STUDIES ON THE SURVIVAL OF *SUNETTA SCRIPTA* IN DIFFERENT SALINITIES

THESIS SUBMITTED TO THE

COCHIN UNIVERSITY OF SCIENCE AND TECHNOLOGY

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

IN

MARINE BIOLOGY

UNDER THE FACULTY OF MARINE SCIENCES

BY

SUPRIYA, R.

DIVISION OF MARINE BIOLOGY, MICROBIOLOGY AND BIOCHEMISTRY

SCHOOL OF MARINE SCIENCES

COCHIN UNIVERSITY OF SCIENCE AND TECHNOLOGY

KOCHI - 682 016

1992

TO MY PARENTS

D E C L A R A T I O N

I hereby declare that the thesis entitled,'STUDIES ON THE SURVIVAL OF *SUNETTA SCRIPTA* IN DIFFERENT SALINITIES', *is an authentic record of research work carried out by me under the supervision and guidance of Prof.* (Dr.) *R. Damodaran in partial fulfilment of the requirements of the Ph. D Degree in the Faculty of Marine Sciences of the Cochin University of Science and Technology and that no part of it has previously found the basis for the award of any degree, diploma or associateship in any university.*

Supriya. R

Kochi - 16
27.7.92

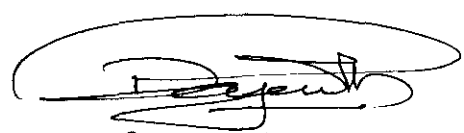
SUPRIYA. R.

All the corrections recommended by the examiners are incorporated

Supriya R

C E R T I F I C A T E

This is to certify that the thesis bound herewith is an authentic record of the research work carried out by Miss. SUPRIYA. R., in the Division of Marine Biology, Microbiology and Biochemistry School of Marine Sciences, Kochi - 16, under my supervision, in partial fulfilment of the requirements for the degree of Doctor of Philosophy of Cochin University of Science and Technology and further that no part thereof has been presented for any other degree.



Prof. R. DAMODARAN

ACKNOWLEDGEMENTS

I wish to express my profound and sincere indebtedness and deep sense of gratitude to Prof. (Dr.) R. Damodaran, Division of Marine Biology, Microbiology and Biochemistry, School of Marine Sciences, for his guidance, critical scrutinization and constant encouragement through out the course of this investigation.

I would like to place on record my deep and sincere gratitude to Dr. T. K. Sivadas, Principal Scientist, Central Institute of Fisheries Technology (CIFT), Kochi, for his valuable help, guidance and co-operation for designing the 'Oyster Activity Monitor'.

I am thankful to Prof. (Dr.) C. V. Kurian, Former Director, School of Marine Sciences, for scrutinizing the manuscripts and for valuable suggestions.

Thanks are also due to Prof. (Dr.) N. R. Menon, Director, School of Marine Sciences, for giving me the necessary facilities and encouragement.

I am also thankful to Dr. A. Mohandas, Head, School of Environmental studies for providing the computer facilities to complete this investigation.

I also thank Shri. K. Krishna Iyer, Scientist, CIFT, Kochi, for the help in statistical analysis of the data.

I express my sincere gratitude to Mr. C. G. Rajendran and Dr. M. V. Mohan, Associate Professors, Rice Research Station, Vyttila, for their valuable help and guidance.

Further, I have to acknowledge the help and co-operation extended to me by Mrs. Vijayabharathy, Mr. Elias and Mr. Gokulan, CIFT, Kochi.

My unfeigned thanks to my friends of School of Environmental Studies, School of Marine Sciences and Department of Industrial Fisheries, for their sincere help.

The work presented here would not have been possible without the financial assistance from Cochin University of Science and Technology and Council of Scientific and Industrial Research, New Delhi.

(SUPRIYA. R.)

ABBREVIATIONS

B C I	:	Body Condition Index
^{o}C	:	Degree (s) Celsius
cm	:	Centi meter (s)
Cl^{-}	:	Chloride ion
C.R.	:	Clearance Rate
Conc.	:	Concentration
DC	:	Direct Current
E	:	East
eg.	:	Example
Fig.	:	Figure
gm	:	Gram (s)
h (hrs)	:	Hour (s)
K^{+}	:	Potassium ion
K_1	:	Gross Growth Efficiency
K_2	:	Net Growth Efficiency
l	:	Liter (s)
LSD	:	Least Significant Variation
m	:	Meter
mg	:	Milli gram
min	:	Minute (s)
mm	:	Milli meter
N	:	North
Na^{+}	:	Sodium ion
nm	:	Nano meter
N.S	:	Not Significant
O_2	:	Oxygen
O.C	:	Oxygen Consumption
O:N	:	Oxygen to Nitrogen ratio
ppt	:	Parts per thousand
SD	:	Standard Deviation
V	:	Voltage
W (wt.)	:	Body Weight
%	:	Percentage
µgm	:	Micro gram (s)

C O N T E N T S

CHAPTER - 1

GENERAL INTRODUCTION

The organisms living in the intertidal region are subjected to widely fluctuating environmental factors and can tolerate more extreme conditions than other marine organisms. According to Kinne (1971) the intertidal organisms are generally eurythermal, euryhaline and are resistant to desiccation. So an organism inhabiting the fluctuating environment must be able, either to compensate or endure changes that occurring in the ambient environmental conditions. When one or more of the components of the environment fluctuates beyond the tolerance zone, the organism fail to maintain homeostasis and death results.

Salinity is one of the major environmental variables affecting the performance and ultimately determines the growth, survival and distribution of organisms in the intertidal region (Kinne 1971). Effect of salinity on the organisms can be by changing the total osmoconcentration, relative proportion of the solutes, coefficients of absorption and saturation of dissolved gases and density and viscosity of the medium (Kinne, 1971). Salinity is one of the major fluctuating environmental parameter along the South-West coast of India due to the impact of South-West monsoon. As a result most of the intertidal animals living in this area have to tolerate wide changes in salinity for their distribution and abundance.

Sunetta scripta (Linne') is an infaunal siphonate clam

inhabiting the sandy intertidal region and is a major component of the intertidal community. The species has got wide distribution along the South-West coast of India. The important clam beds off Kochi are located on the northern side of the Kochi barmouth and in Munampam, around 20 km north of Kochi. Generally *S. scripta* occurs together with *Meretrix casta* forming extensive clam beds which support a moderately lucrative local fishery. The animal is economically important since it is being exploited for flesh and shell. It is consumed by poor people as it forms a cheap source of protein food. The clam meat is also used as a good poultry feed. The shell of the species is thick and is used in lime and cement industry.

Studies on *S. scripta* in the Kochi barmouth area were carried out by Mohan and Damodaran (1981), Thampuran (1986), Thampuran *et al.* (1982), Katticaran (1988), Suresh (1988), and Suresh and Mohandas (1987; 1990a; b; c).

In the barmouth the animal is available throughout the year, and is found to be highly tolerant to a wide range of environmental conditions. The clam bed is located at a depth of 1.5 to 2.5 m. Since the animal is seen in the sublittoral area, the collections were made during low tide with the help of fishermen. During monsoon the salinity over the clam bed showed very low value (1.5 ppt) and in pre-monsoon period the value goes upto 35.1 ppt. The annual temperature range of the area is from 24.25^{o}C to 31.40^{o}C (Katticaran, 1988). The substratum is composed of sand, silt, mud and shell fragments; with silt

predominant during monsoon and sand during rest of the year. Eventhough there exists wide range of salinity, the mass mortality of *S. scripta* in the clam bed was never noticed. But the abundance of larger animals are considerably less during monsoon collections.

The previous studies on *S. scripta* in the laboratory conditions indicated that the species can tolerate 5 to 40 ppt salinity (Thampuran *et al.*, 1982). But eventhough the *S. scripta* has got a wide tolerance to salinity, the species so far has not been found as a component of the benthic community in identical substratum in the estuarine region. Therefore it was decided to study in detail the adaptations of the animal; behavioural, physiological and biochemical, in different salinities, with the hope that it may explain the restriction of *S. scripta* in the marine environment. The salinity tolerance study (Thampuran *et al.*, 1982) revealed that the capacity to tolerate salinity variation varies with size. The smaller clams were found to be better adapted than medium and larger size groups. So the work was designed to study the impact of salinity on different size groups.

Bivalve molluscs can isolate their tissues from the unfavourable environmental conditions by closing their valves (Freeman and Rigler, 1957; Bayne, 1973b; Akberali, 1978). Akberali (1978) observed that the *Scrobicularia* shows the tendency to remain closed when transferred from normal sea water to lowered salinity; the greater the drop in salinity, the

greater the tendency to remain closed. The activity of both intertidal and subtidal species of *Modiolus*, as measured by valve movements, was reduced when the animals were exposed to either a high or low salinity (Pierce, 1971). Valve closure in response to the presence of heavy metal pollutants has also been reported in a number of bivalve molluscs (Davenport, 1977; Manley and Davenport, 1979; Akberali and Black, 1980; Akberali *et al.*, 1981; Manley, 1983). But Davenport (1979) reported that in *Mytilus edulis* the isolation of mantle cavity fluid is not simply produced by valve closure, but comprises a three part sequence. The first part of this sequence is the closure of exhalent siphon, which effectively ceases the irrigation of mantle cavity. Further decline in external salinity results in the closure of inhalent siphon, which is then followed by valve closure. A similar sequence was also reported in *Scrobicularia plana* (Akberali and Davenport, 1981). Mane (1974) reported that when the salinity increased from low, the *Katelysia opima* open their valves and do active valve movement. From the above studies, it is evident that the bivalves can distinguish between favourable and unfavourable conditions and modify the behavioural response so that the animal can minimise the harmful effects of unfavourable condition or exploit favourable conditions.

S. scripta living in its natural environment off Kochi is subjected to wide variations in salinity due to tidal and seasonal changes. As stated earlier even under very low

salinity prevailing on the clam bed, the mass mortality of *S. scripta* does not occur. The animal also showed wide tolerance to salinity in the laboratory conditions (Thampuran *et al.*, 1982). From these it is clear that the animals must have some adaptations at the behavioural level so that it can overcome the stress caused by the lowered salinity. As valve movement is an indication of stress, it was decided to study the valve movement of the animal in different salinities and in different size groups. For this purpose an 'Activity Monitor' was developed in collaboration with Central Institute of Fisheries Technology, Kochi, in which the sensor senses the valve movement without making any stress to the natural behaviour of the animal.

The physiological flexibility of *S. scripta* in relation to salinity variation can be understood by studying the physiological ecology, since it is the study of how an organism adapted to function in its particular environment (Bayne *et al.*, 1976c; d). The various physiological parameters studied are clearance rate, absorption efficiency, oxygen consumption, ammonia excretion and ionic (Na^+, K^+ and Cl^-) regulation. All these parameters are inturn affected by the behavioural modifications. The amount of food uptake from the water passing through the gill can be understood by studying the clearance rate (Bayne *et al.*, 1976c) which is defined as the volume of water cleared of particles in unit time (Bayne *et al.*, 1985). The total food consumed by an animal is not absorbed as such and some part is lost as faeces (Conover, 1966) and the efficiency

with which the consumed food absorbed is depended on environmental factors, type and condition of food and the physiological condition of the animal (Bayne and Widdows, 1978; Widdows *et al.*, 1979b; Bayne *et al* ., 1985). So the efficiency of absorption of food by *S. scripta* can be understood by estimating absorption efficiency in various salinities and in different size groups. The oxygen consumption can be used as a convenient measure of energy transformation (Scott and Major, 1972). Variation in salinity may modify the rate of metabolism of aquatic invertebrates (Kinne, 1971). Influence of salinity on the oxygen uptake of *S. scripta* can be understood by studying the oxygen consumption in different salinities and in different size groups. A small proportion of the total food uptaken is excreted as metabolic waste products. Variation in the metabolism can be followed by estimating the ammonia, since it being the principal excretory products of protein catabolism (Griffiths and Griffiths, 1987), however amino acids and urea (Bayne, 1973a) are also excreted in small quantities.

The changes in the rate of nitrogen excretion are best understood when related to overall metabolic rate by means of Oxygen:Nitrogen (or O:N) ratio. This ratio when calculated by atomic equivalents may be used to indicate the proportion of protein catabolised relative to carbohydrate and lipid (Conover and Corner, 1968). A low value of O:N signifies the considerable protein utilization. Mayzaud (1973) reported a minimum value of 7 when the amino acids results from the protein

catabolism are deaminated and excreted as ammonia. So this ratio is used by many workers (Bayne and Thompson, 1970; Ansell and Sivadas, 1973; Bayne, 1975; Bayne *et al* ., 1985) as an index of stress. In order to understand the impact of salinity on the protein catabolism, O:N ratios of *S. scripta* were calculated in different salinities and in different size groups.

Most of the marine invertebrates are normally in isosmotic with the surrounding medium (Fredericq, 1901). The isosmoticity of blood with the surrounding medium remain true even for marine euryhaline species which can tolerate a wide range of external salinities. This isosmoticity results from the association of a number of adaptations such as morphological, physiological or biochemical. Since the *S. scripta* is having wide tolerance to salinity the ionic concentrations of the blood and cells must change in accordance with salinity. So it was decided to study the ionic (Na^+, K^+ and Cl^-) concentrations in different salinities and size groups.

By studying a single physiological parameter would not give a clear picture of the survival of *S. scripta* in different salinities. So for the understanding of the impact of salinity on the survival, which inturn causes the distribution and abundance of *S. scripta*, converted all the physiological parameters (clearance rate, oxygen consumption and ammonia excretion) into energy equivalents and put together as scope for growth. Scope for growth is more significant and more easily interpreted, than changes in the rates of a single physiological

variable because it represents the energy balance at any given time under specified conditions. The scope for growth can be calculated by solving Winberg equation (Winberg, 1960), i.e, by subtracting the total energy losses (respiration and excretion) from the energy content of the absorbed ration. In otherwords the efficiencies with which ingested and absorbed rations are utilized for maintenance and converted in to body mass. The scope for growth can range from positive values when there is energy available for growth and reproduction to negative values when the animal is severely stressed and utilizing its body reserves for maintenance metabolism (Bayne *et al.*, 1985).

The absorption efficiency of *S. scripta* is found to be influenced by salinity variations. Any influence can be well understood by studying the efficiencies, gross growth efficiency (K_1) and net growth efficiency (K_2). Gross growth efficiency is the efficiency with which the animal utilizes the ingested ration for growth and reproduction and net growth efficiency with which the assimilated ration is utilized (Bayne *et al.*, 1976c). The values of K_1 and K_2 are negative when the animals are stressed (Thompson and Bayne, 1974; Bayne and Widdows, 1978). The food consumed by the animal is not utilized as such and some quantity is lost as faeces. So the estimation of gross growth efficiency as proportion of ingested ration has its own significance. When the ingested energy is equal to the total energy metabolised then K_1 is zero, then ingestion is a measure of maintenance ration (Thompson and Bayne, 1974).

In addition to the above physiological parameters body condition index is also estimated for *S. scripta* because in the natural environment itself the salinity is changing due to the impact of South-West monsoon which inturn affects the normal behaviour and physiology of the animal. So the proportion of internal shell volume which is occupied by the body tissues varies with stress condition as a result of utilization of body reserves for their maintenance metabolism, when the feeding is stopped (Baired, 1966). By estimating the body condition index, the impact of salinity variation on *S. scripta* in its natural environment in different months can be understood.

The periods of valve closure are commonly associated with behavioural inactivity and cessation of pumping activity. The most obvious effects of this is the limitation of time available for feeding, cessation of aerobic respiration and accumulation of end products. Under these conditions the bivalves show the adaptive mechanisms by the shifting off aerobic pathways to sustain basal metabolism (de Zwaan, 1977; Akberali and Trueman, 1985) and hence bivalves have been called facultative anaerobes (de Zwaan, 1977; Hochachka, 1985), capable of surviving indefinitely in the absence of oxygen and capable of active oxidative metabolism in its presence. Under these conditions organic substrates instead of oxygen are the acceptors of electrons. Karnaukhov (1971; 1979) reported that the carotenoid together with haemoproteins and some respiratory enzymes forms a special intracellular organoid, 'carotenoxysome' which is acting

as intracellular reserve of oxygen under conditions when mitochondria are not efficient. Carotenoids provide for an oxygen reserve in the 'carotenoxysome' by acting as accumulator of oxygen or an equivalent electron acceptor (Karnaukhov, 1979). So inorder to understand anaerobic metabolism during valve closure, it was decided to study the caroteniod concentration in soft body parts of *S. scripta* in different salinities and size groups.

All the results of the above observations are given in the five chapters of the thesis. The first chapter gives the general introduction describing the habit and habitat of the animal and also objectives and importance of the present investigation. The second chapter describes the behavioural adaptations of *S. scripta* in different salinities and size groups. The third chapter deals with different physiological parameters and the effect of salinity and size on them and the energy budget as the physiological adaptations and the fourth chapter deals with the biochemical adaptations in different salinities and size groups. The fifth chapter gives the overall conclusions of the present investigation followed by the list of references.

CHAPTER - 2

BEHAVIOURAL ADAPTATIONS

2.1 Introduction

Behavioural modification is one of the most sensitive indication of environmental stress and may directly affect survival. Many bivalves when exposed to lethal environmental stress, rely on behavioural mechanism which enable them to avoid such condition. Mobile species move away from unfavourable condition where as for sedentary species survival depends upon behavioural mechanisms such as burrowing in to the substratum, retracting into the existing burrows or closing of the valves.

Bivalve molluscs are able to isolate and protect themselves from a variety of adverse environmental conditions by closing the valves (Coleman and Trueman, 1971; Gilles, 1972; Bayne, 1973b; Nagabhushanam and Bidarkar, 1975; Akberali *et al.*, 1977; 1981; Akberali, 1978; Davenport, 1979; Akberali and Davenport, 1981; 1982). Valve closure of bivalves prevents drastic changes in osmotic concentration of their body fluids when exposed to short term fluctuations in salinities (Shumway, 1977). Valve closing mechanism allows the animal a period of grace and thus prevents osmotic shock. Although altering the shell movement/closing may help the organism to withstand transient adverse changes in the environment it can not contribute to its long term survival in situations where the change in the environment may be of a permanent nature. This is because

during any period of valve closure the animal incurs penalties related to feeding, reproduction or exchange of gases and metabolites.

Davenport (1979) showed that the closure of the exhalent siphon, thus preventing pumping was the crucial event which largely isolates the mantle cavity of mussel from falling external salinities, the shell valve closure occurred at rather lower salinities to produce virtually a complete isolation. The same was also observed for *Katelysia opima* (Mane, 1974); *Scrobicularia plana* (Akberali and Davenport,1981) and *Donax denticulatus* (Genovea *et al.*, 1988).

The development of electronic and other analytical techniques has led to significant advances in our knowledge of bivalve behaviour. Galtsoff (1964) studied the valve movements of the American oyster *Crassostrea virginica* using slow motion kymograph which is very time consuming. Under ordinary circumstances for most of the molluscan species the opening and closing movements of shell are so small. For long term observations the speed of the kymograph drum should be adjusted, and very slow movement is not possible with the kymograph. Various authors used impedance technique to record heart beat and valve activity of bivalve molluscs and other marine invertebrates in relation to a variety of environmental changes such as tidal exposure (Coleman, 1974; Earll, 1975), feeding (Thompson and Bayne, 1972; Widdows, 1973a), temperature (Stone, 1980; Davenport and Carrion-Cotrina, 1981), oxygen (Brand and

Roberts, 1973), salinity (Davenport, 1977; Akberali, 1978; Akberali and Davenport, 1981) and pollution (Davenport, 1982; Manley, 1983). Davenport (1979) has used stress gauge for monitoring valve movement of *Mytilus edulis* exposed to falling sea water concentrations. It is not clear whether the stress gauge senses the valve movement without making any stress to the natural behaviour of the animal. For the present study an 'Oyster Activity Monitor' was constructed in collaboration with Central Institute of Fisheries Technology, Kochi for studying shell movements of *S. scripta*. The instrument will not cause any stress to the natural behaviour of the animal while recording the valve movement.

The major advantage of the system is the antenna of the sensor which makes only a feather contact with the shell. The force applied on the shell is less than 30 mg. The antenna can move through a distance of about 8 mm which is enough to record valve opening. The paper recordings have shown the movements in the order of 0.05 mm can be detected easily. The method also allows continuous long term measurements as the paper charts are normally 50 m in length. The electronic circuits and controls are able to work at 9 V DC supply obtainable either from a battery or a battery eliminator.

The main aim of this study is to relate behavioural adaptations of *S. scripta* to changes in external salinity by using 'Oyster Activity Monitor'.

2.2 Materials and methods

2.2.1 Acclimation of test animals

Sunetta scripta were collected from Fort Cochin area (between latitude $9^{o}28'$ and $10^{o}00'$ N and longitude $76^{o}13'$ and $76^{o}11'$ E) and brought to the laboratory in plastic bags containing sea water collected from the same site, cleaned and grouped into three size groups; small (1.5 $\pm$ 0.5 cm), medium (2.5 $\pm$ 0.5 cm) and large (3.5 $\pm$ 0.5 cm). They were kept in large plastic basins which are used as acclimation tanks containing sand for three days at 30 ppt sea water. The acclimation period of three days was fixed by estimating oxygen consumption of the animals which was found to be in a steady state. During acclimation they were fed on 3-4 days old blue green algae *Synechocystis salina* cultured in a metal free medium. The water in the acclimation tank was purified using biological filter (Spotte, 1970) (Fig. 1) and changed once in two days. After three days of acclimation 5 animals of each size group were taken for valve movement study. Then the salinity of the water was altered slowly by the addition of water of higher salinity obtained by the evaporation of sea water and deionised water so that salinity reached 40 ppt and 20 ppt respectively within three days. At the end of third day, the animals were used for valve movement study. The water of 20 ppt was further diluted with deionised water to obtain 10 ppt and 5 ppt, taking again three days for dilution at each step.

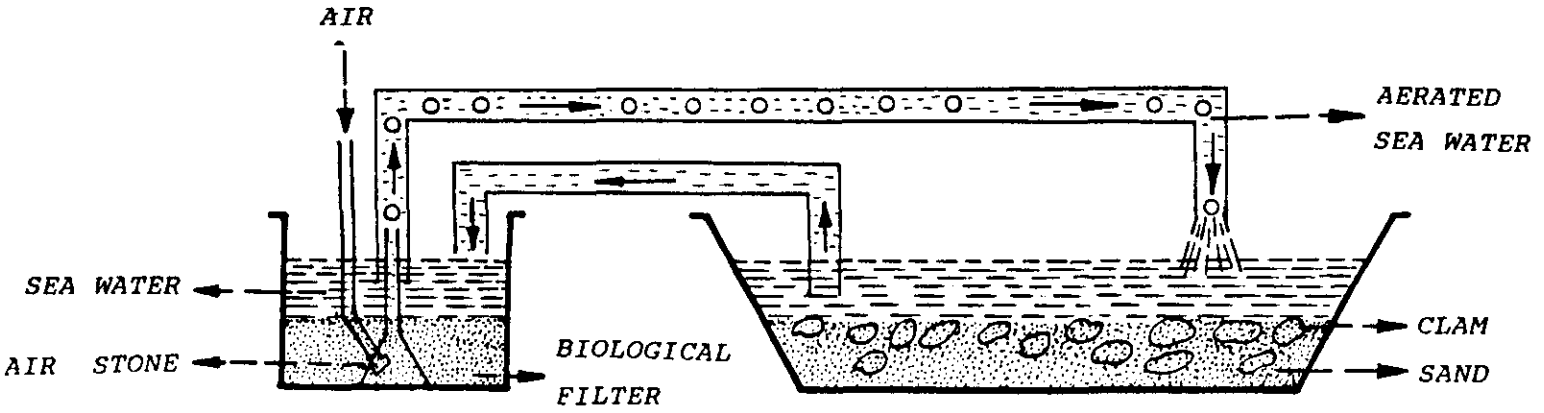


Fig. 1 Acclimation system with biological filter used for acclimating clams in different salinities

The animals thus acclimated were used for the experiment. During these periods they were fed on *S. salina* and gave aeration by biological filter.

Another set of animals of the above three size groups were acclimated at 5 ppt for three days. During acclimation, they were fed on *S.salina* and water was purified using biological filter. After three days, the records were taken continuously for six hours and after that the salinity of the medium was increased by the addition of sea water of higher salinity obtained by the evaporation of sea water, with an interval of 15 minutes. After each addition, salinity of the water was measured (Table 1) and noted the salinity at which the animal showed valve movement, siphon with drawl etc. The Table 1 shows the average salinity of four experiments. The salinity and temperature (28 $\pm$ 2^{o}C) of the water samples were determined by using Salinity Temperature Probe developed by Central Institute of Fisheries Technology, Kochi.

2.2.2 Oyster Activity Monitor

The instrumental system consists of three essential parts as given in the schematic diagram (Fig. 2) namely sensor, electronic unit and paper chart recorder. The sensor consists of a light antenna made of 0.5 mm dia stainless steel wire and is capable of making both ends to move with the fulcrum at the middle. The lower end of the antenna rests on the upper shell

Table 1. Salinity variations during stepwise increase in salinity by addition of sea water (each step is the mean of four experiments)

Salinity increase	Size Groups		
	Small (1.5 cm)	Medium (2.5 cm)	Large (3.5 cm)
Step 1	7.90 ± 0.4320	8.13 ± 0.6238	7.93 ± 0.8539
Step 2	9.73 ± 0.4272	9.80 ± 0.3367	9.88 ± 0.1500
Step 3	11.85 ± 0.6000	12.12 ± 1.0800	14.60 ± 0.7071
Step 4	13.30 ± 0.4163	14.79 ± 0.2000	16.76 ± 0.5400
Step 5	15.58 ± 0.5000	17.48 ± 0.7300	20.33 ± 1.1500
Step 6	15.73 ± 0.4924	21.08 ± 1.7896	22.15 ± 0.4655

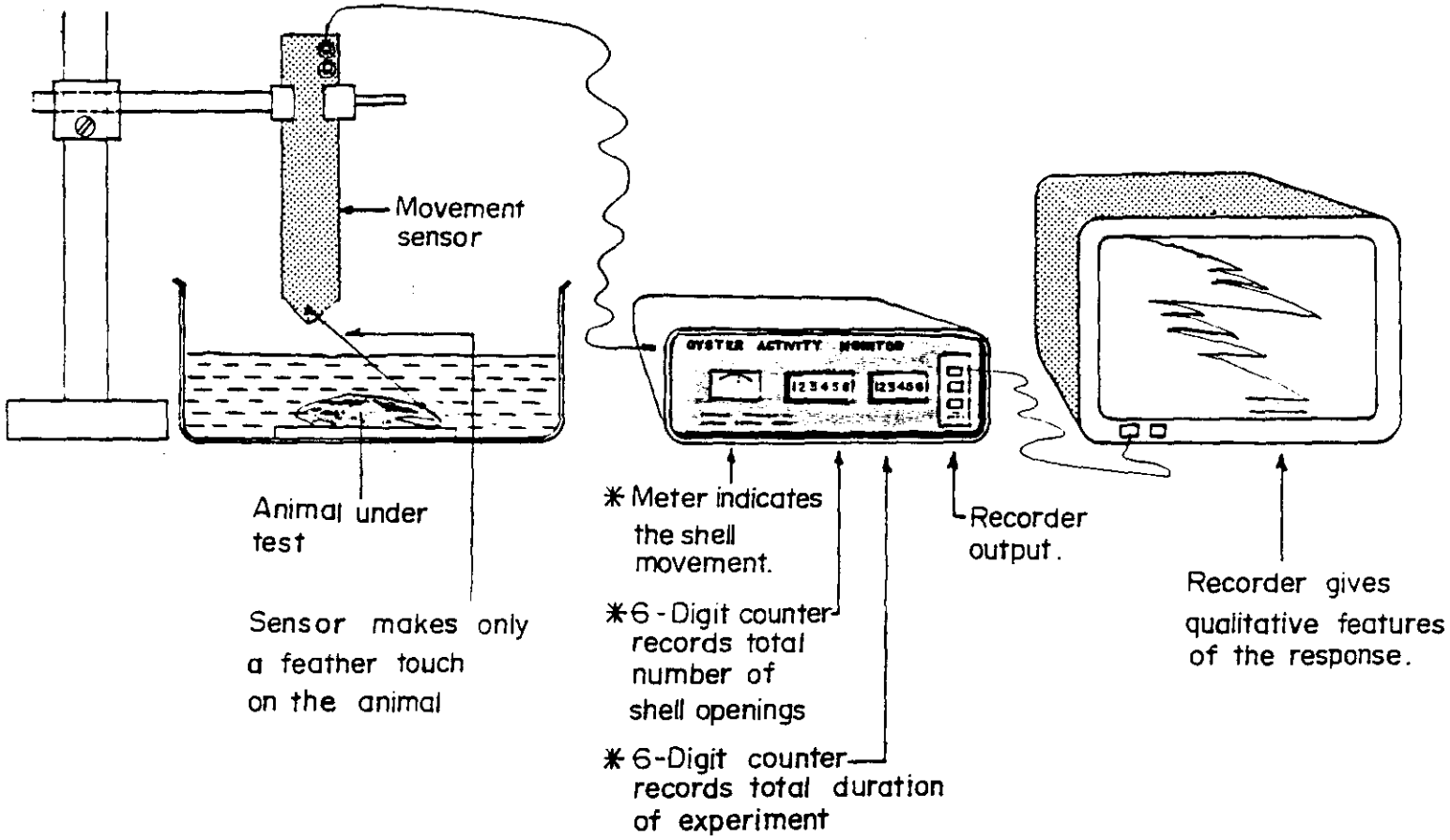


Fig. 2 Schematic diagram of Oyster Activity Monitor

of the animal while the upper end moves between an electro-optic sensing system producing electrical signals proportional to the obstruction caused on the optical path due to the movement of the shell. The electrical signals produced in proportional to the movements of the shell are transmitted to the electronic unit. The electronic unit converts the signals obtained from the sensor into DC voltage and fed to the paper chart recorder for recording.

2.2.3 Experimental setup

S. scripta acclimated at different salinities were placed on a tripod so that the animal is permanently fixed and kept in basin having enough water of desired salinity. The antenna is kept in contact with the upper shell of the animal from behind so that it moves freely upwards without making any stress. The position of the sensor is adjusted precisely such that the signal is properly set which is indicated in the meter of the instrument. Since the antenna is immersed in water, the variations in water level do not affect the recordings. The sensor is connected to the meter and the recorder pin is connected to the recorder port. Now the system is ready for observation.

2.3 Results

S. scripta belonging to three size groups acclimated at different salinities showed the following valve movements.

From the Fig. 3 it can be seen that at four different salinities 40, 30, 20 and 10 ppt, the smaller size group (1.5 cm) animals kept the valves in opened condition throughout the experiment. The animals acclimated at 40 ppt salinity showed regular valve movements with gradual opening and sudden partial closing. The *S. scripta* acclimated at 30 ppt and 20 ppt salinity sea water showed steady valve movements. The initial openings were found to be without much time lag followed by regular and rhythmic movement of valves at much shorter intervals and partial closure of the valves at more or less constant interval. At 10 ppt the magnitude of gradual openings and sudden partial closings was much less. At 5 ppt salinity they kept the valves closed with intermittent attempt to open.

The medium size group (2.5 cm) animals (Fig. 4.) acclimated at different salinities showed different types of valve movement. At 40, 30 and 20 ppt salinity the animals kept the valves in open condition throughout the period of experiment. A constant gaping and rhythmic partial closings of valves were observed only at 30 ppt . When comparing the valve movements at 40 ppt and 30 ppt, the partial closures at 40 ppt were found to be at wide intervals. When the animals were in 20 ppt, the partial openings and closings were much more frequent than in 40

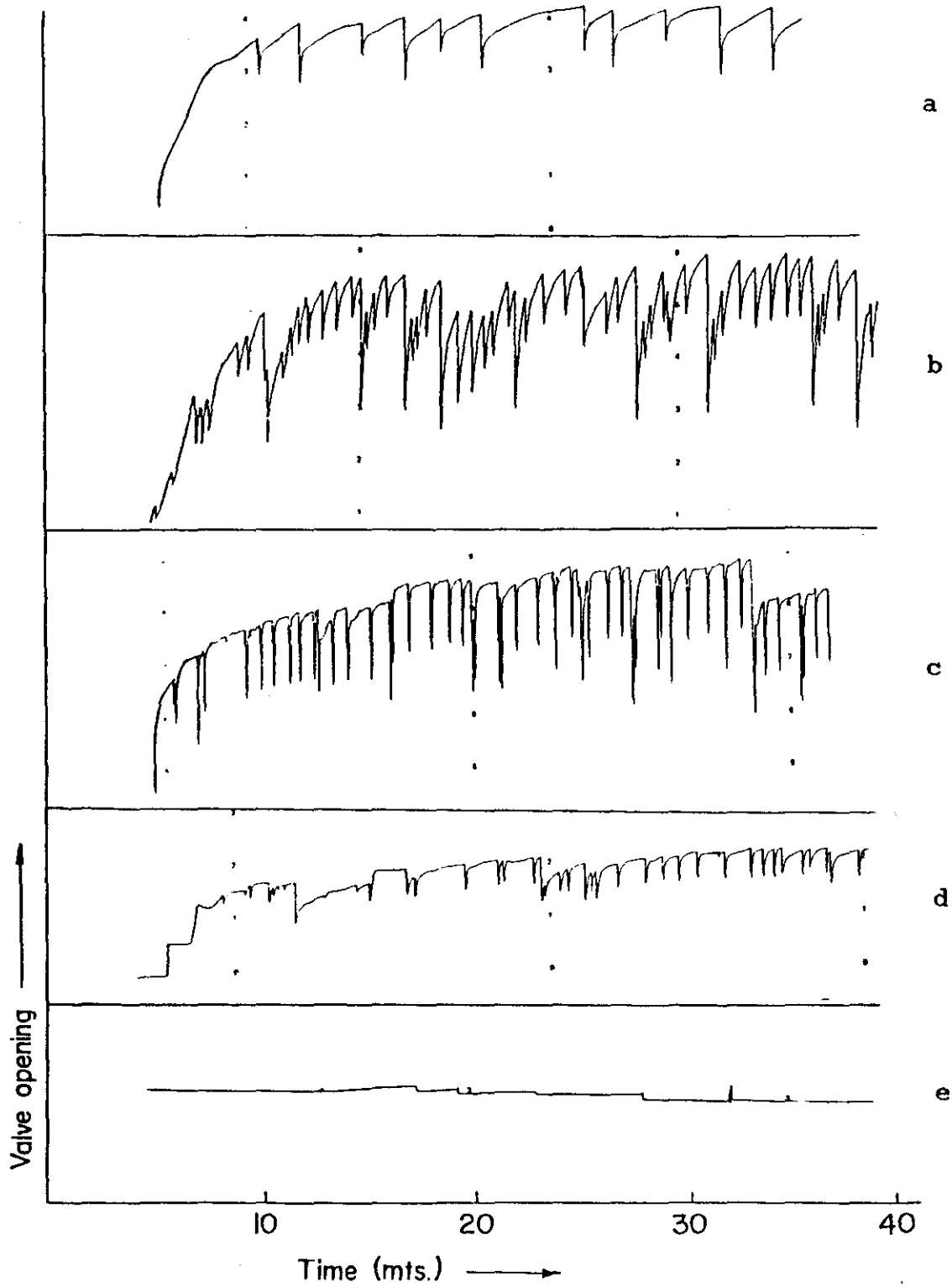


Fig. 3 Shell valve movement of small size group (1.5 cm) acclimated in different salinities a 40, b 30, c 20, d 10 and e 5 ppt

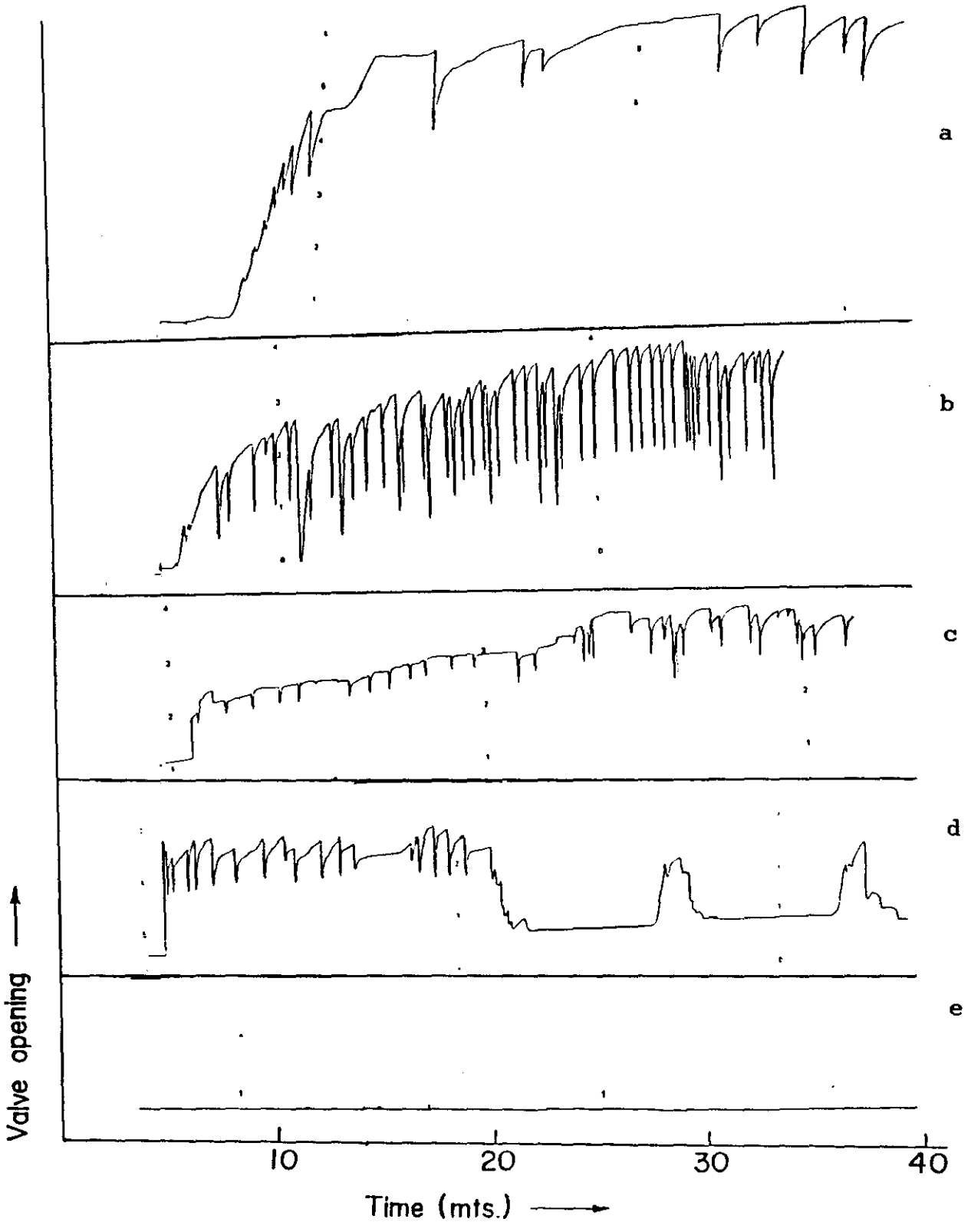


Fig. 4 Shell valve movement of medium size group (2.5 cm) acclimated in different salinities a 40, b 30, c 20, d 10 and e 5 ppt

ppt though the magnitude is less than in 30 ppt. The intervals between partial closings were found to be greater than 30 ppt but less than 40 ppt . At 10 ppt salinity sea water during the initial stages almost regular openings and closings occurred but later the animal kept a prolonged closed condition with intermittent openings. At 5 ppt salinity sea water the animal remained with the valves closed.

