STUDIES ON THE BIOLOGY OF THE CLAM *SUNETTA SCRIPTA* (LINNE), FROM THE SUBTIDAL WATERS OF COCHIN

Thesis submitted to
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By

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To the memory of my father and to my mother.

C E R T I F I C A T E

This is to certify that this thesis is an authentic record of the research work carried out by Miss. CLARA MARGRET KATTICARAN, under my scientific supervision and guidance in the Division of Marine Biology, Microbiology and Biochemistry, School of Marine Sciences, Cochin University of Science and Technology, in partial fulfilment of the requirements for the degree of Doctor of Philosophy of the Cochin University of Science and Technology under the Faculty of Marine Sciences, and no part thereof has been presented for the award of any other degree, diploma or associateship in any University.

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D E C L A R A T I O N

I, Clara Margret Katticaran, do hereby declare that this thesis entitled "STUDIES ON THE BIOLOGY OF THE CLAM, SUNETTA SCRIPTA (LINNE) FROM THE SUBTIDAL WATERS OF COCHIN" is a genuine record of the research work done by me under the scientific supervision of Dr. K.Y. MOHAMMED SALIH, Reader, School of Marine Sciences, Cochin University of Science and Technology and has not previously formed the basis for the award of any degree, diploma, or associateship in any University.

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A C K N O W L E D G E M E N T S

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CONTENTS

PREFACE

Bivalves, particularly those that form large aggregations support a variety of fisheries throughout the world; most are harvested for food, the shells are used in industry, for jewellery, curios and souvenirs. Several estimates have been made of the number of living gastropod molluscs and bivalves in the world, but Boss (1971) concluded there are about 7500 bivalve species and in 1980, about 10% of these provided the major portion of reported bivalve landings (Anon, 1977). A break-down of 1980 world landings shows that total Asian production, Orient, southeast Asia and Indonesia combined was 50.5% of world production (data from FAO statistics).

Among the commercially exploited bivalves in India, clams are of most importance in terms of quantitative abundance. Apart from the introduction of simple clam scooping devices, the fishing method of hand picking has largely remained unchanged over the years. Clam marketing is highly localised in centres of collection. The scenario is changing with the increasing demand for frozen clam meat in foreign markets. Exports which started with a meagre 16 tonnes in 1981-82 showed a 65 fold increase reaching 1033 tonnes in 1984-1985. Clams being a sedentary resource there is danger of the beds being overexploited in a short time. Therefore utmost care is recommended in management of this resource. Though there is a well established system of licensing, controlled by the State department of Mining and Geology, for the black clam Villorita cyprinoides in the Vembanad lake, there seems to be no such regulation for Sunetta scripta fishery in the Cochin barmouth area.

S. scripta is fished by hand as well as from plank-built boats using dredge nets attached to the ends of wooden poles (Kuthukoruvala). The mesh size is large enough to eliminate juvenile clams, but the larger spawning adults are included in the catch during the spawning season. Industrial or domestic sewage pollution in the Cochin barmouth may also negatively affect natural stocks. Seasonal closures, reserve areas, size limits etc . can be implemented to maintain natural stocks but management of bivalve resources presents unique problems, and models used to manage finfish seem unsatisfactory.

Enhancement and culture of bivalves presents an opportunity to maximise and even increase production of many growing areas. Clam culture is less intensive both for capital and labour, involves simple farming and management techniques and is considered an efficient means of protein production. Clams are efficient converters of primary production and growth rate is fast with maximum production in 5-6 months. With culture, production is less influenced by poor recruitment . Stable production facilitates market development. Bivalves are being increasingly used in bio-medical research. Culture practises would ensure uninterrupted supplies of experimental material.

Paucity of biological data restricts the development of efficient management and culture techniques of bivalves. This study was undertaken with a view to provide information on some aspects of biology of the bivalve S. scripta which have hitherto been uninvestigated.

INTRODUCTION

INTRODUCTION

Sunetta scripta (Linné) belongs to Phylum Mollusca one of the larger phyla in the Animal Kingdom. Molluscs, with over 100,000 living species (Abbot, 1954), to say nothing of the more than 20,000 fossil species discovered to date (Solem, 1974), are exceeded in number only by the Arthropoda and possibly by the Protozoa and Nematoda. Before a study of the bivalve, S. scripta is attempted, an account of the Mollusca and Class Bivalvia would be relevant.

There is now a general consensus that the seven recognised existing classes of the Mollusca are derived from a Pre-cambrian ancestor having the grade of organisation found in free-living flatworms and nemerteans (Stasek, 1972; Trueman, 1975; Salvini-Plawen, 1981). Because of the extreme diversity of the molluscan body forms, the concept of the "archetypic mollusc" (the theoretical ancestor exhibiting those features generally considered to be the primitive basis of several molluscan traits) is used as a convenient frame work for the description of some basic characteristics of the phyla.

The archetypic mollusc was probably a rather flat, sluggish, bilaterally symmetrical littoral or sub-littoral animal with a low shield like shell and a broad flat ventral sole. The radula - a toothed chitinous ribbon, borne on a short snout and used to rasp food - and the mantle or pallium - a fold of the epidermis enclosing the viscera and secreting the shell - are two features unique to the phylum. The posterior space between the mantle skirt and the visceral mass, termed the mantle or pallial cavity is of particular significance in molluscan organisation and

housed the paired series of respiratory organs and received waste material from the anus and excretory ducts (Fretter and Graham, 1962). The gut was adapted for microphagous feeding. Paired auricles, kidneys and simple gonads are present. Sexes were separate and fertilisation was external. Spiral cleavage produced a trocophore larva which developed into a characteristic veliger with a velum. The shell of the primitive mollusc was probably little more than a tough dorsal cuticle of conchiolin impregnated with calcareous salts, calcification at one or more centres giving rise to the univalve, bivalve and multivalve condition. Though such an archetype is still perhaps the most likely ancestor of at least the gastropods and bivalves, it has been suggested that a more plausible archetype might be one with a four-fold organisation similar to that in present day Nautilus. From this a reduction to a single pair system would result in the Bivalvia and the Gastropoda, and replication to a many pair system, the Chitons (Russel-Hunter, 1979; Seed, 1983).

The Bivalvia with approximately 31,000 living species (Russel-Hunter, 1979) form the second largest class of the Molusca. The first undisputed bivalve fossils come from the lower Middle Cambrian rock. In recent years the Linnean name for the Class Bivalvia, has been favoured over others such as Acephala, Lamellibrenchia, Pelecypoda, etc. (Cox 1969), clearly implying the distinguishing feature of the class. The name Lamellibranchia now signifies the non-protobranch and non-septibranch molluscs.

Clams, Oysters, Mussels, Scallops and Ship-worms constitute the Class Bivalvia. The Bivalvia are untorted, bilaterally symmetrical,

laterally compressed molluscs lacking a head, radula and associated buccal mass, the sensory functions being delegated to the mantle margins. The single enveloping mantle secretes the shell valves, the two valves connected by a mantle isthmus secreting proportionately less crystalline calcium carbonate and proportionately more elastic tanned protein to form the ligament. Associated with the ligament is a thickening of the dorsal margins of the shell valves to form the hinge plate bearing hinge teeth and sockets. The number and arrangement of the hinge teeth are important taxonomic features of Bivalvia. Variously developed pallial muscles include anterior and posterior adductors that attach to each valve. Closure of the shell results from contracture of the adductor muscles, opposing the elastic properties of the hinge ligament. In the absence of buccal mass, radula, jaws or pharynx, the Bivalvia developed other feeding organs such as labial palps (Protobranchia), ctenidia, adapted for trapping small animals (Septibranchia) and cilliary currents generated by the gill in the rest of the class. A second evolutionary trend was toward a burrowing habit. In adaptation to which, the foot lost the original creeping sole and became axe-shaped forming a wedge-like organ which aided penetration of the sub-stratum (Fretter and Graham, 1976). Although widely distributed in aquatic habitats, the method of locomotion of the Bivalves and their dependance on gills and pelagic development have prevented them from becoming established on land.

The rest of the body organisation conforms to the general molluscan pattern. Paired auricles, kidneys and simple gonads are present. The lips of the mouth are extended to form labial palps. The gut is generally

modified for particulate feeding, with complex ciliary sorting mechanisms and a crystalline style in the stomach. The elaborate ciliary feeding mechanisms developed by the bivalves are unparalleled in the Animal Kingdom.

Shells popularly known as "clams" such as S. scripta, are included in the Family Veneridae. They are characterised by a shallow burrowing habit and absence of byssus in the adult. The success of the Veneridae is based on the possession of short separate siphons and as a consequence their ability to colonise soft sediments at depth.

The immense clam resources of the estuaries and backwaters along the coast of the country have been traditionally exploited by coastal populations. Clam fishing activity picks up during the monsoon when weather conditions are not conducive to sea-fishing. Hand picking is supplemented by clam scooping devices operated from boats. Besides their place in the diet, clam shells from live clams and sub-soil deposits, have found immense uses in the cement, lime, paper, rayon, leather, carbide and fertiliser industries as well as being used as shell-grit for poultry.

In order of abundance Villorita cyprinoides, Meretix casta, Paphia malabarica, Katelysia opima, Meretrix meretrix, Marsia sp. and Tapes sp. are fished from Kerala estuaries. S. scripta is fished from Cochin and Azhikode areas and there are unpublished reports of moderately large beds at Kayamkulam. The accessibility of the clam bed, large size of the clam and the thickness of the shell, make fishing of this species a not unprofitable venture.

The present investigation includes a description of the species, its habit and habitat from the Cochin area.

Reproductive ecology is central to an understanding of the biology of a species and is essential to proper management of a natural resource. This study includes an account of the reproductive patterns in S. scripta during 2 years of the life cycle, with discussions on possible coordinating factors.

The bio-chemical make-up of S. scripta is also studied. Seasonal variations are correlated with reproductive patterns and nutritional cycles.

The Cochin backwaters have come to be recognised as one of the highly polluted estuaries, in the west coast of the sub-continent, with industrial, harbour, agricultural, domestic and urban sewage. In the light of the increasing awareness of the pollution indicator properties of bivalves, it was considered worthwhile to study the seasonal variation of some trace metals in natural populations of S. scripta from Cochin.

Thampuran (1986) has indicated high tolerance to copper in S. scripta. Biochemical responses are likely to be amongst the first manifestations of excess metal accumulation. Lactic acid accumulation, in selected tissues of S. scripta, in relation to oxygen uptake during exposure to copper formed a part of this study. The investigation was extended to include a study of these responses during recovery from exposure.

In an identical experiment, variations in key metabolic substrates, protein and carbohydrate, were investigated during exposure to copper and recovery.

REVIEW OF LITERATURE

The most important works on the molluscan fauna of the Indian region are by Smith (1901), Preston (1909, 1914, 1915 and 1916), Hornell (1910a, 1916), Hornell (1910, 1916, 1917, 1948 and 1951), Gude (1914), Annandale and Kemp (1916), Prashad (1920, 1921a, b and 1932), Winckworth (1927, 1940a, b) Gravely (1927, 1941, 1942), Crichton (1941) Satyamurti (1952, 1956 and 1960) and Cheriyan (1968).

Although the reproductive cycles of numerous bivalves have been described (reviewed by Giese, 1959; Sastry, 1975, 1979; Seed, 1976; Andrew, 1979) there is still only a partial understanding of the complex interaction between exogenous (eg. food availability, temperature, salinity, etc.) and endogenous (nutrient reserves, hormonal cycle, genotype etc). variables that determine the initiation and duration of the various phases of the cycle and thus ensure synchrony of gamete development within a population. The role of temperature in gametogenesis has been reviewed by Loosanoff, (1971) Kinne, (1963, 1964, 1970) and Sastry (1979). Durve (1965) suggested that temperature might not influence the spawning of marine bivalves of Indian coasts, but considered salinity as an important factor in initiation of spawning. Gametogenesis correlated with salinity changes has been studied in various bivalves by Panikkar and Aiyer (1939) and Paul (1942); in Placuna placenta from Kakinada Bay (Sastry, 1955); in Meretrix casta from fish ponds near Mandapam, India (Durve, 1964); in Donax faba (Alagaraswami, 1966) and Donax cuneatus (Rao, 1967) from south east India; in Xenostrobus secularis from west Australia (Wilson, 1968, 1969) in Mytilus viridis (= Perna viridis) and Katelysia opima from

the southwest Indian Coast (Nagabhushanam and Mane, 1975 a and b) and in Meretrix casta from Cochin (Salih, 1977). The role of food (phytoplankton) in regulating gametogenesis has recently been stressed (Giese and Pearse, 1974). Bayne (1975), Zandee et al. (1980) and Pieters et al. (1979) related food availability with production of ripe gametes and spawning in M. edulis. Parulekar and Dalal (1980) correlated gonad growth in Perna viridis with phytoplankton abundance. According to Lubet (1973) gonadal development in Mytilus is controlled by neuroendocrine factors but temperature and food act to synchronize or induce the different stages of gametogenesis. The existence of nuerosecretory cycles have been reported in a number of bivalves (Nagabhushanam and Mane, 1973; Lubet and Mathieu, 1982).

Gametogenesis has been extensively studied in bivalves. Published reports include the work on Mercenaria mercenaria and Cyprina islandica (Loosanoff, 1937, a, b, and 1953), Pinctada margretifera (Tranter, 1958) Argopecten (= Aequipecten) irradians (Sastry, 1963, 1966, 1968 and 1970), Macoma balthica (Lammens, 1967) and Placopecten magellanicus (Naidu, 1970).

The chemical composition of several marine organisms has been studied in detail and a considerable body of information has been accumulated on this subject (Vinogradov, 1953). Basically such information is useful to understand the role of major biochemical constituents such as carbohydrates, lipid and protein in the metabolic activities of the animal. Outstanding contributions in the metabolic transformation of energy stores in bivalves are those of Giese (1966), Hammen (1969) Campbell

and Bishop (1970), Gabbot and Walker (1971), Gilles (1972) and Gabbot and Holland (1973), Gabbot (1976, 1983) and Bayne (1976). Fluctuation in biochemical constituents with respect to reproductive cycles was observed by Lubet (1959); Lubet and Le Feron de long Camp (1969); Widdows and Bayne (1971); Bayne (1973); Gabbot and Bayne (1973); Ansell (1974 a, b and c), Ansell and Bodoy (1979) and de Zwaan and Zandee (1972). In sea urchin and chiton considerable work on this aspect of reproductive biology has been caried out by Greenfield et al. (1958) Giese and Araki (1962), Giese et al. (1959) and Giese (1959). A few studies on the accumulation of biochemical constituents and their transformation during the reproductive period in bivalves have been carried out from the Indian sub-continent and they are chiefly those of Nagabhushanam (1961), Saraswathy and Nair (1969). Suryanarayanan and Alexander (1972), Nagabhushanam and Deshmukh (1974), George and Nair (1975), Nagabhushanam and Talikhedkar (1977), Nagabhushanam and Dhamne (1977), Mane and Nagabhushanam (1977), and Salih, (1979).

Sedentary animals like bivalve molluscs are more susceptible to pollutants and often build up high concentrations of toxic substances. This has long been appreciated for bacterial contamination (Dodgson, 1928) and pollution by heavy metals (Boyce and Herdman, 1897) and since bivalves are major components of littoral fauna, they have been the subject of many toxicological investigations. The concentration of several trace metals in bivalves have been investigated by Vinogradov (1953), Brooks and Rumsby (1965), Segar et al (1973), Bryan (1973), Bryan and Hummerstone

(1978), Zingde et al. (1976), Nambisan et al. (1977), Rajendran and Kurian (1986), Pillai et al. (1986). Bivalve molluscs have been widely advocated and adopted for monitoring levels of metals in the ocean and certain genera and species, notably oysters and mussels have been extensively studied in temperate waters. Goldberg (1975) and Goldberg et al (1978) proposed the use of bivalve molluscs, especially M. edulis in "Mussel Watch" programmes to monitor levels of heavy metals initially in the USA and now internationally. The characteristics of a good indicator organism have been reviewed by Phillips (1977). Of these a simple correlation between metal levels in the organism and its environment and the availability of that organism to relocation were considered particularly important. Direct proportionality of uptake across a variety of epithelial systems (Simkiss and Taylor, 1981) has been claimed for a large number of bivalves; in M. edulis with lead (Schulz - Baldes, 1974); zinc (George and Pirie, 1980) and cadmium (Fowler and Benayoun, 1974); in Tapes decussatus for chromium (Chipman, 1966); in Crassostrea virginica for chromium and lead (Shuster and Pringle, 1969); in Protothaca staminea for copper (Roesijadi, 1980) and in Anodonta nuttaliana for manganese and zinc (Harrison, 1969).

CHAPTER I

DESCRIPTION OF SUNETTA SPECIES, HABIT AND HABITAT

Though Sunetta scripta (Linné) from Cochin barmouth (southwest coast of India), has been the subject of isolated studies, (Mohan and Damodaran 1981; Thampuran, 1986; Suresh and Mohandas, 1987), there has been no formal identification or description of this species, its habitat preferences or distribution in this area. Lists of molluscan fauna, by Preston (1916) and Cheriyan (1968) have not included this clam of significant commercial importance. S. scripta occurs together with Meretrix casta over an extensive clam bed which supports a moderately lucrative local fishery. A preliminary survey of the clam bed in May 1983 indicated a population density of 420 clam/m^2 but this was during the active southwest monsoon periods when there is heavy mortality. Densities are likely to be higher earlier in the year.

Adams and Adams (1858) have described the genus Sunetta Link, and reported eleven species, among them S. scripta, from Senegal, India, Japan and Australia. Gravely (1941) has listed four species of Sunetta from Madras; S. scripta, S. donacina, S. meroe and a last species resembling S. excavata reported from Japanese waters.

Satyamurti (1956) adopting the classification of Thiele (1935) groups S. scripta under the Family Veneridae and Series Veneracea of Class Bivalvia. Satyamurti (1956) also described S. scripta from shell collections of Pamban, Madras. "The shell is smooth and glossy, moderately elongated, evenly rounded in front and obliquely truncated behind, the junction of the posterior and ventral margins being rather angular. The hinge teeth are thin and narrow and the tooth in front of the cardinals

In the left valve and the hollow in the right are considerably elongated. The lunule is long and narrow and deeply excavated. The area behind the umbo is also deeply sunk. The sites of the adductor impressions are marked by slight depressions and the pallial line is deeply sinuate. The inner surface is uniformly whitish and its margin is finely crenulated all around. The outer surface is entirely smooth and polished but bears a bright colour pattern which is subject to variation. It is generally pale yellowish or fleshy white, marked all over with purplish arrowhead markings, which may either be very numerous and close set or few and far apart."

In the present study, a few additional observations were made of the species collected from the barmouth area. The lunule is lanceolate but not as deeply sunk as the escutcheon. The pallial sinus is roughly triangular in outline. The ventral margin of the shell is slightly convex. The umbo is situated slightly behind the midline. The colour of the clams varies from brown through yellow to fleshy white. The older specimens are mostly devoid of chevron markings. The largest specimen measured 52.5 mm of length and 40.7 mm in height.

Except for a few minor ecological variations, the description seems to concur with that of Satyamurti (1956) establishing this clam as Sunetta scripta (Linne) (Fig. 1, 2, 3)

The synonmy of this species reads as follows:-

Donax scripta, Linne, Syst. Nat., EdX, 1758 p683.

Donax scripta Linne, Syst. Nat., EdXII Vol II, 1767 p1127, 109

Cuneas scripta, Rumphius, Mus. Amb. 1739 pl 43 fig 1.m.

Meroe scripta, Reeve, Conch. Icon., XIV, 1864, Meroe, plii Fig 6a and b.

Sunetta scripta, Gravely, Bull: Mad. Govt. Mus(Nat.Hist.), V.No.1 1941 p 50

Habitat:- The clam beds are largely sub-littoral occuring at depths of 1.5 m to 2.5 m. The salinity varies from maxima of 36.46 x 10^{-3}S during the pre-monsoon to minima of 1.30 x 10^{-3}S (Chapter II Fig. 19) during the wet season. Temperature shows less drastic variations ranging from 24.25^{o}C to 31.40^{o}C. The substratum is composed of sand, silt, mud and shell fragments; with silt predominating during the southwest monsoon period and sand during the rest of the year.

Distribution:- The clam bed lies between latitude 9^{o} 28' and 10^{o}00'N and longitudes 76^{o}13' and 76^{o}31'E, on the northern side of the entrance into the Cochin barmouth. (Fig. 4).

