate (μlo_2/g/h)	Sl. No.	Weight (mgs)	Oxygen consumption rate (μlo_2/hr)	Metabolic rate (μlo_2/g/h)
1.	42	16.9	402.38	21.	198	32.5	131.05
2.	52	32.5	164.14	22.	200	25.1	125.5
3.	60	27.4	279.59	23.	218	47.7	218.81
4.	60	79.4	199.50	24.	220	43.2	196.36
5.	69	17.4	228.95	25.	248	63.5	87.71
6.	76	20.9	264.56	26.	251	25.1	125.5
7.	79	18.2	303.33	27.	316	41.7	131.96
8.	86	31.1	361.63	28.	370	31.4	84.86
9.	92	27.4	456.67	29.	398	79.4	199.50
10.	98	14.5	278.85	30.	417	39.8	95.44
11.	103	40.4	348.28	31.	502	38.3	296.90
12.	106	32	301.89	32.	515	63.4	88.67
13.	109	31.9	292.66	33.	604	87.3	144.54
14.	116	52.3	78.77	34.	664	52.3	78.77
15.	118	26.3	381.16	35.	667	51.4	102.39
16.	128	19.2	186.41	36.	710	52.5	73.94
17.	129	19.1	149.22	37.	715	63.4	88.67
18.	129	31.9	247.29	38.	724	60.6	117.67
19.	133	31.9	239.85	39.	838	101.1	120.64
20.	191	28.8	150.79	40.	899	89.7	99.78

Table - 9 Oxygen uptake rate and metabolic rate of Sunetta scripta exposed to 2 ppm of copper in 25 x 10^{-3} salinity.

Sl. No.	Weight (mgs)	Oxygen Consumption Rate ($\mu l o_2$/h)	Metabolic Rate ($\mu l o_2$/g/h)	Sl. No.	Weight (mgs)	Oxygen Consumption Rate ($\mu l o_2$/h)	Metabolic Rate ($\mu l o_2$/g/h)
1.	32	15.9	496.88	21.	147	40.4	274.83
2.	35	17.4	497.14	22.	149	29.6	198.66
3.	38	20.9	550	23.	151	32.8	217.22
4.	40	12.6	315	24.	156	42.0	269.23
5.	50	15.9	318	25.	158	20.4	129.11
6.	50	11.0	220	26.	169	40.6	240.24
7.	50	12.6	252	27.	205	24.0	117.07
8.	58	12.0	206.90	28.	232	49.9	215.09
9.	58	13.8	237.93	29.	249	40.3	161.85
10.	60	25.7	428.33	30.	287	34.0	118.47
11.	66	12.9	195.46	31.	302	25.1	83.11
12.	68	24.3	357.35	32.	309	52.5	169.90
13.	78	12.0	153.85	33.	341	51.0	149.56
14.	79	21.9	277.22	34.	355	29.5	83.10
15.	83	14.5	174.70	35.	380	26.3	69.21
16.	87	12.0	137.93	36.	437	34.7	79.41
17.	100	18.2	182	37.	457	41.7	91.25
18.	110	27.4	249.09	38.	468	55.0	117.52
19.	129	16.2	125.58				
20.	138	20.0	144.93				

Table - 10 Oxygen uptake rate and metabolic rate of Sunetta scripta in the control experiments in 20 x 10^{-3} salinity.

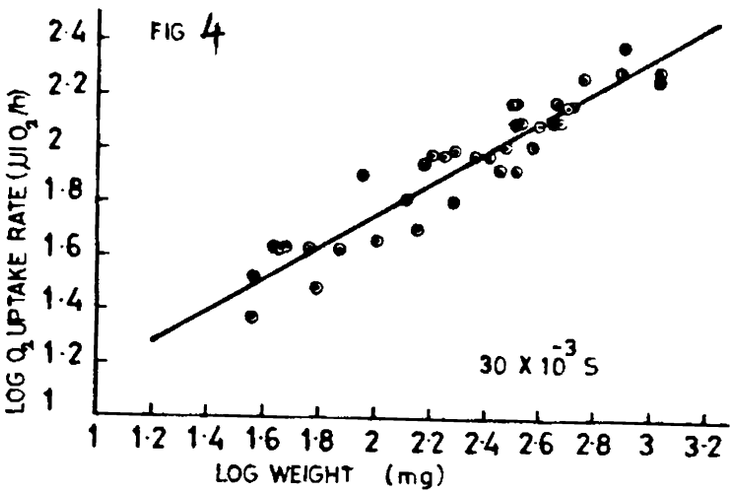
Sl. No.	Weight (mgs)	Oxygen consumption rate ($\mu l o_2/h$)	Metabolic rate	Sl. No.	Weight (mgs)	Oxygen consumption rate ($\mu l o_2/h$)	Metabolic rate	Sl. No.	Weight (mgs)	Oxygen consumption rate ($\mu l o_2/h$)	Metabolic rate
1.	40	31.6	790	21.	133	26.5	199.25	41.	291	60.1	206.53
2.	40	15.9	397.5	22.	143	87.1	609.09	42.	296	82.3	278.04
3.	49	28.1	573.47	23.	144	18.8	130.56	43.	297	43.5	146.47
4.	55	26.6	483.64	24.	144	27.9	193.75	44.	302	64.9	214.90
5.	58	43.2	744.83	25.	147	29.6	201.36	45.	310	81.8	263.87
6.	64	28.2	440.63	26.	181	26.3	145.30	46.	318	98.7	310.38
7.	65	28.8	443.08	27.	191	26.3	137.70	47.	398	39.8	100
8.	75	49.1	654.67	28.	195	86.8	445.13	48.	411	86.6	341.73
9.	78	28.6	366.67	29.	202	41.9	207.43	49.	413	65	157.39
10.	82	29.7	362.20	30.	210	65.1	310	50.	437	33.1	75.74
11.	83	16.6	200	31.	212	82.8	390.57	51.	437	28.8	65.90
12.	91	29.7	362.20	32.	227	65.3	287.67	52.	457	104.7	229.10
13.	97	42.1	434.02	33.	232	66.4	286.21	53.	501	72.4	144.51
14.	102	18.7	180.33	34.	251	66.3	264.14	54.	510	61.3	63.58
15.	110	18.7	170	35.	251	34.7	138.25	55.	603	79.4	131.68
16.	113	18.7	167.80	36.	254	86.8	341.73	56.	631	63.1	100
17.	117	56.1	479.49	37.	281	86.8	308.90	57.	681	43.3	63.58
18.	118	19.8	167.80	38.	284	60	211.27	58.	692	49.6	71.68
19.	120	65.5	545.83	39.	288	24	83.35				
20.	130	19.8	152.31	40.	289	65.1	225.26				

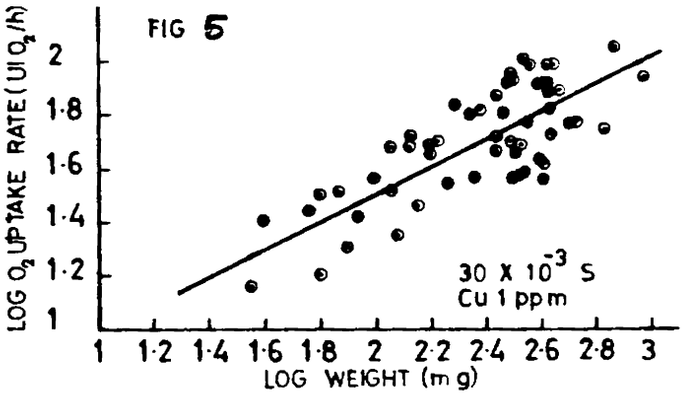
Table - 11 Oxygen uptake rate and metabolic rate of *Sunetta scripta* exposed to 1 ppm of copper in 20 x 10^{-3} salinity.

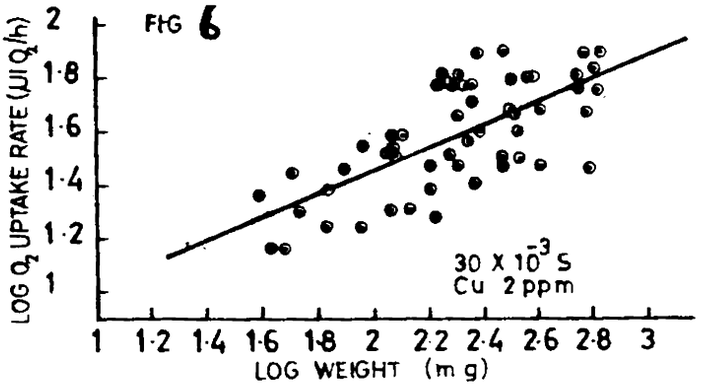
Sl. No.	Weight (mgs)	Oxygen consumption rate ($\mu lo_2/h$)	Metabolic rate	Sl. No.	Weight (mgs)	Oxygen consumption rate ($\mu lo_2/h$)	Metabolic rate	Sl. No.	Weight (mgs)	Oxygen consumption rate ($\mu lo_2/h$)	Metabolic rate
1.	22	16.8	763.64	21.	137	37.4	272.99	41.	632	72	113.92
2.	33	14.5	439.39	22.	158	27.5	174.05	42.	703	43.6	62.02
3.	35	21.9	625.71	23.	174	20.9	120.12	43.	726	31	42.70
4.	40	8.32	208	24.	181	50.5	279.01	44.	832	50.1	60.22
5.	44	13.8	313.64	25.	193	49.7	257.51	45.	990	48.5	48.99
6.	46	17.4	378.26	26.	210	27.9	132.86				
7.	57	29.8	522.81	27.	229	29.6	129.26				
8.	61	12.8	209.84	28.	255	55.4	217.26				
9.	66	16.7	253.03	29.	285	49.4	173.33				
10.	71	29.7	418.31	30.	309	65.1	210.68				
11.	76	12.8	209.84	31.	332	33	99.40				
12.	76	16.6	218.42	32.	334	19.95	59.73				
13.	82	16.7	203.66	33.	343	32.9	95.92				
14.	90	37.1	412.22	34.	349	64.9	185.96				
15.	98	24.2	246.94	35.	372	64.7	173.93				
16.	108	18.8	174.07	36.	411	43.4	105.60				
17.	118	19.7	166.95	37.	487	46.9	96.30				
18.	123	18.7	152.03	38.	551	30.9	56.08				
19.	125	19.8	158.4	39.	560	43.4	77.5				
20.	132	18.7	141.67	40.	567	54.3	95.77				

Table - 12 Oxygen uptake rate and metabolic rate of *Sunetta scripta* exposed to 2 ppm of copper in 20×10^{-3} salinity.

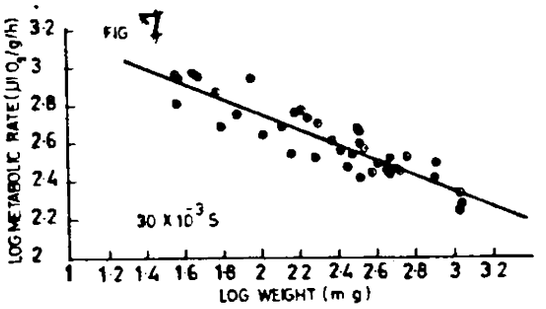
Sl. No.	Weight (mgs)	Oxygen Consumption Rate ($\mu lo_2/h$)	Metabolic Rate ($\mu lo_2/g/h$)	Sl. No.	Weight (mgs)	Oxygen Consumption Rate ($\mu lo_2/h$)	Metabolic Rate ($\mu lo_2/g/h$)
1.	50	21.9	438	21.	209	47.9	229.19
2.	56	16.9	301.79	22.	251	31.6	125.90
3.	59	14.5	245.76	23.	275	50.1	182.18
4.	60	19.1	317.5	24.	288	21.9	76.04
5.	69	21.9	317.10	25.	307	46.2	150.49
6.	71	16	225.35	26.	316	28.8	91.14
7.	79	12.6	159.49	27.	404	32	79.21
8.	85	19.2	225.88	28.	441	60.3	136.74
9.	98	21.8	222.45	29.	501	39.8	79.44
10.	107	16	149.53	30.	501	25.1	50.10
11.	113	19.1	169.03	31.	523	72.2	138.05
12.	116	19.2	165.52	32.	638	33.9	53.14
13.	120	13.7	114.17	33.	658	54.2	82.37
14.	121	27.3	225.62	34.	735	51.3	69.80
15.	130	28.8	221.54	35.	756	39.5	52.25
16.	145	14.5	100	36.	782	37.1	47.44
17.	159	20	125.79	37.	794	55.7	70.15
18.	164	32	195.12	38.	877	40.8	46.52
19.	191	16.6	86.91				
20.	200	25.1	125.5				

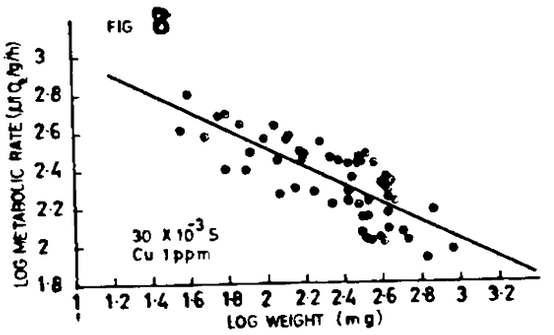


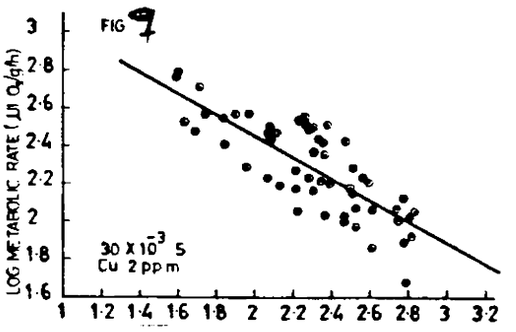




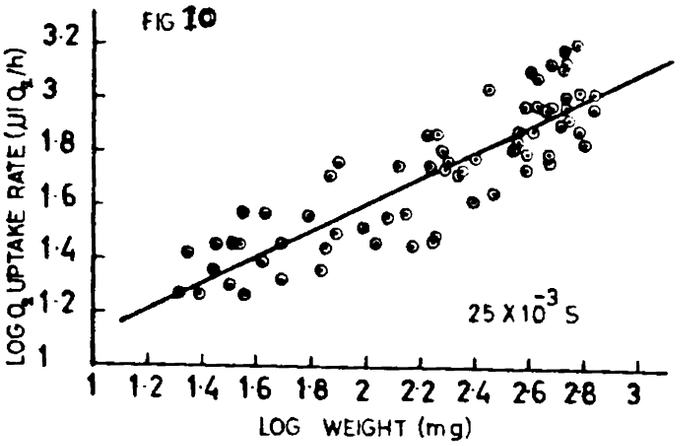
Figs. 4, 5, 6 :- Relationship between oxygen consumption rate and dry weight in the control animals and those exposed to 1 and 2 ppm of Cu(II) in 30 x 10^{-3} salinity.

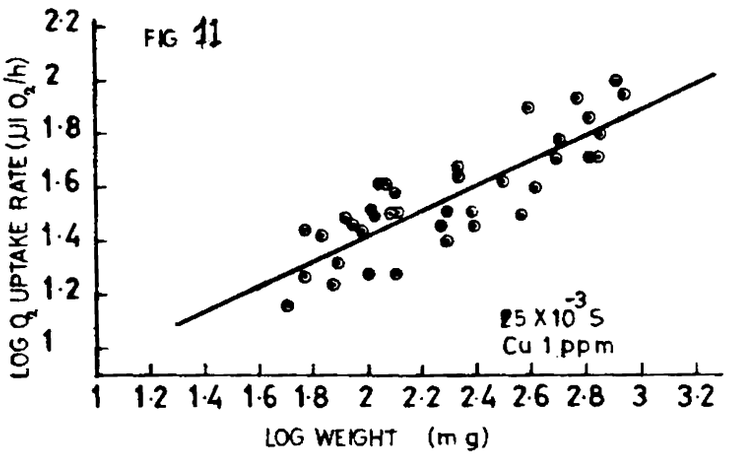


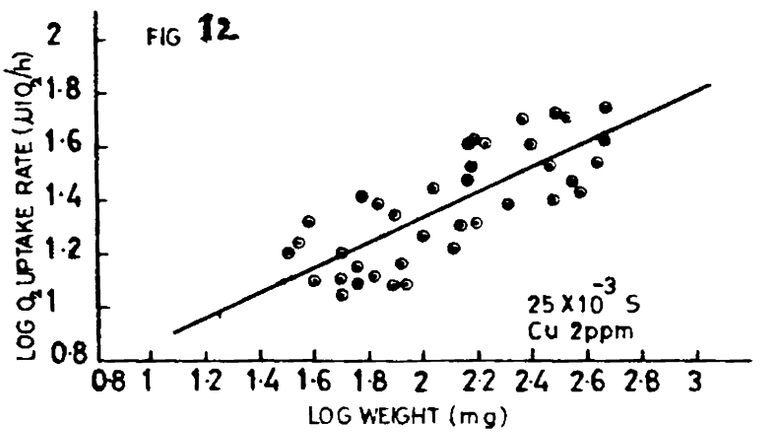




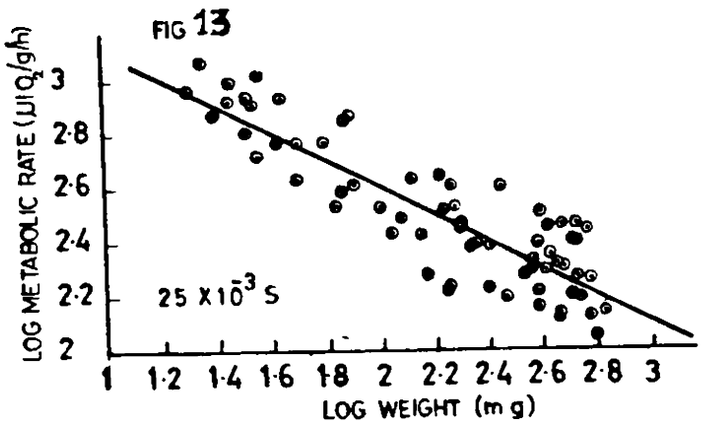
Figs. 7, 8, 9 :- Relationship between metabolic rate and dry weight in the control animals and those exposed to 1 and 2 ppm of Cu(II) in 30 x 10^{-3} salinity.

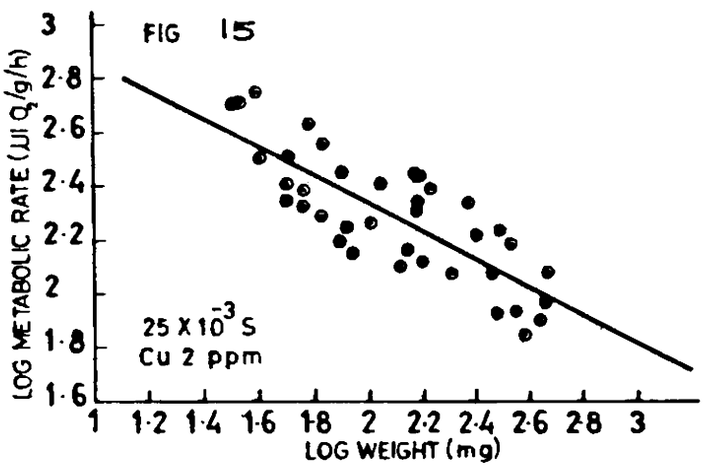


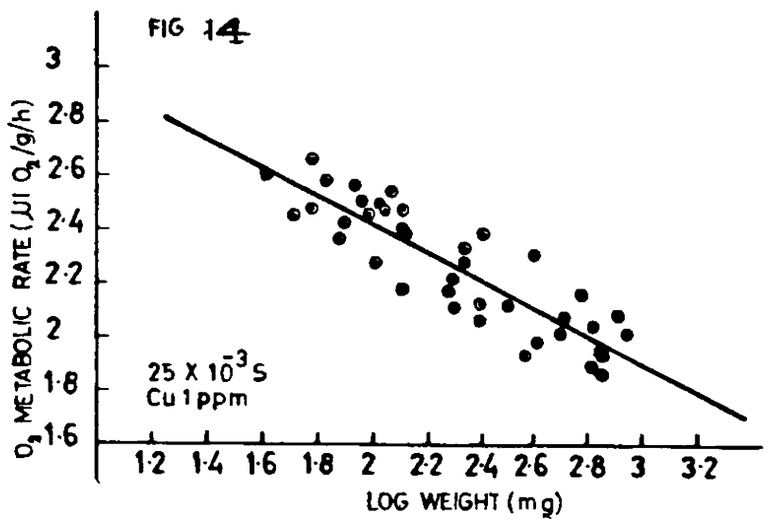




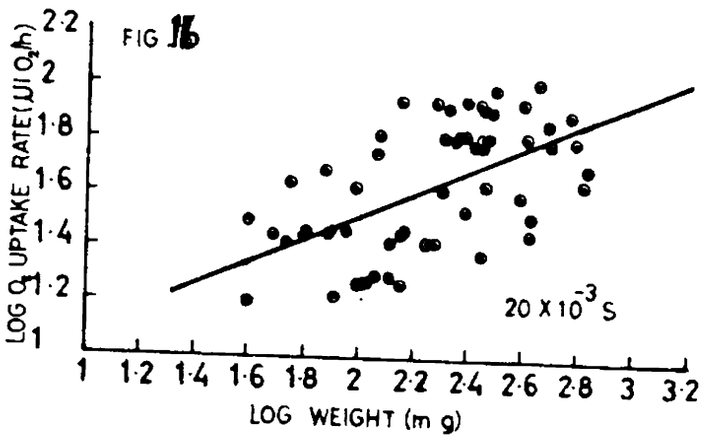
Figs. 10, 11, 12 :- Relationship between oxygen consumption rate and dry weight in the control animals and those exposed to 1 and 2 ppm of Cu(II) in 25 x 10^{-3} salinity.

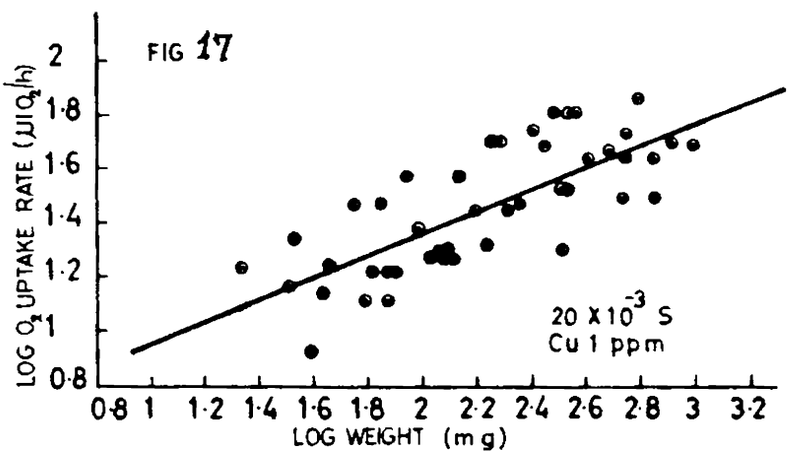


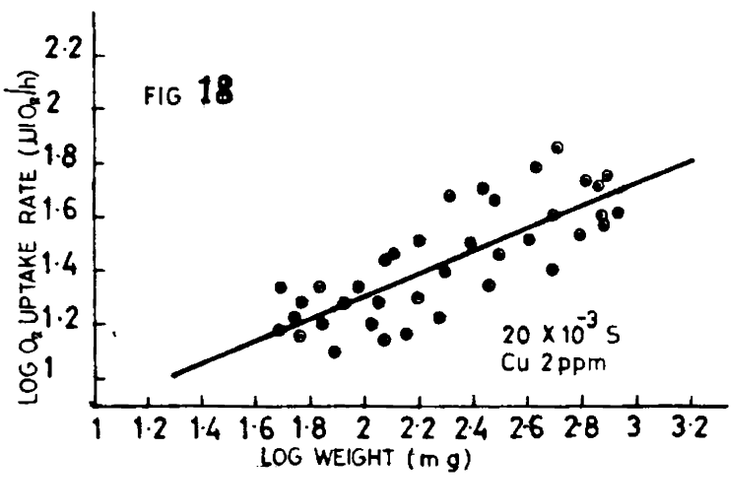




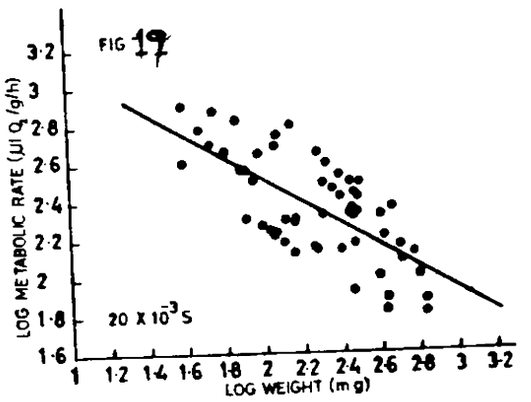
Figs. 13, 14, 15 :- Relationship between metabolic rate and dry weight in the control animals and those exposed to 1 and 2 ppm of Cu(II) in 25 x 10^{-3} salinity.

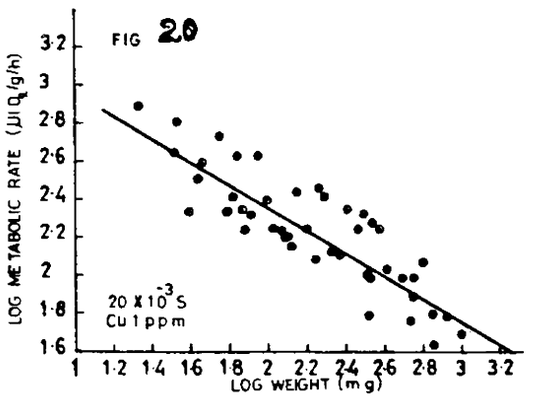


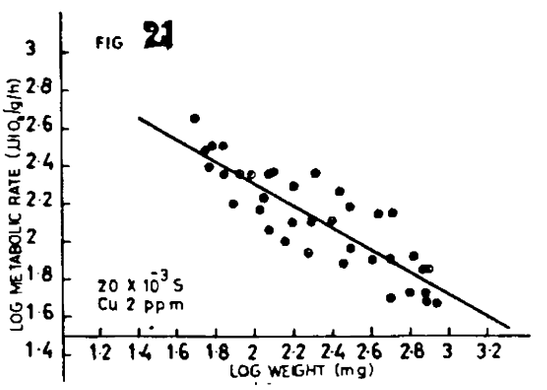




Figs. 16, 17, 18 :- Relationship between oxygen consumption rate and dry weight in the control animals and those exposed to 1 and 2 ppm of Cu(II) in 20 x 10^{-3} salinity.







Figs. 19, 20, 21 :- Relationship between metabolic rate and dry weight in the control animals and those exposed to 1 and 2 ppm of Cu(II) in 20 x 10^{-3} salinity.

4.4 DISCUSSION

In Sunetta scripta the highest rate of oxygen consumption was observed in 30 x 10^{-3} salinity. The oxygen consumption rate increases with 0.5910th power of the body weight and the metabolic rate decreases with 0.4090th power of the body weight. It was observed that oxygen consumption rate increased with increasing body weight while the weight specific oxygen consumption decreased with increasing body weight under all the experimental conditions (Tables 16 - 24).

In bivalves the 'b' value is reported to vary from 0.24 to 0.95. Rotthauwe (1958) obtained a 'b' value of 0.660 for Mytilus edulis which obeyed the law of surface proportional metabolism. Srinivasan (1965) has reported the weight exponent for Martesia fragilis as 0.55. In Perna perna a 'b' value of 0.625 was reported by Bayne (1967). For Congeria sallei, Mangapati Rao et al (1974) had obtained a 'b' value of 0.59. The 'b' values obtained for Nausitora hedleyi acclimated in 5 x 10^{-3} and 20 x 10^{-3} salinities were 0.5959 and 0.6043 respectively and the value for Teredo furcifera acclimated in 30 x 10^{-3} salinity was 0.5461 (Mohan & Cheriyan 1981). Hamburger et al (1983)

had obtained a 'b' value of 0.887 for Mytilus edulis ranging in size 0.1 mg - 10 mg tissue dry weight whereas a lower 'b' value of 0.663 for larger animals. They concluded that the 'b' exponent is constant at about 0.9 throughout the early pelagic larval stage as well as during the juvenile stage upto a size of 1 mg - 10 mg tissue dry weight, above which the 'b' value decreases to about 0.7. From the foregoing account it can be observed that the weight exponent for Sunetta scripta approximates to many of the earlier data. But it is seen that the 'b' value of Sunetta scripta obtained under the control experiments in 30×10^{-3} salinity does not strictly come within the range proposed by Von Bertalanffy (1957). However, many more metabolic types other than those proposed by Von Bertalanffy (1957) have been reported by various workers like Kuenzler (1961), Kennedy & Mihursky (1972), Mohan (1979).

The inverse proportionality of metabolic rate to body weight observed in the present study is in support of many of the earlier data. Von Brand et al (1948) reported that the rate of oxygen consumption in snails was inversely proportional to the size of the animals calculated on the basis of weight. Similar observations were

made by Nagabhushanam (1966) in Martesia striata. Davies (1966) reported in Patella vulgata, the respiratory rate of high and low level measured at 15°C was inversely proportional to the size of specimens, when calculated on a basis of fresh and dry weight. Ranade (1973) also noted the increasing rate of oxygen uptake with increasing body weight in the clams Meretrix meretrix and Katelysia opima.

Salih (1978) and Mohan (1979) have also made similar observations in the clam Meretrix casta and shipworms Nausitora hedleyi and Teredo furcifera respectively. It was reported by Deshmukh (1979) that the respiratory rate of the clam Meretrix meretrix decreased as the body weight increased. Famme (1980 b) also observed higher weight specific oxygen consumption rate in smaller specimens of Mytilus edulis when compared to the large ones.

It is seen that in Sunetta scripta the metabolic rates varied from 173.69 to 928.26 $\mu l O_2/g/h$ in animals of dry weight 1072 mg and 46 mg respectively, in the control experiments in 30×10^{-3} salinity. The differences in the experimental conditions and also the variations in the environmental history of the animals experimented, should be

considered while comparing the metabolic rates obtained under the present study with those of other bivalves. Moreover some authors have expressed the metabolic rates on a wet weight basis. In Cardium edule metabolic rate of 370 $\mu l O_2/g/h$ was observed (Vahl 1972 a). A seasonal variation of metabolic rate from 81 to 256 $\mu l O_2/g/h$ has been noticed in Chlamys islandica by Vahl (1978). Bayne et al (1973) have reported that the metabolic rate of Mytilus edulis in winter and summer as 263 $\mu l O_2/g/h$ and 164 $\mu l O_2/g/h$ respectively. Shafee (1976) has determined a metabolic rate of 800 $\mu l O_2/g/h$ in 35 x 10^{-3} S. at 28 $\pm$ 1°C for Mytilus viridis. The respiration rate expressed on a dry weight basis ranged between 179 and 241 $\mu l O_2/g/h$ in Katherina tunicata (Stickle & Sabourin 1979).

Mohan (1979) had reported the metabolic rates of Nausitora hedleyi in the acclimation media of 5 x 10^{-3} and 20 x 10^{-3} salinities as 175.2 $\mu l O_2/g/h$ and 186.1 $\mu l O_2/g/h$ respectively and that for Teredo furcifera in the acclimation medium of 30 x 10^{-3} S as 54.41 $\mu l O_2/g/h$. It is seen that the metabolic rate of 221.11 $\mu l O_2/g/h$ obtained for Sunetta scripta (for 1 g of the animal) in the control experiments in 30 x 10^{-3} salinity is comparable to many of the above data.

Of the three different levels of oxygen consumption rate, the metabolic rates obtained under the present study could be considered as the standard rate since the experiments were conducted on unfed specimens. Thompson and Bayne (1972) have made a clear distinction between 'active' metabolism related to feeding in the short term, a 'routine' metabolism associated with long term feeding and standard metabolism attained after prolonged starvation. Thompson and Bayne (1972) in their studies relating to oxygen consumption and dry flesh weight in Mytilus edulis, obtained the weight exponent for standard metabolic rates as 0.62 while in the active respiration of starved animals the weight exponent was determined to be 0.80. Von Bertalanffy (1964) has pointed out that in some cases the weight exponent is greater for fed than for starved animals. Bayne et al (1973) had determined four different values for 'b' from the experiments conducted on Mytilus edulis during winter and summer. The standard rates for winter and summer varied from 0.724 and 0.670 respectively while the routine rates varied from 0.774 and 0.702 for winter and summer respectively. Similar observations were made by Lomte and Nagabhushanam (1971) in the fresh water mussel Parreysia corrugata. The reasons for the low metabolic

rates for standard metabolism could be explained by the report of Dral (1968) that under conditions of low food availability Mytilus may employ less than half the total surface of the gill in moving water through mantle cavity and hence the area available for gas exchange will be diminished, lowering the effectiveness and ultimately causing a reduction in the oxygen uptake. It is also explained by Thompson & Bayne (1972) that the presence of particulate matter in the gut of the organism maintain a high oxygen requirement and is responsible for the increased oxygen uptake.

It is also evident from the present studies that lower salinities have a depressing effect on the oxygen consumption rate of Sunetta scripta which results in the lowering of the metabolic rate. The 'b' values under the control experiments varied from 0.59 in 30 x 10^{-3} salinity to 0.48 in 25 x 10^{-3} and 0.41 in 20 x 10^{-3} salinities.

Changes in metabolic rates were observed by Potts and Parry (1964) while reviewing the metabolic response of various aquatic poikilotherms to osmotic stress. In Mytilus edulis the oxygen consumption was seen to increase with decreasing salinities by Lagerspetz & Sirkka (1959).

A decrease in the oxygen consumption with decreasing salinity was noted by King (1965), in Maja verrucosa, Libinia emarginata, Carcinus meditterraneus and Calinectus sepidus.