When the larger size group (3.5 cm) animals (Fig. 5) were acclimated at different salinities the behaviours were found to be different from that of small and medium size groups animals. The animals kept the valves in open condition throughout the experiments at 40, 30 and 20 ppt salinity sea water. A constant gaping and partial closure of valves were observed at 30 ppt salinity sea water. In 40 ppt salinity a prolonged open condition was associated with partial closure at wide interval. In 20 ppt also the partial closure was at wide interval and also the width of the gap was much less than in 40 ppt. In both of the salinities (40 ppt and 20 ppt) the animals kept the exhalent siphon inside the valves. The animals kept in 10 ppt salinity sea water showed closed condition with regular attempt to open. At 5 ppt salinity a complete closure of valves occur.

When considering the three size groups together a regular and rhythmic movements of valves were observed at 20 ppt and 30 ppt salinities for smaller size group and 30 ppt salinity for medium and larger size groups animals. When the ambient medium alters from 30 ppt salinity the animals showed behavioural

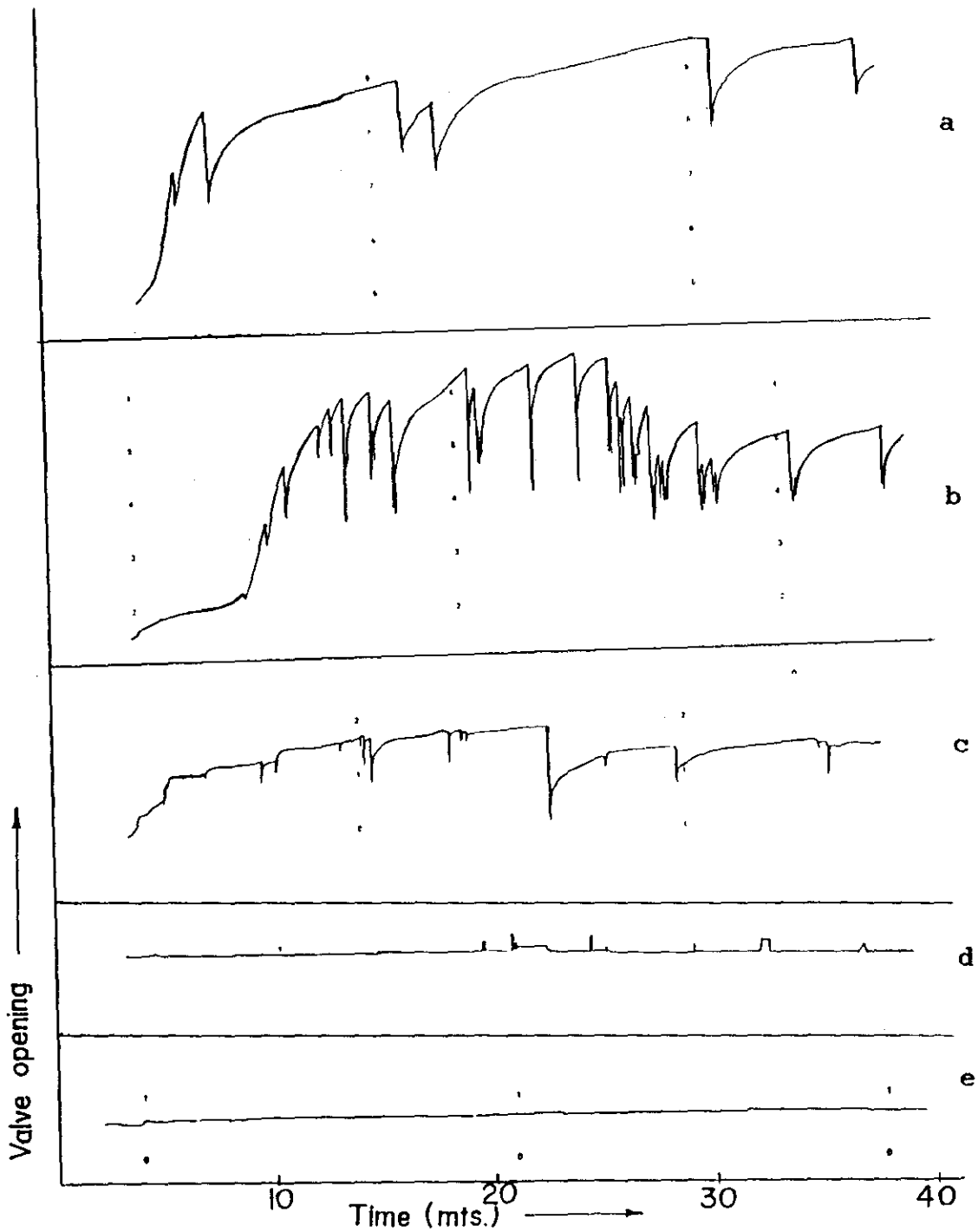


Fig. 5 Shell valve movement of large size group (3.5 cm) acclimated in different salinities a 40, b 30, c 20, d 10 and e 5 ppt .

change by altering the pattern of valve movement and in extreme salinities by closing.

When the above three size groups animals acclimated at 5 ppt were subjected to gradual increase in salinity the smaller size group was found to open their valves at lower salinities compared to medium and larger size groups (Fig. 6). Salinity required to induce the valve opening in small, medium and larger size groups (average of four experiments) were 15.58, 17.48 and 20.33 ppt respectively (Table 1). The siphons came out at 15.73 ppt for smaller, 21.08 ppt for medium and 22.15 ppt for larger size groups. When observing the valve movement of the three size groups, the intervals between the closings were found to be decreasing as the size of the animal increases (Fig. 6). Valve closure was complete or nearly complete only in the case of large size group. But for small and medium size groups it was only partial (Fig.6).

2.4 Discussion

Since *S. scripta* tolerate a wide range of salinity, the animal must have adaptive capabilities at the behavioural levels. In extreme dilution of the ambient medium the animal closes the valves and isolated its tissues from the ambient conditions. Valve movements were found to be reduced in unfavourable salinities.

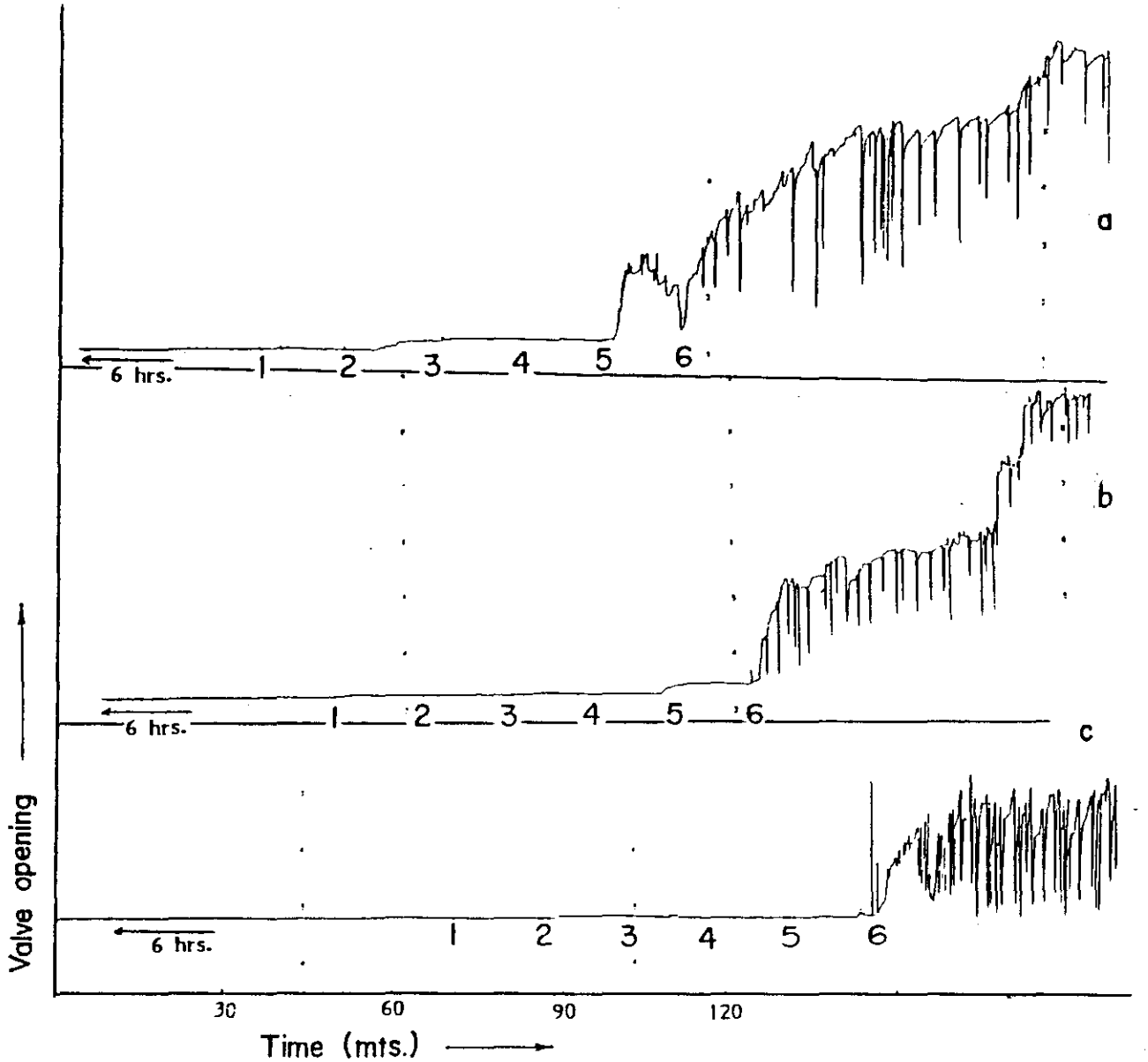


Fig. 6 Shell valve movement of three different size groups subjected to rising salinities from 5 ppt. a small b medium and c large 1,2,3,4,5,6 etc. steps of additions of increasing sea water salinity

Observations of Fig. 3,4 and 5 showed the valve movements varying in different salinities and complete closures were recorded only in 5 ppt which was reported as the most unfavourable salinity of the animal (Thampuran *et al.*, 1982). In favourable salinities (20 and 30 ppt) for smaller size group (1.5 cm) animals the valve movements were rhythmic partial closures and openings after the initial gaps. Same valve movement was noticed at 30 ppt for medium sized (2.5 cm) animals. With slight variation in time component similar valve movement was noticed for larger sized (3.5 cm) animals in 30 ppt. This indicates that there is a rhythmic partial closure of valves in optimum salinity after the initial gap. The rhythmic partial closures and openings of the valves may be due to the muscular activity associated with water pumping and was exhibited as a regular process only in favourable salinities. In extreme of tolerance range or in resistance range the valve movement at greater intervals indicates reduced pumping activity. In extreme salinities (eg. 10 ppt) the pumping activity totally ceases for larger animals and much reduced for medium and smaller size groups. Previous studies (Thampuran *et al.*, 1982) had showed greater tolerance of salinity variation by smaller animals and least tolerance by larger animals. The fact that even at 10 ppt salinity, the smaller animals are not totally cutting them off from the environment may be an important fact which enhances their survival in lower salinities.

The isolation of mantle cavity is not only produced by valve closure but also by closure of the exhalent siphon. In unfavourable salinities *S. scripta* showed closure of exhalent siphon which effectively ceases the irrigation of the mantle cavity. Same type of behaviour was also reported by Davenport (1979) and Akberali and Davenport (1981). In extreme conditions the intermittent testing of the external medium occurs and is found to be advantageous since it allows the *S. scripta* to monitor the situation. This type of testing behaviour was also reported for *Mytilus edulis* (Coleman and Trueman, 1971), *Modiolus* (Pierce, 1971) and *Donax denticulatus* (Trueman, 1983). These observations were cleared when the animals acclimated at very low salinity (5 ppt) were subjected to gradual increase in salinity (Table 1). Fig. 6 indicate that when the animals were subjected to very low salinity (5 ppt) they totally cut off from the ambient medium, as also observed in Fig. 3,4 and 5. But once a near favourable or favourable salinity was obtained after a prolonged unfavourable condition they do active pumping most probably to repay the oxygen debt they had already built. Here also smaller animals resumed their ventilatory activity at lower salinities than the medium, and the medium sized one earlier than the large (Fig. 6). All the above observations clearly indicate that the behavioural component is playing an important role in the survival of *S. scripta* in varying salinities.

CHAPTER - 3

PHYSIOLOGICAL ADAPTATIONS

Physiological responses of animals towards environmental changes has evoked extensive research at organismal, tissue or cellular levels. In providing an assessment of the condition of an individual the physiological responses have three important attributes. 1. they represent an integration of many cellular and biochemical processes that can alter in response to changes in the environment. 2. they represent non-specific (general) response to the sum of environmental stimuli which are complementary to more specific responses at the biochemical level, and 3. they are capable of reflecting deterioration in the environment before effects manifest themselves in the population or the community level (Widdows, 1985a). An attempt has been made in this chapter to understand the physiological flexibility of *S. scripta* in relation to salinity by studying the physiological parameters such as clearance rate, absorption efficiency, oxygen consumption, ammonia excretion, O:N ratio and ionic (Na^+, K^+ and Cl^-) regulation.

3.1 CLEARANCE RATE

3.1.1 Introduction

The bivalves are predominantly 'suspension' or 'filter' feeders. They obtain their food by filtering of suspended particles from the water passing through the gills. The

filtration rate or clearance rate is the volume of the water cleared of particles in unit time (Bayne *et al.*, 1985).

There are various studies regarding the clearance rate of bivalves (Thompson and Bayne, 1972; Brand and Taylor, 1974; Bayne *et al.*, 1977 ; Shumway and Youngson, 1979) and the feeding mechanisms of various suspension feeding organisms were summarised by Pandian (1975a). Different types of live algal suspensions were used by Winter (1973), *Dunaliella euchlora*; Thompson and Bayne (1974), *Tetraselmis suecica*; Vahl (1972), mixture of *Isochrysis galbana* and *Monochrysis lutheri* and Sanina (1976), *Chlamydomonas* sp. and *Scenedesmus quadricauda*.

Like other physiological functions filtration rate is affected by a number of environmental factors such as salinity (Loosanoff, 1950; Nagabhushaṇam, 1956; Bohle, 1972; Mane, 1975; Alagarswami and Victor, 1976; and Widdows, 1985b), temperature (Dame, 1972; Widdows, 1973b; Wilson and Seed, 1974; Schulte, 1975; and Bayne *et al.*, 1976c), food concentration (Winter, 1969; 1970; 1973; 1978; Tenore and Dunstan, 1973; Wilson and Seed, 1974; Schulte, 1975; Sanina, 1976; Epifanio and Ewart, 1977; Navarro and Winter, 1982; Wright *et al.*, 1982) and tides (Widdows and Bayne, 1971; Langton and Gabbott, 1974; Mathers, 1976; Morton. 1977). In addition to the environmental factors clearance rate is also affected by the size of the organism (Walne, 1972; Winter 1973; Thompson and Bayne 1974; Bayne *et al.*, 1975).

Since *S. scripta* shows the behavioural modifications in various salinities, the clearance rate is likely to vary with salinity. So the aim is to delineate the effect of salinity and size on the clearance rate of *S. scripta*.

3.1.2 Materials and methods

3.1.2.1 Acclimation of test animals

Sunetta scripta were collected from the area as described in chapter 2 during the pre-monsoon period when salinity was around 30 ppt. Clams were immediately brought to the laboratory in polythene bags containing sea water collected from the same site, cleaned and grouped into three size groups, small (1.5 $\pm$ 0.5 cm), medium (2.5 $\pm$ 0.5 cm) and large (3.5 $\pm$ 0.5 cm). The animals were acclimated in 30 ppt as given in chapter 2. After the acclimation of three days, the salinity of the medium was altered slowly by the addition of filtered water of higher salinity (prepared by the evaporation of sea water) and deionised water so that salinity reached 35 ppt and 25 ppt respectively within three days. Again after three days the process was repeated so that the experimental salinities of 40, 45, 20, 15, 10 and 5 ppt were obtained. Throughout the acclimation period *S. scripta* were fed on *Synechocystis salina* and feeding was stopped 24 hrs before the commencement of experiments. The salinity of the water samples was determined by modified Mohr Knudson method (Strickland and Parsons, 1972).

3.1.2.2 Experimental setup

The filtration rates were determined using an indirect method by monitoring the reduction in particle concentration during definite time interval (Bayne, *et al.*, 1985). The experiments were done in wide mouthed conical flask of 2 litre capacity and completely covered with black paper so as to cut off light for reducing cell division and prevents the aggregation of algal cells, since the blue green algae *S. salina* used for the study is found to be photosensitive. The animals which were acclimated at different salinities were carefully introduced into the conical flask (two numbers of same size group) containing 2 litres of filtered (42 Whatman filter paper) sea water of corresponding salinities and allowed to recover from the handling shock. After observing the animals are actively filtering, added a known quantity of algal culture solution (3 days old culture) so that the algal concentration will be 0.8 mg dry weight/l. This concentration was fixed by conducting piolet experiments and was found to be the optimum concentration for feeding. The solution was allowed to mix thoroughly by gently bubbling air into the vessel without causing apparent disturbance to the animal. After thorough mixing the initial sample of 10 ml was pipetted out and after two hours the next sample was taken. Abel (1976) stated that the equation used for computation of the rate of clearance assumes that the clearance 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. Hence this experiments were restricted for a period of two hours. The samples were analysed for fluorescence units using Fluorescence Spectrophotometer at emission 286 nm and excitation at 574 nm and converted this units into dry weights (Widdows, personal communication). A concentration of 0.8 mg dry weight/l gives 170 fluorescence units. The experiments were repeated at different salinities ranging from 5 ppt to 45 ppt with an interval of 5 ppt in three size groups. Filtration (clearance) rate was calculated in litres/hour (l/h) using Quayle's equation (1948).

$$m = \frac{M}{n\ t} \quad \log_e \frac{Co}{Ct}$$

where

m = Filtration rate (l/h)

M = Volume of the test solution (sea water) (l)

n = Number of animals per test vessel

t = Time interval between sampling (h)

Co = Initial concentration of algal suspension

Ct = Final concentration of algal suspension

3.1.2.3 Data analysis

The rate of most physiological processes are dependent on individual body size (reviewed by Bayne *et al* 1976c; d). So the relationship between body weight and physiological measurements were described by the simple allometric equation after the $\log_{10}$ transformation of the values.

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

where Y = physiological rate

W = dry tissue weight

a and b are the intercept and slope of the Y vs W regression respectively. The data were analysed statistically (Snedecor and Cochran, 1968).

Physiological rate of clams for a standard dry body weight (1 gm) were calculated for comparison using the relationship given by Bayne and Newell (1983).

$$Ys = \left(\frac{Ws}{We}\right)^{b} x\ Ye$$

where Ys = standard value for physiological variable

Ws = standard weight (1 gm)

We = dry weight of the experimental animal

Ye = measured value for physiological variable

b = the corresponding weight exponent

3.1.3 Results

The rate of clearance of algal cells (l/h) for standard sized (1 gm dry weight) animals of different size groups at different salinities are given in the Table 2 and Fig. 7. The values varied from 0.7692 to 2.4127 l/gm/h for smaller size group, 0.7294 to 2.3421 l/gm/h for medium and 0.5701 to 2.3303 l/gm/h for larger size group (Table 2). The weight specific clearance rate showed an inverse relationship with increasing body size and decrease and increase of salinity from 30 ppt. The regressions of log clearance rate vs log dry body weight at different salinities are calculated (Fig. 8 a-g) and r, a and b values are given in the Table 3. The relationship between logarithm of clearance rate (C. R.) and logarithm of dry body weight (W) can be represented in the form of linear equation

$$\log C.\ R. = \log a + b \log W$$

The regression coefficients were analysed using analysis of covariance (Table 4) and the results showed significant ($p<0.01$) variation in clearance rate in different salinities.

3.1.4 Discussion

The filtration (clearance) rate is a parameter of great ecological significance, since it is the component of energy

Table 2. Clearance rate (l/h) of different size groups in different salinities ($\bar{x} \pm SD$) calculated for a standard sized (1 gm dry weight) animal

Salinity (ppt)	Size Groups		
	Small (1.5 ± 0.5 cm)	Medium (2.5 ± 0.5 cm)	Large (3.5 ± 0.5 cm)
5	0.0000	0.0000	0.0000
10	0.0000	0.0000	0.0000
15	0.7692 ± 0.2886	0.7294 ± 0.2204	0.5701 ± 0.2022
20	1.7742 ± 0.3282	1.2210 ± 0.2410	1.1181 ± 0.3504
25	2.0691 ± 0.4103	2.0480 ± 0.3760	1.9540 ± 0.1943
30	2.4127 ± 0.5230	2.3421 ± 0.3714	2.3303 ± 0.4168
35	2.2501 ± 0.3024	2.2006 ± 0.4055	2.1911 ± 0.4565
40	1.9057 ± 0.2585	1.3030 ± 0.2982	1.2912 ± 0.3756
45	1.5427 ± 0.6403	1.2367 ± 0.2199	1.2299 ± 0.4951

Table 3. Values of r, a and b for clearance rate at different salinities

salinity (ppt)	number of observations	r	a	b
15	30	0.8741	0.3772	0.8259
20	30	0.9068	0.7797	0.7537
25	39	0.9721	0.5742	0.9102
30	40	0.9750	0.5343	0.9499
35	35	0.9667	0.4465	0.9723
40	33	0.9320	0.4394	0.9006
45	31	0.9581	-0.7043	1.2891

Table 4. Analysis of covariance of clearance rate in different salinities

						Deviations from regression		
Salinity	df	x^2	y^2	xy	Reg.Coeff	df	ss	ms
15	29	2.8293	2.5259	2.3368	0.8259	28	0.5959	0.0213
20	29	3.4939	2.4144	2.6337	0.7538	28	0.4291	0.0153
25	38	3.8602	3.3849	3.5138	0.9103	37	0.1864	0.0050
30	39	5.2828	5.0148	5.0184	0.9500	38	0.2476	0.0065
35	34	3.2772	3.3159	3.1868	0.9724	33	0.2170	0.0066
40	32	4.0671	3.7981	3.6630	0.9006	31	0.4990	0.0161
45	30	3.6102	6.5359	4.6540	1.2891	29	0.5363	0.0185
Pooled	231	26.4207	26.9899	25.0065	0.9465	230	3.3219	0.0144
						224	2.7113	0.0121
				Difference between slopes		6	0.6106	0.1018

Comparison of slopes (6,224) = 8.4132 **

** $p < 0.01$

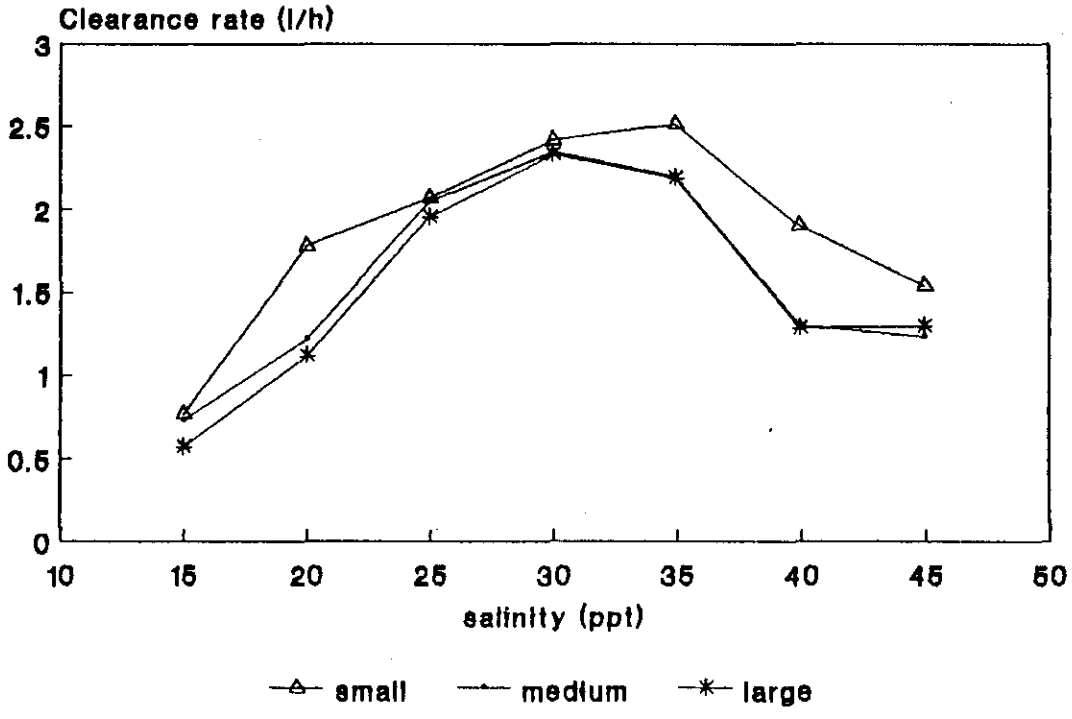


Fig. 7 Weight specific clearance rate (l/gm/h) of different size groups in different salinities (ppt).

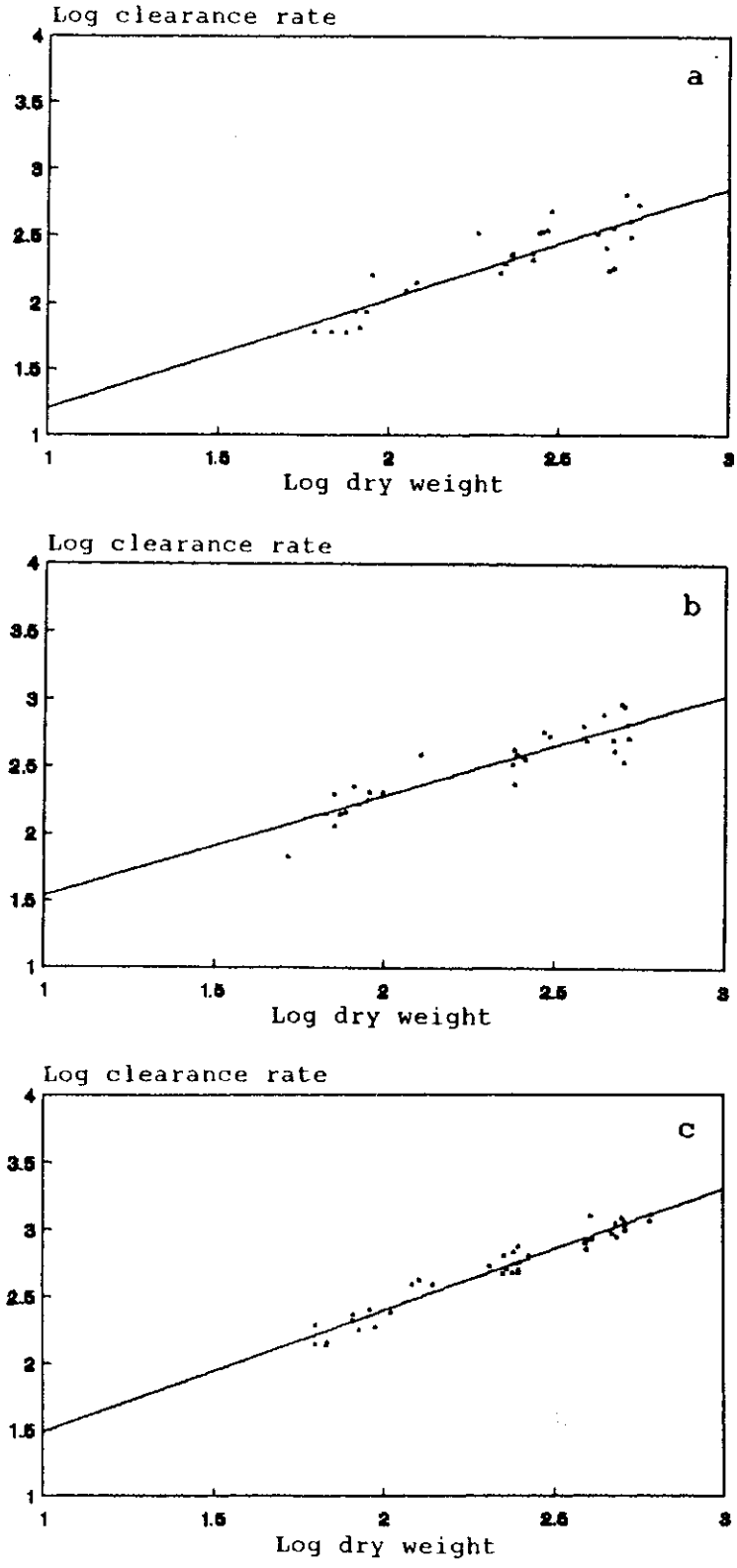
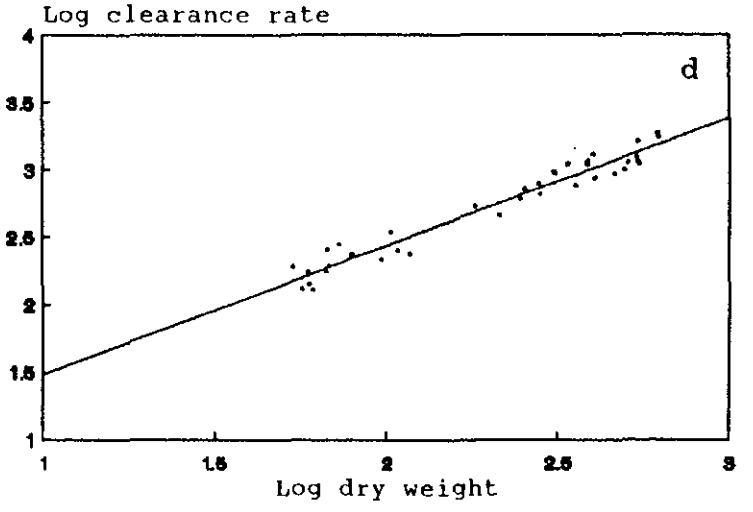


Fig.8 a,b,c Regression line showing clearance rate (ml) and dry body weight (mg) in different salinities. a 15, b 20, c 25 ppt



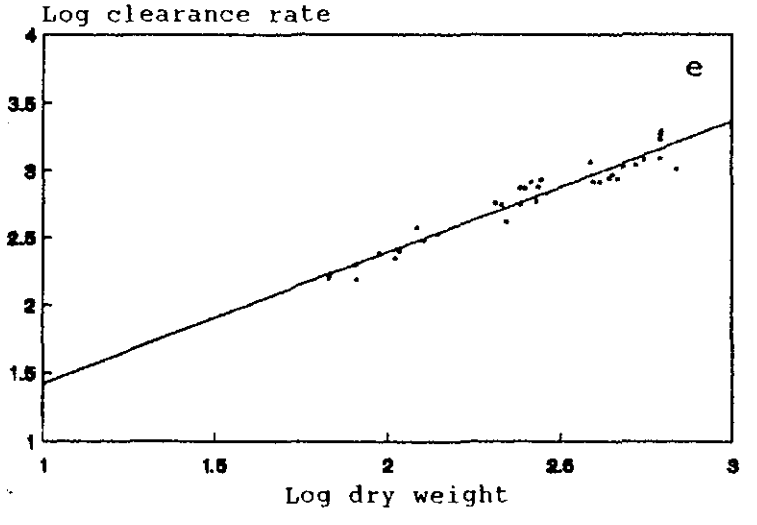
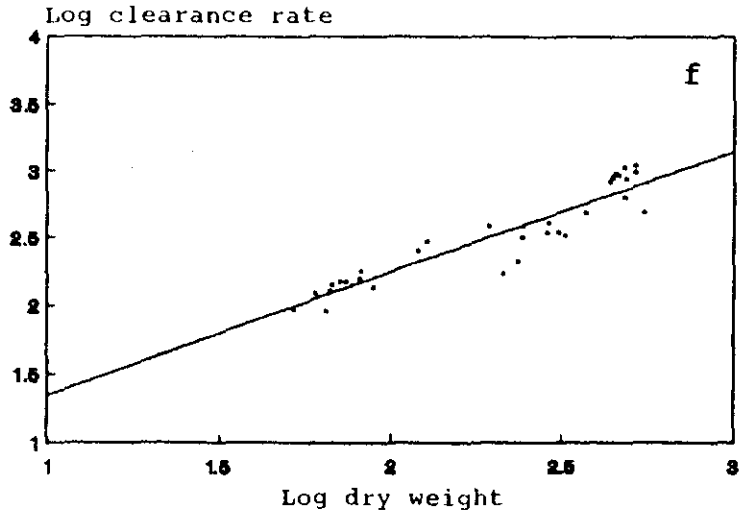


Fig. 8 d,e Regression line showing clearance rate (ml) and dry body weight (mg) in different salinities. d 30, e 35 ppt



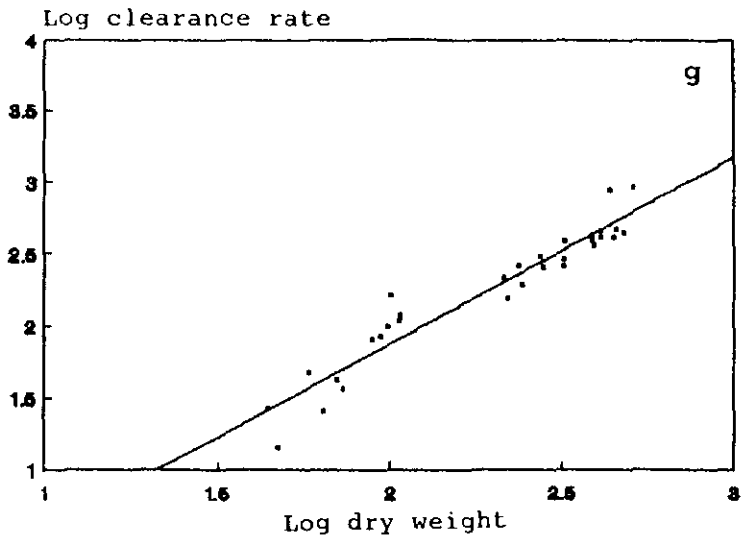


Fig. 8 f,g Regression line showing clearance rate (ml) and dry body weight (mg) in different salinities. f 40, e 45 ppt

budget which is primarily affected by stress. Having determined the filtration rate and knowing the concentration of suspended particles in water, it is possible to calculate the amount of food retained by the gills and ingested by the animal, as long as no pseudofaeces are produced. The *S. scripta*, when subjected to various salinities, it modifies the rate of filtration which gives an idea about the physiological flexibility of the organism and it controls the net energy balance in response to changes brought about in physiology or metabolism.

The 'b' values estimated at 15, 20, 25, 30, 35, 40 and 45 ppt are 0.8259, 0.7537, 0.9102, 0.9499, 0.9723, 0.9006, 1.2891 respectively (Table 3). In all the salinities except 45 ppt the 'b' values obtained are indicating a proportionality between surface area (0.67) and body weight (1.0). These values are found to be higher than the values reported by Walne (1972), Winter (1973), Bayne *et al.* (1976a) and Bayne and Newell (1983). The weight exponents for filtration rate showed wide variations among different animals. It varied from negative values to a value of 0.76 in mussels (Winter, 1969; Walne, 1972). Navarro and Winter (1982) quantified the relationship between filtration rate and body size of *M. chilensis* at three different algal densities and the 'b' values varied between 0.58 and 0.62. In the present investigation the analysis of covariance showed significant variation in different salinities.

The rate of filtration and body weight showed a linear relationship ie, the filtration rate increased with increasing

body weight under all experimental conditions. But the weight specific filtration rate showed a decreasing trend with increasing body weight. Same trend was also reported by Walne (1972) and Krishnakumar (1987). In the present investigations the weight specific filtration rate of smaller size group is found to be 2.4127 l/gm/h and that of medium and larger size groups are 2.3421 l/gm/h and 2.3303 l/gm/h respectively at 30 ppt (Table 2). When compared to these values Allen (1962), Theede (1963), Thompson (1984) and Clarke and Griffiths (1990) estimated lower values of clearance rate. But higher values were reported by Widdows *et al.* (1980) and Widdows and Johnson (1988). Widdows *et al.* (1980) obtained clearance rate ranged between 2.54 to 5.21 l/h for 1.6 gm mean dry weight and 3.19 to 5.91 l/h for 2.2 gm mean dry weight and former at 30 ppt and later at 29.5 ppt salinity.

Although there is a large amount of data in the literature on the biology of filter feeding bivalves, differences in experimental procedures and techniques make it difficult to compare and contrast the effects of particular environmental factors on the functioning of filtration and biodeposition.