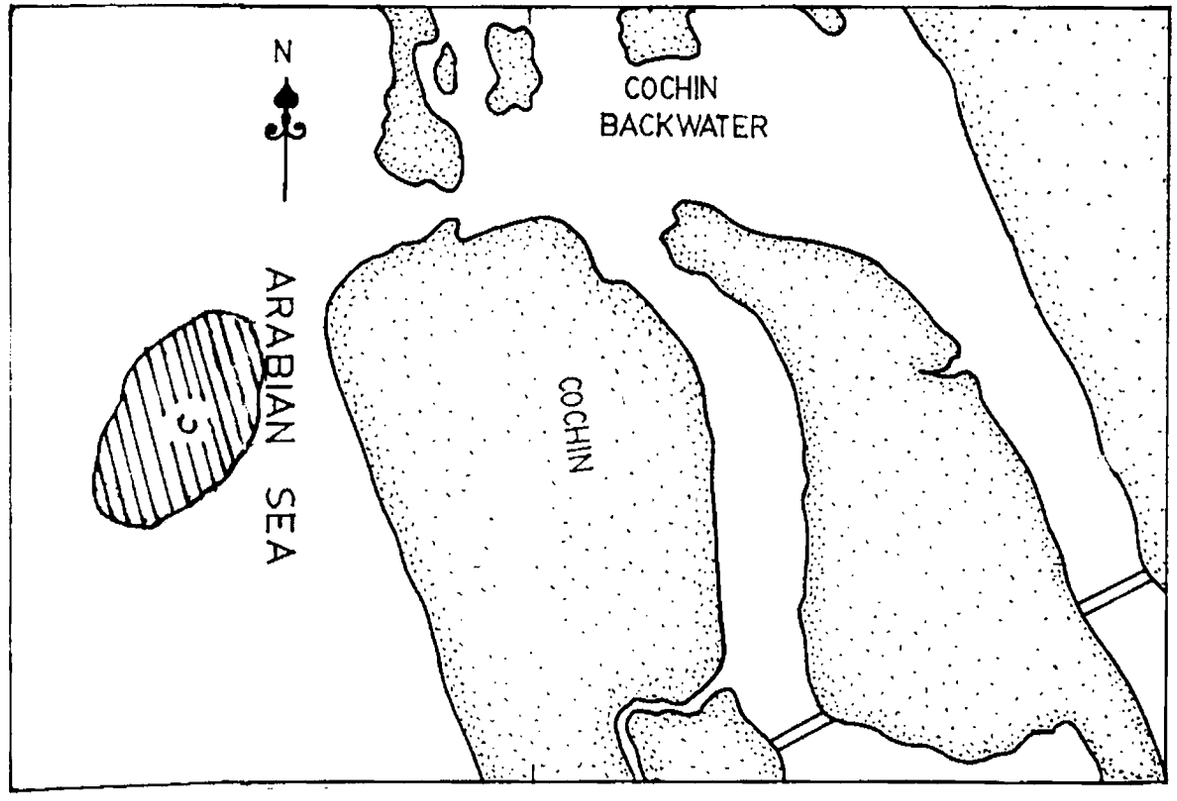

Figure 4. Map showing location of clam bed. (C)

CHAPTER II

THE REPRODUCTIVE BIOLOGY OF SUNETTA SCRIPTA

Very little is known of the reproductive biology of S. scripta from Cochin, where it is fished commercially, or for that matter elsewhere along the east and west coasts of the sub-continent. Knowledge of reproductive behaviour and factors determining breeding are central to the understanding of life history, ecology and suitability for culture of an organism. "Reproductive difficulty" has been considered a major factor limiting species distribution (Hutchins, 1974; Fritchman, 1962). Such information would also be useful should legislation become necessary with regard to fishing. Rand (1973) and Giese and Pearse (1974) have postulated year-round spawning activity for tropical marine organisms though various exogenous factors which initiate and synchronise breeding activity also restrict it to certain periods of the year. For shallow tropical marine seas and estuaries, salinity has been considered a "natural spawning stimulus" (Panikkar and Aiyer, 1939; Stephen 1980a) in gametogenic cycles, comparable with the role of temperature in temperate waters. Stephen (1980 a) envisaged a "Hornell's rule" homologous to "Orton's Rule" (Thorson, 1946) and "Crisp's Rule" (Qasim, 1956) to signify a relationship between salinity and reproduction.

Studies on the southeast and west coasts have established the reproductive cycles of many species in estuarine and marine environment (Panikkar and Aiyer, 1939; Durve, 1965; Nagabhushanam and Dhamne, 1977; Salih, 1977, Stephen, 1980a; Ajithakumar, 1984). The major objectives

of this study were to (1) establish the gametogenic pattern during an annual period, (2) to elucidate the probable influence of salinity and/or temperature on reproduction.

MATERIALS AND METHODS

Fortnightly samples were collected from the clam bed off Cochin bar-mouth with a hand-operated bottom dredge from 1983 July to 1985 June. At each sampling approximately 35 clams between 25 mm and 40 mm were examined. The macroscopic appearance of the individual gonad is the same for each phase of both sexes and is given with the description of the gonad. Sex and arbitary stage of maturity were ascertained from fresh gonad smears of individual clams. Histological examination of the gonad was made to determine gametogenesis because external appearance did not acurately reflect gametogenic activity.

Portions of the gonadal tissue, always from the centre of the organ from approximately 18 individuals were fixed in Bouin's fluid, dehydrated in ethanol and embedded in paraffin wax, m.p. 60-62^{O}C. Sections were made at 7 μ m thickness and stained in Mayer's haemalum and counterstained in eosin. At least 2 slides were processed from each piece of gonad for examination.

Size frequency methods based on samples of oocyte diameter measurements were also used to determine stages of the reproductive cycle. Measurements were made on about 20 oocytes each of 6 females from smears placed in sea water on a slide. The lesser diameter of the stalked oocytes and the mean diameter of the rounded oocytes were

measured with an ocular micrometer and classified as $\geq 45\,\mu$m (mature) and between 15 and 14 μm (developing). The percentage frequency of oocytes in the size groups were calculated and a frequency polygon was drawn. This method overlooks the proportion of smaller oocytes in the samples which remain attached to the follicle wall, but gives an indication of stages of the reproductive cycle, especially spawning time. Measurements of oocyte diameter were undertaken to indicate breeding periodicity, as measurement of gametogenic activity in males, was less objective, and hence considered not as reliable.

RESULTS AND DISCUSSION

Description of the Gonad

The gonad is the largest and most conspicuous organ in the ripe clam, forming a continuous mass around the folds of the digestive tract and the adjacent digestive gland. The wall of the gonad is made up of an outer epithelial and an inner muscular layer. Histological examination of the gonad shows numerous follicles with connective tissue in between. The gonads begin development from the posterior end and as a rule, newly formed follicles in the anterior portion of the visceral mass, contain more cells in the early stages of gametogenesis. The amount of connective tissue varies depending upon the state of maturity of the gonad. Transverse muscle fibres are also seen in the connective tissue between the follicles. Contraction of gonad musculature probably assists in gamete release (Nagabhushanam and Dhamne, 1977).

The spent gonads appear flaccid, watery, transparent and are pale brown or colourless. When mature, the gonads are cream-coloured, soft and smears from female gonads are faintly granular in texture.

Sex ratios

In this study, gonadal smears from a total of 557 individuals of S. scripta were examined. The total sex ratio was 173 males: 201 female : 179 indifferent : 4 parasitized.

It is difficult to determine sex ratios during the spent and early gametogenic phase, because females often retain oocytes and thus the observed sex ratios would be biased in favour of females with many males being classed as indifferent. To overcome this bias the sex ratio was determined by computing only those samples during the mature and spawning periods when majority of the clams could be sexed from smear examination.

Chi-square tests were performed against a 1:1 sex ratio (Zar, 1974). Non-significance was found and the null hypothesis of 1:1 sex-ratio was accepted.

Sex change often occurs in bivalves as a consequence of age, rapid rate of growth or change in environmental condition (Nagabhushanam and Dhamne, 1977).

After spawning the clams passed through a short period of recovery consisting in part of resorption of unspawned material. In females this period is especially short and oogenesis began even before the older

oocytes were discharged. The presence in the follicles of well defined sex cells through all periods of the gonadal cycle indicates stability of sex, and sex reversal rarely if ever occurs. During the period of investigation no case of hermaphroditism was observed in S. scripta.

Parasitic Castration

The gonads of 4 individuals of S. scripta (Fig 5) were found to be infected with the larval stages of trematodes. In all cases infection had caused complete castration of the host clam. All the infected individuals were identified as females from the presence of degenerating oocytes in follicles. Infection was observed only in the months of December, January and March when all the clams are mature and temperature and salinity are high. Infected individuals appeared healthy and were indistinguishable externally from uninfected individuals, by morphology and behaviour. The digestive gland appeared normal and there was no indication of impairment of function in the sectioned material. The specific identity of this parasite which sterilises its host was not determined.

A sporozoan parasite was also observed in the leydig tissue of the gonad in the early stages of gametogenesis. The tissue in sectioned material apeared unaffected. The spores were absent in the mature gonads.

Cheng (1967) reviewing parasitism in the marine molluscs has included an instance of parasitic castration in Ostrea edulis by sporocysts of Bucephalus haimeanus Dupouy and Martinez (1973) reported proliferation of sporocysts of Protoeces maculatus utilising storage substances (glycogen)

of the connective tissue, resulting in arrest of the gametogenic cycle in Mytilus galloprovincialis. Stephen (1977) has reported parasitic castration in Crassostrea madrasensis by the larval trematode Bucephalus sp. An unidentified haplosporidian infection was also observed in the gills and connective tissue. Parasitic castration of Turbo intercostalis was reported by Joll (1980) as resulting from infection by larval stages of trematodes.

Size at first maturity

All animals between 10 mm and 26 mm shell length were grouped into 2 mm length classes of sexually mature and immature stages based upon examination of gonad histology.

As indicated in Table I an increased frequency of developed gonads was noticed with increased shell length. The smallest clam with mature gonads was a female of 20.6 mm shell length. More than 75% of the clams of shell length greater than 22 mm were found to be mature, though none of the clams below 26 mm had spent gonads. In juvenile clams visceral mass consisted mainly of the digestive gland and connective tissue occupied the spaces between the loops of the intestine.

Growth in tropical species is especially rapid and juveniles reach sexual maturity well within the first year of life. Individuals can avoid possible problems arising from variability in patterns of growth by being able to spawn at an early age (Creese, 1980), even though it would be selectively advantageous to grow as large as possible before beginning to reproduce. In S. scripta it would appear that the early post-settlement period is devoted solely to somatic growth, gonad development

TABLE I RELATIVE NUMBERS OF IMMATURE TO MATURE GONADS AGAINST SHELL LENGTH IN S. SCRIPTA

Shell Length in mm	Number with immature gonads	Number with mature gonads	% with mature gonads
14.1-16.0	12	0	0
16.1-18	8	0	0
18.1-20	16	0	0
20.1-22	6	4	40
22.1-24	2	9	81.8
24.1-26	3	23	88.4

being initiated on reaching a certain length in order to maximise reproductive output.

Description of gonadal development

Indifferent phase

In early development gonads of male and female clams are indistinguishable even in histological examination (Fig. 6). The term indistinguishable - henceforth referred to as indifferent - applies to specimens that could not be sexually differentiated ie. with low levels of spermiogenic or ovogenic activity and correspondingly low levels of recognisable sex cells. Either spematogonia or oocytes were required to determine sex with certainty. Most of the gonad consists of interfollicular connective tissue or Leydig cells (Fig. 7). The Leydig cells are specialised for lipid and glycogen storage (Gabott, 1983; Stephen, 1980a). In species with storage specialised connective tissue, gonad quantity cannot be taken as a measure of gonad maturity, as increases in gonad index may be due to increased food supply and thus increased storage.

The follicle wall cells of the early follicle are broad and compact with basophilic nuclei 2μm in diameter. Large stem cells (Franter, 1958; Dredge, 1982) are dispersed around the follicle wall. The stem cells average 7μm in diameter with nuclei - with 1 or more nucleoli - occupying most of the cell volume.

The early follicle also contained vacuolated follicle cells similar to cells reported from Mercenaria mercenaria (Porter, 1964),

Venus straitula (Ansell, 1961) Mya arenaria (Ropes and Stickney, 1965), Mercenaria mercenaria (Keck et al., 1975) and Teredo navalis (Coe, 1943). A nutritive (Coe, 1943) and a phagocytic (Keck et al., 1975) function have been atributed to the follicle cells. Keck et al., (1975) also mention their role in the expansion of the gonad follicles. The collapse of the folicle cells forms a central lumen in the follicle and in this cavity gametogenesis is completed. Lammens (1967) tested the inner follicle cells and obtained positive results with Sudan Black B indicating probable nutritive function. The stem cells and the follicle cells seem to be derived from transforming areas on the follicle wall (Fig. 8). In some female follicles early gametogenic stages are observed even before complete cytolysis of residual oocytes of previous spawning.

Male - Early spermatogenesis

Gametogenesis proper in male clams begins only after the follicles are cleared of residual matter from previous spawning. Cell division proceeds till spermatogonia are formed when there is a break in continuity (Tranter, 1958). The gonad rests here before meoisis. The spermatogonia (7μm diameter) form 5-7 cell thick layers in the folicle (Fig 9). The gonad is composed mainly of connective tissue cells.

Male - Late maturing

After an initial lag phase cell division is accelerated. There is a rapid turnover of cells, the stages between primary spermatocyte and spermatozoa being of very short duration. Synchronization of spawning in male and female is necessary to achieve reasonable fertilisation success

and increased pace of spermatogenic activity serves to bring male and female cycles into phase. On examination the gonad follicles appear distended with spermatogonia, spermatocytes, spermatids and spermatozoa at the core (Fig. 10). The spermatocytes (4 μm) consists almost entirely of nuclear material in various phases of meoitic prophase, (Tranter, 1958) with clear cytoplasm and a faintly staining cell membrane. Spermatids are slightly smaller than spermatocytes (Fig. 11) but are more distinctly basophilic. The central core of the follicle is occupied by spermatozoa and as gametogenesis proceeds the outer band of spermatogonia recede to form a lightly staining band. The spermatozoa consist of a comma-shaped uniformly basophilic spermhead (3.5 μ m length) and a distinctly eosinophilic tail. Even at high magnification an acrosome was not observed.

Male - Mature

The male gonad was termed mature when the follicle contained mainly spermatozoa (Fig.12). In keeping with the rapid increase of follicular contents, the follicle wall expands and the connective tissue is occluded so that walls of adjacent follicles are apposed to each other (Fig. 12). The spermatozoa are arranged in radial bands with the tails directed towards the ciliated ducts.

Male-Spent

Spawning in males is more complete than in females. The spent phase is characterised by contracted follicles, a noticeable reduction of ripe gametes and extremely slight gametogenic activity. Connective tissue reappears to occupy the space between the follicle. The follicle is invaded by phagocytes which cytolyse the residual unspawned spermatozoa.

The early stages of spermatogenesis were not observed. The spent phase in S. scripta is of very short duration and the follicle quickly redevelops to repeat the cycle.

Female - Early Oogenesis

In the female, gametogenesis begins even before the residual oocytes are absorbed. Webber and Giese (1969) noted initiation of gametogenesis immediately after spawning in black abalone Haliotis cracherodii. The earliest identifiable female follicles contain oogonia, primary oocytes and follicle cells. The primary oocyte is larger (5 μ m diameter) than the spermatocyte. The chromosomes occur in a densely staining clumps (Figs. 13, 14). The follicles are still shrunken and Leydig tissue predominates in the gonad.

Female - Pre-vitellogenesis

The pre-vitellogenic oocyte (Fig. 15) develops in the lumen of the follicle - probably nourished by the follicle cells - before they become attached and vitellogenesis begins. The nucleolus reappears and grows rapidly. The oocyte has an average diameter of 11.5 μ m and the nucleus occupies more than half the cell volume. The cytoplasm is basophilic but stains less heavily than the nucleus.

Female - Vitellogenesis

The oocytes become attached to the follicle walls by means of a broad base. The nucleus migrates to the proximal end and the oocyte gradually attains a pear-shaped appearance (Fig 16). The short axis of the oocyte measures 48 μ m and the nuclear diameter 28 μ m.

As yolk accumulates the cytoplasmic volume increases and it loses its basophilic nature though the nucleus is still the clearer of the two. An amphinucleolus develops which is probably related to the vitellogenic activity in the oocyte (Raven, 1961). Young and De Martini (1970) and Joll (1980) considered the appearance of an amphinucleolus as an indication of ribosomal RNA production. Vitellogenesis commenced soon after the nucleus entered the vegetative phase.

Female - Mature

The gonad was considered mature when the number of detached oocytes was greater than the number of attached oocytes. The free oocyte is between 45 and 50 μ m in diameter (Fig. 17). Ripe oocytes from live clams placed in sea-water quickly become spherical and lose the germinal vesicle. Whereas the early follicle is predominated by oogonia and primary oocytes, in mature follicles the early stages are noticeably fewer in number. Growth of the tightly packed follicles obliterates the interfollicular connective tissue.

Female - Spent

Spawning in females probably takes longer than in males as mature oocytes are obtained in samples from October to March (Fig. 18). Spawning *per se* was not observed. A partially spent phase described by Mason (1958) and Shafee and Lucas (1980) in *Pecten maximus* and *Clamys varia* (L.) was also not detected, though females are not complete spawners and residual oocytes are always found in the spawned gonad.

Phagocytosis appears to be the primary method of residual cell destruction. Eosinophilic masses are noticed along the walls of some spent and early developing gonads. These residual masses often have a cellular appearance and may represent clumps of phagocytes (Fig. 18).

Salinity and Temperature of the clam bed

Temperature in the clam bed maintains fairly steady levels varying from pre-monsoon maxima of 31.40°C, to minima during the monsoon of 24.20°C.

Salinity variations are much more obvious with predominantly 3 phases (Fig. 19): (1) a period of rising salinity from mid-September to end of November, (2) a period of relatively stable salinity from mid-December to early March and (3) a period of decreasing salinity from end of May to end of August. During the period of stable salinity, values are high and fairly steady between 36 x 10^{-3} and 28 x 10^{-3}S. The onset of the S.W. monsoon results in increased freshwater discharge through the barmouth. A rapid decline in salinity causes oligohaline conditions to be established by June with values as low as 1.30 x 10^{-3}S. With cessation of monsoon the higher pre-monsoon values are gradually established.

The Annual Reproductive Cycle

The period just after spawning concluded, forms a convenient point to begin description of the developmental cycle of S. scripta populations, from one gametogenic cycle to the next. Gametogenesis is initiated soon after spawning and in some females even before the follicles are cleared of cell debris. Nagabhushanam and Dhamne (1977) found sexually

Figure 1. External view of shell of S. scripta showing lunule (L), escutcheon (E) and hinge ligament (H).

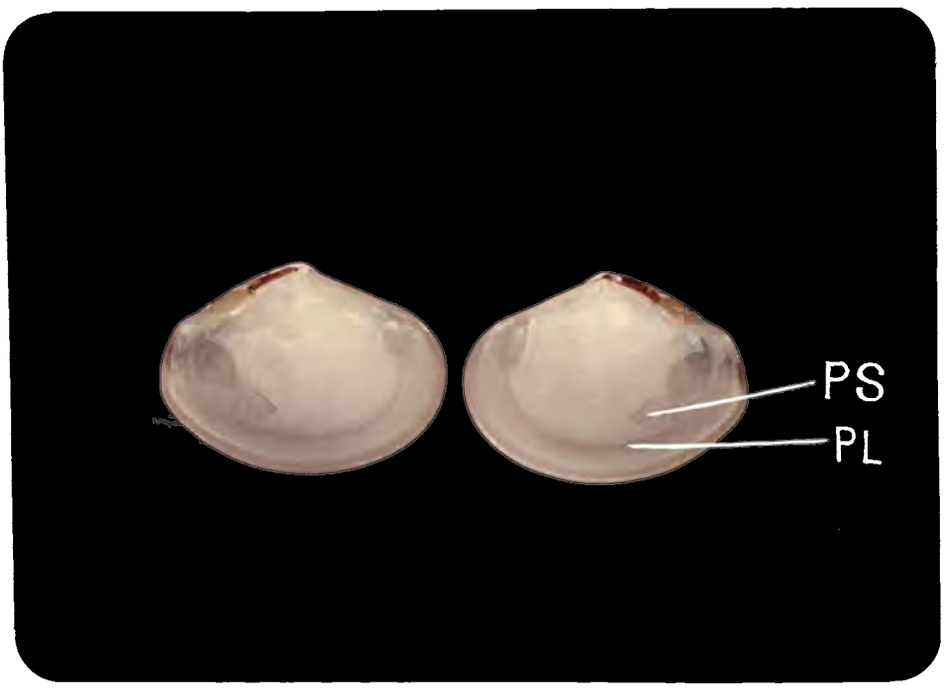

Figure 2. Internal view of shell of S. scripta showing pallial line (PL) and pallaial sinus (PS).