Ramamurthi (1965) while studying the relationship between metabolism and osmotic stress in the two fresh water molluscs, Pila globosa and Lamellidens marginalis found that there was a decrease in total metabolism in response to different salinities. In Mytilus perna the oxygen uptake was observed to decrease with decreasing salinities (Bayne 1967). Van Whinkle (1968) while investigating the effect of salinity on the gill tissue oxygen consumption in different bivalves, concluded that the oxygen consumption in Crassostrea virginica and Mytilus edulis was relatively constant in salinities ranging from 5×10^{-3} to 30×10^{-3} while in Mercenaria mercenaria and Modiolus demissus the oxygen consumption increased in lower salinities. It was observed by Lomte and Nagabhushanam (1971) that in the fresh water mussel Parreysia corrugata the oxygen consumption decreased for a rise in the external NaCl concentration from 0.1% to 0.7%. Bayne (1973) while investigating the respiratory rates of the bivalves Geolina ceylonica, Anadora granosa

and Mytilus edulis at different salinities has concluded that Mytilus edulis alone exhibited a regulatory response in the respiratory rate at lower salinities. According to Shafee (1976) the 'b' values were seen to vary from 0.70 to 0.90 in Mytilus viridis in the different experimental salinities. Salih (1978) reported that the 'b' values varied from 0.42 to 0.75 for a salinity variation of 5×10^{-3} to 40×10^{-3} S in Meretrix casta. Newell (1976) has observed an increase in oxygen consumption in many marine invertebrates when exposed to low salinities. Stickle & Sabourin (1979) while investigating the respiration rate of Mytilus edulis and Katherina tunicata under varied salinity conditions concluded that the stepwise acclimated mussels showed an inverse relationship with salinity ($30 \times 10^{-3} - 10 \times 10^{-3}$). According to Lang (1968) & Bayne (1973) a primary inhibition of oxygen consumption occurs following a salinity change. When a marine osmoconformer is exposed to a salinity variation, a period of osmotic adjustment usually follows (Potts & Parry 1964). It has been observed that a salinity change causes a reduction in oxygen consumption, but also that in the course of some weeks it may re-adjust to its original level (Schlieper 1955). As observed by Bayne (1973) and

Gilles (1972) the bivalves exhibit valve closure for isolating the tissues from the ambient conditions. It has been already mentioned that the valve closure brings about reduced oxygen uptake. In those cases where shell closure was not complete the oxygen consumption was reduced in low salinities by the direct inhibition of the gill cilia (Van Whinkle, 1972).

Results obtained in respiration studies where ambient medium contained copper indicate that copper has a depressing effect on the oxygen consumption of Sunetta scripta (Figs. 22 - 24). In the animals exposed to 1 ppm of copper in 30 x 10^{-3} salinity the oxygen consumption rate was seen to increase with .5129th power of the body weight and the metabolic rate decreased with .4871th power of the body weight. With 2 ppm of copper in the same salinity the oxygen consumption rate increases with .4327th power of the body weight and the metabolic rate decreases with .5684th power of the body weight. The reduction in the oxygen consumption and metabolic rate values is evident from (Table 16 - 18). Baker (1969) had observed that copper had a damaging effect on the gill tissue of many marine animals. In many of the marine animals especially in bivalves respiration is effected by means of the water

current drawn into the animal's body under the influence of the ctenidial cilia. Hence any damage caused to the gill tissue will adversely affect the animal by the impairement of the respiratory efficiency. Inhibitory action of metals was observed by Fromm & Stockes (1962), Thurberg et al (1974), Dawson et al (1977) and Tort et al (1982) by Cr, Ag, Cd & Hg and Zn respectively. Cadmium depressed the gill tissue O_2 consumption while copper had no effect in both the green crab and the rock crab (Carcinus maenas & Cancer irroratus) experimented by Thurberg et al (1973). MacInnes & Thurberg (1973) have noted the inhibitory action of copper, silver, arsenic, and zinc when exposed individually and an elevated action when exposed to Cd alone or to a combination of Cd & Cu on the mud snail Nassarius obsoletus. Reduction of oxygen consumption of the excised gill tissue was observed on exposure to chromium in Mytilus edulis by Capuzzo & Sasner (1977). A reduction in the oxygen consumption of Mytilus galloprovincialis was observed when the sea water contained magnesium, copper, nickel, and molybdenum ions caused respiratory and cardiovascular depression (Scott & Major 1972). They found that oxygen consumption was reduced by 12% on exposure to 0.3 mg l^{-1} copper.

Brown and Newell (1972) while studying the toxic effects of copper and zinc on the metabolism of Mytilus edulis observed that copper had a depressing effect on the oxygen uptake while zinc carried no effects. Delhaye and Cornet (1975) found that a lower concentration of 0.25 mg 1^{-1} copper reduced oxygen consumption by about 50% in Mytilus edulis. Copper was proved to be a respiratory depressant by Mathew & Menon (1983) in their studies on the tropical bivalves Meretrix casta and Perna viridis. All the four metals studied by them (Ag, Cu, Zn & Pb) were known to reduce the metabolic rate in these bivalves at sub-lethal concentrations.

It is observed that in Sunetta scripta the oxygen consumption rate values showed more reduction in 2 ppm concentrations than in 1 ppm and the animals were seen to secrete mucus when exposed to the pollutant. The above result is in agreement with the findings of Scott & Major (1972) who observed that reduction in the oxygen consumption rate increased in Mytilus edulis as the concentration of copper increased in the experimental media. They also observed that the experimental mussels secreted mucus when exposed to copper. The secretion of mucus though a protective mechanism adopted by

molluscs against adverse conditions may also reduce the oxygen uptake by blocking the gills. As suggested by Brown & Newell (1972) reduction in the oxygen consumption was caused by suppressed ciliary activity rather than a direct effect on the respiratory enzymes. It is observed that Sunetta scripta adopts valve closure mechanism when subjected to a chemical or a mechanical stimulus. According to Shapiro (1964) the reduction in the oxygen consumption resulted either from valve closure or from a direct metabolic effect. The rate of ciliary activity is correlated with the rate of oxygen consumption (Gray 1928). According to Sleigh (1969) lateral cilia of the ctenidia stops beating when the exhalent siphon closes while Coleman (1973) and Davenport (1979) proposed some ciliary activity, but decreased effective irrigation of the mantle cavity during valve closure. According to Famme (1980) during closure, due to the decreased ciliary activity, the effective irrigation of the mantle cavity decreases whereas the effective uptake of oxygen is retained due to decreased diffusion distances in the water in the closed condition. Further, both partial or complete shell valve closure is known to reduce the heart rate as stated by Bayne et al (1976). The reduction in the heart

beat will bring about a reduced convection within the tissues which could influence the rate of oxygen uptake (Famme 1980). Hence the shell closing mechanism exhibited by most of the bivalves may also help them in lowering the metabolic rate by a reduced oxygen uptake. As observed by Capuzzo & Sasner (1977) the reduction in the oxygen consumption of the excised gill tissues of Mytilus on exposure to Cr was due to the inhibition of ciliary activity. It is stated that there may be some chemo sensory mechanism in the animals which is responsible for detecting increased levels of metals and conveyed through the branchial nerve, results in a reduction in the rate of ciliary beating (Howell et al 1984), which may ultimately interfere with the normal respiratory processes.

In the control animals in 25 x 10^{-3} salinity, the oxygen consumption rate increases with 0.4847th power of the body weight and the metabolic rate decreases with 0.5083th power of the body weight. Here also the oxygen consumption rate and metabolic rate values showed progressive reduction as the concentration of copper increased (Table 19 - 21). In the animals exposed to 1 ppm of copper in 25 x 10^{-3} salinity the oxygen consumption rate

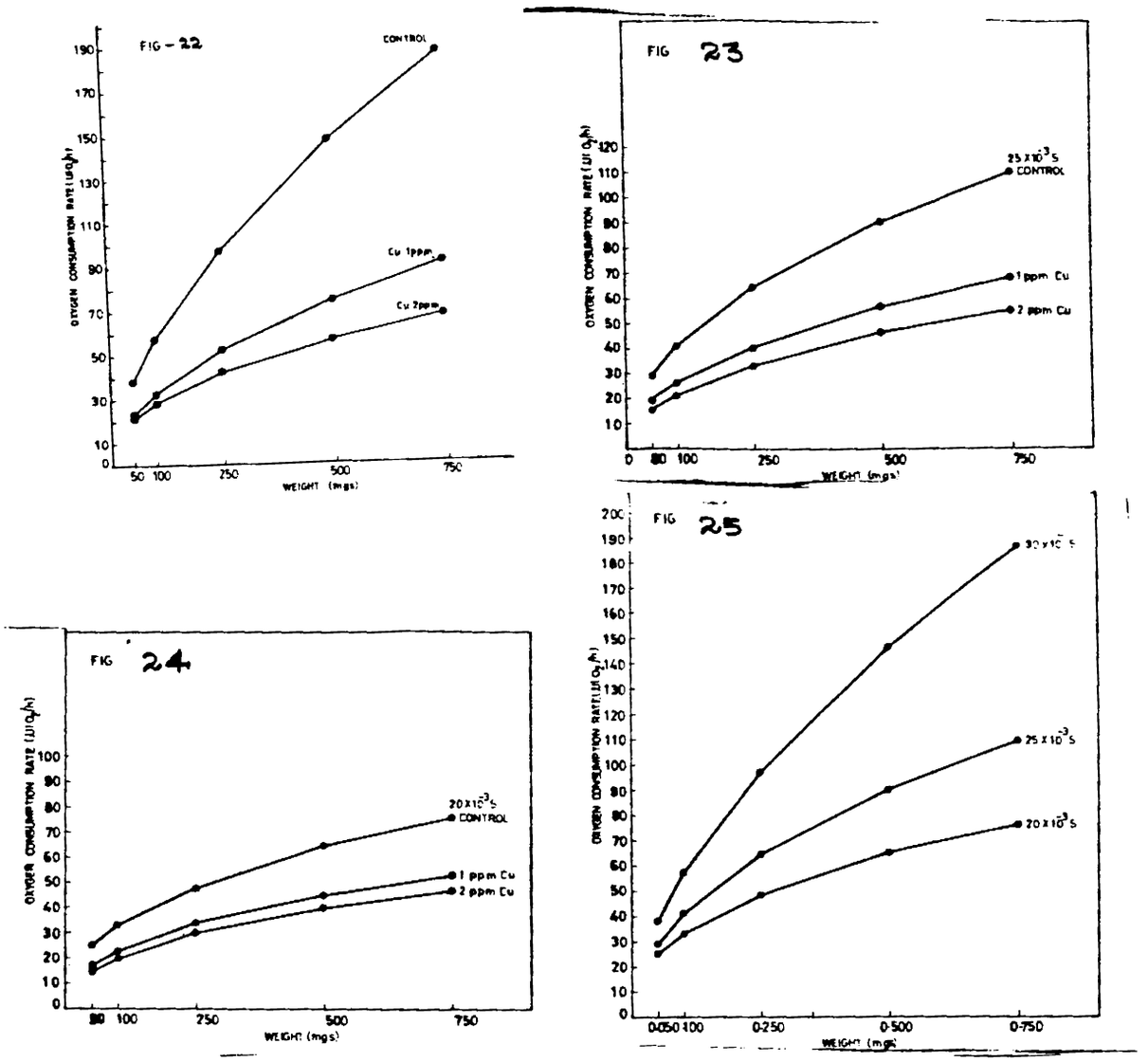
increased with 0.4742th power of the body weight and the metabolic rate decreased with 0.5019th power of the body weight. In the same salinity regime with 2 ppm of copper the oxygen consumption rate was seen to increase with 0.4714th power of the body weight while the metabolic rate decreased with .5295th power of the body weight.

The same trend was observed in 20 x 10^{-3} salinity also. The inhibitory effect of copper is evident from the oxygen consumption and metabolic rate values obtained on exposure to 1 and 2 ppm copper concentrations (Tables 22 - 24). In the control animals in 20 x 10^{-3} S the oxygen consumption rate increases with .4158th power of the body weight and the metabolic rate decreases with .5859th power of the body weight. In the animals exposed to 1 ppm copper concentration the oxygen consumption is seen to increase with .4096th power of the body weight while the metabolic rate decreased with .6039th power of the body weight. The oxygen consumption increases with .4172th power of the body weight and the metabolic rate decreases with .5843th power of the body weight in the animals exposed to 2 ppm copper in 20 x 10^{-3} salinity. The effect of copper on the animals of different sizes in the three salinities is also evident from figures 26 - 28.

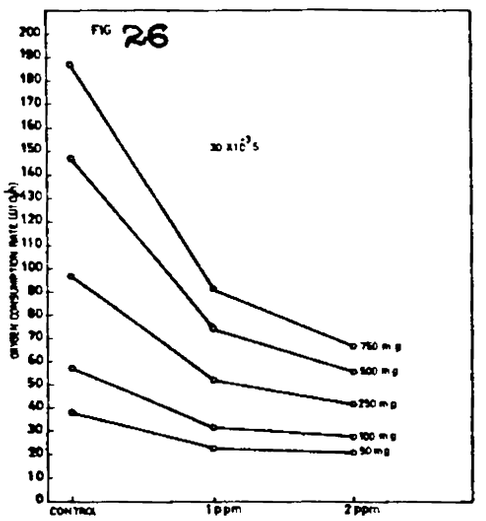
On comparing the 'b' values obtained under the control and dosed experiments in the three different salinities it can be noted that the reductions were not great enough to show any significant statistical difference in any of the three salinities (Tables 25, 26 and 27). An attempt was also made to compare the 'b' values obtained under the control experiments in the three different salinities. Even though the oxygen consumption rates are recorded to decrease with decreasing salinities as evident from Tables 16, 19 & 22 and Figs.25 and 29, no significant statistical difference was observed between the three regression coefficients (Table 28). This is a significant finding which shows that the oxygen consumption and metabolic rates show the same trend under the different experimental conditions but with reduced or increased rates as the case may be. However, the effect of copper on the metabolism of these bivalves towards reduced activity, would be harmful to the population as a whole under chronic stress. For the purpose of study, here the animals were artificially ventilated by keeping the valves forcibly open, while investigating the effect of salinity or copper on the oxygen uptake of Sunetta scripta. Hence, in the natural

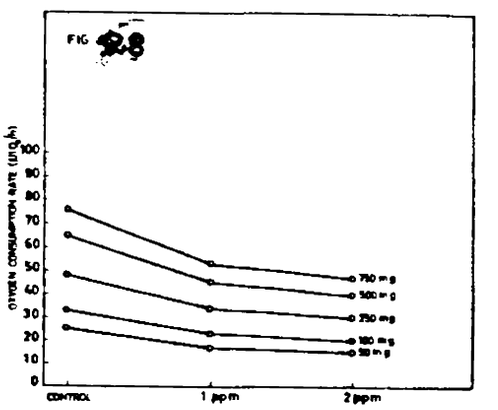
habit the inhibitory effect of copper or salinity on the respiratory rate of the animals may be magnified due to the valve closure mechanism exhibited by these bivalves.

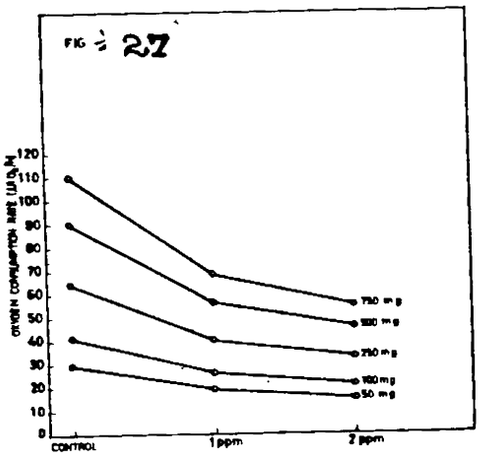
However, the high resistance capacity of the clam Sunetta scripta towards environmental stresses like salinity and pollutant copper indicate its usefulness as a bioassay organism in pollution. The clam being an edible one can cause serious health hazard as it can survive under heavy pollution stress.

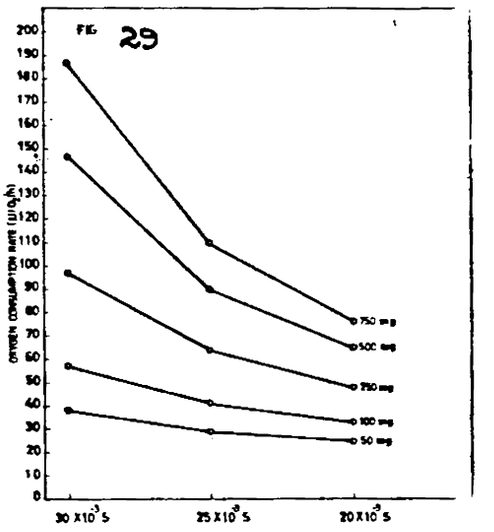


Figs. 22, 23, 24, 25 :- Trend of variation in the oxygen consumption rate between the control and dosed experiments in the salinities 30 x 10^{-3}, 25 x 10^{-3} and 20 x 10^{-3} and between the three controls in animals of dry weight 50, 100, 250, 500 and 750 mg.









Figs. 26, 27, 28, 29 :- Effect of copper (1 and 2 ppm) on the oxygen consumption rate in the animals of size 50, 100, 250, 500 and 750 mgs dry weight in the three salinities (30, 25 and 20 x 10^{-3}).

Table - 16 Oxygen uptake rate and metabolic rate for 50, 100, 250, 500 and 750 mg body weight of S. scripta in the control experiments in 30 x 10^{-3} salinity.

Body Wt. (mgs)	O_2 Uptake rate ($\mu l O_2/h$)	Metabolic rate ($\mu l O_2/g/h$)
50	37.65	753
100	56.70	567
250	97.44	389.76
500	146.79	293.58
750	186.55	248.73

Table - 17 Oxygen uptake rate and metabolic rate for 50, 100, 250, 500 and 750 mg body weight of S. scripta exposed to 1 mg l^{-1} of Cu(II) in 30 x 10^{-3} salinity.

Body Wt. (mgs)	O_2 Uptake rate ($\mu l O_2/h$)	Metabolic rate ($\mu l O_2/g/h$)
5 0	22.68	453.6
100	32.37	323.7
250	51.78	207.12
500	73.90	147.8
750	90.98	121.31

Table - 18 Oxygen uptake rate and metabolic rate for 50, 100, 250, 500 and 750 mg body weight of *S. scripta* exposed to 2 mg l^{-1} of Cu(II) in 30 x 10^{-3} salinity.

Body Wt. (mgs)	O_2 Uptake rate ($\mu l O_2/h$)	Metabolic rate ($\mu l O_2/g/h$)
50	20.83	416.6
100	28.12	281.2
250	41.80	167.2
500	56.43	112.86
750	67.26	89.68

Table - 19 Oxygen uptake rate and metabolic rate for 50, 100, 250, 500 and 750 mg body weight groups of *S. scripta* in the control experiments in 25 x 10^{-3} salinity.

Body Wt. (mgs)	O_2 Uptake rate ($\mu l O_2/h$)	Metabolic rate ($\mu l O_2/g/h$)
50	28.73	574.6
100	40.46	404.6
250	63.62	254.48
500	89.60	179.2
750	109.47	145.96

Table - 20 : Oxygen uptake rate and metabolic rate for 50, 100, 250, 500 and 750 mg weight groups of *S. scripta* exposed to 1 mg l^{-1} of Cu(II) in 25 x 10^{-3} salinity.

Body Wt. (mgs)	O_2 Uptake rate ($\mu l O_2/h$)	Metabolic rate ($\mu l O_2/g/h$)
50	18.83	376.6
100	26.15	261.5
250	40.38	161.52
500	56.10	112.2
750	67.99	90.65

Table - 21 Oxygen uptake rate and metabolic rate for 50, 100, 250, 500 and 750 mg weight group of *S. scripta* exposed to 2 mg l^{-1} of Cu(II) in 25 x 10^{-3} salinity.

Body Wt. (mgs)	O_2 Uptake rate ($\mu l O_2/h$)	Metabolic rate ($\mu l O_2/g/h$)
50	15.41	308.2
100	21.37	213.7
250	32.91	131.64
500	45.64	91.28
750	55.25	73.67

Table - 22 Oxygen uptake rate and metabolic rate for 50, 100, 250, 500 and 750 mg weight groups of *S. scripta* in 20 x 10^{-3} salinity.

Body Wt. (mgs)	O_2 Uptake rate ($\mu l O_2/h$)	Metabolic rate ($\mu l O_2/g/h$)
50	24.83	496.6
100	33.11	331.1
250	48.43	193.72
500	64.57	129.14
750	76.41	101.88

Table - 23 Oxygen uptake rate and metabolic rate for 50, 100, 250, 500 and 750 mg weight groups of *S. scripta* exposed to 1 mg l^{-1} of Cu(II) in 20 x 10^{-3} salinity.

Body Wt. (mgs)	O_2 Uptake rate ($\mu l O_2/h$)	Metabolic rate ($\mu l O_2/g/h$)
50	17.38	347.6
100	23.09	230.9
250	33.60	134.4
500	44.64	89.28
750	52.70	70.27

Table - 24 Oxygen uptake rate and metabolic rate for 50, 100, 250, 500 and 750 mg weight groups of *S. scripta* exposed to 2 mg l^{-1} of Cu(II) in 20 x 10^{-3} salinity.

Body Wt. (mgs)	O_2 Uptake rate ($\mu l O_2/h$)	Metabolic rate ($\mu l O_2/g/h$)
50	15.10	302
100	20.16	201.6
250	29.55	118.2
500	39.46	78.92
750	46.73	62.31

Table - 25. Analysis of co-variance at 30 x 10^{-3} salinity.

Salinity - 30 x 10^{-3} (Control)			30 x 10^{-3} S. 1 ppm Cu			30 x 10^{-3} S. 2 ppm Cu		
Σxy =	176.8989		Σxy =	226.0872		Σxy =	206.0962	
Σy =	74.15		Σy =	94.72		Σy =	88.46	
Σy^2 =	147.2027	Σxy = 3.8063	Σy^2 =	162.7722	Σxy = 3.1581	Σy^2 =	141.99	Σxy = 2.4663
Σx =	88.7056	Σy^2 = 2.5126	Σx =	131.7993	Σy^2 = 2.5601	Σx =	128.9088	Σy^2 = 2.2548
Σx^2 =	213.5107	Σx^2 = 6.4401	Σx^2 =	316.3546	Σx^2 = 6.1572	Σx^2 =	302.4392	Σx^2 = 5.6985
n =	38		n =	56		n =	56	

Source	n	df	Σx^2	Σxy	Σy^2	b	df	S_s	ms
Within Control	38	37	6.4401	3.8063	2.5126	0.5910	36	0.263	0.0073
Within Cu I	56	55	6.1572	3.1581	2.5601	0.5129	54	0.9403	0.0174
Within Cu II	56	55	5.6985	2.4663	2.2548	0.4328	54	1.1874	0.0220
	150	147	18.2958	9.4307	7.3275		144	2.3907	
Pooled Within	150	147	18.2958	9.4307	7.3275		146	2.4664	0.0169
				Difference in Slopes			2	0.0757	0.0379
				Comparison of slopes	F	$\frac{0.0379}{0.0169}$ =	2.2426		

'F' is not significant.

Table - 26: Analysis of co-variance at 25 x 10^{-3} salinity.

Salinity - 25 x 10^{-3} (Control)			25 x 10^{-3} S. 1 ppm Cu			25 x 10^{-3} S. 2 ppm Cu		
Σxy = 265.4215			Σxy = 146.2731			Σxy = 111.3001		
Σy = 115.54	Σxy = 4.5823		Σy = 62.39	Σxy = 2.7914		Σy = 52.1800	Σxy = 2.135	
Σy^2 = 203.7132	Σy^2 = 2.6891		Σy^2 = 99.0837	Σy^2 = 1.7709		Σy^2 = 73.3360	Σy^2 = 1.6846	
Σx = 149.9289	Σx^2 = 9.4535		Σx = 91.9902	Σx^2 = 5.8861		Σx = 79.4993	Σx^2 = 4.5292	
Σx^2 = 349.4134			Σx^2 = 217.4410			Σx^2 = 170.8486		
n = 67			n = 40			n = 38		

Source	n	df	Σx^2	Σxy	Σy^2	b	df	Ss	ms
Within Control	49	48	9.4535	4.5823	2.6891	0.4847	47	0.468	0.00996
Within Cu I	40	39	5.8861	2.7914	1.7709	0.4742	38	0.4471	0.0118
Within Cu II	38	37	4.5292	2.135	1.6846	0.4714	36	0.6782	0,0188
	127	124	19.8688	9.5087	6,1446		121	1.5933	0.0132
Pooled Within	127	124	19.8688	9.5087	6.1446		123	1.594	0.013
					Difference in slopes		2	.0007	0.0004
					Comparison of Slopes F	=	0.0308		

'F' is not significant.

Table - 27 Analysis of co-variance at 20×10^{-3} salinity.

Salinity - 20×10^{-3} (Control)

Σxy = 217.2810
Σy = 94.64
Σy^2 = 157.5808
Σx = 131.6067
Σx^2 = 304.7331
n = 58

Σxy = 2.5352
Σy^2 = 3.1544
Σx^2 = 6.1068

20×10^{-3} S. 1 ppm Cu

Σxy = 149.6553
Σy = 65.60
Σy^2 = 97.9946
Σx = 100.3720
Σx^2 = 232.0220
n = 45

Σxy = 3.3352
Σy^2 = 2.3644
Σx^2 = 8.1434

20×10^{-3} S. 2 ppm Cu

Σxy = 129.0983
Σy = 54.65
Σy^2 = 80.1685
Σx = 88.1694
Σx^2 = 210.0795
n = 38

Σxy = 2.2968
Σy^2 = 1.5732
Σx^2 = 5.5047

Source	n	df	Σx^2	Σxy	Σy^2	b	df	Ss	ms
Within Control	58	57	6.1068	2.5352	3.1544	.4151	56	2.1019	0.0375
Within Cu I	45	44	8.1434	3.3352	2.3644	.4096	43	0.9984	0.0232
Within Cu II	38	37	5.5047	2.2968	1.5732	.4172	36	0.6149	0.0171
	141	138	19.7549	8.1672	7.092		135	3.7152	0.0275
Pooled Within	141	138	19.7549	8.1672	7.092		137	3.7155	0.0271
						Difference in slopes	2	.0003	0.00015
						Comparison of slopes F =		0.0055	

'F' is not significant.

Table-28 : Analysis of co-variance of the control experiments in 30×10^{-3}, 25×10^{-3} and 20×10^{-3} salinities.

30×10^{-3} S. Control	25×10^{-3} S. Control	20×10^{-3} S. Control
Σxy = 3.8063	Σxy = 4.5823	Σxy = 2.5352
Σy^2 = 2.5126	Σy^2 = 2.6891	Σy^2 = 3.1544
Σx^2 = 6.4401	Σx^2 = 9.4535	Σx^2 = 6.1068

Source	n	df	Σx^2	Σxy	Σy^2	b	df	Ss	ms
Within Control	38	37	6.4401	3.8063	2.5126	0.5910	36	0.263	0.0073
Within Cu I	49	48	9.4535	4.5823	2.6891	0.4847	47	0.468	0.0096
Within Cu II	58	57	6.1068	2.5352	3.1544	0.4151	56	2.1019	0.0375
	145	142	22.0004	10.9238	8.3561		139	2.8329	0.0204
Pooled Within	145	142	22.0004	10.9238	8.3561		141	2.9321	0.0208
				Difference in slopes			2	0.0992	.0496
				Comparison of slopes F =		2.3846			

FILTRATION RATE

The bivalves noted for their sedentary filter feeding habit obtain their food from the finely dispersed suspended matter present in the ambient water. The term "suspension feeding" or "filter feeding" implies the filtration and retention of small suspended particles from water that passes through the specialised structures namely the gill cilia. The suspended organic material ranges in size from macroscopic particles down to colloidal dimensions. In filter feeders the intake of food depends on the efficiency of the filter, concentration of food particles present in the surrounding water and the pumping rate. The feeding performance of a suspension feeding bivalve can be measured by the rate at which the particles are removed from suspensions. The rate of filtration which provides a useful index of feeding activity is also suggested by Abel (1976) as a reliable sublethal toxicity index.

Many attempts have been made to estimate the rate of propulsion in lamelli branches using either direct or indirect measurement techniques. In the direct method the inhalent and the exhalent water currents were

separated and the water flow in the latter was measured. Galtsoff (1926) estimated the rate of flow in oysters and other molluscs using the direct method. Many authors like Loosanoff & Engle (1947), Tammes & Dral (1955), Orinna (1964), Coughlan & Ansell (1964), Davids (1964) etc. have worked on this aspect using the direct method. But this method is not commonly employed due to certain disadvantages. Here the water pumped which may or may not have been filtered is separated and measured. Also, the animals may not behave normally when subjected to the stress of the collecting device.

The indirect approach is often preferred because of its simplicity and accuracy. In such systems, filtration rate is calculated from the exponential decrease in particle concentration which occurs during the feeding period. Many workers have determined the rate of water pumped using Jorgensen's (1943) formula. Some authors have used modified versions of the formula derived by Jorgensen (1943). In the present study, the rate of filtration is computed using Quayle's (1948) equation. Coughlan (1969) suggests that the different forms of the exponential, relationship used by various workers are essentially the same.

Influence of size on the filtration rate of filter feeders had been the focus of attention of several reviews. Fox et al (1937) had investigated the relation of filtration rate to size in Mytilus californianus. The relationship of the rate of pumping to body size was determined by Jorgensen (1943, 1949) in mussels. Willemsen (1952) had studied the feeding activity in Mytilus edulis and Cardium edule in relation to dry weight. Size related filtration rate was investigated by Segal et al (1953) in Mytilus californianus. Rice & Smith (1958) and Coughlan & Ansell (1964) had determined the effect of body size on the filtering activity of the clam Venus mercenaria. Srinivasan (1968) has estimated the filtration rate of Martesia fragilis belonging to different sizes and has presented as an index of metabolic rate. Hughes (1969) had worked on the similar aspect in Serolicularia plana. Filtration rate in relation to total weight was studied by Ali (1970) in Hiatella arctica. The relationship between filtration rate and body size was determined by Walne (1972) in five species of bivalves including Crassostrea, Mytilus and Ostrea. Widdows (1978) had worked on the effect of body size, food concentration and season on the physiology of Mytilus edulis. Mohlenberg & Riisgard (1979) determined

the filtration rate and relation to dry weight in 13 species of suspension feeding bivalves. The filtration rate in relation to dry weight in Argopecten irradians and Crassostrea virginica was worked out by Palmer (1980).

Like any other physiological functions the rate of filtration that is the volume of water drawn into the animal's body per unit time is influenced by a number of environmental factors like salinity, temperature, dissolved oxygen and concentration of suspended matter. Influence of temperature on the filtering activity of marine organisms was investigated by several authors [Galtsoff (1928), Walne (1972), Mathers (1974), Schulte (1975) & Menon (1974)]. Many investigations have also been carried out on the effect of particle concentration on the clearance rate of filter feeders [Loosanoff & Engle (1947), Davids (1964), Ali (1970), Mortan (1971), Winter (1973), Mathers (1974), Schulte (1975), Winter (1978), Widdows (1978), Riisgard & Mohlenberg (1979), Palmer (1980) & Riisgard & Randlov (1981)].

Among the different environmental factors influencing the feeding activity of estuarine and marine organisms, salinity deserves more attention since the animals are

more liable to be affected by salinity stress than any others in the marine and estuarine realms. But the information available on this aspect is limited. Hopkins (1936) studied the adaptation of the feeding mechanism of the oyster, Ostrea gigas to changes in salinity. The pumping rates of Crassostrea virginica was determined in the different salinity regimes by Loosanoff (1950), in lamellibranchs by Cole & Hepper (1954). Nagabhushanam (1956) investigated the effect of different salinities on the filtration rate of Martesia striata. In the clam Meretrix casta the rate of clearance was studied by Durve (1963) in relation to size and salinity. Bohle (1972) investigated the rate of filtration in Mytilus edulis in relation to salinity. In the clam Katelysia opima the filtration rate was observed to be influenced by pH, temperature and salinity (Mane 1975). Alagarswami & Victor (1976) studied the rate of filtration in the pearl oyster Pinctada fucata in the normal sea water ($34x10^{-3}$ S), in dilutions ($14x10^{-3}$ S and $20x10^{-3}$ S) and in higher concentrations ($44x10^{-3}$ S, $50x10^{-3}$ S and $57x10^{-3}$ S). Krishnamurthy & Ramamurthy (1968) studied the effect of salinity, temperature and pH on the feeding activity of the bivalve Arca granosa with a view to determining the various factors responsible for their distribution in the estuary.

Both mechanical and chemical factors are known to influence the beat frequency of the lateral cilia, contraction of gill musculature and adductor muscles, activity of the mantle margin, etc. which determine the rate of water flow through the gill (Loosanoff & Engle 1947). Abel (1976) advocates the advantages of using filtration rate measurements in determining the effects of environmental pollutants. He opines that the method is non-destructive, economical and also provides more accurate information than is possible from lethal toxicity tests.

Effect of toxic pollutants on the rate of filtration of filter feeders is not investigated in great detail so far. Winter (1972) had conducted long term experiments for investigating the impact of ferric hydroxide flakes on the filter feeding behaviour of Mytilus edulis L. The toxicity of copper and zinc on the filtration rate of Mytilus edulis was determined by Abel (1976). Capuzzo & Sasner (1977) investigated the impact on the filtration rate and ciliary activity of Mya arenaria and Mytilus edulis in response to the uptake of chromium. The toxicity of the heavy metals Cu, Ag, Zn & Pb on the rate of filtration of the bivalves Perna viridis and

Meretrix casta was studied by Mathew & Menon (1984). Howell *et al* (1984) had determined the effect of different concentrations of copper on the rate of filtration and ciliary beating in *Mytilus edulis*. Above studies indicate that the rate of filtration is adversely affected by the environmental contaminants like the heavy metals. The clam *Sunetta scripta* occurring near the Cochin barmouth is subjected to salinity stress as well as there is a potential danger of they being subjected to heavy metal pollutants in their natural habitat. Hence with a view to determining the impact of both the salinity and copper stress on the feeding activity of *Sunetta scripta* the animals of different sizes were experimented in the three selected salinity regimes with different copper concentrations.

It is well known that ciliary beating is responsible for both feeding and respiration in bivalves. As a consequence, any change in the ciliary beating will be reflected both in the respiratory and feeding behaviour of the animal. Hence a correlative study of the oxygen consumption and filtration rates will be highly useful.

Information regarding the relationship between oxygen consumption and filtration rate remains meagre since very few authors have worked on this aspect. Gauld (1951) measured the feeding rate and oxygen uptake in the copepod Calanus finmarchicus. Jorgensen (1952) related filtration rate to oxygen uptake in the filter feeders like the oyster, Ostrea virginica and the ascidians Ciona intestinalis and Molgula manhattensis. This is confirmed by various authors and the data was reviewed by Jorgensen (1960, 1966). Srinivasan (1968) has made a correlative study of the oxygen consumption and filtration rate in Martesia fragilis belonging to different size groups. Vahl (1972 a) determined the ration of litres of water pumped/ml of oxygen consumed in Cardium edule belonging to different sizes. The relation between water transport and oxygen uptake was studied by Vahl (1972 b) in Chlamys opercularis. In Mytilus edulis the pumping and oxygen consumption rates were determined in animals of different sizes by Vahl (1973). McLusky (1973) studied the oxygen consumption and filtration rate of the scallop Chlamys opercularis in animals belonging to a wide size range maintained at four different temperatures. Riisgard et al (1980)

studied the rate of water processing, oxygen consumption and efficiency of particle retention in pre and post metamorphic Mytilus edulis. The effect of body size on the oxygen consumption and clearance rate of Mytilus edulis veliger larvae was investigated by Riisgard & Randlov (1981).