At 15, 20, 25, 35, 40 and 45 ppt the weight specific filtration is found to be less than that at 30 ppt (Table 2). But by scrutiny of the results of weight specific filtration rate at above and below 30 ppt, the animals maintained above 30 ppt showed greater capacity to filter than those kept below 30 ppt in all three size groups. This may be due to their greater tolerance to higher salinities when compared to lower

salinities. Same type of adaptation was also observed in *M. edulis* by Widdows (1985b). The rate of adaptation of *M. edulis* to an abrupt rise in salinity from 15 to 30 ppt is more rapid than the rate of adaptation to a decline in salinity from 30 to 15 ppt (Widdows 1985b). In the present observations the clearance rate declined sharply below 25 and above 35 ppt for medium and large size groups. For smaller size group a sudden decrease was only below 20 ppt (Fig. 7) and such a sudden decrease was not observed above 30 ppt. These observations were in agreement with the salinity tolerance of different size groups. The smaller size groups are found to be more tolerant than medium and larger size groups. Below 15 ppt all the size groups showed no filtration.

In the nine salinities studied, at 10 and 5 ppt no filtration was noticed (Table 2) which is due to the reduced movement/closure of valves and cessation of pumping activity. At 5 ppt all the three size groups showed complete closure of valves with or without insignificant openings. But at 10 ppt valve movement nearly ceases for larger size group and much reduced for medium and smaller size groups. In these salinities the animals showed retraction of siphons and closure of valves. This will inturn ceases the pumping activity and hence no food uptake. These types of depressing effect of lower salinity on filtration rate were also observed by Cole and Hepper (1954), Nagabhushanam (1956) and Widdows (1985b). Foster-Smith (1976) considered the various possibilities for control over pumping

activity, confirming that closure of exhalent siphon was the most common means of regulating pumping. For *S. scripta*, in this investigations at 20 ppt the medium and larger size group animals kept their siphon in withdrawed condition. This behaviour would account for the cessation of pumping activity. Above 35 ppt *S. scripta* showed reduced filtration. In the present investigations *S. scripta* are observed to filter more efficiently in 30 ppt than 25 and 35 ppt. Eventhough the animals are filtering at 25 and 35 ppt, a rhythmic opening of valves associated with muscular activity and water pumping is seen as a regular process only at 30 ppt.

3.2 ABSORPTION EFFICIENCY

3.2.1 Introduction

A large and variable proportion of the particulate matter ingested by the organisms is refractory and not available as an energy source. The efficiency with which an organism absorbs ration by the digestive system is the absorption efficiency (Conover, 1966). Because of the difficulty to recover all the faeces produced by an aquatic filter feeder, direct estimation of absorption efficiency by comparing the organic or energy content of the food and faeces is impractical. Hence, Conover (1966) proposed a method by which absorption efficiency may be determined from the ash-free dry weight to dry weight ratios of

food and faecal samples. This method is based on the premise that only the organic component of the food is significantly affected by digestion.

The factors affecting absorption efficiency in bivalves have been considered by Bayne *et al.* (1976c), Winter (1978), Widdows *et al* (1979a), Vahl (1980) and Kiorboe and Mohlenberg (1981). Bayne *et al.* (1976c) concluded that increase in temperature slightly depressed absorption efficiencies in *Mytilus* species. Elvin and Gonor (1979) observed that exposure to elevated temperatures during low tide in spring and summer could enhance absorption efficiency of *Mytilus californianus*. In the laboratory experiments using pure algal cultures, the absorption efficiency is observed to decline rapidly at high cell concentrations (Thompson and Bayne, 1974; Widdows, 1978a; Griffiths, 1980). The presence of inorganic particles in suspension may 'dilute' organic matter present (Widdows, *et al.*, 1979a; Vahl, 1980) and bring about a reduction in absorption efficiency. In addition to the above factors salinity can also influence absorption efficiency (Widdows 1985b). The present study is aimed to find out the absorption efficiency of different size groups of *S. scripta* and also to understand the impact of salinity on this parameter.

3.2.2 Materials and methods

Faeces of *S. scripta* in different salinities were collected with the help of a pipette into washed, ashed and pre-weighed

glass fibre filter (4.5 cm) and washed the filter with distilled water. A known volume (5 liters) of the sea water containing algal culture was also filtered through a washed ashed and pre-weighed GFC glass fibre filters (4.5 cm) (Strickland and Parsons, 1972) and salts were washed out of filters with distilled water. All the filters were dried at 90°C for 24 hours and weighed before and after ashing at 450°C for 3 hours in a muffle furnace. The absorption efficiency (e) was calculated by the ratio method of Conover (1966) and represents the efficiency with which clams absorb material cleared from suspension.

The Conover ratio for absorption efficiency was calculated as follows:

$$e = \frac{F-E}{(1-E)F}$$

where F = ash free dry weight : dry weight ratio of food

E = ash free dry weight : dry weight ratio of faeces

Data were analysed by the methods given by Snedecor and Cochran (1968).

3.2.3 Results

The mean values of the absorption efficiencies of different size groups at salinities are given in Table 5. Each value is the mean of five experiments. The efficiency of smaller size

group ranged between 0.56 to 0.63 and that of medium and larger size groups are 0.53 to 0.62 and 0.52 to 0.60 respectively. The maximum value of 0.63 is obtained for smaller size group in 25, 30 and 35 ppt and the maximum value of 0.62 and 0.60 for medium and larger size groups respectively are obtained in 30 and 35 ppt. The absorption efficiency is found to be decreasing when the salinity decreases or increases from optimum/nearly optimum conditions. The absorption efficiency of clams in all the salinities are found to be decreasing as the size increases (Fig. 9).

The absorption efficiency of different salinity and size group was analysed using anova technique with repeated number of observations. The anova is given in Table 6. From the table it follows that there is significant difference between salinity and between size at 1% level of significance. The least significant difference among salinity is 0.0292 and that of size group is 0.0191. The absorption efficiency of all the three size groups was found to be maximum in 25 to 35 ppt followed by 40 and 45 ppt, 20 and 15 ppt. Among the size groups the absorption efficiency is more in small followed by medium and large.

3.2.4 Discussion

In the various experiments the absorption efficiency varied

Table 5. Absorption efficiencies of different size groups in different salinities ($\bar{x} \pm SD$, n = 5)

Salinity (ppt)	Size Groups		
	Small (1.5 ± 0.5 cm)	Medium (2.5 ± 0.5 cm)	Large (3.5 ± 0.5 cm)
15	0.56 ± 0.054	0.53 ± 0.025	0.52 ± 0.028
20	0.58 ± 0.055	0.55 ± 0.035	0.54 ± 0.058
25	0.63 ± 0.032	0.60 ± 0.042	0.58 ± 0.049
30	0.63 ± 0.028	0.62 ± 0.058	0.60 ± 0.041
35	0.63 ± 0.031	0.62 ± 0.049	0.60 ± 0.057
40	0.60 ± 0.034	0.58 ± 0.032	0.52 ± 0.022
45	0.60 ± 0.031	0.58 ± 0.043	0.52 ± 0.033

Table 6. Anova for absorption efficiencies of different size groups and salinities

Source	ss	df	ms	F
Total	0.2923	104	-	-
Salinity	0.0902	6	0.0150	9.375**
Size	0.0441	2	0.0221	13.813**
Error	0.1580	96	0.0016	-

LSD for Salinity = 0.0292

LSD for Size = 0.0191

** ($p < 0.01$)

Size	Mean	Rank
Small	0.6043	1
Medium	0.5829	2
Large	0.5543	3

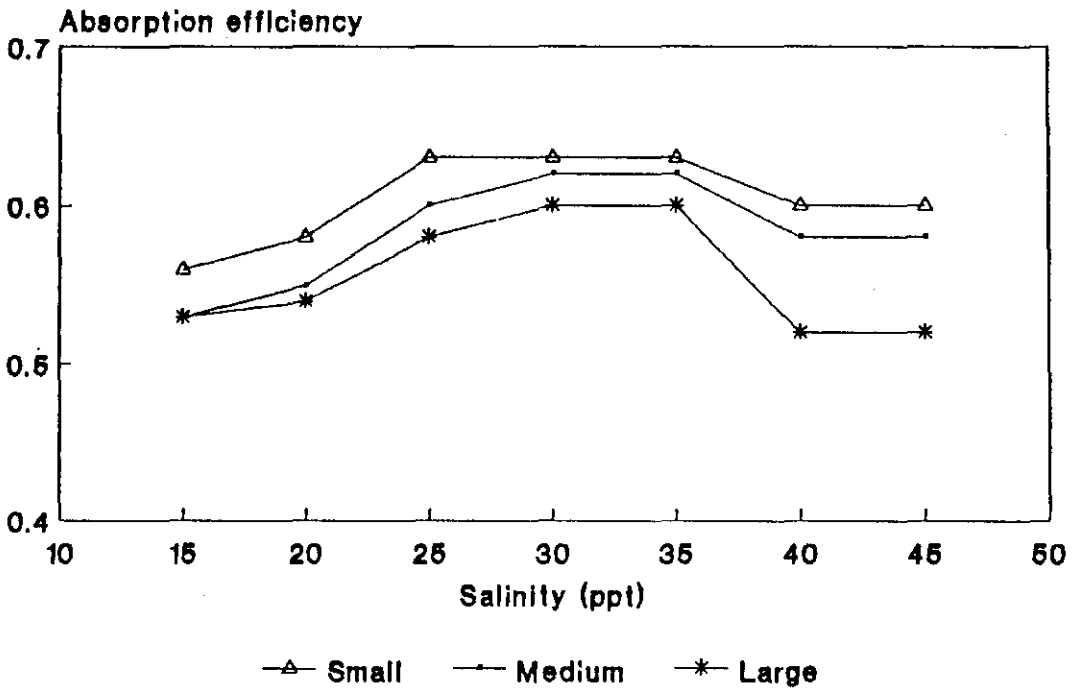


Fig. 9 Absorption efficiencies of different size groups in different salinities (ppt)

between 0.52 to 0.63 (Table 5). The values for *S. scripta* are well within the range described by various authors in different lamellibranchiate bivalves (summarised by Winter, 1978; Bayne and Newell, 1983).

In the present investigation the anova technique revealed that the absorption efficiency is significant at 1% level of significance among size groups and salinities (Table 6). In all the size groups the absorption efficiency is maximum in optimum/nearly optimum salinities. When the salinity alters from optimum (either increase or decrease), correspondingly there is decrease in values. The decrease of efficiencies in the unfavourable salinities indicates the loss of energy in the form of faeces, while the energy available for growth and reproduction decreases correspondingly. Widdows (1985b) estimated the absorption efficiency as a result of abrupt changes between 30 ppt and 15 ppt and found that there was no significant differences in the absorption efficiencies.

Among size groups the least significant difference is 0.0191 and the absorption efficiency is more in small size group followed by medium and large size groups. This is in well agreement with the salinity tolerance of different size groups. Among the three different size groups, the smaller size group is found to be more tolerant than medium and large size groups (Thampuran, *et al.*, 1982.). The decrease of efficiencies in unfavourable salinities may be due to the impact of salinity stress on the activity of digestive enzymes. Same was also

reported by Moore *et al.*, (1980).

3.3 OXYGEN CONSUMPTION

3.3.1 Introduction

Knowledge regarding the limits of respiratory function are important for understanding the physiological adaptation of a species. The estimation of oxygen consumption offers a useful method to assess stress since it is an index of energy expenditure to meet the demands of environmental alterations (Thompson and Bayne, 1972).

The oxygen consumption of an animal is influenced by a number of intrinsic and extrinsic factors like size, ration, season, salinity, and pollutants. The relationship between body size and metabolic rate has been studied for adult molluscs and larvae (Zeuthen, 1947; 1953; Von Bertalanffy, 1957). Some of the studies on suspension and deposit feeders are: *Pactinopecten yessoensis* (Fuji and Hashizume, 1974), *Scrobicularia plana* (Hughes, 1970), *Crassostrea virginica* (Dame, 1972), *Ostrea edulis* (Newell *et al.*, 1977; Rodhouse, 1978), *Cerastoderma edule* (Boyden, 1972a, b; Newell, 1977), *Mytilus edulis* (Bayne *et al.*, 1973; 1975; Famme, 1980a; b), *Mytilus californianus* (Bayne *et al.*, 1976a; b), *Modiolus demissus* (Kuenzler, 1961), *Aulacomya ater* (Griffiths and King, 1979), *Crepidula fornicata* (Newell and Kofoed, 1977) and *Barbatia obliquata* (Prasada Rao and

Veerasalingam, 1981).

It is well known that salinity has considerable influence on the metabolic activities of animals. Marine and brackish water invertebrates subjected to variations in salinity exhibit different respiratory behaviour (Kinne, 1971). The effect of salinity on the oxygen consumption have been described for *Mytilus galloprovincialis* (Bouxin, 1931), *Mytilus edulis* (Langerspetz and Sirkka, 1959; Shafee, 1976), *Martesia striata* (Nagabhushanam, 1962); *Geolina ceylonica*, *Anadara granosa* and *Mytilus edulis* (Bayne, 1973b); *Katelysia opima* and *Meretrix meretrix* (Ranade, 1973); *Meretrix casta* (Salih, 1978), *Nausitora hedleyi* (Mohan, 1979); *Mytilus edulis* and *Katherina tunicata* (Stickle and Sabourin, 1979); *Meretrix meretrix* (Deshmukh, 1979) and *Sunetta scripta* (Thampuran, 1986). Kinne (1964a; b), Ghiretti (1966), Remane and Schlieper (1971) and Newell (1979) have reviewed the effect of salinity on the oxygen consumption of molluscs.

The aim of this study is to understand and delineate the effect of size and salinity on the oxygen consumption of *S. scripta*.

3.3.2 Materials and methods

For oxygen consumption studies animals which were subjected for the estimation of clearance rate were used. Studies were done using respirometer designed by Mohan and Cherian (1980).

The duration of the sampling was 2 hours. A control was also ran under identical condition without the animal. The temperature and pH were maintained at constant level. The water samples were collected using sampling bottle of 10 ml capacity and were analysed for dissolved oxygen content using Winkler's micro method (Welsh and Smith, 1953).

Oxygen consumption (O.C) (ml O_2/h) = Initial oxygen content (ml O_2/l) - Final oxygen content (ml O_2/l) x (Volume of respirometer - Volume of the animal) x $\frac{60}{\text{Time interval (min)}}$

The data were analysed as given in "clearance rate".

3.3.3 Results

The rate of oxygen consumption (ml O_2/gm/h) for different size groups are shown in Table 7 and Fig. 10. From the table it can be noted that the rate of oxygen consumption for a standard sized (1 gm dry body weight) animal decreases with increase in body size and with increase and decrease of salinity from 30 ppt. When the logarithms of the dry body weight (W) (gm) were plotted aginst the logarithms of oxygen consumption (O.C) (ml O_2/h) the points are found to cluster around a straight line, hence follow the relationship

$$\log O.C = \log a + b \log W$$

Table 7. Dissolved oxygen consumption (ml O2/h) of different size groups in different salinities ($\bar{x}$ ± SD) calculated for standard sized (1 gm dry weight) animal

Salinity (ppt)	Size Groups		
	Small (1.5 ± 0.5 cm)	Medium (2.5 ± 0.5 cm)	Large (3.5 ± 0.5 cm)
5	0.4477 ± 0.2677	0.4132 ± 0.1371	0.3514 ± 0.1340
10	0.3423 ± 0.1621	0.3972 ± 0.1324	0.3164 ± 0.1374
15	0.5976 ± 0.2920	0.6244 ± 0.2381	0.6285 ± 0.1537
20	0.7672 ± 0.5078	0.6995 ± 0.2106	0.6700 ± 0.2070
25	1.0062 ± 0.6833	0.9790 ± 0.2547	0.9619 ± 0.1911
30	1.0310 ± 0.5236	0.9706 ± 0.5123	0.9389 ± 0.3675
35	1.0114 ± 0.6051	0.9246 ± 0.1749	0.9487 ± 0.2039
40	0.5545 ± 0.3216	0.5566 ± 0.2123	0.4957 ± 0.1086
45	0.5902 ± 0.3783	0.5614 ± 0.3159	0.5617 ± 0.1760

Table 8. Values of r, a and b for dissolved oxygen consumption in different salinities

Salinity	Number of observations	r	a	b
5	28	0.8049	-0.6963	1.1072
10	28	0.7865	0.6352	0.6309
15	33	0.8403	-0.0391	0.9322
20	31	0.8558	-0.1756	0.9940
25	39	0.8681	-0.0016	0.9858
30	35	0.9226	-0.5469	1.2251
35	36	0.8426	0.3585	0.8638
40	34	0.8032	0.4041	0.7600
45	31	0.8636	-0.3352	1.0121

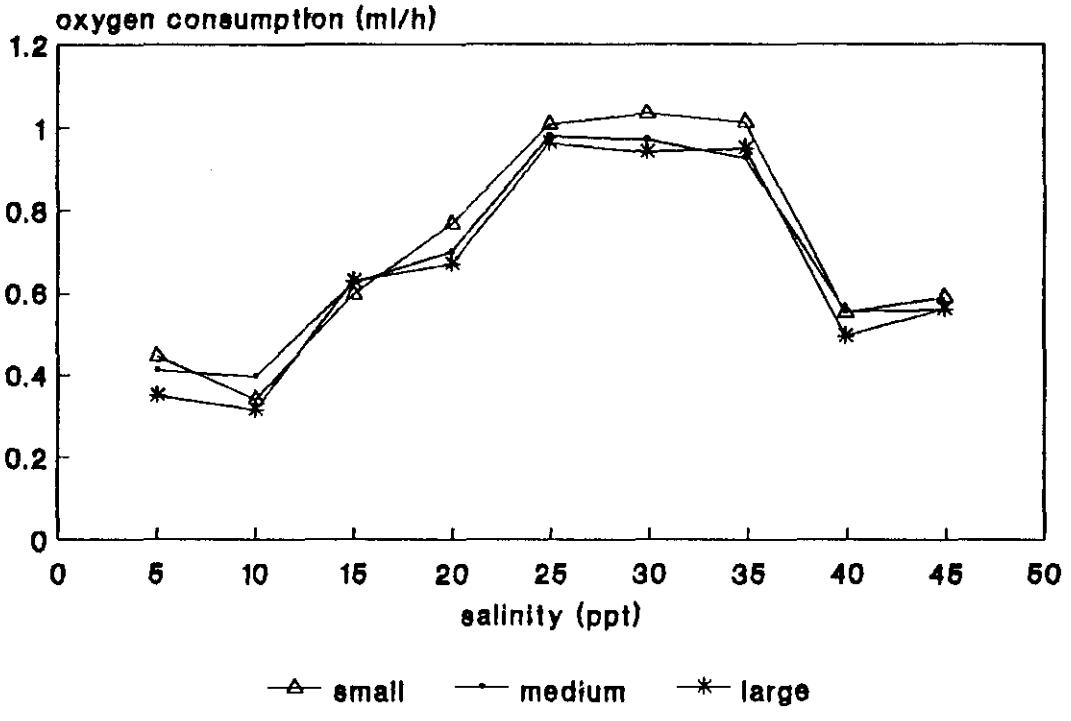


Fig. 10 Weight specific oxygen consumption ($ml O_2/gm/h$) of different size groups in different salinities (ppt)

The regression of log oxygen consumption vs log dry weight at different salinities were calculated and shown in the Figure 11 a-i. The r, a and b values for different salinities were given in the Table 8. To compare the regression coefficients analysis of covariance was employed (Table 9). The slopes were significant ($p<0.01$) only at 30 ppt.

3.3.4 Discussion

A scrutiny of the results in the present study shows that the 'b' values obtained for *S. scripta* at 5, 10, 15, 20, 25, 30, 35, 40 and 45 ppt are 1.1072, 0.6309, 0.9322, 0.9940, 0.9858, 1.2251, 0.8638, 0.7600 and 1.0121 respectively (Table 8). The values ranging from 0.6309 to 1.2251 revealed that the regression coefficient was significantly different only at 30 ppt (Table 9). Von Bertalanffy (1957) has identified three metabolic types, (1) rate of respiration proportional to surface area (b = 0.67) (2) respiration rate proportional to weight (1.00) (3) intermediate group (b = 0.67-1.00). In the present study at 40, 35, 25, 20, and 15 ppt the 'b' values were between proportionality weight and surface area (0.67 to 1.00). The 'b' value >1 were obtained at 5, 30, and 45 ppt and <0.67 at 10 ppt. Existence of metabolic type other than those proposed by Von Bertalanffy (1957) has been reported by Kuenzler (1961) and Kennedy and Mihursky (1972). The values obtained in the present study were comparable with the values of Rodhouse (1978). He

Table 9. Analysis of covariance of rate of dissolved oxygen consumption in different salinities

						Deviations from regression		
Salinity	df	x^2	y^2	xy	Reg.Coeff	df	ss	ms
5	27	2.7626	5.2280	3.0588	1.1072	26	1.8413	0.0708
10	27	3.8128	2.4538	2.4053	0.6308	26	0.9364	0.0360
15	32	2.8815	3.5467	2.6862	0.9322	31	1.0426	0.0336
20	30	3.6301	4.8968	3.6083	0.9940	29	1.3102	0.0452
25	38	3.8602	4.9780	3.8052	0.9858	37	1.2269	0.0332
30	34	3.7695	6.6461	4.6179	1.2251	33	0.9889	0.0300
35	35	3.6340	3.8193	3.1391	0.8638	34	1.1077	0.0226
40	33	4.4669	3.9991	3.3948	0.7600	32	1.4190	0.0443
45	30	3.3335	4.5783	3.3738	1.0121	29	1.1637	0.0401
Pooled	286	32.1511	40.1461	30.0894	0.9359	285	11.9860	0.0421
						277	11.0365	0.0398
				Difference between slopes		8	0.9494	0.1187

Comparison of slopes (8,277) = 2.979 **

** $p < 0.01$

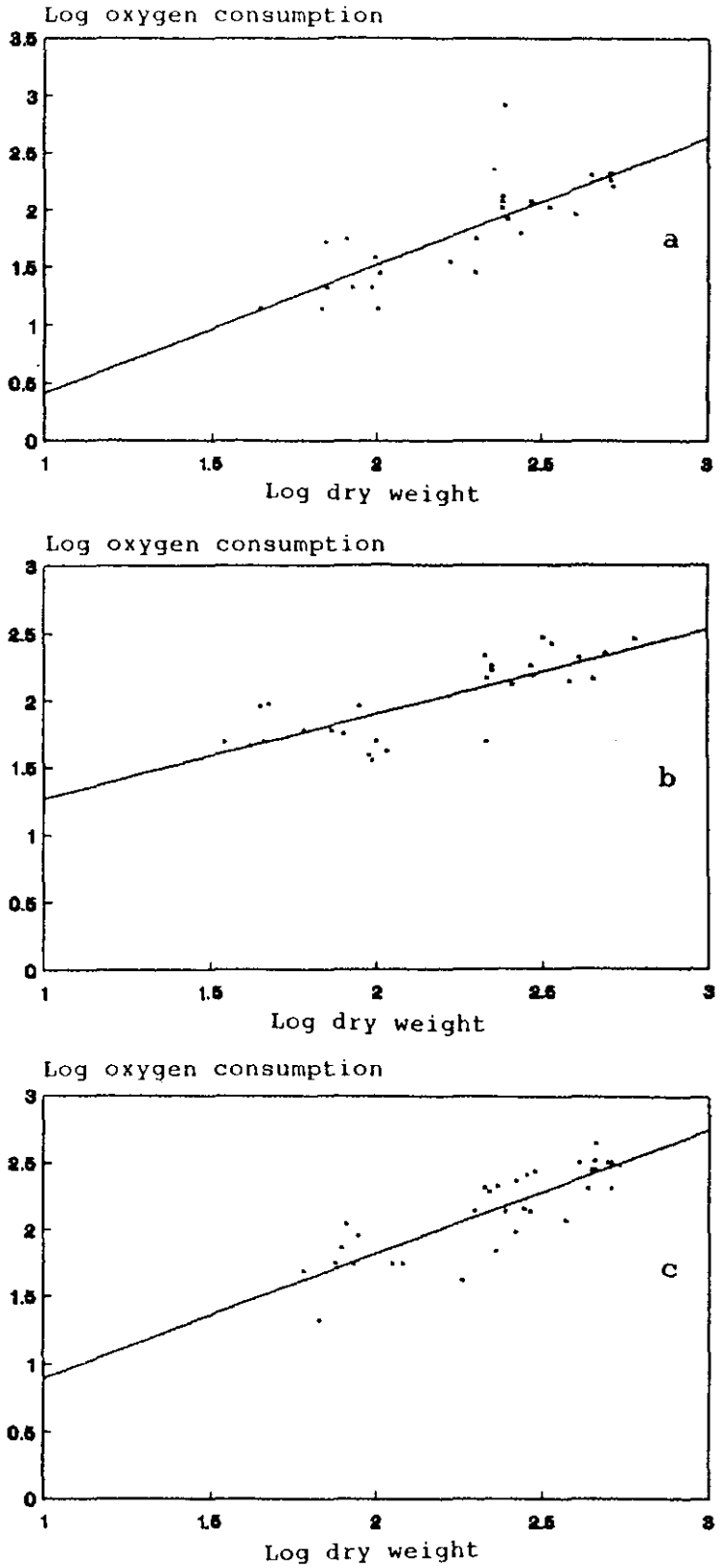


Fig. 11 a,b,c Regression line showing oxygen consumption (mlO_2/h) and dry body weight (gmx1000) in different salinities.
a 5, b 10, c 15 ppt

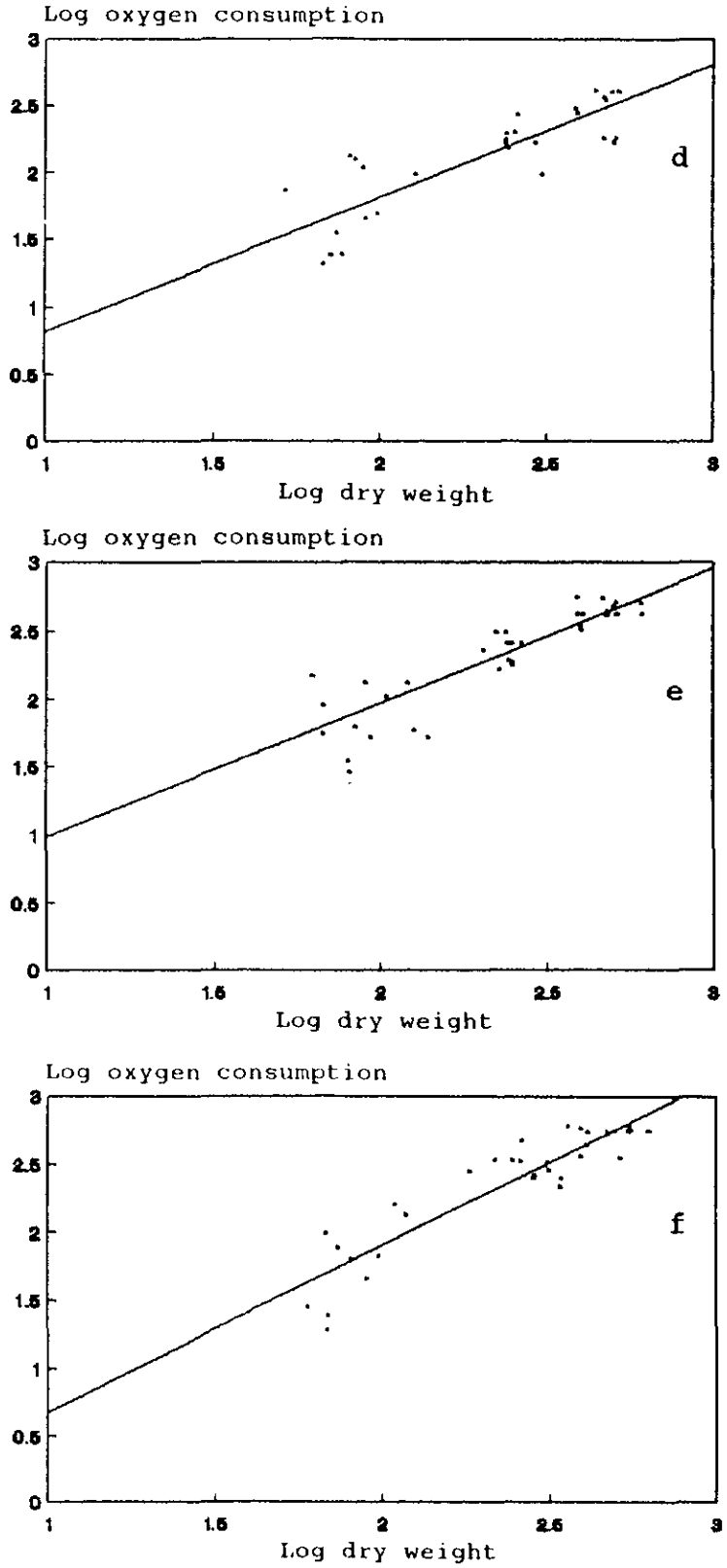
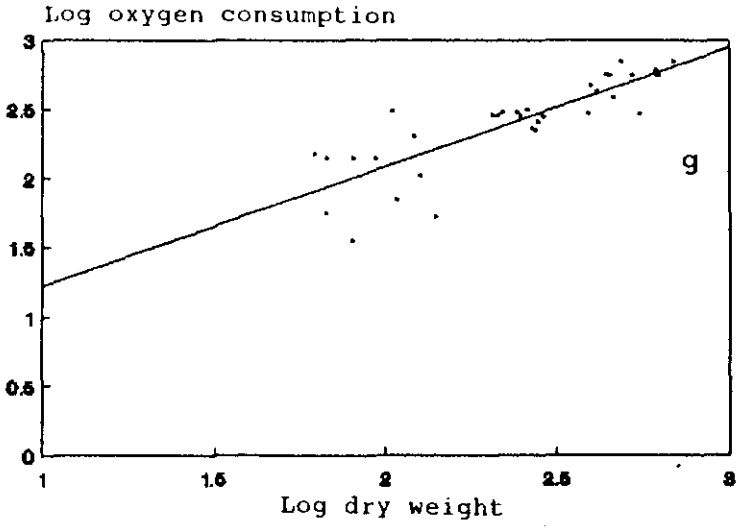
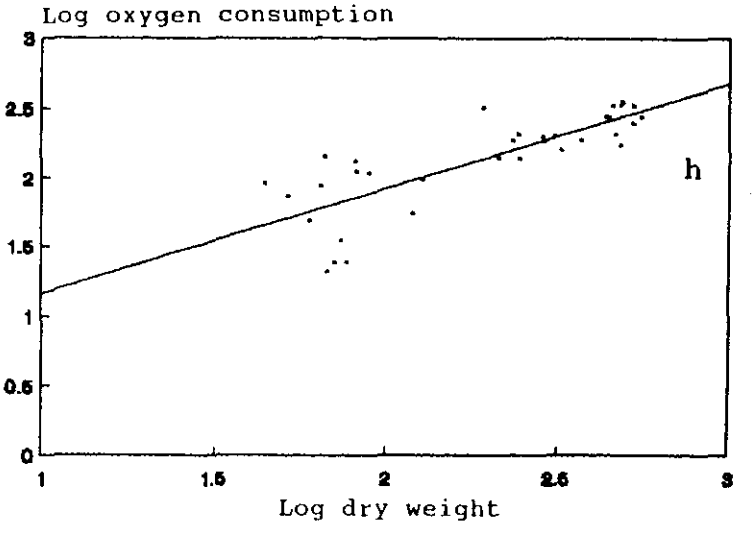


Fig. 11 d, e, f Regression line showing oxygen consumption (mlO_2/h) and dry body weight (gmx1000) in different salinities.
a 20, b 25, c 30 ppt





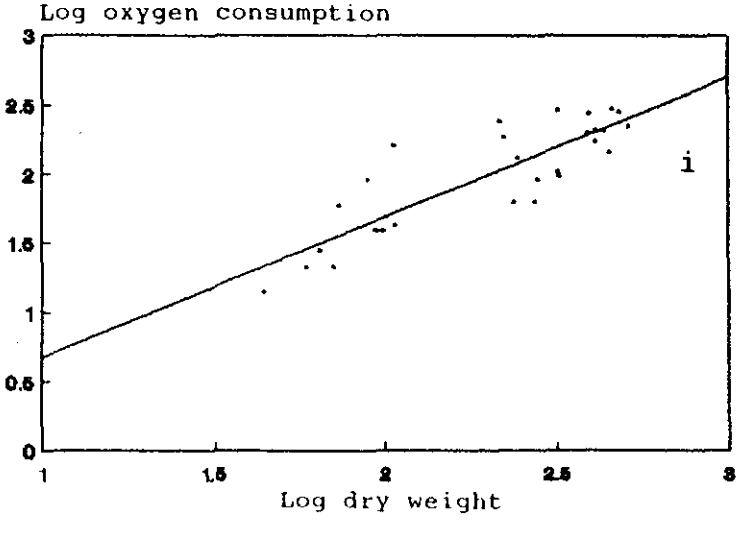


Fig. 11 g, h, i Regression line showing oxygen consumption (mlO_2/h) and dry body weight (gmx1000) in different salinities.
a 35, b 40, c 45 ppt

got 'b' value between 0.899 and 1.090 for *Ostrea edulis*. The value reported for *Crassostrea virginica* (Dame, 1972) vary from 0.603 to 0.734 and that for *Mylilus edulis* (Bayne, *et al.*, 1973) ranged between 0.670 and 0.744. A mean exponent value of 0.7 was reported for molluscs by Bayne and Newell (1983). Zeuthen (1953) and Rao and Bullock (1954) reported that the 'b' values change within a group of animals and is not constant for the same species under different environmental conditions and at different developmental stages

The weight specific oxygen consumption of different salinities were compared and the results concluded that the weight specific oxygen consumption for smaller clams were greater than the medium and larger clams in almost all the salinities studied (Table 7). These findings are in general agreement with the observations of the earlier workers on the size dependent respiration in molluscs (Zeuthen, 1955; Davies, 1967; Ganapati and Ramasastry, 1972; Kennedy and Mihursky, 1972; Mangapathy Rao *et al.*, 1974). In *Martesia striata* the oxygen consumption decreased with increase of size (Nagabhushanam, 1966). The same trend was also reported by Salih (1978) in *Meretrix casta*, Deshmukh (1979) in *Meretrix meretrix* and Mohan (1979) in *Nausitora hedleyi* and *Teredo furcifera*. Famme (1980a) also observed high weight specific oxygen consumption in smaller specimens of *Mytilus edulis* when compared to larger ones.

When comparing the weight specific oxygen consumption with other animals, the values were found to be higher in all the

nine salinities (Table 7). Bayne *et al.*(1973) and Vahl (1978) observed a seasonal variation of 0.081 to 0.256 ml/h in *Chlamys islandica* and 0.263 to 0.164 ml/h in *Mytilus edulis* respectively. Shafee (1976) reported a higher value of 0.8 ml/h at 35 ppt for *Mytilus edulis*. The values obtained for *S. scripta* in this study are comparable with the values reported by Widdows *et al.* (1980) and Hawkins *et al.* (1986b). Hawkins *et al.* (1986b) obtained a value of 1.09 ml/h for *Perna viridis*. Baby and Menon (1986) reported a much higher value of 1.7599 ml/h for *Perna indica*. Since *S. scripta* is an infaunal species it is likely that it need only less oxygen when compared to *Perna indica* which is an epifaunal species.

Analysis of the results of the experiment shows that oxygen consumption of *S. scripta* are size dependent in all nine salinities ranging from 5 to 45 ppt at 5 ppt interval. At 35, 30, and 25 ppt the oxygen consumption for smaller group of 1 gm dry body weight are 1.0114, 1.0310 and 1.0062 ml O_2/h respectively. For medium and larger size groups (1 gm) the corresponding values are 0.9246, 0.9706, 0.9790 ml O_2/h and 0.9487, 0.9389 and 0.9619 ml O_2/h respectively (Table 7). From these values it can be seen that there is no significant variation in the oxygen consumption at 35, 30 and 25 ppt within the same size group. But when taking the three size groups together within the same salinity (35, 30 and 25 ppt) the weight specific oxygen consumption is found to be decreasing with increasing body weight. But at 45 and 5 ppt the weight specific

oxygen consumption is found to be increasing when compared with the 40 and 10 ppt respectively (Fig. 10). This may be due to the increased energy demands due to higher osmotic stress. From the Fig. 10 it was further observed in *S. scripta* that in all the three size groups the consumption rises sharply as the dilution increased from 40 ppt to 35 ppt and a steady state upto 25 ppt. After 25 ppt a sharp decline occurs for smaller size group as the salinity approached down to 10 ppt. For medium and larger size groups there is a sharp decline upto 20 ppt and after that upto 15 ppt the decrease is less significant. Below 15 ppt a sharp decrease occurs. From these it can be concluded that the smaller clams are more sensitive in all the salinities than medium and larger ones.

The estuarine and marine organisms subjected to variations in salinity exhibit various metabolic types. Kinne (1971) reported four different types of respiratory behaviour for marine and brackish water invertebrates 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. Since *S. scripta* exhibited an increase in extreme salinities (5 and 45 ppt), it may be due to the euryhaline

nature. Increased oxygen uptake in response to extreme salinity is common among invertebrates (Newell, 1979) and presumably reflects elevated costs incurred within ranges of salinity tolerance. In *M. edulis* (Lagerspetz and Sirkka, 1959) and *Perna viridis* (Hawkins *et al.*, 1987) reported an increased oxygen consumption with decreased salinities.

In conclusion the oxygen consumption of *S. scripta* was found to be maximum in 30 ppt which was reported as the optimum salinity and when the salinity alters from the optimum (unfavourable salinities) the oxygen consumptions were found to be reduced. But in extreme salinity conditions (5 and 45 ppt) the oxygen consumptions were found to increase which can be due to the additional energy requirement.

3.4 AMMONIA EXCRETION

3.4.1 Introduction

A small proportion of the total food uptake by the animal is excreted as metabolic waste products. No organism limits its nitrogen excretion to one product, but the aquatic invertebrates commonly excrete much of their nitrogen in the form of ammonia (Bayne *et al* ., 1985). Using a variety of clams, mussels and oysters held in aquaria, Hammen (1968) found that although ammonia was the major nitrogenous excretory product, some amount of amino acid was also excreted in appreciable amounts in some species. This amino acid excretion is seemed to be proportional

to the relative surface area:mass ratio as well as transaminase levels in the tissues (Hammen, 1968). *Mytilus edulis* excretes 80-90% of the total nitrogen as ammonia, approximately 5-10% as amino-nitrogen (Bayne and Scullard, 1977; Livingstone *et al.*, 1979) and 5% as urea (Bayne, 1973a). Most attention has focused on ammonia excretion as it formed the major end product of protein catabolism. Bayne *et al.* (1976d) reviewed ammonia excretion in marine mussels and various excretory products of different organisms were summarised by Pandian (1975b).