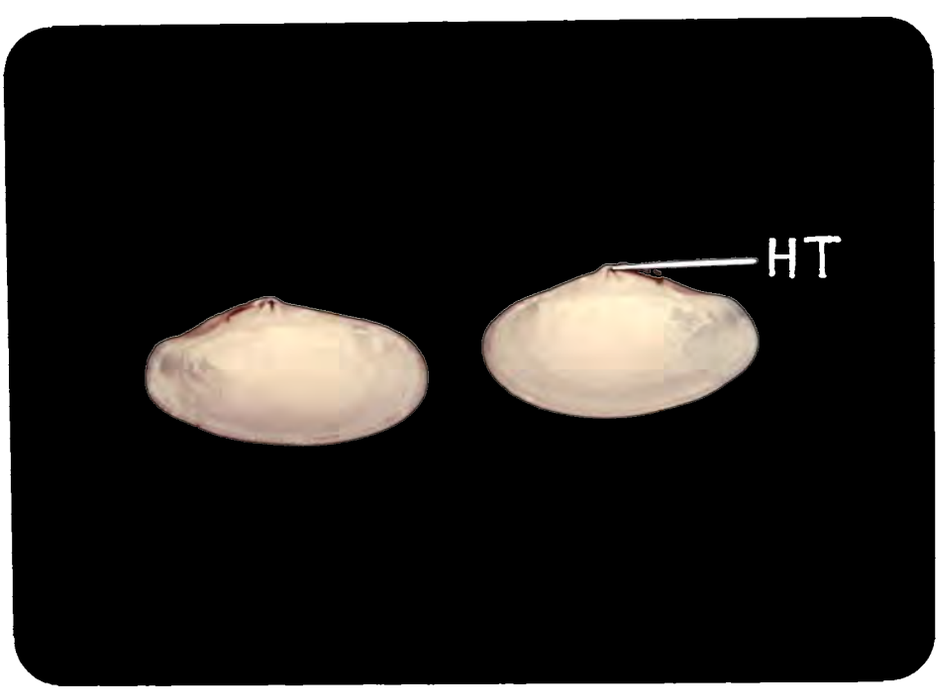

Figure 3. Internal view of shell of S. scripta showing hinge teeth (HT).

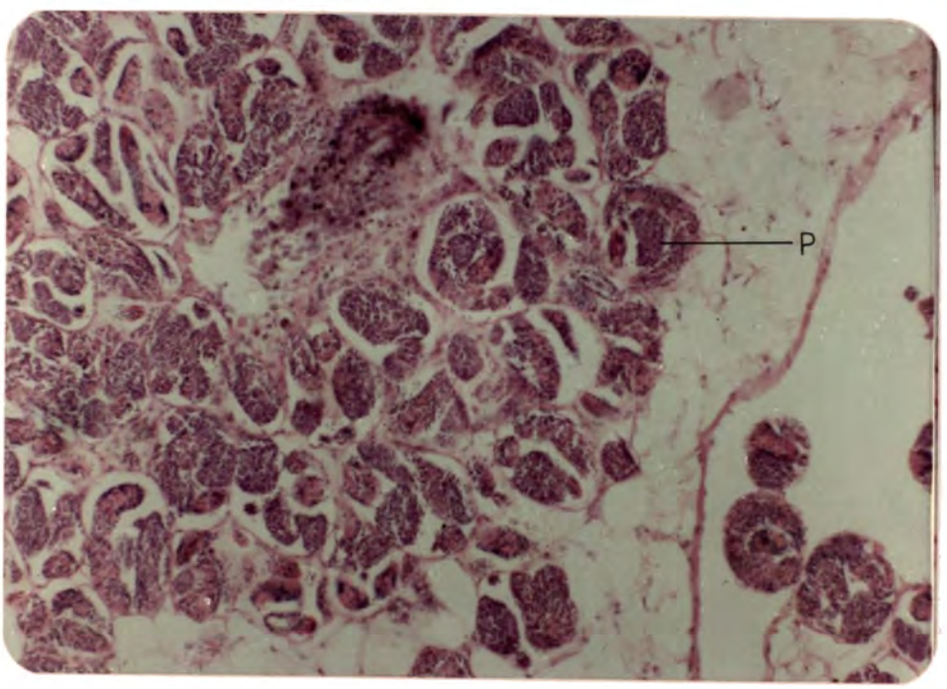

Figure 5. Section of female gonad overrun by a parasitic infection. Specific identity unknown, (P = parasite).

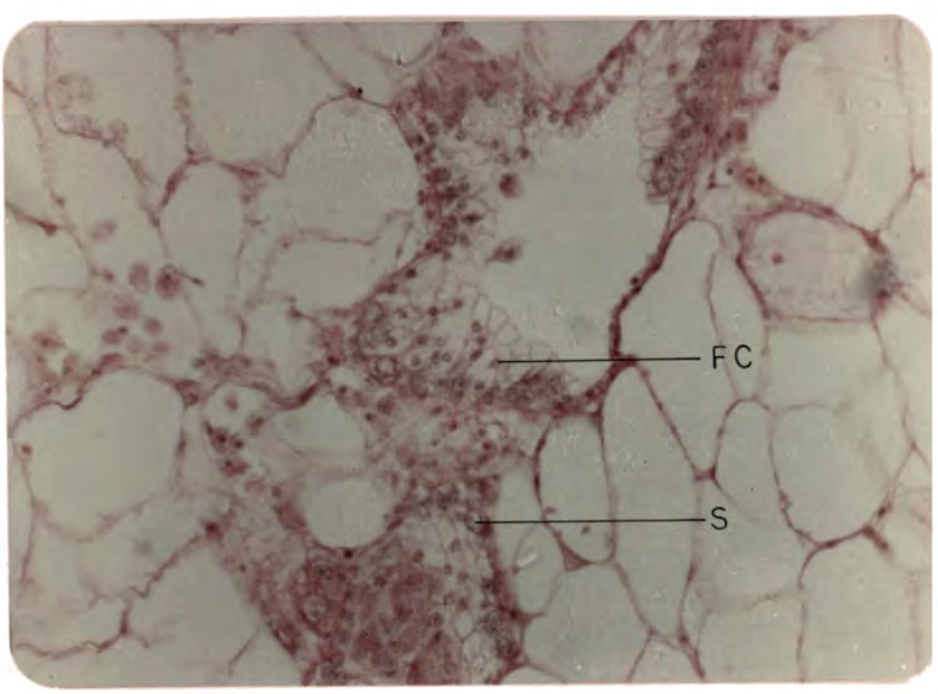

Figure 6. Section of gonad in early gametogenesis. Indifferent stage. Follicle is filled with follicular cells (FC) and stem cells, (S).

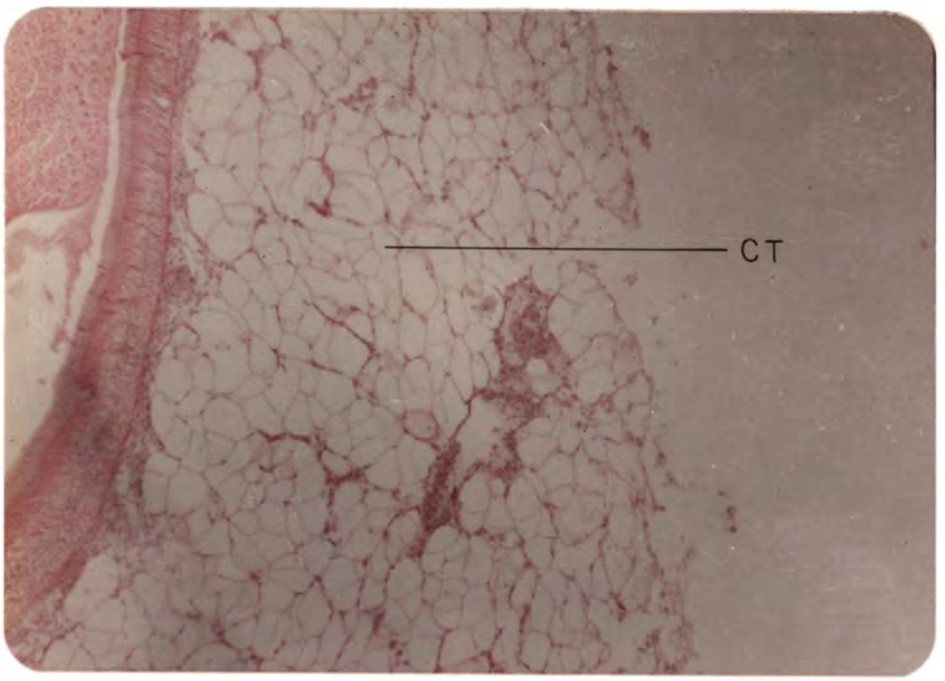

Figure 7. Section of gonad showing early follicle development. Connective tissue (CT) is predominant in the gonad.

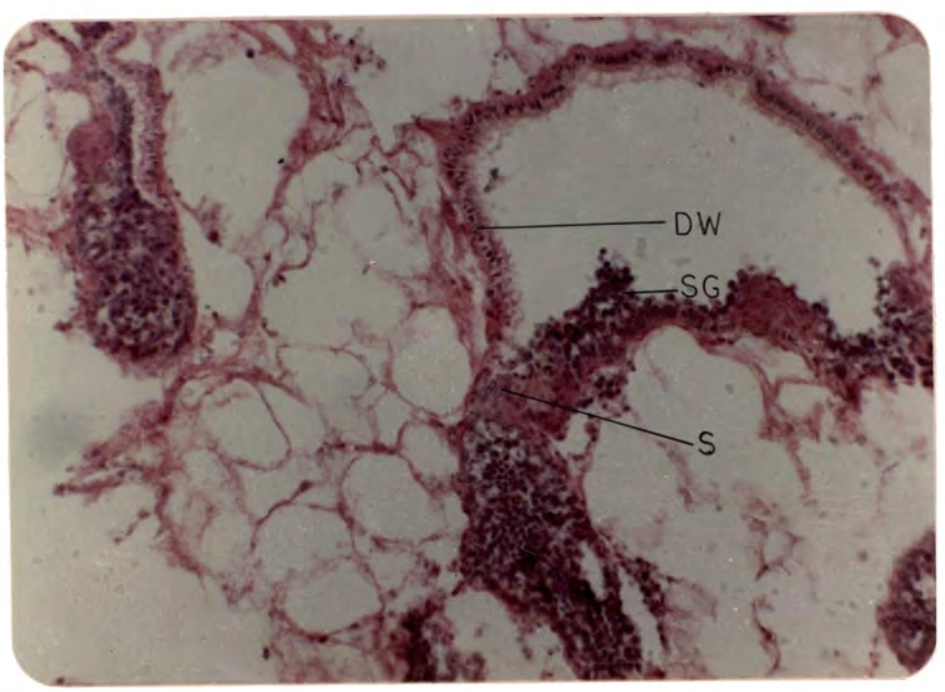

Figure 8. Section of early male gonad. Spermatogonia (SG) and stem cells and ciliated duct wall (DW) are indicated.

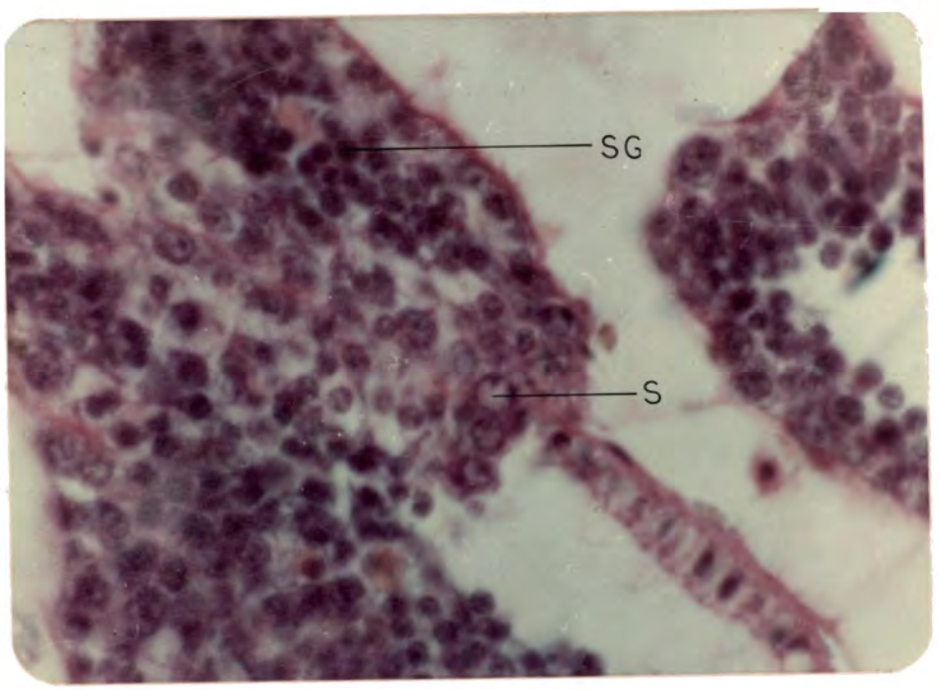

Figure 9. Section of early male gonad showing spermatogonia (SG) and stem cells (S).

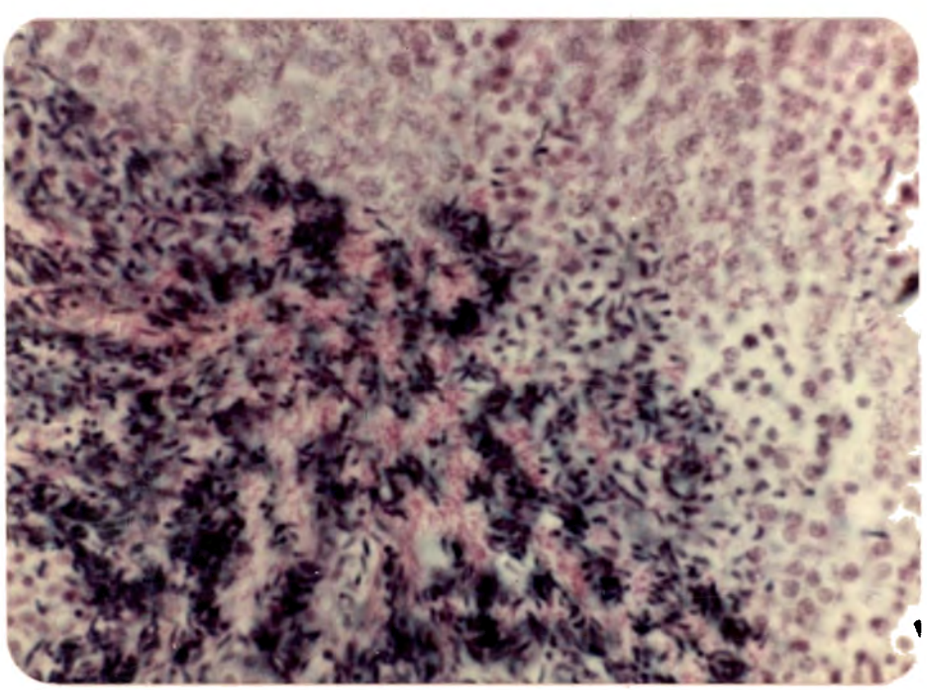

Figure 10. Section of late maturing male gonad.

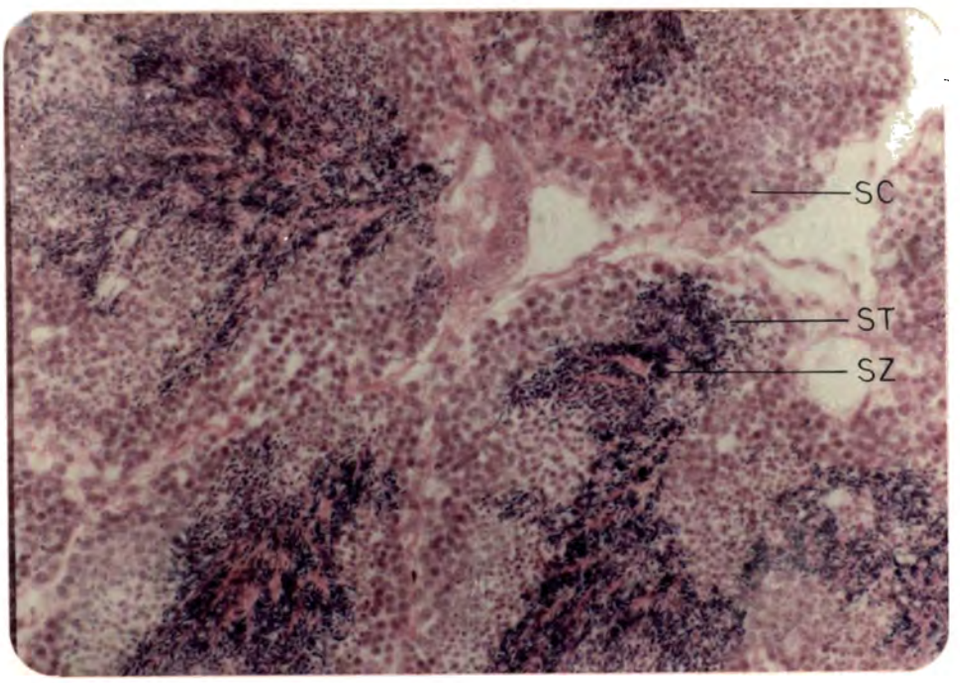

Figure 11. Section of late maturing male gonad. Spermatocyte (SC), spermatids (ST) and spermatozoa present (SZ).

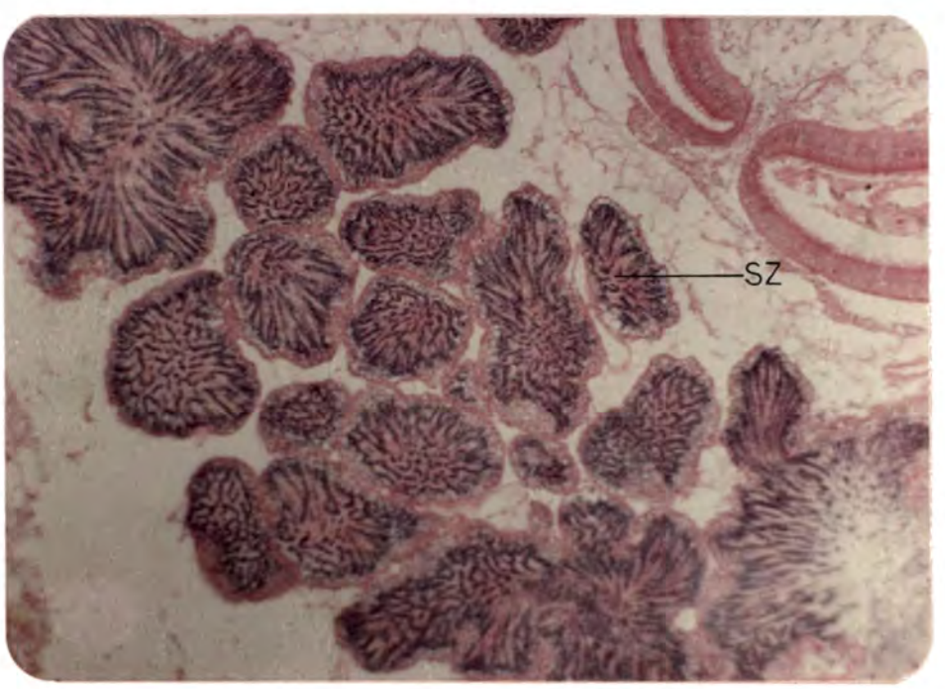

Figure 12. Section of mature male gonad. Radially arranged spermatozoa (SZ). Early stages reduced to narrow bands at follicle wall.

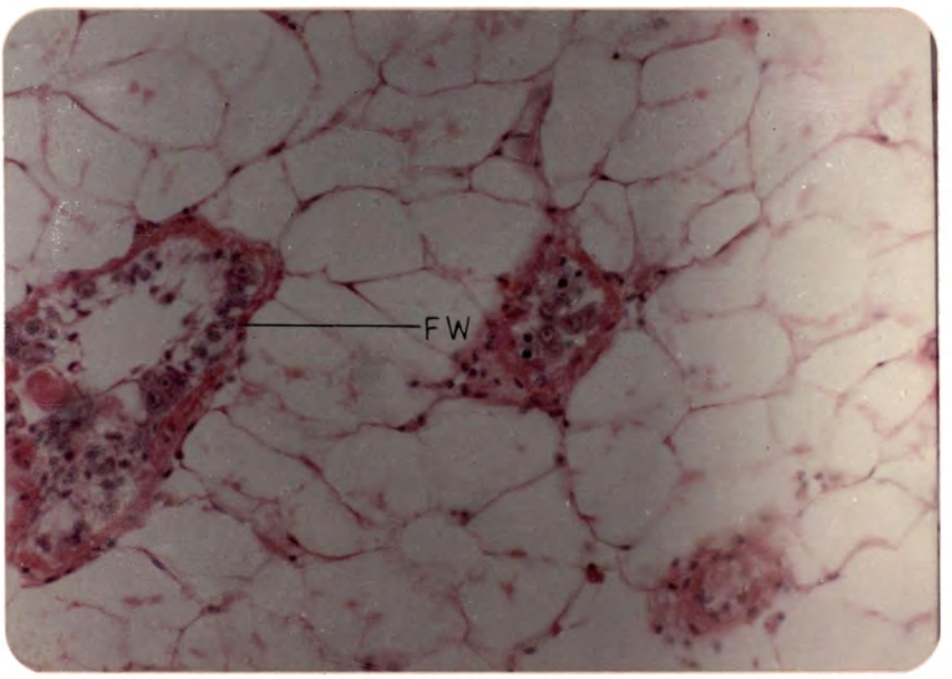

Figure 13. Section of female gonad showing early oogenesis. Follicle wall (FW) is prominent. Red eosinophilic mass probable represents cell debris from previous spawning. Extensive interfollicular tissue.