5.2 MATERIALS AND METHODS

Animals were collected and acclimated as mentioned in Chapter 2. Animals were given 2 to 3 days acclimation period during which they were not fed. The method described by Abel (1976) was adopted with minor modification for the present study. The filtration rates were determined by an indirect method, monitoring the reduction in particle concentration per unit volume during a time interval. The method yields accurate information and can also be carried out with minimum of equipments and materials. The simplicity and reliability makes it a more acceptable technique than the complicated ones. The experiments were conducted in wide mouthed conical flasks completely covered with black paper so as to shut off light.

As in oxygen consumption rate studies small glass pieces were introduced in between the shell valves for preventing the clams from valve-closure due to salinity or copper stress. Animals were then carefully introduced into the test vessels containing 500 ml of sea water and allowed to recover from handling for 20-30 min. At this stage a definite quantity of the solution of the dye, Neutral Red (BDH) was added to the test vessels to give a concentration of approximately 1 mg/l in the experimental media. According to Abel (1976) the initial dye concentration of 1 ppm used in the experiments were observed to give maximum values and the efficiency of extraction was found to decrease with increasing concentration. The dye solution was gently mixed by stirring giving least disturbance to the animal. The initial sample was immediately taken by pipetting out 10 mm from the test solution. At fixed intervals samples were extracted and the dye concentration in the samples were determined spectrophotometrically at 425 mm in a Hitachi 200-20 model spectrophotometer.

The filtration rate was computed by Quayle's (1948) equation,

$$m = \frac{M}{nt} \log_e \frac{Co}{Ct}$$

where m = Filtration rate in ml min^{-1}

M = Volume of test solution in ml

n = Number of animals in the test vessel

Co = Dye concentration in initial sample

Ct = Dye concentration in final sample

t = Time between samplings in minutes.

Clearance rates of the animals ranging in size 0.030-0.760 gms were studied in three salinity regimes ($20x10^{-3}$ S, $25x10^{-3}$ Sand 30 x 10^{-3} S). Abel (1976) states that the equation used for the computation of the rate of filtration assumes that the filtration rate is constant over the time 't' and hence it is advisable to reduce the time interval as far as possible for lessening the experimental error. The bivalves in general are noted for their sedentary habit and low rate of metabolism and the clam, Sunetta scripta is not an exception to this. The optimum time interval between sample extractions for this particular clam was found to be 1 hr. Experiments were conducted on single animals for a period of 3 hrs and the average value was taken.

The effect of copper on the filtration rate of Sunetta scripta was studied by exposing them to lower

concentrations like 0.05 and 0.1 ppm of copper. High concentrations used in oxygen consumption rate studied were found to arrest filtration entirely. The test organisms were pre-exposed to the copper concentrations for 24 hrs before exposing them to the toxicant in the test medium. Control experiments were always run along with the toxicity tests. As in respiration studies the dry weight was considered as the size of the animal.

A standard graph was prepared with a series of concentrations of the dye and the clearing rates were read out from the graph.

The relationship with filtration rates and dry weight under the control and experiments was determined by semi-log linear regression analysis and the data presented on semi-logarithmic plots.

5.3 RESULTS

1. Effect of copper on the filtration rate of *Sunetta scripta* in relation to body weight in 30 x 10^{-3} S.

1.a Filtration rate in the control experiments in 30x10^{-3} S.

The rate of filtration of the control animals of different sizes in 30 x 10^{-3} S is presented in Table 29.

Body weight of the animals ranged between 0.046 and 0.619 gm while the filtration rate varied from 69.36 to 245.38 ml/hr respectively. It can be noted that the rate of filtration increases with the increase in body size. A semilogarithmic plot of the filtration rate and body weight is given in Fig.30. The filtration rate and body weight were observed to have a linear relationship. The estimated values of regression coefficient 'b' and log 'a' are 0.8584 and 1.8577 respectively.

1.b Filtration rate of the animals exposed to 0.05 ppm of Cu(II) in 30 x 10^{-3} S.

In animals ranging in size 0.040 and 0.770 gm dry weight the rate of filtration varied from 65.21 to 234.71 ml/hr. The filtration rate of the animals of different weights of the above size range exposed to 0.05 ppm of copper (II) in 30 x 10^{-3} salinity is presented in Table 30. In Fig.31 logarithm of filtration rate is plotted against the body weight of the animals. The estimated values of 'b' and log 'a' are 0.6503 and 1.8491 respectively.

1.c Filtration rate of the animals exposed 0.1 ppm of Cu(II) in 30 x 10^{-3} salinity.

The rate of filtration of Sunetta scripta of different sizes exposed to 0.1 ppm of copper in 30 x 10^{-3} salinity is presented in Table 31. The filtration rate varied from 59.33 to 162.92 ml/hr in animals whose dry weight ranged between 0.073 and 0.680 gm. A semilogarithmic plot of filtration rate against body weight is shown in Fig.32. The 'b' and log 'a' values estimated are 0.6026 and 1.8077 respectively.

2. Effect of copper on the filtration rate of Sunetta scripta in relation to body weight in 25 x 10^{-3} S.

2.a Filtration rate in control experiments in 25x10^{-3} S.

In animals ranging in size 0.064 to 0.752 gm dry weight the rate of filtration varied from 71.64 ml/hr to 211.94 ml/hr and the data is presented in table 32. A semilogarithmic plot of filtration rate and body weight is given in Fig.33. The estimated value of regression coefficient 'b' and log 'a' are 0.8025 and 1.8492 respectively.

2.b Filtration rate of the animals exposed to 0.05 ppm of Cu(II) in 25 x 10^{-3} salinity.

In animals of the size range 0.059 to 0.715 gm dry weight the rate of filtration varied from 54.91 to 174.62 ml/hr. The filtration rate of the animals of different weights of the above size range exposed to 0.05 ppm Cu in 25 x 10^{-3} salinity is presented in Table 33. In Fig.34 logarithm of filtration rate is plotted against body weight of the animals. The estimated values of 'b' and log 'a' are 0.6023 and 1.7503 respectively.

2.c Filtration rate of the animals exposed to 0.1 ppm of Cu(II) in 25 x 10^{-3} salinity.

The rate of filtration of the animals of different weights exposed to 0.1 ppm of copper in 25 x 10^{-3} salinity is presented in Table 34. The filtration rate varied from 48.13 to 173.50 ml/hr animals ranging in size 0.052 to 0.732 gm dry weight. In Fig.35 the semilogarithmic plot of the rate of filtration against body weight is shown. The estimated values of 'b' and log 'a' are 0.5538 and 1.7397 respectively.

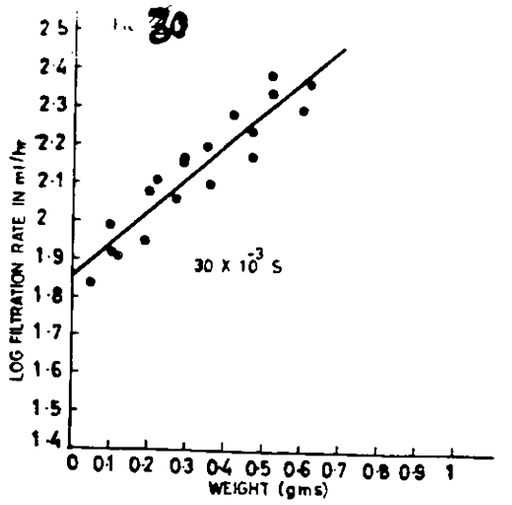
3. Experiments on the effect of copper (II) on the filtration rate of Sunetta scripta in relation to body weight in 20 x 10^{-3} salinity.

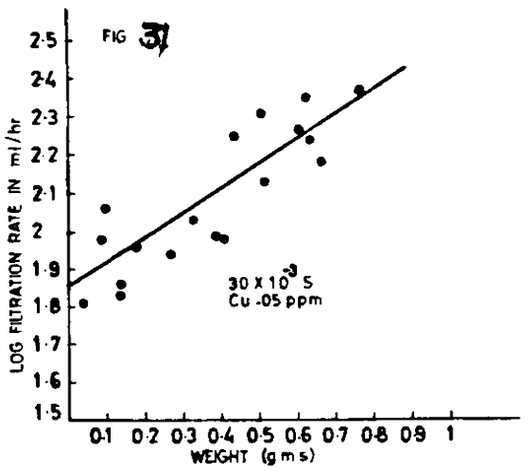
3.a Filtration rate of the animals in the control experiments in 20 x 10^{-3} salinity.

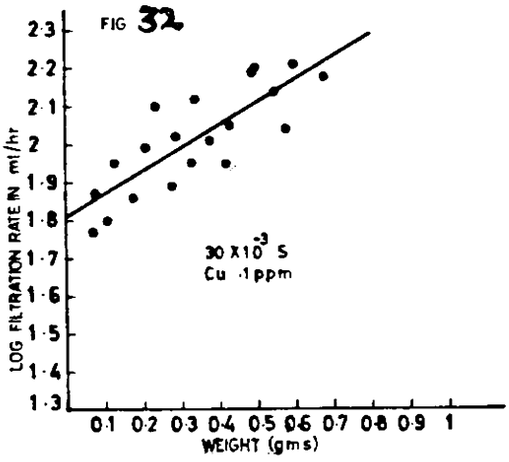
In animals of the size range 0.072 to 0.549 gm dry weight the rate of filtration varied from 56.75 to 147.54 ml/hr. The filtration rates of the animals of different weights of the above size range under the control experiments in 20 x 10^{-3} salinity are given in Table 35. A semilogarithmic plot of filtration rate and body weight is shown in Fig.36. The values of 'b' and log 'a' are estimated to be 0.7011 & 1.7553 respectively.

3.b Filtration rate of the animals exposed to 0.05 ppm of Cu(II) in 20 x 10^{-3} salinity.

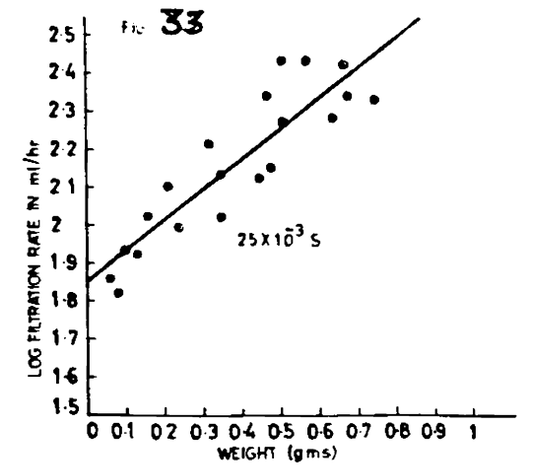
The filtration rate of the clams of different sizes exposed to 0.05 ppm of copper (II) in 20 x 10^{-3} salinity is presented in Table 36. Body weight of the animals ranged between 0.42 & 0.682 gm dry weight while the filtration rate varied from 32.71 to 101.06 ml/hr. In Fig.37 logarithm of the rate of filtration is plotted

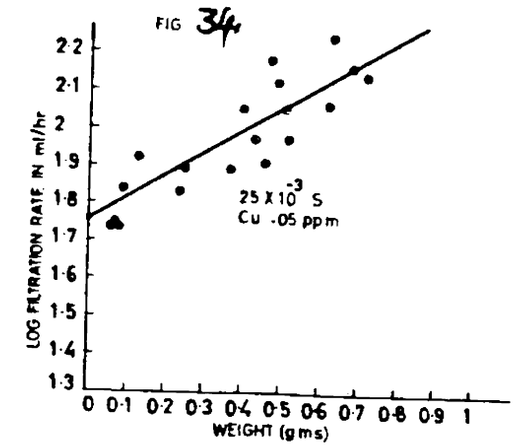


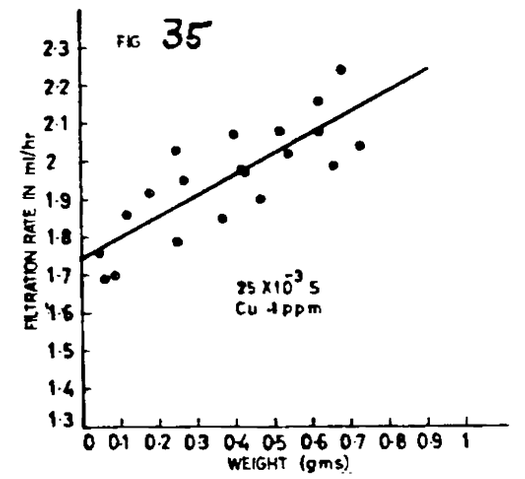




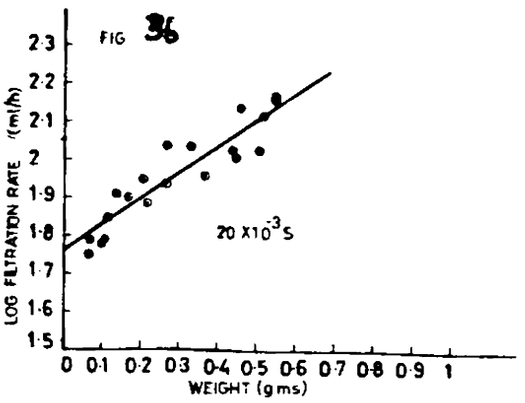
Figs. 30, 31, 32 :- Relationship between rate of filtration and dry weight in the control animals and those exposed to .05 and .1 ppm of Cu(II) in 30 x 10^{-3} salinity.

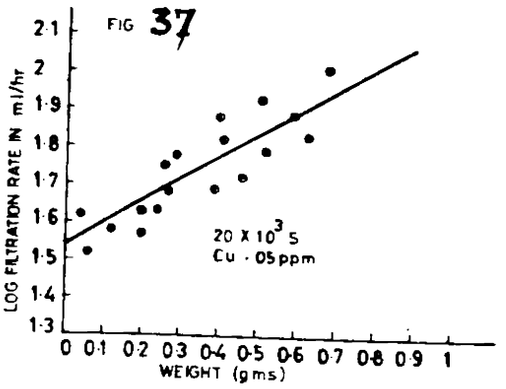


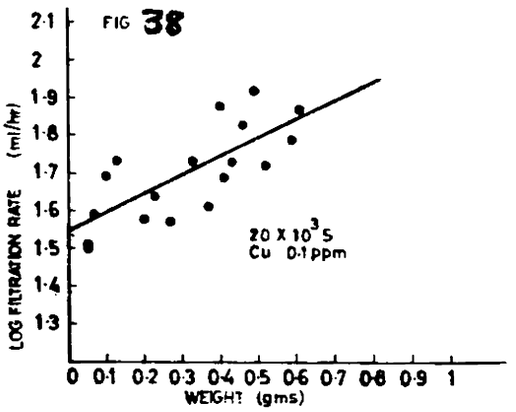




Figs. 33, 34, 35 :- Relationship between rate of filtration and dry weight in the control animals and those exposed to .05 and .1 ppm of Cu(II) in 25 x 10^{-3} salinity.







Figs. 36, 37, 38 :- Relationship between rate of filtration and dry weight in the control animals and those exposed to 0.05 and .1 ppm of Cu(II) in 20 x 10^{-3} salinity.

Table - 29 - Rate of filtration in ml/h in Sunetta scripta under the control experiments at salinity of 30 x 10^{-3}.

	Wt.(Gms)	F.R.(ml/h)	F.R.ml/mg/hr
1.	0.046	69.36	1.51
2.	0.059	81.77	1.39
3.	0.100	83.25	0.83
4.	0.103	98.56	0.96
5.	0.117	80.48	0.69
6.	0.189	88.49	0.47
7.	0.198	120.29	0.61
8.	0.218	129.83	0.60
9.	0.271	115.90	0.43
10.	0.286	144.18	0.50
11.	0.292	147.05	0.50
12.	0.352	156.59	0.44
13.	0.363	125.40	0.35
14.	0.424	191.31	0.45
15.	0.467	146.25	0.31
16.	0.471	174.82	0.37
17.	0.517	245.38	0.47
18.	0.521	217.77	0.42
19.	0.598	198.70	0.33
20.	0.619	235.67	0.38

Table-30 - Rate of filtration in Sunetta scripta exposed to 0.05 ppm of copper at 30 x 10^{-3} salinity.

	Wt.(Gm)	F.R. (ml/h)
1.	0.040	65.21
2.	0.088	95.80
3.	0.099	113.48
4.	0.137	67.67
5.	0.144	71.94
6.	0.182	91.93
7.	0.225	138.52
8.	0.273	87.96
9.	0.329	162.33
10.	0.332	107.45
11.	0.388	97.03
12.	0.412	95.96
13.	0.437	176.23
14.	0.506	203.94
15.	0.521	136.27
16.	0.611	188.00
17.	0.633	221.41
18.	0.641	174.66
19.	0.670	151.29
20.	0.770	234.71

Table - 31 - Rate of filtration in Sunetta scripta exposed to 0.1 ppm of copper at a salinity of 30×10^{-3}

	Wt.(Gm)	F.R. (ml/h)
1.	0.073	59.33
2.	0.075	73.37
3.	0.107	63.43
4.	0.129	88.43
5.	0.183	72.55
6.	0.212	98.44
7.	0.236	124.98
8.	0.281	76.81
9.	0.291	104.34
10.	0.334	99.33
11.	0.337	131.38
12.	0.379	105.97
13.	0.421	89.33
14.	0.427	111.05
15.	0.491	155.14
16.	0.498	159.72
17.	0.549	138.85
18.	0.577	108.54
19.	0.601	162.92
20.	0.680	152.51

Table-32 - Rate of filtration in Sunetta scripta under the control experiments at a salinity of 25×10^{-3}

	Wt. (Gm)	F.R. (ml/h)
1.	0.064	71.64
2.	0.084	66.79
3.	0.103	84.59
4.	0.130	83.59
5.	0.162	105.29
6.	0.208	126.88
7.	0.241	98.22
8.	0.323	160.43
9.	0.352	135.55
10.	0.354	103.56
11.	0.451	131.44
12.	0.473	221.24
13.	0.476	142.40
14.	0.510	187.96
15.	0.513	270.44
16.	0.569	271.80
17.	0.644	190.06
18.	0.674	262.22
19.	0.680	220.04
20.	0.752	211.94

Table-33 - Rate of filtration in *Sunetta scripta* exposed to 0.05 ppm of copper at a salinity of 25×10^{-3}

	Wt.(Gm)	F.R. (ml/h)
1.	0.059	54.91
2.	0.072	56.52
3.	0.079	55.26
4.	0.088	68.96
5.	0.127	83.59
6.	0.243	67.94
7.	0.245	77.38
8.	0.246	109.88
9.	0.373	76.94
10.	0.404	112.82
11.	0.434	92.86
12.	0.460	80.76
13.	0.469	151.57
14.	0.487	131.15
15.	0.513	113.32
16.	0.521	93.99
17.	0.617	114.89
18.	0.634	174.62
19.	0.680	145.38
20.	0.715	139.06

Table-34 - Rate of filtration in Sunetta scripta exposed to 0.1 ppm of copper at a salinity of 25 x 10^{-3}.

	Wt. (Gm)	F.R. (ml/h)
1.	0.052	57.70
2.	0.058	48.13
3.	0.088	49.52
4.	0.115	71.83
5.	0.182	83.19
6.	0.246	61.99
7.	0.254	106.12
8.	0.271	89.74
9.	0.373	71.39
10.	0.404	117.17
11.	0.417	96.01
12.	0.427	93.00
13.	0.465	79.45
14.	0.517	121.14
15.	0.544	103.68
16.	0.616	120.11
17.	0.621	143.42
18.	0.661	97.77
19.	0.675	173.50
20.	0.732	110.79

Table-35 - Rate of filtration in Sunetta scripta under the control experiments at a salinity of 20×10^{-3}.

	Wt. (gm)	F.R. (ml/h)
1.	0.072	56.75
2.	0.073	61.00
3.	0.099	60.91
4.	0.111	62.26
5.	0.117	71.33
6.	0.138	80.77
7.	0.171	78.73
8.	0.210	89.28
9.	0.221	78.40
10.	0.265	109.95
11.	0.274	87.76
12.	0.331	110.71
13.	0.371	90.26
14.	0.437	106.26
15.	0.451	103.21
16.	0.462	137.31
17.	0.506	107.23
18.	0.517	131.75
19.	0.549	147.54
20.	0.549	143.93

Table-36 - Rate of filtration in Sunetta scripta exposed to 0.05 ppm of copper at a salinity of 20 x 10^{-3}.

	Wt.(Gm)	F.R. (ml/h)
1.	0.042	41.74
2.	0.059	32.71
3.	0.109	52.79
4.	0.121	38.32
5.	0.196	37.16
6.	0.204	42.95
7.	0.209	42.19
8.	0.241	42.19
9.	0.256	55.95
10.	0.266	47.74
11.	0.291	59.55
12.	0.390	48.56
13.	0.404	75.21
14.	0.410	66.74
15.	0.462	52.18
16.	0.506	84.26
17.	0.519	61.49
18.	0.588	78.50
19.	0.627	67.83
20.	0.682	101.06

Table-37 - Rate of filtration in Sunetta scripta exposed to 0.1 ppm of copper at a salinity of 20×10^{-3}

	Wt. (Gm)	F.R. (ml/h)
1.	0.051	32.05
2.	0.053	32.05
3.	0.054	31.58
4.	0.070	39.00
5.	0.104	48.65
6.	0.129	54.05
7.	0.201	38.29
8.	0.208	61.18
9.	0.225	43.33
10.	0.274	37.28
11.	0.331	53.13
12.	0.371	40.35
13.	0.395	76.59
14.	0.406	49.39
15.	0.426	53.90
16.	0.464	67.59
17.	0.491	83.24
18.	0.521	52.27
19.	0.586	61.17
20.	0.610	73.91

against body weight of the animals. The estimated values of 'b' and log 'a' are 0.5856 and 1.5395 respectively.

3.c Filtration rate of the animals exposed to 0.1 ppm of Cu(II) in 20 x 10^{-3} salinity.

The filtration rate of the animals exposed to 0.1 ppm of Cu in 20 x 10^{-3} salinity ranging in size 0.051 to 0.610 gm dry weight varied from 31.58 to 83.24 ml/hr (Table 37). A semilogarithmic plot of filtration rate against body weight is shown in Fig.38. The estimated values of 'b' and log 'a' are 0.5149 and 1.5392 respectively.

5.4 DISCUSSIONS

The animals were observed to filter more efficiently in 30 x 10^{-3} salinity than in 20x10^{-3} salinity or 25 x 10^{-3} salinity. The rate of filtration and body weight showed a linear relationship ie. the filtration rate increased with increasing body weight under all the experimental conditions. In 30 x 10^{-3} salinity, in the control animals, the filtration rate increases with 0.8584th power of the body weight.

The weight exponents for filtration rate determinations showed wide variations among the different animals. It varied from negative values to a value of 0.76 in mussels (Bayne *et al* 1976). The weight exponent for *Modiolus modiolus* was estimated to be 0.74 for animals ranging in size 0.43 - 3.95 gms (Winter, 1969). 'b' value of 0.25 was estimated by Walne (1972) in *Mytilus edulis* while Winter (1973) had determined the value of 'b' as 0.74 for a different size range. For *Mytilus edulis*, Vahl (1973) had obtained a 'b' value of 0.60 for animals ranging in size 0.01 - 1 gm dry flesh weight. Thompson & Bayne (1974) report the weight exponent for *Mytilus edulis* weighing less than 1 gm dry weight as 0.38 and suggested that it decreases for larger animals. Bayne *et al* (1975) had determined 'b' values of 0.46 and 0.42 for *Mytilus californianus* at high and low ration respectively. Srinivasan (1968) observed that the total water filtration increase by a factor 0.44 only upto 30 mg level in *Martesia fragilis*.

It was observed that in the clam *Sunetta scripta* the rate of filtration varied from 69.36 to 245.38 ml/hr for animals ranging in size 0.46 to 0.619 gm in 30×10^{-3} s. The rate of filtration showed a linear relationship with

size. A linear relationship with the rate of filtration and body size was also observed by Srinivasan (1968) in Martesia fragilis upto a body weight of 30 mg wet weight. He observed that the animals weighing 3.25 mg filtered at the rate of 14.03 ml/h and it gradually increased with increasing body weight. Fox et al (1937), Chipman & Hopkins (1954) and Durve (1963) made similar observations on Mytilus californianus, Pecten irradians and Meretrix casta respectively. Walne (1972) also came to the conclusion that the filtration of a species increases with increasing size. Capuzzo & Sasner (1977) while investigating the effect of Cr. in Mya arenaria and Mytilus edulis concluded that the weight dependency of filtration rate was not affected by any treatment. Similarly, the increased filtration rates with increasing size observed in Sunetta scripta was also found to be unaffected either by the impact of salinity or copper (Figs. 39 - 42).

In Sunetta scripta in 30×10^{-3} S. the rate of filtration per mg of the body weight was determined to be 1.51 ml/mg/h in animals of weight 46 mg which decreased to 0.38 ml/mg/h animals of 619 mg body dry weight (Table 29).

In Martesia fragilis the rate of filtration varied from 4.316 ml/mg/h to 1.539 ml/mg/h in animals of size 3.25 and 28 mg wet weight respectively (Srinivasan 1968). The above observations show that the rate of filtration per mg of the body weight is higher in smaller animals. Bayne et al (1976) suggest that the reduction in the filtration activity of larger mussels observed by Schlieper et al (1958) is a significant factor in the decreased growth efficiencies of larger mussels.

In Sunetta scripta the rate of filtration was observed to be 1.51 l/g/h for an animal of 0.46 gm dry weight in 30 x 10^{-3} salinity. In Crassostrea virginica the rate of filtration was observed to be 1 lit/gm/hr for a weight ranging 12-14 gm wet weight (Loosanoff & Nomejko 1946). Chipman & Hopkins (1954) determined a similar rate ie. 0.99 l/gm/h wet weight in the bay scallop Pecten irradian weighing 3.3 gm wet weight. A lower rate of 0.17 l/gm/hr was obtained by Rao (1953) in Mytilus californianus of 1.5 gm wet weight. A similar rate was obtained by Willensen (1952) for Mytilus edulis of comparable weights. Jorgensen (1960) reported the filtration rate in Mytilus edulis of 1 gm

weight to be 1.48 l/g/hr. A very high filtration rate of value of 4.316 l/gm/hr observed in Martesia fragilis of wet weight 0.0032 gm may be due to the very small size of the individuals as explained by Srinivasan (1968). In the present study the filtration rate per gram of the animal is estimated to be 520.12 ml/g/hr for Sunetta scripta of 1 gm body weight in the control animals in 30×10^{-3} salinity. From the foregoing account it can be concluded that the rates obtained for Sunetta scripta is comparable to the rates obtained for many other bivalves. Decreasing rate of filtration with increasing body size is also shown in many of the above works.

In Sunetta scripta in 25×10^{-3} salinity, the weight exponent for the filtration rate was observed to be 0.8025 in the control animals (Fig.33), while in 20×10^{-3} salinity the filtration rate increased with 0.7011th power of the body weight (Fig.36). A reduction in the rate of filtration with decreasing salinities can be noted on comparison of the calculated values (Fig.42). Similar observations were made by several authors. Depressing effect of low salinities on the filtration rate of lamellibranchs was observed by Dodgson (1928),

Cole & Hepper (1954) and Nagabhushanam (1956). Hopkins (1936) had observed normal pumping rates in the oyster Ostrea gijas in salinities ranging from 25 to 39 ppm while the efficiency declined below 13 ppm. Even though the clam, Meretrix casta is able to withstand wide variations in salinity the rate of filtration decreased in extreme low and high salinities (Durve 1963). Krishnamurthy & Ramamurthy (1968) found that the feeding rate is directly related to salinity. The maximum rate recorded was in 28×10^{-3} salinity and the minimum rate was in 10×10^{-3} salinity while investigating the impact of different salinities (10×10^{-3}, 15×10^{-3}, 20×10^{-3}, 25×10^{-3} and 28×10^{-3} salinities) on the feeding rate of the bivalve Arca granosa. Alagarswami & Victor (1976) observed the maximum rate of filtration in Pinctada fucata in the normal sea water (34×10^{-3} S), very poor rates in dilutions (14×10^{-3} S & 20×10^{-3} S) higher rates than in dilutions in higher concentrations (44×10^{-3} S, 50×10^{-3} S and 57×10^{-3} S). In Mytilus edulis, Bohle (1972) had observed reduced filtration rates in lower salinities. Another observation made under the present study is that in Sunetta scripta the impact of salinity was reflected more in the larger specimens showing greater reductions

in lower salinities (Fig.46).

In the clam, Sunetta scripta exposed to 0.05 ppm and 0.1 ppm of copper in 30 x 10^{-3} salinity, the rate of filtration was observed to increase with 0.6503th and 0.6026th power of the body weight respectively. Reducing action of copper on the filtration rate of Sunetta scripta is evident from the above observations (Figs,39, 40 & 41). The inhibitory effect of heavy metals on the filtering activity of animals has been observed by various authors. Reduction of filtration rate and disturbed ciliary activity were observed in response to uptake of dissolved chromium by Capuzzo & Sasner (1977) in Mya arenaria and uptake of both dissolved and particulate chromium in Mytilus edulis. They also observed that larger specimens showed greater reductions in filtration rate in Mya arenaria on exposure to 1 mg/l of chromium. In similarity with the above finding in Sunetta scripta the copper induced reductions were found to be greater in larger specimens in all the different salinities experimented (Figs.43 - 45). Abel (1976) observed the inhibitory effect of copper and silver respectively in Mytilus edulis. It was reported by Abel (1976) that

copper concentrations of 0.15 mg/l reduced filtration rate by 50% in Mytilus edulis. Howell et al (1984) also observed a fall in the filtration rate of Mytilus edulis when exposed to copper. According to Howell et al (1984) the impact of copper leading to a reduction in the filtration rate of the animals is not due to the direct action of copper on the gill cilia or on the valve closure response but suggest a chemosensory mechanism mediated via the branchial nerve which result in reduced rate of ciliary beating.

Both in 25×10^{-3} and 20×10^{-3} salinities, Sunetta scripta showed the same trend when exposed to the copper concentrations (Figs.40, 41). In animals exposed to 0.05, and 0.1 ppm of copper in 25×10^{-3} S the rate of filtration was observed to increase with 0.6023th and 0.5538th power of the body weight respectively (Figs.34 and 35). In 20×10^{-3} S when exposed to 0.05 and 0.1 ppm copper concentrations the weight exponents for filtration rates were observed to be 0.5856 and 0.5149 respectively (Figs.37 & 38).

On comparison of the filtration rates obtained under the three different experimental salinities, it can

be noted that the rate of reduction in filtration rate induced by copper was greater in higher salinities while in low salinities the effect of copper was less pronounced when compared to the control values. This can be explained as due to the cumulative depressing action of both salinity and copper acting on the animals.

In a correlative study of the filtration and oxygen consumption rate, it is observed that in Sunetta scripta the rate of filtration increases with increasing oxygen uptake maintaining a near linear relationship in animals upto a weight of 500 mg beyond which this was not maintained in the control animals in 30 x 10^{-3} salinity (Fig.47). Srinivasan (1968) also observed increased rate of oxygen uptake with increasing filtering rates in Martesia fragilis in animals upto the level of mature adult of 20 mg weight. He suggests that the higher filtration and respiration efficiency of the smaller animals is shown by the above observation. The above findings also support the fact that increased metabolism and activity is always exhibited by the smaller individuals. A correlation of filtration and oxygen consumption rates in the animals in 25 x 10^{-3} S and 20 x 10^{-3} S shows that as the salinity is lowered the

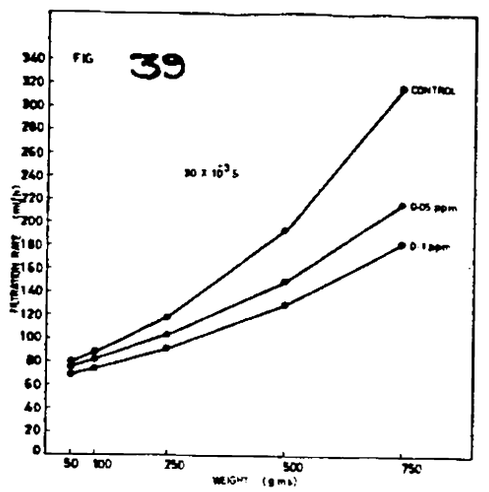
size range showing a linear relationship is also reduced (Figs.48, 49). This again supports for lowered metabolic rates observed in Sunetta scripta in low salinities.

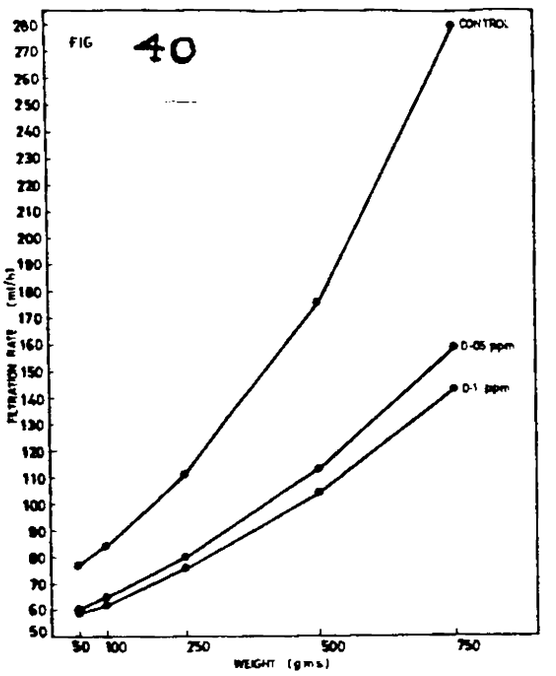
When filtration rate is expressed as litres of water filtered for each ml of oxygen consumed in animals of different sizes, the rates varied from 2.11 to 1.22 in the control experiments in 30 x 10^{-3} salinity (Table 38). Srinivasan (1968) found that in Martesia fragilis the rates varied from 1.06 to 4.465 in experimental animals ranging in size 7.20-34.50gm wet weight. It can be noted from both the above observations that the higher rates of filtration relative to oxygen consumption observed in young ones is seen to decrease as the animal grows. The young forms will need more food for the extra energy required for growth and hence the higher rates of filtration relative to oxygen consumption in the young, than in the mature adults as suggested by Jorgensen (1952). In Lasea subsa the ratio between oxygen uptake and filtration rate was found to be 1:4 (Ballentine & Mortan 1956). It is found that filtering rates per ml of oxygen consumed by Sunetta scripta is comparable to the above findings. Jorgensen found that the oyster, Ostrea virginica and the

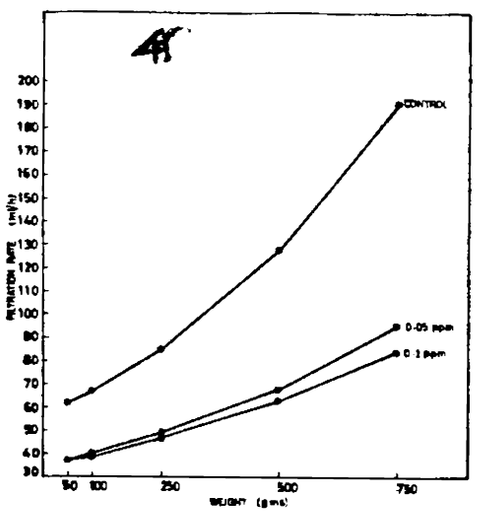
ascidians, Ciona intestinalis and Molgula manhattensis filter about 10 to 20 litres of water for each ml of oxygen consumed. While reviewing the weight exponents for the filtration rate and oxygen uptake, determined by various authors under different experimental conditions, Bayne et al (1976) suggest that the reason for most of the low exponents recorded for filtration rate than those of oxygen uptake may be explained by the relationship between growth efficiency and size. In the present study since the experiments were conducted on unfed specimens, the metabolic rates obtained are the standard rates and hence the weight exponents for oxygen uptake are lower when compared to those recorded for filtration rate.