Following study was conducted to understand the effect of size and salinity on the ammonia excretion of *S. scripta*.

3.4.2 Materials and methods

The oxygen consumption and ammonia excretion were estimated simultaneously. A sample of 10 ml was collected and estimated the ammonia content using phenolhypochlorite method (Solorzano, 1969). The rate of ammonia excretion was calculated using the following equation and the control value was deduced.

$$\text{Ammonia excretion } (\mu\text{gm } NH_4\text{-N/h}) = \text{Final concentration } (\mu\text{m}) - \text{Initial concentration } (\mu\text{m}) \times \frac{14}{1000/V} \times \frac{1}{t}$$

where

V = Volume of the sea water in which the animal is incubated

t = Incubation time

The data were analysed as given in "clearance rate".

3.4.3 Results

The rate of ammonia excretion (μgm NH_4-N/h) for standard sized (1 gm dry weight) animals of three different size groups at different salinities are given in Table 10 and Fig. 12. The values varied from 116.8650 to 242.7360 μgm NH_4-N/h for smaller size group, 108.9071 to 286.9273 μgm NH_4-N/h for medium size group and 113.7688 to 242.8053 μgm NH_4-N/h for larger size group. The ammonia nitrogen-excretion at different salinities showed no specific trend with salinities. The regression of log ammonia-nitrogen excreted vs log dry body weight at different salinities are calculated (Fig. 13 a-i) and the r, a and b values are given in Table 11. The relationship between logarithm of ammonia-nitrogen (NH_4-N) excreted and logarithm of dry body weight (W) can be represented in the form of linear equation

$$\log NH_4\text{-N} = \log a + b \log W$$

To compare the regression coefficients analysis of covariance was employed (Table 12) and the results showed no significant variations in ammonia excretion at different salinities.

Table 10. Ammonia excretion (µgm NH4-N/h) of different size groups in different salinities ($\bar{x} \pm SD$) calculated for standard sized (1 gm dry weight) animal

Salinity (ppt)	Size Groups		
	Small (1.5 ± 0.5 cm)	Medium (2.5 ± 0.5 cm)	Large (3.5 ± 0.5 cm)
5	204.3500 ± 70.1337	218.3775 ± 117.6991	235.9392 ± 46.0069
10	216.1521 ± 79.3601	286.9273 ± 108.9242	242.8053 ± 71.2581
15	192.7260 ± 127.0582	156.2004 ± 83.0696	154.7982 ± 43.2413
20	209.0060 ± 128.3660	197.9845 ± 56.7587	178.8401 ± 56.1403
25	128.5020 ± 69.2239	126.9290 ± 35.0234	133.6531 ± 51.2373
30	116.8650 ± 77.8870	129.2012 ± 39.2762	124.7460 ± 99.1375
35	122.0943 ± 66.0430	108.9071 ± 57.1744	113.7688 ± 33.5024
40	242.7360 ± 71.7574	226.6631 ± 126.8495	208.6071 ± 43.4326
45	193.5803 ± 138.8200	210.2740 ± 81.2803	203.6009 ± 60.9431

Table 11. Values of r, a and b for ammonia-nitrogen excretion in different salinities

Salinity	Number of observations	r	a	b
5	31	0.8566	1.3649	0.9778
10	28	0.8550	1.4191	0.9738
15	35	0.8005	1.1738	0.9877
20	31	0.9104	0.6821	1.1895
25	40	0.8548	1.3605	0.9061
30	38	0.8501	0.3423	1.2251
35	36	0.8608	0.9022	1.0384
40	35	0.8398	0.9752	1.1024
45	36	0.8470	1.5084	0.9154

Table 12. Analysis of covariance of rate of ammonia excretion in different salinities

						Deviations from regression		
Salinity	df	x^2	y^2	xy	Reg.Coeff	df	ss	ms
5	30	3.1197	4.0657	3.0505	0.9778	29	1.0828	0.0373
10	27	3.9981	4.8409	3.8936	0.9739	26	1.0491	0.0404
15	34	3.4187	5.2045	3.3766	0.9877	33	1.8695	0.0566
20	30	3.6301	6.1971	4.3179	1.1895	29	1.0611	0.0366
25	39	4.2706	4.7982	3.8694	0.9061	38	1.2923	0.0340
30	37	4.3982	9.1332	5.3882	1.2251	36	2.5322	0.0703
35	35	3.5149	5.1157	3.6499	1.0384	34	1.3256	0.0390
40	34	4.5000	7.7541	4.9607	1.1024	33	2.2855	0.0693
45	35	4.9485	5.7802	4.5297	0.9154	34	1.6339	0.0481
Pooled	301	35.7988	52.8896	37.0365	1.0346	300	14.5726	0.0486
						292	14.1320	0.0484
				Difference between slopes		8	0.4406	0.0551

Comparison of slopes (8,292) = 1.138 (N S)

N S Not significant

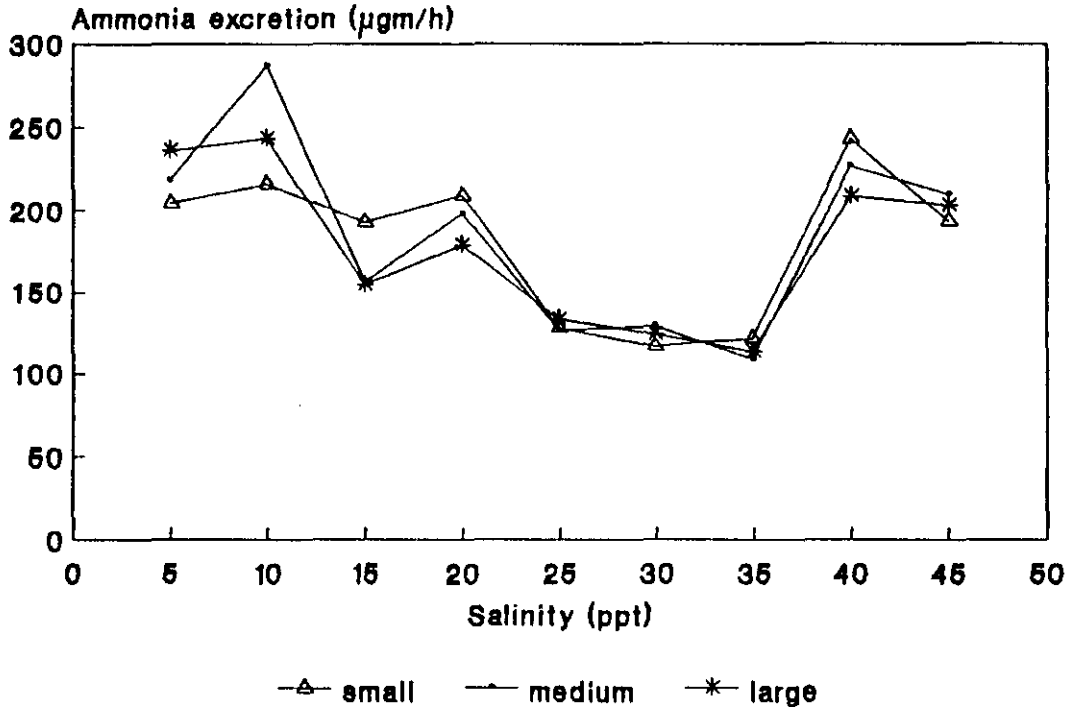
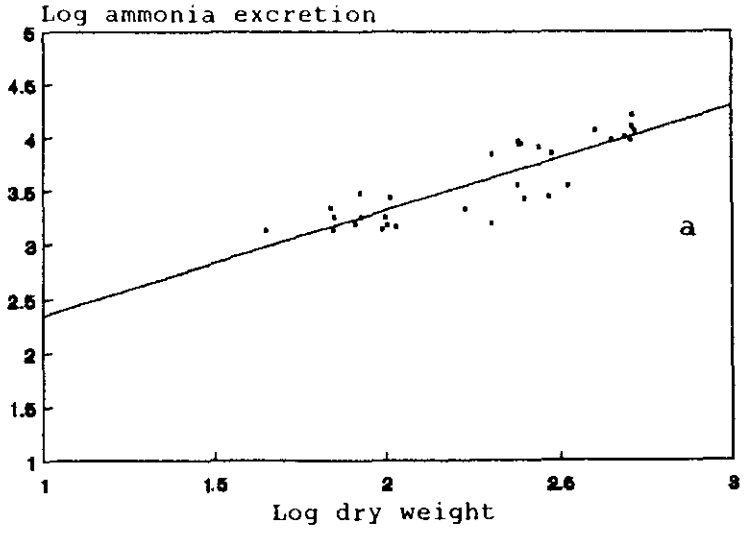
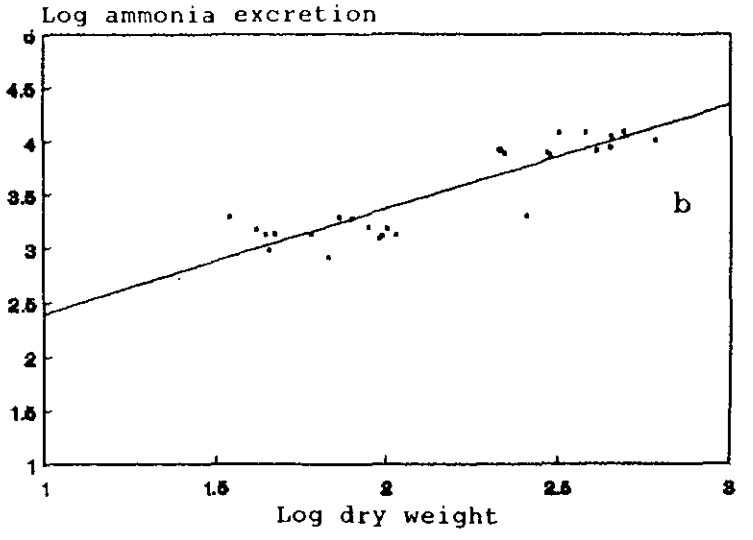


Fig. 12 Weight specific ammonia-nitrogen excretion (µgm NH_4-N/gm/h) of different size groups in different salinities (ppt)





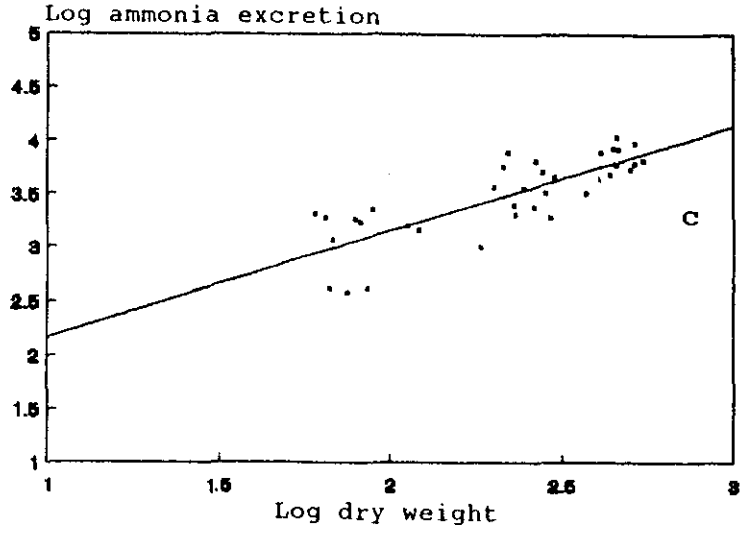
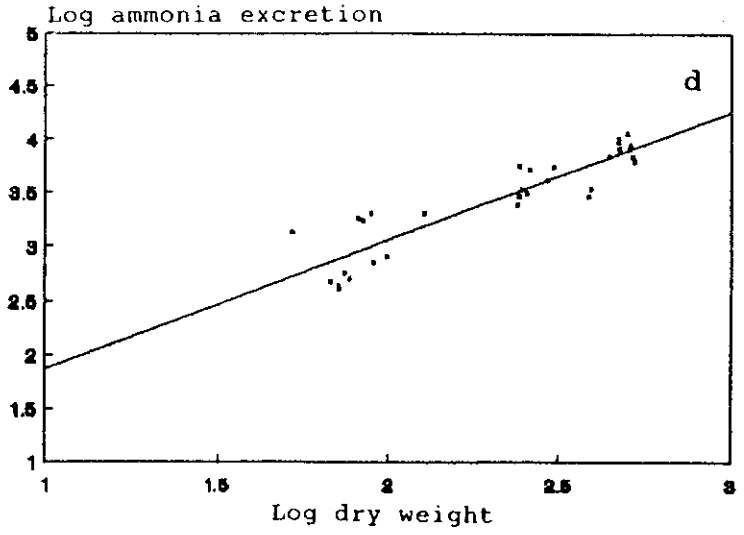
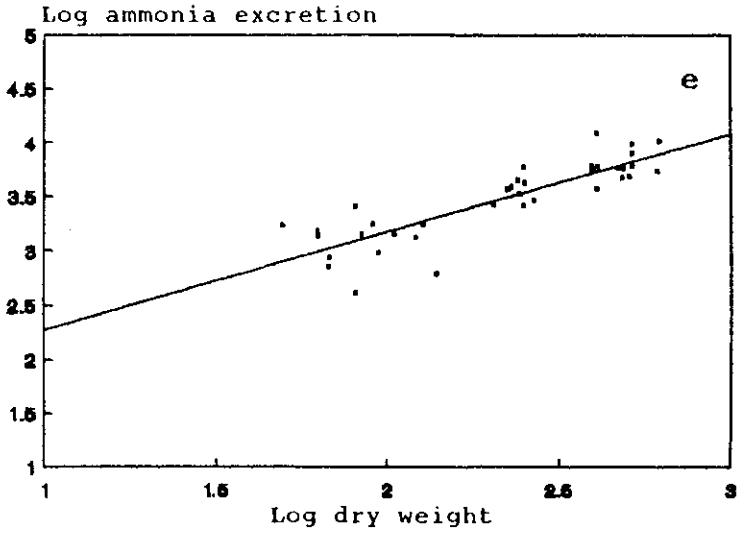


Fig. 13 a, b, c Regression line showing ammonia excretion (µgm/hx100) and dry body weight (mg) in different salinities. a 5, b 10, c 15 ppt





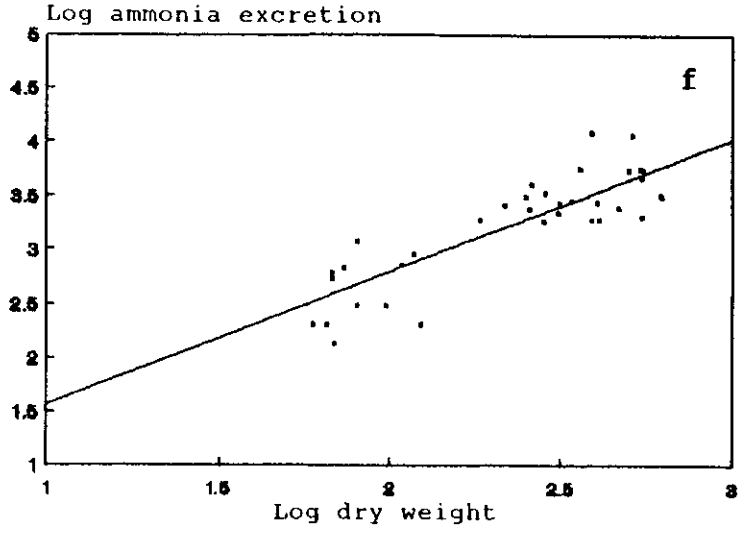


Fig. 13 d, e, f Regression line showing ammonia excretion (µgm/hx100) and dry body weight (mg) in different salinities. a 20, b 25, c 30 ppt

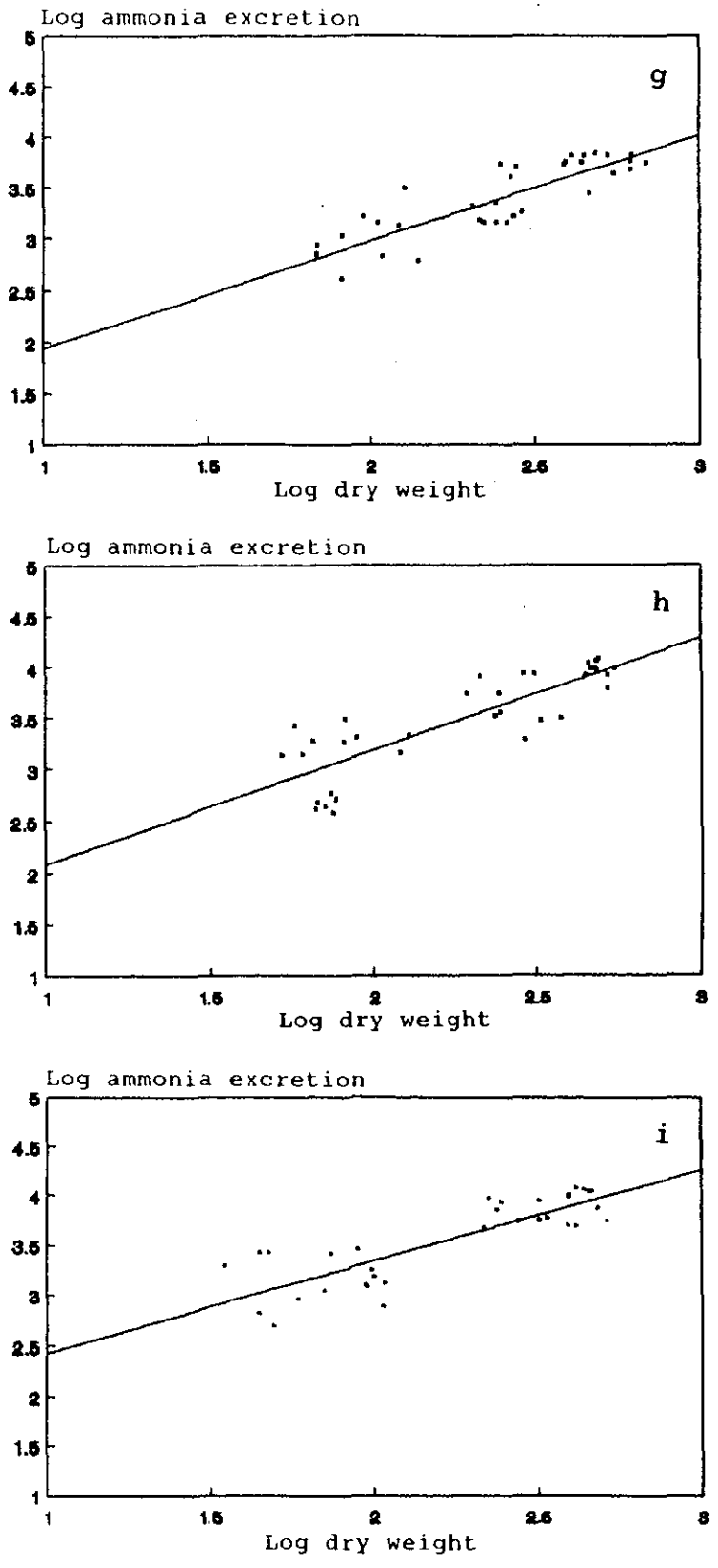


Fig. 13 g, h, i Regression line showing ammonia excretion (µgm/hx100) and dry body weight (mg) in different salinities. a 35, b 40, c 45 ppt

3.4.4 Discussion

In the present investigation 'b' value for ammonia-nitrogen excretion is found to be vary from 0.9061 to 1.2251. At 20, 30, 35 and 40 ppt the values are >1.00 and at 5, 10, 15, 25 and 45 ppt <1.00 (Table 11). To compare the regression coefficient, analysis of covariance was employed (Table 12) and the results shows that there is no significant variation in slopes. The values obtained in the present study are falling between the range of values reported for bivalves, 0.40 to 1.48 (Griffiths and Griffiths, 1987) A wide range of values for *M. edulis* was reported by Bayne and Scullard (1977), 0.482 to 1.480 and Thompson (1984), 0.021 to 1.312. When comparing with the 'b' values of *S. scripta*, comparatively less value was reported for *P. viridis* (0.4155) by Krishnakumar and Damodaran (1986) and *Donax serra* (0.55) by Brown *et al.*(1989).

In all the nine salinities studied, the range of ammonia-nitrogen produced by 1 gm dry weight varied from 116.8650 to 242.7360 µgm NH_4-N/h for smaller size group, 108.9071 to 286.9273 µgm NH_4-N/h for medium size group and 113.7688 to 242.8053 µgm NH_4-N/h for larger size group. These values were found to be higher when compared with the values reported for *M. edulis* (Widdows *et al.*, 1980). Widdows *et al.* (1980) reported the values ranged between 31.22 to 42.39 µgm NH_4-N/h. Widdows and Johnson (1988) determined ammonia excretion of *M. edulis* as a response to petroleum hydrocarbons

and copper and the results varied from 0.81 to 20.54 µgm NH_4-N/h. Krishnakumar *et al.* (1990) reported mean value for ammonia excretion of 55.8 and 193.9 µgm NH_4-N/h for *P. viridis* as a result of exposure to mercury and copper respectively. Mathew (1990) reported a value of 2.2 x 10^{-5}mg NH_4-N/h/mg for *Donax incarnatus* at 30 ppt.

The results of ammonia-nitrogen excretion of *S. scripta* show that there is no specific trend in ammonia-nitrogen excretion at different salinities (Fig. 12). This variability may be due to the disproportionate reliance on protein catabolism for energy production by the individual animals. The minimum value of 116.8650 µgm NH_4-N/h for smaller size group was recorded in 30 ppt and the minimum value of 108.9071 µgm NH_4-N/h for medium and 113.7688 µgm NH_4-N/h for larger size group were recorded in 35 ppt (Table 10). By studying the salinity tolerance the optimum salinity recorded for all the three size groups was 30 ppt (Thampuran, *et al.*, 1982) and the minimum amount of ammonia-nitrogen excretion is in optimum (30 ppt) /nearly optimum (25 and 35 ppt) conditions. It was also confirmed by monitoring the valve movement of different size groups at different salinities (chapter 2). A regular and rhythmic partial closures and openings of the valves were noticed only at 30 ppt indicating active ventilation which occur only in favourable salinities. But at 45, 15 and 5 ppt lower values were recorded when compared with 40, 20 and 10 ppt for all the three size groups (Table 10). This can be due to the

lowered movement/closure of valves and also due to the recycling of amino acids for protein synthesis (De Zwaan and Van Marrewijk, 1973). The increased catabolism enhances the demands on protein turnover and may be met by increasing the recycling of amino acids for protein synthesis (Hawkins *et al.*, 1986a). In these circumstances there would be no net change in the rate of ammonia-nitrogen excretion. But Andrews and Reid (1972) suggested that during prolonged periods of valve closure, detoxification of ammonia into urea occurs and De Zwaan and Van Marrewijk (1973) postulated the conversion of ammonia into alanine (involving alanine dehydrogenase). Speeg and Campbell (1969) opined an alternative mechanism that the free amino acid produced may react with hydrogen ions formed by the action of carbonic anhydrase on bicarbonate in the presence of urease, so releasing carbonate ions for deposition in the shell as calcium carbonate.

There is little information available on the range in which environmental factors may affect either the balance between the various nitrogenous end products or the rates of excretion. Hawkins *et al.* (1986a) estimated increased ammonia-nitrogen production due to the increased catabolism of proteins as a result of starvation and reduced filtration activity in *M. edulis*. Emerson (1969) investigated the effect of reduced salinity which causes increased ammonia-nitrogen excretion in *Macoma inconspicua*. Same was also reported by Allen and Garrett (1971) in *Mya arenaria* and Bayne (1975) in *M. edulis*. Allen

and Garrett (1971) estimated an increase from 3.22 µgm NH_4-N/d at 34 ppt to a maximum of 64.4 µgm NH_4-N/d at 17 ppt in *Mya arenaria*. Ansell and Sivadas (1973) have documented differences in excretion rates with differences in animal size in *Donax vittatus*. These studies suggest a marked variability in the rates of nitrogen excretion by bivalves and where ever recordings of oxygen consumption have been made there is an evidence that these two physiological process do not always vary in the same direction nor to the same extent in response to changes in the environment.

3.5 OXYGEN:NITROGEN RATIO

3.5.1 Introduction

A general response of bivalve molluscs to stress is the closure of valves and cessation of pumping activity and food intake. Under these circumstances the metabolic requirement is met by the utilization of nutrient reserves. Generally changes in carbohydrate, protein and lipid stores occur only in response to condition of extreme stress (Bayne and Thompson, 1970). A ratio between oxygen consumed and nitrogen excreted (O:N, calculated in atomic equivalents) provides an index of the relative utilization of protein in energy metabolism (Conover and Corner, 1968; Corner and Cowey, 1968; Bayne *et al.*, 1976e; Bayne and Newell, 1983; Widdows, 1978b; 1985a, b; Thompson,

1984; Krishnakumar and Damodaran, 1986; Hawkins *et al.*, 1987; Krishnakumar, 1987; Krishnakumar *et al.*, 1990).

So the aim of this study is to understand the effect of size and salinity on the level of oxidative and protein metabolism in *S. scripta*.

3.5.2 Materials and methods

By using the oxygen consumption and ammonia excretion data from the previous sessions, the O:N ratios were calculated in different size groups and salinities. The calculations were done by the equation given by Bayne *et al.* (1985).

$$O : N = \frac{\text{ml } O_2/h \times 1.428}{16} : \frac{\text{mg } NH_4\text{-N/h}}{14}$$

3.5.3 Results

The O:N ratio for small, medium and large size groups are given in the Table 13 and Fig. 14. The range of values for smaller, medium and larger size groups are 2.2880 to 20.5784, 2.3410 to 17.0133 and 1.7457 to 24.1115 respectively. The maximum and minimum value of 24.1115 and 1.7457 are estimated for larger size group. The O:N ratio did not show any dependence on size group, but were found to be decreasing when the salinity altered from 30 ppt. except for medium (Table 13).

Table 13. Oxygen to Nitrogen (O:N) ratio of different size groups in different salinities calculated for standard sized (1 gm dry weight) animal ($\bar{x} \pm SD$)

Salinity (ppt)	Size Groups		
	Small (1.5 ± 0.5 cm)	Medium (2.5 ± 0.5 cm)	Large (3.5 ± 0.5 cm)
5	2.2880 ± 1.3913	2.4354 ± 1.6374	1.7457 ± 0.8042
10	4.5328 ± 2.2291	2.4816 ± 0.9218	2.2900 ± 1.0545
15	5.3810 ± 2.4182	6.1623 ± 3.1541	5.3022 ± 1.3678
20	7.3269 ± 1.1046	6.6528 ± 1.6143	7.6687 ± 2.9471
25	10.9800 ± 3.7900	9.0160 ± 2.1858	9.2260 ± 2.8329
30	20.5784 ± 5.4332	15.3000 ± 3.6653	24.1115 ± 10.0380
35	13.2572 ± 3.7220	17.0133 ± 3.2181	12.1964 ± 2.7828
40	6.4320 ± 1.5929	5.2911 ± 2.1869	3.9210 ± 0.9118
45	3.5240 ± 0.8027	2.3410 ± 1.2036	3.4430 ± 1.2090

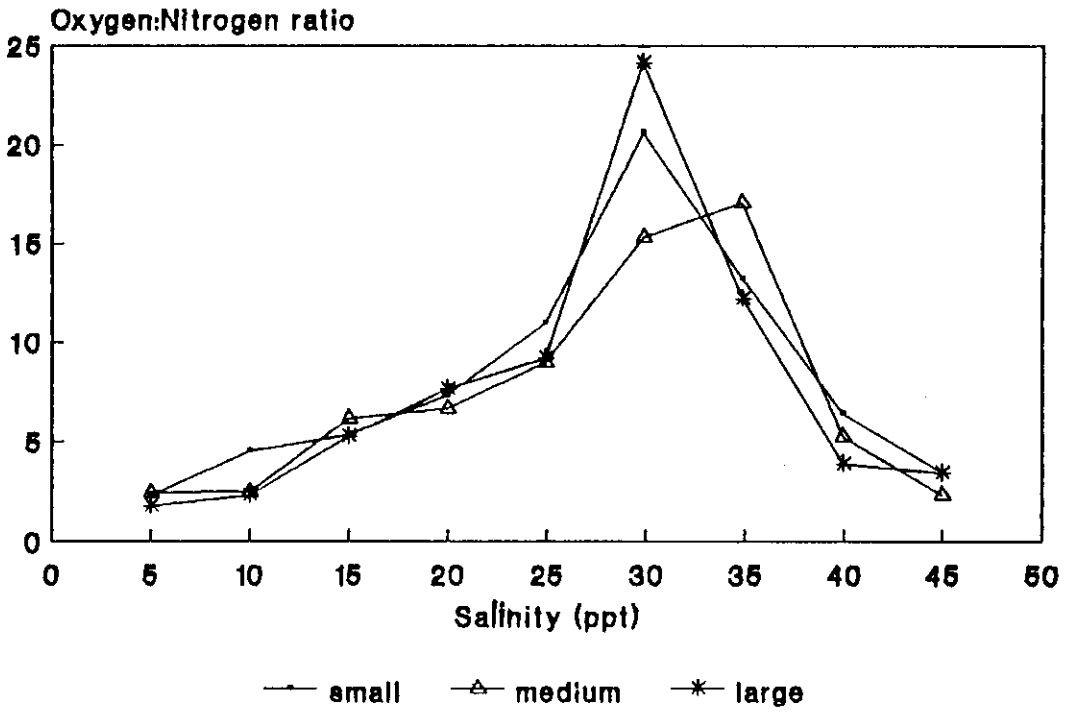


Fig. 14 Weight specific oxygen to nitrogen (O:N) ratio of different size groups in different salinities (ppt)

3.5.4 Discussion

The O:N ratio provides a useful index for understanding the 'the level of activity' of the oxidative and protein metabolism (Mayzaud, 1973) which decides the efficiency of an animal to maintain itself in a community. The atomic equivalent of O:N ratio may be used to indicate the proportion of the protein catabolized relative to carbohydrate and lipid.

Present investigation showed a clear cut stress effect on the O:N ratios with salinity (Table 13, Fig. 14). The maximum values of O:N ratios were obtained in optimum (30 ppt)/nearly optimum (25 and 35 ppt) salinity conditions. The maximum value of 24.1115 and 20.5784 for larger and smaller size groups respectively were in 30 ppt but for medium size group a maximum value of 17.0133 was at 35 ppt. The widely publicized assumption is that an O:N ratio value of 30 or less is generally indicative of a stressed condition cannot be accepted in the present instance since the maximum value obtained in the optimum salinity is 24.1115. In all the cases the values were below 30. When the salinity alters from the optimum (30 ppt), the O:N ratios were found to be decreasing. The same trend was observed in all the three size groups. The minimum O:N ratio recorded in the present investigation is 1.7457 at 5 ppt. The basic concept that could be put forward to explain the low O:N ratio is that the mussels rely heavily on the catabolism of proteins than of non-protein substrates to meet the increased demands for energy

during stress. For *M. edulis* the O:N ratio values above 50 as representative of a healthy mussel and below 30 of stressed ones (Widdows, 1985a). Bayne (1973a) investigated the seasonal changes of O:N ratio in *M. edulis*. He gave a relatively constant value of 100 signifying the predominance of carbohydrate and/or lipid catabolism over the utilization of protein in energy metabolism. Theoretical calculations show that complete catabolism of proteins as the sole energy substrate would give an O:N ratio of 9.33. During periods of minimal or negative growth (<20 and >35 ppt) the O:N ratios of *S. scripta* were reduced to theoretically minimal or less values. Utilization of ammonia-nitrogen in synthetic pathways or failure to oxidise the carbon skeletons of the amino acids will result in deviation from the theoretical expectations of O:N ratio. Since O:N ratio may vary with gametogenic cycle, nature of food and nutrient reserves, the interpretation of the O:N ratio should be based on relative change rather than absolute value (Shirely and Stickle, 1982; Widdows 1985a). This makes it necessary to collect information on this index under different ecological and physiological condition to obtain base line values which can be used as a stress index.

3.6 PHYSIOLOGICAL ENERGETICS

The physiological flexibility of an organism in relation to the environmental demand can be followed by studying the

physiological energetics of the animals. An energetic approach can provide an integration and means of assessing the overall performance in terms of the 'costs' and 'benefits' and the effectiveness of various behavioural, physiological and metabolic responses to environmental change (Shick *et al.*, 1988).

Most of the marine animals are capable of some degree of compensation for environmental changes within its zone of tolerance and zone of resistance (Blackstock, 1984; Akberali and Trueman, 1985). But prolonged unfavourable conditions may excert stress on the animal and chances of survival are significantly reduced. According to Bayne (1975) "stress is a measurable alteration of a physiological (behavioural, biochemical or cytological) steady state which is induced by an environmental change, and which renders the individuals (or the population) more vulnerable to further environmental change". Such alteration in the functional state may result in the improvement of an organism's fitness or a deterioration in well being (Bayne *et al.*, 1985).

The four physiological responses proposed for routine use in environmental monitoring are (Bayne, 1975; Bayne *et al.*, 1975; IMCO *et al.*, 1980).

1. Scope for growth - a measure of the energy status of an organism.
2. Growth efficiency - the efficiency with which an individual converts food into body tissues.

3. Oxygen:Nitrogen ratio - a measure of the balance between catabolic processes.
4. Body condition indices - indicative of alterations in the nutritional status of the animal.

All these physiological stress indices are derived from the integrations of basic biological process.

3.6.1 SCOPE FOR GROWTH

3.6.1.1 Introduction

Growth and reproduction are fundamental properties of all living organisms and is the basis for a population to establish in a particular environment. Warren and Davis (1967) defined the "scope for growth" as "the difference between the energy of the food an animal consumes and all other energy utilizations and losses". This is not measured directly but rather is derived by subtraction of energy respired and excreted from the energy absorbed from food. Alterations in the amount of matter or energy incorporated into growth and reproduction can be obtained by the balanced equation of Winberg (1960).

$$C - F = A = R + U + P$$

$$P = A - (R + U)$$

Where P = Energy incorporated into somatic growth and gamete production.

C = Food energy consumed.

F = Energy lost as faeces.

A = Energy absorbed from food.

R = Energy respired.

U = Energy excreted.

This energy budget provides an integration of these basic biological process (feeding, food absorption, respiration and excretion) as an index of energy available for growth and reproduction.

The direct measurement of growth and production is difficult in many species. This is especially true in bivalve molluscs, because 1. a major portion of the total production can be lost in the form of gametes by single or gradual spawning 2. it is impracticable to measure weight changes in animals with shells and variable amounts of sea water 3. it is not sufficient to measure changes in shell length because there is no apparent tight coupling between shell growth and tissue growth (Hilbish, 1986). Under these circumstances the scope for growth is an useful stress index because it reveals the whole organism's response to the environmental stress, both natural and anthropogenic. Brett (1979) opined that the environment acts not directly on growth but on the mechanisms of energy supply and demands, that influence the scope for growth. The scope for growth can range from positive values when there is energy available for somatic growth and production of gametes, to negative values when the animal is severely stressed and

utilizing its body reserves for maintenance metabolism.

Many workers have determined "scope for growth" of aquatic organisms. Warren and Davis (1967) were the first to use scope for growth as a measure of examining the bioenergetics of production in fish in response to environmental temperature change. Scope for growth in response to seasonal cycle was reported in *Mytilus edulis* (Widdows, 1978b; Bayne and Widdows, 1978), *Crassostrea virginica* (Dame, 1972), *Cardium edule* (Newell, 1977). Buxton *et al.* (1981) studied scope for growth in juvenile *Ostrea edulis* as a function of acclimation and exposure to temperature. The use of this index for assessing the physiological condition of mussels in response to temperature, ration and body size in laboratory and field were described by Widdows and Bayne (1971), Thompson and Bayne (1974), Bayne *et al.* (1975; 1978; 1979), Widdows (1978b), Griffiths and King (1979), Shafee (1979) and Widdows *et al.*, 1981). Gilfillan (1975) and Gilfillan *et al.* (1977) have shown that scope for growth (estimated in terms of carbon flux rather than energy units) declined in three bivalve species (*Mytilus edulis, Modiolus demissus* and *Mya arenaria*) as a result of exposure to oil. Scope for growth was studied in response to salinity changes in *Mytilus edulis* by Stickle and Sabourin (1979) and Shumway and Youngson (1979). Bayne *et al.* (1979; 1981) and Bayne and Worral (1980) compared estimates of growth, from the physiological measurements in the laboratory and from the two naturally occurring populations of mussels and showed

very good agreement between these two estimates. Evidence of this agreement enables confidence to be placed upon scope for growth measurements, as an index of true physiological condition.

The main physiological components of energy equation are

(1) food energy consumed

The amount of energy consumed from the food depends upon the food availability and feeding rates, the efficiency of digestion and absorption. Total energy consumed can be calculated from the clearance rate in the case of filter feeders which is defined as the volume of water cleared of particles per unit time (Bayne *et al.*, 1985). Measurement of feeding, digestion and assimilation process provide estimates of the amount of food consumed and assimilated by the animal. This forms an important component of the bioenergetic equation and is generally influenced by stress (Bayne *et al.*, 1985).

(2) energy loss due to respiration

Respiration represents a measure of that part of the food in take or of available body reserves which is required to provide energy to support life process. Energy losses by respiration can be expressed in terms of oxygen utilization, carbon dioxide liberation or heat production (Bayne *et al.*, 1985). Among these the oxygen consumption is a convenient measure of energy transformation (Scott and Major, 1972). Crisp (1971) gave an oxycalorific equivalent of 20.33 Joules for 1 ml

of oxygen which can be used to convert oxygen consumption (ml O_2) to energy equivalents. The metabolic energy expenditure is affected by a number of environmental and endogenous factors (Newell, 1973; 1979; Newell and Roy, 1973; Widdows, 1978a).