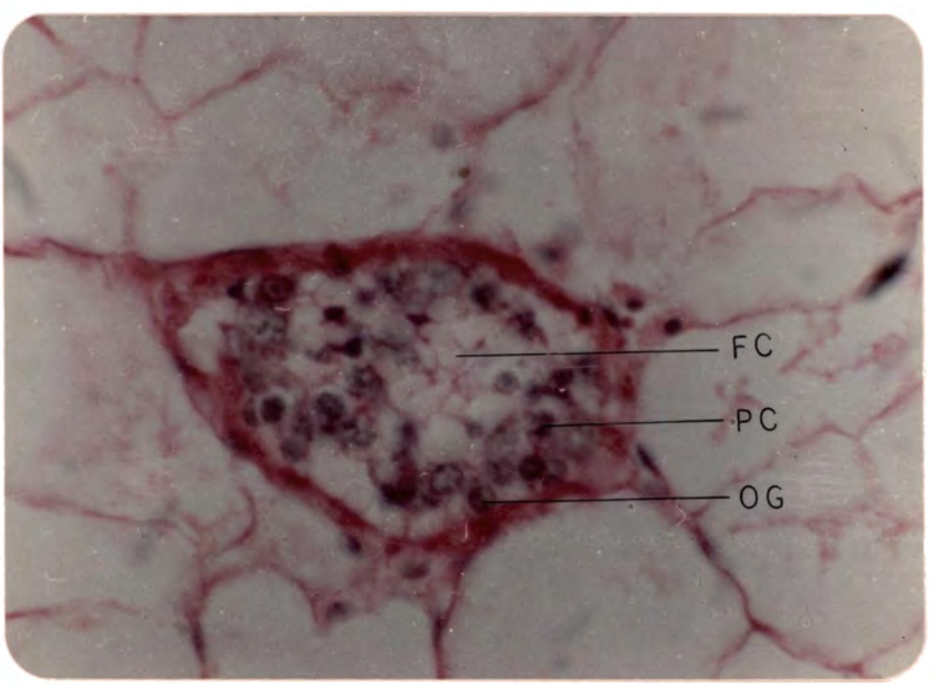

Figure 14. Section of female gonad at early oogenesis. Follicle contains follicular cells (FC) oogonia (OG) and primary oocytes (PC).

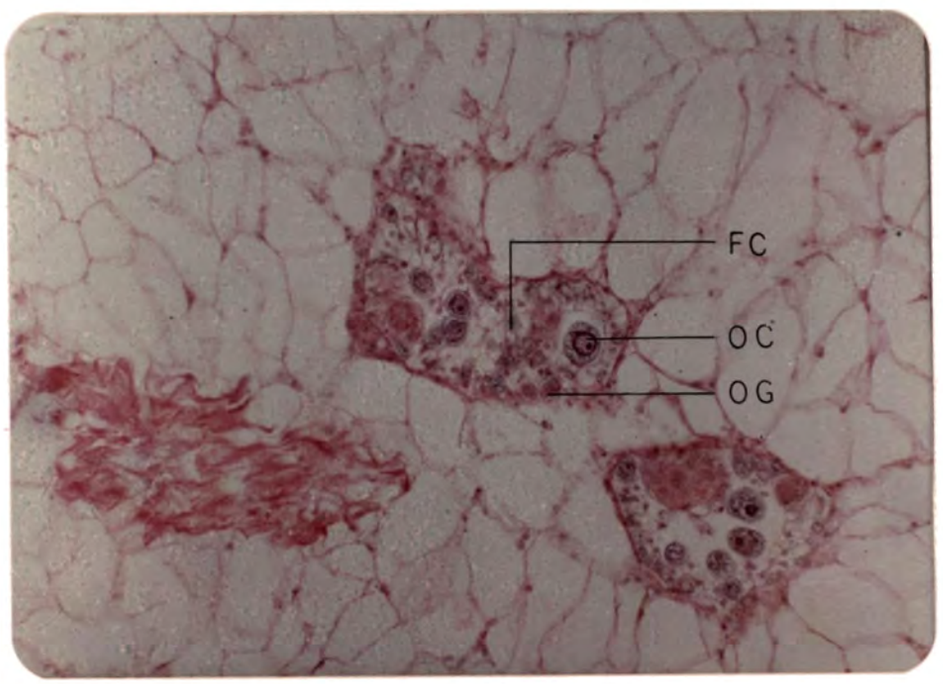

Figure 15. Section of gonad of pre-vitellogenic female. Follicles contain follicular tissue (FC), oogonia (OG) and oocytes (OC) at start of vegetative phase.

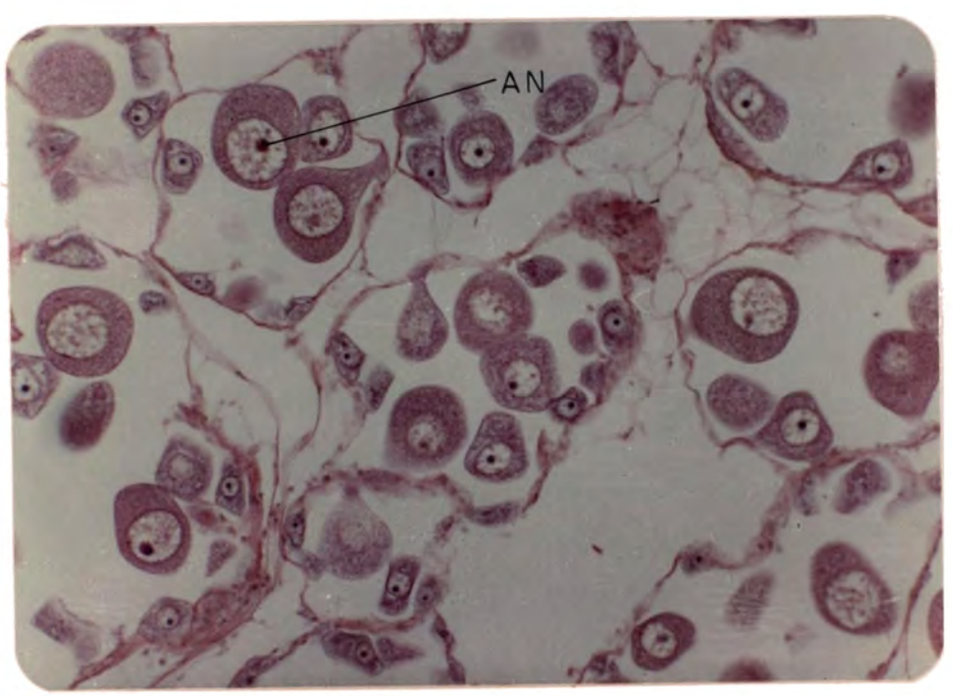

Figure 16. Section of vitellogenic female gonad. Amphinucleolus (AN) is present.

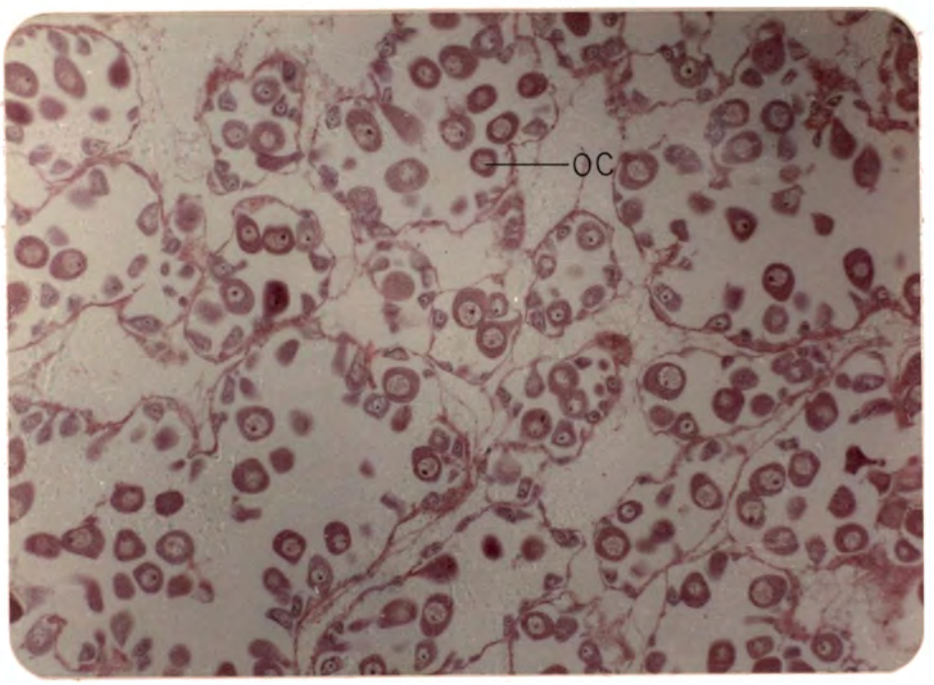

Figure 17. Section of gonad of mature spawning female. Free oocytes (OC) predominate. Little interfollicular connective tissue.

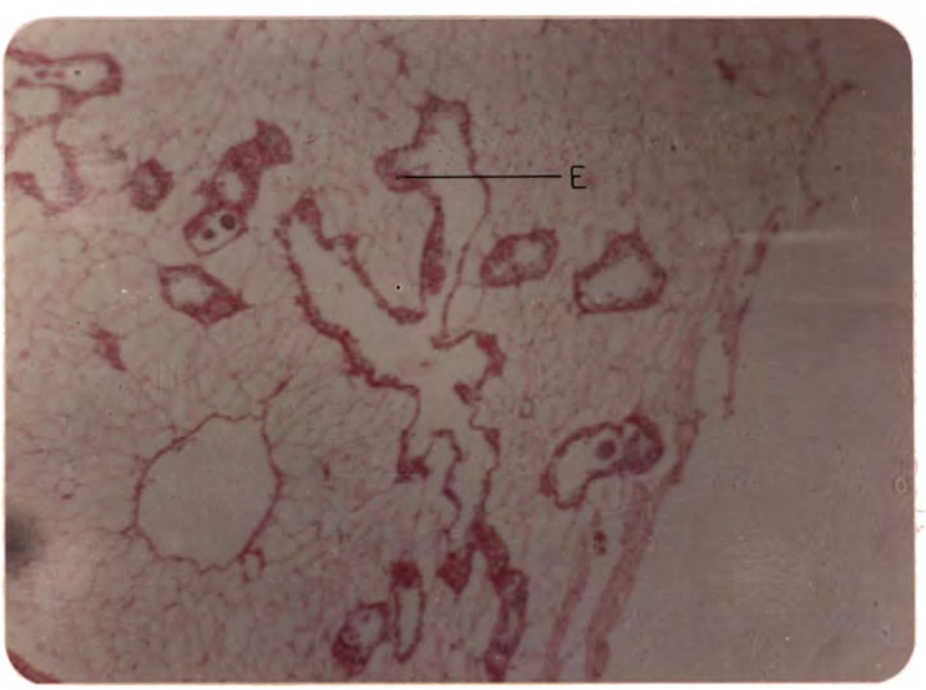

Figure 18. Section of gonad of spent female. Follicle contains eosinophilic masses (E) and cell debris.

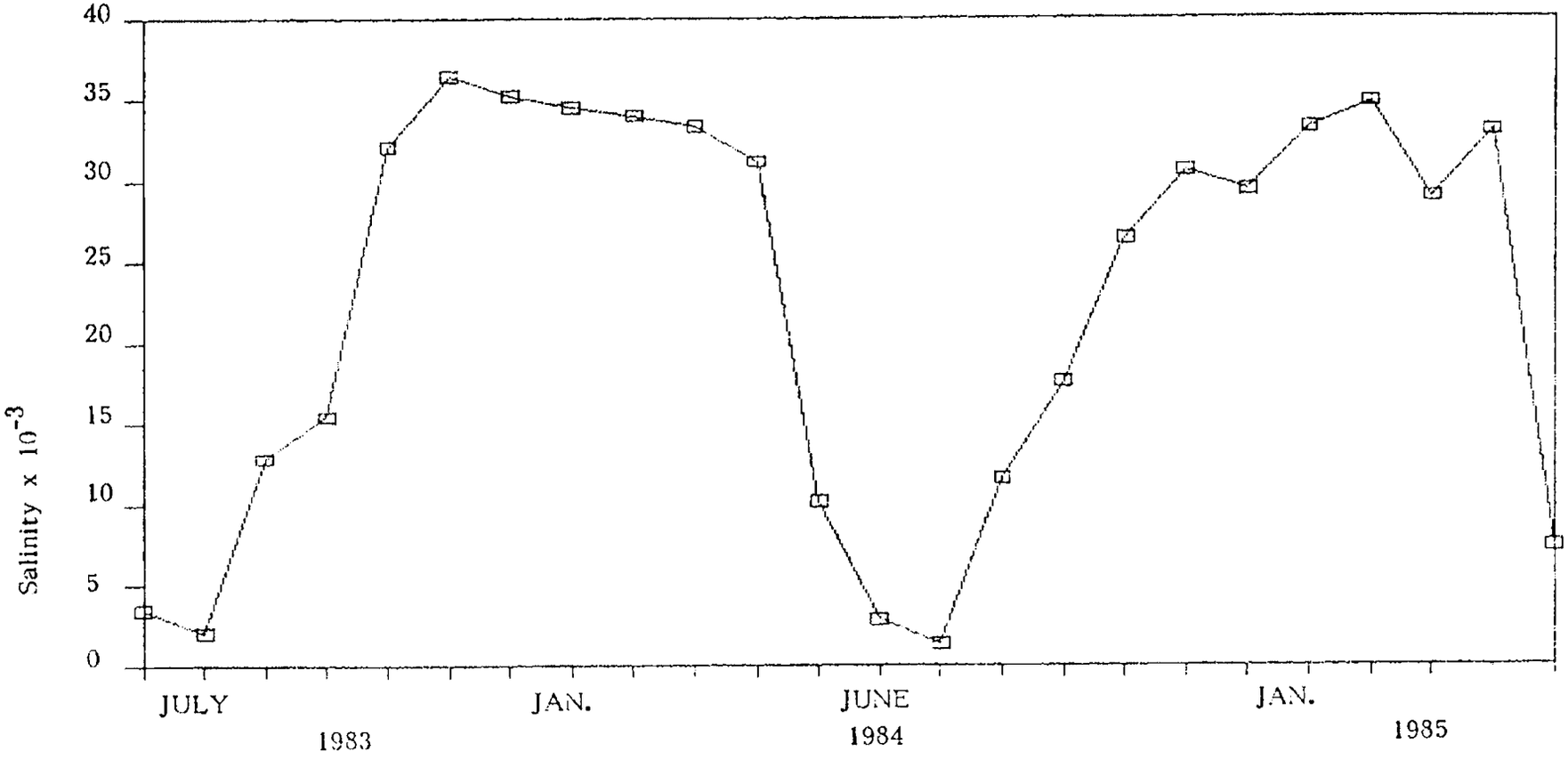

Figure 19. Monthly variations in salinity in the clam bed near the Cochin barmouth.

mature animals in Pahia laterisulca populations 4-6 weeks after a first spawning. Stephen cited by Braley (1982) found a relative lack of a quiescent phase in mussels and oysters from coastal India. This is possible because of a stable food supply (Braley, 1982) which restores depleted food reserves quickly.

Early gametogenic stages appear in S. scripta from mid-April and a period of indifferent sex extends to mid-July when the gonad follicles consist of the earliest germ cells (Table II). While early gametogenesis is slow, somatic growth is accelerated to reconstitute reserve stores which will fuel further developments in the cycle (Stephen, 1980a).; The spent and early gametogenic phase extends to late August/mid-September with females in pre-vitellogenic phase and male follicles containing spermatogonia. From mid-September to mid-October extends a period of active cell proliferation in males and vitellogenic activity in females. This accelerated growth serves to bring male and female cycles into phase and by mid-October all the animals examined were in late developing or mature condition. This condition was maintained till late November when all the animals observed were mature. Spent gonads appeared in the samples from mid-December and constituted a sizeable proportion from late February to March/May. Spawning per se was not observed. A partially spent phase described by Mason (1958), and Shafee and Lucas (1980) in Pecten maximus and Chlamys varia, was also not detected, though in females residual oocytes are always found in the spawned gonad. The spawning period therefore extends over 4 months from November to March. A possible interpretation is that early spawners have undergone

TABLE IIFORTNIGHTLY VARIATIONS IN NUMBER OF GONADS OF S. SCRIPTA CLASSFIED INTO DEVELOPMENTAL STAGES AFTER MICROSCOPIC EXAMINATION.

	MALE					FEMALE				
	I	E	L	M1	S1	EO	P	V	M2	S2	N
13 July 83	14	-	-	-	-	3	-	-	-	-	17
29 July	9	4	-	-	-	6	3	-	-	-	22
27 August	2	7	-	-	-	2	5	-	-	-	16
12 September	-	11	-	-	-	1	8	-	-	-	20
29 September	-	9	1	-	-	-	6	3	-	-	19
10 October	-	-	7	1	-	1	1	8	-	-	18
29 October	-	-	3	6	-	-	-	4	3	-	16
13 November	-	-	-	7	-	-	-	2	9	-	18
30 November	-	-	-	6	-	-	-	2	7	-	15
15 December	-	-	-	8	1	-	-	1	6	-	16
31 December	-	-	-	9	-	-	-	-	10	-	19
15 January 84	-	-	-	6	1	-	-	-	7	2	16
31 January	-	-	-	4	4	-	-	-	6	3	17
12 February	-	-	-	5	3	-	-	-	4	4	16
24 February	-	-	-	4	1	-	-	-	2	6	13
15 March	-	-	-	2	7	-	-	-	3	5	17
24 March	-	-	-	1	5	-	-	-	-	8	14
16 April	11	-	-	-	2	-	-	-	-	4	17
3 May	14	-	-	-	1	-	-	-	-	3	18
15 May	15	-	-	-	-	1	-	-	-	1	17
30 May	14	1	-	-	-	3	-	-	-	-	18
15 June	13	-	-	-	-	2	-	-	-	-	15
28 June	11	4	-	-	-	2	1	-	-	-	18
12 July	4	5	-	-	-	3	7	-	-	-	19
29 July	1	7	-	-	-	2	5	-	-	-	15
14 August	1	8	-	-	-	1	4	1	-	-	15
30 August	-	7	-	-	-	-	6	2	-	-	15
13 September	-	10	2	-	-	-	8	3	-	-	23
26 September	-	8	3	-	-	-	5	4	1	-	21
19 October	-	-	8	3	-	-	-	2	7	-	20
24 October	-	-	2	11	-	-	-	3	4	-	20
15 November	-	-	1	6	-	-	-	2	7	-	16
21 November	-	-	-	8	-	-	-	-	6	-	14
16 December	-	-	-	5	-	-	-	-	9	-	14
24 December	-	-	-	5	1	-	-	-	8	-	14
5 January 85	-	-	-	7	-	-	-	-	9	1	17
18 January	-	-	-	11	3	-	-	-	6	2	22
7 February	-	-	-	10	2	-	-	-	7	1	20
21 February	-	-	-	4	3	-	-	-	6	4	17
15 March	-	-	-	2	4	-	-	-	1	5	12
29 March	-	-	-	1	6	-	-	-	2	5	14
13 April	4	-	-	-	2	1	-	-	-	6	13
25 April	5	-	-	1	-	-	-	-	1	9	16
18 May	7	-	-	-	-	-	-	-	-	8	15
29 May	12	-	-	-	-	2	-	-	-	1	15
15 June	11	-	-	-	-	4	-	-	-	-	15

I = Immature; E = Early gametogenesis; L = Late maturing; M1 = Mature male; S1 = Spent male; EO = Early oogenesis; P = Pre-vitellogenesis; V = Vitellogenesis; M2 = Mature female; S2 = Spent female; N = Number.

multiple or prolonged spawning, gamete replacement occuring too quickly to preclude detection of the early stage.

In the case of extended or "dribble spawning", in the event of a catastrophy in an unpredictable environment, which could kill or prevent settlement of the vulnerable larvae only a portion of the potential recruits would be lost (Newell et al., 1982).

Certain fundamental properties of the reproductive strategy of a species may be maintained across a wide range of environmental qualities; for example, such properties as mean egg size, the unit energetic costs of gamete production and the relationship between maximum rate of increase of age related reproductive effort and the weight related change in energetic surplus (Bayne and Newell, 1983). Other properties of reproduction are more vulnerable to environmental changes, including the timing of gametogenesis and spawning, the fecundity of the individual, and the maximum reproductive effort attainable in the individual life span.

Sastry (1979) described the reproductive cycle of a species as a genetically controlled response to components of the environment especially temperature, salinity, light and food and endogenous factors within the organism.

In higher latitudes, variations in temperature have been recognised as the main stimulus for maturation and spawning (Galstoff, 1930, 1932; Loosanoff, 1937a; Chipperfield, 1953; Giese, 1959; Bayne, 1975). However in a tropical marine environment, temperature is relatively stable-variations

between maxima and minima being too insignifant to elicit major physiological responses.

Rand (1973) and Giese and Pearse (1974) maintained that spawning in tropical species occurred throughout the year. However in tropical estuaries and coastal waters salinity variations cause restriction of spawning periods (Stephen , 1980a). Sastry (1979) reviewing gametogenesis in tropical bivalves found both restricted and extended spawning activity.