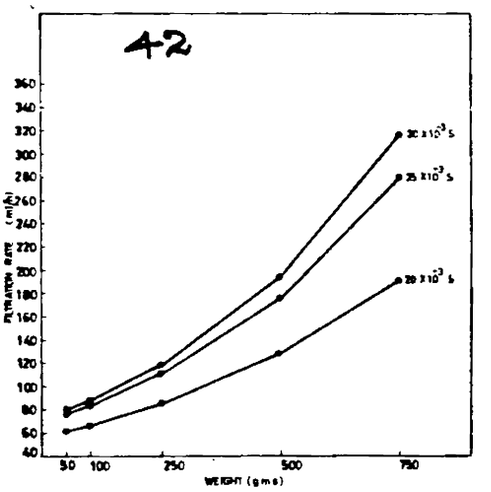
The capacity of Sunetta scripta to tolerate very high concentrations of copper has already been established in the previous experiments. Although the animals are able to survive under such polluted conditions the inhibitory effect of copper on the respiration of the clams is evident from the previous chapter. Depressing action of copper on the filtration rate of the animal is also made clear in the present study. Hence it can be concluded

that Sunetta scripta is able to survive but the population may get effected if they are subjected to chronic pollution by copper. According to Capuzzo & Sasner (1977) reduction of filtration rates under stress conditions is a more serious problem for a population of bivalves than a lowered metabolic activity in an individual organism.

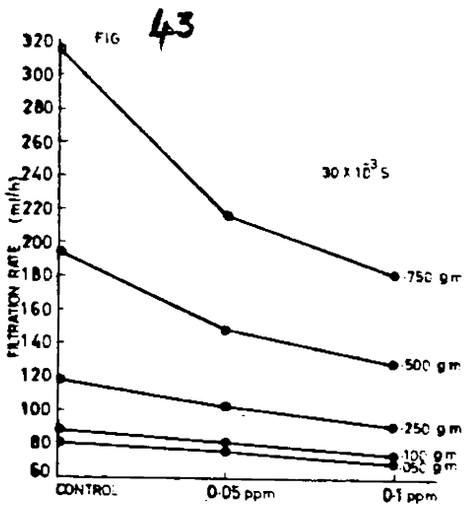


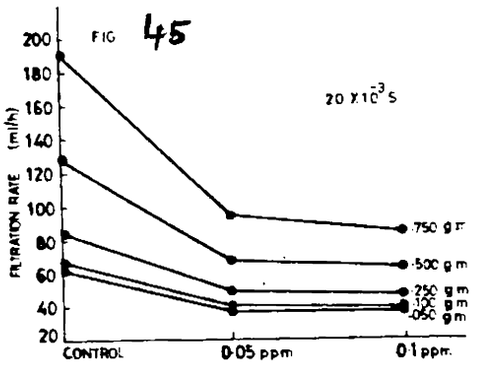


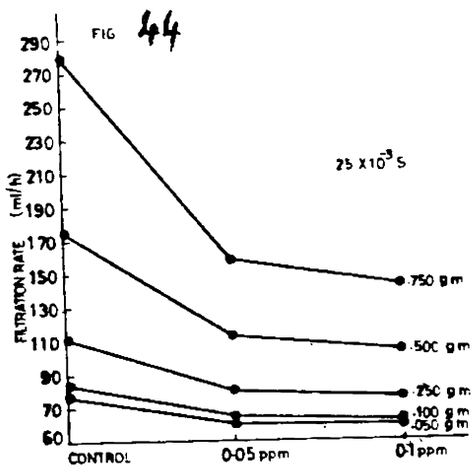


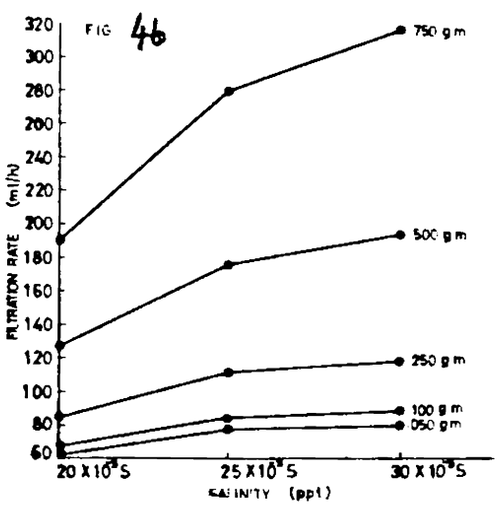


Figs. 39, 40, 41, 42 :- Trend of variation in the filtration rate between the control and dosed experiments in 30, 25 and 20 x 10^{-3} salinities and between the three controls in animals of dry weight .050, .100, .250, .500 and .750 gms.

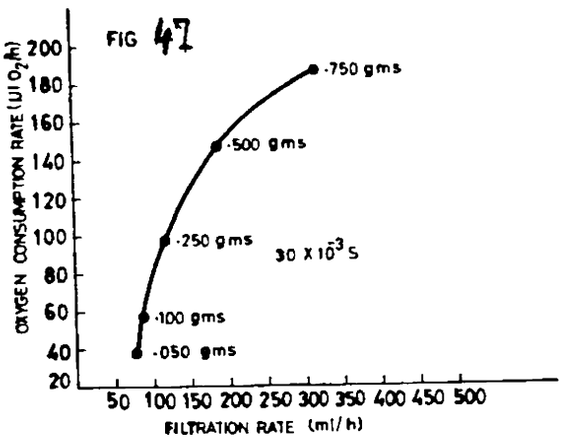


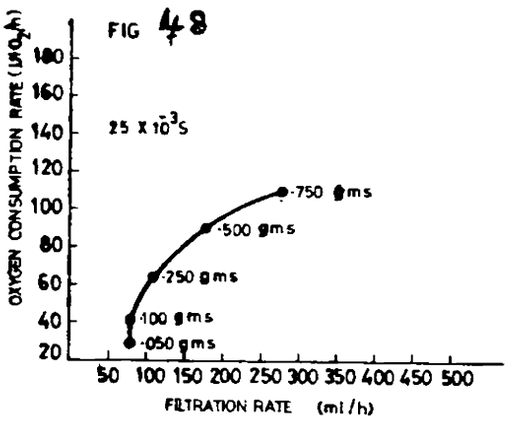


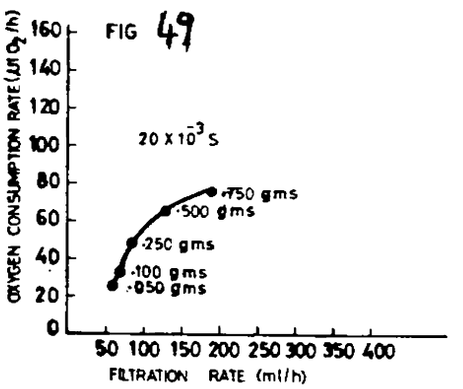




Figs. 43, 44, 45 & 46. Effect of copper (.05 and .1 ppm) on the rate of filtration in the animals of size .050, .100, .250, .500 and .750 gms dry wt. in the three salinities (30, 25 and 20 x 10^{-3}).







Figs. 47, 48, 49 :- Oxygen consumption versus filtration rate in animals of size .050, .100, .250, .500 and .750 gms dry wt. in 30, 25 and 20 x 10^{-3} salinities.

Table-38 - Relationship between rate of filtration and oxygen consumption in 30 x 10^{-3} salinity.

No.	Dry wt. mgs	Filtration rate 1/cc oxygen consumed
1	50	2.11
2	100	1.54
3	250	1.22
4	500	1.32
5	750	1.70

BIOACCUMULATION

Bioaccumulation, an important physiological phenomenon exhibited by many of the aquatic invertebrates especially bivalves deserve much attention in view of the public health hazards and also of the assessment of environmental quality. Phillips (1977) has made a detailed review regarding the use of biological indicator organisms for monitoring trace metal pollution in marine and estuarine environments. Many of the bivalve molluscs fit the role of indicators of pollution in coastal marine and estuarine realms. Their capacity to concentrate contaminants in body tissues to levels considerably higher than in the ambient waters, sedentary mode of life as adults and availability of sufficient tissue for analyses make them useful indicators of pollution. Goldberg (1975) has proposed the bivalves especially mussels as suitable indicators of trace metal pollution in marine environments. Bayne (1978) suggest that when most of the pollutants may be present in sea water in concentrations between 10^{-15} gg^{-1} and 10^{-12} gg^{-1}, concentrations in bivalve tissue may vary between 10^{-9} gg^{-1} and 10^{-4} gg^{-1}. The toxic

effects of accumulated heavy metals are well documented in several reviews (Clarke 1947, Scott & Major 1972, Eisler, 1977). Both the larvae and the adults may be affected by the metal which even cause mortality (D'Agostino & Finney 1974, Wisely & Blick 1967, Martin & Stephenson 1977). The biological property of metals to be bioaccumulated injures not only an individual organism but in the long run affects populations and being bioaccumulated through the food chain, poses a threat to human health.

The uptake of metals by marine and estuarine organisms had been the focus of attention of several reviews. The bioaccumulation of metals varies from metal to metal and differs among the various organisms (Waldichuk 1974). Boyce & Herdman had reported the green-sick condition of the oysters caused by the abnormal accumulation of copper as early as 1897. The ability of the bivalve molluscs to concentrate copper and other metals above ambient water levels was observed by several workers [Vinadograv (1953), Galtsoff (1964), Skidmore (1964), Ikuta (1967), Brooks & Rumsby (1965), Brooks & Rumsby (1967), Pringle *et al* (1968), Shuster & Pringle (1969), Arthur & Leonard (1970), Romeril (1971),

Nickless et al (1972), Eisler et al (1972), Pentreath (1973), Cunningham & Tripp (1973), Ireland (1973), Bryan (1973), Schulz-Baldes (1973, 1974), Eustace (1974), Nielsen (1974), Smith et al (1975), Phillips (1976), Pentreath (1976), Phillips (1977), Nambisan et al (1977), Boyden (1977), D'Silva & Kureishy (1978), Davies & Pirie (1978), D'Silva & Qasim (1979), Lakshmanan & Nambisan (1979)7.

Brooks & Rumsby (1965) had studied the relative uptake of eleven elements by an oyster, mussel and a scallop. The rate of uptake of cadmium by the oyster was determined by Brooks & Rumsby (1967).

In Mytilus edulis, Scott & Major (1972) measured the rate of uptake of copper over a period of 72 hr. Vernberg & Vernberg (1972) had observed the heavy metal copper being bioaccumulated in the fiddler crab, Uca pugilator, when exposed to the metal salt solutions. Nambisan et al (1977) had investigated the copper bioaccumulation by Meretrix casta when exposed to 0.5, 1.0, 5.0, and 10.0 ppm concentrations of the metal. The rates of copper and zinc accumulation were determined in the green mussel, Mytilus viridis exposed to

.005 and .01 ppm of copper and 0.1 and 0.2 ppm of zinc for a period of five weeks by D'Silva & Kureishy (1978). D'Silva & Qasim (1979) had investigated the accumulation and depuration of copper in the rock oyster, Crassostrea cucullata exposed to different concentrations of the metal for a period of 7 weeks. The accumulation of copper in oysters was studied by Ikuta (1967). Pringle et al (1968) & Shuster & Pringle (1969) had worked on the accumulation of copper by estuarine molluscs and the American oyster, Crassostrea virginica respectively.

The uptake of metals are known to be influenced by the different environmental variables like salinity (Phillips, 1976). According to Bryan (1971) the fresh water outfalls form the source of most of the trace metals in the marine environment. Hence salinity should be considered as one of the important parameters influencing the uptake of trace metals by marine organism (Phillips 1977). There is only scant information regarding the effect of salinity on the uptake of metals by marine organisms. O'Hara (1973) had determined the rates of cadmium uptake in the fiddler crab Uca pugilator under different stable salinities. Olson & Harrel (1973)

had investigated the toxicity of copper to the clam Rangia cuneata in different salinity media. Phillips (1976) had studied the effects of stable salinities on the uptake of zinc, cadmium, lead and copper by the common mussel Mytilus edulis.

The net uptake of zinc by Mytilus edulis under different natural and artificial salinity stresses was studied by Phillips (1979). Wolfe & Coburn (1970) had observed the effect of salinity on the trace metal concentrations in the bivalve molluscs.

The effect of body size on the trace metal content in bivalve molluscs was investigated by Boyden (1974). The net uptake of metals are also known to be influenced by various factors like size (Boyden 1977), temperature (Schulz-Baldes 1973), season (Bryan 1973, Pentreath 1973 & Phillips 1976), position of the animal in the water column (Nielsen 1974, Phillips 1976), etc.

With the addition of industrial units every year the pollution of the marine environment has become inescapably a world problem now. Currently authorities concerned with environmental management and pollution control bodies have started to recognize the need for

using bioassays in detecting changes in the environmental quality. The bivalve molluscs, possessing many of the characteristics of an ideal indicator species are recognized as fit for toxicity studies. It is in this context that a work on the copper bioaccumulation of the bivalve mollusc, Sunetta scripta was conceived. Since the natural bed of the clam exhibits wide variations in salinity, the study was undertaken with a view to determining the effect of different salinities on the uptake of copper by the clams belonging to different size groups.

6.2 MATERIALS & METHODS

The location of the clam bed, collection and acclimation of specimens in the laboratory and preparations for the experiments are described earlier (Chapter 2 Salinity Tolerance). Specimens of Sunetta scripta acclimated in the habitant salinity were kept in the test salinities for an acclimatization period of two days. With a view to determining the influence of lower salinities on the uptake kinetics of the metal, experiments were conducted in three different salinity viz. 10×10^{-3} S, 20×10^{-3} S and 30×10^{-3} S. The above choice is on the grounds that the clams in the natural habitat are

exposed to very low saline conditions during the monsoon period which extends from May to September. For studying the effect of lower and higher concentrations of the metal, in the pattern of uptake, .05, 1.0 and 2.0 ppm of copper were used.

The test animals belonging to each of the three size groups (20-25mm, 30-35mm and 40-45mm) were kept in the filtered sea water of the desired salinity for two days for acclimatizing them to the test conditions. The test solution was changed once every 24 hours. A group of animals which served as the control were dissected and the different tissues like gills, mantle, adductor muscle, foot and viscera were separated for metal analysis before exposing to the action of the pollutant. During the 6-day accumulation period, batches of animals were sacrificed every 2 days for the determination of the copper content in the different body parts. The adhering water from the dissected tissues was removed by carefully pressing within filter paper folds and dried at 80°C in an oven. The dry weight of the tissue samples were determined.

For the determination of the copper content, the

sample material was dried at 80°C to constant weight. A weighed portion (0.1 - 1.0 g) of the sample was taken in a kjeldhal flask and 10 ml of conc. HNO_3 and 5 ml of conc. H_2SO_4 were added. It was heated gently in the beginning till charring occurred and then, was heated strongly. Oxidising condition was maintained in the mixture by adding small amounts of conc. HNO_3. While heating, SO_3 fumes were seen to be copiously evolved and the heating was continued to remove all the organic matter. When no more of SO_3 fumes were produced and the digest became colourless, heating was stopped. The solution was cooled and diluted to 25 ml in a volumetric flask using deionized water (AOAC 1975). Blanks were prepared with the same concentration of acids, but without the tissue.

The copper concentrations were determined by Atomic Absorption Spectrophotometric method. The analysis was done directly using a Perkin Elmer 2380 model Atomic Absorption Spectrophotometer using sir acetylene flame. The samples were aspirated directly into the flame and the corresponding readings were noted. The average of these separate readings was taken as the value. The details of the instrument settings are given in Table - 39a.

Table - 39a

Instrumental settings for Atomic Absorption Spectrophotometric Estimation of Copper

Element	Wave length (nm)	Lamp Current	Spectral width	Concentration range	Fuel
copper	324.7	30m A	0.7	1-4 ppm	air-acetylene

6.3 RESULTS

The results of the analysis of copper concentrations in different tissues of the animal exposed to 0.05 ppm, 1.0 ppm and 2.0 ppm of copper over a period of 144 hr in

30×10^{-3} S, 20×10^{-3} S and 10×10^{-3} salinities are presented in Tables 39, 40 & 41 in Figures 50 - 76.

An increase in copper level was observed in all the tissues on exposure to copper concentrations. The background level of copper was always the highest in the gill tissues. The order of distribution of copper in the various tissues in the control samples were gills > mantle viscera > foot > adductor muscle. In general the gill tissue had the highest rate of uptake under the different experimental conditions. The rate of uptake was almost the same in mantle and viscera with slightly higher rates in the mantle. Adductor muscle had the next higher rate with foot showing the least rate of uptake. The efficiency of accumulation of the different tissues under different experimental conditions can be assessed from the concentration factors.

Accumulation of copper at a salinity of 30×10^{-3}.

Copper was found to be concentrated in all the different tissues studied during 6 day accumulation period. The copper content of the five different tissues (gills, mantle, foot, adductor muscle and viscera) in animals of three size groups exposed to different concentrations of

copper during the 6 day accumulation period in 30 x 10^{-3} salinity and their respective concentration factors are given in the Table 39b.

The efficiency of accumulation was found to be highest in the gill tissue in all the three size groups and copper concentrations. In 30 x 10^{-3} salinity in the animals of the size range 20mm to 25mm exposed to 0.05 ppm concentration of copper had increased from a background level of 197.15 µg g^{-1} in the gill tissue to 242.54 µg g^{-1}. Different tissues, in the order of decreasing copper concentrations was gills, mantle, viscera, foot, adductor muscle. The concentration factors obtained in the various tissues viz. the gills, mantle, viscera, foot and adductor muscle were 4850.71, 1679.73, 1524.76, 893.35 and 882.32 respectively. It can be noted that the uptake was linear in all the tissues studied in the case of animals of size 20mm to 25mm exposed to 0.05 ppm copper (Fig.50).

In 30mm to 35mm sized clams exposed to 0.05 ppm of copper, the concentration of copper found in the gill tissue was 153.09 µg g^{-1} and in the foot 51.77 µg g^{-1} at 6 days of accumulation. The concentration factors

ranged from 3061.86 to 1035.48 in the different tissues. Tissue copper levels followed the order gills > mantle adductor muscle > viscera > foot. A linear uptake was observed only in the gills and the mantle while in the adductor muscle, viscera and foot the uptake was curve-linear (Fig.51).

In animals of size 40mm to 45mm the gill tissue having a concentration factor of 3167.6 exhibited the highest efficiency of uptake when exposed to copper concentration of .05 ppm. The lowest efficiency was observed to be in the adductor muscle with a concentration factor of 594.6. Copper concentration in the gills ranged between 118.52 $\mu g\ g^{-1}$ and 158.38 $\mu g\ g^{-1}$ while in the adductor muscle the copper concentration increased from a background level of 16.83 $\mu g\ g^{-1}$ to 29.73 $\mu g\ g^{-1}$ of copper. The order of distribution with respect to the concentration factor of copper being, gills > mantle viscera > foot > adductor muscle. Excepting viscera, which had a linear pattern of uptake, in all the other tissues the pattern of uptake was curve-linear as evident from Fig.52.

On exposure to 1.0 ppm copper, the metal was distributed in all the body components studied in all the three size groups, but differed in magnitude. The copper content in the gill tissue of small sized clams (20mm to 25mm) in 30 x 10^{-3} salinity went up to 236.96 $\mu g\ g^{-1}$ at the end of 6 days from a background level of 169.92 $\mu g\ g^{-1}$ showing the highest rate of uptake. The lowest rate of uptake had occurred in the foot where the copper content varied from 12.53 $\mu g\ g^{-1}$ to 17.95 $\mu g\ g^{-1}$. The order of distribution being: gills > mantle viscera > adductor muscle > foot with respect to C.F. From Fig.53 it is seen that the pattern of uptake was curve-linear in all the five different tissues.

In the animals of size range 30mm to 35mm, the rate of uptake was observed to be highest with the gills in 1 ppm of copper. The gill tissue content of copper increased to 323.47 $\mu g\ g^{-1}$ from a control value of 258.78 $\mu g\ g^{-1}$. The highest and the lowest concentration factors ranged from 323.47 (gills) to 18.21 (foot). The ranking followed the order gills > mantle viscera > adductor muscle > foot. The uptake pattern was curve-linear with time in all the tissues studied (Fig.54).

The gill tissue concentration of copper had increased from a background level of 301.46 $\mu g\ g^{-1}$ to 365.03 $\mu g\ g^{-1}$ showing an increase of 21.09% in animals ranging in size 40mm to 45mm exposed to 1 ppm copper. The efficiency of accumulation was found to be highest in the gill tissue (365.03) and lowest in foot (25.26). Tissues in the order of increasing concentrations of copper was gills > mantle > viscera > adductor muscle > foot. In Fig.55 it is shown that a curve-linear pattern of uptake was exhibited by all the tissues.

In the size range 20mm to 25mm the tissue concentration of copper increased in all the tissues after an accumulation period of 144 hr on exposure to 2 ppm of copper in 30 x 10^{-3} salinity. The highest concentration was found in the gill tissue (174.34 $\mu g\ g^{-1}$) and the lowest in the foot (22.33 $\mu g\ g^{-1}$). The order of distribution being, gills > mantle > viscera > adductor muscle > foot with respect to the concentration factor. It can be seen that the magnifications were 67.41% and 6.38% in the gills and foot respectively. The uptake pattern in all the five different tissues had followed a curve-linear relationship with time as evident from Fig.56.

In the medium sized clams (30mm to 35mm) maintained in 2 ppm copper solution, even though the gill tissue had the largest amount of the accumulated metal, copper was seen to be concentrated in all other tissues with different magnifications. In the gill tissue the copper concentrations had increased from a control value of 182.55 $\mu g\ g^{-1}$ to 249.25 $\mu g\ g^{-1}$ showing a 36.54% increase in the tissue copper level. The concentration factors ranged from 124.63 in the gills to 16.00 in the foot. The order of distribution with respect to the concentration factor of copper being gills > mantle > viscera > adductor muscle > foot. Except in viscera and foot, the uptake pattern in the other three tissues was linear with time (Fig.57).

In the clams of size 40mm to 45mm exposed to 2 ppm copper concentration gills had the highest concentration (161.19 $\mu g\ g^{-1}$) of accumulated copper while adductor muscle had the lowest (15.79 $\mu g\ g^{-1}$). The magnifications were 48.37% and 44.33% in the gills and foot respectively. Tissue copper levels followed the order gills > mantle viscera > foot > adductor muscle. Concentration factors of 80.59, 24.42, 17.07, 7.96 and 7.89 were attained in the gills, mantle, viscera, foot and adductor muscle respectively.

It is shown in Fig.58 that in all the tissues, the pattern of uptake was curve-linear.

2. Accumulation of copper at a salinity of 20×10^{-3}.

The uptake of copper in the various tissues under the different experimental conditions in 20×10^{-3} salinity is given in the Table 40. Copper was found to be concentrated to a very high level in all the tissues in the animals ranging in size 20mm to 25mm exposed to 0.05 mg l^{-1} of copper in 20×10^{-3} S, The efficiency of accumulation was found to be highest in the gill tissue with a concentration factor of 5704.96. It was followed by mantle, viscera, adductor muscle and foot with concentration factors of 3101.08, 1944.84, 1777.09 and 808.70 respectively. In the gill tissue the concentration of copper had increased from a background level of 166.97 µg g^{-1} to 285.25 µg g^{-1} while in the foot having the lowest C.F., the values ranged from 18.37 µg g^{-1} to 40.44 µg g^{-1} in the tissue copper level, the tissues in the order of decreasing copper content being gills > mantle > viscera > adductor muscle > foot. Copper was taken up linearly in the gill tissue while in all others a curve-linear relationship was observed (Fig.59).

It is seen that in the size group 30mm to 35mm exposed to 0.05 ppm Cu in all the five different tissues have taken up large amounts of the metal. In the gill tissue which showed the greatest accumulation efficiency the copper level had increased from a control value of 202.17 $\mu g\ g^{-1}$ to 309.65 $\mu g\ g^{-1}$ showing 53.16% increase. Here the adductor muscle had the lowest rate of uptake in which the tissue copper level had increased from 17.47 $\mu g\ g^{-1}$ to 21.87 $\mu g\ g^{-1}$. The various tissues like the gills, mantle, viscera, foot and adductor muscle had concentration factors of 6193.07, 2080.21, 2139.99, 1118.12 and 437.31 respectively. The ranking followed the order gills > viscera > mantle > foot > adductor muscle. A linear pattern of uptake was observed in the gills, mantle and viscera while in the other two tissues the uptake was curve-linear as seen in Fig.60.

The tissue copper levels had considerably increased in all the five tissues during the 6-day accumulation period in the large sized clams (40mm to 45mm) in 0.05 ppm copper. Gill tissue had attained concentration of 298.92 $\mu g\ g^{-1}$ after 144 hr of exposure showing 52.38% increase from the background level of copper. The highest

accumulation efficiency was exhibited by the gill having a concentration factor of 5978.34. Others like the mantle, viscera, foot and the adductor muscle had the concentration factors of 2491.23, 1152.42, 789.05 and 1163.29 respectively. Tissue copper levels followed the order gills > mantle > adductor muscle > viscera > foot. In all the tissues, the pattern of uptake was curve-linear (Fig.61).

In 20 x 10^{-3} S on exposure to 1 ppm of copper in animals of size 20mm to 25mm, copper was seen to be accumulated in all the body tissues studied, but differing in magnitudes. The copper content in the gill tissue went up to 391.86 µg g^{-1} at the end of 6 days from a control value of 237.64 µg g^{-1} showing the highest rate of uptake having a C.F. of 391.87. In the foot where the lowest rate of uptake is observed the copper concentration was raised to the level of 33.38 µg g^{-1} from a background concentration of 10.26 µg g^{-1}. The order of distribution being gills > mantle > viscera > adductor muscle > foot with respect to C.F. Except in the foot and adductor muscle where the metal ions were linearly taken up into the body tissues, the rest of the body tissues had a curve-linear pattern of uptake which

can be observed from Fig.62.

The tissue concentration of copper increased in all the tissues after an accumulation period of 144 hr in the clams of size 30mm to 35mm on exposure to 1 ppm copper. The highest concentration was found in the gill tissue (326.60 $\mu g\ g^{-1}$) and the lowest in the foot (36.23 $\mu g\ g^{-1}$). It can be seen that the magnifications were 72.88% and 51.34% in the gills and foot respectively. The gill tissue with a concentration factor of 326.70 exhibited the highest efficiency of accumulation among others, the order of distribution being gills > mantle > viscera > adductor muscle > foot. The uptake pattern in the gills, mantle and foot followed a curve-linear relationship with time while in the viscera and adductor muscle linear uptake was observed as evident from Fig.63.

In the size group 40mm to 45mm, it is seen that all the five different tissues have concentrated copper on exposure to a copper concentration of 1 ppm. In the gill tissue which showed the highest accumulation efficiency the copper level had increased from a control value of 208.83 $\mu g\ g^{-1}$ to 324.18 $\mu g\ g^{-1}$ showing a 55.24% increase.

Here the adductor muscle had the lowest rate of uptake in which the tissue copper level had increased from 20.67 $\mu g\ g^{-1}$ to 23.66 $\mu g\ g^{-1}$ showing an increase of 14.47% only. The gill tissue showing the highest accumulation efficiency had a concentration factor of 324.18, but in the foot the value was only 20.66. The distribution followed the order gills > mantle > viscera > adductor muscle > foot with respect to C.F. A linear pattern of uptake was observed in the mantle while in all others the uptake was curve-linear (Fig.64).

In the smallest size group 20mm to 25mm exposed to a copper concentration of 2 ppm in 20 x 10^{-3} salinity, the rate of uptake was observed to be highest with the gills. The gill tissue content of copper increased to 370.04 $\mu g\ g^{-1}$ from a control value of 215.48 $\mu g\ g^{-1}$. The efficiency of accumulation was found to be highest in the gill tissue (185.02) and lowest in the adductor muscle (8.57). The ranking followed the order: gills > mantle > viscera > foot > adductor muscle. The uptake pattern was curve-linear in all the tissues studied as seen in Fig.65.

In the clams of size 30mm to 35mm exposed to 2 ppm of copper, gills had the highest concentration (393.66 $\mu g\ g^{-1}$) of accumulated copper while foot had the lowest (43.72 $\mu g\ g^{-1}$). The concentration factors ranged from 196.83 (gills) to 21.86 (foot) in the five different tissues. The tissues in the order of decreasing copper content being: gills > mantle > viscera > adductor > muscle > foot. Copper was seen to be taken up linearly in the mantle and viscera while in others a curve-linear relationship was observed (Fig.66).

In 40mm to 45mm size group exposed to 2 ppm of copper, the rate of uptake was observed to be highest with the gills. The gill tissue content of copper increased to 365.18 $\mu g\ g^{-1}$ from a control value of 238.16 $\mu g\ g^{-1}$ showing 53.33% increase. The highest efficiency of accumulation was seen in the gill which had a concentration factor of 182.59 and the lowest in the adductor muscle with a C.F. of 8.02. The ranking followed the order gills > mantle > viscera > foot > adductor muscle. The uptake pattern was curve-linear in all the tissues except in the mantle where a linear uptake was observed (Fig.67).

3. Accumulation of copper at a salinity of 10 x 10^{-3} salinity.

Distribution pattern of copper in the five different tissues of Sunetta scripta exposed to different copper concentrations in 10 x 10^{-3} salinity is shown in Table 41. In 10 x 10^{-3} salinity media containing 0.05 ppm copper, the metal was distributed in all the body components in animals of size group 20mm to 25mm, but with different magnitudes. The highest levels were found with the gill (177.09 µg g^{-1}) and viscera (74.73 µg g^{-1}) during 6 days of exposure. Foot (28.85 µg g^{-1}) and adductor muscle (39.92 µg g^{-1}) accumulated comparatively lesser amounts of the metal. The order of distribution being: gill > mantle > viscera > adductor muscle > foot with respect to C.F. Copper was seen to be taken up linearly into the gill and foot during the accumulation period while the mantle, viscera and adductor muscle had a curve-linear relationship is evident from Fig.68.

The efficiency of accumulation was observed to be highest in the gill tissue where the copper content showed 39.41% increase from the background level in the animals ranging in size 30mm to 35mm exposed to .05 ppm.

The background level of copper in the gill was 149.25 $\mu g\ g^{-1}$ and the metal content was 208.07 at 6 days. In foot having the lowest accumulation, efficiency the copper concentration varied from 13.08 $\mu g\ g^{-1}$ to 17.91 $\mu g\ g^{-1}$, the order of distribution being gills > viscera > mantle > adductor muscle > foot. A curve-linear pattern of uptake was observed in all tissues except in the gill where the uptake was linear (Fig.69).

In .05 ppm copper concentration, the gill tissue concentration of copper had varied from a background level of 229.77 $\mu g\ g^{-1}$ to 203.32 $\mu g\ g^{-1}$ in the largest size group (40mm to 45mm) studied. A concentration factor of 4066.44 was observed with the gill tissue which showed the highest efficiency among others. On other body tissues like the mantle, viscera, foot and the adductor muscle, concentration factors of 1220.63, 1039.88, 122.96 and 342.80 were obtained respectively. The order of distribution with respect to C.F. being gills > mantle > viscera > adductor muscle > foot. It can be noted from Fig.70 that in all the five different tissues the pattern of uptake was curve-linear.

On exposure to 1 ppm copper concentration in 10×10^{-3} salinity, gills had the highest concentration (419.70 µg g^{-1}) of accumulated copper while adductor muscle had the lowest (59.33 µg g^{-1}) in the animals of size 20mm to 25mm. Tissue copper levels followed the order gills > mantle > viscera > foot > adductor muscle. The gill tissue having the highest concentration efficiency had a concentration factor observed in the adductor muscle was 59.33. Excepting viscera all the other tissues exhibited curve-linear pattern of uptake is shown in Fig.71.

In the animals of size range 30mm to 35mm exposed to 1 ppm of copper, all the tissues under investigation were seen to accumulate copper but differed considerably in magnitudes. In the gill tissue the copper level increased from 120.04 µg g^{-1} to 207.35 µg g^{-1} at the end of 6 days. Highest accumulation efficiency was observed in the gill tissue having a concentration factor of 207.35. In the foot and adductor muscle, the efficiency was very low as can be observed from the concentration factors of 5.11 and 6.29 respectively. Tissue copper levels followed the order gills > mantle > viscera > adductor muscle > foot. The pattern of uptake was

curve-linear in all the tissues (Fig.72).

In the largest size group (40mm to 45mm) exposed to 1 ppm copper concentration, the rate of uptake was highest with the gills. The gill tissue content of copper increased to 203.20 $\mu g\ g^{-1}$ from a control value of 127.69 $\mu g\ g^{-1}$. In foot having the lowest rate of uptake the copper concentration ranged from 15.79 $\mu g\ g^{-1}$ to 18.29 $\mu g\ g^{-1}$. The concentration factors in the various tissues ranged from 203.20 (gill) to 18.29 (foot). The order of distribution being, gill > mantle > viscera > adductor muscle > foot. All the tissues had curve-linear pattern of uptake as shown in Fig.73.