(3). energy loss due to excretion

A small proportion of the total energy absorbed by the animal is excreted as metabolic waste products. The energy lost as excreta therefore forms a negative component of the basic energy equation (Bayne *et al.*, 1985). Among a variety of bivalves ammonia comprised about 90% of total measure of nitrogen excretion (Bayne *et al.*, 1976d; Bayne and Newell, 1983). Therefore the rate of ammonia excretion has to reflect the rate of protein catabolism (Widdows, 1978b). So the estimation of ammonia excretion will give an idea about the loss of energy through excretion.

3.6.1.2 Materials and methods

The mean data of clearance rate, absorption efficiency, oxygen consumption and ammonia excretion were taken from the previous sessions for the calculation of scope for growth. To calculate scope for growth, all the physiological components of energy equation were first converted in to energy equivalents (Joules/h) as given by Bayne *et al.* (1985).

1. Energy consumed (C):

C = Clearance rate (l/h) x particulate organic matter (mg/l)

x energy content of particulate organic matter (J/h)

The energy content of particulate organic matter (algal) was taken as 23.5 J/mg dry weight (Slobodkin and Richman, 1961).

2. Energy absorbed from the seston (A):

$A = C\ (J/h) \times e$ where e = absorption efficiency

3. Energy respired (R):

$R = vO_2\ (ml\ O_2/h) \times 20.33$

$(1\ ml\ O_2/h = 20.33J)$

4. Energy excreted (U):

$U = NH_4\text{-}N$ excretion ($\mu gm\ NH_4/h$) x 0.0249

$(1\ \mu gm\ NH_4\text{-}N/h = 0.0249\ J)$

On the basis of these energy equivalents, the scope for growth were calculated using the following equation (Winberg, 1960).

$$P\ (J/h) = A - (R + U)$$

where P = scope for growth

A = energy absorbed from food

R = energy respired

U = energy excreted

3.6.1.3 Results

The energy values obtained for clearance rate, absorption efficiency, oxygen consumption, and ammonia excretion in the case of three size groups of *S. scripta* are given in Table 14a,b,c. The values have been calculated for three different size groups (1 gm dry tissue weight) in nine different salinities. The scope for growth for different size groups showed negative trend with increasing body size (Table 14a,b,c). As illustrated in Fig. 15 a,b,c for smaller size group animals positive scope for growth was noticed between 25 and 45 ppt. For medium and larger size groups the range was between 25 and 35 ppt and 30 and 35 ppt respectively. Considering all the salinities and all the size groups, the maximum scope for growth of 4.7059 (J/gm/h) is obtained for smaller size group (Table 14a).

3.6.1.4 Discussion

An energetic approach, based on the integration of feeding rate, food absorption, oxygen consumption, excretion, growth and reproduction provides a useful means of assessing the overall performance of an animal. As applied to mussels, the use of basic energy equation (Winberg, 1960) requires the measurement of respiration rate, clearance rate, excretion rate and assimilation efficiency, with all values expressed in energy units (Joules) (Thompson and Bayne, 1974). The net energy

Table 14 a,b. The calculation of energy budget and scope for growth of different size groups in 9 different salinities. a Small (1.5 ± 0.5 cm) b Medium (2.5 ± 0.5 cm) (1 gm dry weight)

Salinity (ppt)	CR (l/h)	POM (mg/l)	EC (C) (J/h) CRxPOMx23.5	AE e	EA (J/h) A = Cxe	ER R (J/h)	EE U (J/h)	SFG (J/h) P=A-(R+U)	
5	0	0.8	0	0	0	9.1017	5.0883	-14.1900	
10	0	0.8	0	0	0	6.9590	5.3822	-12.3412	
15	0.7692	0.8	14.4610	0.56	8.0981	12.1492	4.7989	-8.8500	
20	1.7742	0.8	33.3550	0.58	19.3459	15.5972	5.2042	-1.4555	
25	2.0691	0.8	38.8991	0.63	24.5064	20.4560	3.1997	0.8507	a
30	2.4127	0.8	45.3588	0.63	28.5760	20.9602	2.9099	4.7059	
35	2.2501	0.8	42.3019	0.63	26.6502	20.5618	3.0401	3.0483	
40	1.9057	0.8	35.8272	0.60	21.4963	11.2730	6.0441	4.1792	
45	1.5427	0.8	29.0028	0.60	17.4017	11.9988	4.8201	0.5828	
5	0	0.8	0	0	0	8.4004	5.4376	-13.8370	
10	0	0.8	0	0	0	8.0751	7.1445	-15.2196	
15	0.7294	0.8	13.7127	0.53	7.2677	12.6941	3.8894	-9.3158	
20	1.2210	0.8	22.9548	0.55	12.6251	14.2208	4.9298	-6.5255	
25	2.0480	0.8	38.5024	0.60	23.1014	19.9031	3.1605	0.0378	b
30	2.3421	0.8	44.0315	0.62	27.2995	19.7323	3.2171	4.3501	
35	2.2006	0.8	41.3713	0.62	25.6502	18.7971	2.7118	4.1413	
40	1.3030	0.8	24.4964	0.58	14.2079	11.3157	5.6439	-2.7517	
45	1.2367	0.8	23.2450	0.58	13.4850	11.4133	5.2358	-3.1641	

CR Clearance Rate; POM Particulate Organic Matter; EC Energy Consumed; AE Absorption Efficiency EA Energy Absorbed; ER Energy Respired; EE Energy Excreted; SFG Scope For Growth

Table 14 c. The calculation of energy budget and scope for growth of large size (3.5 ± 0.5 cm) group in 9 different salinities (1 gm dry weight)

Salinity	CR (l/h)	POM (mg/l)	EC (J/h) CRxPOMx23.5	AE e	EA (J/h) A = Cxe	ER R (J/h)	EE U (J/h)	SFG (J/h) P=A-(R+U)
5	0.00	0.8	0.00	0.00	0.00	7.1440	5.8749	-13.0189
10	0.00	0.8	0.00	0.00	0.00	6.4324	6.0459	-12.4783
15	0.5701	0.8	10.7179	0.52	5.5841	12.7774	3.8545	-11.0478
20	1.1181	0.8	21.0203	0.54	11.3509	13.6211	4.4531	-6.7233
25	1.9540	0.8	36.7352	0.58	21.3064	19.5554	3.3280	-1.5770
30	2.3303	0.8	43.8096	0.60	26.2858	19.0878	3.1062	4.0918
35	2.1911	0.8	41.1927	0.60	24.7156	19.2871	2.8328	2.5957
40	1.2912	0.8	24.2746	0.52	12.6228	10.0776	5.1943	-2.6491
45	1.2299	0.8	23.1221	0.52	12.0235	11.4194	5.0697	-4.4656

CR Clearance Rate; POM Particulate Organic Matter; EC Energy Consumed; AE Absorption Efficiency EA Energy Absorbed; ER Energy Respired; EE Energy Excreted; SFG Scope For Growth

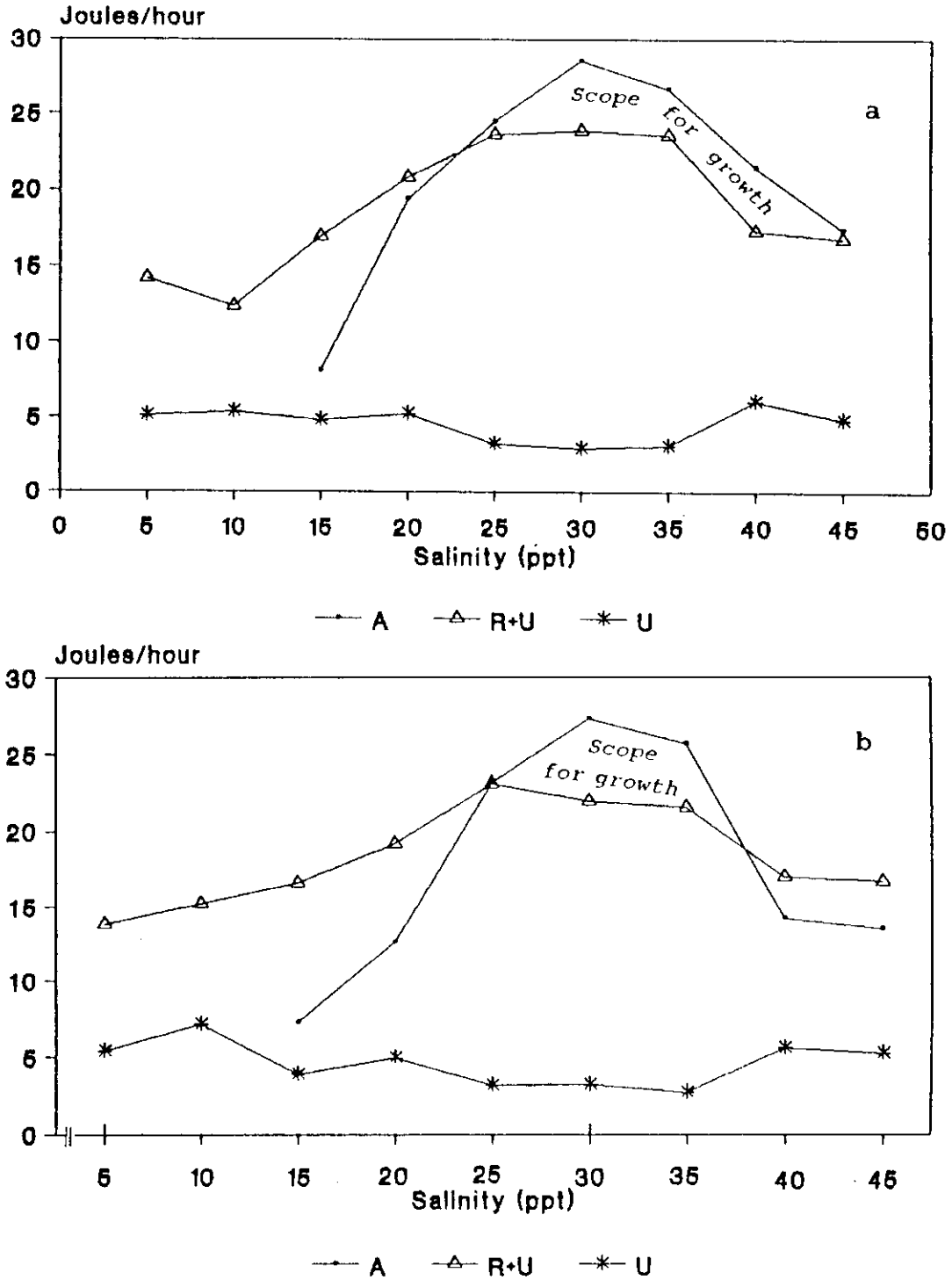


Fig. 15 a,b Scope for growth of (a) small (1.5 ± 0.5 cm) and (b) medium (2.5 ± 0.5 cm) size groups acclimated in different salinities (ppt)

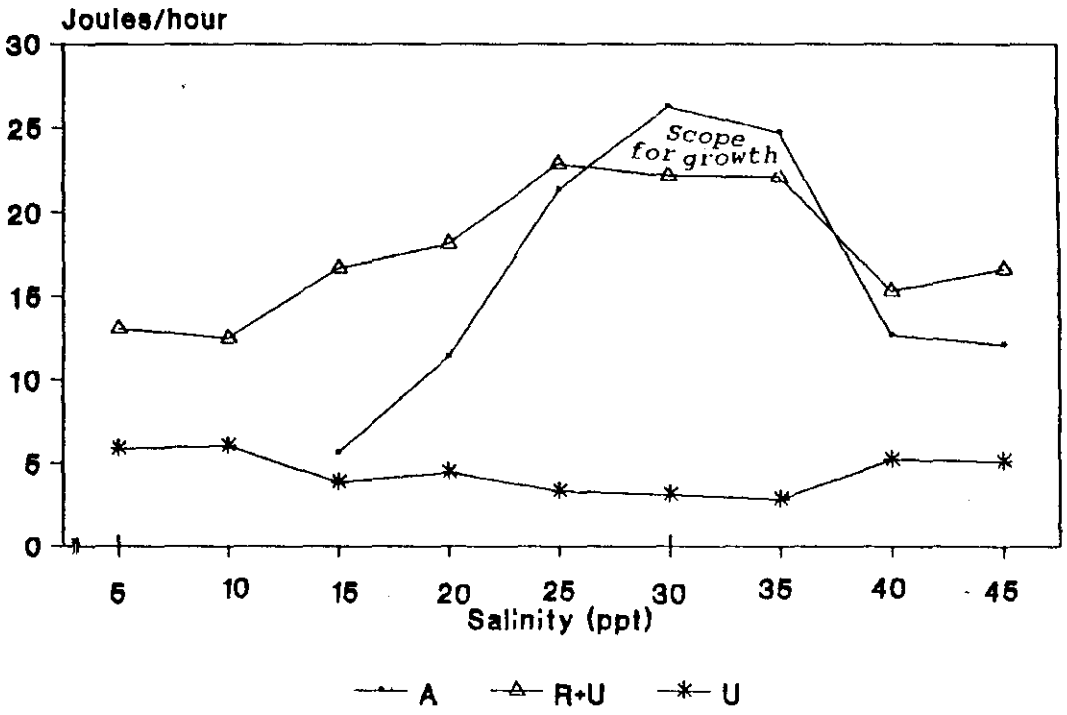


Fig.15 c Scope for growth of large (3.5 ± 0.5 cm) size group acclimated in different salinities (ppt)

available for growth and reproduction, the "scope for growth" represents the energy balance of an animal under specified conditions. The scope for growth provides an index of energy balance with out distinction between somatic growth and gamete production. The manner in which growth was altered have more significance than the change in the rate of any single physiological function. The scope for growth will be positive when surplus energy available for growth or it will be negative due to the utilization of animals' own endogenous energy reserves. In other words, when utilization of energy exceeds the energy input, the index becomes negative and under these conditions body reserves must be utilized (Gabbott and Bayne, 1973; Thompson *et al.*, 1974; Dare and Edwards, 1975; Widdows, 1978a) and if this situation is prolonged the animal must eventually die (Bayne *et al.*, 1979). According to Fry (1947) the region of negative scope for growth in which an organism cannot survive indefinitely, can be refereed to as the zone of resistance and the region of positive scope for growth, the zone of tolerance. When the absorbed ration exactly balances the sum of the metabolic demand and the energy losses due to excretion, growth and growth efficiency are zero, the ration level is called maintenance ration (Widdows, 1978b)

A major component in the maintenance of viable organism is the demand for protein synthesis. During stress condition the degradation of proteins into amino acids occurs due to the increased demand for energy. This inturn causes the depletion

of body proteins. Under these conditions some quantity of amino acids are recycled to the metabolic pool for further protein synthesis and are associated with high energy costs (De Zwaan and Van Marrewijk, 1973). According to Hawkins *et al.* (1986a) under stress condition as much as 30% of the normal energy demand is due to the turnover of proteins.

All the process that comprise the energy balance equation are capable of variation in response to changes in the environment. The animal must be able to balance its gains from the environment against its metabolic losses in order to allocate an optimal distribution of surplus energy to somatic growth and reproduction.

Each physiological response of *S. scripta* vary with salinity change. The sequence of events are similar in salinity increase and in salinity decrease from 30 ppt. When considering the energy budget and scope for growth of *S. scripta*, the positive component of the energy equation is the energy consumed and absorbed from the food and the negative component comprises the energy loss through respiration and excretion. The energy absorbed from the food decreases above and below 30 ppt. The maximum amount of energy consumed for the three size groups were in 30 ppt (45.3588, 44.0315 and 43.8096 J/gm/h for small, medium and large size groups respectively). The maximum energy absorption is also in 30 ppt for the three size groups (28.5760, 27.2995 and 26.2858 J/gm/h for small, medium and large size groups respectively) (Table 14 a,b,c). In 5 and 10 ppt, for all

the three size groups there is no filtration rate and hence no food uptake and energy absorption (Table 14 a,b,c). In lower salinities closure/withdrawal of exhalent siphon and closure of valves/partial closure occurs. This behaviour would account for the cessation of the pumping and feeding activity and consequent energy absorption. Respiratory and excretory loss in all the nine salinities for the three size groups are given in Table 14 a,b,c. The weight specific energy loss due to respiration is found to be higher in smaller size group when compared to medium and larger size groups. In addition to this the energy loss due to respiration were found to be decreasing with increase and decrease of salinity from 30 ppt except 5 and 45 ppt for all the three size groups. But there is no specific trend in energy loss due to excretion and salinity change.

In the present investigations the maximum scope for growth of 4.7059 J/gm/h is found to be for smaller size group in 30 ppt and the scope for growth is found to be decreasing with increasing body size (Table 14 a,b,c). This maximum value obtained is found to be comparable with the values reported by Widdows (1985b) and Widdows *et al.* (1990). The higher scope for growth for smaller size group is due to the higher proportion of ingestion rate and absorption when compared with respiration and excretion. The reduced scope for growth for medium and large size groups is because as animals grow in size, the increase in feeding rate (and hence the ration obtained) is disproportionate to the increase in metabolic rate. Bayne *et al.* (1973) reported

at any given ration smaller mussels have a greater scope for growth per unit of body size than larger ones and their optimum scope for growth occurs at a higher ingested ration in relation to body size. Thompson (1984) opined in *Mytilus edulis* that scope for growth is dependent on body weight. Initially there was a rapid improvement in scope for growth as body weight increased but the rate of change fell considerably in larger mussels. In the case of the scallop *Placopecten megallanicus* the scope for growth expressed per unit body weight declined as body weight increased (Mac Donald and Thompson, 1986). Vahl (1981) has recorded a similar phenomenon in the different age classes of the Icelandic scallop, *Chlamys islandica.*

The present study reveals that the scope for growth of the three size groups are found to be salinity dependent and the values ranged from negative to positive values. For smaller size group positive scope for growth is obtained above 25 upto 45 ppt, but for medium and larger size groups it is only above 25 upto 35 ppt and 30 to 35 ppt respectively (Fig. 15 a,b,c). In all the other salinities the scope for growth was negative. The higher scope for growth is obtained at 30 ppt for all the three size groups. When the salinity alters from 30 ppt (either decrease or increase), correspondingly there is a decrease in scope for growth. The negative scope for growth is due to the increase in the negative component of energy equation. In otherwords, the energy loss due to respiration and excretion is found to be greater than the energy absorbed from the food. In

10 and 5 ppt for all the three size groups, there is no food uptake and hence no energy absorption and there occurs only energy loss through respiration and excretion Fig. 15 a,b,c). Widdows (1985b) reported the reduced scope for growth for *M. edulis* in lower salinity. He acclimated *M. edulis* by the method of Livingston *et al.* (1979) and concluded that the scope for growth depressed between 20 and 15 ppt. In response to salinity changes below 20 ppt, respiration, clearance rate, and scope for growth for *M. edulis* declines (Shumway and Youngson, 1979; Stickle and Sabourin, 1979). Stickle and Bayne (1982) reported negative values of scope for growth for *Thais lapillus* below 20 ppt.

S. scripta is affected by salinity variations due to the South-West monsoon which inturn affects the distribution and abundance of this species. The simple salinity tolerance study (Thampuran *et al.*, 1982) revealed that the animal is capable of tolerating wide range of salinity (5 to 40 ppt) for short periods (15 days). Highest percentage of mortality was recorded in 5 ppt salinity and the mortality rate increased as the age of clams advanced (33.31%, 19.99% and 13.32% in larger, medium and smaller clams respectively over a period of 15 days). It was seen that the 100% survival for larger clams in 25 to 35 ppt range of salinities while the medium and small size groups were in 20 to 35 ppt and 15 to 40 ppt respectively for a period of 15 days (Thampuran *et al.*, 1982). But the present investigations show that the positive scope for growth obtained for smaller,

medium and larger size groups are 25 to 45 ppt, 25 to 35 ppt and 30 to 35 ppt respectively. Below and above these salinities, there is negative scope for growth and the maximum scope for growth is at 30 ppt for all the three size groups. This reveals that the optimum salinity for growth is 30 ppt in all the three size groups and the adaptation of *S. scripta* to an increased salinity from 30 ppt is greater than decrease in salinity from 30 ppt. The studies show that the animal is capable of tolerating lowered salinities for short term fluctuations, but it can not tolerate long term fluctuations, because during this time there is little or no food uptake due to the closure of valves and cessation of pumping activity, and the energy demands are met by the utilization of body reserves.

Eventhough the animal is having wide range of tolerance in salinity, successfully established population can be seen only in the marine zone close to the estuary. The species never colonised in the estuarine habitat though similar substratum are available. This is because the lowest salinity for positive scope for growth is 25 ppt and that too only for smaller and medium size groups. For larger size group it is only above 30 ppt. The salinity of the estuarine area is controlled mainly by the South-West monsoon. During monsoon the salinity goes to very low values in the estuary and this condition prevails for 3 to 4 months. Thus the period in which the animal has to maintain itself in the resistance zone is considerably long, and recur annually. This can be the main reason why *S. scripta*

occurs only in marine environment, though it is capable of tolerating wide range of salinities. Thus salinity is acting as a limiting factor for *S. scripta* which restrict the potential for energy acquisition and thereby setting limits for the establishment of a population.

3.6.2 GROSS AND NET GROWTH EFFICIENCIES

3.6.2.1 Introduction

In ecological context, growth relationships are best described as efficiencies. The most commonly used indices in molluscan energetics are those of gross and net growth efficiencies. The gross growth efficiency K_1 is the proportion of the ingested ration for scope for growth (Bayne and Widdows, 1978). The scope for growth as a proportion of the absorbed ration represents the net growth efficiency (K_2; Ivlev, 1961) and is a measure of the efficiency with which absorbed ration is converted into body tissues (Paloheimo and Dickie, 1965; 1966; Thompson and Bayne, 1974; Widdows, 1978b; Ansell, 1982). The values of K_1 and K_2 are negative when the animals are stressed (Thompson and Bayne, 1974; Bayne and Widdows, 1978). The important factors affecting the gross and net growth efficiencies are food concentration (Paloheimo and Dickie, 1966; Conover and Lalli, 1974; Brett, 1979), Temperature (Widdows 1978b; Newell and Branch, 1980) and size (Rodhouse, 1978).

Since the food intake and absorption efficiency of *S. scripta* is found to be varying with salinity, the salinity may effect gross and net growth efficiencies. This study is aimed to delineate the effect of salinity and size on the growth efficiencies of *S. scripta*.

3.6.2.2 Materials and methods

The gross and net growth efficiencies were calculated using the following equations:

Gross growth efficiency $K_1 = \frac{A - (R+U)}{C}$ (Bayne and Widdows, 1978)

Net growth efficiency $K_2 = \frac{A - (R+U)}{A}$ (Bayne *et al.*, 1985)

where A = energy absorbed from food (J/h)

R = energy respired (J/h)

U = energy excreted (J/h)

C = energy consumed (J/h)

The data of A, R, U and C for the three different size groups in different salinities were taken from Table 14 a, b, c.

3.6.2.3 Results

The gross growth efficiency of different size groups in different salinities are given in Tables 15 and Fig. 16. The values ranged between ⁻0.6120 to 0.1166 for small size group. For medium and large size groups the values range from ⁻0.6794 to 0.1001 and ⁻1.0308 to 0.0934 respectively. The efficiency is higher for smaller size group when compared to medium and large size groups. For smaller size group the positive values of gross growth efficiency is obtained between 25 and 45 ppt. But for medium size group the positive values are between 25 and 35 ppt and that of large size group is in 30 and 35 ppt. In all the other salinities, for the three size groups the values are found to be negative.

The net growth efficiency of the three size groups in different salinities are given in Table 16 and Fig. 17. The values ranged between ⁻1.0928 to 0.1944 for small size group. The range of values for medium and large size groups are ⁻1.2818 to 0.1615 and ⁻1.9784 to 0.1557 respectively. The smaller size group showed positive values between 25 and 45 ppt. But for medium and large size groups the range got restricted between 25 to 35 ppt and 30 and 35 ppt respectively. In all the other salinities, the values are negative for the three size groups. Both the growth efficiencies showed maximum values in 40 ppt for smaller size group and that of medium and large size groups are 35 and 30 ppt respectively.

Table 15. Gross growth efficiency of different size groups in different salinities

Salinity (ppt)	Size Groups		
	Small (1.5 ± 0.5 cm)	Medium (2.5 ± 0.5 cm)	Large (3.5 ± 0.5 cm)
15	-0.6120	-0.6794	-1.0308
20	-0.0436	-0.2843	-0.3198
25	0.0219	-0.0010	-0.0429
30	0.1037	0.0988	0.0934
35	0.0721	0.1001	0.0630
40	0.1166	-0.1123	-0.1091
45	0.0201	-0.1361	-0.1931

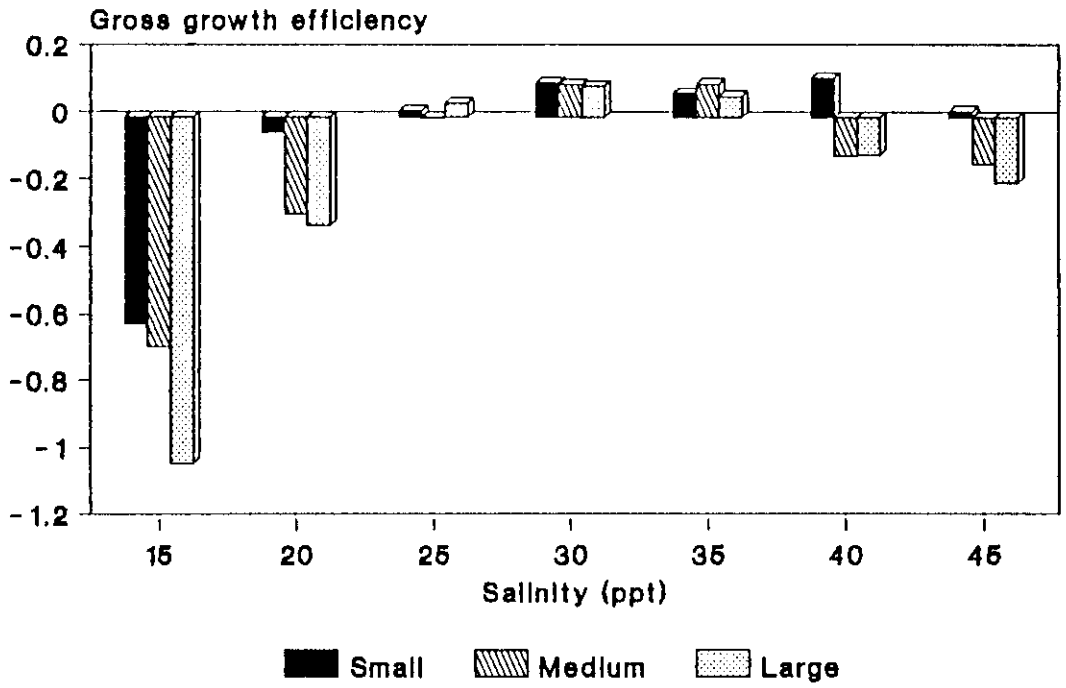


Fig. 16 Gross growth efficiency of different size groups acclimated in different salinities (ppt). (Small, 1.5 ± 0.5 cm; Medium, 2.5 ± 0.5 cm; Large, 3.5 ± 0.5 cm)

Table 16. Net growth efficiency of different size groups in different salinities

Salinity (ppt)	Size Groups		
	Small (1.5 ± 0.5 cm)	Medium (2.5 ± 0.5 cm)	Large (3.5 ± 0.5 cm)
15	-1.0928	-1.2818	-1.9784
20	-0.0752	-0.5169	-0.5923
25	0.0347	0.0016	-0.0740
30	0.1647	0.1593	0.1557
35	0.1144	0.1615	0.1050
40	0.1944	-0.1937	-0.2099
45	0.0335	-0.2346	-0.3714

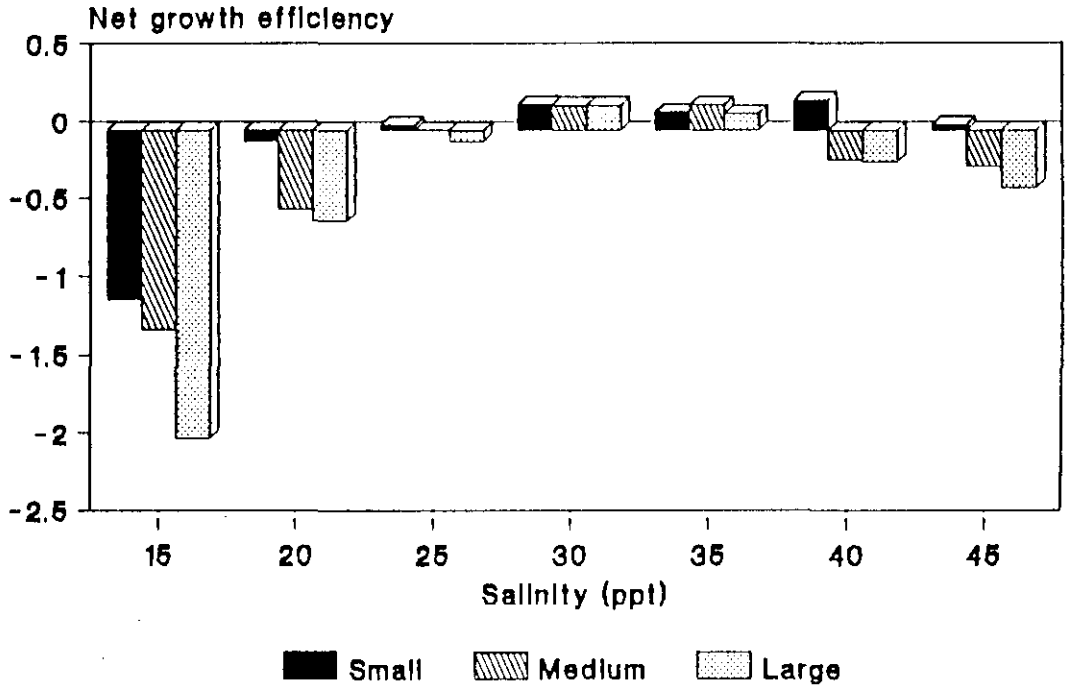


Fig. 17 Net growth efficiency of different size groups acclimated in different salinities (ppt). (Small, 1.5 ± 0.5 cm; Medium, 2.5 ± 0.5 cm; Large, 3.5 ± 0.5 cm)

3.6.2.4 Discussion

The results shown in Table 15 and 16 and Fig. 16 and 17 indicate that gross and net growth efficiencies for smaller size group is higher than medium and larger size groups. They could also maintain this efficiencies in a wider range of salinity than medium and large size groups. The higher growth efficiencies for smaller size group can be due to higher weight specific filtration rate. Jorgenson (1976) and Brown *et al.* (1989) opined that the growth efficiencies will inevitably decline when the food uptake increases less rapidly than respiration with increasing size. Bayne and Widdows (1978) reported that negative relationship between body size and the ratio of weight specific clearance rate to respiration rate, reduces the growth efficiencies of large animals.

Comparing the scope for growth and growth efficiencies of these different size groups, the scope for growth was found to be maximum in 30 ppt for all the three size groups. But the growth efficiencies were maximum in 40 ppt for smaller size group, 35 ppt for medium size group. For larger size group both were maximum in 30 ppt. The higher growth efficiencies exhibited by smaller size group can be mainly due to substantial reduction in oxygen consumption, i.e, energy expenditure in relation to consumption and assimilation. Eventhough there is a reduction in consumption and assimilation rate than in 35 and 30 ppt, the reduction in oxygen consumption gives the animal

sufficient surplus energy to maintain a higher growth efficiency in 40 ppt, even after taking into account the increased ammonia excretion. The experiments being short-term it may need further varification to know whether the animal will be able to maintain this efficiency for a larger period because the K_1 and K_2 were found to be lower in 35 ppt for smaller size group.

The higher growth efficiency of medium size group in 35 ppt is again due to substantial reduction in oxygen consumption compared to 30 ppt. Here also consumption and assimilation is not showing a proportionate reduction which allows the animal to maintain a higher growth efficiencies.

3.7 IONIC REGULATION

3.7.1 Introduction

Among the mussels there is a wide spectrum of salinity tolerance from stenohaline to euryhaline (Pierce, 1970). While considering the physiological adaptation to varying salinity, there are three different degrees of control of body fluid composition 1. control of the composition of various ions 2. extra cellular anisosmotic control 3. intra cellular isosmotic regulation. These are inter-related and together controls the cell volume (Duchateu and Florkin, 1956). Ionic regulation is the maintenance of ionic concentrations in the body fluids which are different from the concentrations to be expected, should occur passive equilibrium between the internal and external

media (Robertson, 1964). Among the solutes encountered in living organisms, inorganic ions are important. They participate as cofactors in many enzyme reactions, provide chemical gradients, and influence the permeability of biological membranes to other solutes.

The haemolymph of marine molluscs constitute about 30-80% of the soft parts. The difference between the haemolymph of marine molluscs and the surrounding sea water are often small. Marine species acclimated to a diluted medium exhibits two types of responses 1. their blood remain isosmotic with the environmental medium down to the lower limiting salinity 2. their blood remain hyperosmotic to the surrounding medium (Kinne, 1971). In species without extracellular fluid anisosmotic regulation, the blood remains near isosmotic in all the salinities encountered. In some molluscan species, the blood has been found hyperosmotic in salinities lower than 15 ppt (Freeman and Rigler, 1957; Todd, 1964).

Pierce (1970) examined four species of *Modiolus*, among these two are poikilosmotic euryhaline and other two are poikilosmotic stenohaline. In all the cases the extra cellular fluids were hyperosmotic to the medium. Pierce (1970) concluded that the hyperosmoticity of body fluid is not a function of species' habitat nor is it an active process, but is due to passive Gibbs-Donnan equilibrium caused by proteins in solution in the blood. This osmotic difference will result in the influx of water into the animal, unless it is opposed by a hydrostatic

pressure in the body fluids. Potts (1954) reported that the permeability characteristic of body are of crucial importance to survival at low salinities. Bivalves with their large surface area (mantle, convoluted gills etc.) exposed directly to the medium will have higher permeabilities which will impose an upper limit on the extent to which their blood can be maintained hyperosmotic to the medium without incurring a very large metabolic cost.

Among the bivalves in general, behavioural responses probably contribute most to their adaptation to fluctuations in salinity. On exposure to extremes of environmental salinity, many marine and brackish water bivalves promptly seal themselves off by closure of valves. In this way they keep the equilibrium with their environment, not only of their internal body fluids, but also of water in their mantle cavities which buffers from their environment (Gilles, 1972; Freeman and Rigler, 1957; Davenport, 1979; Shumway and Youngson, 1979; Widdows, 1985b). However, this mechanism can only help the organism to wait for better condition during a relatively short period of time.

The ions which regulate the extracellular ionic concentrations of bivalves are sodium, potassium, chloride, calcium, magnesium, sulphate and phosphate. Among these the major ions are sodium, potassium and chloride (Potts, 1958; Bricteux-Gregoire *et al.*, 1964). So the ionic regulation of *S. scripta* in different salinities can be followed by estimating the concentration of the major ions such as Na^+, K^+ and Cl^- of

the haemolymph. This study was undertaken (1) to establish the ion concentration as a function of different salinities (2) to determine the ability of this species to regulate Na^+, K^+ and Cl^- concentrations in the haemolymph and (3) to study whether ionic regulatory ability has any variation as a function of size, since the tolerance to salinities depends on size.

3.7.2 Materials and methods

The animals of different size groups (small, 1.5 ± 0.5 cm; medium, 2.5 ± 0.5 cm; and large, 3.5 ± 0.5 cm) were acclimated in salinities ranging from 5 to 45 ppt as given in chapter 3.

After proper acclimation five animals of the same size groups were pooled, opened the valves and the mantle fluid were drained out into absorbent paper. The haemolymph were collected from adductor sinus with the help of a syringe. Then the pooled haemolymph was centrifuged at 6000 rpm for 30 minutes and the serum was separated from blood cells. This separated serum was diluted with distilled water and samples were analysed for Na^+ and K^+ by Flame Photometric method (Robinson and Ovenston, 1951; Oser, 1965) using Flame Photometer (Elico, Type-22). The chloride content of the serum was estimated using Chloride Meter (Elico Chloride Meter Model EE-34) which is designed for automatic colorimetric titration of biological fluids.

3.7.3 Results

The Na^+ concentration estimated in different size groups and salinities of sea water are given in the Table 17. In all the size groups the minimum value is in 5 ppt and that of maximum in 45 ppt. In 25, 30 and 35 ppt the smaller size group is hypoionic to sea water. But the medium and large size groups showed hypoionic state only in 35 and 40 ppt (Table 17). The hyperionic state of smaller size group is below 20 ppt and above 40 ppt. Below 30 ppt and in 45 ppt the medium and large size groups exhibited hyperionic state (Table 17) (Fig. 18).

Table 18 shows the Cl^- values obtained for the different size groups acclimated in different salinities and the corresponding sea water salinities. In none of the salinities and size groups showed isosmotic condition with sea water. Small, medium and large size groups, in all the salinities, showed hyperionic state (Fig 19). This hyperionic state is much more pronounced for smaller size group than medium and large size groups (Fig 19). Among the size groups, the Cl^- concentration is found to be decreasing as the size of the animal increases.