Gonad development in S. scripta from the bed under study coincides with ambient salinity changes and appears to be so adapted. The annual breeding pattern shows a single gametogenic cycle with a single but protracted spawning period. Analysis of gametogenic activity in each of the salinity seasons indicates a possible relationship between reproduction and variations in salinity - (Fig. 20,21) - (1) a recovery and slow early gametogenic phase occuring during the low salinity period, (2) a gametogenically active phase associated with the period of rising salinity and (3) spawning activity associated with high and relatively stable salinity.

There is ample support for the regulatory role of salinity in breeding cycles of tropical euryhaline organisms (Hornell 1910; Malpas, 1933; Paul, 1942; Sandison, 1966a and b; Hill, 1967; Wilson, 1969).

Stephen (1980a) proposed "Hornell's Rule" to describe the relationship of salinity and breeding in tropical marine environments. Wilson (1969) reported inhibition of gametogenesis at very low salinities in the mussel Xenostrobus securis. Loosanoff (1952) found that Crassostrea virginica

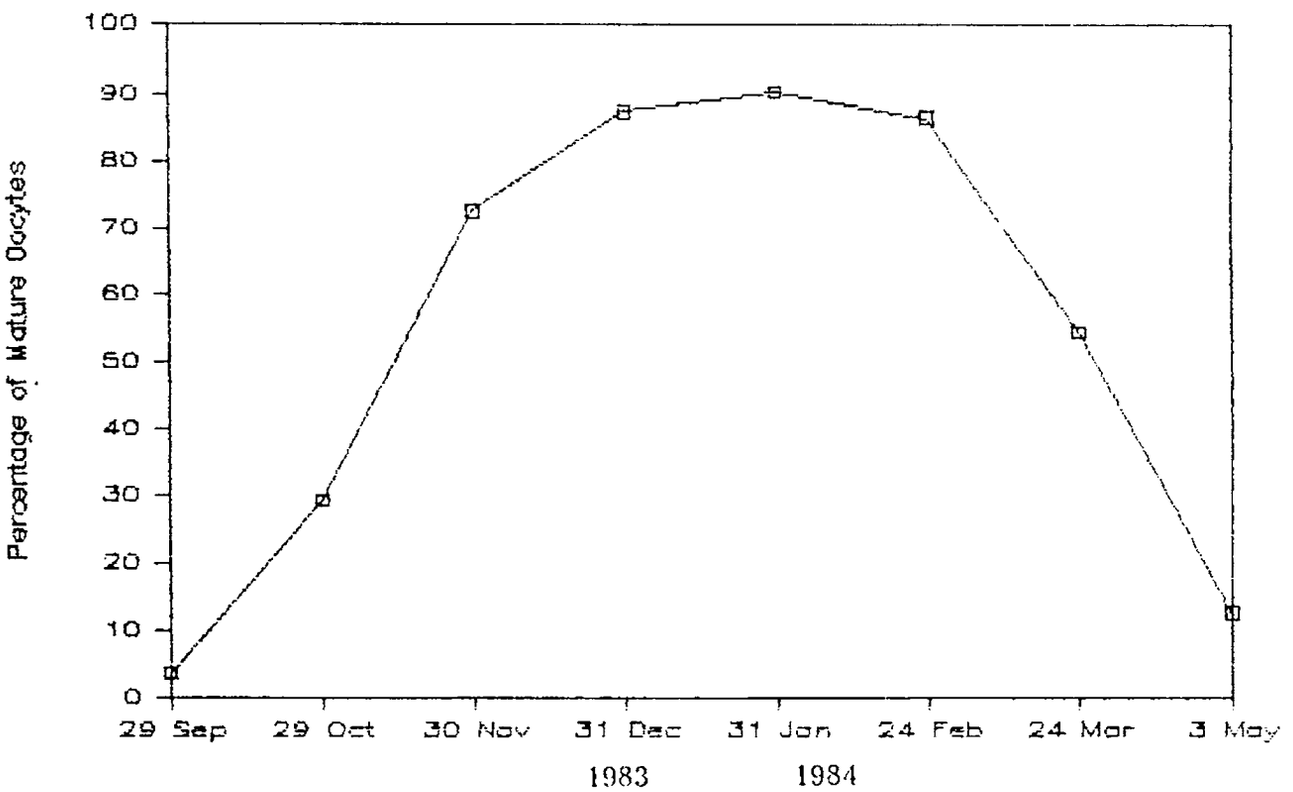

Figure 20. Variations in percentage frequency of the mature oocytes in the breeding period of S. scripta.

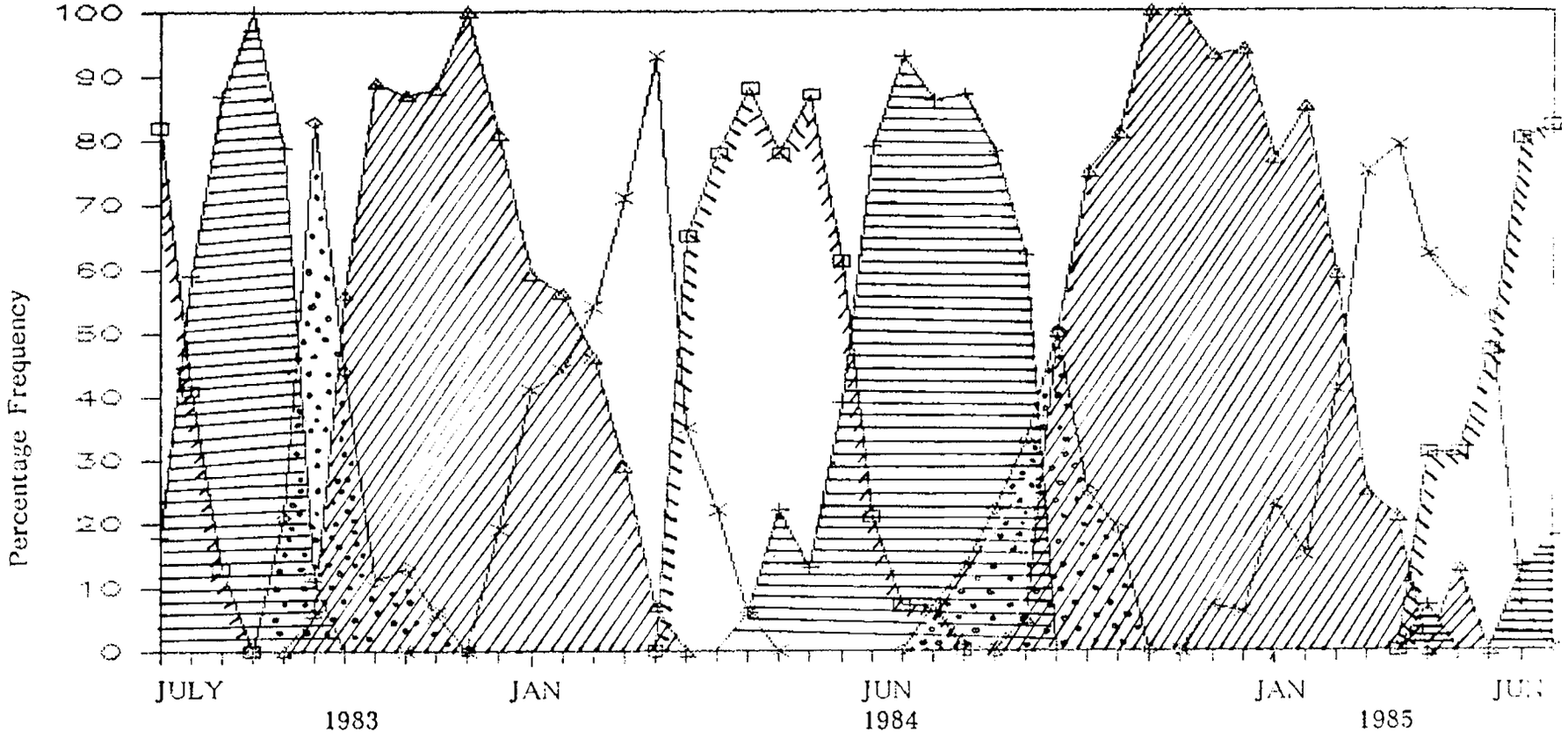

Figure 21. Frequency polygon of gonadal stages in S. scripta. (= early gametogenesis, = late maturing, = mature, = spent, = indifferent).

from Long Island Sound developed spermatozoa at a salinity of 7.5×10^{-3}S but eggs did not develop normally. Active gametogenesis in S. scripta corresponds to rising ambient salinities from approximately 1.5×10^{-3} to 33×10^{-3}S. The possible existence of a threshold or triggering salinity value needs further experimental investigation. Alagaraswami (1966) has also found active gametogenesis in Donax faba from the east coast during rising salinities following the monsoon. Spawning in S. scripta takes place in high but stable salinities. (Figs. 20 and 21). Salinity variations acting as a "natural spawning stimulus" were described by Panikkar and Aiyar (1939). Webber and Giese (1969) discussing the role of spawning stimuli suggested that after initiation gametogenesis may continue until the gonad has increased to a size that inhibited it. Giese and Pearse (1974) considers the role of gametes to be very important in epidemic spawners. Stephen (1980) suggests the presence of conspecific gametes as a final stimulus.

Nutrient levels in the habitat cannot be underestimated as a factor affecting gametogenesis. Qasim (1956) formulated "Crisp's Rule" to signify the relationship between spawning season and availability of food for larvae. Shafee and Lucas (1980) observed that the reproductive energy of a bed of inter-tidal oyster, Crassostrea virginica varied from 0-48% of the total production depending upon the season. Though tropical marine environments have typically stable food levels, gametogenic cycles may be adjusted to coincide with phytoplankton peaks in the region. Organisms with a short life span, depend upon the offspring of a single

spawning for propagation of the species. Hence spawning times are selected to optimize survival of the larvae to adulthood.

Gopinathan et. al., (1975) observed 3 phytoplankton peaks in the barmouth - (1) January - February, (2) June - July and (3) September-October. The first peak coincides with the major spawning period so that larvae and spat are ensured a food supply as plankton and detritus. The second and most prominent peak appears during the early gametogenic period when somatic rather than germinal growth is emphasised. The third peak coincides with the period of active gametogenesis when depleted food reserves are supplemented by direct mobilisation of ingested food to the gonad. Broom and Mason (1978) found three annual spawning peaks in Chlamys opercularis in suspended cage culture as compared to the single spawning of scallops in an outdoor tank. A good supply of food in the suspended cage is suggested as a reason. Newell et al (1982) points out that gamete production is ultimately dependent on the nutrients available for gametogenesis, either in terms of a nutrient reserve or food recently ingested.

Though it is difficult to demonstrate a simple causal influence of exogenous factors on reproduction, the weight of evidence supports the idea that a threshold salinity or possibly a rate of change of salinity is a very important factor influencing gametogenesis (reviewed by Sastry, 1979; Stephen, 1980a). However the role of salinity may be indirect affecting plankton production and hence food available to the population. Butler (1949) attributed the influence of depressed salinity on gametogenesis

in Crassostrea virginica to variations in food availability rather than depression of sexual activity by low saline water. Blake and Sastry (1979) however found that in scallops (Agropecten irradians irradians), in the vitellogenic phase, the nuerosecretory cycle is independent of nutrition. Regardless of the supply of nutrients, interaction between gonads and ganglia ensured completion of the gametogenic cycle at a rate dependant upon ambient temperatures. Webber and Giese (1969) have suggested that gametogenesis may solely be dependant on internal neurosecretory rythms which are activated by, but do not need continuous input from exogenous factors. The existence of neurosecretory cycles have been demonstrated in a number of bivalves (Ajithakumar 1984, Nagabhushanam, 1963; Nagabhushanam and Mane, 1973; Lubet and Mathieu, 1982).

In conclusion it may be stated that though gametogenic cycles in S. scripta seem to correspond to ambient salinity patterns, the influence of a phytoplankton production cycle cannot be under-rated. An over-riding neurosecretory cycle may also be implicated. Only when gametogenesis can be induced off-season, by manipulation of the environment in controlled experiments, can definite conclusions be drawn to establish the influence of a causal factor.

CHAPTER III

BODY DISTRIBUTION AND SEASONAL CHANGES IN BIOCHEMICAL COMPOSITION OF *SUNETTA SCRIPTA*

Bivalvia have been the subject of biochemical investigations not only in their importance as food for man but also because of their significant role in the economy of many benthic areas. Seasonal metabolic cycles in bivalves are a reflection of complex interactions between food availability, environmental parameters, growth and reproductive activities (Bayne, 1976, Gabbot, 1983). In general, energy storage in the form of protein, lipid and glycogen occurs during nutrient abundance prior to gametogenesis and is subsequently utilised in the production of gametes when metabolic demand is high (Gabbot, 1975; Bayne, 1976).

Ansell and Trevallion (1967) defined seasonal activities of boreal bivalves affecting the seasonal cycle of gross biochemical composition to include (1) a winter period of inactivity when gametogenic activity is slow and stored reserves supply metabolic needs (2) a short period of renewal of activity in spring to restore reserves when there is rapid gametogenesis, and (3) a reproductive period in summer when somatic and germinal growth and spawning proceed together in response to environmental changes, particularly to enhancement of food supply. The relative importance of the different substrates, their sites of storage and timing of utilisation in relation to season vary between species as well as between populations of the same species (Giese, 1969; Bayne, 1976; Barber and Blake.(1981).

Although there is now an extensive literature on the seasonal cycles in tissue weight and biochemical composition associated with

growth and reproduction in boreal bivalves, information on tropical species is more limited. The vastly differing conditions of the tropics give rise to varying metabolic strategies in organisms. Most of the studies on the biochemistry of Indian bivalves have been concerned with the biochemical composition, seasonal changes in composition and calorific values of pooled homogenised animals with little or no distinction of sex, gonad condition or environmental parameters (Venkataraman and Chari, 1951; Durve and Bal, 1961; Saraswathy and Nair, 1969; George and Nair, 1975; Sivankutty and Shynamma, 1975; Krishnakumari et al., 1977; Nagabhushanam and Mane, 1978; Shafee, 1978; Lakshmanan and Nambisan, 1980 and Jayabal and Kalyani, 1986). Giese (1969) points out that such data have limited use in a study of the relation of the biochemistry of the animal to its nutritional or reproductive stages.

During the two years of this study, the population of S. scripta underwent two complete reproductive cycles. Following a spawning, the population consists mainly of clams of indifferent sex. Gametogenesis is slow till September when germinal development attains prominence. Mature clams appear in October. Spawning begins in late November and continues till March-May after which there is a short refractory period of gonadal rest, when the clams are termed spent. The present investigation involves a comprehensive study of the seasonal distribution of moisture, lipid, protein and carbohydrate fractions of the whole body and component organs and energy levels of the whole body of S. scripta.

MATERIALS AND METHODS

Sampling (described earlier) was conducted on a monthly basis spread over 2 years 1983-1985. Following collection, the clams were left overnight in filtered sea water to allow clearance of any mud from the mantle cavity and the gut. The clams selected were adults within the shell length range of 25-40 mm, thus including the maximum size reached by specimens from this location. S. scripta is gonochoristic and for most of the year sexes were easily distinguishable from microscopic examination of gonad smears. The two sexes were thus treated separately except during the early gametogenic stage when sexes were indistinguishable from smear examination and hence were termed indifferent.

Biochemical estimations were made on whole clams - male, female and indifferent - as well as on body components—foot, mantle, adductor muscle, gill, digestive gland and gonad.

The clams were forced open with scalpel, washed with a minimum quantity of glass distilled water and blotted dry. To estimate moisture content the flesh (whole clams and component organs) was weighed immediately on removal from shell and dried in an air oven at 80°C overnight till to constant weight. The difference between the dry weight and fresh weight expressed as percentages of fresh weight (F Wt) denoted moisture levels. The dried tissue samples were used for determination of organic compounds and calorific values. All the biochemical data were determined on mixtures of dried tissues from 10 individuals, which has the advantage of giving an average value for the species while avoiding the excessive amount of time required for individual determination.

Calorofic values were determined by the method of Karzinkin and Tarkovskaya (1960) based on iodate oxidation of the organic fraction in the sample. A known weight of dried sample was oxidised with a mixture of potassium iodate and concentrated sulphuric acid. Unreacted iodine was back titrated with sodium thiosulphate (0.1 N). The calorific values were calculated by the equation mentioned by the authors and the results are expressed as K cal g^{-1} dry weight.

Protein in the tissues was determined by the method of Lowry et al., (1951). A weighed sample of the tissue was subjected to alkali while being warmed in a water bath. The solution was treated first with an alkaline solution of copper sulphate and then with Folin's reagent. The intensity of the blue colour of the resulting solution measured spectrophotometrically at 750 nm was proportional to the protein concentration of the sample. Bovine Serum Albumin was used to prepare the standard curve.

For total carbohydrate levels weighed tissue samples were extracted with 5% Trichloroacetic acid containing 0.1% silver sulphate. The extract was warmed with concentrated sulphuric acid and the rose-coloured furfural formed was estimated photometrically at 520 nm. This method was proposed by Kemp and vanKitz (1953) and considered suitable by Raymont and Krishnaswamy (1960) for the estimation of aquatic organism. The standard curve was prepared using glucose.

The method of Barnes and Blackstock (1973) was used to estimate lipid levels. Weighed tissue samples were extracted with chloroform

methanol and the lipid extract treated with sulphuric acid, phosphoric acid and vanillin. The optical density of the red-coloured complex was estimated at 520 nm. This method was approved by the authors as particularly suitable for determining seasonal changes in lipid content of organisms.

The data for organic constituents is depicted as mg g^{-1} dry weight (DW). Statistical limits were not indicated since only duplicate determinations were made except when gross discrepancies were noticed in which case another pair of determinations were carried out. The disadvantage of expressing results as level is that changes in one biochemical component are reflected by reciprocal changes in all other components. But changes in biochemical level indicate the relative importance of different reserves.

RESULTS

The results of the analysis are presented in Tables III-VII and Figs. 22-30. Protein levels (Table III; Figs. 22, 23, 24) in whole tissue showed peak values during the spawning period (401-462 mg g^{-1}DW) and low levels (209-330 mg g^{-1}DW) in the post-spawning and early gametogenic phase. Male clams had higher protein levels. Carbohydrate values showed high levels (269-289 mg g^{-1} DW) during the spawning phase of the first gametogenic cycle and relatively lesser (161-199 mg g^{-1} DW) values during the same period of the succeeding gametogenic cycle. Enhanced carbohydrate values were noticed during the post-spawning and early gametogenic phase of female and indifferent clams in 1984. Lipid levels were low varying from 66-139 mg g^{-1} DW. Values were usually below 100 mg g^{-1} DW. but higher values were observed during the spawning period of the second

TABLE III MONTHLY VARIATION IN MEAN TOTAL PROTEIN LEVELS IN WHOLE TISSUES AND COMPONENT ORGANS OF S. SCRIPTA (mg g^{-1} DW).

	July 83	August	September	October	November	December	Jany.84	February	March	April	May	June
MANTLE	379.05	352.24	327.14	312.19	316.62	307.31	341.07	493.92	427.37	424.99	326.62	322.85
FOOT	472.40	454.84	413.33	439.23	425.79	478.10	441.31	417.07	420.57	380.93	384.17	370.47
DIGESTIVE GLAND	349.03	312.19	367.89	344.66	350.37	417.17	330.30	313.24	359.50	369.34	393.49	305.72
GONAD	235.35	258.33	259.82	327.20	257.67	286.20	219.78	221.10	317.33	264.36	243.27	293.25
ADDUCTOR MUSCLE	425.07	440.38	407.32	439.00	487.71	446.26	478.57	492.99	432.69	429.44	479.93	438.01
GILL	340.75	334.37	349.19	374.25	362.09	402.54	446.16	423.60	438.07	359.55	378.82	387.71
MALE			349.39	305.65	462.33	431.38	440.76	468.01	410.22			
FEMALE			305.37	321.78	437.67	373.70	420.22	433.41	394.69			301.57
INDIFFERENT	350.94	390.38							416.04	313.19	360.00	330.16

	July	August	September	October	November	December	Jany.85	February	Mar ch	April	May	June
MANTLE	409.37	419.79	447.72	429.06	443.52	425.57	375.25	407.14	444.28	501.00	351.33	385.60
FOOT	410.36	452.52	471.37	450.68	435.94	403.24	447.15	395.75	407.03	452.87	465.70	389.25
DIGESTIVE GLAND	328.87	357.43	384.26	353.68	407.48	401.75	355.44	332.29	348.64	359.56	376.24	302.14
GONAD	254.16	286.52	314.26	304.17	445.88	318.92	343.58	351.49	304.16	233.92	276.37	367.54
ADDUCTOR MUSCLE	444.56	516.91	467.33	476.70	461.25	487.69	489.93	392.17	415.83	453.11	481.38	490.52
GILL	394.01	358.95	382.56	407.36	454.34	401.62	434.86		395.64	374.94	442.42	459.00
MALE			445.56	440.57	409.09	454.71	450.71	450.82	384.80	320.39	435.86	
FEMALE	299.95	403.37	405.73	402.33	410.66	401.00	422.16	343.53	376.40	416.38		
INDIFFERENT	291.37	353.73								395.27	398.60	362.81

TABLE IV MONTHLY VARIATION IN MEAN TOTAL CARBOHYDRATE LEVELS IN WHOLE TISSUE AND COMPONENT ORGANS OF S. SCRIPTA (mg g^{-1} DW).