In 10×10^{-3} saline media containing 2 ppm of copper, the copper content in the gill tissue went up to 279.75 $\mu g\ g^{-1}$ from a background value of 141.70 $\mu g\ g^{-1}$ at the end of 6 days in the smallest size group (20mm to 25mm) experimented. This was equivalent to 97.42% increase. The lowest rate of uptake was observed in adductor muscle where there was only 47.02% increase in the tissue copper level compared to the background concentration. The gill tissue had the highest accumulation efficiency (139.88) and adductor muscle (20.81) the lowest.

The order of distribution being gills > mantle > viscera > foot > adductor muscle. It can be seen from Fig.74 that a curve-linear pattern of uptake was observed throughout.

On exposure to 2 ppm copper the highest accumulation efficiency was observed in the gill tissue in the 30mm to 35mm size group, in which the metal content at the end of 6 days was 278.71 ug g^{-1}. This was equal to 74.58% increase from a background level of 159.65 ug g^{-1}. Concentration factors of 139.36, 28.73, 34.33, 10.09 and 14.05 were observed in the gills, mantle, viscera, foot and adductor muscle respectively. In the gills, mantle, adductor muscle and foot, the pattern of uptake was curve-linear while in viscera a linear uptake of the metal ions was observed as shown in Fig.75.

In the animals ranging in size 40mm to 45mm maintained in sea water continuing 2 ppm of copper, the copper concentration in the gill tissue had increased to 235.53 µg g^{-1} from a control value of 139.94 µg g^{-1} which is equal to 68.31% increase. Considerable difference can be noticed in the accumulation efficiencies

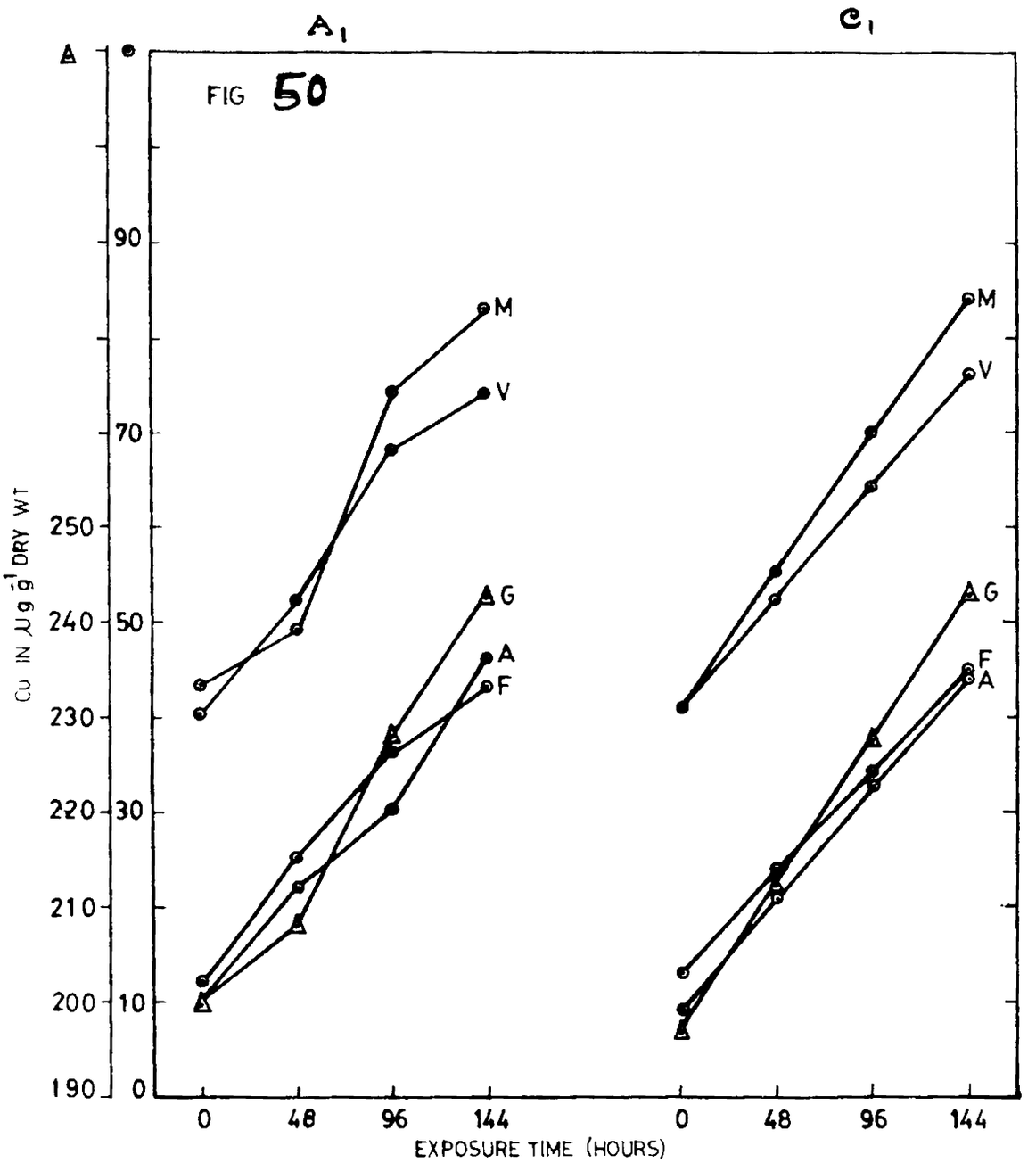


Fig.50 Concentration of Cu (μg/g dry wt.) in S. scripta exposed to 0.05 ppm of Cu.
(S = 30 x 10^{-3}; Size = 2-2.5cm)

A_1 = Actual; C_1 = Calculated.
M = Mantle; V = Viscera; G = Gill; F = Foot;
A = Aductor muscle

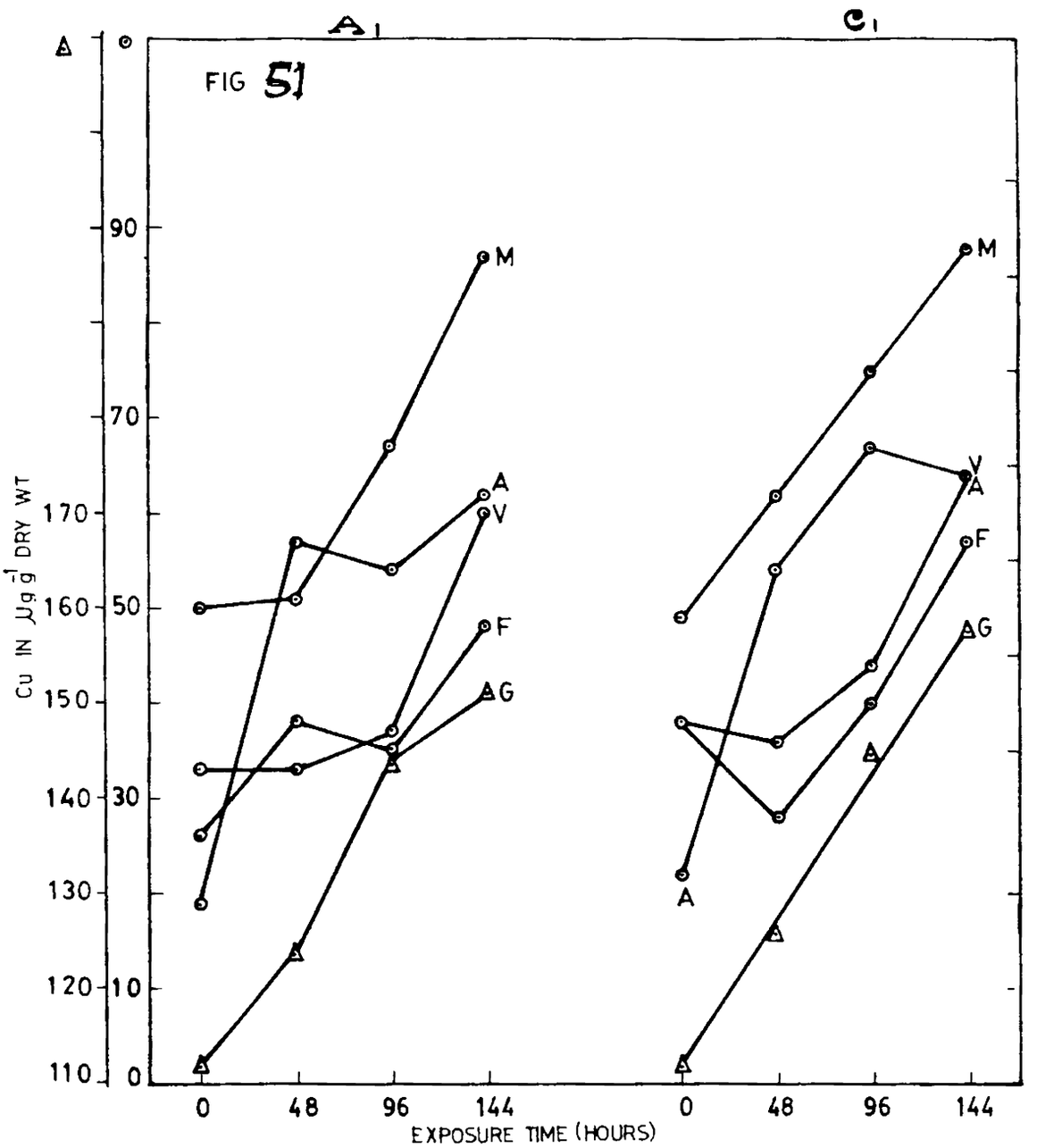


Fig.51 Concentration of Cu (µg/g dry wt.) in S. scripta exposed to 0.05 ppm of Cu.
($S = 30 \times 10^{-3}$; Size = 3-3.5cm)

A_1 = Actual; C_1 = Calculated.

M = Mantle; V = Viscera; G = Gill; F = Foot;
A = Aductor muscle

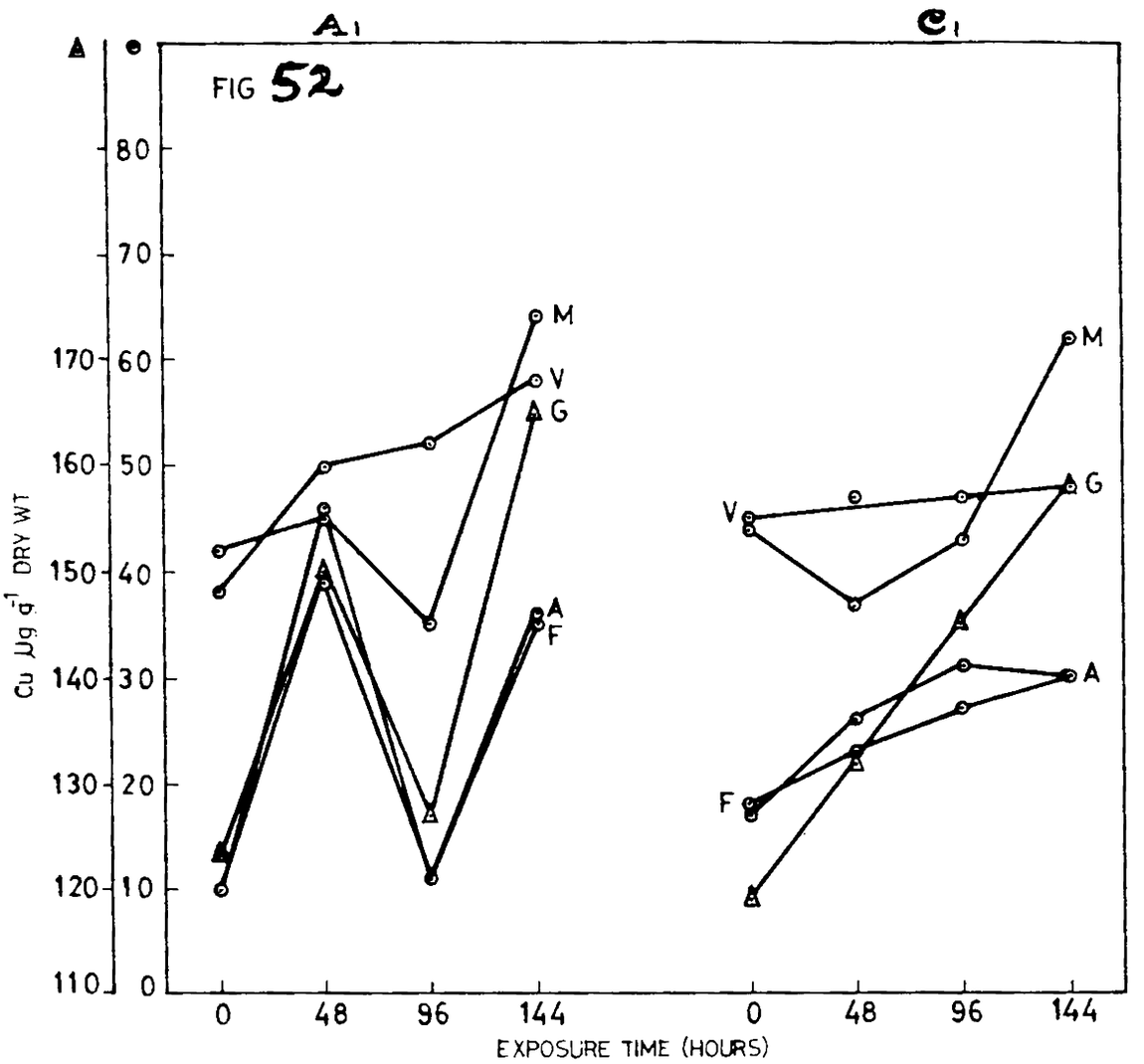


Fig.52 Concentration of Cu (µg/g dry wt.) in S. scripta exposed to 0.05 ppm of Cu.
($S = 30 \times 10^{-3}$; Size = 4-4.5cm)

A_1 = Actual; C_1 = Calculated.
M = Mantle; V = Viscera; G = Gill; F = Foot;
A = Aductor muscle

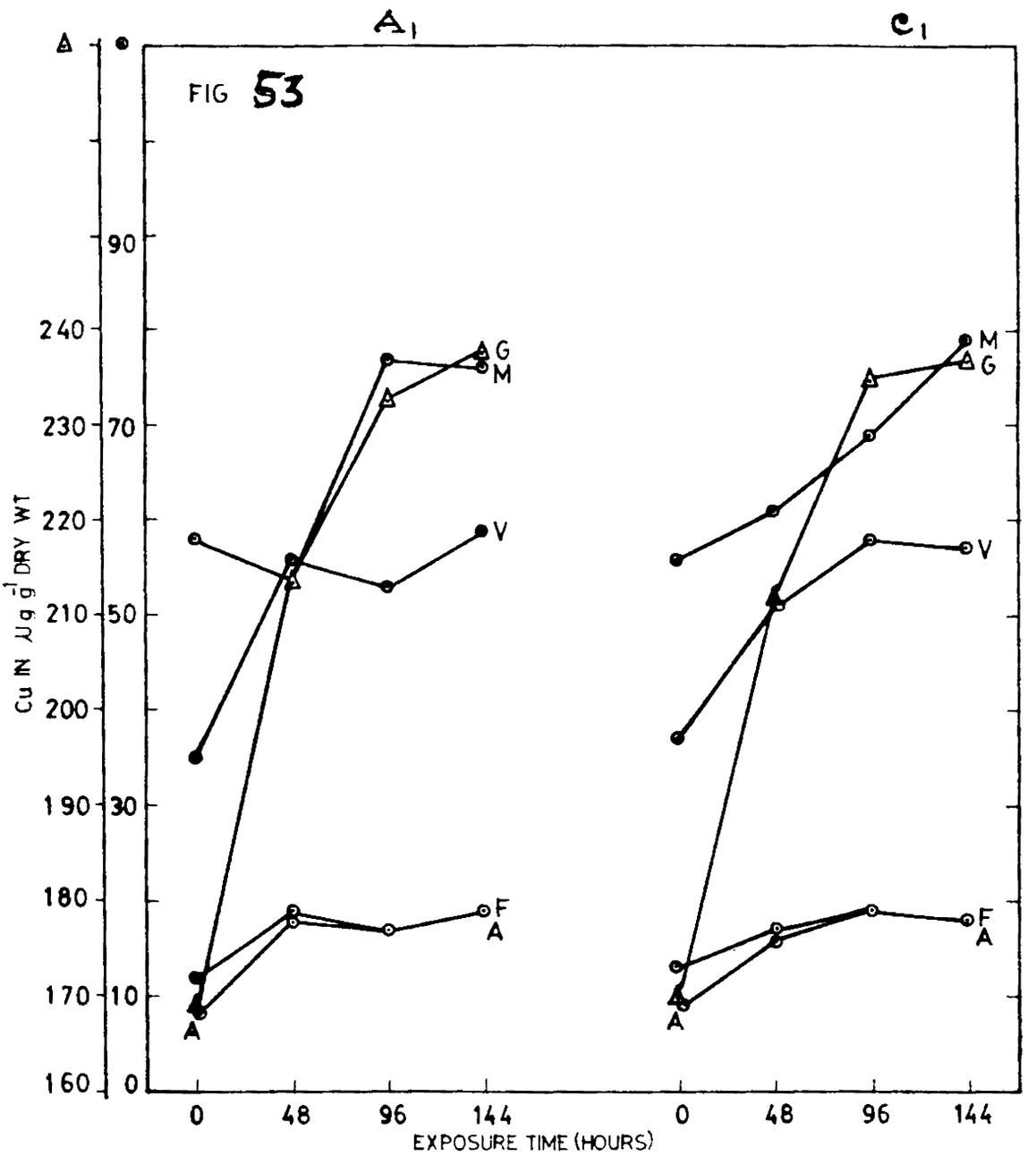


Fig.53 Concentration of Cu (µg/g dry wt.) in S. scripta exposed to 1.0 ppm of Cu.
($S = 30 \times 10^{-3}$; Size = 2-2.5cm)

A_1 = Actual; C_1 = Calculated.
M = Mantle; V = Viscera; G = Gill; F = Foot;
A = Aductor muscle

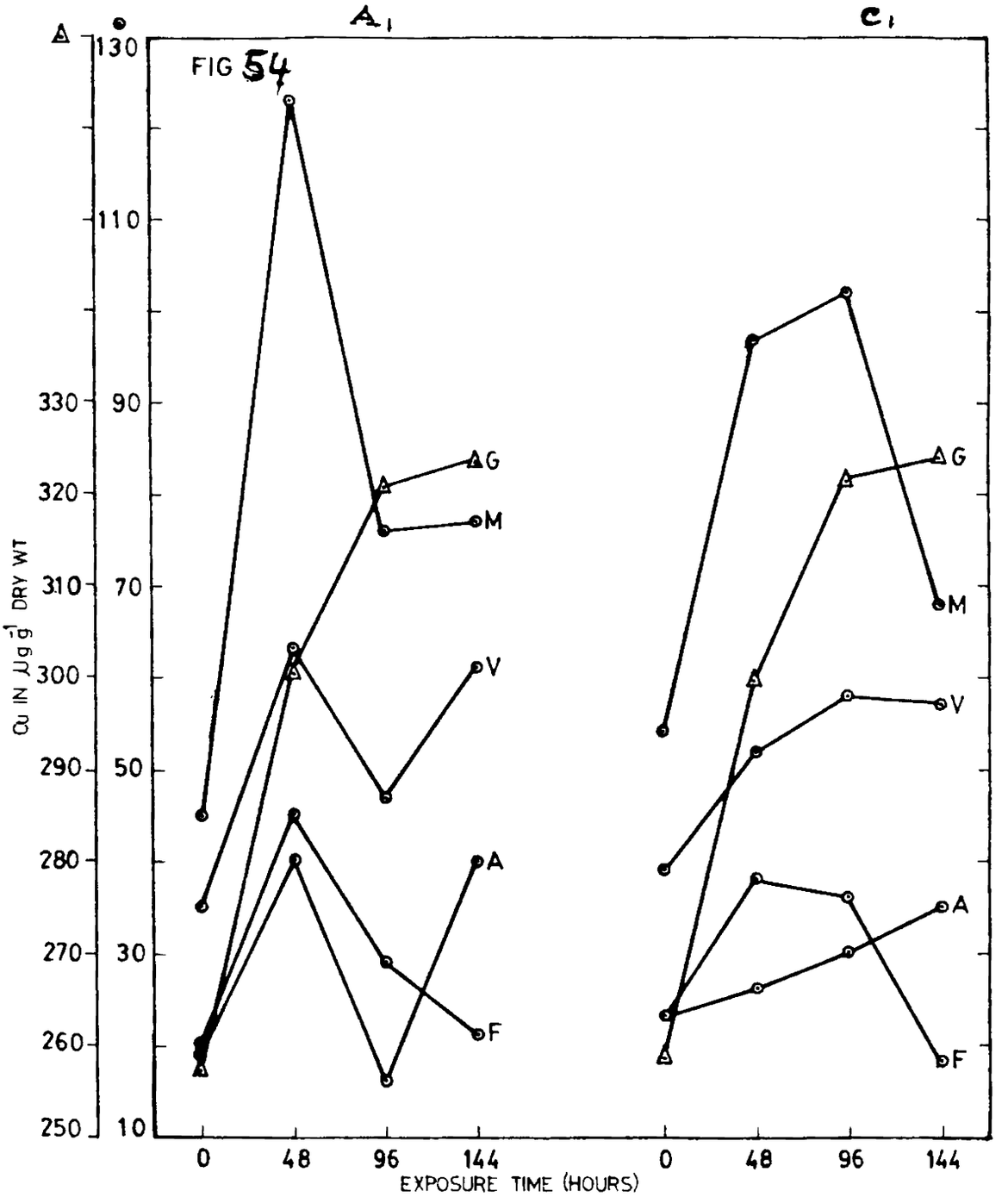


Fig.54 Concentration of Cu (µg/g dry wt.) in S. scripta exposed to 1.0 ppm of Cu.
($S = 30 \times 10^{-3}$; Size = 3-3.5cm)

A_1 = Actual; C_1 = Calculated.
M = Mantle; V = Viscera; G = Gill; F = Foot;
A = Aductor muscle

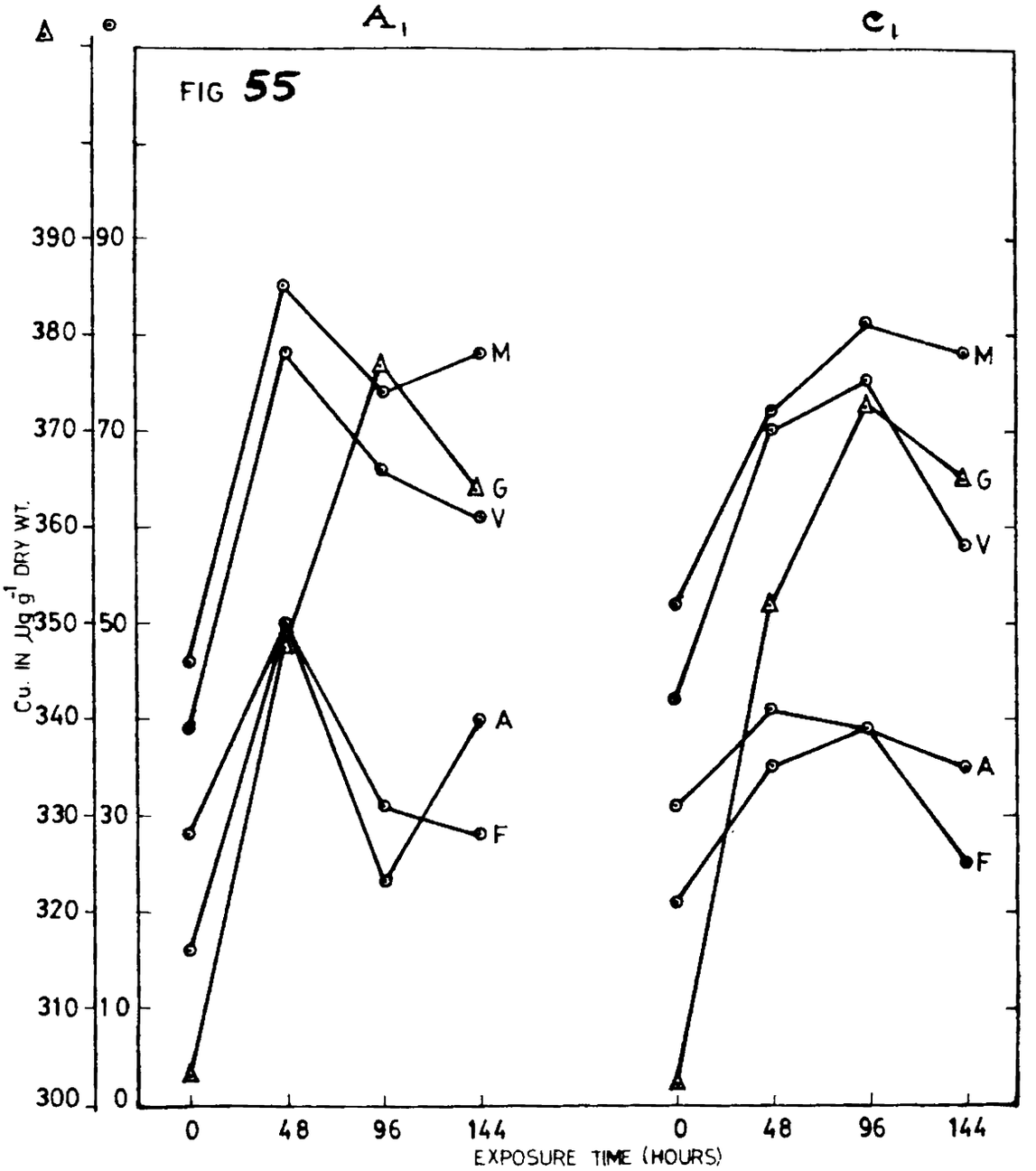


Fig.55 Concentration of Cu (µg/g dry wt.) in S. scripta exposed to 1.0 ppm of Cu.
($S = 30 \times 10^{-3}$; Size = 4-4.5 cm)

A_1 = Actual; C_1 = Calculated.

M = Mantle; V = Viscera; G = Gill; F = Foot; A = Aductor muscle

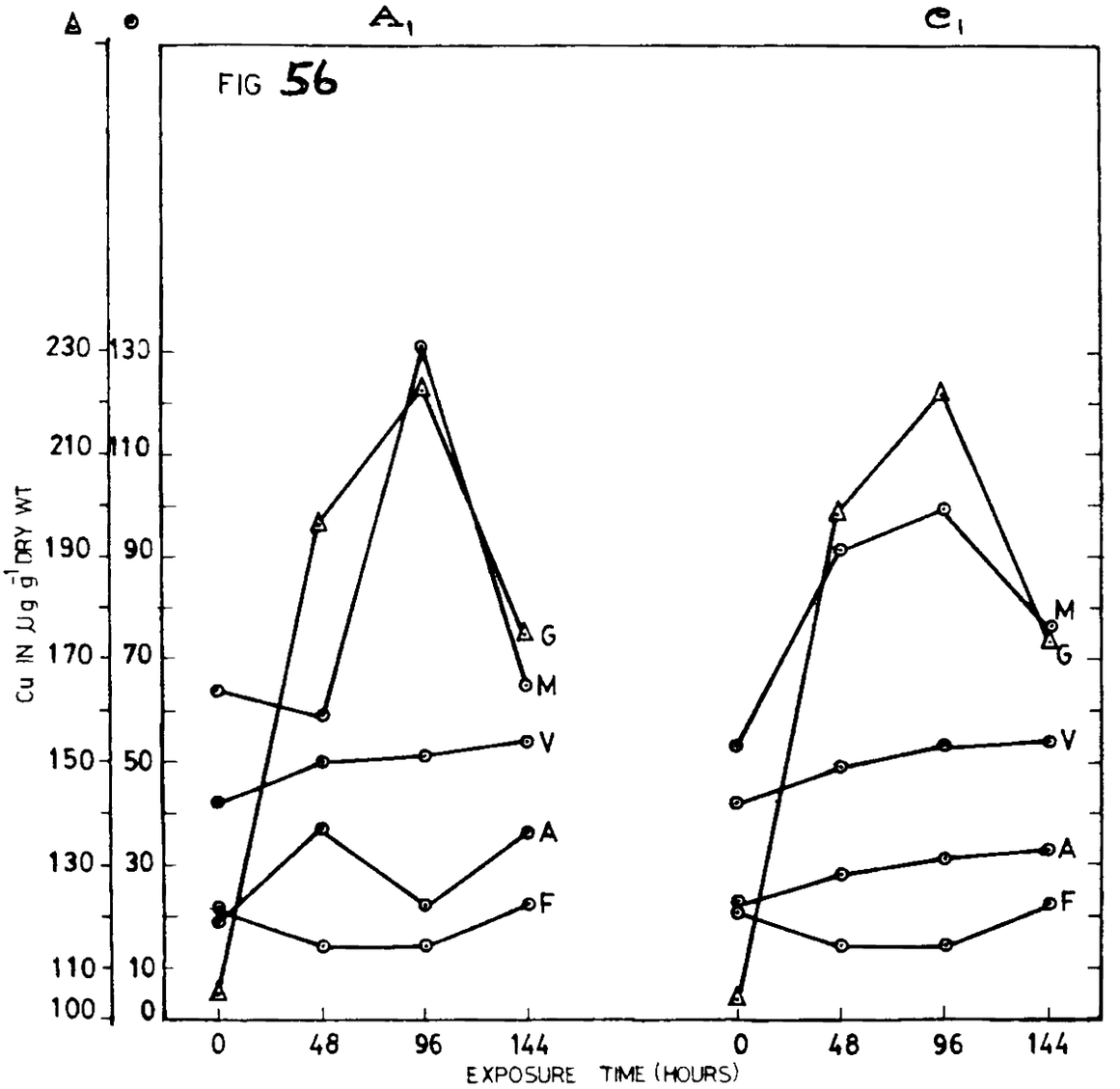


Fig.56 Concentration of Cu (µg/g dry wt.) in S. scripta exposed to 2.0 ppm of Cu.
(S = 30 x 10^{-3}; Size = 2-2.5cm)

A_1 = Actual; C_1 = Calculated.
M = Mantle; V = Viscera; G = Gill; F = Foot;
A = Aductor muscle

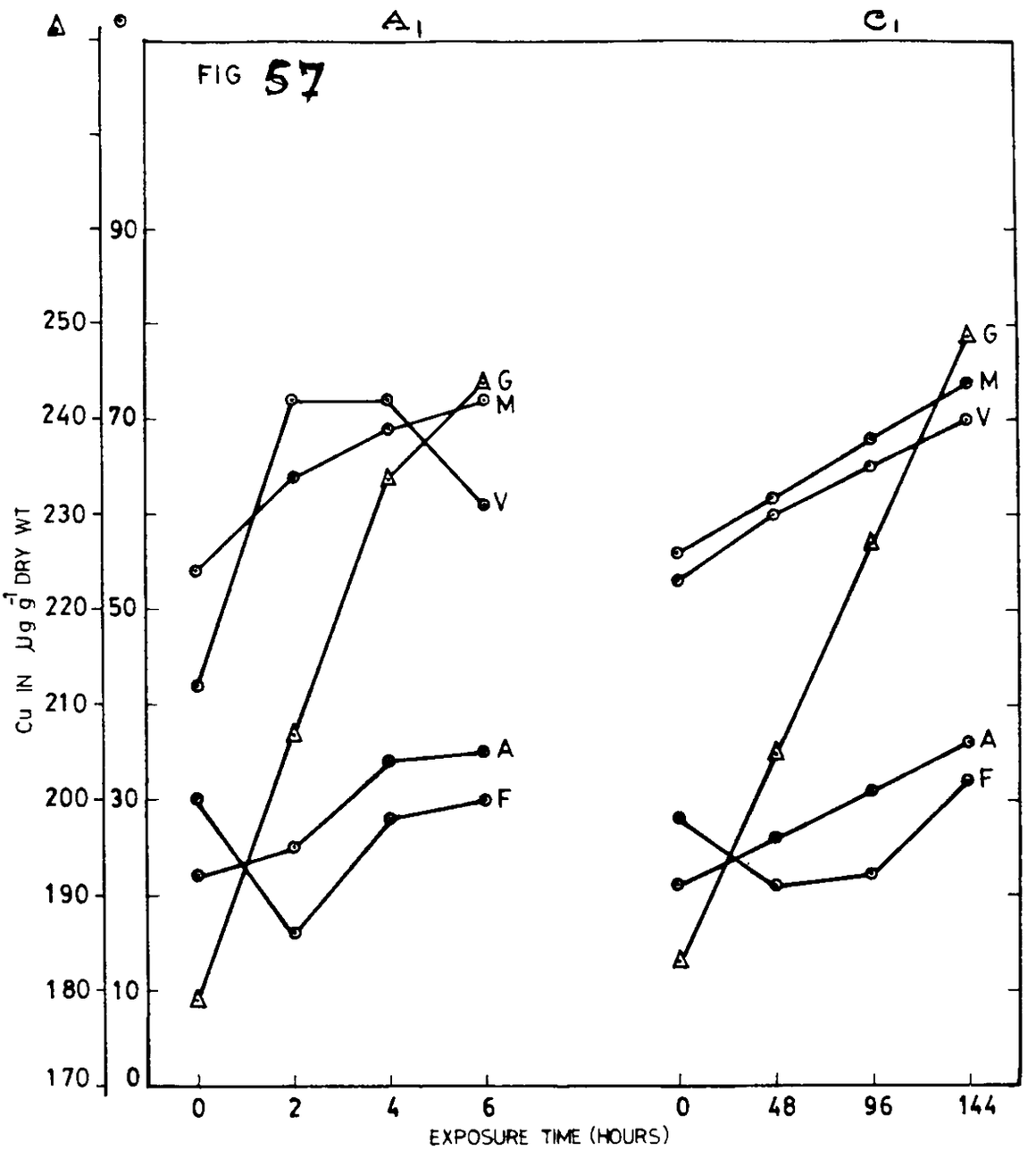


Fig.57 Concentration of Cu (µg/g dry wt.) in S. scripta exposed to 2.0 ppm of Cu.
($S = 30 \times 10^{-3}$; Size = 3-3.5cm)

A_1 = Actual; C_1 = Calculated.