The different estimations of K^+ concentration for small, medium and large size groups at salinities ranging from 5 ppt to 45 ppt are given in Table 19. In all the salinities, the three different size groups showed hyperionic state when compared with sea water (Fig. 20). But among size groups the ionic

Table 17. Na+ concentration in different size groups ($\bar{x} \pm SD$) and different sea water salinities (μequivalents/ml)

Salinity (ppt)	Sea water	Size Groups		
		Small (1.5 ± 0.5 cm)	Medium (2.5 ± 0.5 cm)	Large (3.5 ± 0.5 cm)
5	70.50	232.61 ± 9.40	215.94 ± 8.15	213.04 ± 7.39
10	140.48	268.84 ± 14.58	241.16 ± 10.39	239.13 ± 11.19
15	216.00	303.62 ± 14.84	255.07 ± 12.48	253.51 ± 12.67
20	275.00	313.04 ± 14.83	285.65 ± 14.78	278.96 ± 11.18
25	341.30	337.83 ± 13.74	406.38 ± 18.24	374.26 ± 10.30
30	408.00	372.61 ± 10.57	457.25 ± 12.22	445.70 ± 8.09
35	480.83	454.35 ± 12.76	478.19 ± 23.40	480.67 ± 10.73
40	545.00	574.71 ± 10.38	528.41 ± 13.85	508.17 ± 13.16
45	626.80	636.90 ± 12.20	650.17 ± 13.43	678.26 ± 10.04

Table 18. Cl- concentration in different size groups ($\bar{x} \pm SD$) and different sea water salinities (μ equivalents/ml)

Salinity (ppt)	Sea water	Size Groups		
		Small (1.5 ± 0.5 cm)	Medium (2.5 ± 0.5 cm)	Large (3.5 ± 0.5 cm)
5	81.34	213 ± 10.59	208 ± 10.33	208 ± 7.88
10	163.20	249 ± 8.76	248 ± 10.33	227 ± 6.75
15	240.10	308 ± 11.40	295 ± 13.50	281 ± 7.38
20	318.00	380 ± 13.30	322 ± 12.29	322 ± 7.89
25	392.80	478 ± 16.20	456 ± 20.11	450 ± 7.75
30	480.10	599 ± 13.70	503 ± 14.94	495 ± 11.79
35	560.80	664 ± 13.50	569 ± 12.87	568 ± 7.89
40	630.60	745 ± 11.80	696 ± 32.04	667 ± 16.35
45	720.10	792 ± 10.30	812 ± 30.11	788 ± 7.89

Table 19. K+ concentration in different size groups ($\bar{x} \pm SD$) and different sea water salinities (μ equivalents/ml)

Salinity (ppt)	Sea water	Size Groups		
		Small (1.5 ± 0.5 cm)	Medium (2.5 ± 0.5 cm)	Large (3.5 ± 0.5 cm)
5	1.45	4.25 ± 0.72	4.15 ± 0.51	3.85 ± 0.44
10	2.90	7.09 ± 0.96	5.47 ± 0.50	5.00 ± 0.40
15	4.37	10.32 ± 0.46	5.62 ± 0.34	5.13 ± 0.54
20	5.83	10.93 ± 0.64	8.16 ± 0.60	7.95 ± 0.30
25	7.20	12.82 ± 0.35	10.43 ± 0.76	9.64 ± 0.28
30	8.70	13.05 ± 0.53	11.52 ± 0.38	11.41 ± 0.54
35	10.20	14.85 ± 1.12	13.21 ± 0.69	13.03 ± 0.34
40	14.66	20.30 ± 0.51	15.29 ± 1.20	15.26 ± 0.94
45	17.11	20.94 ± 0.37	20.88 ± 1.95	18.51 ± 1.29

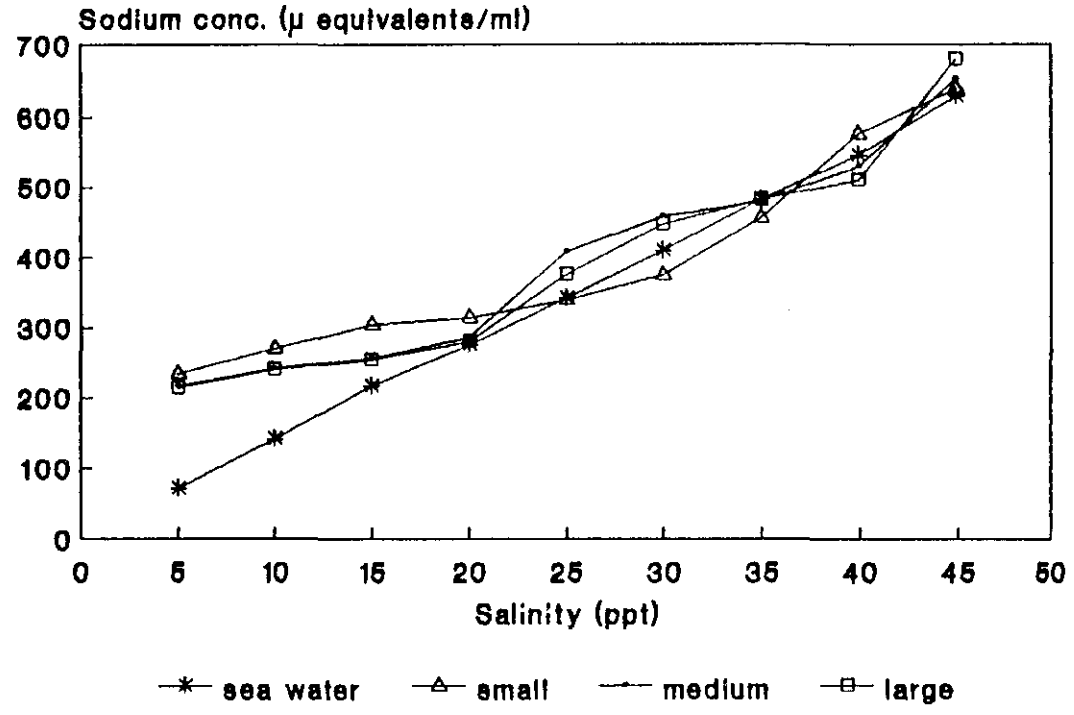


Fig. 18 Sodium ion concentration (μ equivalents/ml) of different size groups acclimated in different salinities (ppt) Small, 1.5 ± 0.5 cm; Medium, 2.5 ± 0.5 cm; Large, 3.5 ±0.5 cm)

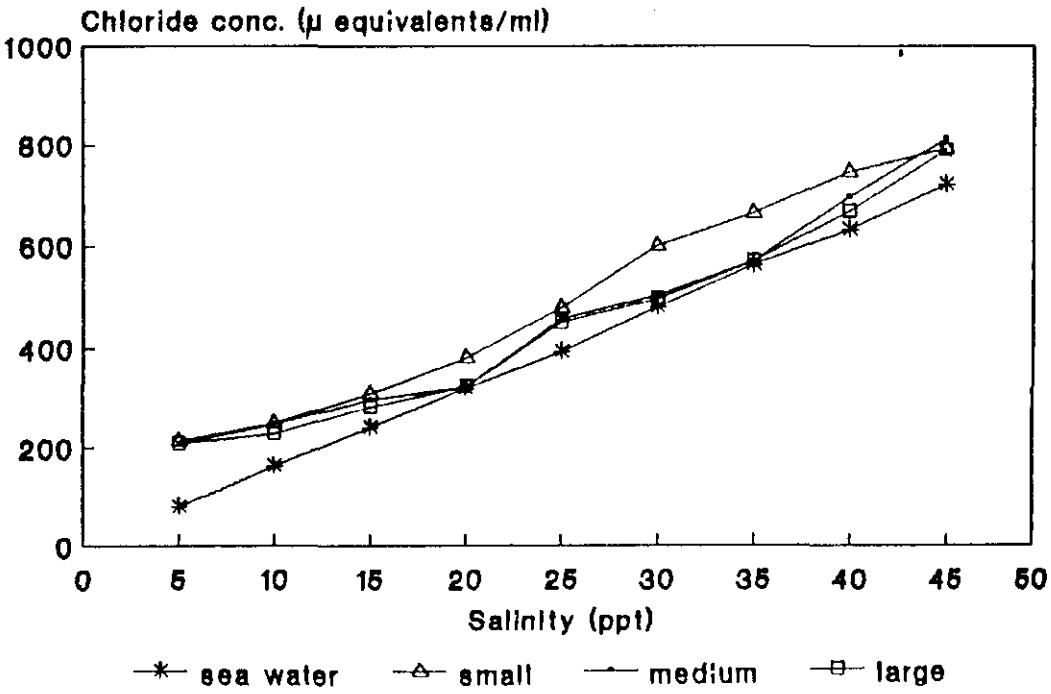


Fig. 19 Chloride ion concentration (μ equivalents/ml) of different size groups acclimated in different salinities (ppt) Small, 1.5 ± 0.5 cm; Medium, 2.5 ± 0.5 cm; Large, 3.5 ±0.5 cm)

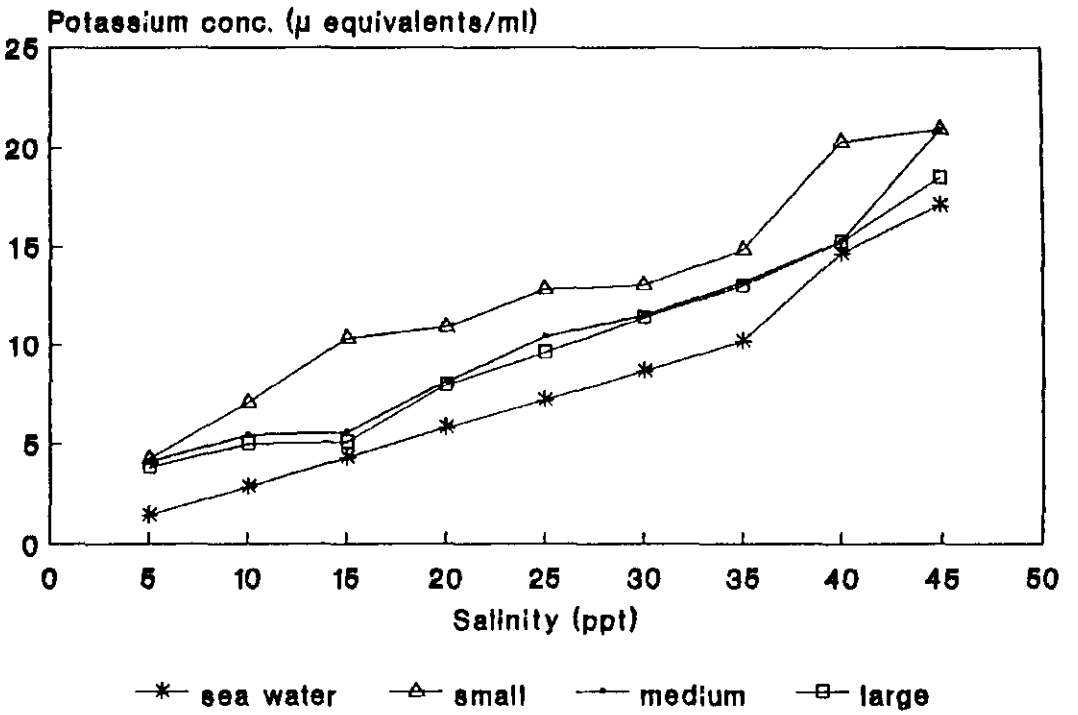


Fig. 20 Potassium ion concentration (µ equivalents/ml) of different size groups acclimated in different salinities (ppt) Small, 1.5 ± 0.5 cm; Medium, 2.5 ± 0.5 cm; Large, 3.5 ±0.5 cm)

concentration is found to be decreasing as the size increases (Fig 20).

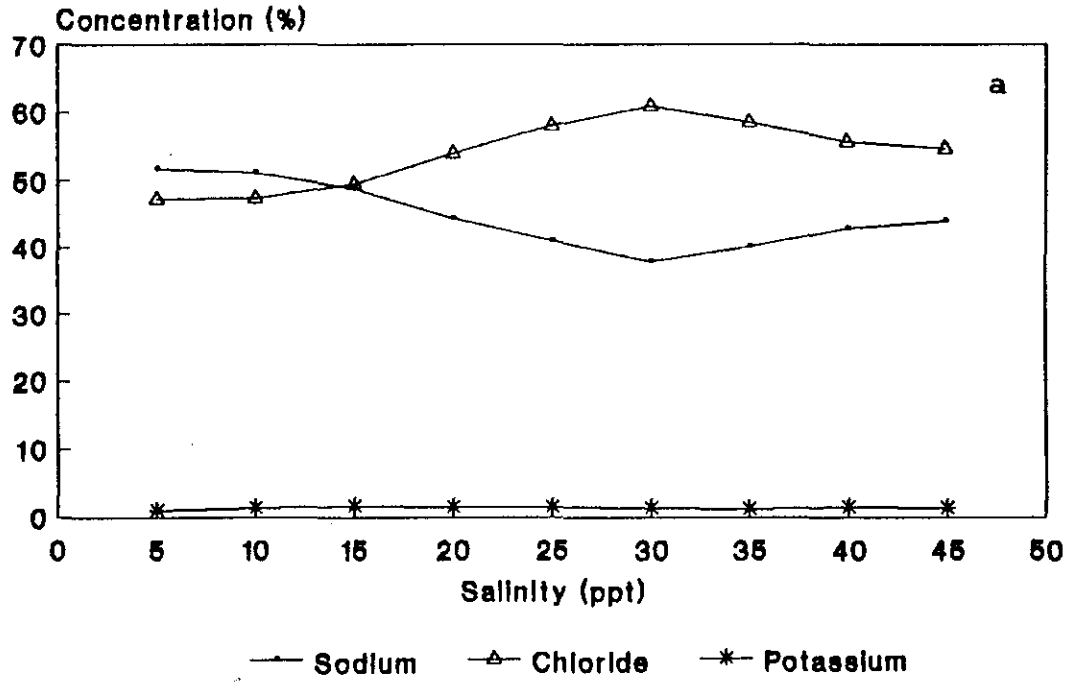
3.7.4 Discussion

The results obtained in the present investigations shows that the Cl^- and K^+ concentrations of body fluid are found to be hyperionic to sea water in all the size groups and salinities (Fig. 19,20). The values showed direct relationship between ionic concentrations of sea water and haemolymph. When the salinity of the water decreases or increases there is a corresponding decrease or increase in Na^+, Cl^- and K^+ concentrations (Fig. 18,19, 20). Among the size groups the smaller size group showed more hyperionic state for Cl^- and K^+ than medium and large size groups. But in the case of Na^+, the hyperionic state is not observed in all the salinities. For smaller size group the Na^+ is found to be hypoionic to sea water in 25 to 35 ppt. But the medium and large size groups showed hypoionic state in 35 and 40 ppt (Table 17). When compared to sea water the hyperionic state of Na^+ is more pronounced in lowered salinities for all the three size groups (Fig. 18). The difference between the Na^+ and Cl^- of sea water and that of haemolymph is more in 5 ppt than the other salinities. The hyperionic condition of various ions can be due to anisosmotic extracellular regulation. The hyperionic state of ions were also reported by Krogh (1965), Pierce (1970; 1971), Deaton

(1981), Widdows (1985b) and Deaton *et al.* (1989).

There are various conclusions put forward for the hyperionic state of ions. Pierce (1970) reported that hyperosmoticity of body fluid is due to a passive Gibbs-Donnan equilibrium caused by the proteins in solution in the blood. Another observation is that the difference between ionic concentrations of medium and blood is an indication of active extracellular osmotic control (Wilson, 1968; Bedford and Anderson, 1972). In addition to these Potts (1954) reported the importance of the permeability characteristics of body in the survival of animals. He reported that the bivalves with their large surface area (mantle, convoluted gills etc.) exposed directly to the medium will impose blood hyperosmotic to the medium without incurring very large metabolic cost.

Assuming the Na^+, $K^+$and Cl^- are contributing nearly 100% of the total inorganic ions, their percentage contribution in haemolymph was followed in salinities in the three size groups. It was found that the K^+ concentration remains more or less steady in all the salinities from 5 to 45 ppt (Fig. 21 a,b,c). While the Na^+ concentration showed a decrease when the salinity increased, a corresponding change in Cl^- was noticed with the salinity decreased. The trend was really pronounced in smaller size group though exhibited by medium and large size group (Fig. 21 a,b,c) The reason for Na^+ accumulation in lower salinities and Cl^- accumulation in higher salinities needs further investigation.



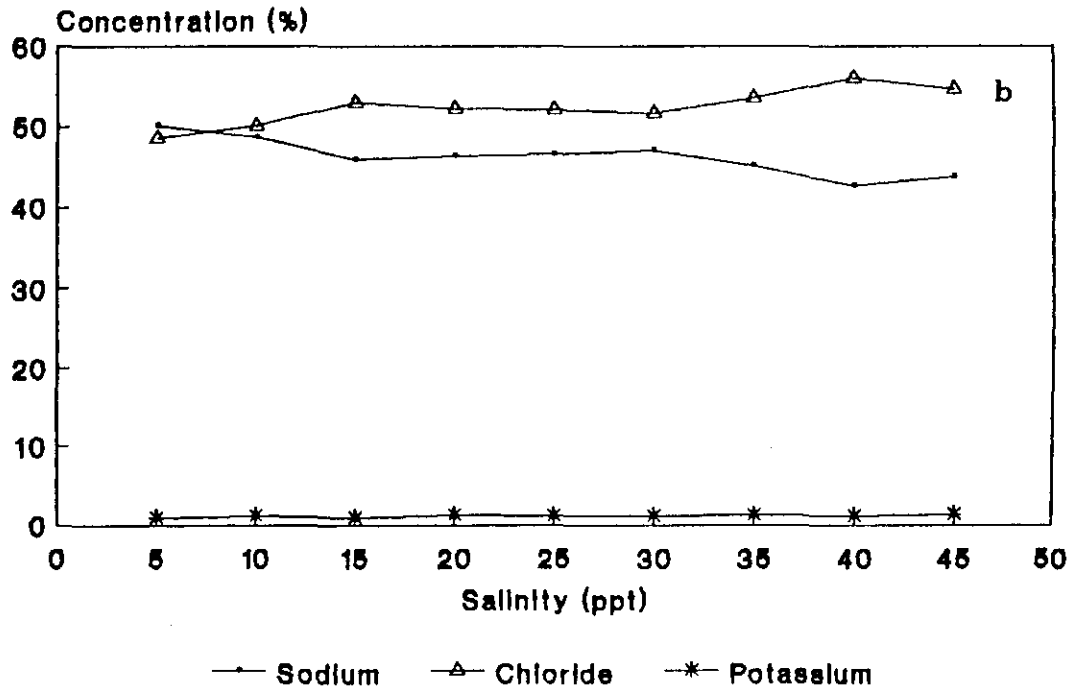


Fig. 21 a,b Percentage concentrations of Na^+, Cl^- and K^+ ions for smaller (1.5 ± 0.5 cm) and medium (2.5 ± 0.5 cm) acclimated in different salinities (ppt)

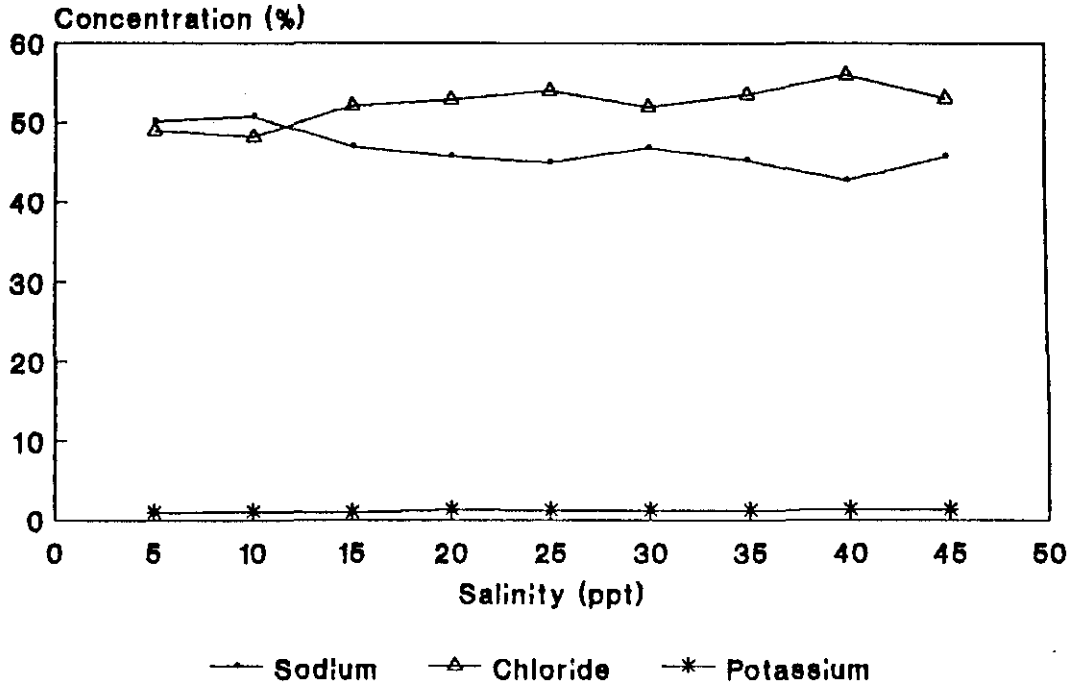


Fig. 21 c Percentage concentrations of Na^+, Cl^- and K^+ ions for large (3.5 ± 0.5 cm) acclimated in different salinities

Although initial changes in the cellular osmolarity are attributed to changes in the levels of inorganic ions, intracellular amino acids (taurine and glycine) play a major role in the total osmolarity of *S. scripta* in various salinities (George, personal communication). Same was also reported by Hochachka and Somero (1973), Gilles (1975) and Hoyaux *et al.* (1976).

3.8 BODY CONDITION INDEX

3.8.1 Introduction

The variations in meat content of bivalve molluscs are depending upon their physiological conditions and impact of environmental parameters. There is a marked seasonal cycle in body condition index of bivalves and this will depend upon the balance between food availability, rates of feeding and rate of catabolism. An increase in body condition index reflects an increase in the organic constituents associated with growth.

Since *S. scripta* is an inhabitant of sandy intertidal regime, it is affected by tidal and seasonal changes in salinity. This variation in salinity causes the reduction in time available for feeding. In addition to this, during monsoon season the animal is severely affected by the very low salinity prevailing on the clam bed (1.5 ppt). During this period energy needed for the maintenance metabolism may be met from the body

reserves. The salinity also plays a major role in the gametogenic condition of *S. scripta* (Katticaran, 1988) which inturn affects the body condition index. So the proportion of internal shell volume which is occupied by the body tissues is likely to vary with salinity condition and hence the estimation of body condition index has its own significance. The body condition index shows the relation between internal shell volume and the total soft tissue mass (Baired, 1966). The body condition index of various mussels and oysters were estimated by Bayne and Thompson (1970); Walne (1970); Gabbott and Stephenson (1974); Gee *et al.* (1977) and Bayne *et al.* (1985). So this study is aimed to understand the effect of salinity on the body condition index of *S. scripta*.

3.8.2 Materials and methods

S. scripta were collected (from January 1988 to December 1989) from the area as given in chapter 2, were brought in to the laboratory in polythene bags containing sea water collected from the same site. The animals were cleaned and grouped into three size groups, small (1.5 $\pm$ 0.5 cm), medium (2.5 $\pm$ 0.5 cm) and (3.5 $\pm$ 0.5 cm). The body condition index (B C I) was calculated using the following equation (Widdows 1985b).

$$B\ C\ I = \frac{\text{Dry tissue mass (gm)}}{\text{Shell cavity volume (ml)}} \times 1000$$

The dry tissue mass was determined by dissecting out the soft body parts and dried at 90°C for 24 hours to constant weight and shell cavity volume was calculated by subtracting empty shell volume from the total displacement volume of the completely closed clam.

3.8.3 Results

Body condition index of three different size groups of *S. scripta* are calculated as monthly mean values for two years (1988 to 1989) and the results are given in Table 20 and Fig. 22. Changes in body condition index are found to be related to the environmental salinity prevailing in the area. The maximum values of body condition index were obtained in January associated with the fattening of tissues in all the three size groups. Variations in body condition index is more pronounced in large and medium size groups than smaller size group (Fig. 22). During South-West monsoon the salinity of the environment decreased due to the influx of fresh water. During the two years of observations the salinity showed wide variations due to the impact of monsoon. The various salinities recorded during the study can be classified into three phases. The first one is the higher salinity period which extended from January to April. During this time the salinity ranged between 32.1 to 34.3 ppt (1988) and 33.1 to 35.1 ppt (1989). The second is the decreasing salinity period which extended from May to August and

Table 20 Monthly variations in Salinity (ppt) and Body condition index of three different size groups

Months	Salinity (ppt)		Size Groups					
			Small (1.5 ± 0.5cm)		Medium (2.5 ± 0.5cm)		Large (3.5 ± 0.5cm	
	1988	1989	1988	1989	1988	1989	1988	1989
JAN	34.3	35.1	140.8	153.2	246.8	248.8	288.4	284.6
FEB	34.0	34.3	142.6	148.6	229.6	219.6	280.8	287.6
MAR	33.6	34.2	140.6	141.8	174.6	184.6	248.8	250.6
APR	32.1	33.1	138.8	140.6	135.9	120.8	200.6	218.6
MAY	25.0	26.8	118.7	120.8	136.3	121.6	138.7	122.6
JUN	20.3	23.3	116.2	108.8	140.3	120.6	122.3	112.8
JUL	15.1	14.3	100.2	96.6	136.1	128.8	120.3	118.8
AUG	2.6	1.5	112.2	118.8	100.8	93.6	88.7	80.6
SEP	3.0	2.1	114.8	112.6	180.8	171.6	80.8	69.8
OCT	5.6	4.8	122.8	125.8	212.6	198.1	130.8	128.8
NOV	15.3	17.1	144.8	139.8	222.8	216.6	152.2	148.8
DEC	29.3	31.0	150.6	158.1	238.6	241.6	240.6	260.6

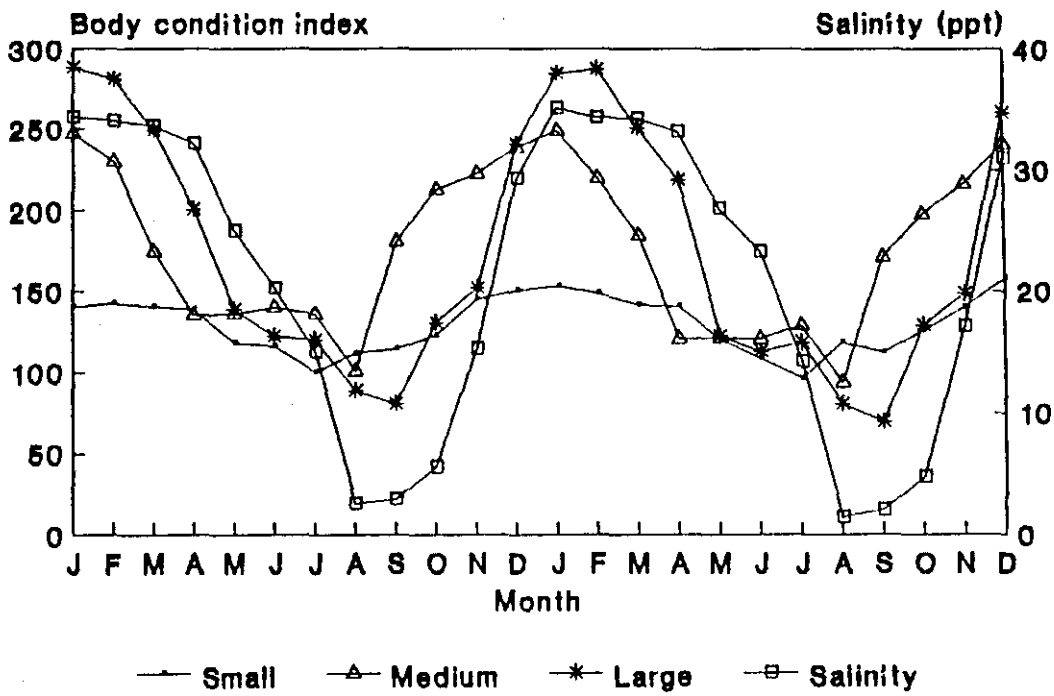


Fig. 22 Monthly variations in salinity (ppt) and body condition index of three different size groups. Small, 1.5 ± 0.5 cm; Medium, 2.5 ± 0.5 cm; Large, 3.5 ±0.5 cm)

salinity decreased from 20.3 to 2.6 ppt (1988) and 23.3 to 1.5 ppt (1989). The third is the rising salinity period, extends from September to December and the values varied from 3.0 to 29.3 ppt (1988) and 2.1 to 31 ppt (1989) (Table 20).

3.8.4 Discussion

The meat content of the bivalve molluscs is depended mainly upon the food availability, environmental and reproductive conditions of the animal. The reproductive cycles of *S. scripta* is related to salinity (Katticaran, 1988) and gametogenic condition inturn affects body condition index. The observations of gametogenic activity indicated a relationship between the three salinity periods (Katticaran, 1988), (a) recovery and slow early gametogenic activity occur during the low salinity period (b) the rising salinity period is associated with gametogenically active phase (c) high and stable salinity period induces the spawning activity.

The maximum values of body condition index for medium and large size groups are obtained in January when there prevails higher salinity conditions (Fig. 22). This is due to the higher food availability and feeding rate. During this time the deposition of maximum amount of fat and the proliferation of reproductive elements occur (Katticaran, 1988) and inturn causes the increased body condition index. Eventhough at the end of January prevails high salinity, a decline in body condition

index occurs for medium and large size groups due to spawning. The very low values of body condition index for medium size is in August but for large is in September. This is due to the compound impact of gametogenic spent condition and the lowered salinity. The lowered salinity due to the influx of fresh water during South-West monsoon inhibit the feeding mechanisms in *S. scripta*. So the body condition index decreases due to the lack of feeding. This condition inturn affects the utilization of body reserves for maintenance metabolism. But on late September the salinity increases and feeding, somatic growth accelerates and it inturn causes the increasing of the body condition index.

For smaller size group the variations in body condition index during the two years observations is less when compared with medium and large size groups (Fig. 22). On the onset of rising salinity period the body condition index is found to be increasing. But during the decreasing salinity due to South-West monsoon, inhibits the feeding rate and the body condition index is also found to be decreasing. This can be due to the utilization of body reserves. Seasonal variations in body condition index were also given for *Ostrea edulis* (Gabbott and Stephenson, 1974); *Mytilus edulis* (Gee *et al* 1977); *Perna viridis* (Ramachandran, 1980) and *Villorita cyprinoides* (Reddy, 1983) and *Donax incarnatus* (Thippeswamy and Mohan Joseph, 1987). Increase of body condition index starts much earlier in smaller size group than medium and large size groups. For smaller size group it starts from July and for medium and large size groups,

August and Séptember respectively. This is in well agreement with the salinity tolerance of size groups as smaller size group is found to be more tolerant than medium and large size groups.

From the above results it can be concluded that for medium and large size groups both the salinity and gametogenic condition plays a major role in the body condition index while for smaller size group it is due to the impact of salinity because none of the animals below 20 mm are reported for sexual maturity (Katticaran, 1988). The annual reduction in the body condition index in the case of *S. scripta* population therefore is partly due to salinity and partly due to spawning. Reduction in the body condition index of small size group during low saline period is an indication that salinity plays a role in maintaining the body condition index of the animal.

CHAPTER - 4

BIOCHEMICAL ADAPTATIONS

4.1 Introduction

It is well known that bivalves are able to withstand periods of shell closure and resultant lack of oxygen (Crenshaw and Neff, 1969; Moon and Pritchard, 1970; Coleman and Trueman, 1971; Akberali and Trueman, 1979; Davenport, 1979; 1982; Widdows *et al.* (1979a). The change over from aerobic to anaerobic respiration in bivalves normally occurs when the oxygen tension of the mantle cavity falls to low levels, after the bivalve has closed its valves in response to environmental stress. Bivalves have been called facultative anaerobes (De Zwaan, 1977; Hochachka,1985), capable of surviving indefinitely in the absence of oxygen and capable of active oxidative metabolism in its presence. In these organisms under anoxic conditions organic substrates instead of oxygen, are the acceptors of electrons (Karnaukhov, 1979). Studies on the biochemical pathways operating in bivalve molluscs during anaerobiosis has shown that these pathways differ from the classical ones described in vertebrates (Stokes and Awapara, 1968; Hochachka and Mustafa, 1972; De Zwaan and Zandee, 1972). Under aerobic conditions, like vertebrates the intertidal bivalves catabolise glycogen (glucose) to pyruvate which is then fully oxidised to carbondioxide and water (Hammen, 1969). But under anaerobic conditions the end product of vertebrate is lactate but in

bivalve molluscs it is succinate and alanine (Stokes and Awapara, 1968; Hammen, 1969; De Zwaan and Van Marrewijk, 1973; Hammen, 1975). Presumably some lactate is formed under anaerobic conditions but this is of minor importance compared to the production of succinate and alanine.

The conservation of fermentable substances and avoidance of self pollution by accumulation of undesirable end products are the major problems related to anaerobic tolerance (Hochachka, 1985) and good anaerobes solve this problem by coupling metabolic arrest with channel arrest or the fundamental factors of cells by 1. metabolic arrest capacity (reversed Pasteur effect) 2. low permeability membrane 3. equal ATP synthesis and utilization and 4. stable membrane function (Hochachka, 1985). The complex biochemical process behind anoxic energy production, and reduced metabolic rates are not yet completely revealed (Hochachka, 1985).

Several experimental results suggested a possible correlation between the presence of cytosomes and anoxic tolerance. Electron microscopic analysis revealed considerable structural alteration of cytosomes after several days of anoxia (Zs-Nagy, 1967) and some respiratory enzyme activity found in cytosomes (Zs-Nagy, 1976a). The acid phosphatase activity of cytosomes which increases especially during prolonged anoxia was interpreted as a sign of lytic process occurring in the cytosomes (Zs-Nagy, 1977). The occurrence of cytosomes were reported in all the basic tissues of molluscs especially in the

epithelial tissues of intestine, kidney, statocyst, siphon, gonads and pedal gland (Zs-Nagy, 1973). Karnaukhov (1971; 1979) suggested the term 'carotenoxysomes' for molluscan granules rich in carotenoids.

Regarding the role of cytosomes or carotenoxysomes, two different hypotheses have been put forward by Zs-Nagy(1977) and Karnaukhov (1979). The energy yielding process of cytosomes has been called "anoxic endogenous oxidation" (Zs-Nagy,1971a,b; 1973; 1974; 1977). In this process an intrinsic cytosomal electron acceptor substance (Zs-Nagy and Ermini, 1972) which is having high positive redox potential for electron acceptance (Zs-Nagy and Ermini, 1972). This substance can be fatty acids (Zs-Nagy and Ermini, 1972; Hochachka and Somero, 1973). But according to Karnaukhov (1971; 1979), Karnaukhov and Fedorov (1977) and Karnaukhov *et al.* (1977), the carotenoids together with haemoproteins and some respiratory enzymes form an intracellular organoid 'carotenoxysome' which acts as an intracellular oxygen reserve (accumulator) in cytosomes. The use of conjugated double bonds for oxygen accumulation results in the loss of colour of carotenoid (Karnaukhov, 1969; 1970; 1971; 1973a; b). This colourless oxygenated carotenoid may serve as an electron acceptor equivalent of molecular oxygen and can be considered analogous with the oxidised form of cytochrome oxidase (a_1+a_3) of mitochondrial respiratory chain. The carotenoxysomes will not be active during normal mitochondrial activity since they are connected to mitochondria by regulatory

mechanisms. At low levels or if (a_1+a_3) is inhibited, mitochondria loose their activity and the NADH oxidative system localised in the carotenoxysome is activated. This will result in an increase in the number of carotenoxysomes (and concentration of carotenoid consequently) in body of molluscs having anaerobiosis or anoxic tolerance.

Karnaukhov (1979) classified the molluscan species studied into three groups on the basis of different degrees of tolerance of environmental pollution accompanied by a decrease in dissolved oxygen concentration. Group I includes the pure water inhabitants which have been living recently only on the shore. Species which populate sea area with moderate pollution belong to Group II and a low concentration of carotenoids is characteristic of Group II. The Group III includes species inhabiting the strongly polluted area and are characterised by high carotenoid concentration.

Sunetta scripta is capable of tolerating a wide range of salinity and it shows reduced valve movement/closure at unfavourable/extreme ambient salinity conditions. These reduced movement/shell closure leads to the anaerobic metabolism and the production of undesirable end products leads to the changes in the overall functioning of the animal. So the objective of this study is to investigate the relationship between carotenoid concentration in the whole soft body of *S. scripta* in relation to salinity.

4.2 Materials and methods

4.2.1 Acclimation of test animals

S. scripta were collected the area as given in chapter 2, were brought into the laboratory and grouped into three size groups (Small, 1.5 ± 0.5 cm; Medium, 2.5 ± 0.5 cm; Large, 3.5 ± 0.5 cm) and acclimated for five days in 30 ppt as given in chapter 3. During acclimation period they were fed with *S. salina*. On each day 40 animals of each size group were transferred into filtered (42 Whatman) sea water of 30 ppt for 24 hours for emptying the gut. After that the carotenoid contents were extracted. After 5 days, half of the animals acclimated in 30 ppt were transferred into 25 ppt and another half into 35 ppt and again acclimated for five days. On each day 40 animals of each size group were transferred into filtered sea water of same salinity for one day and estimated the carotenoid content. The process of acclimation is repeated by changing the acclimation salinity to 20, 15, 10, 5, 40 and 45 ppt.

4.2.2 Estimation of carotenoid

The carotenoid content of the animals which were kept in filtered sea water were estimated using the standard procedures given by Karnaukhov *et al.* (1977) and Karnaukhov and Fedorov (1977). Five animals of each size group were pooled and treated

as one sample. Eight samples were took for each size group. The soft tissues of the pooled animals were dissected out, wiped with filter paper and the wet weight were determined. The weighed tissues were ground with chilled acetone in a glass mortar. The acetone extract was filtered under reduced pressure through a sintered glass funnel and the solid residues were returned to the mortar for further extraction. The extraction was repeated until the acetone extract was colourless. The volume of acetone extracts were noted and optical density was measured using Hitachi spectrophotometer (Model 200-20) at 455 nm.

The carotenoid concentration in mg/100 gm of wet tissue weight was calculated from the equation (Karnaukhov, *et al.*, 1977)

$$\text{Carotenoid mg/100 gm} = \frac{0.4\ DV}{P}$$

where D = the optical density of the extract

V = total volume of the extract in ml

P = the total wet weight in gm of the tissue from which the carotenoid was extracted.

4.3 Results

The results of the experiments on the three size groups at different salinities during the five days of acclimation are

given in Table 21a,b,c. The lowest concentration of carotenoids are at 30 ppt in all the days for the three size groups (Fig. 23 a,b,c). The maximum value was obtained for larger size group at 5 ppt on the third day. But on the fourth day onwards the concentrations were found to be decreasing and again a lower value at the fifth day (Table 21 a,b,c; Fig. 23 a,b,c). The trend is found to be same in all the three size groups.