	July 83	August	September	October	November	December	Jany.84	February	March	April	May	June
MANTLE	111.23	88.15	87.10	76.14	55.08	68.06	59.59	54.65	53.05	63.76	125.29	109.27
FOOT	96.95	128.79	150.85	134.16	116.41	123.14	120.00	81.05	138.07	122.56	133.13	131.25
DIGESTIVE GLAND	157.93	131.38	180.26	157.14	239.16	243.30	140.28	174.11	152.48	250.93	224.53	243.35
GONAD	487.16	297.96	227.62	243.75	410.02	519.09	296.73	330.27	508.75	479.16	441.65	550.66
ADDUCTOR MUSCLE	117.85	101.63	124.58	105.39	112.04	84.95	89.28	71.11	75.04	93.08	105.34	142.35
GILL	72.69	32.00	45.10	29.37	26.04	35.89	26.35	26.92	23.01	38.76	58.39	69.62
MALE			155.36	200.54	211.55	269.79	156.60	157.20	154.23			
FEMALE			140.92	178.12	223.46	289.01	165.90	155.84	157.91			217.49
INDIFFERENT	195.19	130.60							112.34	126.70	286.51	256.95

	July	August	September	October	November	December	Jany.85	February	March	April	May	June
MANTLE	127.57	64.65	49.39	43.78	57.69	41.55	64.03	32.36	65.98	68.27	4.14	80.76
FOOT	141.38	110.92	67.59	69.50	115.40	98.82	73.04	35.58	99.15	145.15	98.12	125.03
DIGESTIVE GLAND	241.07	154.22	146.29	112.30	162.80	235.62	128.90	105.88	131.80	200.70	165.88	133.80
GONAD	443.43	295.71	155.34	223.57	400.09	207.81	237.25	183.48	402.88	295.87		374.00
ADDUCTOR MUSCLE	141.13	71.73	68.55	63.93	103.39	83.51	43.51	54.51	101.50	76.33	77.37	79.59
GILL	86.33	60.23	41.05	43.41	41.40	31.87	56.42	29.28	23.97	54.76	49.35	36.24
MALE			148.05	94.82	122.30	161.87	154.80	146.35	155.30	194.73		
FEMALE	240.77	235.70	153.91	96.15	125.00	199.06	153.03	154.80	177.05	207.80		124.68
INDIFFERENT	216.55	208.35								183.50	135.44	123.07

TABLE V MONTHLY VARIATION IN MEAN TOTAL LIPID LEVELS IN WHOLE TISSUES AND COMPONENT ORGANS OF S. SCRIPTA (mg g^{-1} DW).

	July	August	September	October	November	December	Jany.84	February	March	April	May	June
MANTLE	97.98	88.48	123.09	48.86	52.66	85.94	62.87	90.42	99.45	77.72	65.65	60.03
FOOT	85.34	85.07	66.66	51.82	66.23	83.57	77.57	78.70	118.04	71.82	51.44	58.64
DIGESTIVE GLAND	165.28	223.36	230.21	224.17	118.15	118.59	109.06	128.08	164.80	241.03	109.55	97.40
GONAD	100.85	110.92	124.51	148.51	66.40	86.32	88.62	48.59	49.67	55.18	44.93	49.25
ADDUCTOR MUSCLE	49.35	72.34	49.73	42.21	52.71	65.37	55.74	61.83	74.32	52.11	35.82	47.19
GILL	115.51	130.16	125.60	132.76	128.47	106.07	105.90	111.11	124.11	112.79	98.03	101.01
MALE			121.84	57.86	74.34	89.55	63.60	83.71	110.15			
FEMALE			118.08	82.59	75.97	86.12	97.12	97.58	122.43			83.71
INDIFFERENT	112.03	118.81							106.36	90.61	69.73	85.04

	July	August	September	October	November	December	Jany.85	February	March	April	May	June
MANTLE	68.58	72.32	61.16	118.01	89.82	60.49	123.01	132.37	108.48	110.71	88.35	109.29
FOOT	71.81	79.09	69.51	83.72	93.65	123.61	103.21	119.83	136.10	105.58	64.19	109.09
DIGESTIVE GLAND	195.20	177.36	92.92	238.54	155.75	179.76	162.95	128.81	153.07	147.56	196.75	205.05
GONAD	46.76	55.67	49.32	172.06	82.34	94.80	157.20	93.70	99.35	107.61	127.78	131.30
ADDUCTOR MUSCLE	35.49	50.88	27.19	74.51	58.15	78.89	85.55	71.02	68.29	71.41	80.93	82.53
GILL	84.71	109.22	66.75	125.03		127.40	150.00	156.30	151.33	82.84	109.13	169.06
MALE			66.52	118.49	94.34	81.84	114.08	120.77	124.64	88.57		
FEMALE	78.17	95.33	74.79	131.57	93.71	102.92	131.07	129.35	139.08	105.24		124.62

TABLE VI MONTHLY VARIATION OF MOISTURE LEVELS IN WHOLE TISSUES AND COMPONENT ORGANS OF S. SCRIPTA (% FW).

	July	August	September	October	November	December	Jan.84	February	March	April	May	June
MANTLE	85.373	80.996	84.500	86.258	82.195	82.396	83.529	81.833	83.574	81.833	80.845	80.759
FOOT	81.901	80.666	74.255	76.306	77.657	74.461	76.919	74.410	74.831	73.316	73.012	77.880
DIGESTIVE GLAND	84.067	75.316	74.755	76.257	69.971	70.071	73.895	66.998	70.426	68.953	66.221	67.013
GONAD	74.399	77.398	80.757	82.072	70.372	70.118	74.611	64.823	70.907	66.590	72.618	75.234
ADDUCTOR MUSCLE	81.239	79.033	78.909	79.689	76.077	76.134	78.006	74.648	77.222	75.004	73.907	73.942
GILL	85.405	84.436	83.938	83.923	82.403	80.563	83.366	81.173	84.275	83.959	82.454	81.275
MALE			82.455	79.387	75.112	77.650	79.967	78.379	79.417			
FEMALE	80.608	81.950	76.932	75.598	80.195	78.129	81.319					76.177
INDIFFERENT	82.525	82.669							80.233	78.655	75.866	77.831

	July	August	September	October	November	December	Jan.85	February	March	April	May	June
MANTLE	84.133	84.088	85.786	84.845	85.576	85.729	87.840	86.163	83.469	83.471	84.791	87.423
FOOT	82.254	78.419	77.214	77.111	75.484	76.369	76.374	80.011	73.758	75.618	76.445	77.588
DIGESTIVE GLAND	77.205	66.032	64.115	73.910	73.855	76.922	76.574	75.011	71.046	70.613	73.589	73.160
GONAD	81.483	79.909	80.237	75.717	78.457	77.424	75.580	71.349	72.625	69.991	71.476	72.294
ADDUCTOR MUSCLE	79.531	79.938	77.713	79.332	76.672	79.907	89.112	78.265	76.021	75.407	77.697	74.158
GILL	84.739	85.528	84.763	83.021	84.484	81.135	87.334	88.220	81.534	82.605	84.138	81.738
MALE			81.790	80.691	80.907	80.374	79.319	80.393	77.566	76.892		
FEMALE	82.239	83.913	83.995	81.920	81.567	80.626	78.334	80.046	77.776	80.374		80.242
INDIFFERENT	82.340	83.786								73.995	80.956	80.847

TABLE VII MONTHLY VARIATIONS IN CALORIFIC LEVEL (KCAL g^{-1}DW) OF S. SCRIPTA.

	MALE	FEMALE	INDIFFERENT
July 83			3.532
Aug			3.565
Sep	3.722	3.914	
Oct	3.881	3.817	
Nov	3.176	3.215	
Dec	3.451	3.782	
Jan 84	3.739	3.809	
Feb	3.692	3.721	
Mar	3.677	3.637	3.702
Apr			3.336
May			3.942
Jun		3.729	3.874
Jul		3.785	3.694
Aug		3.826	3.850
Sep	3.638	3.726	
Oct	3.241	3.439	
Nov	3.505	3.717	
Dec	3.585	3.604	
Jan. 85	3.560	3.579	
Feb	3.697	3.755	
Mar	3.612	3.680	
Apr	3.557	3.611	3.621
May			3.331
Jun		3.423	3.372

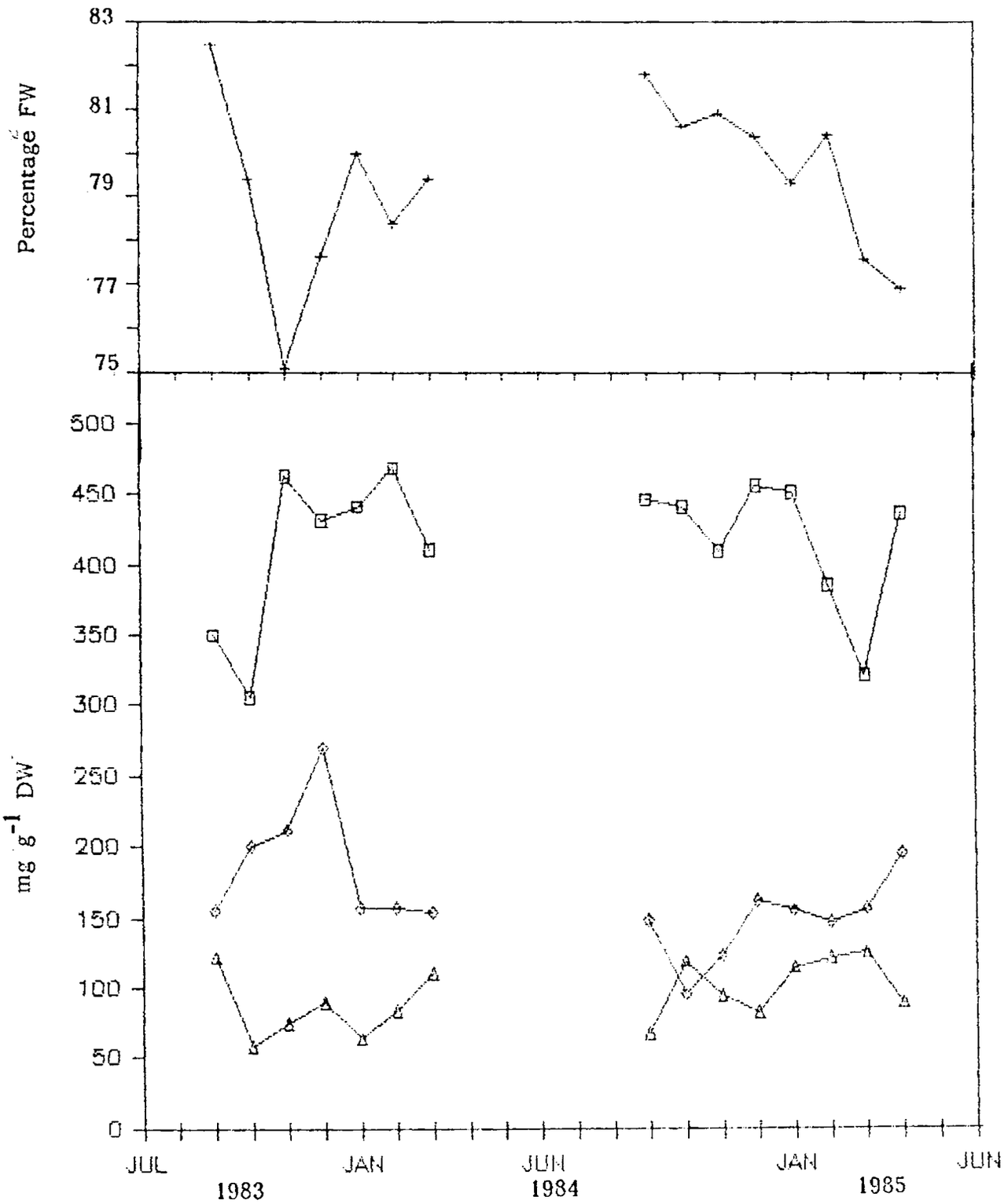

Figure 22. Monthly variations in total protein (□), carbohydrate (◊), lipid (△) and moisture (+) in male S.scripta.

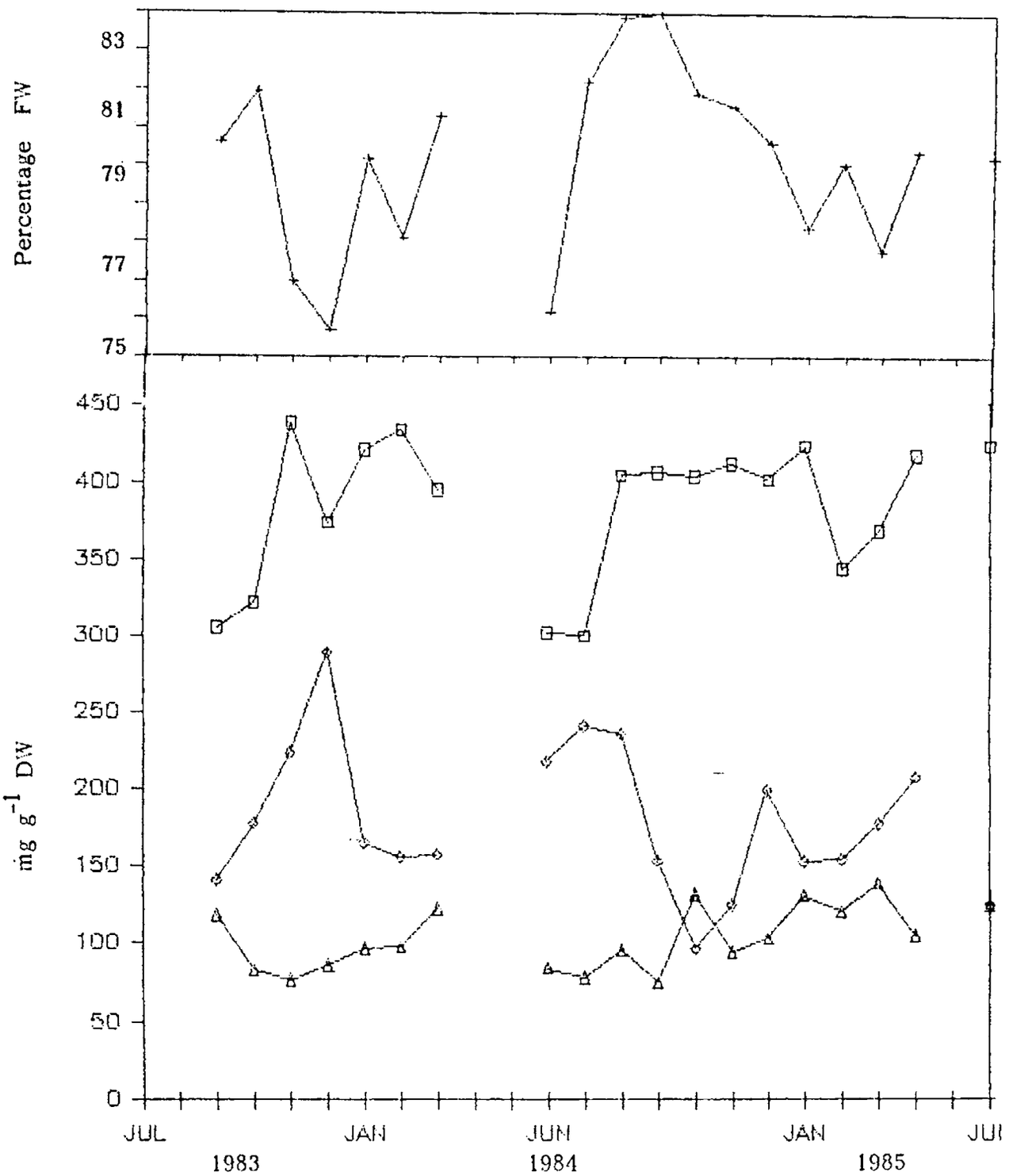

Figure 23. Monthly variations in total protein (□), carbohydrate (◇) lipid (△), and moisture (+) in female S.scripta.

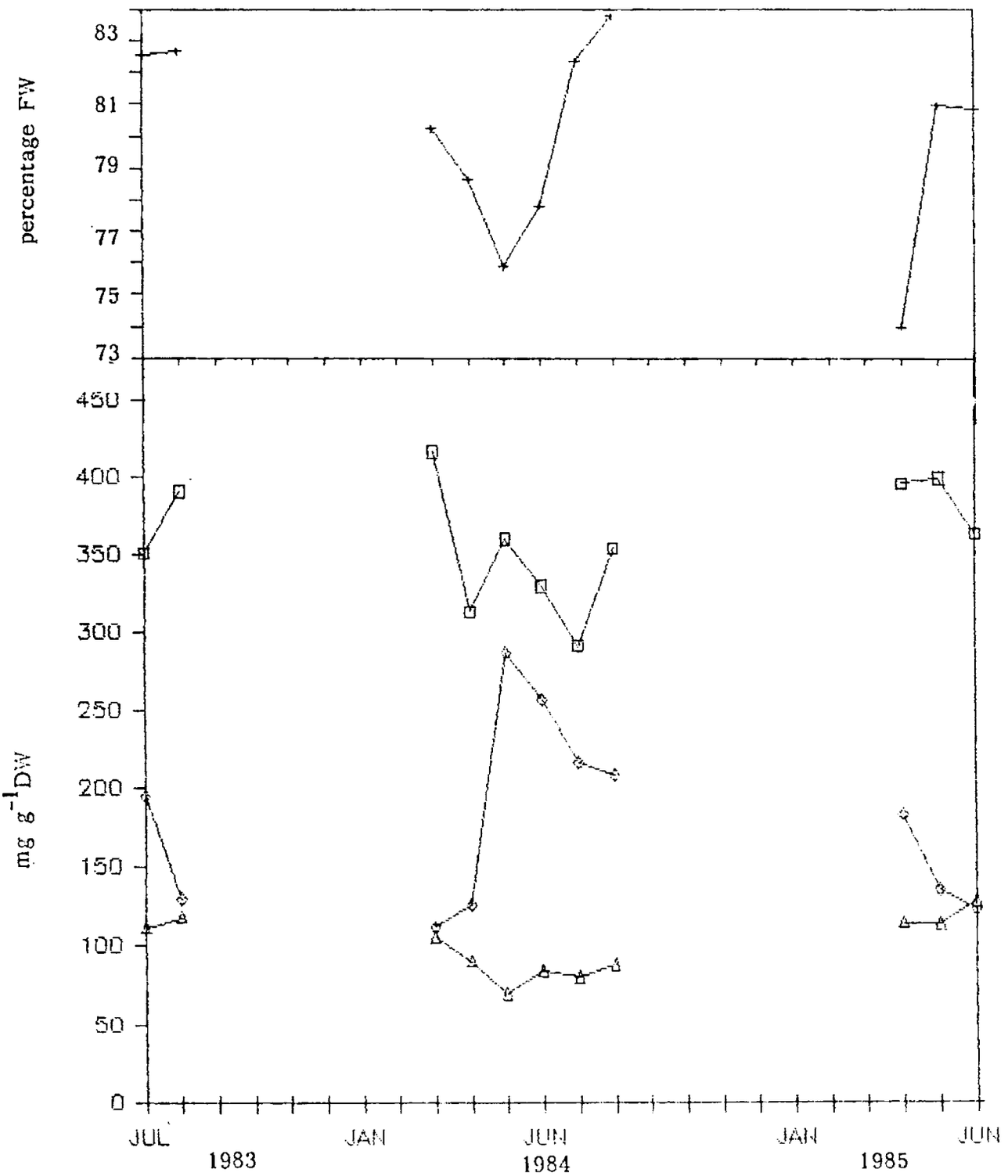

Figure 24. Monthly variations in total protein (□), carbohydrate (◇), lipid (△) and moisture in indifferent S.scripta.