M = Mantle; V = Viscera; G = Gill; F = Foot; A = Aductor muscle

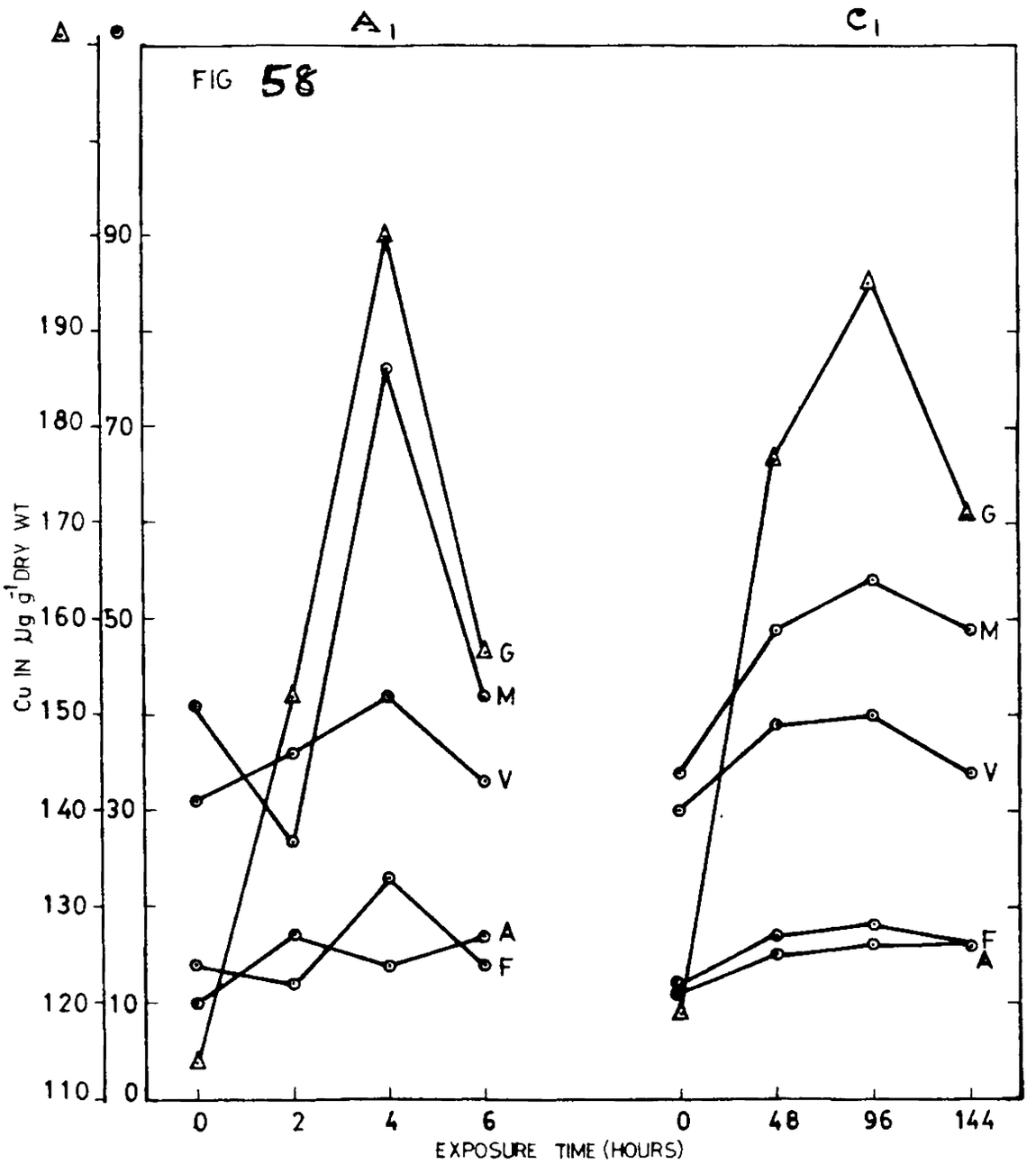


Fig.58 Concentration of Cu (µg/g dry wt.) in S. scripta exposed to 2.0 ppm of Cu.

(S = 30 x 10^{-3}; Size = 4-4.5cm)

A_1 = Actual; C_1 = Calculated.

M = Mantle; V = Viscera; G = Gill; F = Foot; A = Aductor muscle

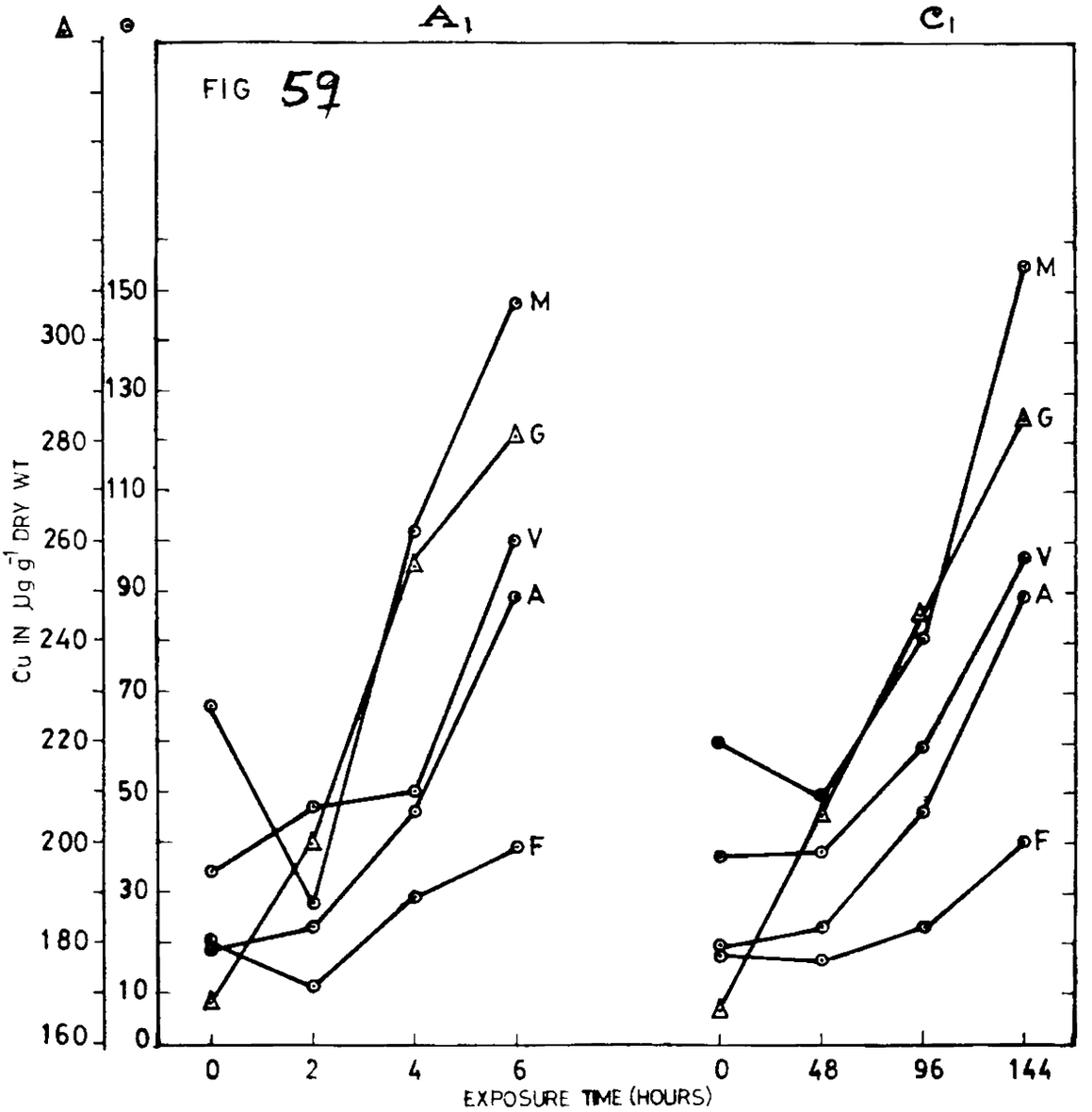


Fig.59 Concentration of Cu (µg/g dry wt.) in S. scripta exposed to 0.05 ppm of Cu.
($S_o = 20 \times 10^{-3}$; Size = 2-2.5cm)

A_1 = Actual; C_1 = Calculated.

M = Mantle; V = Viscera; G = Gill; F = Foot;
A = Aductor muscle

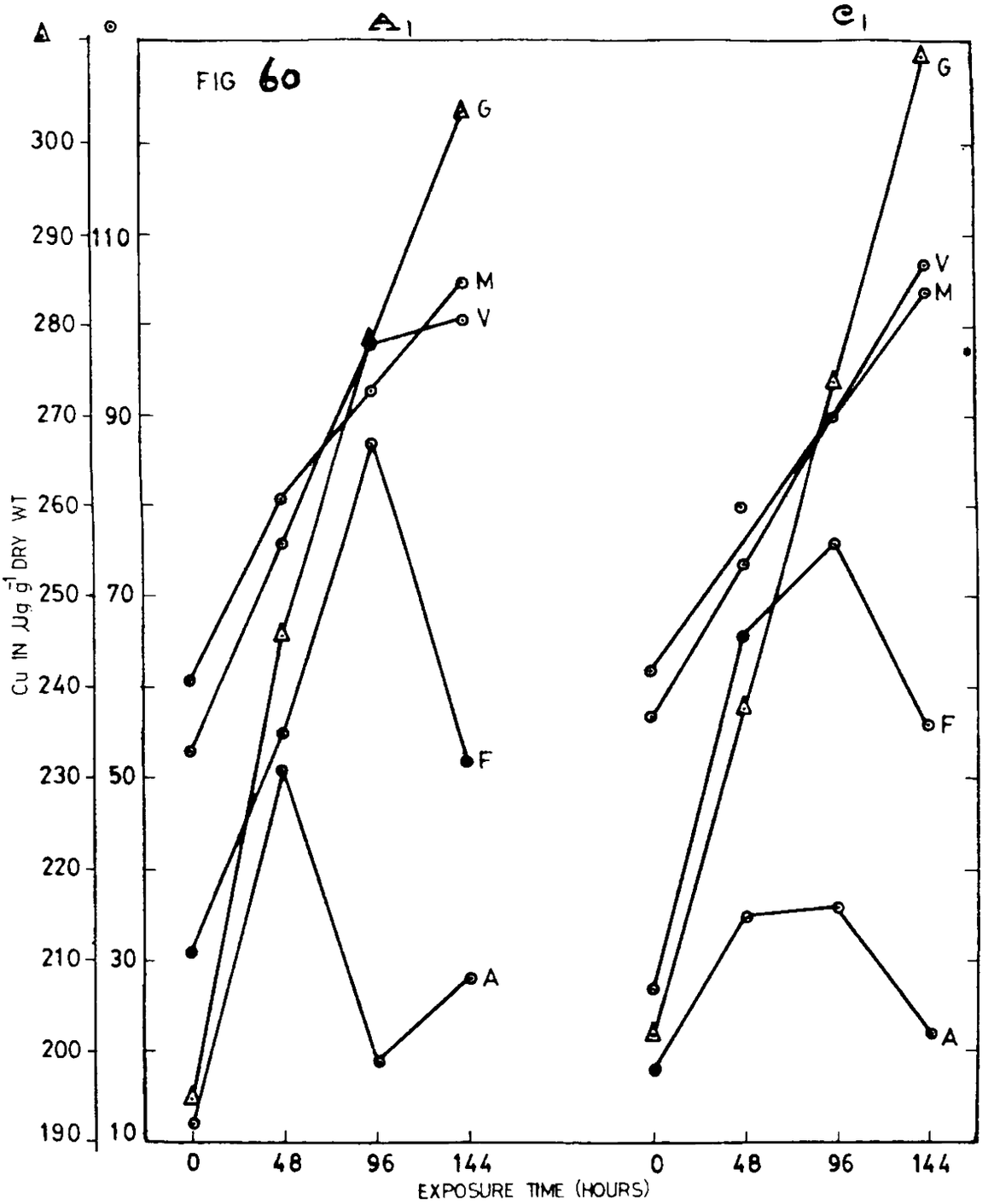


Fig.60 Concentration of Cu (µg/g dry wt.) in *S. scripta* exposed to 0.05 ppm of Cu.

($S = 20 \times 10^{-3}$; Size = 3-3.5cm)

A_1 = Actual; C_1 = Calculated.

M = Mantle; V = Viscera; G = Gill; F = Foot; A = Aductor muscle

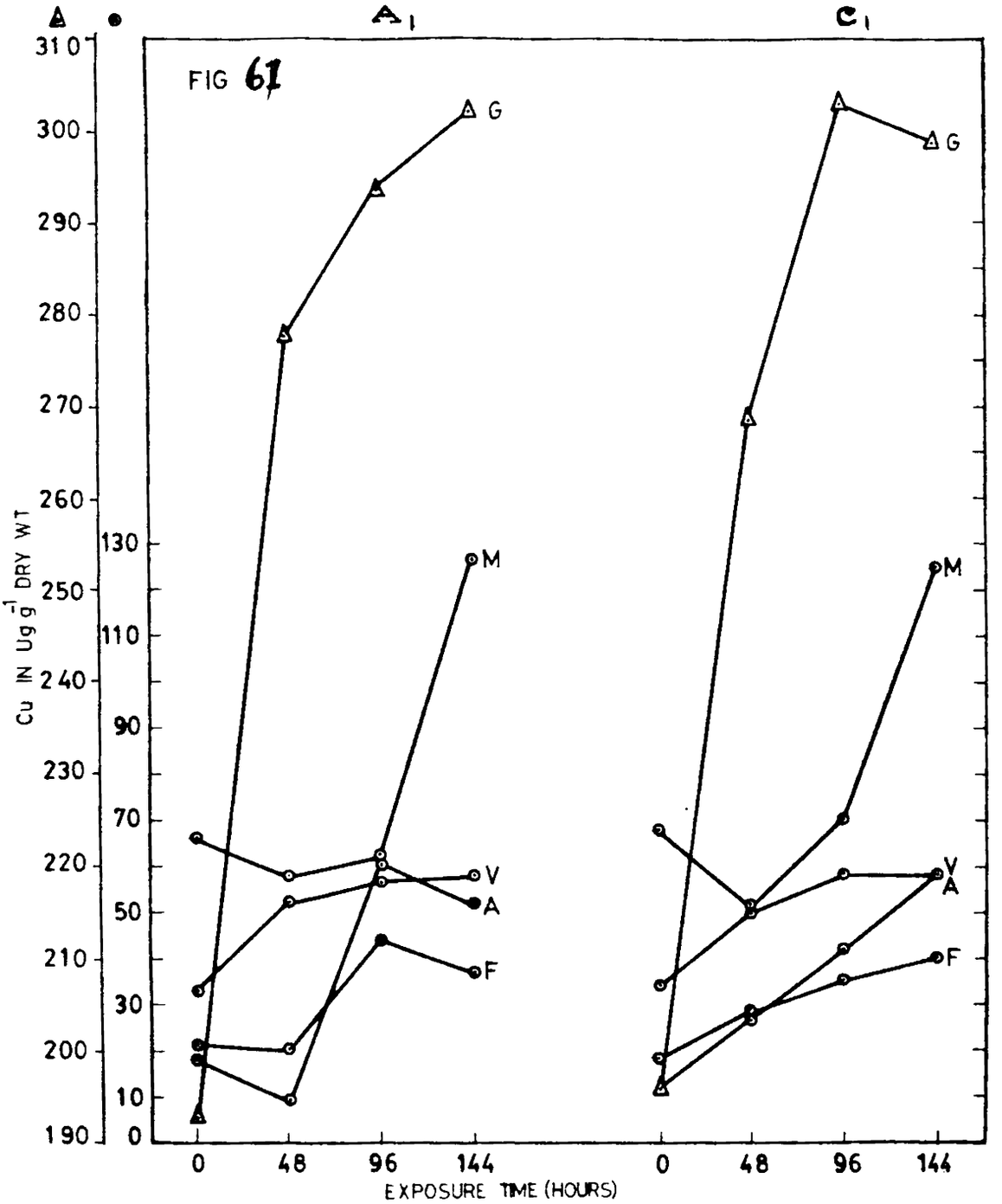


Fig.61 Concentration of Cu (µg/g dry wt.) in S. scripta exposed to 0.05 ppm of Cu.

(S = 20 x 10^{-3}; Size = 4-4.5cm)

A_1 = Actual; C_1 = Calculated.

M = Mantle; V = Viscera; G = Gill; F = Foot;
A = Aductor muscle

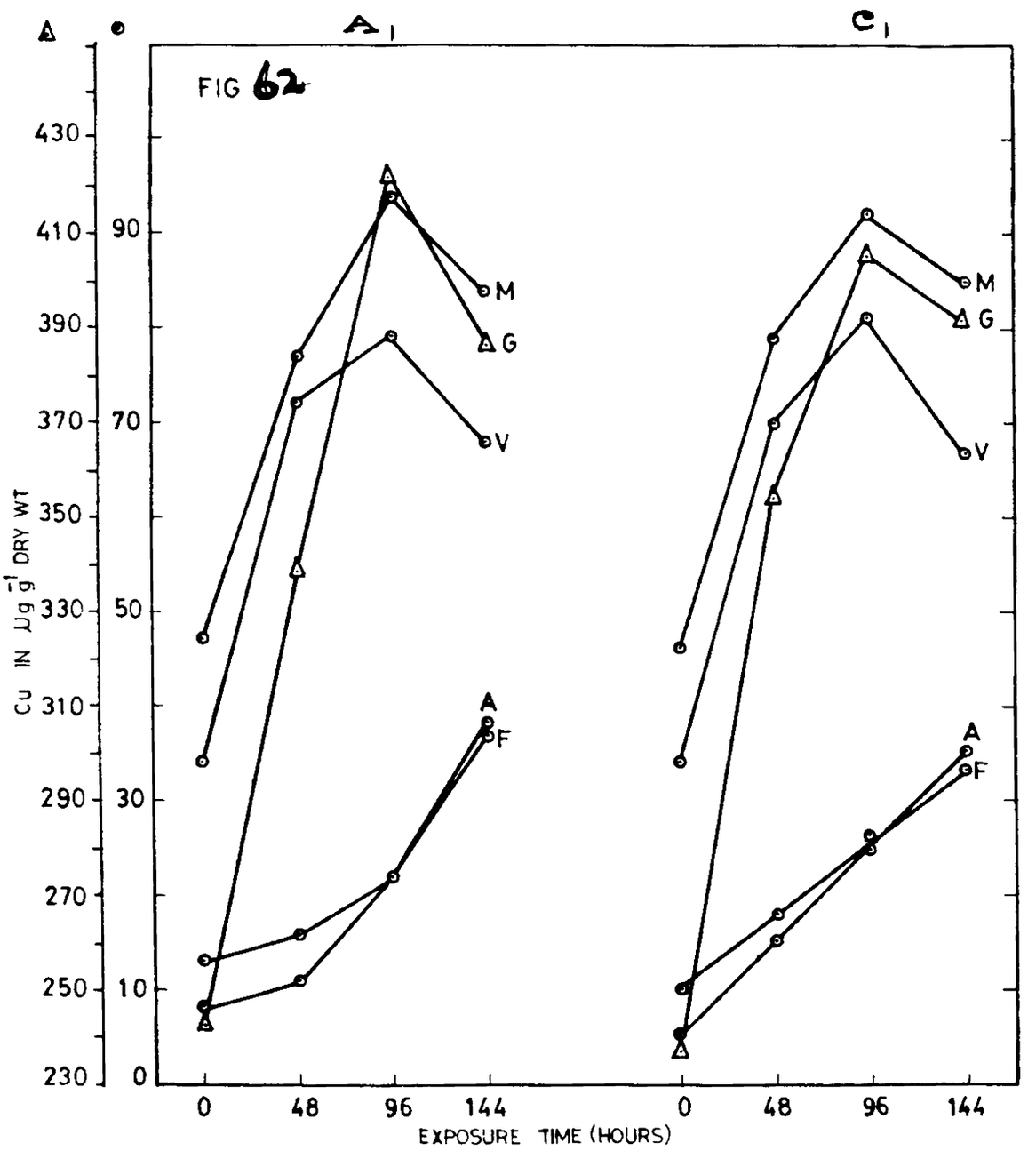


Fig.62 Concentration of Cu (µg/g dry wt.) in S. scripta exposed to 1.0 ppm of Cu_{-3}
(S = 20 x 10 ; Size = 2-2.5cm)

A_1 = Actual; C_1 = Calculated.
M = Mantle; V = Viscera; G = Gill; F = Foot;
A = Aductor muscle

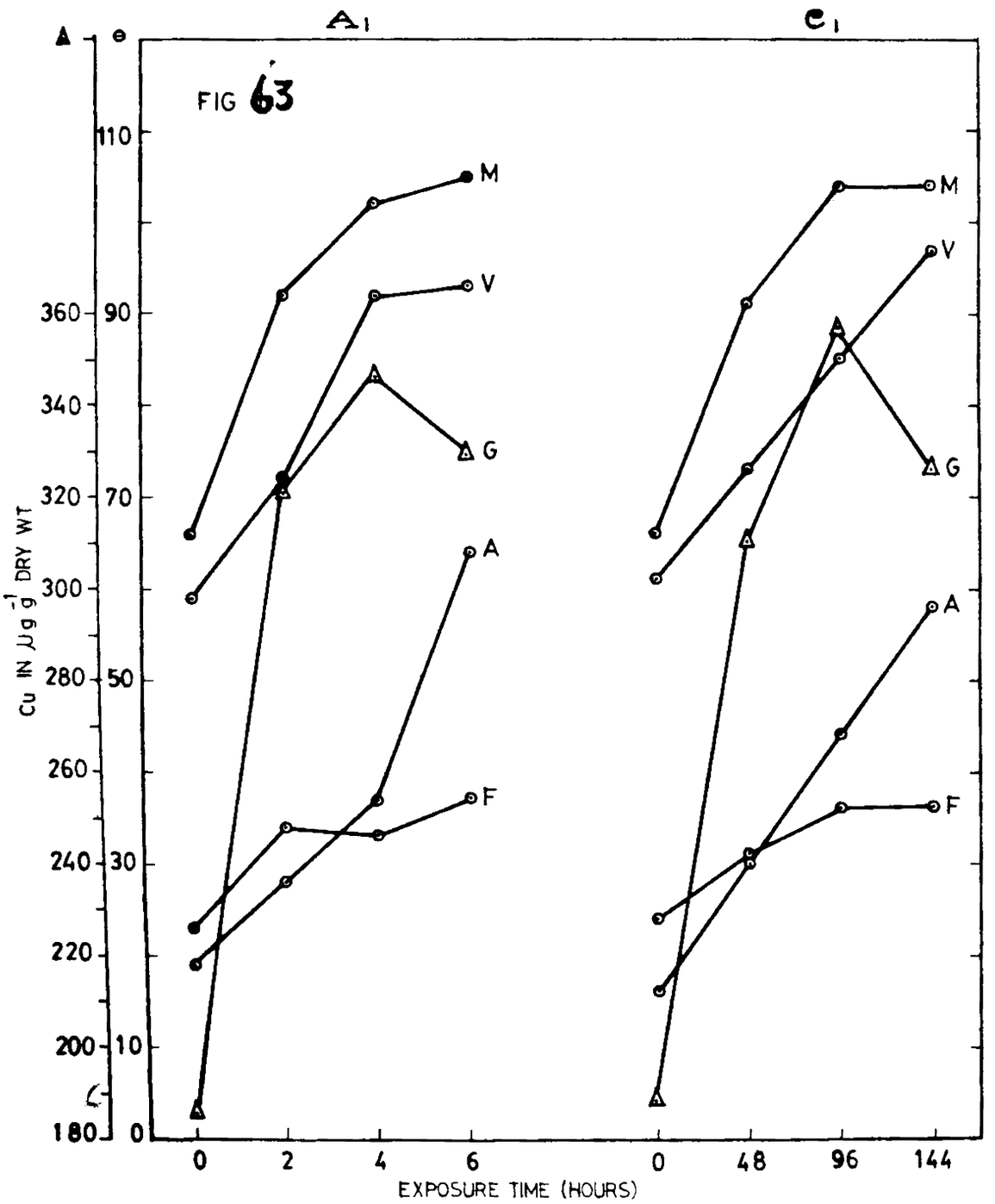


Fig.63 Concentration of Cu (µg/g dry wt.) in S. scripta exposed to 1.0 ppm of Cu.
($S = 20 \times 10^{-3}$; Size = 3-3.5cm)

A_1 = Actual; C_1 = Calculated.
M = Mantle; V = Viscera; G = Gill; F = Foot;
A = Aductor muscle

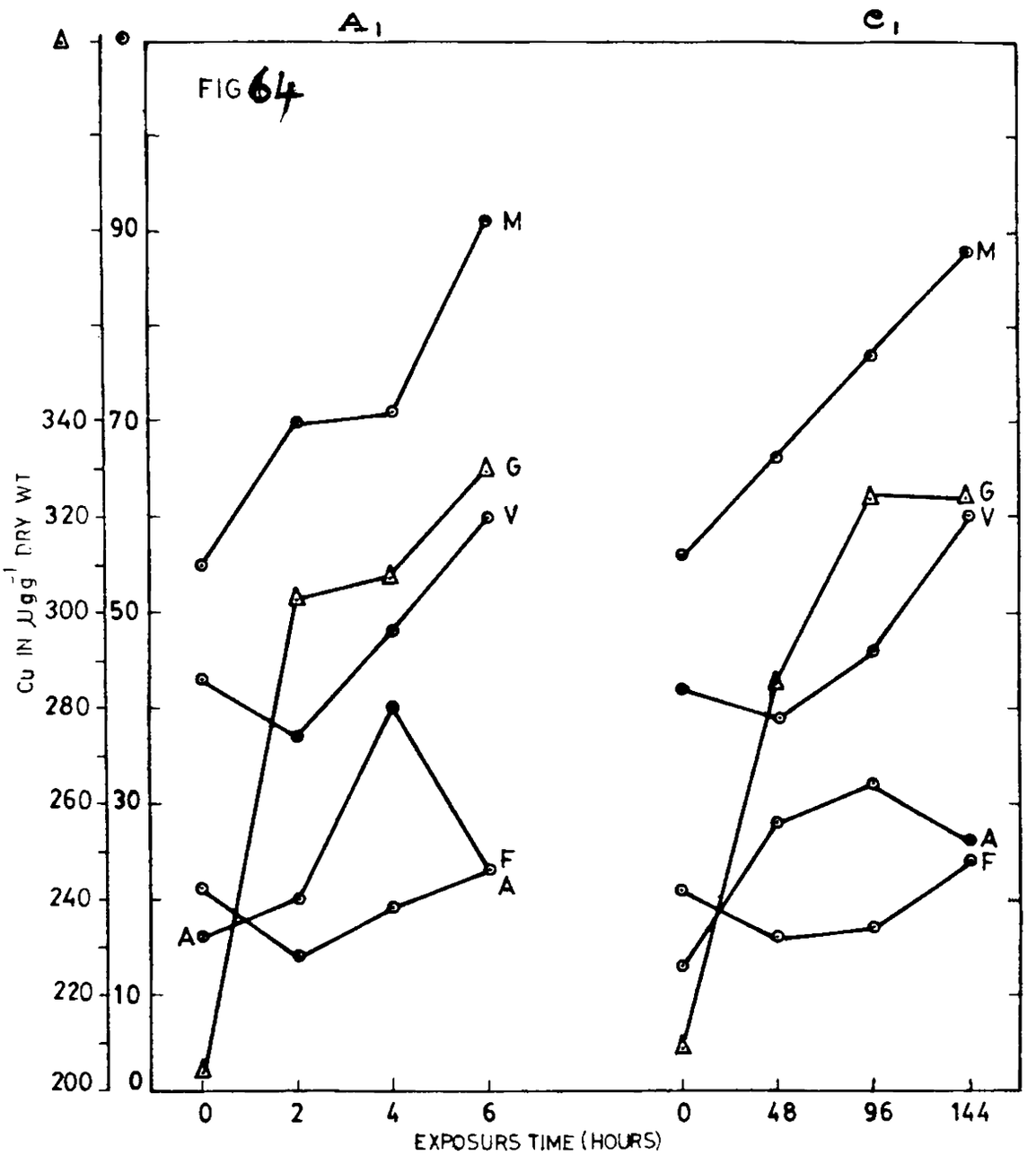


Fig.64 Concentration of Cu (µg/g dry wt.) in S. scripta exposed to 1.0 ppm of Cu.
($\bar{S}$ = 20 x 10^{-3}; Size = 4-4.5cm)

A_1 = Actual; C_1 = Calculated.
M = Mantle; V = Viscera; G = Gill; F = Foot;
A = Aductor muscle

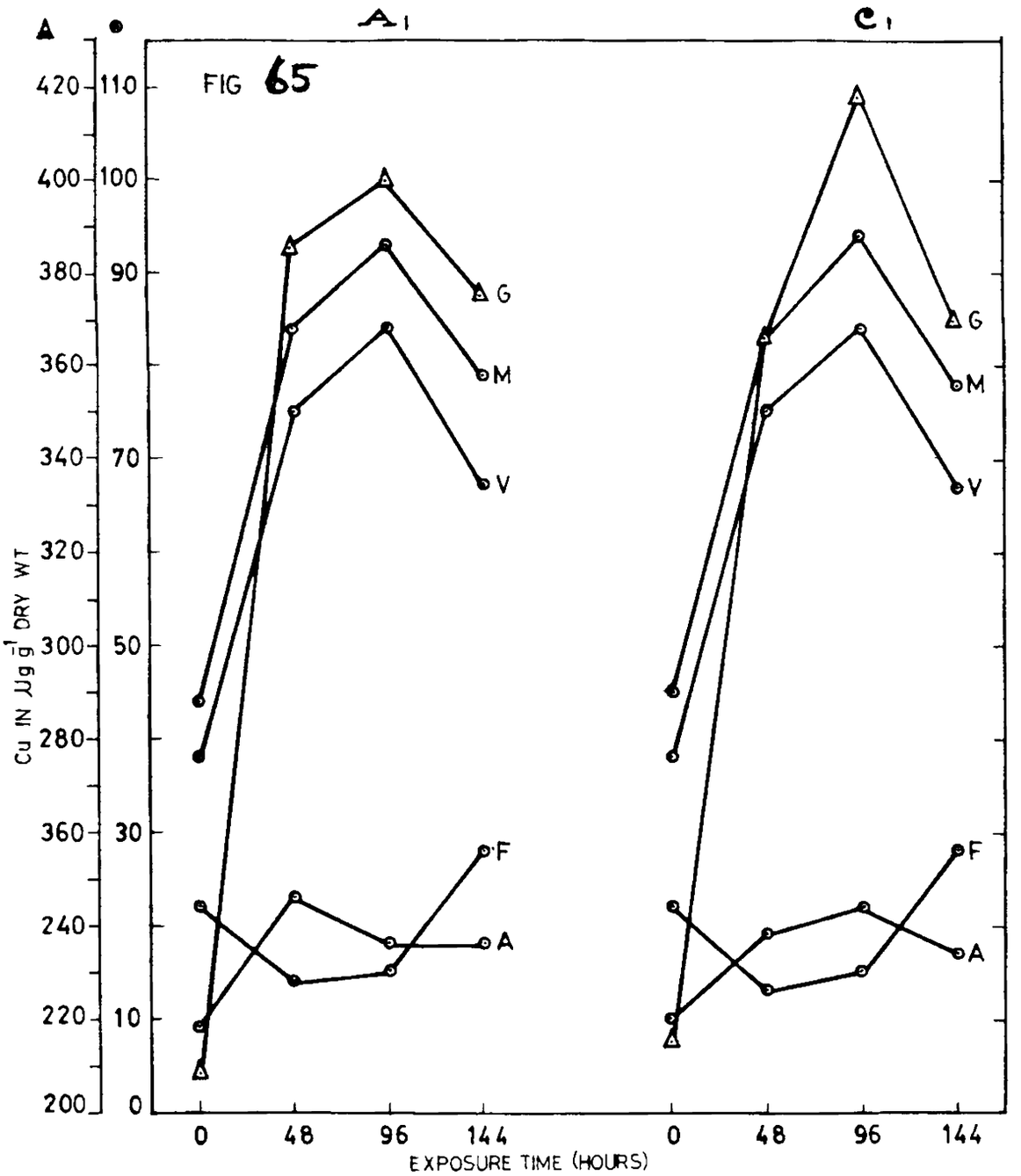


Fig.65 Concentration of Cu (µg/g dry wt.) in S. scripta exposed to 2.0 ppm of Cu. ($S = 20 \times 10^{-3}$; Size = 2-2.5cm)

A_1 = Actual; C_1 = Calculated.
M = Mantle; V = Viscera; G = Gill; F = Foot;
A = Aductor muscle

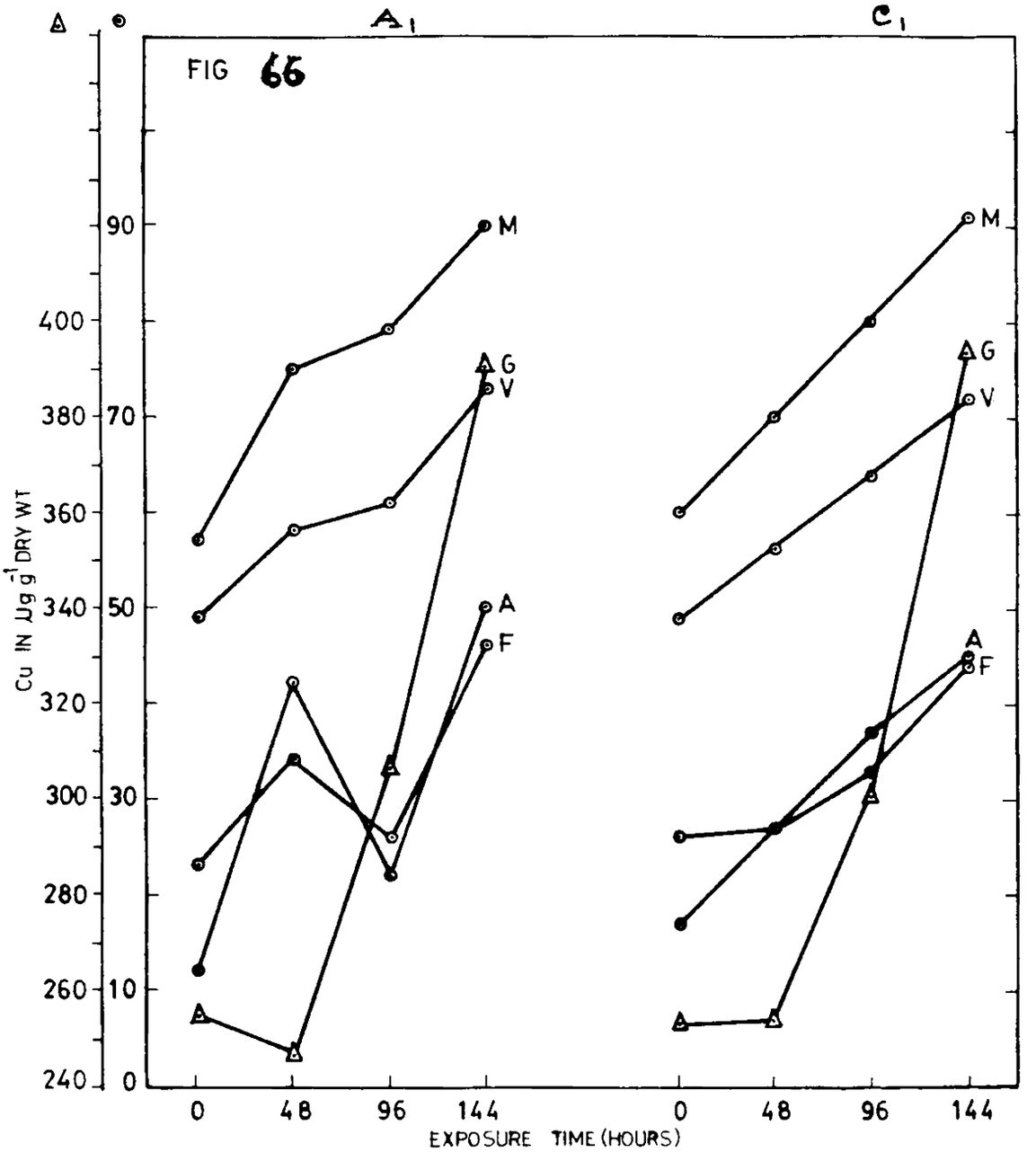


Fig.66 Concentration of Cu (µg/g dry wt.) in S. scripta exposed to 2.0 ppm of Cu.
(S = 20 x 10^{-3} ; Size = 3-3.5cm)