4.4 Discussion

While considering the carotenoid concentration in the tissue of *S. scripta* in different salinities, the total carotenoid was found to be higher in all the salinities other than 30 ppt. This increase of carotenoid was more pronounced as the salinity decreased from 30 ppt. This can be mainly due to the reduced ventilatory activity exhibited by the animal in lower salinities and consequent lack of oxygen availability. When the animals are subjected to higher or lower than 30 ppt salinities, carotenoid content was found to be increasing upto third day and decreasing after that (Fig. 23 a,b,c). The results indicate that in higher/lower salinities the animal is depending upon carotenoids as an electron acceptor as normal oxygen supply is impaired. Most probably the animal is able to adjust to the environmental alterations and depend on other mechanisms for survival within a period of three days which can be the reason for the decrease in carotenoid concentration after

Table 21 a,b. Mean values of carotenoid concentration (mg/100 gm wet tissue weight) for small (1.5 + 0.5 cm) and Medium (2.5 + 0.5 cm) size groups acclimated in different salinities (ppt)

Salinity (ppt)	Days of acclimation				
	1	2	3	4	5
5	1.0689	1.5758	3.5731	2.0010	0.9082
10	0.9843	1.5021	1.5136	0.8230	0.6560
15	0.9067	1.1209	1.1365	0.6321	0.2053
20	0.4159	0.9882	1.0475	0.4321	0.1900
25	0.2045	0.4182	0.5954	0.3240	0.1299
30	0.1402	0.2225	0.2842	0.1523	0.1155
35	0.1520	0.2537	0.4323	0.4002	0.1584
40	0.4344	0.5322	0.6017	0.4321	0.2368
45	0.6105	0.6841	0.7093	0.5108	0.3487

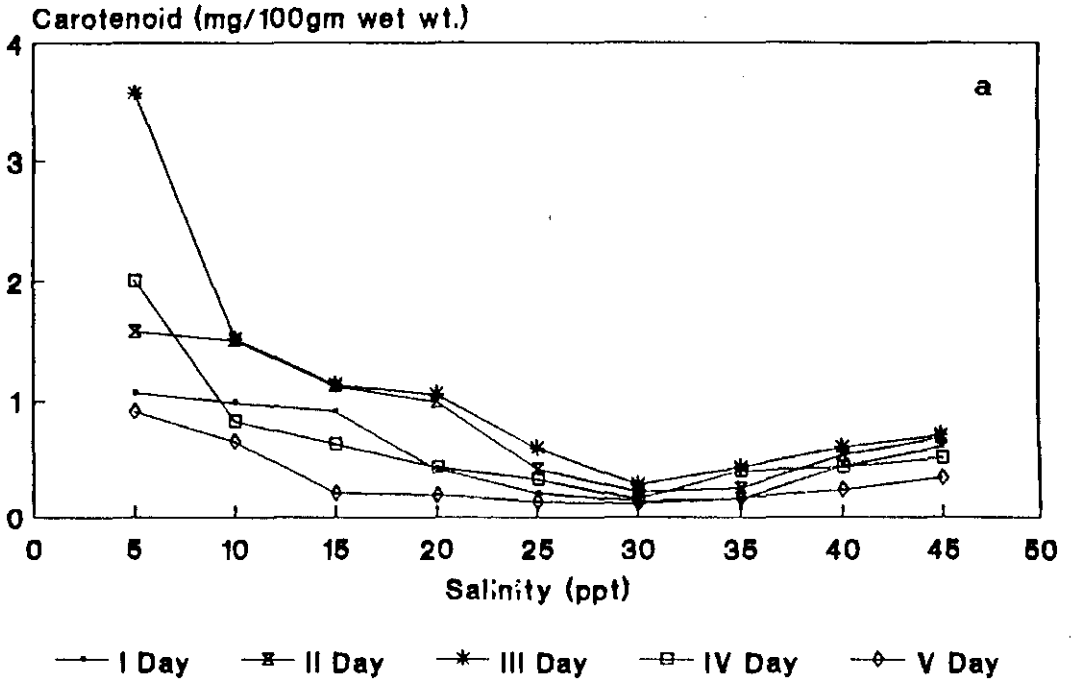
a

Salinity (ppt)	Days of acclimation				
	1	2	3	4	5
5	3.4926	4.8300	4.9142	3.2182	1.0317
10	2.6243	3.0803	3.6161	2.0081	0.7491
15	1.6624	2.1502	2.8560	1.1231	0.2148
20	1.5560	2.0558	2.5473	1.1028	0.2061
25	1.0231	1.1738	1.3188	0.8321	0.1575
30	0.4748	0.5483	0.8220	0.5367	0.1343
35	0.7594	1.5482	2.3337	1.4281	0.1825
40	1.4112	2.7237	3.2833	1.8024	0.2997
45	2.1147	3.4369	3.7313	1.9810	0.3786

b

Table 21 c. Mean values of carotenoid concentration (mg/100 gm wet tissue weight) for large size group (3.5 + 0.5 cm) acclimated in different salinities (ppt)

Salinity (ppt)	Days of acclimation				
	1	2	3	4	5
5	5.9741	6.2376	8.7267	4.2187	1.4929
10	5.2379	5.8416	7.3585	3.8280	1.0538
15	3.4425	4.3357	4.5635	2.6601	0.2799
20	2.8266	3.9714	4.1226	2.0080	0.2513
25	1.8669	2.0069	3.3911	2.1800	0.1711
30	1.5925	1.6371	1.8669	1.0028	0.1438
35	2.1744	2.3443	3.4425	2.1002	0.2185
40	4.0335	4.3357	4.7345	2.8208	0.3289
45	4.2184	4.5611	4.8337	2.3128	0.4062



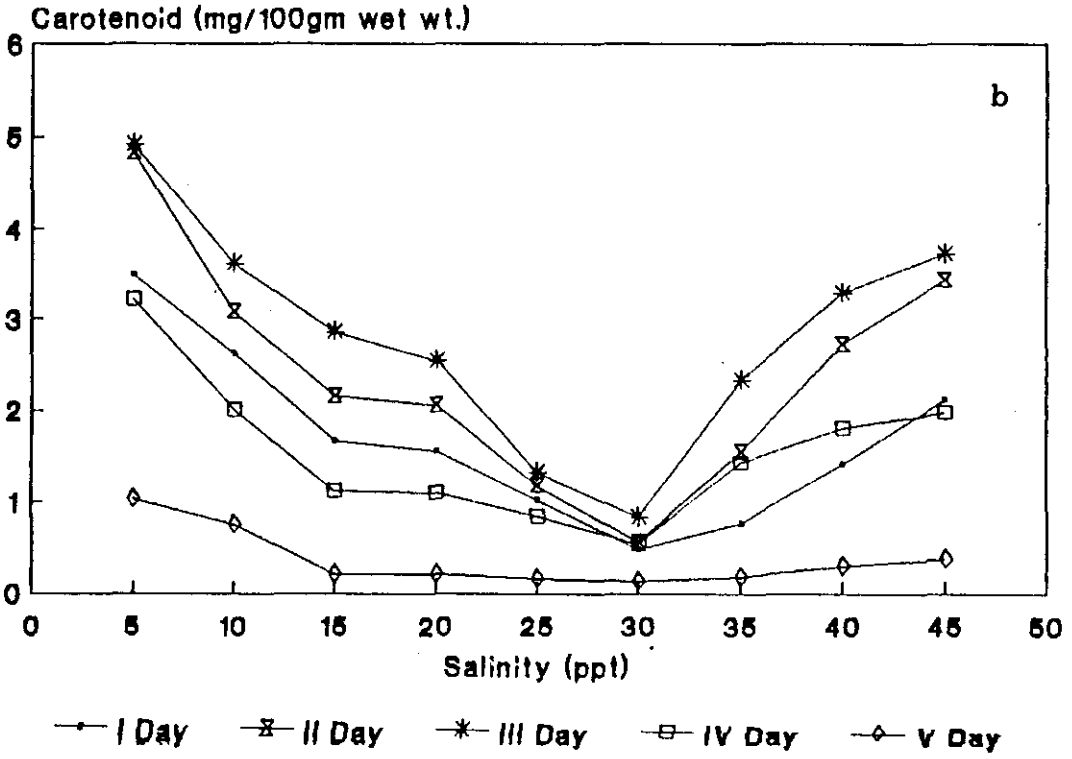


Fig. 23 a,b Carotenoid concentration (mg/100 gm wet tissue weight) for Small (1.5 ± 0.5 cm) and Medium (2.5 ± 0.5 cm) size groups acclimated in different salinities (ppt) for 5 days

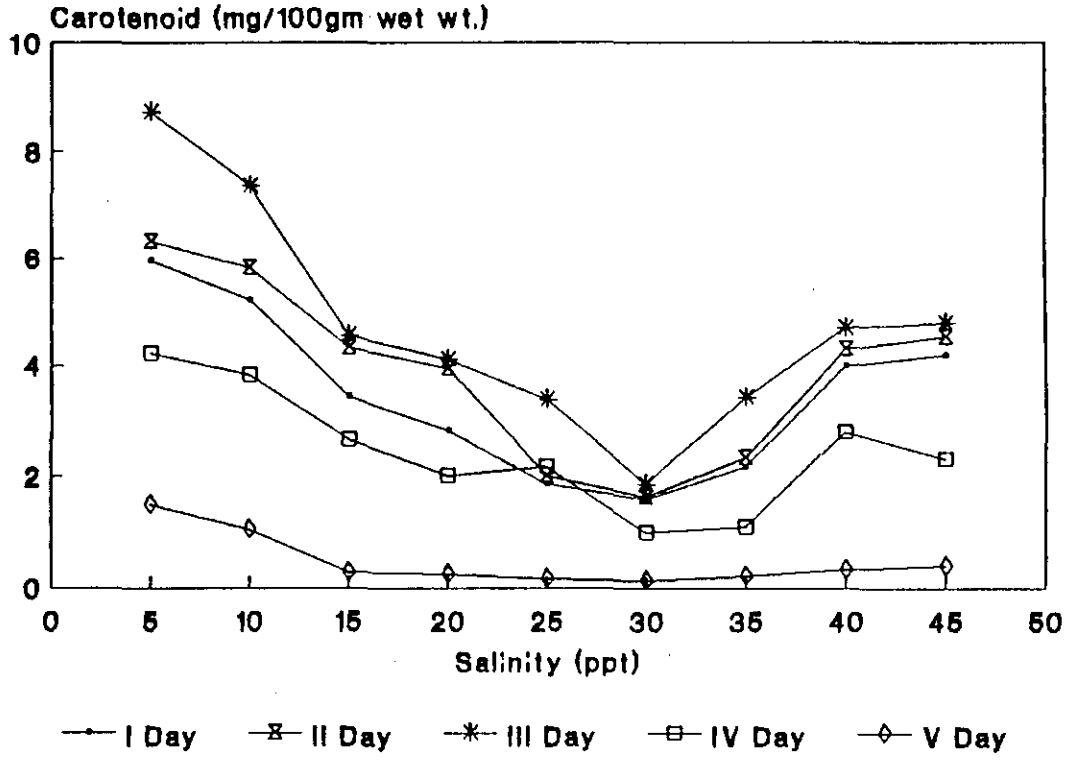


Fig.23c Carotenoid concentration (mg/100 gm wet tissue weight) for large (3.5 ± 0.5 cm) size group acclimated in different salinities for 5 days

the third day. Same type of observations were obtained for the estuarine clam *Villorita cyprinoides* var *Cochinensis* for the sub-lethal effects of copper and mercury (Sathyanathan *et al.*, 1988).

The increase of carotenoid concentration were reported in molluscs under their adaptation to hypoxic condition (Karnaukhov, *et al.*, 1977). The same trend was reported for the concentration in the body of molluscs under the influence of respiratory inhibitors (KCN) and pollutants (mineral oil) Karnaukhov (1971) and Karnaukhov *et al.* (1977). When compared to *Villorita cyprinoides* (Sathyanathan *et al.*, 1988) and *Perna viridis* (Krishnakumar, 1987) *S. scripta* is having high carotenoid content and may be a higher tolerance to anoxic condition.

It is worthwhile to consider the fact that a steady state of oxygen consumption was obtained in the case of the animal, when acclimated in different salinities after a period of three days (chapter 2). Therefore first three days appears to be the period during which the animal adjust itself to the altered environmental salinity.

CHAPTER - 5

SUMMARY AND CONCLUSIONS

Sunetta scripta is an infaunal siphonate clam inhabiting the sandy intertidal region and is a dominant species of the intertidal community off Kochi. The species has got wide distribution along the South-West coast of India. *S. scripta* in its natural bed of Kochi area is subjected to wide fluctuations in salinities due to South-West monsoon and tidal fluctuations. It appears that monsoon is a major ecological factor that affect the species than tides. During monsoon the salinity prevailing over the clam bed is very low due to the large quantity of fresh water discharge from Vembanad lake and this persists for 3 to 4 months. Even under this severe low salinity condition, mass mortality of the clams do not occur. The species also showed wide tolerance to salinity in laboratory conditions. Inspite of the wide tolerance to salinity, the species never formed a component of the benthic community in the identical substratum in the estuarine area. So the following studies were conducted to find out the impact of salinity on the distribution and abundance of this species.

The animal is ecologically and economically important and is used as an indicator organism in pollution monitoring studies.

To understand the impact of salinity on *S. scripta*, behavioural, physiological and biochemical approaches were carried out. As previous report indicated differences in

salinity tolerance in different size groups, studies were conducted in three size groups (small 1.5 ± 0.5 cm; medium 2.5 ± 0.5 cm; large 3.5 ± 0.5 cm). The behavioural studies on three different size groups were carried out by monitoring valve movements in salinity ranging from 5 to 40 ppt using an `Oyster Activity Monitor'. The results showed that in favourable salinities (20 and 30 ppt for smaller size group and 30 ppt for medium and larger size groups) the animal exhibited rhythmic partial closure and opening. This indicates the muscular activity which leads to ventilation is carried out only in favourable salinities. In 5 ppt, smaller size group animals and in 10 ppt medium and large size groups of animals, showed closed condition with an attempt to open occasionally which may be for testing of the ambient medium. A completely closed condition was observed for the three size groups in 5 ppt. In all the salinities except 30 ppt the valve movements were found to be reduced. In 40 and 20 ppt the larger size group animals kept the exhalent siphon in withdrawed condition. But this condition was not observed for small and medium size groups. When the animals acclimated at very low salinity (5 ppt) were subjected to increasing salinities, the salinity required for valve opening was less for smaller size group than medium and large size groups. From these results it could be concluded that behavioural component is playing a major role in the survival of *S. scripta* in different salinities and also the smaller size group has got higher tolerance than medium and large size

groups.

The various physiological parameters studied were clearance rate, absorption efficiency, oxygen consumption, ammonia excretion, O:N ratio and ionic regulation. These studies were done in salinities ranging from 5 to 45 ppt at an interval of 5 ppt. All these physiological parameters were found to be positively correlated with size of the animal. But the weight specific physiological rates were found to be inversely correlated with the size of the animal. The maximum clearance rate of *S. scripta* for the three size groups was obtained in 30 ppt and decreased as the salinity increased or decreased from 30 ppt. In 10 and 5 ppt no filtration was noticed in all the three size groups. Among size groups, weight specific clearance rate was found to be decreasing as the size of the animal increased.

The absorption efficiency studies on different size groups showed that the efficiency was higher in smaller size group than in medium and larger size groups. The maximum value of absorption efficiency for smaller size group was obtained in 25 to 35 ppt and that of medium and large size groups was in 30 to 35 ppt. These results indicated that the efficiency is higher in optimum/nearly optimum salinities. When the salinity deviated from the optimum, the ~~the~~ absorption efficiency was also found to drecease in all the three size groups.

The dissolved oxygen consumption was found to be higher in 30 ppt and decreasesd when salinity increased or decreased from

30 ppt. The trend was found to be the same in all the size groups. But in 45 and 5 ppt a slight increase of oxygen consumption occurs. This can be due to the additional energy requirement to meet osmotic stress. The weight specific oxygen consumption was found to be higher for smaller size group than for medium and large size groups in all the salinities.

The weight specific ammonia-nitrogen excretion at different salinities showed no specific trend with salinity. This variability may be due to disproportionate reliance on protein catabolism by the species in various salinities. Eventhough there was no specific trend with the salinities, minimum quantity of ammonia excretion for the three different size groups was in optimum (30 ppt)/nearly optimum (25 and 35 ppt) salinities. But in 45 and 15 ppt lower values were recorded when compared with 40 and 20 ppt for all the three size groups. This may be due to the recycling of amino acids for protein synthesis for compensating the increased utilization of proteins for energy production.

The Oxygen:Nitrogen ratio was found to be decreasing when the salinity was altered from 30ppt indicating the catabolism of proteins for energy production. The higher values of O:N ratios were obtained in optimum/nearly optimum salinities for all the three size groups. Below 20 and above 35 ppt the O:N ratio was found to be reduced and approximate to theoretically minimum values. Among size groups the O:N ratio did not show any clear cut relationship. The minimum and maximum values of O:N ratio

were obtained for larger size group.

The scope for growth for different size groups showed negative trend with increasing body size. The maximum scope for growth was obtained for smaller size group. The higher scope for growth for smaller size group indicates the higher proportion of ingestion and absorption when compared with respiration and excretion than medium and large size groups. The scope for growths were positive in optimum and nearly optimum salinities indicating the surplus energy available for growth. In unfavourable and extreme salinities the scope for growths were found to be negative due to utilization of body reserves for maintenance metabolism. The results of scope for growth studies revealed that the optimum salinity for growth was 30 ppt in all the three size groups. The species is found to be better adapted to survive in salinity greater than 30 ppt than in low salinity. The range of salinity for positive scope for growth for smaller size group was from 25 ppt to 45 ppt. For medium and larger size groups the positive values were observed from 25 to 35 ppt and 30 to 35 ppt, respectively. In the estuarine area of Kochi, the salinity is controlled mainly by the South-West monsoon and the low saline condition persists for 3 to 4 months. This low saline condition prevailing for prolonged period restricts positive scope for growth. Colonization of the species in the estuarine area, appears to be restricted mainly due to this environmental condition.

When considering the ecological efficiencies of the animal,

the gross and net growth efficiencies have its own importance because it gives an idea about the utilization of ingested (gross growth efficiency) and absorbed (net growth efficiency) energy for scope for growth. The estimations of net and gross growth efficiencies reveals that the efficiencies were higher for smaller size group when compared to medium and large size groups. They could also maintain these efficiencies in a wide range of salinity than medium and large size groups. This higher growth efficiencies for smaller size group can be due to the higher weight specific clearance rate. When comparing the scope for growth of these different size groups, the scope for growth was found to be maximum in 30 ppt for all the size groups. But the growth efficiencies showed maximum in 40 ppt for smaller size group and 35 ppt for medium. For larger size group scope for growth and growth efficiencies were in 30 ppt. The higher growth efficiencies exhibited by smaller size group can be due to the substantial reduction in energy expenditure in relation to consumption and assimilation which gives the animal sufficient surplus energy to maintain a higher growth efficiencies in 40 ppt. The higher growth efficiencies of medium size group in 35 ppt can also be due to the substantial reduction in oxygen consumption as there is no proportionate reduction in consumption and assimilation when compared to 30 ppt, while there is marked reduction in oxygen consumption.

The ionic concentrations (Na^+, K^+ and Cl^-) estimated did not show any isoionic condition with sea water. The Cl^- and K^+

concentrations of body fluid were found to be hyperionic to sea water in all the salinities and size groups. This hyperionic state was found to be higher for smaller size group than medium and large size groups. But in the case of Na^+ the hyperionic state was not observed in all the salinities. In optimum/nearly optimum salinities the animals kept the Na^+ hypoionic to sea water. The hyperionic state of Na^+ was more pronounced in lowered salinities for all the size groups. When the concentration of these three ions is taken as 100%, the percentage concentration of K^+ was found in a steady state in all the salinities for the three size groups. But in lowered salinities, percentage concentration of Na^+ was found to be higher than Cl^- and in higher salinities the Cl^- was higher. The trend was found to be the same for the three size groups. But the magnitude of difference between the percentage concentration of Na^+ and Cl^- was more pronounced for smaller size group than for medium and large size groups. The above results clearly indicate that the animals are capable of regulating ionic concentrations in a wide range of salinities either by increasing Na^+ or Cl^- concentration.

The values of body condition index clearly showed a close relationship with salinity conditions. The maximum body condition index was obtained in higher and stable salinity conditions. The lowered saline condition during monsoon season restricts the feeding rate of the animals which inturn causes the utilization of body reserves for maintenance metabolism.

During this time body condition index was found to be decreased. In addition to this, salinity was reported as a major factor affecting the gametogenic condition of *S. scripta*. Spawning takes place during April-May and after the period the animal encounters prolonged low saline period. This can be the reason for low body condition index during the entire monsoon period. Even smaller size group, which are not involved in gametogenic activity, has got a reduction in body condition index during the low saline period. This is an indication that salinity plays a role in maintaining the body condition index of the species.

The biochemical studies (carotenoid concentration) of *S. scripta* indicate that the animals are capable of overcoming short term variations in salinity by increasing the carotenoid content. In unfavourable/extreme salinity conditions the carotenoid content was found to increase due to the anoxic condition created by the reduced movement/closure of the valves. This increase of carotenoid was more pronounced as the salinity decreased from 30 ppt. When the animals were subjected to higher or lower than 30 ppt salinities, carotenoid content was found increased upto the third day and decreased after that. This results indicate that in unfavourable/extreme salinities the animal is depending upon carotenoid as an electron acceptor as normal oxygen supply is impaired. Most probably the animal is able to adjust to the environmental alterations and depend on other mechanisms for survival within a period of three days which can be the reason for the decrease in carotenoid content

after third day. The trend was found to be the same in all the size groups.

In conclusion, the behavioural, physiological and biochemical studies in different salinities and size groups indicate that salinity is a limiting factor in the distribution and abundance of *S. scripta* and is restricting the species to marine environment.

REFERENCES

ABEL, P. D. 1976. Effects of some pollutants on the filtration rates of *Mytilus*. *Mar. Pollut. Bull.*, 7, 228-231.

AKBERALI, H. B. 1978. Behaviour of *Scrobicularia plana* (da Costa) in water of various salinities. *J. Exp. Mar. Biol. Ecol.*, 33, 227-249.

AKBERALI, H. B. AND BLACK, J. E. 1980. Behavioural responses of the bivalve *Scrobicularia plana* (da Costa) to short-term copper (Cu^{2+}) concentrations. *Mar. Environ. Res.*, 4, 97-107.

AKBERALI, H. B. AND DAVENPORT, J. 1981. The response of the bivalve *Scrobicularia plana* (da Costa) to gradual salinity changes. *J. Exp. Mar. Biol. Ecol.*, 53, 251-259

AKBERALI, H. B. AND DAVENPORT, J. 1982. The detection of salinity changes by the marine bivalve molluscs *Scrobicularia plana* (da Costa) and *Mytilus edulis* (L.). *J. Exp. Mar. Biol. Ecol.*, 58, 59-71.

AKBERALI, H. B. AND TRUEMAN, E. R. 1979. pO_2 and pCO_2 changes in the mantle cavity of *Scrobicularia plana* (Bivalvia) under normal and stress conditions. *Estuarine and Coastal Marine Sciences* 9, 499-507

AKBERALI, H. B. AND TRUEMAN, E. R. 1985. Review: Effects of Environmental stress on marine bivalve molluscs. *Adv. Mar. Biol.*,22, 101- 198.

AKBERALI, H. B., HONG, T. M. AND TRUEMAN, E. R. 1981. Behavioural and siphonal tissue responses of *Scrobicularia plana* (Bivalvia) to zinc. *Mar. Environ. Res.*, 5, 251-264.

AKBERALI, H. B., MARRIOTT, K. R. M. AND TRUEMAN, E. R. 1977. Calcium utilization during anaerobiosis induced by osmotic shock in a bivalve mollusc. *Nature* (London). 266, 852-853.

ALAGARSWAMI, K. AND VICTOR, A. C. C. 1976. Salinity tolerance and rate of filtration of the pearl oyster *Pinctada fucata*. *J. Mar. Biol. Ass. India.*, 18, 1, 149-158.

ALLEN, J. A. 1962. Preliminary experiments on the feeding and excretion of bivalves using *Phaeodactylum* Labelled with ^{32}P *J. Mar. Biol. Ass. U.K.*, 42, 609-623.

ALLEN, J. A. AND GARRETT, M. R. 1971. The excretion of ammonia and urea by *Mya arenaria* L. (Mollusca:Bivalvia). *Comp. Biochem. Physiol.*, 39A, 633-642.

ANDREWS, T. R. AND REID, R. G. B. 1972. Ornithine cycle and uricolytic enzymes in four bivalve molluscs. *Comp. Biochem. Physiol.*, 42B, 475-491.

ANSELL, A. D. 1982. Experimental studies of a benthic predator-prey relationship. II Energetics of growth and reproduction and food conversion efficiencies in long-term cultures of the gastropod drill *Polinices alderi* (Forbes) feeding on the bivalve *Tellina tenuis* (da Costa) *J. Exp. Mar. Biol. Ecol.*, 61, 1-29.

ANSELL, A. D. AND SIVADAS, P. 1973. Some effects of temperature and starvation on the bivalve *Donax vittatus* (da Costa) in experimental laboratory populations. *J. Exp. Mar. Biol. Ecol.*, 13, 229-262.

BABY, K. V. AND MENON, N. R. 1986. Oxygen uptake in the brown mussel, *Perna indica* (Kuriakose and Nair) under sublethal stress of Hg, Cd and Zn *Indian J. Mar. Sci.*, 15, 127-128.

BAIRED, R. H. 1966. Factors affecting growth and condition of mussels (*Mytilus edulis* L.) *Fish. Invest., Minist. Agric. Fish. Food., Lond., Ser.* 2, 25, 1-33.

BAYNE, B. L. 1973a. Physiological changes in *Mytilus edulis* L. induced by temperature and nutritive stress. *J. Mar. Biol. Ass. U.K.*, 53, 39-58.

BAYNE, B. L. 1973b. The responses of three species of bivalve molluscs to declining oxygen tension at reducd salinity. *Comp. Biochem. Physiol.*, 45A. 793-806.

BAYNE, B. L. 1975. Aspects of physiological condition in *Mytilus edulis* (L.) with special reference to the effects of oxygen tension and salinity. In: *Proce. 9th Europ. Mar. Biol. Symp.*, (Ed.) H. Barns, University press, Aberdeen, 213-238.

BAYNE, B. L. AND NEWELL, R. D. 1983. Physiological energetics of marine molluscs. In: *The Mollusca*, Vol.4, Physiology, Part

I (eds.) Saleddin, A.S.M. and Wilbur, M., Academic press. New york. 407-515.

BAYNE, B. L. AND SCULLARD, C. 1977. Rates of nitrogen excretion by species of *Mytilus* (Bivalvia:Mollusca) *J. Mar. Biol. Ass. U.K.*, 57, 355-364.

*BAYNE, B. L. AND THOMPSON, R. J. 1970. Some physiological consequences of keeping *Mytilus edulis* in the laboratory. *Helgolander*wiss. Meeresunters. 20, 526-552.

BAYNE, B. L. AND WIDDOWS, J. 1978. The physiological ecology of two populations of *Mytilus edulis* L. *Oecologia, Berl.*, 37, 137-162.

BAYNE, B. L. AND WORRAL, C. M. 1980. Growth and production of mussels *Mytilus edulis* from two populations. *Mar. Ecol. Prog. Ser.*, 3, 317-328.

BAYNE, B. L., BAYNE, C. J., CAREFOOT, T. C. AND THOMPSON, R. J. 1976a. The physiological ecology of *Mytilus californianus* Conrad. 1. Metabolism and energy balance. *Oecologia*, 22, 211-228.

BAYNE, B. L., BAYNE, C. J., CAREFOOT, T. C. AND THOMPSON, R. J. 1976b. The physiological ecology of *Mytilus californianus* Conard. Adaptations to low oxygen tension and air exposure. *Oecologia*, 22, 229-250.

BAYNE, B. L., BROWN, D. A., BURNS, K., DIXON, D. R. IVANOVICI, A., LIVINGSTONE, D. R., LOWE, D. M., MOORE, M. N., STEBBING, A. R. D. AND WIDDOWS, J. 1985. The effects of stress and pollution on marine animals. Praeger Special Studies. Praeger Scientific Publishers 314 pp

BAYNE, B. L., CLARKE, K. R. AND MOORE, M. N. 1981. Some practical considerations in the measurement of pollution effects on bivalve molluscs and some possible ecological consequences. *Aquat. Toxicol.*, 1, 159-174.

BAYNE, B. L., GABBOTT, P. A. AND WIDDOWS, J. 1975. Some effects of stress in the adult on the eggs and larvae of *Mytilus edulis* L. *J. Mar. Biol. Ass. U. K.*, 55, 675-689.

BAYNE, B. L., HOLLAND, D. L., MOORE, M. N., LOWE, D. M. AND

WIDDOWS, J. 1978. Further studies on the effects of stress in the adult on the eggs of *Mytilus edulis J. Mar. Biol. Ass. U.K.*, 58, 825-841.

BAYNE, B. L., MOORE, M. N., WIDDOWS, J., LIVINGSTONE, D. R. AND SALKELD, P. 1979. Measurement of the responses of individuals to environmental stress and pollution: Studies with bivalve molluscs *Phli. Trans. R. Soc. Lond.*, B. 563-581.

BAYNE, B. L., THOMPSON, R. J. AND WIDDOWS, J. 1973. Some effects of temperature and food on the rate of oxygen consumption by *Mytilus edulis* L. In: *Effects of Temperature on Ectothermic Organisms* (ed.) W. Wieser. Springer-Verlag, Berlin. 191-193.

BAYNE, B. L., THOMPSON, R. J. AND WIDDOWS, J. 1976c. physiology I. In :*Marine Mussels: their Ecology and Physiology* (ed.) Bayne, B. L. Cambridge University Press, Cambridge, 121-206.

BAYNE, B. L., WIDDOWS, J. AND NEWELL, R. I. E. 1977. Physiological measurements on estuarine bivalve molluscs in the field. In : *Biology of Benthic Organisms*(eds.) B. K. Keegan, P. O Ceidigh and P. J. S. Boaden, Pergamon, New York, 57-68.

BAYNE, B. L., WIDDOWS, J., AND THOMPSON, R. J. 1976d. Physiology: II. In :*Marine Mussels: their Ecology and Physiology* (ed.) Bayne, B. L., Cambridge University Press, Cambridge, 207-260.

BAYNE, B. L., WIDDOWS, J. AND THOMPSON, R. J. 1976e. Physiological integrations In: *Marine mussels: their ecology and physiology.* (ed.) B.L. Bayne, Cambridge University press, Cambridge. 261-291.

BEDFORD, W. B. AND ANDERSON, J. W. 1972. The physiological responses of the estuarine clam, *Rangia cuneata* (Gray). I. Osmoregulation. *Physiol. Zool.*, 45, 255-260.

BLACKSTOCK, J. 1984. Biochemical and metabolic regulatory responses of marine invertebrates to natural environmental

change and marine pollution. *Oceanogr. Mar. Biol. Ann. Rev.*, 22, 263-314.

BOHLE, B. 1972. Effects of adaptation to reduced salinity on filtration activity and growth of mussels (*Mytilus edulis*). *J. Exp. Mar. Biol. Ecol.*, 10, 41-49.

*BOUXIN, H. 1931. Influences des variations rapides de la salinite' sur la consommation d'oxygene chez *Mytilus edulis* var. *galloprovincialis* (Lmk.). *Bull. de l'Institut Oce'anographique*. 569, 1-11.

BOYDEN, C. R. 1972a. The behaviour, survival and respiration of the cockles *Cerastoderma edule* and *C. glaucum* in air. *J. Mar.Biol. Ass. U.K.*, 52, 661-680.

BOYDEN, C. R. 1972b. Aerial respiration of the cockle *Cerastoderma edule* in relation to temperature. *Comp. Biochem. Physiol.*, 43A, 697-712.

BRAND, A. R. AND ROBERTS, D. 1973. The cardiac responses of the scallop *Pecton maximus* (L) to respiratory stress. *J. Exp. Mar. Biol. Ecol.*, 13, 29-43.

BRAND, A. R. AND TAYLOR, A. C. 1974. Pumping activity of *Arctica islandica* (L) and some other common bivalves. *Mar. Behav. Physiol.*, 3, 1-15.

BRETT, J. R. 1979. Environmental factors and growth. In: *Fish Physiology* (ed.) W.S. Hoar, D.R. Randall and J.R. Brett. Vol.8, Academic press, New york. 599-675.

*BRICTEUX-GREGOIRE, S., DUCHÂTEAU-BOSSON, G., JEEUNIAUX, C. AND FLORKIN, M. 1964. Constituants osmotiquement actifs des muscles adducteurs de *Mytilus edulis* adaptée a l'eau de mer ou a l'eau saumâtre. Archives. *Internationales de Physiologie et de Biochemic*. 72, 116-123.

BROWN, A. C., STENTON-DOZEY, J. M. E. AND TRUEMAN, E. R. 1989. Sandy-Beach Bivalves and Gastropods : A Comparison between *Donax serra* and *Bullia digitalis*. *Adv. Mar. Biol.*, 25, 179-247.

BUXTON, C. D., NEWELL, R. C. AND FIELD, J. G. 1981. Response-surface analysis of the combined effects of

exposure and acclimation temperatures on filtration, oxygen consumption and scope for growth in the oyster *Ostrea edulis*. *Mar. Ecol. Prog. Ser.*, 6, 73-82.

CLARKE, B. C. AND GRIFFITHS, C. L. 1990. Ecological energetics of mussels, *Choromytilus meridionalis* under simulated intertidal rock pool conditions. *J. Exp. Mar. Biol., Ecol.* 137, 63-77.

COLE, H. A. AND HEPPER, B. T. 1954. Use of neutral red solution for the comparitive study of filtration rates of lamellibranchs. *J. Cons.*, 20, 197-203.

COLEMAN, N. 1974. A review of heart rate in the bivalve molluscs. *Oceano. Mar. Biol. Ann. Rev.*, 12, 301-313.

COLEMAN, N. AND TRUEMAN, E. R. 1971. The effect of aerial exposure on the activity of the mussels *Mytilus edulis* (L.) *and Modiolus modiolus* (L.). *J. Exp. Mar. Biol., Ecol.* 7, 295-304.

CONOVER, R. J. 1966. Assimilation of organic matter by zooplankton. *Limnol. Ocanogr.*, 11, 338-345.

CONOVER, R. J. AND CORNER, E. D. S. 1968. Respiration and nitrogen excretion by some marine zooplankton in relation to their life cycles. *J. Mar. Biol. Ass. U.K.*, 48, 49-75.

CONOVER, R. J., LALLI, C. M. 1974. Feeding and growth in *Clione limacina* (Phipps), A pteropod mollusc. Assimilation, metabolism and growth efficiency. Vol.II *J. Exp. Mar. Biol. Ecol.*, 16, 131-154.

CORNER, E. D. S. AND COWEY, C. B. 1968. Biochemical studies on the production of marine zooplankton. *Biol. Bull.*, 43, 393-426.

CRENSHAW, M. A. AND NEFF, J. M. 1969. Decalcification at the mantle-shell interface in molluscs. *Amer. Zool.*, 9, 881-885.

CRISP, D. J. 1971. Energy flow measurements. In: *Methods for the study of marine benthos*, IBP Handbook No.16 (ed.) N.A. Holme and A.D. McIntyre, Blackwell Scientific Publications, Oxford. 197-279.

DAME, R. F. 1972. The ecological energies of growth, respiration and assimilation in the American Oyster, *Crassostrea virginica*. *Mar. Biol.*, 17, 243-250.

DARE, P. J. AND EDWARDS, D. B. 1975. Seasonal changes in flesh weight and biochemical composition of mussels (*Mytilus edulis* L.) in the Conwy estuary North Wales. *J. Exp. Mar. Biol. Ecol.*, 18, 89-97.

DAVENPORT, J. 1977. A study of the effects of copper applied to specimens of *Mytilus edulis* (L.) exposed to steady and fluctuating salinity levels. *J. Mar. Biol. Ass. U.K.*, 57, 63-74.

DAVENPORT, J. 1979. The isolation response of mussels (*Mytilus edulis* L.) exposed to falling seawater concentrations. *J. Mar. Biol. Ass. U. K.*, 59, 123-132.

DAVENPORT, J. 1982. Environmental simulation experiments on marine and estuarine animals. *Adv. Mar. Biol.*, 19, 133-256.

DAVENPORT, J. AND CARRION-COTRINA, M. 1981. Responses of the mussel *Mytilus edulis* L. to simulated subarctic tide pool conditions. *J. Therm. Biol.*, 6, 257-265.

DAVIES, P. S. 1967. Physiological ecology of *Patella*. II Effect of environmental acclimation on the metabolic rate. *J. Mar. Biol. Ass. U.K.*, 47, 61-74.

DEATON, L. E. 1981. ion regulation in fresh water and brackish water bivalve molluscs. *Physiol. zool.*, 54, 109-121.

DEATON, L. E., DERBY, J. G. S., SUBHEDAR, N. AND GREENBERG, M. J. 1989. Osmoregulation and salinity tolerance in two species of bivalve molluscs: *Limnoperna fortunei* and *Mytilopsis leucophaeta*. *J. Exp. Mar. Biol. Ecol.*, 133, 67-79.

DESHMUKH, R. S. 1979. On the oxygen consumption of the estuarine mollusc, *Meretrix meretrix* under various conditions. *J. Mar. Biol. Ass. India.*, 21, 1-9.

DE ZWAAN, A. 1977. Anaerobic energy metabolism in bivalve molluscs. *Oceanogr. Mar. Biol. Ann. Rev.*, 15, 103-187.

DE ZWAAN, A. AND VAN MARREWIJK, W. J. A. 1973. Anaerobic glucose

degradation in the sea mussel *Mytilus edulis* L. *Comp. Biochem. Physiol.*, 44B, 429-439.

DE ZWAAN, A. AND ZANDEE. 1972. The utilization of glycogen and accumulation of some intermediates during anaerobiosis in *Mytilus edulis Comp. Biochem. Physiol.* 43B, 47-54.

*DUCHÂTEAU, D. AND FLORKIN. 1956. Systems intracellulaires dácides amine's libres et osmore'gulation des Crustace's. *J. de. Physiologie.*, Paris. 8, 520

*EARLL, R. 1975. "Some Aspects of Heart rate and Activity in Bivalve Molluscs in the Laboratory and the Natural Environment." Ph.D Thesis, Univ. Manchester.