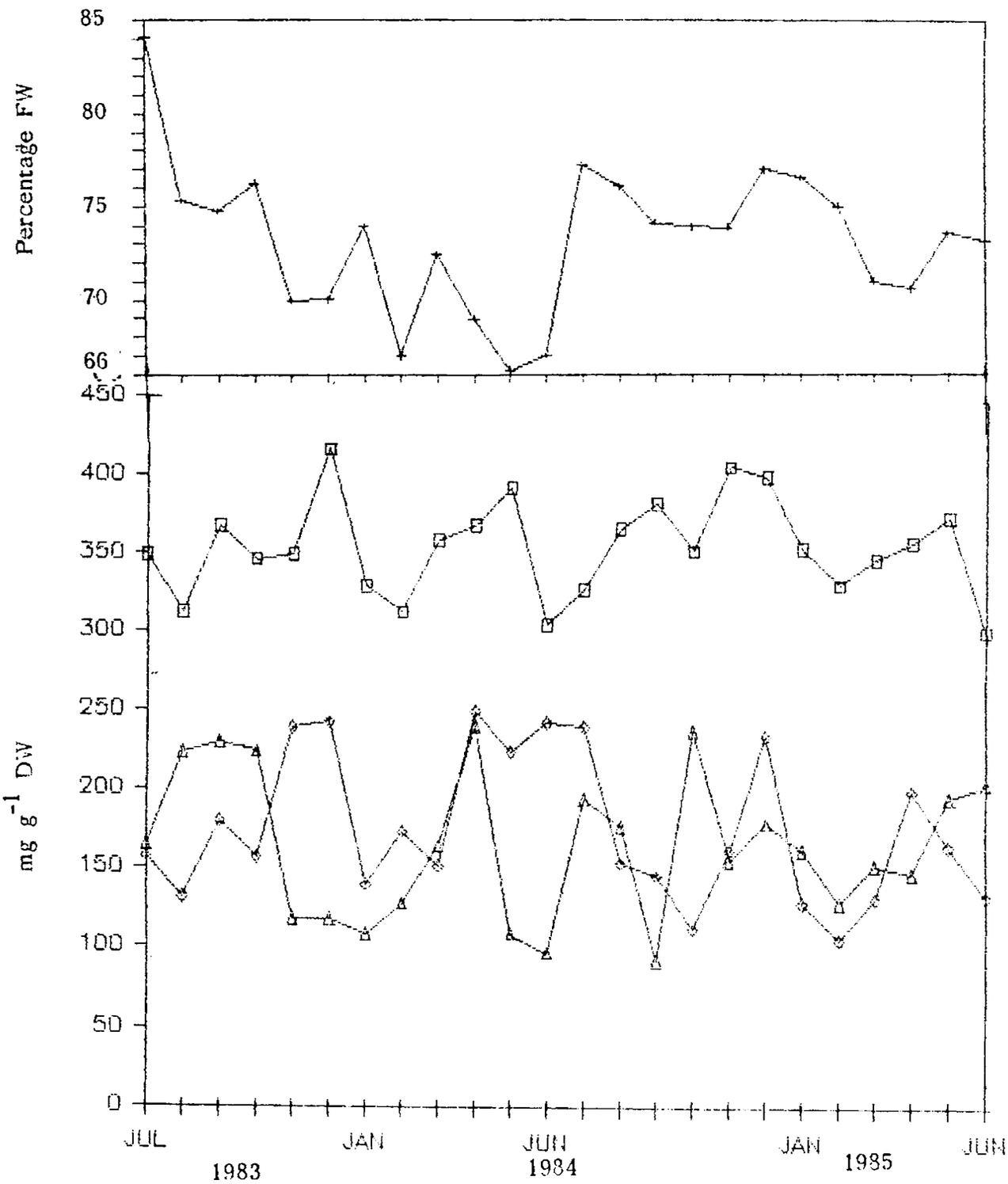

Figure 25. Monthly variations in total protein (□), carbohydrate (◇), lipid (△) and moisute (+) in digestive gland of S.scripta.

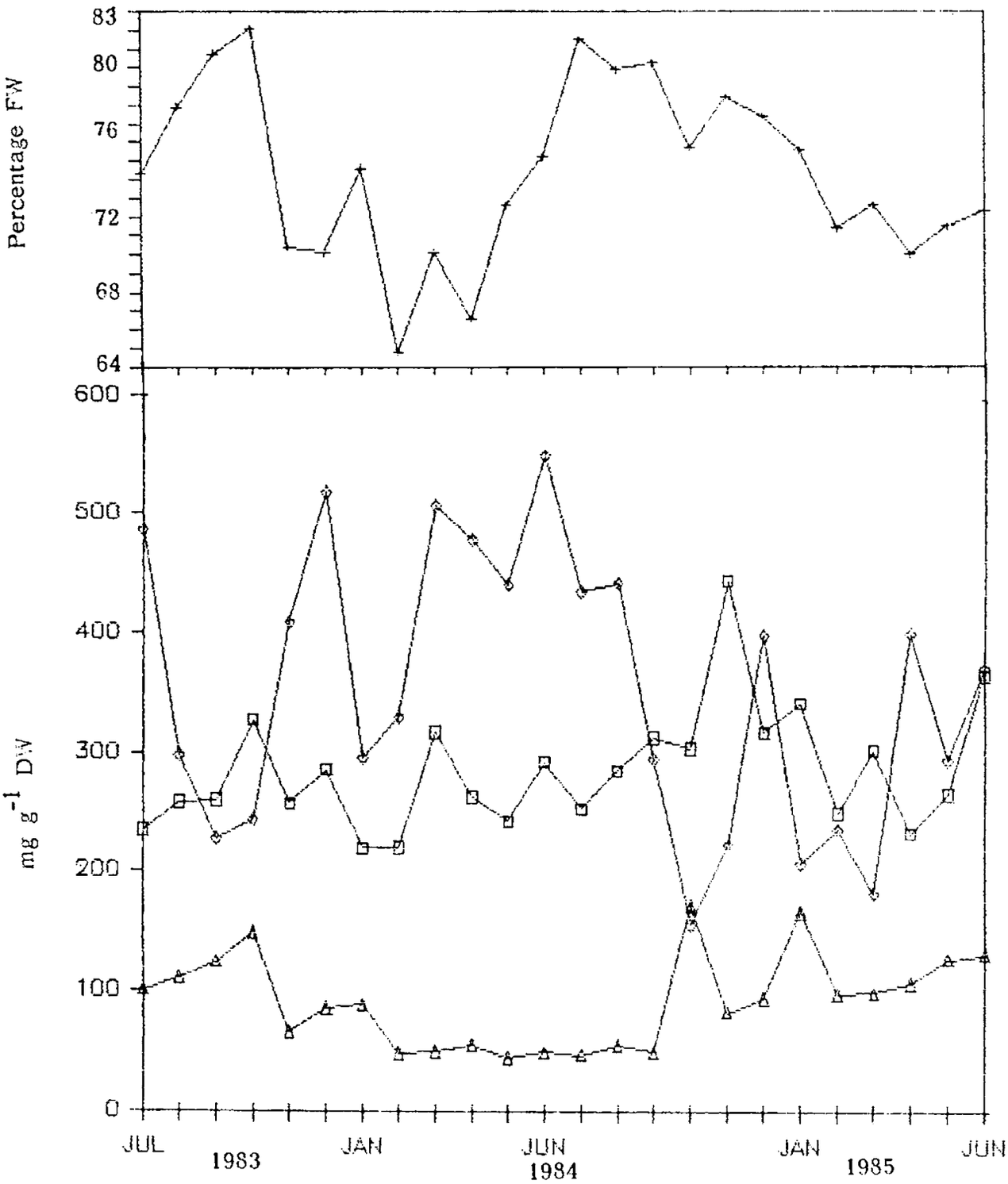

Figure 26. Monthly varations in total protein (□), carbohydrate (◇), lipid (△) and moisture (+) in the gonad of S. scripta.

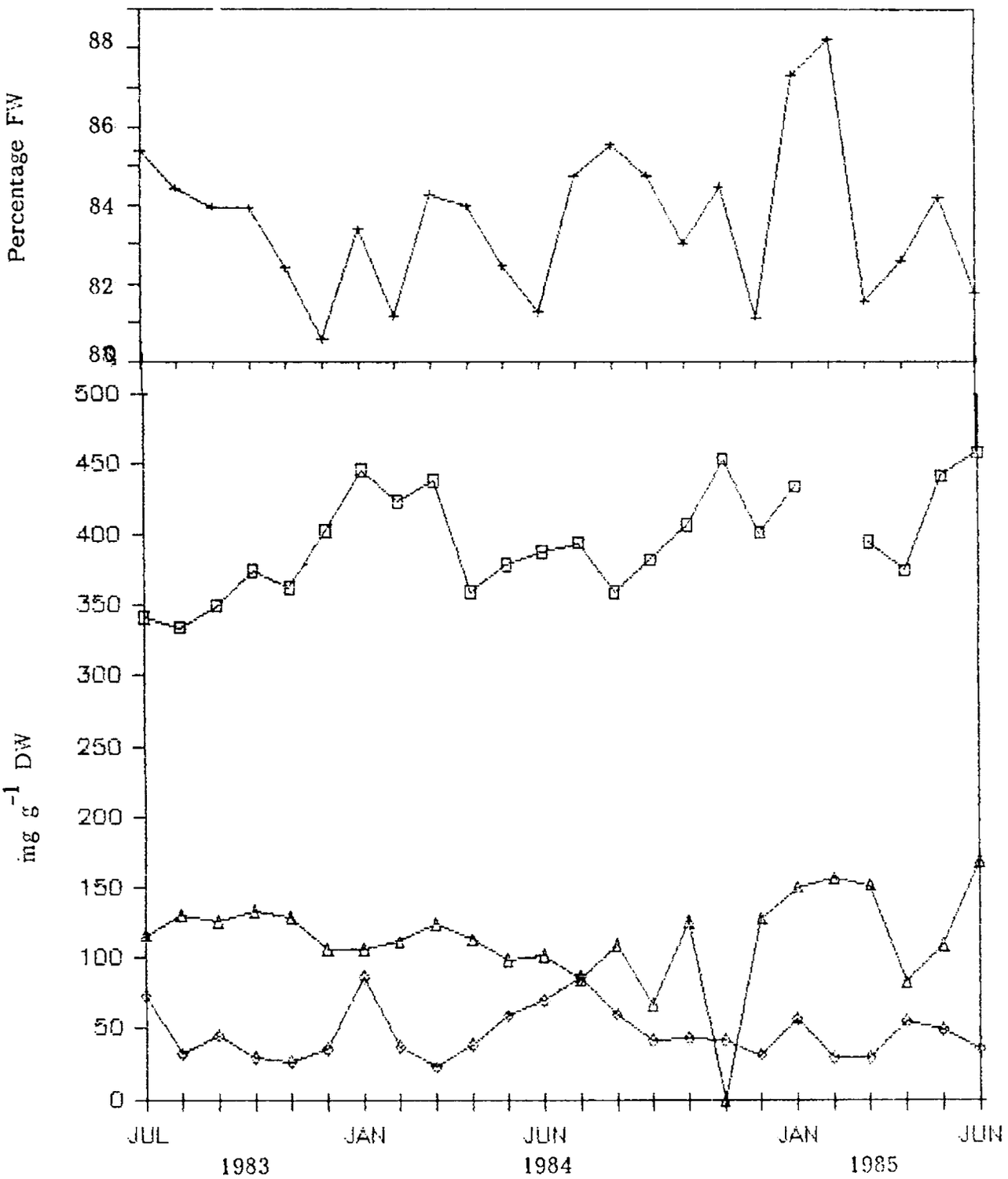

Figure 27. Monthly variations in total protein (□), carbohydrate (◇), lipid (△) and moisture (+) in the gill of S. scripta.

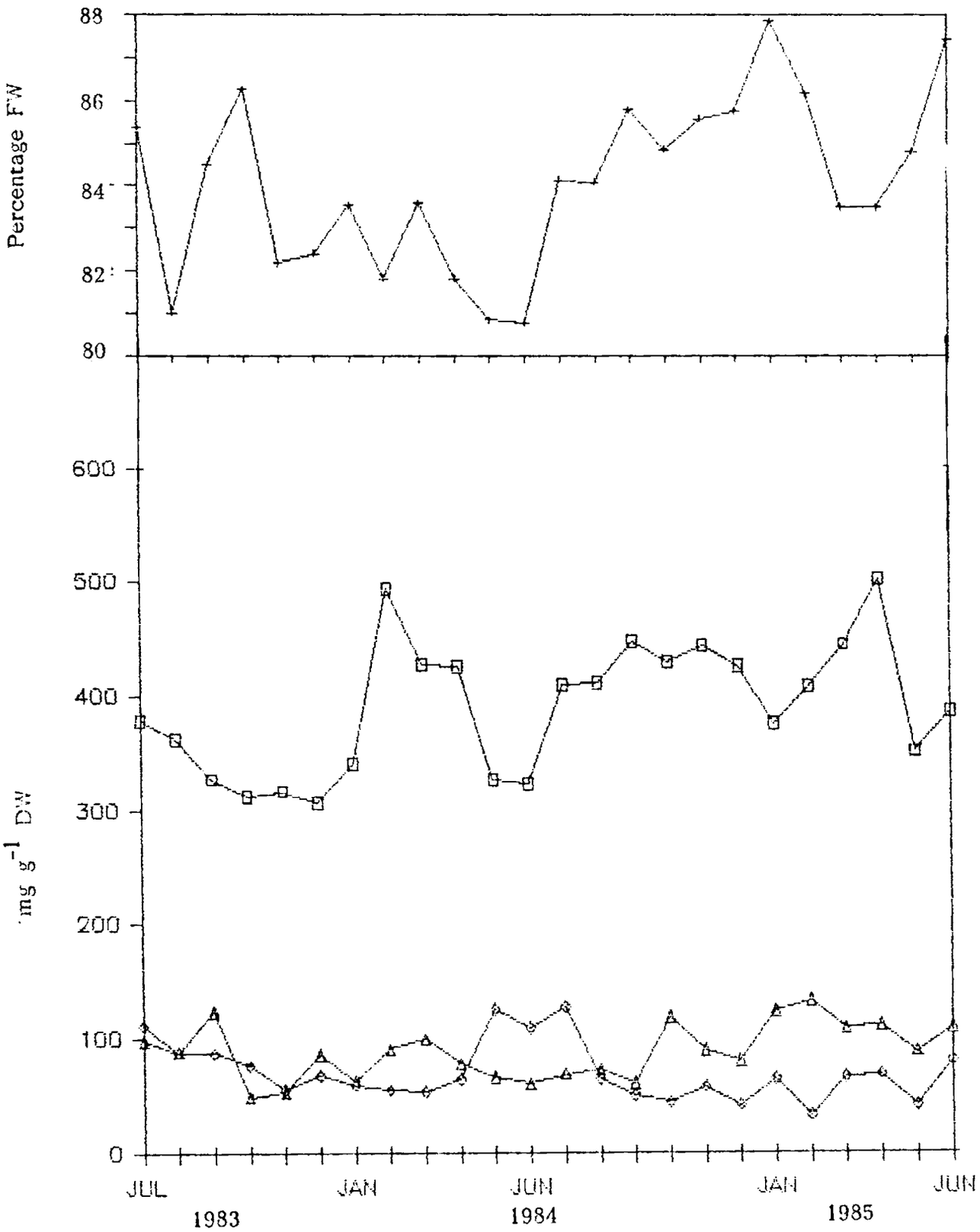

Figure 28. Monthly variations in total protein (□), carbohydrate (◇), lipid (△) and moisture (+) in the mantle of S. scripta.

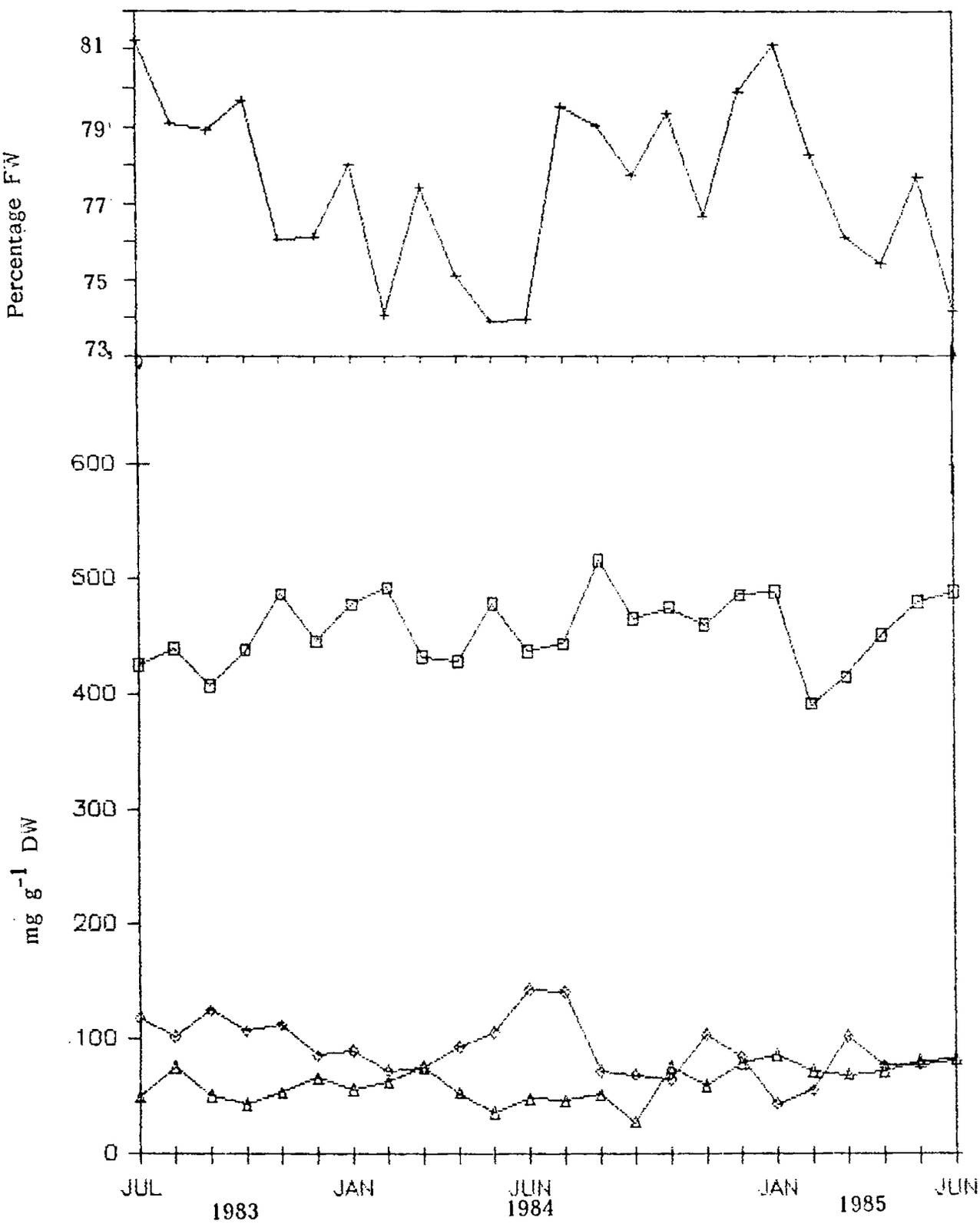

Figure 29. Monthly variations in total protein (□), carbohydrate (◇), lipid (△) and moisture (+) in the adductor muscle of S. scripta.

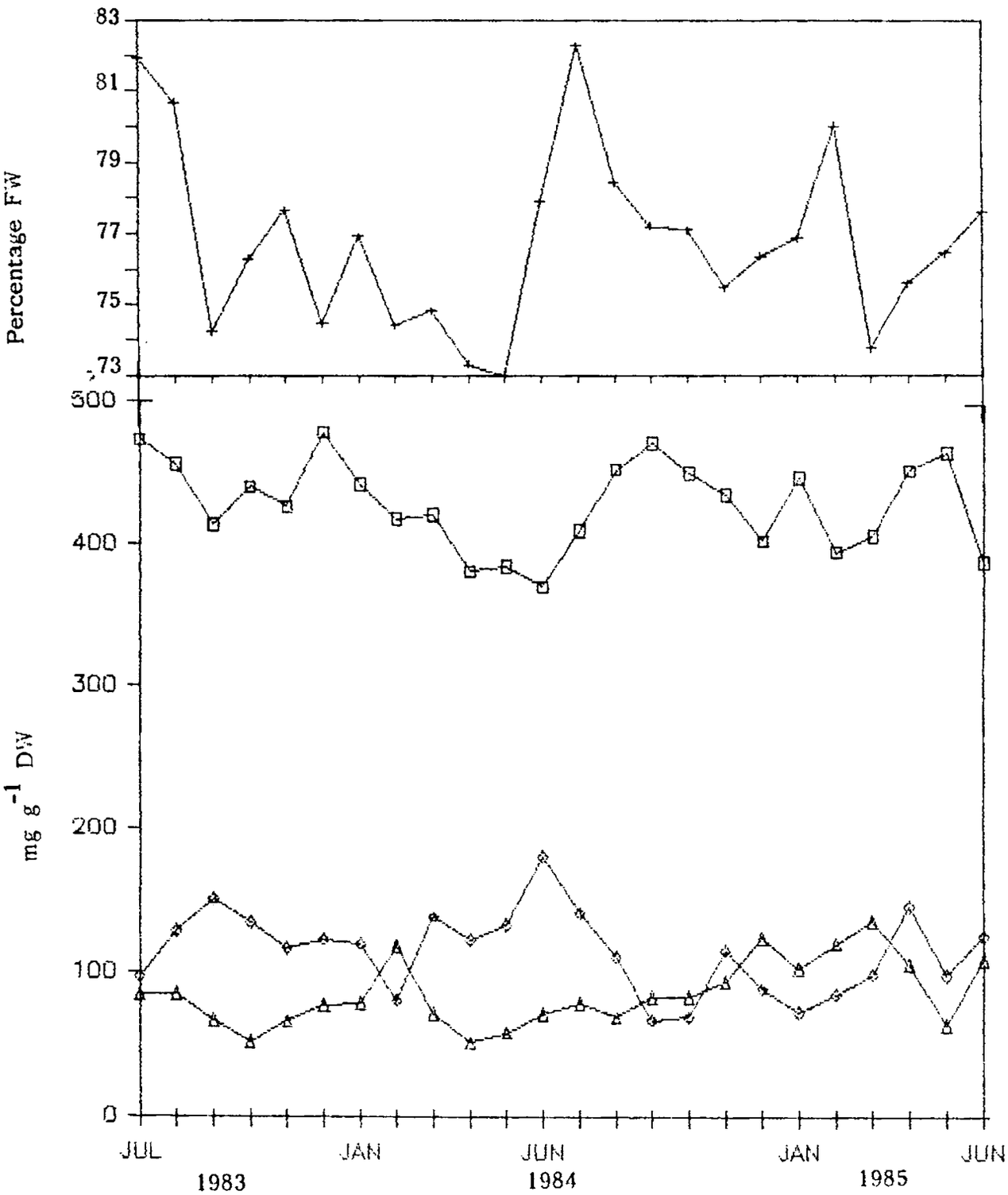

Figure 30. Monthly variations in total protein (□), carbohydrate (◆) lipid (▲) and moisture (+) in the foot of S. scripta.

gametogenic cycle. Moisture levels in whole clams declined from pre-spawning maxima of 80-83% fresh weight to low levels of 75% Fresh weight during the spawning period. The combined calorific value of all the tissues of the whole animal, showed little seasonal fluctuation most of the values falling between 3.1 and 3.9 K cal g^{-1} DW. There were no consistent differences in calorific values between the sexes.