A_1 = Actual; C_1 = Calculated.
M = Mantle; V = Viscera; G = Gill; F = Foot;
A = Aductor muscle

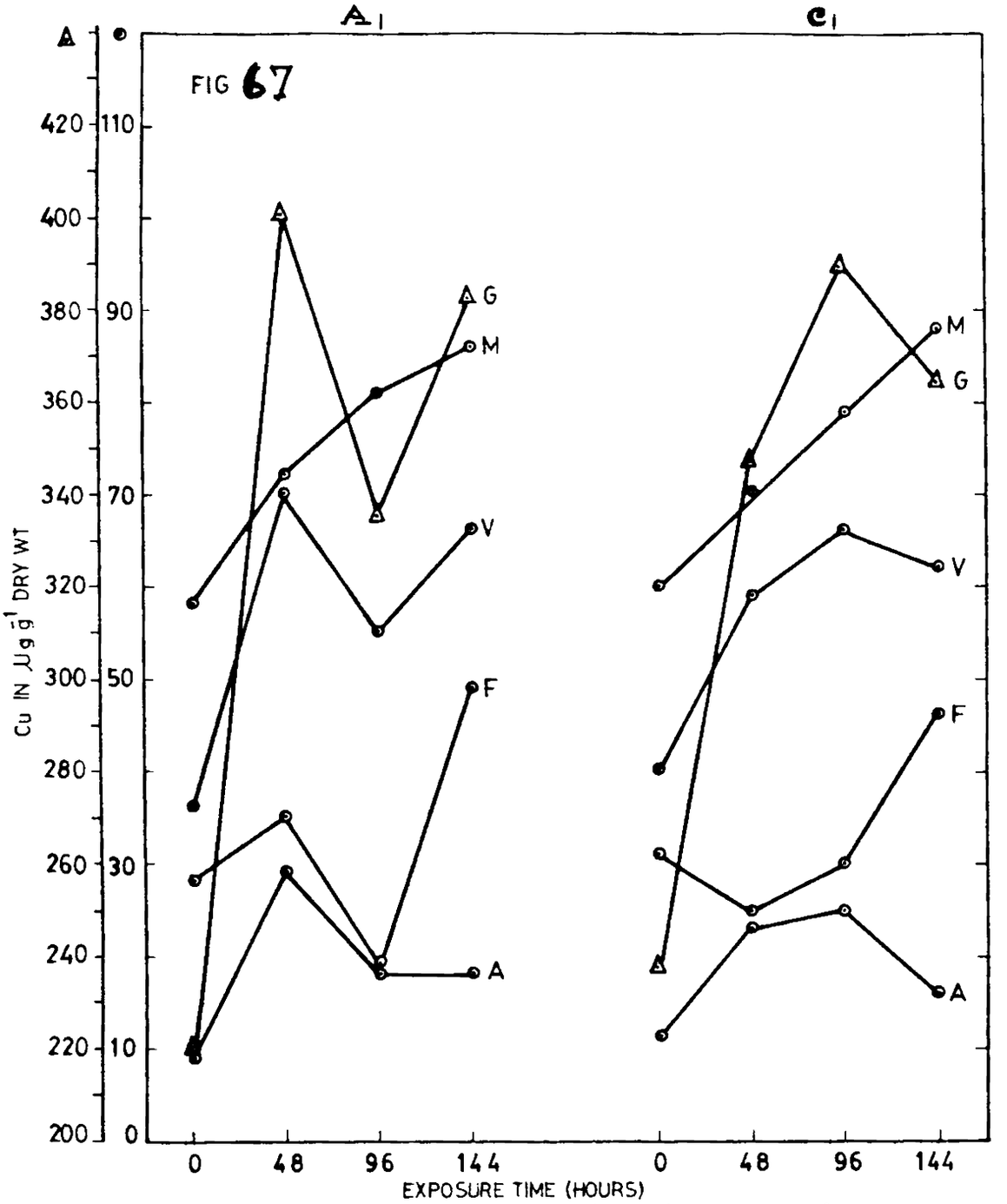


Fig.67 Concentration of Cu (µg/g dry wt.) in *S. scripta* exposed to 2.0 ppm of Cu.
($S = 20 \times 10^{-3}$; Size = 4-4.5cm)

A_1 = Actual; C_1 = Calculated.
M = Mantle; V = Viscera; G = Gill; F = Foot;
A = Aductor muscle

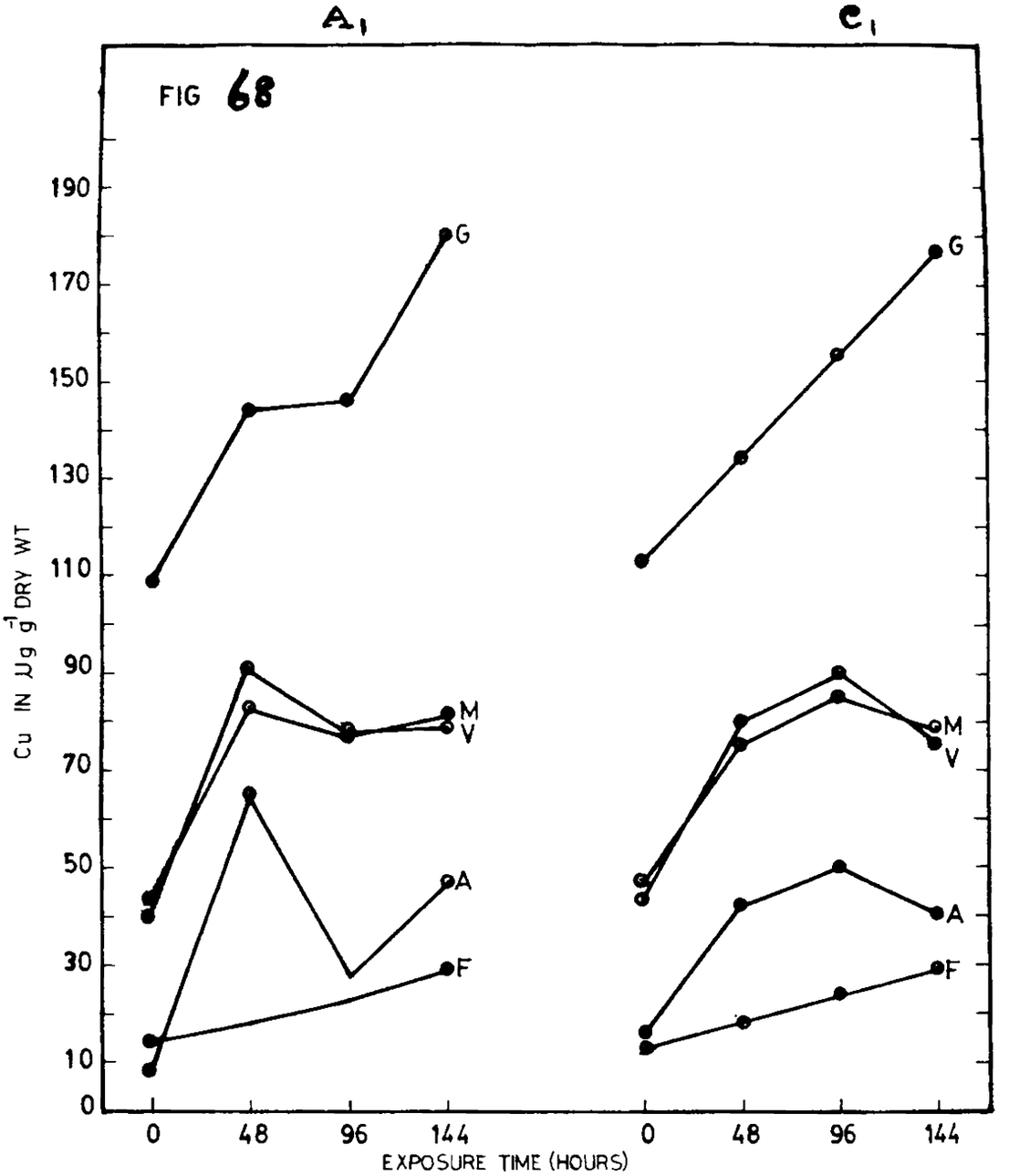


Fig.68 Concentration of Cu (µg/g dry wt.) in S. scripta exposed to 0.05ppm of Cu.
(S = 10×10^{-3}; Size = 2-2.5cm)

A_1 = Actual; C_1 = Calculated.
M = Mantle; V = Viscera; G = Gill; F = Foot;
A = Aductor muscle

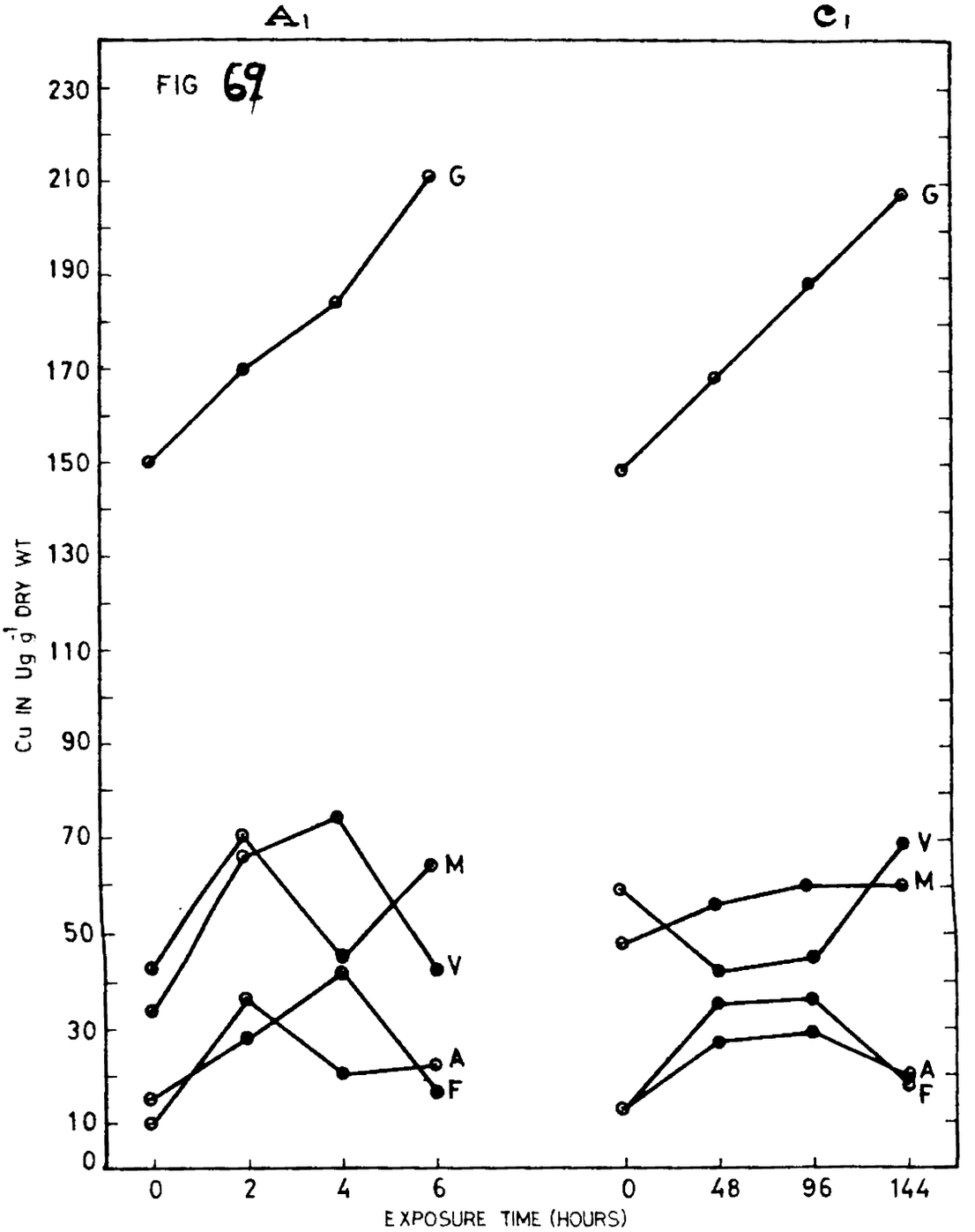


Fig.69 Concentration of Cu (µg/g dry wt.) in S. scripta exposed to 0.05 ppm of Cu.
($S = 10 \times 10^{-3}$; Size = 3-3.5cm)

A_1 = Actual; C_1 = Calculated.
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A = Aductor muscle

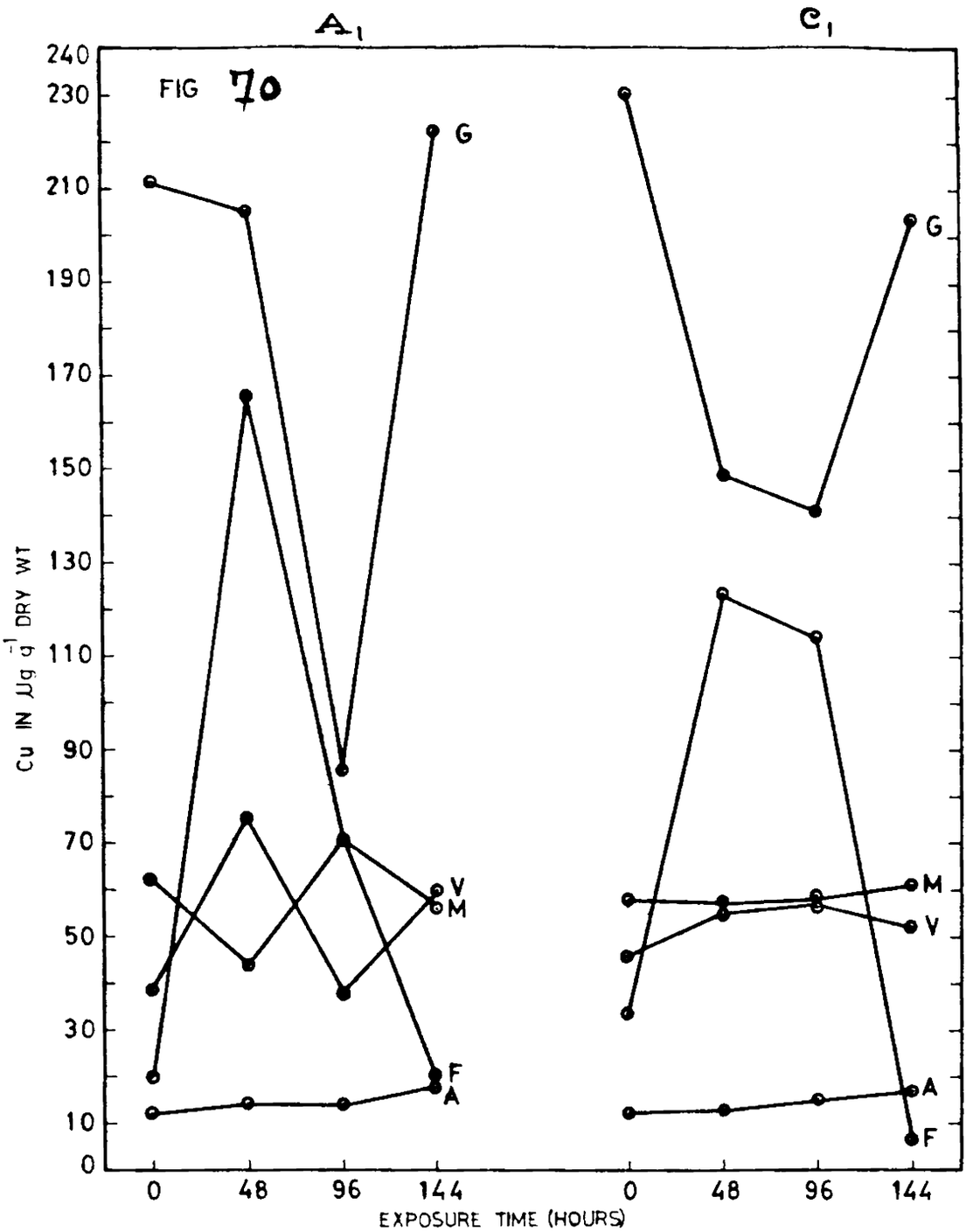

Fig.70 Concentration of Cu (µg/g dry wt.) in S. scripta exposed to 0.05 ppm of Cu.
($S = 10 \times 10^{-3}$; Size = 4-4.5cm)

A_1 = Actual; C_1 = Calculated.
M = Mantle; V = Viscera; G = Gill; F = Foot;
A = Aductor muscle

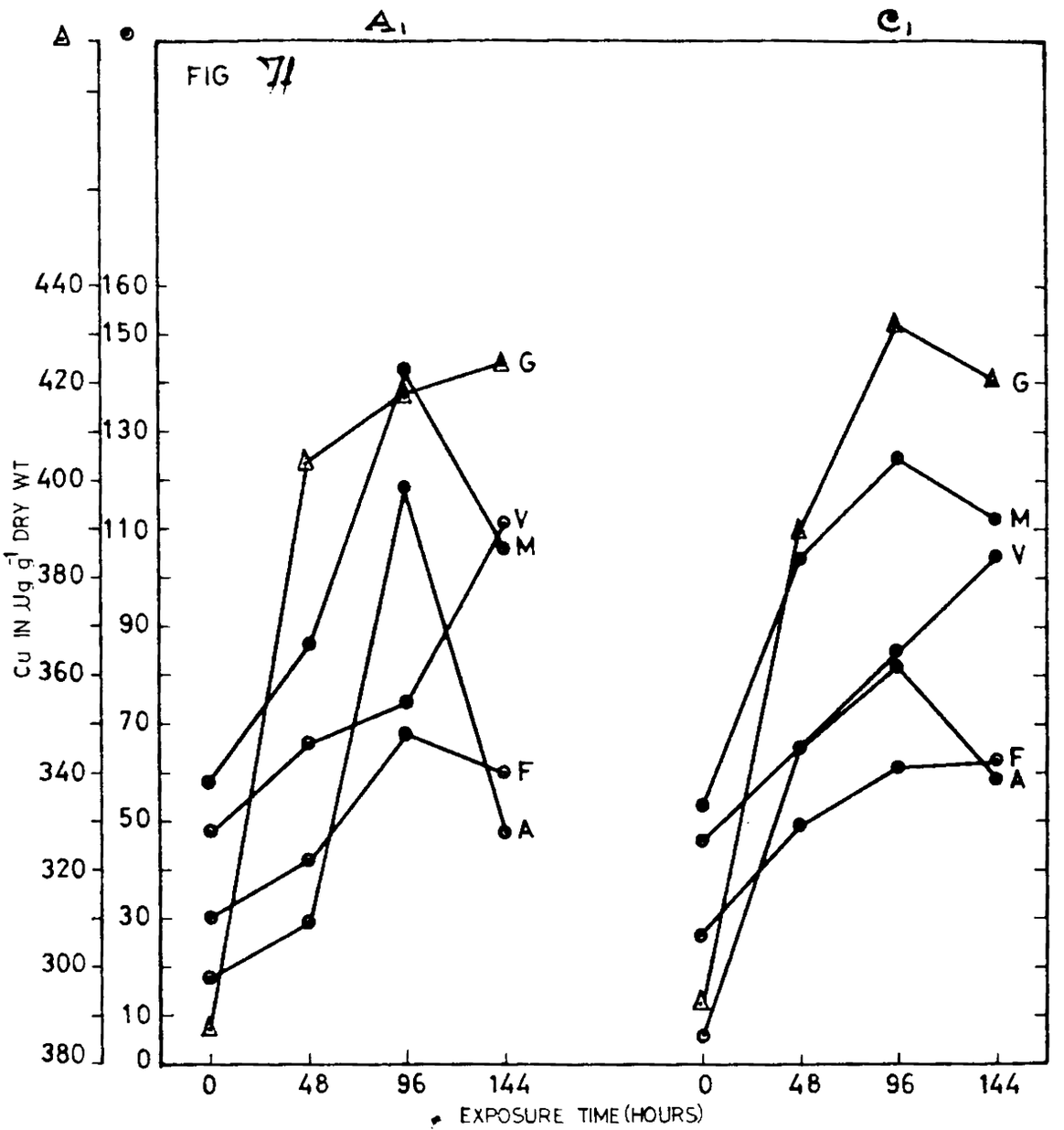

Fig.71 Concentration of Cu (µg/g dry wt.) in S. scripta exposed to 1 ppm of Cu. ($S = 10 \times 10^{-3}$; Size = 2-2.5cm)

A_1 = Actual; C_1 = Calculated.

M = Mantle; V = Viscera; G = Gill; F = Foot; A = Aductor muscle

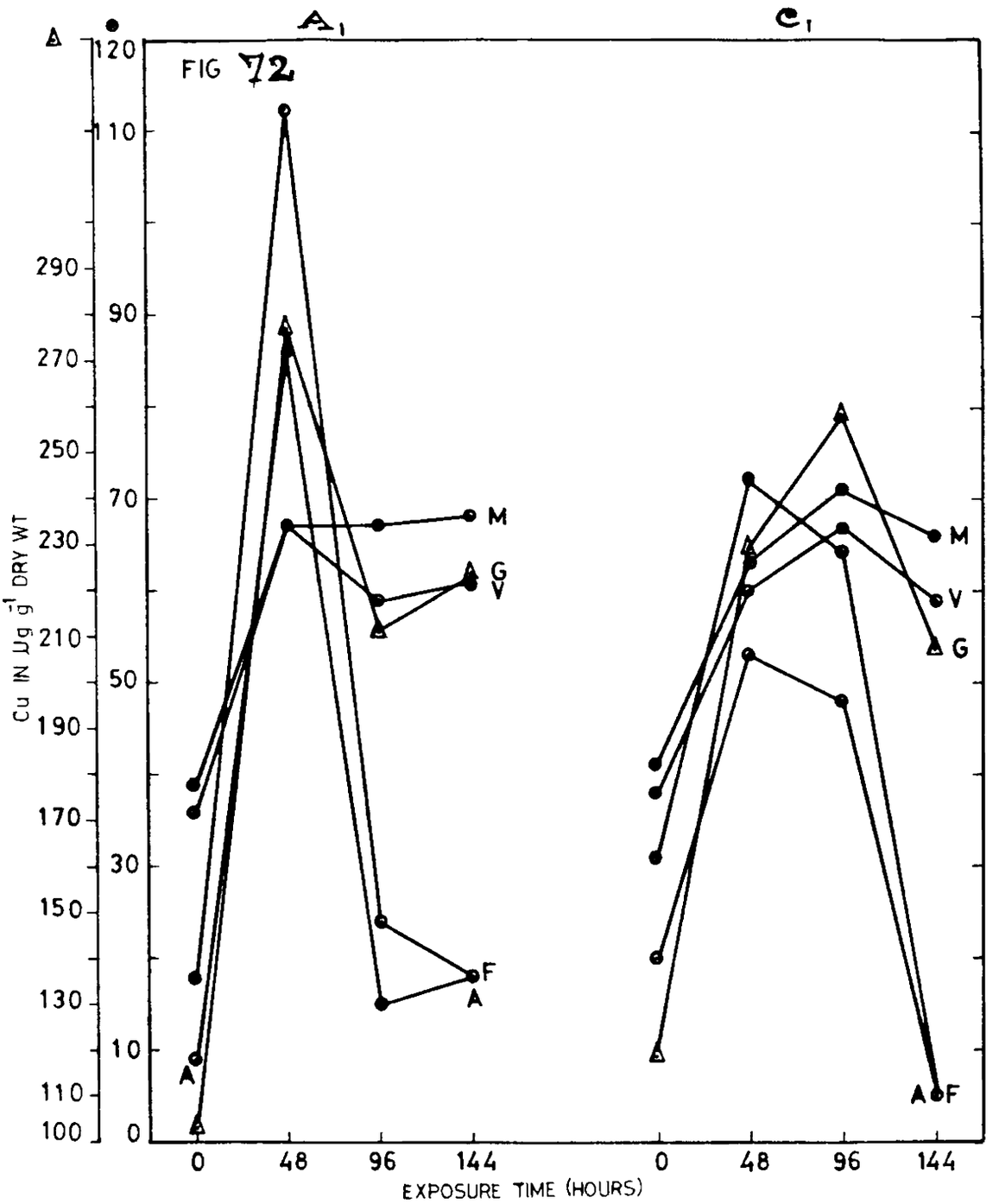

Fig.72 Concentration of Cu (µg/g dry wt.) in S. scripta exposed to 1 ppm of Cu. ($S = 10 \times 10^{-3}$; Size = 3-3.5cm)

A_1 = Actual; C_1 = Calculated.

M = Mantle; V = Viscera; G = Gill; F = Foot; A = Aductor muscle

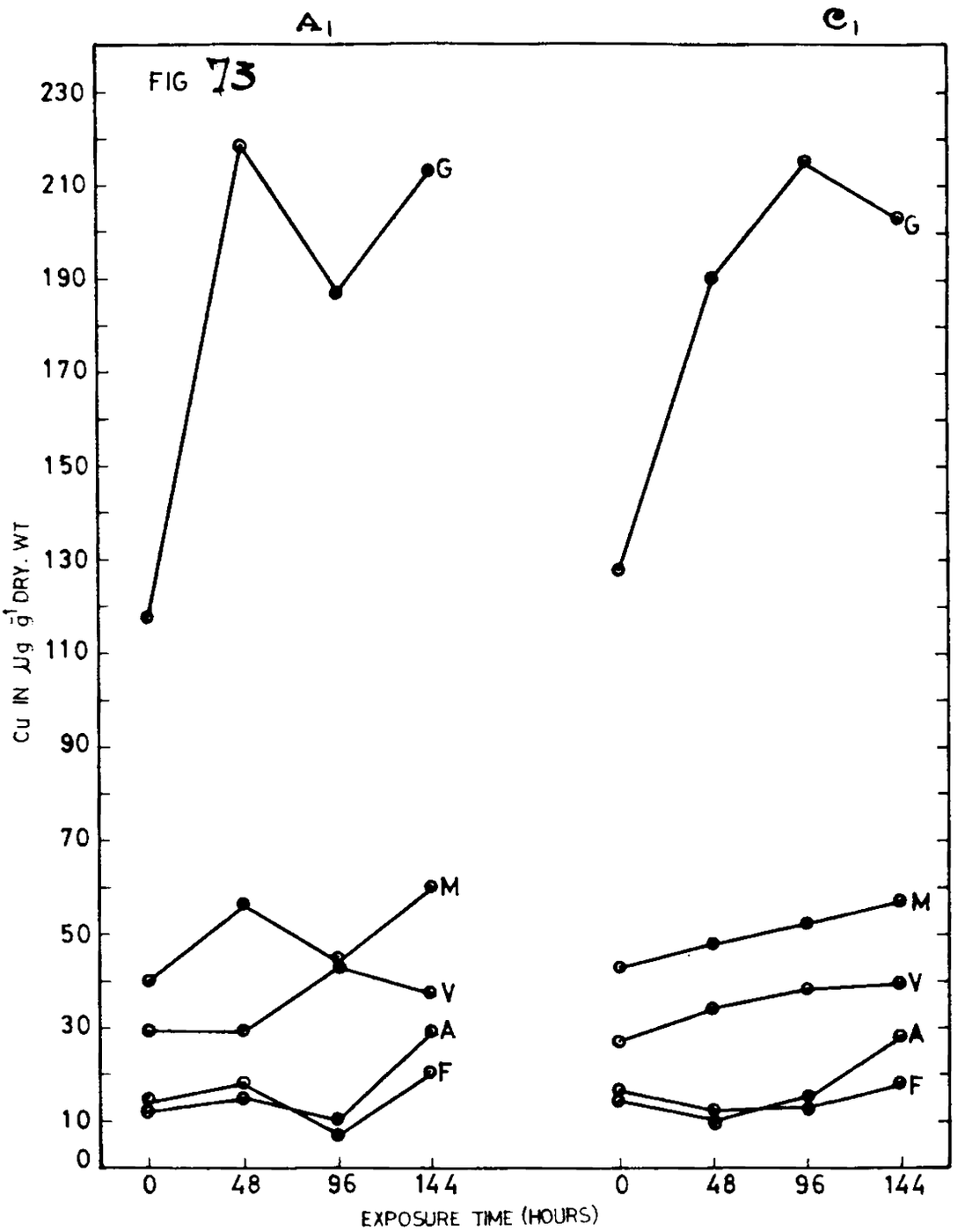

Fig.73 Concentration of Cu (µg/g dry wt.) in S. scripta exposed to 1 ppm of Cu. ($S = 10 \times 10^{-3}$; Size = 4-4.5cm)

A_1 = Actual; C_1 = Calculated.

M = Mantle; V = Viscera; G = Gill; F = Foot; A = Aductor muscle

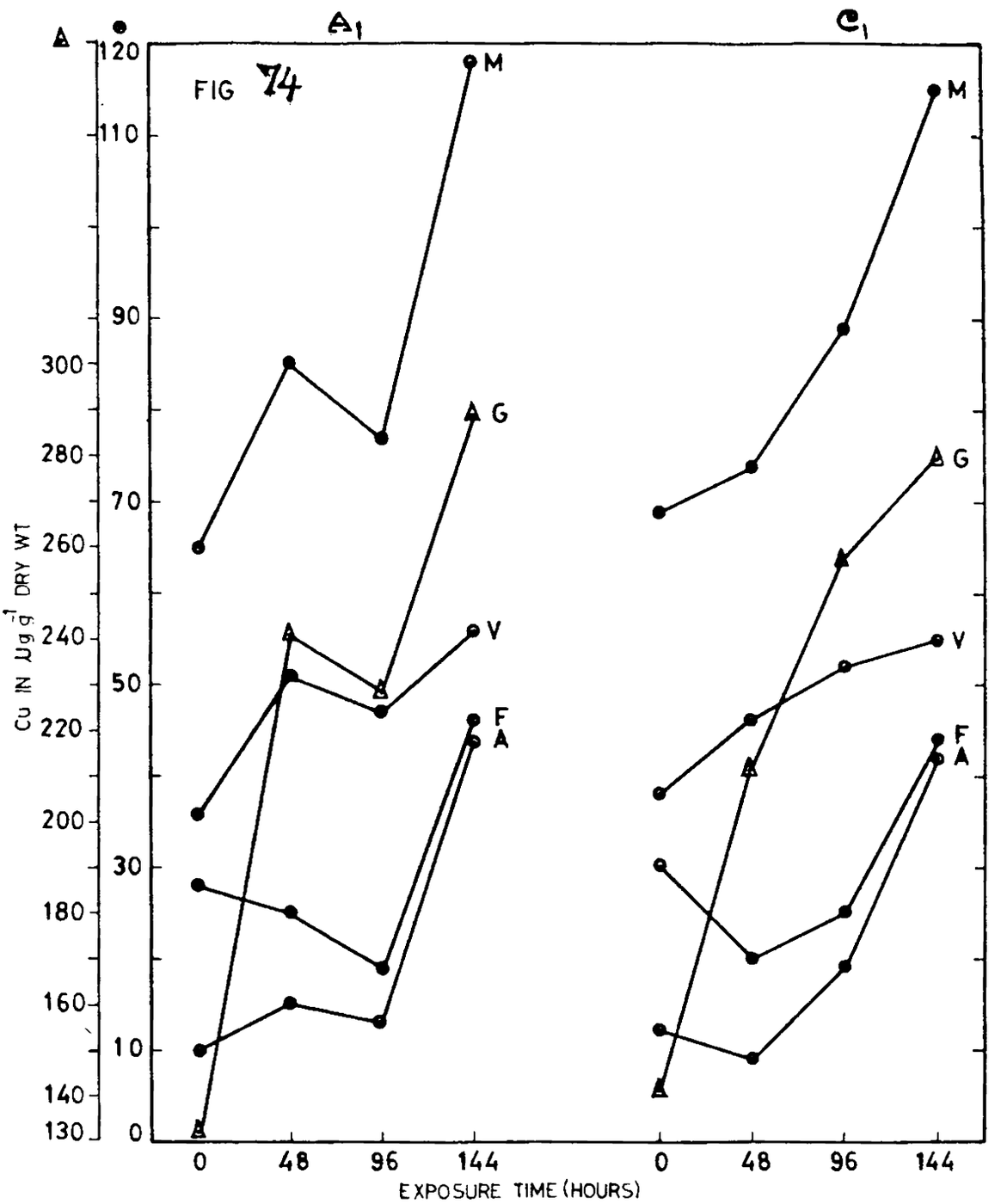

Fig.74 Concentration of Cu (µg/g dry wt.) in S. scripta exposed to 2 ppm of Cu. ($S = 10 \times 10^{-3}$; Size = 2-2.5cm)

A_1 = Actual; C_1 = Calculated.