ELVIN, O. W. AND GONOR, J. J. 1979. The thermal regime of an intertidal *Mytilus californianus* Conrad population on the Central Oreon Coast. *J. Exp. Mar. Biol. Ecol.*, 39, 265-279.

EMERSON, D. N. 1969. Influence of salinity on ammonia excretion rates and tissue constituents of euryhaline invertebrates. *Comp. Biochem. Physiol.*, 29, 1115-1133.

EPIFANIO, C. E. AND EWART, J. 1977. Maximum ration of four diets for the oyster *Crassostrea virginica* Gmelin, *Aquaculture*, 11, 13-29.

FAMME, P. 1980a. Oxygen-dependence of the respiration by the mussel *Mytilus edulis* L. as a function of size. *Comp. Biochem. Physiol.*, 67, 171-174.

FAMME, P. 1980b. Effect of shell valve closure by the mussel *Mytilus edulis* L. on the rate of oxygen consumption in declining oxygen tension. *Comp. Biochem. Physiol.*, 67, 167-170.

FOSTER-SMITH, R. L. 1976. Some mechanisms for the control of pumping in bivalves. *Mar. Behaviour. Physiol.*, 4, 41-60.

*FREDERICQ, L. 1901. Sur la concentration mole'culaire du sang et des tissus chez les animaux aquatiques. *Bull. Acad. Belg. Cl. Sci.*, 428 pp.

FREEMAN, R. F. H. AND RIGLER, F. H. 1957. The responses of *Scrobicularia plana* (da Costa) to osmotic pressure changes. *J. Mar. Biol. Ass. U.K.*, 36, 553-567.

FRY, F. E. J. 1947. Effects of the environment on animal activity. Publications of the Ontario Fisheries Research Laboratory, 68, 1-62.

*FUJI, A. AND HASHIZUME, M, 1974. Energy budget for a Japanese common scallop *Patinopecten yessoensis* (Jay) in Mutsu Bay. *Bull. Fac. Hokkaido Univ.*, 25, 7-19.

GABBOTT, P. A. AND BAYNE, B. L. 1973. Boichemical effects of temperature and nutritive stress on *Mytilus edulis* L. *J. Mar. Biol. Ass. U.K.*, 43, 269-286.

GABBOTT, P. A. AND STEPHENSON, R. R. 1974. A note on the relationship between the dry weight condition index and the glycogen content of adult oysters (*Ostrea edulis* L.) kept in the laboratory. *J. Cons. Int. Explo. Mer.*, 35, 359-361.

GALTSOFF, P. S. 1964. The American oyster *Crassostrea virginica* Gmelin. *Fish. Bull.* 64, U. S. Government Printing Office, Washington, D. C. pp 480.

GANAPATI AND RAMASASTRY, R. V. 1972. Oxygen consumption and metabolic rate in relation to body size in the intertidal gastropod, *Turbo intercostalis* (Menke) *Proc. Indian Nat. Sci. Acad.*, 38, 555-559.

GEE, J. M., MADDOCK, L. AND DAVEY, J. T. 1977. The relationship between infestation by *Mytilicola intestinalis*, Steuer (Copepoda, Cyclopoidea) and the condition index of *Mytilus edulis* in South-West England. *J. Cons. Int. Explor. Mer.*, 37, 300-308.

GENOVEA DE MAHIEU, ALBERTO MARTIN AND JOSE'L RADA 1988. Physiological and behavioural aspects related to salinity changes in *Donax denticulatus* (Bivalvia : Donacidae). *Comp. Biochem. Physiol.*, 89, 487-493.

GHIRETTI, F. 1966. Respiration. In: *Physiology of Mollusca* (eds.) Willus, K.M. and Yonge, C.M.). Academic press, New York, 175-208.

GILFILLAN, E. S. 1975. Decrease of net flux in two species of mussels caused by extracts of crude oil. *Mar. Biol.*, 29,

53-58.

GILFILLAN, E. S., MAYO, D. W., PAGE, D. S., DONOVAN, D. AND HAUSON, S. 1977. Effects of varying concentrations of petroleum hydrocarbons in sediments on carbon flux in *Mya arenaria* In: *Physiological Responses of Marine Biota to Pollutants*. (eds.) F.J. Vernberg, A. Calabrese, F.P. Thurberg and W.B. Vernberg, Academic press, New York, 299-314.

GILLES, R. 1972. Osmoregulation in three molluscs *Accanthochitona discrepans* (Brown) *Glycymeris glycymeris* (L.) and *Mytilus edulis* (L). *Biol. Bull., Mar. Biol. Lab., Woods Hole.* 142, 25-35.

GILLES, R. 1975. Mechanisms of iono and osmoregulation. In: *Marine Ecology* (ed. O. Kinne), Vol II. Part 1. Wiley Interscience, New York, 259-347.

GRIFFITHS, R. J. 1980. Natural food availability and assimilation in the bivalve *Chromytilus meridionalis. Mar. Ecol. Prog. Ser.*, 3, 151-156.

GRIFFITHS, C. L. AND GRIFFITHS, R. J. 1987. Bivalvia In: *Animal Energetics* (ed.) T.J. Pandian and F.J. Vernberg. Academic press, New York, 2, 1-88.

GRIFFITHS, C. L. AND KING, J. A. 1979. Some relationships between size, food availability and energy balance in the ribbed mussel *Aulacomya ater. Mar. Biol.*, 51, 141-149.

HAMMEN, C. S. 1968. Aminotransferase and amino acid excretion of bivalve molluscs and brachiopods. *Comp. Biochem. Physiol.*, 26, 697-705.

HAMMEN, C. S. 1969. Metabolism of the oyster, *Crassostrea virginica. Amer. Zool.*, 9, 309-318.

HAMMEN, C. S. 1975. Succinate and lactate oxidoreductases of bivalve molluscs. *Comp. Biochem. Physiol.*, 50B, 407-412.

HAWKINS, A. J. S., BAYNE, B. L. AND DAY, A. J. 1986a. Protein turnover, physiological energetics and heterozygosity in the blue mussel *Mytilus edulis*: the basis of variable age specific growth. *Proc. R. Soc. London.* 229B, 161-176.

HAWKINS, A. J. S., BAYNE, B. L., MENON, N. R. AND DAMODARAN, R. 1986b. The mussel *Perna viridis* and *Perna indica* as transplantable indicators of pollution: Comparison of their metabolic response to reduction of both oxygen tension and salinity. In: *Proc.of the nat. sem. on mussel watch., Cochin Univ. Sci. Tech.*, 1, 51-64.

HAWKINS, A. J. S., MENON, N. R., DAMODARAN, R. AND BAYNE, B. L. 1987. Metabolic responses of the mussels *Perna viridis* and *Perna indica* to declining oxygen tension at different salinities. *Comp. Biochem. Physiol.*, 88A, 4, 691-694.

HILBISH, T. J. 1986. Growth trajectories of shell and soft tissues in bivalves: Seasonal variation in *Mytilus edulis* L. *J. Exp. Mar. Biol. Ecol.* 96, 103-113.

HOCHACHKA, P. W. 1985. Theorems of environmental adaptations. C.M.F.R.I., Special Publication No. 26, Cochin, India, 153pp.

HOCHACHKA, P. W. AND MUSTAFA, 1972. Invertebrate facultative anaerobiosis. *Science*, 178, 1056-1060.

HOCHACHKA, P. W. AND SOMERO, G. N. 1973. *Strategies of biochemical adaptation.* W. B. Saunders, Philadelphia, 358pp.

HOYAUX, J., GILLES, R. AND JENTAUX, C. H. 1976. Osmoregulation in molluscs of the intertidal zone. *Comp. Biochem. Physiol.*, 53A, 361-365.

HUGHES, R. N. 1970. An energy budget for a tidal flat population of the bivalve *Scrobicularia plana*. (Da Costa). *J. Anim. Ecol.*, 6, 167-178.

*IMCO/FAO/UNESCO/WHO/IAEA/UN/UNEP. 1980. Joint group of experts on the Scientific Aspects of Marine Pollution (GESAMP). Monitoting Biological variables to Marine Pollution. *Rep. Stud.* GESAMP. 12, 20 pp.

IVLEV, V. S. 1961. Experimental ecology of the feeding of fishes. Yale University press, New Haven, 302pp.

JORGENSON, C. B. 1976. Growth efficiencies and factors controlling size in some mytilid bivalves, especially

Mytilus edulis L. A review and interpretation, *Ophelia*, 15, 175-192.

*KARNAUKHOV, V. N. 1969. Carotenoids in oxidative metabolism of animal cells. *Abstr. 2nd U.S.S.R. Biochem. Miting. Sect.*, 8, 30 pp, Tashkent (In Russian).

KARNAUKHOV, V. N. 1970. Dissertation. Institute of Biophysics. Acad. U.S.S.R. *Pushchino*, U.S.S.R. (In Russian).

*KARNAUKHOV, V. N. 1971. On a role of caroteniods in intracellular oxygen storage. *Dokl. Akad. Nauk.*, U.S.S.R. 196, 1221-1224. (In Russian).

KARNAUKHOV, V. N. 1973a. Functions of caroteniods in animal cells. *Nauka*. Moscow (In Russian).

KARNAUKHOV, V. N. 1973b. On the nature and function of yellow aging pigment lipofuscin. *Exp. Cell. Res.*, 80, 474-483.

KARNAUKHOV, V. N. 1979. The role of filtrator molluscs rich in carotenoids in the self-cleaning of fresh waters. *Symp. Biol. Hung.*, 19, 151-167

KARNAUKHOV, V. N. AND FEDOROV, G. G. 1977. The role of Carotenoids and Vitamin A in animal adaptation to high altitude. *Comp. Biochem. Physiol.*, 57A, 377-381.

KARNAUKHOV, V. N., MILIVIDOVA, N. Y. AND KARGOPOLOVA, I. N. 1977. On a role of carotenoids in tolerance of sea mollusca to environment pollution. *Comp. Biochem. Physiol.*, 56A, 189-193.

KATTICARAN, C. M. 1988. Studies on the biology of the clam *Sunetta scripta* (Linne), from the subtidal waters of Cochin. Ph. D. Thesis. Cochin University of Science and Technology. 155 pp.

KENNEDY, V. S. AND MIHURSKY, J. A. 1972. Effect of temperature on the respiratory metabolism of three Chesapeake Bay bivalves. *Chesapeake Sci.*, 13, 1-22.

KINNE, O. 1964a. The effects of Temperaure and salinity on marine and brackish water animals. II. Salinity and temperature-Salinity combinations. *Oceano. Mar. Biol. Ann.*

Rev., 2, 281-339.

*KINNE, O. 1964b. Non-genetic adaptation to temperaure and salinity. *Helgolander Wissenschaftliche Meeresunersuchungen*, 9, 433-458.

KINNE, O. 1971. Salinity: Animals-invertebrates. In: Marine Ecology Vol. I, Environmental factors, Part 2, (ed.) Kinne, O. Wiley-Interscience, New York, 821-925.

KIORBOE, T. AND MOHLENBERG, F. 1981. Particle selection in suspension feeding bivalves. *Mar. Ecol. Prog. Ser.*, 5, 291-296.

KRISHNAKUMAR, P. K. 1987. Physiological effects of some heavy metals on *Perna viridis* (Linnaeus). Ph. D. Thesis. Cochin University of Science and Technology, Cochin, 126 pp.

KRISHNAKUMAR, P. K. AND DAMODARAN, R. 1986. Effect of body size on the physiology of green mussel *Perna viridis* (Linnaeus) *Nat. Sem. Mussel Watch.*, 13-14 Feb., Univ. Cochin. 77-85.

KRISHNAKUMAR, P. K., ASOKAN, P. K. AND PILLAI, V. K. 1990. Physiological and cellular responses to Copper and Mercury in the green mussel *Perna viridis* (Linnaeus). *Aquatic Toxicol.* 18, 163-174.

KROGH, A. 1965. *Osmotic Regulation in Aquatic Animals.*, Dover Publications, INC, New York. 53-64.

KUENZLER, E. J. 1961. Structure and energy flow of a mussel population in a Georgia Salt Marsh. *Limnol. Oceanogr.*, 6, 191-204.

*LANGERSPETZ, K. AND SIRKKA, A. 1959. Uersuche uber den Sauerstoffverbrauch von *Mytilus edulis* aus dem Brackwasser der finnischen Kuste., *Kieler* Meeresforschungen, 15, 89-96.

LANGTON, R. W. AND GABBOTT, P. A. 1974. The tidal rhythm of exracellular digestion and the response to feeding in *Ostrea edulis*. *Mar. Biol.*, 24, 181-197.

LIVINGSTONE, D. R., WIDDOWS, J. AND FIETH, P. 1979. Aspects of nitrogen metabolism of the common mussel *Mytilus edulis:* Adaptation to abrupt and fluctuating changes in salinity. *Mar. Biol.*, 53, 41-55.

LOOSANOFF, V. L. 1950. On the behaviour of oyster transferred from low to high salinities. *Anat. Res.*, 108, 91.

MAC DONALD, B. A. AND THOMPSON, J. 1986. Influence of temperature and food availability on the ecological energetics of the giant scallop *Placopecten magellanicus* 1. Physiological ecology, the gametogenic cycle and scope for growth. 93, *Mar. Biol.* 37-48.

MANE, U. H. 1974. The adaptations of the estuarine clam *Katelysia opima* to the salinity fluctuations. *Rivista di Biologia.*, 27, 73-107.

MANE, U. H. 1975. A study on the rate of water transport of the clam, *Katelysia opima* in relation to environmental conditions. *Hydrobiologia.*, 47, 439-451.

MANGAPATI RAO, K., RAMACHANDRA RAJU, P. AND GANTI, S. S. 1974. Studies on respiration in relation to body size and oxygen tension in the pelecypod, *Congeria sallei* (Reeluz). *Proc. Ind. Acad. Sci.*, XXX, 163-171.

MANLEY, A. R. 1983. The effects of copper on the behaviour, respiration, filtration and ventilation activity of *Mytilus edulis*. *J. Mar. Biol. Ass. U.K.*, 63, 205-222.

MANLEY, A. R. AND DAVENPORT, J. 1979. The behavioural response of some marine bivalves to heigtened sea water copper concentrations. *Bull. Environ. Contamin. Toxicol.*, 22, 739-744.

MATHERS, N. F. 1976. The effects of tidal currents on the rhythms of feeding and digestion in *Pecten maximus* L. *J. Exp. Mar. Biol. Ecol.*, 24, 271-283.

MATHEW, P. 1990. Sub-lethal effects of heavy metals on *Perna indica* (Kuriakose and Nair) and *Donax incarnatus* Gmelin. Ph. D Thesis. Cochin Univ. Sci. Tech., Cochin. 171pp.

MAYZAUD, P. 1973. Respiration and nitrogen excretion of zooplankton II. Studies of the metabolic characteristics of starved animals. *Mar. Biol.*, 21, 19-28.

MOHAN, M. V. 1979. Studies on the Teredinids of Cochin Harbour. Ph. D. Thesis. Univ. of Cochin. pp 196.

MOHAN, M. V. AND CHERIAN, P. V. 1980. Oxygen consumption of the

green mussel *Perna viridis* L. in relation to body weight and declining oxygen tension. *Bull. Dept. Mar. Sci.*, Univ. of Cochin. 11, 1, 75-84.

MOHAN, M. V. AND DAMODARAN, R. 1981. Allometric relatioship in the clam *Sunetta scripta* L. *Indian J. Mar.Sci.*,10, 198-200.

MOON, T. W. AND PRITCHARD, A. W. 1970. Metabolic adaptation in vertically separated populations of *Mytilus californianus* Conard. *J. Exp. Mar. Biol. Ecol.*, 5, 35-46.

MOORE, M. N., KOEHN, R. K. AND BAYNE, B. L. 1980. Leucine amino peptidase (amino peptidase 1), N-acetyl-β-hexosaminidase and lysosomes in the mussel, *Mytilus edulis*, in response to salinity changes. *J. Exp. Zool.*, 214, 239-249.

MORTON, B. S. 1977. The tidal rhythm of feeding and digestion in the Pacific Oyster, *Crassostrea gigas* (Thurberg). *J. Exp. Mar. Biol. Ecol.*, 26, 135-151.

NAGABHUSHANAM, R. 1956. The rate of water filtration in the marine wood boring molluscs, *Martesia striata*. *Proc. Indian. Acad. Sci.*, 47B, 223-227.

NAGABHUSHANAM, R. 1962. Some aspects of the general biology of *Martesia striata* L. *Bull. Nat. Inst. Sci.*, India. 19, 126-130.

NAGABHUSHANAM, R. 1966. On the oxygen consumption of the wood boring mollusc, *Martesia striata* under various conditions. *Proc. 2nd All India Cong. Zool.*, 2, 154-159.

NAGABHUSHANAM, R. AND BIDARKAR, K. S. 1975. Adaptations of the common Rock Oyster, *Crassostrea cucullata* to low salinities at Ratnagiri. *Bull. Dept. Mar. Sci., Univ. Cochin.* 7, 409-417.

NAVARRO, J. M. AND WINTER, J. E. 1982. Ingestion rate, assimilation efficiency and energy balance in *Mytilus chilensis* in relation to body size and different algal concentrations. *Mar. Biol.*, 67, 3, 255-266.

NEWELL, R. C. 1973. Factors affecting the respiration of intertidal inveretebrates. *Amer. Zool.*, 13, 513-528.

NEWELL, R. C. 1979. Biology of intertidal animals, 3rd ed.,

Marine Ecological Surveys Ltd. Faversham, Kent, 781pp

NEWELL, R. I. E. 1977. The eco-physiology of *Cardium edule* L. Ph. D. Thesis, Univ. of London, 215pp.

NEWELL, R. C. AND BRANCH, G. M. 1980. The effects of temperature on the maintenance of metabolic energy balance in marine invertebrates. *Adv. Mar. Biol.*, 17, 329-396.

NEWELL, R. C. AND KOFOED, L. H. 1977. The energetics of suspension-feeding in the gastropod, *Creppidula fornicata L. J. Mar. Biol. Ass., U.K.*, 51, 161-180.

NEWELL, R. C. AND ROY, A. 1973. A statistical model relating the oxygen consumption of a mollusk (*Littorina littorea*) to activity, body size and environmental conditions. *Physiol. Zool.*, 46, 4, 252-275.

NEWELL, R. C., JOHNSON, L. G. AND KOFOED, L. H. 1977. Adjustment of the components of energy balance in response to temperature change in *Ostrea edulis*. *Oecologia*, 30, 97-110.

OSER, B. L. 1965. In: "*Hawk's*" *Physiological Chemistry*. 14thEd. Tata Mc Graw Publishing Co. Ltd., Bombay. 1141pp.

PALOHEIMO, J. E. AND DICKIE, L. M. 1965. Food and growth of fishes. I. A growth curve derived from experimental data. *J. Fish. Res. Bd., Can.*, 22, 521-542.

PALOHEIMO, J. E. AND DICKIE, L. M. 1966. Food and growth of fishes. II Effects of food and temperature on the relation between metabolism and body weight. *J. Fish. Res. Bd., Can.*, 23, 869-908.

PANDIAN, T. J. 1975a. Mechanisms of heterotrophy; Intake of energy and matter. In: *Marine Ecology* (ed.) Kinne, O. Vol 2. Physiological Mechanisms. Part 1. 64-106.

PANDIAN, T. J. 1975b. Mechanisms of heterotrophy; Intake of energy and matter. In: *Marine Ecology* (ed.) Kinne, O. Vol 2. Physiological Mechanisms. Part 1. 179-207.

PIERCE, S. K. 1970. Water balance in the genus *Modiolus* (Mollusca: Bivalvia:Mytilidae). Osmotic concentrations in changing salinities. *Comp. Biochem. Physiol.*, 36, 521-533.

PIERCE, S. K. 1971. Volume regulation and valve movements by marine mussels. *Comp. Biochem. Physiol.*, 39, 103-117.

POTTS, W. T. W. 1954. The inorganic composition of blood of *Mytilus edulis* and *Anondonta cygnea*. *J. Exp. Biol.*, 31, 376-385.

POTTS, W. T. W. 1958. The inorganic and aminoacid composition of some lamellibranch muscles. *J. Exp. Biol.*, 35, 749-764.

PRASADA RAO, D. G. V. AND VEERASALINGAM, M. 1981. Oxygen consumption in relation to body size in the bivalve, *Barbatia obliquata*. *Globios*, 8, 84-86.

*QUAYLES, D. B. 1948. Biology of *Venerupis pullastra* (Montagu). Ph. D Thesis. Univ. Glasgow. Scotland.

RAMACHANDRAN, K. 1980. Studies on the biology of the green mussel, *Perna viridis* (Linnaeus). M. FSc. Thesis, Univ. Agri. Sci., Bangalore, 219 pp.

RANADE, M. R. 1973. Effects of temperature and salinity on the oxygen consumption in clams. *J. Bombay. Nat. Hist. Soc.*, 70, 128-146.

RAO, K. P. AND BULLOCK, T. H. 1954. Q_{10} as a function of size and habitat temperature in poikilotherms. *Amer. Nat.*, 88, 33-44.

REDDY, D. K. N. 1983. Studies on the population ecology and reproductive biology of the clam *Villorita cyprinoides* (Gray). M. FSc. Thesis. Univ. Agri. Sci., Bangalore, 100pp.

REMANE, A. AND SCHLIEPER, C. 1971. *Biology of Brackish Water*. (ed.) Wiley-Interscience, New York, 372pp.

ROBERTSON, J. D. 1964. Osmotic and ionic regulation. In: *Physiology of mollusca* (ed.) K. M. Wilbur and C. M. Yonge Vol. I Academic Press, New york. 283-311.

ROBINSON, A. M. AND OVENSTON, T. C. J. 1951. A simple flame photometer for international standard operation and notes on some new liquid spectrum filters. *Analyst* , 76, 416-424

RODHOUSE, P. G. 1978. Energy transformations by the oyster *Ostrea edulis* L. in a temperate estuary. *J. Exp. Mar. Ecol.*, 34,

1-22.

SALIH, K. Y. M. 1978. Salinity tolerance of *Meretrix casta* (Chemnitz). *Bull. Dept. Mar. Sci.*, Univ. Cochin. 9, 105-123.

SANINA, L. V. 1976. Rate and intensity of filtration in some Caspian sea bivalve molluscs. *Oceanology*. (U.S.S.R.). 15, 496-498.

SATHYANATHAN, B., MURALEEDHARAN NAIR, S., JACOB CHACKO AND NAMBISAN, P. N. K. 1988. Sublethal effects of Copper and Mercury on some biochemical constituents of the estuarine clam, *Villorita cyprinoides* var. cochinensis (Hanley). *Bull. Environ. Contam. Toxicol.*, 40, 510-516.

SCHULTE, E. H. 1975. Influence of algal concentration and temperature on the filtration rate of *Mytilus edulis*. *Mar. Biol.*, 30, 331-341.

SCOTT, D. M. AND MAJOR, C. W. 1972. The effect of copper (II) on survival, respiration and heart rate in the common blue mussel *Mytilus edulis*. *Biol. Bull., Mar. Biol. Lab., Woods Hole, Mass.* 143, 679-688.

SHAFEE, M. S. 1976. Effect of salinity and time of exposure to air on the metabolism of green mussel *Mytilus viridis*. *Ind. J. Mar. Sci.*, 5, 130-132.

SHAFEE, M. S. 1979. Ecological energy requirements of the green mussel, *Perna viridis* L. from Ennore estuary, Madras. *Oceanol. Acta.*, 2, 69-74.

SHICK, J. M., WIDDOWS, J. AND GNAIGER, E. 1988. Calorimetric Studies of Sessile Intertidal Animals. *Amer. Zool.*, 28, 161-181.

SHIRLEY, T. C. AND STICKLE, W. B. 1982. Responses of *Leptasterias hexactis* (Echinodermata:Asteroida) to low salinity II. Nitrogen metabolism, Respiration and Energy budget. *Mar. Biol.*, 69, 155-163.

SHUMWAY, S. E. 1977. The effects of salinity fluctuations on the osmotic pressure and Na^{+}, Ca^{2+} and Mg^{2+} ion concentration in the haemolymph of bivalve mollusc. *Mar. Biol.*, 41,

153-178.

SHUMWAY, S. E. AND YOUNGSON, A. 1979. The effects of fluctuating salinity on the physiology of *Modiolus demissus* (Dillwyn). *J. Exp. Mar. Biol. Ecol.*, 40, 167-181.

SLOBODKIN, L. B. AND RICHMAN, S. 1961. Calories/gm in species of animals. *Nature* (London). 191, pp 299.

SNEDECOR, G. W. AND COCHRAN, W. G. 1972. Statistical methods. The Iowa State University Press, Iowa, 593 pp.

SOLORZANO, L. 1969. Determination of ammonia in natural water by the phenol-hypochlorite method. *Limnol. Oceanogr.*, 14, 799-801.

SPEEG, K. V. AND CAMPBELL, J. W. 1969. Arginase and urea metabolism in terrestrial snails. *Ame. J. Physiol.*, 216, 1003-1012.

SPOTTE, H. STEPHEN. 1970. Water management in the closed systems. In: *Fish and Invertebrate Cultures.* John Wiley and Sons. 3-59.

STICKLE, W. B. AND BAYNE, B. L. 1982. Effects of temperature and salinity on the oxygen consumption and nitrogen excretion in *Thais (Nucella) lapillus. J. Exp. Mar. Biol. Ecol.*, 58, 1-17.

STICKLE, W. B. AND SABOURIN, T. D. 1979. Effects of salinity on the respiration and heart rate of the common mussel, *Mytilus edulis* L. and the black chiton, *Katherina tunicata* (Wood). *J. Exp. Mar. Biol. Ecol.*, 41, 257-268.

STOKES, T. AND AWAPARA. J. 1968. Alanine and Succinate as end-products of glucose degradation in the clam *Rangia cuneata. Comp. Biochem. Physiol.*, 25, 883-892.

STONE, N. M. 1980. *Growth Line Patterns in Bivalve Molluscs : The Mechanism and Periodicity of Line Formation.* Ph.D Thesis Univ. Manchester.

STRICKLAND, J. D. H. AND PARSON, T. R. 1972. A practical handbook of sea water analysis. *Rev. Ed. Bull. Fish. Res. Bd., Can.* 167, 1-331.

SURESH, K. 1988. Haematology of some marine and estuarine

molluscs of commercial importance. Ph. D Thesis, Cochin University of Science and Technology, 176 pp.

SURESH, K. AND MOHANDAS, A. 1987. Hemolymph lactic acid levels in *Sunetta scripta* (Bivalvia) exposed to sublethal concentrations of Mercury and Copper. *J. Invertebr.Pathol.*, 49, 265-272.

SURESH, K. AND MOHANDAS, A. 1990a. Hemolymph acid phosphatase pattern in copper-stressed bivalves. *J. Invertebr.Pathol.*, 55, 118-125.

SURESH, K. AND MOHANDAS, A. 1990b. Number and types of hemocytes in *Sunetta scripta* and *Villorita cyprinoides* var cochinensis (Bivalvia) and leucocytosis subsequent to bacterial challenge. *J. Invertebr. Pathol.*,55, 312-318.

SURESH, K. AND MOHANDAS, A. 1990c. Effect of sublethal concentrations of copper on hemocyte number in bivalves. *J. Invertebr. Pathol.*, 55, 325-331.

TENORE, K. R. AND DUNSTAN, W. M. 1973. Comparison of feeding and biodeposition of three bivalves at different food levels. *Mar. Biol.*, 21, 190-195.

THAMPURAN, L. 1986. Physiological effects of Copper (II) on *Sunetta scripta* (L.) Ph. D Thesis, Cochin Univ. Sci. Tech., Cochin.

THAMPURAN, L., KRISHNAN NAMBISAN, P. N. AND DAMODARAN, R. 1982. Studies on *Sunetta scripta* L. Salinity tolerance and copper toxicity. *Bull. Dept. Mar. Sci., Univ. Cochin.* 13, 46-52.

*THEEDE, H. 1963. Experimentelle Untersuchungen uber die Filtrations leistung der Miesmuschel *Mytilus edulis* L. *Kieler Meeresforschungen*, 19, 20-41.

THIPPESWAMY, AND MOHAN JOSEPH, M. 1987. Seasonal variability in the condition of wedge clam *Donax incarnatus* (Gmelin). The First Indian Fisheries Forum, *Proc. Asian Fish. Soc.*, Indian Branch, Mangalore, Dec 4-8, 247-249.

THOMPSON, R. J. 1984. The reproductive cycle and physiological ecology of the mussel *Mytilus edulis* in a subarctic,

non-estuarine environment. *Mar. Biol.*, 79, 277-288.

THOMPSON, R. J. AND BAYNE, B. L. 1972. Active metabolism associated with feeding in the mussel *Mytilus edulis* (L.) *J. Exp. Mar. Biol. Ecol.*, 9, 111-124.

THOMPSON, R. J. AND BAYNE, B. L. 1974. Some relationships between growth, metabolism and food in the mussel, *Mytilus edulis*. *Mar. Biol.*, 27, 317-326.

THOMPSON, R. J., RATCLIFFE, N. A. AND BAYNE. B. L. 1974. Effects of starvation on structure and function in the digestive gland of the mussel *Mytilus edulis* L. *J. Mar. Biol. Ass. U.K.*, 54, 699-712.

TODD, M. E. 1964. Osmotic balance in *Hydrobia ulvae* and *Potamopyrgus jenkinsi* (Gastropoda:Hydrobiidae). *J. Exp. Biol.*, 41, 665-667.

TRUEMAN, E. R. 1983. Observations of the tropical surf clam *Donax denticulatus* to changes of temperature and salinity. *J. Molluscan Studies*. 49, 242-243.

VAHL, O. 1972. Particle retention and relation between water transport and oxygen uptake in *Chlamys opercularis* L. *Ophelia*. 10, 67-74.

VAHL, O. 1978. Seasonal changes in oxygen consumption of the Iceland scallop, *Chlamys islandica*. (O. F. Muller) from 70°N. *Ophelia*, 17, 143-154.

VAHL, O. 1980. Seasonal variations in seston and the growth rate of the Iceland scallop, *Chlamys islandica*. (O. F. Muller) from Balsfjord 70°N. *J. Exp. Mar. Biol. Ecol.*, 48, 195-204.

VAHL, O. 1981. Energy transformations by the Iceland scallop, *Chlamys islandica* (O.F. Muller) from 70°N. The age specific energy budget and net growth efficiency. *J. Exp. Mar. Biol. Ecol.*, 53, 281-296.

VON BERTALANFFY, L. 1957. Quantitative laws in metabolism and growth. *Quarterly. Rev. Biol.*, 32, 217-231.

WALNE, P. R. 1970. The seasonal variation of meat and glycogen content of seven populations of oysters, *Ostrea edulis* L.

and a review of the literature. *Fishery Investi.*, London, Ser.2, 26, 35pp.

WALNE, P. R. 1972. The influence of the current speed, body size and water temperature on the filtration rate of the five species of bivalves. *J. Mar. Biol. Ass., U.K.* 52, 345-374.

WARREN, G. E. AND DAVIS, G. E. 1967. Laboratory studies on the feeding, bioenergetics and growth of fish. In: The biological basis of fresh water fish production. (ed.) S.D. Gerking Blackwell Scientific Publications, Oxford. 175-214.

*WELSH, J. H. AND SMITH, R. I. 1953. *Laboratory Excercises in Invertebrate Physiology*. Burges Publishing Co., Minneapolis.

WIDDOWS, J. 1973a. Effect of temperature and food on the heart beat, ventilation and oxygen uptake of *Mytilus edulis*. *Mar. Biol.* 20, 269-276

WIDDOWS, J. 1973b. The effects of temperature on the metabolism and activity of *Mytilus edulis*. *Netherlands J. Sea. Res.*, 7, 387-398.

WIDDOWS, J. 1978a. Combined effects of body size, food concentration and season on the physiology *Mytilus edulis* L. *J. Mar. Biol. Ass. U. K.*, 58, 109-124.

WIDDOWS, J. 1978b. Physiological indices of stress in *Mytilus edulis*. *J. Mar. Biol. Ass. U. K.*, 58, 125-142.

WIDDOWS, J. 1985a. Physiological responses to pollution. *Mar. Pollut. Bull.*, 16, 129-134.

WIDDOWS, J. 1985b. The effects of fluctuating and abrupt changes in salinity on the performance of *Mytilus edulis*. In: *Marine Biology of Polar Regions and Effects of Stress on Marine Organisms*. (eds.) Gray, J. S. and Christiansen, M. E. John Wiley and Sons, London. 555-566.

WIDDOWS, J. AND BAYNE, B. L. 1971. Temperature acclimation of *Mytilus edulis* with reference to its energy budget. *J. Mar. Biol. Ass. U. K.*, 51, 827-843.

WIDDOWS, J. AND JOHNSON, D. 1988. Physiological energetics of

Mytilus edulis: Scope for growth. *Mar. Ecol. Prog. Ser.*, 46, 113-121.

WIDDOWS, J., BAYNE, B. L., LIVINGSTONE, D. R., NEWELL, R. I. E. AND DONKIN, P. 1979a. Physiological and biochemical responses of bivalve molluscs to exposure to air. *Comp. Biochem. Physiol.* 62A, 301-308.

WIDDOWS, J., BAYNE, B. L., DONKIN, P., LIVINGSTONE, D. R., LOWE, D. M. MOORE, M. N. AND SALKELD, P. N. 1981. Measurement of the responses of mussels of environmental stress and pollution in Sullom Voe: A base line study. *Proc. Roy. Soc.* Edin., B. 80, 323-338.

WIDDOWS, J., BURNS, K. A., MENON, N. R., PAGE, D. S. AND SORIA, S. 1990. Measurement of Physiological Energetics (SFG) and Chemical Contaminants in Mussels (*Arca zebra*) transplanted along a contamination gradient Bermuda. *J. Exp. Mar. Biol. Ecol.*, 138, 99-117.

WIDDOWS, J., FIETH, P. AND WORRAL, C. M. 1979b. Relationship between seston, available food and feeding activity in the common mussel *Mytilus edulis*. *Mar. Biol.*, 59, 195-207.

WIDDOWS, J., PHELPS, D. K. AND GALLOWAY, W. 1980. Measurement of physiological condition of mussels transplanted along a pollution gradient in Narragansett Bay. *Mar. Environ. Res.*, 4, 181-194.

WILSON, B. R. 1968. Survival and reproduction of the mussel *Xenostrobus securis* (Lam.) in a Western Australian Estuary. 1. Salinity tolerance. *J. Nat. History.*, 2, 307-328.

WILSON, J. H. AND SEED, R. 1974. Laboratory experiments on pumping and filtration in *Mytilus edulis* L. using suspensions of colloidal graphite. *Ir. Fish. Invest. Ser.* (Mar.)., 14, 1-20.

WINBERG, G. G. 1960. Rate of metabolism and food requirements of fishes. *Fish. Res. Bd. Can.* Transl. Ser., 194, 1-253.

WINTER, J. E. 1969. Uber den Einflub der Nahrungskonzentration und anderer Faktoren auf Filtrierleistung und

Nahrungsausnutzung der Muscheln *Arctica islandica* und *Modiolus modiolus*. *Mar. Biol.* 4, 87-135.

WINTER, J. E. 1970. Feeding and food utilization in *Arctic islandica* L. and *Modiolus modiolus* L. at different food concentrations. In: *Marine Food Chains* (ed.) J.H. Steele. Univ. California, Press., Berkeley, California. 196-206.

WINTER, J. E. 1973. The filtration rate of *Mytilus edulis* and its dependence on algal concentration, measured by a continuous automatic recording apparatus. *Mar. Biol.*, 22, 317-328.

WINTER, J. E. 1978. A review on the knowledge of suspension feeding in lamellibranchiate bivalves, with special reference to artificial aquaculture systems. *Aquaculture*, 13, 1-33.

WRIGHT, R. T., COFFIN, R. B., ERSING, C. P. AND PEARSON, D. 1982. Field and laboratory measurement of bivalve filtration of natural marine bacterio-plankton. *Limnol. Oceanogr.*, 27, 91-98.

*ZEUTHEN, E. 1947. Body size and mtabolic rate in the animal kingdom, with special regard to the marine microfauna. *Comptes Rendus des Travaux du Laboratocre Carlsberg, S'erie Chimique*, 26, 17-161.

ZEUTHEN, E. 1953. Oxygen uptake as related to body size in organisms. *Quart. Rev. Biol.*, 28, 1-12.

ZEUTHEN, E. 1955. Comparative physiology of respiration *Ann. Rev. Physiol.* 17, 459-482.

ZS-NAGY, I. 1967. Histological, histochemical and electron microscopical studies on the cytosomes of the nerve cells in *Anodonta cygnea* L. (Mollusca, Lamellibranchiata). *Annal. Biol.*, Tihany, 34, 25-39.

ZS-NAGY, I. 1971a. Pigmentation and energy dependent Sr^2 accumulation of molluscan neurons under anaerobic conditions. *Annal. Biol.*, Tihany. 38, 117-125.

ZS-NAGY, I. 1971b. The lipochrome pigment of molluscan neurons, a specific electron acceptor. *Comp. Biochem. Physiol.*, 40A,

595-602.

ZS-NAGY, I. 1973. Carbohydrate and ATP production in the tissues of *Anodonta cugnea* L. (Mollusca, pelecypoda) under normal and anoxic conditions. *Acta. Biochem. Biophys.* Hung., 8, 143.

ZS-NAGY, I. 1974. Some quantitative aspects of oxygen consumption and anaerobic metabolism of molluscan tissues. A review. *Comp. Biochem. Physiol.*, 49A, 399-405.

ZS-NAGY, I. 1977. Cytosomes (Yellow Pigment Granules) of Molluscs as cell organells of anoxic energy production. *Intl. Rev. Cytol.*, 49, 331-377.

ZS-NAGY, I. AND ERMINI, M. 1972. Oxidation of $NADH_2$ by the lipochrome pigment of the bivalve *Mytilus galloprovincialis* (Mollusca, Pelecypoda). *Comp. Biochem. Physiol.*, 43B, 39-46.

---

* NOT REFERRED ORIGINAL

TAXONOMIC INDEX

* NOT INCLUDED