M = Mantle; V = Viscera; G = Gill; F = Foot; A = Aductor muscle

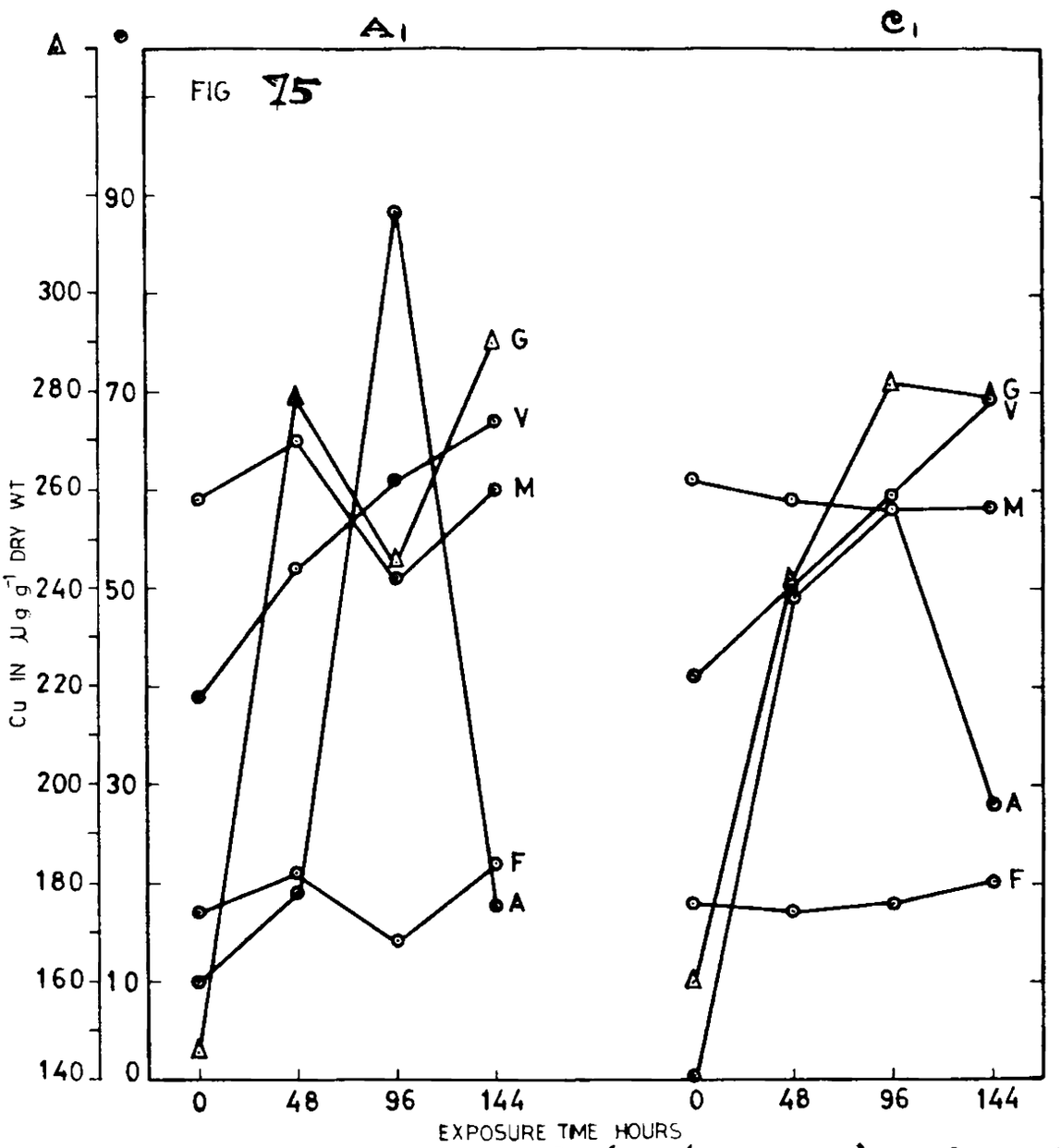

Fig.75 Concentration of Cu (µg/g dry wt.) in *S. scripta* exposed to 2 ppm of Cu. ($S = 10 \times 10^{-3}$; Size = 3-3.5cm)

A_1 = Actual; C_1 = Calculated.

M = Mantle; V = Viscera; G = Gill; F = Foot; A = Aductor muscle

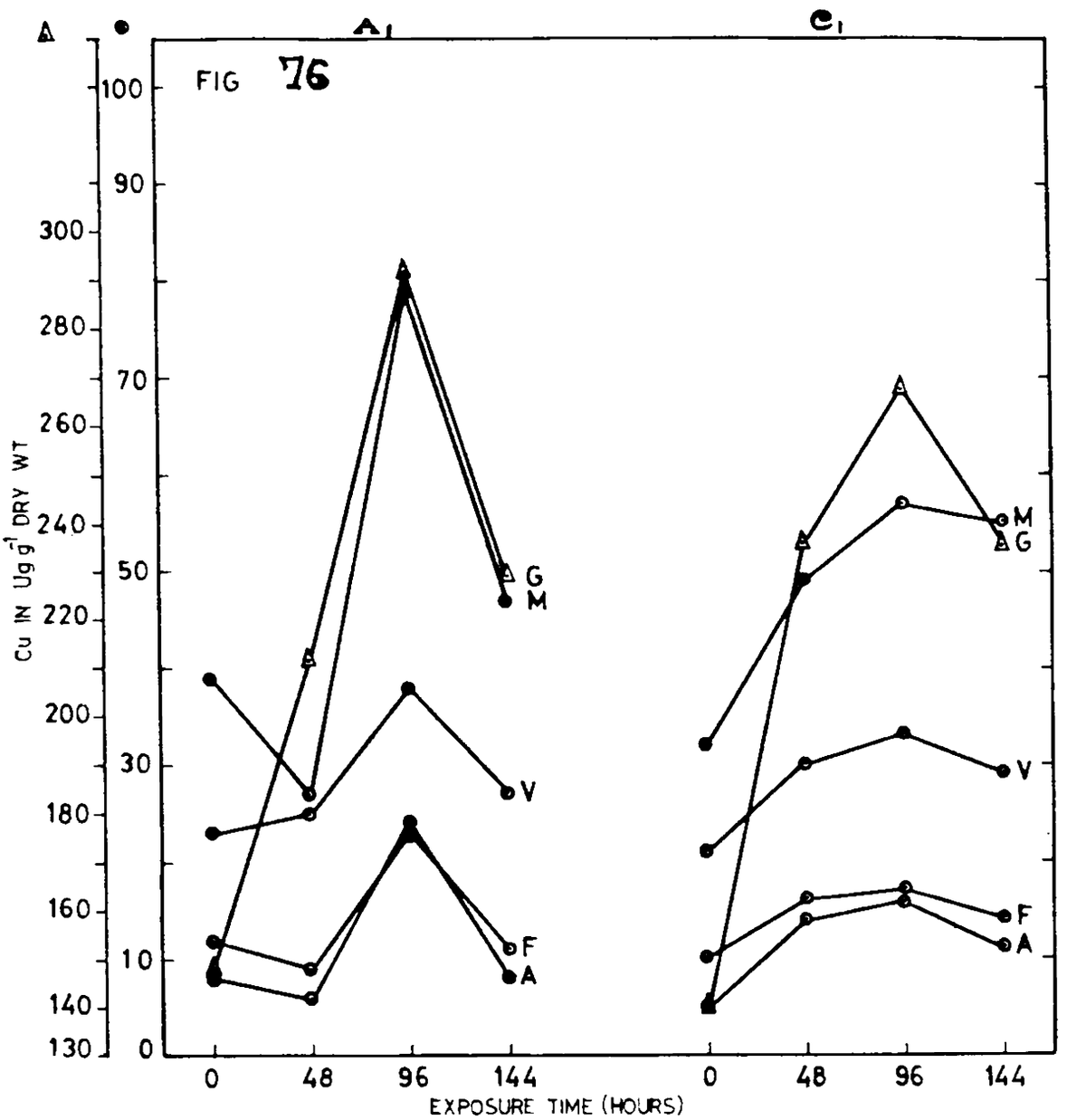

Fig.76: Concentration of Cu (µg/g dry wt.) in S.scripta exposed to 2 ppm of Cu. ($S = 10 \times 10^{-3}$; Size = 4-4.5cm)

A_1 = Actual; C_1 = Calculated.
M = Mantle; V = Viscera; G = Gill; F = Foot; A = Aductor muscle

Table - 396 Uptake pattern of Cu(II) in the various tissues of Sunetta scripta exposed to copper concentration at 30 x 10^{-3} salinity.

Tissue concentration of copper (ug g^{-1} dry wt.)

Tissue	Time of exposure to 0.05 ppm of copper					Time of exposure to 1 ppm of copper					Time of exposure to 2 ppm of copper				
	Control	2 days	4 days	6 days	C.F.at 6 days	Control	2 days	4 days	6 days	C.F.at 6 days	Control	2 days	4 days	6 days	C.F.at 6 days
20-25 mm															
Gills	197.15	212.28	227.41	242.54	4850.8	169.92	212.22	234.57	236.96	236.96	104.14	198.54	221.94	174.34	87.17
Mantle	40.60	55.06	69.52	83.99	1679.8	55.52	61.40	69.05	78.47	78.47	52.64	91.27	98.90	75.55	37.78
Viscera	40.48	52.40	64.32	76.24	1524.8	36.78	51.20	58.06	57.35	57.35	42.07	48.69	52.54	53.63	26.82
Foot	13.31	23.76	34.21	44.67	893.4	12.53	16.88	18.68	17.95	17.95	20.99	13.54	13.99	22.33	11.17
Adductor muscle	9.24	20.87	32.49	44.12	882.4	9.10	15.52	18.61	18.35	18.35	21.89	27.77	31.31	32.52	16.26
30-35 mm															
Gills	112.21	125.84	139.47	153.09	3061.8	258.78	300.14	321.70	323.47	323.47	182.55	204.78	227.02	249.25	124.63
Mantle	44.15	57.08	70.02	82.95	1659.0	53.79	97.38	102.22	68.30	68.30	55.79	61.82	67.85	73.87	36.94
Viscera	33.18	30.62	39.22	58.99	1179.8	38.66	52.24	58.41	57.17	57.17	52.56	59.48	65.15	69.55	34.78
Foot	32.55	28.27	34.67	51.77	1035.4	22.45	37.52	36.11	18.21	18.21	28.09	21.05	22.35	32.01	16.01
Adductor muscle	21.53	49.32	61.90	59.28	1185.6	23.27	25.97	29.92	35.11	35.11	21.37	26.29	31.22	36.14	18.07
40-45 mm															
Gills	118.52	131.51	144.79	158.38	3167.6	301.46	351.94	373.13	365.03	365.03	108.64	167.24	184.75	161.19	80.60
Mantle	44.34	36.78	42.54	61.63	1232.6	52.24	72.11	80.76	78.17	78.17	33.87	49.32	54.31	48.84	24.42
Viscera	44.95	46.63	46.86	47.73	954.6	41.49	69.57	74.95	57.65	57.65	29.95	38.46	39.85	34.14	17.07
Foot	18.34	23.25	27.10	29.90	598	31.05	41.17	39.24	25.26	25.26	12.38	16.76	17.95	15.93	7.97
Adductor muscle	16.83	26.22	30.52	29.73	594.6	21.44	34.61	39.09	34.87	34.87	10.94	14.50	16.12	15.79	7.90

Table 40 Uptake pattern of Cu(II) in the various tissues of Sunetta scripta exposed to copper concentrations at 20×10^{-3} salinity.

Tissue concentration of copper (ug g^{-1} dry wt.)

Tissue	Time of exposure to 0.05 ppm of copper					Time of exposure to 1 ppm of copper					Time of exposure to 2 ppm of copper				
	Control	2 days	4 days	6 days	C.F.at 6 days	Control	2 days	4 days	6 days	C.F.at 6 days	Control	2 days	4 days	6 days	C.F.at 6 days
20-25 mm															
Gills	166.97	206.39	245.82	285.25	5705.00	237.64	354.68	406.09	391.87	391.87	215.48	367.26	418.78	370.04	185.02
Mantle	59.56	49.26	81.09	155.05	3101.00	45.92	78.86	91.88	84.98	84.98	44.74	82.61	93.73	78.1	39.05
Viscera	36.65	38.44	58.64	97.24	1944.8	34.18	69.77	80.82	67.33	67.33	38.40	74.70	84.06	66.47	33.24
Foot	18.37	16.01	23.37	40.44	808.8	10.26	17.96	25.67	33.38	33.38	21.64	13.36	15.37	27.66	13.83
Adductor muscle	18.89	22.98	46.30	88.86	1777.2	4.77	14.83	24.89	34.95	34.95	9.82	19.19	21.63	17.14	8.57
30-35 mm															
Gills	202.17	238.00	273.83	309.65	6193.00	188.97	311.39	357.30	326.70	326.70	252.74	253.73	300.70	393.66	196.83
Mantle	61.45	80.13	94.32	104.01	2080.2	66.42	90.95	103.48	104.01	104.01	59.70	70.03	80.36	90.70	45.35
Viscera	57.28	73.85	90.43	107.00	2140.00	60.72	72.87	85.01	97.16	97.16	48.72	56.31	63.90	71.49	35.75
Foot	27.34	66.08	75.60	55.91	1118.2	23.94	31.42	35.52	36.23	36.23	25.54	27.01	33.07	43.72	21.86
Adductor muscle	17.47	34.60	36.01	21.87	437.4	15.79	30.00	44.21	58.42	58.42	16.88	27.20	36.61	45.11	22.56
40-45 mm															
Gills	196.17	268.94	303.19	298.92	5978.40	208.83	286.09	324.54	324.18	324.18	238.16	347.46	389.79	365.18	182.59
Mantle	68.39	50.74	69.46	124.56	2491.20	55.56	66.20	76.84	87.47	87.47	60.12	69.52	78.92	88.31	44.16
Viscera	33.55	50.31	58.33	57.62	1152.40	41.74	39.29	45.52	60.41	60.41	39.72	58.76	66.11	61.76	30.88
Foot	18.30	28.26	35.31	39.45	789.00	20.67	15.87	16.87	23.66	23.66	30.88	24.58	29.51	45.66	22.83
Adductor muscle	12.21	26.97	42.29	58.17	1163.4	13.32	28.14	32.23	25.60	25.60	11.00	22.83	24.51	16.04	8.02

Table - 41 Uptake pattern of Cu(II) in the various tissues of Sunetta scripta exposed to copper concentrations at 10 x 10^{-3} salinity.

Tissue concentration of copper (ug g^{-1} dry wt.)

Tissue	Time of exposure to .05 ppm of copper					Time of exposure to 1 ppm of copper					Time of exposure to 2 ppm of copper				
20-25 mm	Control	2 days	4 days	6 days	C.F.at 6 days	Control	2 days	4 days	6 days	C.F.at 6 days	Control	2 days	4 days	6 days	C.F.at 6 days
Gills	112.69	134.16	155.62	177.09	3541.8	292.82	389.75	432.04	419.70	419.70	141.70	211.62	257.64	279.75	139.88
Mantle	46.09	74.48	85.17	78.16	1563.2	52.48	103.75	123.62	112.07	112.07	68.86	73.57	88.78	114.50	57.25
Viscera	43.78	79.74	90.06	74.73	1494.6	45.52	65.02	84.52	104.02	104.02	37.46	46.22	51.95	54.65	27.33
Foot	13.26	18.46	23.65	28.85	577.0	27.42	49.25	60.83	62.15	62.15	30.22	19.75	24.48	44.43	22.22
Adductor muscle	15.47	41.77	49.92	39.92	798.4	6.05	64.66	82.42	59.33	59.33	12.18	8.68	18.49	41.61	20.81
30-35 mm															
Gills	149.25	168.86	188.46	208.07	4161.4	120.04	229.16	258.27	207.35	207.35	159.65	242.26	281.95	278.71	139.36
Mantle	47.83	55.74	59.62	59.49	1189.8	40.60	62.85	71.40	66.26	66.26	61.21	58.80	57.55	57.46	28.73
Viscera	59.36	41.63	44.98	69.41	1388.2	38.35	59.78	66.54	58.66	58.66	40.55	49.92	59.29	68.67	34.33
Foot	13.08	34.47	36.08	17.91	358.2	31.36	72.39	63.64	5.11	5.11	18.07	16.97	17.67	20.18	10.09
Adductor muscle	12.78	26.99	28.93	18.61	372.2	20.26	52.77	48.12	6.29	6.29	0.269	48.71	57.99	28.10	14.05
40-45 mm															
Gills	229.77	149.31	140.5	203.32	4066.4	127.69	189.60	214.78	203.20	203.20	139.94	236.23	268.10	235.53	117.77
Mantle	57.59	56.72	57.87	61.03	1220.6	42.70	47.48	52.30	57.15	57.15	31.50	49.35	57.07	54.67	27.33
Viscera	46.08	55.41	57.38	51.99	1039.8	27.13	33.98	37.88	38.83	38.83	21.12	30.09	32.68	28.91	14.46
Foot	34.38	123.43	114.02	6.148	122.96	15.79	12.11	12.94	18.29	18.29	9.87	15.49	16.68	13.46	6.73
Adductor muscle	12.20	12.80	14.45	17.14	342.8	13.55	9.96	14.68	27.69	27.69	5.44	13.92	15.72	10.82	5.41

of different tissues. The concentration factors ranged from 117.77 (gills) to 5.41 (aductor muscle). The ranking followed the order gills > mantle > viscera > foot > aductor muscle. All the tissues had curve-linear pattern of uptake (Fig.76).

6.4 DISCUSSION

In the copper bioaccumulation studies on *Sunetta scripta*, it is seen that all the different tissues studied accumulated copper, but with different magnitudes. The gill tissue had considerably very high concentrations of the metal when compared to others. The efficiency of accumulation was observed to be highest with gills at all times. Nambisan *et al* (1977) had observed the highest accumulation of copper in the gill tissues of the bivalve *Meretrix casta*. Lakshmanan & Nambisan (1979) while investigating the accumulation of mercury by the mussel *Perna viridis* found that the gills were the major site of mercury accumulation. The higher accumulation of metals

in the gills may be attributed to the filter feeding habit of these bivalves. The feeding and respiration in bivalve molluscs are carried out through the water drawn into the animals body using the gill cilia. Hence the gill tissue comes in direct contact with the ambient water containing the metal for a longer duration than any other tissue. Again the absorption of the metal ions to the mucus sheets may also add to the greater concentrations in the gill tissues. Smith *et al* (1975) suggest that the poly valent ions adhere to the mucus sheets of the oysters. Koringa (1952) found that cations can be absorbed on the mucus of the gills of *Crassostrea virginica*. Since the mucus sheets are known to take part in the feeding of bivalve molluscs, they may also contribute to the increased concentrations observed in the gill tissues. Brooks & Rumsby (1965) also came to the conclusion that the gills form the major site of accumulation of trace metals in the scallops (*Pecten novae - zelandiae*), mussels (*Mytilus edulis aoteanus*), and oysters (*Ostrea sinuata*). In *Crassostrea virginica* the highest concentration of the metal was observed in the gills (Cunningham & Tripp, 1975). Vernberg & Vernberg (1972) also showed that greater amounts of metal were concentrated in the

gills of Fiddler crab, Uca pugilator. Pentreath (1976) had observed higher accumulation of Hg^{203} in the gills of plaice Pleuronectes platessa when exposed to $Hg^{203}Cl_2$.

It is seen in all cases that next to gills, the preferred site of deposition of the metal is the mantle which is more or less closely followed by viscera. The lowest efficiency is exhibited by 'foot' while in the adductor muscle the concentration factor is observed to be higher when compared to foot. The higher concentration factors observed in adductor muscle may be due to the fluid trapped inside the adductor simneses. Thus in general, the order of distribution with respect to concentration factor is gills > mantle > viscera > adductor muscle > foot, while the background levels of copper in the five different tissues in the order of decreasing concentrations is as follows: gills > mantle viscera > foot > adductor muscle.

Another important observation revealed from the present study is that the rate of uptake was seen to increase as the concentration of copper increased and the concentration factor decreased with increasing concentration

of the metal in the medium showing greater uptake efficiency at the lower concentrations. The deactivating effect of high concentration of metal ions on the organisms and the lower bioavailability of the metal at higher concentrations may be considered as the factors responsible for the above finding. Also, the phenomena of hydrolysis and precipitation are the two important characteristics of the chemistry of copper. Hence at higher concentrations copper is liable to be precipitated and these precipitated particles will not be available to the filter feeders.

In the oyster, Ostrea sinuata, Brooks & Rumsby (1967) noted that the fractionation factors (ratio of concentrations of elements in the animal compared to those in sea water) for cadmium decreased steadily with increasing concentrations of the element in sea water. Pringle et al (1968) while studying the accumulation of trace metals by estuarine molluscs found that the uptake of metals was directly proportional to the external concentration. The rate of uptake also depends on other factors like (i) species differences (ii) environmental concentration level to which the animal may be subjected and duration of exposure (iii) temperature, salinity,

dissolved oxygen and physiological conditions of the animal. Schulz-Baldes (1974) who had studied extensively on lead uptake and lead loss in the mussel Mytilus edulis found that a constant rate of Pb concentration of the medium was taking place. Smith et al (1975) also made similar observations on three species of clams (Unionidae) Nambisan et al (1977) found that the rate of uptake in Meretrix casta is linearly dependent on the copper concentration of the medium. In the green mussel Mytilus viridis the rate of uptake of copper and zinc was dependent on the concentration of the metals present in the medium (D'Silva & Kureishy 1978). Davies & Pirie (1978) found that a linear relationship existed between the mercury content of the mussels and the mean water mercury concentrations for a period of 20 days exposure while during longer exposure the relationship between rate of uptake and water concentration was not holding good. D'Silva & Qasim (1979) found that in the rock oyster Crassostrea cucullata the initial rate of uptake was directly related to the metal concentration in the medium. In Perna viridis the rate of uptake of Hg was seen to increase with increasing concentration of the metal in the medium and the greater uptake efficiency was observed at lower

concentrations (Lakshmanan & Nambisan 1979). The above observations give support to the present data.

In the copper bioaccumulation of Sunetta scripta, the influence of salinity on the uptake rate of copper is also observed. In the three different salinities employed for investigating the effect of salinity on the net uptake of the metal it is observed that the highest and lowest rates of uptake occured in 20 x 10^{-3} S and 30 x 10^{-3} S respectively. In 10 x 10^{-3} S the lowest salinity experimented, the rate of uptake was lower than in 20 x 10^{-3} S but higher than in 30 x 10^{-3} S. O'Hara (1973) has shown that the net uptake of Cd by the fiddler crab Uca pugilator is greater in low salinities. Phillips (1976) observed an increase in the net uptake of Cd by Mytilus edulis at low salinities while that of Pb decreased, while the uptake of Zn was unaffected, he also observed that the net uptake of Cu by M. edulis was highly erratic when exposed to different salinity - temperature regimes. This is attributed to the atypical uptake kinetics of copper as suggested by Scott & Major (1972). Phillips (1976) suggested that the uptake of copper is responsive to many interacting environmental

variables and hence may lead to erratic results. Phillips (1977) while investigating the short term effects of salinity on the net uptake of metals by mussels observed that the net uptake of both Cd & Cu was greater in mussels from lower salinity; he has suggested three possible modes of action by which salinity affects the trace metal content of an organism: (i) according to Bryan & Hummerstone (1973) & Phillips (1976) many metals are rendered more available in water of low salinities because of the higher capacity of fresh water than salt water to maintain metals in the water column either in solution or suspension. (ii) Linkage of ion fluxes across the body surface of an organism (Wolfe & Coburn 1970, Bryan & Hummerstone 1973) or changes in the physiological functioning of an organism like 'drinking' or 'water filtration' (Phillips 1977) may also be responsible. (iii) The bivalve molluscs may respond to changes in salinity by the mechanism of valve-closure (Phillips 1977).

The lower uptake rate observed in 10×10^{-3} S in the present study when compared to 20×10^{-3} S, may be considered as due to the valve closure mechanism exhibited by

the clams in lower salinities. Phillips (1977) states that when the animal remains with the valves closed no water can be pumped through the mantle cavity and hence no uptake of metals from food or solution takes place. Hence in an estuarine bivalve mollusc the effects of salinity fluctuations may not be clearly recorded. Moreover, the uptake of metals from food is more significant than from solutions as far as bivalves are concerned (Preston 1971, Pentreath 1973, Schulz-Baldes 1974). But it has been reported by Myers et al (1975) & Styron et al (1976) that the metal absorption by phytoplankton is affected by salinity which indicates that the uptake of metals from food is indirectly affected by this parameter (Phillips 1977). The fact that the uptake of metals from food is indirectly affected by salinity is also brought out from the experiments of Bohle (1972).

From the accumulation studies on different size groups of Sunetta scripta, it is seen that the smallest size group (20mm to 25mm) exhibited slightly higher rates of uptake and that the uptake rates decreased with the increasing size of the clams. Simpson (1979) states that the incorporation of metals into animal tissues is

influenced by two important factors (i) the sum total of external environmental changes and (ii) changes in the animal themselves which include the body weight of the animals. Boyden (1974) has shown that the concentration of zinc and lead in Mytilus edulis is related to the body weight of the animal. He had observed an inverse relationship with body size and metal concentration. Phillips (1976) found that the net weight of the animals was influenced by season and that concentrations or trace metals reciprocated these weight variations. The influence of body size on the accumulation of metals was also observed by Boyden (1977). Simpson (1979) states that the body weight of animals is an important factor while considering the physiological condition of the animals in relation to the body concentration of trace metals.

It was observed during the course of the experiments that the effect of environmental variables like metal concentration and salinity and body changes like 'size' on the uptake of copper by Sunetta scripta is more reflected in the smaller size group among the different sizes studied and in the gill tissue among the various tissues investigated. During the accumulation period the

animal is able to take up and also eliminate metal ions from its tissues. Compared to the smaller size group the larger size group exhibited greater capacity to eliminate copper from its tissues. Hence the results were observed to be erratic in the larger size groups. The greater reflection of the environmental conditions and other factors in the 'gill tissue' when compared to others might be explained by the phenomenon of 'trans location' observed in invertebrates.

When the ambient water of a bivalve mollusc contain high levels of metals, the metal levels in the tissues of bivalves rise quickly; thereafter, the metals are slowly lost (Scott & Major 1972, Majori & Petronio 1973, Clarke 1947). Scott & Major (1972) observed that Mytilus edulis is capable of accumulating copper and also eliminating the metal either by the increased secretion of mucus and subsequent binding of Cu ions or via the faeces in the form of metabolic wastes. The above observations holds good in the present study. The high affinity of heavy metals to biological tissues in general and their slow elimination is one of the important characteristics which deserve attention.

In the animal body these metals are capable of reacting with a variety of binding sites. According to Pringle et al (1968) the metals may be bound to organic molecules (protein molecules). Wolfe (1970) found that the major portion of Zn was associated with proteins perhaps metallothioneins in Crassostrea virginica. It was demonstrated by Coombs (1972) that Zn & Cu were bound to amino groups is oysters and that they do not exist as free Cu or Zn. George et al (1978) while investigating the mechanism of zinc and copper immobilization in the green-sick and normal oysters (Ostrea edulis) using electron probe X-ray micro analysis observed that copper and zinc are immobilized in membrane-limited vesicles within the oyster amoebocytes. However nothing much is known at present about the nature and composition of the metal complexes inside the animal body and the different detoxification mechanisms adopted by the various aquatic organisms.

SUMMARY

In the modern world, when industrial pollution has become a topic of increasing concern, attention is focussed on heavy metals which forms one of the major component of the industrial effluents. Many of the aquatic invertebrates especially bivalves capable of concentrating heavy metals and other contaminants have been proved useful in assessing the pollution of the marine environment. Hence, in the present study a bivalve mollusc is chosen for investigating the heavy metal toxicity.

The bivalve, Sunetta scripta collected from the clam bed near the Cochin barmouth is employed for investigating the toxic effects of the heavy metal pollutant copper (II) on the physiology of the animal. Choice of the pollutant is on the grounds that Cochin being a harbour is most likely to be contaminated by copper due to vessel-related activities.

The animals in the natural habitat may be subjected to salinity variations, both seasonal and tidal fluctuations.

With a view to determining the impact of salinity stress on the survival of the animals, experiments were conducted on small (20-25 mm) medium (30-35mm) and large (40-45mm) size groups in the different salinities ranging from 5×10^{-3} to 40×10^{-3}, at 5×10^{-3} intervals. The animals were seen to survive without any significant mortality in any of the salinity media showing high tolerance to salinity variations. Even though the mortality was low in all the different salinities, the optimum salinity range was found to be 25×10^{-3} S to 35×10^{-3} S. The greater capacity of the smaller size group to adapt to salinity changes when compared to the large ones is also made evident.

The acute toxicity of copper to the bivalve was determined by means of static bioassays. In all the three size groups studied, there was no mortality upto 6 ppm concentration at any of the salinities proving the animal's high tolerance towards the heavy metal copper (II). There was a progressive increase in mucus secretion as the concentration of copper increased in the experimental media. The clams exhibited valve closure in all the copper concentrations tested and they remained inactive.

Oxygen consumption which is considered as a convenient measure of energy transformation, is one of the indicators of sub-lethal toxic stress employed in the present study for investigating copper toxicity. Oxygen consumption rate experiments conducted on animals of different sizes showed a linear relationship with size while the metabolic rate was seen to decrease with increasing body weight. Influence of salinity on the oxygen consumption rate of the animals was investigated by the experiments conducted at 20×10^{-3}, 25×10^{-3} and 30×10^{-3} salinities. Maximum rate of oxygen consumption was recorded in 30×10^{-3} salinity which is considered as the optimum salinity for Sunetta scripta while the rate was seen to decrease in lower salinities. Regression coefficients obtained under the control experiments in 30×10^{-3}, 25×10^{-3} and 20×10^{-3} salinities are 0.59, 0.48 and 0.41 respectively. From the results of the experiments conducted with different copper concentrations (1 and 2 mg l^{-1}) it can be observed that copper has a depressing effect on the oxygen consumption rate of the animals and that the values decreased with increase in the concentration of copper. It is observed that the copper induced reductions in oxygen consumption rate were more pronounced in higher salinities and became less

obvious in lower salinities when compared to the control values. This is explained as due to the cumulative depressing action of both salinity and copper acting on the animals.

The rate of filtration, a useful index of feeding activity which is another suggested sub lethal toxicity index is also employed in the present study for investigating the toxic effects of copper toxicity. On experimenting the animals ranging in weight .030 - .760 g in the three different salinities (20×10^{-3}, 25×10^{-3} and 30×10^{-3}) the clams were observed to filter more efficiently in 30×10^{-3} salinity. The rate of filtration and body weight showed a linear relationship while the filtration rate (per mg of the body weight) is observed to decrease with increasing body weight. In the control experiments in 30×10^{-3} salinity 'b' value of 0.8584 was observed. As in oxygen consumption, the rate of filtration also showed increased reduction with the decrease in salinity. The 'b' values were observed to vary from .8025 in 25×10^{-3} salinity to .7011 in 20×10^{-3} salinity under the control experiments.

On exposure to .05 and .1 mg l^{-1} of copper in 30×10^{-3} salinity the filtration rates were observed to decrease with increasing concentration of copper. The same trend was observed in 25×10^{-3}, 20×10^{-3} salinities where the filtering activity showed greater reductions with the increase in copper concentrations. Here also on comparison of the filtration rates obtained under the three different experimental salinities the copper induced reduction in filtration rate was greater in higher salinities than in low salinities, when compared to the control values, which may be due to the salinity and copper stress acting together on the animals.

A linear relationship between filtration rates and oxygen consumption rates was observed in control experiments in 30×10^{-3} S upto a weight of about 500 mg dry weight beyond which this was not maintained. The above finding is in support of the fact that higher filtration and respiration efficiency, due to the increased rate of metabolism is exhibited by smaller individuals. When filtration rate is expressed as litres of water filtered for each ml of oxygen consumed in animals ranging in size 50 - 750 mg dry weight, the rates varied from 2.11 to 1.22 l respectively. The higher rates of filtration

relative to O_2 consumption observed in animals of smaller size group is a clear indication of the increased metabolic rates exhibited by young forms. Even though Sunetta scripta can withstand very high concentrations of copper, copper is seen to inhibit both respiration and filtration at sub lethal levels which will effect the survival of the populations.

Bioaccumulation which is one of the important biological properties of metals is also investigated. It was observed that, of the five different tissues investigated (gills, mantle, viscera, foot & aductor muscle) the gill tissue had considerably very high concentrations of the metal in all the three size groups (20-25 mm, 30-35 mm and 40-45 mm) at all times. The efficiency of accumulation was also observed to be highest with the gills under almost all the experimental conditions. Next to the gills, mantle had the greatest amount of accumulated metal which is followed by viscera. The lowest efficiency is exhibited by 'foot' while aductor muscle is seen to be more efficient when compared to 'foot'. In general, the order of distribution with respect to concentration factor is gills > mantle > viscera > aductor muscle > foot, while the background levels of copper in the five different tissues

in the order of decreasing concentration is as follows gills > mantle > viscera > foot > aductor muscle.

It is seen that the rate of uptake of copper in Sunetta scripta is dependent on the concentration of metal present in the ambient medium where the efficiency of accumulation of the different tissues was observed to decrease with increasing concentration of the metal in the medium. This is attributed to the numbing effect of high concentration of metal ions on the organisms, lower bioavailability of the metal at higher concentrations and also to the precipitation of copper at higher concentration.

Salinity, an important environmental parameter is also known to exert its influence on the copper-bioaccumulation of S. scripta. Among the three salinity regimes (20×10^{-3}, 25×10^{-3} and 30×10^{-3}) the highest rate of uptake occurred in 25×10^{-3} salinity and the lowest, in 30×10^{-3} salinity. The higher rate of uptake in lower salinities may be explained by the greater availability of metals in lower salinities because of the higher capacity of fresh water than salt water to maintain metals in the water column either in solution or suspension.

Linkage of ion fluxes across the body surface of an organism, changes in the physiological functioning of an organism like 'drinking' or 'water filtration' and the mechanism of valve closure exhibited by bivalves are other suggested modes of action by which salinity affects the trace metal content of an organism. The lower rate of uptake observed in S. scripta in 10×10^{-3} salinity when compared to 20×10^{-3} may be considered as due to the valve closure mechanism exhibited by these bivalves in lower salinities. Even though 'body size' of the organism is not seen to exert a significant influence on the rate of uptake of copper, the uptake rates were observed to decrease slightly with increasing size of the clams. However, the impact of the different variables like metal concentrations, salinity and body size on the uptake rate of Sunetta scripta is reflected more in the smaller size group than in the larger ones and, in the gill tissue among the various tissues investigated. During the accumulated period it is observed that the animal is not only able to take up copper from the ambient medium but also eliminate copper ions from its tissues. This capacity of reducing the tissue metal concentrations is observed to be pronounced more in the

larger animals than the small ones. Hence in the smaller size group the metal ions were taken up more linearly when compared to the large ones and the results were observed to be erratic in the larger size group. The greater reflection of the different variables in the gill tissue may be explained by the phenomenon of 'translocation' observed in invertebrates.

The animal's capacity to concentrate copper to very high levels in body tissues makes it fit for monitoring the effects of pollution load as well as for chemical analysis. The clam meat being consumed by man, especially by lower income class, and also used as a poultry feed, deserves attention from the point of view of human health.

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