Generally carbohydrate was the dominant organic constituent of the gonad (Table IV; Fig. 26) Peak values were observed in June-July (374-550 mg g^{-1} DW.) and lowest values in September to October/November (155-296 mg g^{-1} DW). Protein and lipid were found in moderate high levels early in the spawning period and showed declining trends with the progression of spawning. The lowest total carbohydrate levels coincided almost exactly with protein and lipid peaks at the commencement of spawning. Moisture levels in the gonad showed pre-spawning maxima (81-82% of Fresh weight) and a reduction in level with advancement of the breeding season.

The digestive gland is the only other somatic component which showed indications of a seasonal cycle (Fig. 25). The digestive gland showed the highest lipid level of any organ in the clam with a maximum value of 241 mg g^{-1} DW. High values were observed in the post-spawning and early gametogenic phases and low levels during spawning. Carbohydrate levels were moderately high and showed enhanced values during low lipid periods and a pronounced peak during the post-spawning early gametogenesis phase from April-July 1983. Moisture levels showed low values during spawning and higher levels during the pre-spawning and post-spawning periods.

Biochemical levels of all the other organs examined showed little correlation with seasons or stage of gametogenic development (Figs. 27-30). Protein was the predominant organic constituent of all the organs. The mantle showed large random fluctuations between maxima and minima but the pattern of variation was not consistent during the second year of study. Lipid and carbohydrate values were low but the gill showed surprisingly high lipid levels with values remaining above 100 mg g^{-1} DW during most of the study period. Moisture levels showed slight fluctuations with highest levels in the gill and mantle. There appeared to be slight enhancement of moisture levels in the foot, adductor muscle and mantle in the post-spawning period.

DISCUSSION

Seasonal metabolic changes in S. scripta are influenced by hydrographic factors, nutrient levels and gametogenic development. High nutrient levels in shallow tropical waters more than amply meet the metabolic requirements of the clam and thus precludes the necessity of large reserve stores to support gametogenesis. Thus of all the component organs analysed, only the gonad and the digestive gland (Tables III-VI, Figs. 25,26) with high nutrient turnover rates qualify as storage tissue. Glycogen in the gonad, and lipid and glycogen in the digestive gland are maintained at high levels, and in periods of utilisation, are renewed quickly. Biochemical profiles of the other components generally show little fluctuation. The gonad and digestive gland constitute the major fraction of the visceral mass of the clam and consequently levels in whole tissue roughly follow trends in these organs.

S. scripta is a shallow burrowing Venerid, which can probably feed either as a suspension feeder or as a detritus feeder. A preliminary examination of the gut material over a few months of the study period showed detritus, diatoms, dinoflagellates and fine sand. It is thus rather surprising that such a high lipid diet should result in carbohydrate reserves. Moss and Lawrence (1972) found major reserves of carbohydrate in the filter-feeding echinoid Mellita quinquiesperforata whereas echinoids surviving on macrophytes high in carbohydrate, stored mainly lipids. Metabolism in the lamellibranchs is hinged on a glycogen economy (Giese, 1966), carbohydrate being the most metabolically active fraction in most species. The most prominent carbohydrate stored in bivalves is glycogen with large amounts being stored in the mantle (Walne, 1970; de Zwaan and Zandee, 1972), adductor muscle tissue (Taylor and Venn, 1979) gonad (Sastry, 1979; Gabbot, 1983) and digestive gland (Sastry and Blake, 1971; Thompson, 1977; Vasallo, 1973; Barber and Blake, 1981). This generalisation is especially true of northern species which show changes in body weight mainly due to carbohydrate and glycogen content (Gabbot, 1976). According to Shul'man (1974) glycogen and protein are main respiratory substrates in animals with low levels of energy metabolism often also with a poor oxygen supply or anaerobiosis. The advantages of glycogen as a source of energy are its ready mobilisation and its ability to yield energy under anaerobic conditions.

In the absence of localised nutrient storage depots, bivalves store energy rather generally in various body components. The gonad

in S. scripta serves as the primary reserve for gametogenesis. The high turnover rates of total carbohydrate in the gonad indicate that it is the locus of intense biochemical synthesis. In bivalves (Eble, 1969) glycogen is largely stored in vesicular (Leydig) cells which are distributed close to active metabolic tissues such as digestive gland and the developing gonad. Because of its high carbohydrate level the indifferent gonad condition, composed mainly of connective tissue, is recognised as a stage for the accumulation of nutrient reserves. Bargeton-Conteaux (1942) suggested that glycogen and lipids accumulated in the storage cells during the recovery period were used as nutrients for gamete formation. Lubet et al., (1976) found the connective tissue matrix of Mytilus edulis to consist of vesicular cells storing large amounts of glycogen and adipogranular cells containing lipid droplets and protein granules. In S. scripta energy storage is represented by the proliferation of vesicular connective tissue cells during the post-spawning and early gametogenic phases coinciding with large increases in total carbohydrate in the gonad, digestive gland and whole clam tissue. In the post-spawning stage the metabolic energy demand is low. This situation together with increased feeding to utilise the phytoplankton production peak allows the accumulation of carbohydrate reserves in the gonad and carbohydrate and lipid in the digestive gland. In contrast to the adult, lipid and protein form the main energy reserves of marine bivalve larvae (Holland, 1978) and the role of carbohydrate is negligible, Gabbot (1976) considers the conversion of prestored glycogen into lipid reserves in the developing eggs as a storage cycle analogous to the glucose-fatty acid cycle in vertebrates. Krebs (1972) found the metabolic cost

of such a conversion to be quite small. For each 2 carbon fragment of glucose which is converted to a 2-carbon fragment of fatty acid, 2 moles of ATP are consumed. There are clear advantages in storing fat in the eggs and larvae (Gabbot, 1976). Fat is a more concentrated energy form and it confers buoyancy to the eggs and larvae because of its lower density. Gametogenesis proceeds slowly till September and there is not a 1:1 correlation between nutrient losses in the clam and protein and lipid gains in the gonad, so it is presumed that the stored reserves are mainly used for somatic growth during this period. Active feeding in the post-monsoon period restores food reserves and ingested food may also be directly incorporated into developing gametes. The period of active somatic growth followed by active gametogenesis indicates that the metabolic energy demand of the clam always remains high and is more than compensated by the high eutrophication of the clam beds.; Carbohydrate levels in the maturing gonad showed a declining trend with advancement of gametogenesis with lowest levels at early spawning. It is probable that carbohydrate may have been converted to lipid and protein reserves of the gametes. Spawning in S. scripta is spread over a period of 5 months and there is a gradual depletion of protein and lipid in the gonad due to release of gametes. In the case of component organs it is not known if variations in levels of organic constituents are sex specific as the investigations reported here were carried out without discriminating sex of the clam. But lipid and carbohydrate levels were generally higher in females, which may be attributed to a higher biochemical budget required for egg production.

In the digestive gland of S. scripta, lipid is the most important fraction in terms of metabolic participation, since it is the constituent most rapidly turned over in this study. Great differences are known in the lipid content of bivalves. In Donax incarnatus lipid contents are about 4.5% of the dry weight (Balasubramanian et al., 1979) whereas in Siliqua patula lipids make up 42% of the dry weight (Lewin et al., 1979). Owen (1966) and Weel (1974) have stressed the importance of the digestive gland as the site of carbon assimilation, storage and transfer in bivalves. Lipid in the digestive gland (Fig. 25) showed an extended peak during the post spawning period. High values for lipid in the mussels of indifferent sex have been reported from M. viridis (Wafar et al., 1976) at Goa. The depletion of stored lipid reserves in the digestive gland of S. scripta can be explained as a consequence of energy demands of growth and reproduction. Sastry (1968) and Gabbot and Bayne (1973) have suggested that initiation of the oocyte growth phase is dependant upon the accumulation and transfer of nutrient reserves from the digestive gland to the gonad. From the data on M. viridis (Mane and Nagabhushanam, 1977) an inverse relationship can be deduced between lipid content of the digestive gland and gonad. Lipid is accumulated in the digestive gland of Argopecten (Barber and Blake, 1981) prior to gametogenesis and its utilisation is associated with the initiation of oogenic activity. Since lipid storage occurs at periods of phytoplankton augmentation it is possible that lipid accumulation is the direct result of ingestion of lipid-rich phytoplankton. Seasonal fatty acid changes in soft tissues and gonads of Chlamys techuelcha have been found to be related to phytoplankton fatty acid composition (Pollero et al., 1979).

The gill is the only other component which showed high levels of lipid i.e. ≥ 100 mg g^{-1} (Table VI ; Fig. 27) during most of this study. Giese ——→ (1969) has also reported such unexpected levels in Tivela stultorum. This situation can be explained as an adaptation to facilitate the higher metabolic levels in the gill. The gill also lacks storage tissue and hence it would be advantageous to store fat which is a concentrated energy form and more fully utilised. According to Shul'man (1974) a prerequisite for the utilisation of fat in energy metabolism is adequate oxygenation. Gills in Bivalvia have greater access to oxygen than other tissues.

Protein was the major fraction of all the somatic tissues of the clam (Table III) and like the other organic fractions, showed little seasonal variation. In the gonad carbohydrate levels were often higher than protein levels (Table IV ; Fig. 26). According to various authors, protein would show a decrease in relation to any spawning and would follow a reverse pattern with respect to carbohydrate (Lubet, 1959; Gabbot, 1975; Pieters et al.,1979).This relationship is evident in S. scripta where lowest total carbohydrate levels coincided almost exactly with protein nd lipid peaks at the commencement of spawning.

Mobilisation of protein reserves for utilisation during gametogenesis has been reported in the clam Tapes philippinarum (Adachi, 1979) and M. edulis (Gabbot and Bayne, 1973). In S. scripta, protein does not serve as an energy source, but is the major structural material of the cell. Fluctuations of protein in the mantle (Fig. 28) may be due to variable feeding activities.

The figures for total protein, lipid, carbohydrate and ash (ref. next chapter) when summed up always give a total of less than 100%. Ansell (1974a) has suggested that the reasons for this lack of summation are probably complex. In this study the reason for this discrepancy may be due to the low values obtained for protein. The Lowry method used in this analysis has been observed to produce lower results than the classical biuret analysis when using Bovine Serum Albumin as a test standard. (Bio-Rad Laboratory, Technical Bulletin 1015, 1977). Belisle and Stickle (1978) have also reported this anomaly from studies on Thais haemostoma.

Various authors have reported low moisture levels at periods of increased glycogen - in Crassostrea gryphoides (Durve and Bal, 1961), Nausitora hedleyi (Saraswathy and Nair, 1969) and in C. madrasensis (Stephen, 1980b). An inverse relationship between organic constituents and moisture is observed in S. scripta during the spawning period, but the moisture levels show a parallel increase with carbohydrate reserves in the post-spawning phase. Galstoff (1964) reported an increase of water content upto 92% in O. edulis in low salinity and the increase was ascribed to loss of salts and gain of water by the oysters. A rise in moisture level with a decrease in ambient salinity was noticed in Meretrix meretrix (Deshmukh, 1972) and Katelysia opima (Nagabhushanam and Mane, 1973).

Energy levels in S. scripta remain constant throughout the study period and depletions due to carbohydrate utilisation are made up by protein and lipid increases. This concurs with the observation that energy levels in organisms follow the pattern of variation of ambient nutrient levels which in this analysis is always high. In bivalves, reserve

storage is accompanied by a proliferation of tissue rather than an increase in concentration of cellular constituents. Thus analysis of energy content of a standard animal mayhave revealed a seasonal cycle of variation.

The manner in which nutrient reserves are utilised for gametogenic development in marine bivalves is not completely defined. What is common to seasonal cycles of northern species, regardless of the actual season of spawning is the inverse relationship between previously stored lipid, glycogen and protein reserves and gametogenesis. The mechanism for nutrient storage insulates the gametogenic process from an uncertain food supply and provide some control over the variability of oocyte growth (Bayne, 1976). The importance of different body components as energy storage sites relative to the storage cycle is highly adaptive and is the result of genetic divergence or non-genetic adaptation to environmental variation. Patterns of nutrient storage and utilisation may also show much variation between species as well as between population of the same species. Thus in Chlamys septemradiata (Ansell, 1974b) growth of the gonad in spring is directly supported by feeding activities of bivalves at that time, while in contrast, in Pecten maximums (Comely, 1974) from the same family and same area, growth of the gonad which develops in winter is supported by reserves built up during the previous spring and summer. High nutrient levels in shallow tropical marine environments decrease the necessity of the more elaborate storage cycles observed in boreal species. Phytoplankton production probably lies constantly above the metabolic demand of the organism so that somatic growth and gametogenesis can proceed together. Thus organic fractions of S. scripta

show only slight variations, about two to three fold in gonad compared to the five-fold variation in lipid content of Donax vittatus reported by Ansell (1972) from Scotland. The levels of organic constituents are low in the post spawning phase, possibly contributing to the fact that most clams, unable to meet further maintenance requirements during the low salinity stress period, show large scale mortality. Calow (1979) defined the physiological cost of reproduction as "the extent to which nutrients are used to support reproduction when they are also required to support other aspects of metabolism". He concluded that there is a negative correlation between the energy invested in reproduction and the subsequent survival and reproductive capacity of the parents. In S. scripta it can be safely assumed that it is not so much nutrient depletion as the inability to withstand hyposaline conditions caused by the onset of the monsoon that effect the survival of the adult clams to a second gametogenic cycle. Peak phytoplankton production in June-July reported by Gopinathan et al., (1975) eliminates the possibility ofnutrient stress

Assessment of meat quality of ,the bivalves is dependent on ash and water content and the level of storage products. Low quality meat has high ash and water content (Haven, 1962; Shaw et al., 1967) while high quality is associated with relatively low ash and water contents, but high levels of glycogen or gametes (Quayle, 1969 and Walne, 1970). In the study area, clams were harvested for consumption mainly during the monsoon months when sea fish are not available because of rough sailing conditions. Clams during this period attain the indifferent gonad

condition with extensive proliferation of glycogen rich connective tissue. The late gametogenic and early spawning period are characterised by high organic levels and low moisture and thus of high meat quality. Fishing during this period would reduce the brood stock but since the clam beds are regularly exploited to supply lime industries, marketing of the otherwise discarded clam meat is also recommended.

CHAPTER IV

TRACE METAL SEASONALITY IN SUNETTA SCRIPTA

Simkiss et al. (1982) list three reasons to recommend biological monitoring. Firstly, metals are concentrated in organisms to easily detectable levels; secondly, the accumulated doses indicate biologically available forms (rather than pollutant abundance per se) and finally, because levels in organisms represent a "time integrated" picture of the sum of environmental variations in the recent history of a particular ecosystem. In most bivalves studied, experimental tissue concentrations of trace metal levels tend to reflect those in the water and sediment although direct proportionality is not necessarily found (Shuster and Pringle, 1969; Ayling, 1974; Luoma and Bryan, 1978). Factors which affect metal concentration in molluscs are size, sex, reproductive condition, seasonal variation and available chemical form in sea water (Watling and Watling, 1976; Boyden, 1977; Coombs, 1977; Majori et al., 1978). In an attempt to explain the bioaccumulation kinetics of trace metals by marine organisms various mechanisms have been postulated or suggested in the literature (Romeril, 1971; Fowler and Benayoun, 1974; Coombs, 1977; George, 1980). Absorptive processes include absorption from solution, suspended particles, sediments and from food (Bryan, 1979). Metabolism of toxicants involves basically two processes - detoxication and storage, and detoxification and excretion (Bryan, 1979).

It was the purpose of this study to analyse the accumulation of copper, chromium, manganese, nickel, cobalt and arsenic in S. scripta, to determine accumulation ratios if any and to explain seasonal variations as influenced by environmental parameters and physiological condition.

Cu, Cr, Mn, Ni, Co and As are all biologically essential elements (Da Silva, 1978) but are toxic in excess and can be termed pollutants S. scripta is considered for nomination as a sentinel organism because it satisfies such essential criteria as (1) it is a representative population of the estuarine ecosystem, (2) it is one of the main contributors to the biomass of the ecosystem it represents, and (3) it forms a part of the ecological suite of species representing the trophic level of primary herbivores.

MATERIALS AND METHODS

The method of sample collection and culture were as described in Chapter III. Clams were segregated on basis of sex as male, female, and indifferent. Analysis was carried out on a composite of ten individual clams. Pooling of individuals permits processing of large numbers of clams in a given period of time, resulting in more reliable means and hence more accurate results. Secondly it increases the amount of tissue available for analysis. This practice has the disadvantage that differences between individual clams are often masked. As a result the general picture of accumulation is that of the average.

The clams were opened with stainless steel scalpels, the tissues were washed with a minimum quantity of glass distilled water, dried at 80^{o}C for 24 h and crushed into a fine powder. Dried tissue samples (200-300 mg) were incinerated at 550^{o}C in a muffle furnace for 24 h in silica crucibles to constant weight. The residue in the crucible represented the ash content in the sample.

The pale greenish ash was treated with concentrated HCℓ, evaporated nearly to dryness, dissolved in glass distilled water and made upto 25 ml. All equipments used for analytical work was washed in 10% nitric acid and rinsed in glass-distilled water before use. Determination of metal concentrations in the clam tissues was by atomic absorption spectrophotometry (AAS). Appropriate wave lengths for copper (324.7 nm), manganese (279.5 nm), chromium (357.9 nm), arsenic (193.7 nm), cobalt (240.7 nm), nickel (232.00 nm) and an air-acetylene flame were used.

Two homogenates were prepared from each sample and two digests from each homogenate. Difficulties arise in making comparisons because metal levels are often reported in $\mu g\ g^{-1}$ fresh weight. Therefore data reported in this paper and used for comparison are of metal level relative to dry weight ie. $mg\ g^{-1}$ or $\mu g\ g^{-1}$ dry weight.

Values for metals were compared statistically to estimate correlations between metals and betwen ash and metals (Zar, 1974).

RESULTS

The flux patterns of ash and trace metal levels are depicted in Fig. 31 to 35 and Tables VIII to XIII.

Ash:- The patterns of variation of ash levels, as also levels of trace metals, during the 2 years of study were not consistent. Low levels were found from November to January of the first year coinciding with gametogenic activity and spawning. In the second year the depression in ash levels was of short duration i.e. in December and the lowest values were higher than the values of the previous year. During the first year,

TABLE VIII MONTHLY VARIATION IN MEAN TISSUE CONCENTRATION OF ASH (mg g^{-1} DW) OF S. SCRIPTA.

	MALE	FEMALE	INDIFFERENT
Jul 83			66.172
Aug			63.936
Sep	68.010	78.974	
Oct	66.733	67.586	
Nov	58.963	62.393	
Dec	51.168	46.335	
Jan 84	70.247	64.932	
Feb	77.285	66.438	
Mar	105.119	97.447	88.806
Apr			84.237
May			43.221
Jun		69.566	72.271
Jul		52.033	62.840
Aug		64.492	70.273
Sep	87.796	80.404	
Oct	79.312	82.359	
Nov	90.835	116.204	
Dec	75.323	86.844	
Jan 85	78.614	107.861	
Feb	82.458	88.632	
Mar	74.132	92.774	
Apr	68.368	57.991	64.224
May			75.781
Jun		65.570	65.664

TABLE IX MONTHLY VARIATION IN MEAN TISSUE CONCENTRATION OF MANGANESE ($\mu g\ g^{-1}$ DW) OF S. SCRIPTA

	MALE	FEMALE	INDIFFERENT
Jul 83			222.9
Aug			215.4
Sep	91.0	113.9	
Oct	116.5	116.9	
Nov	40.6	42.1	
Dec	28.7	26.6	
Jan 84	52.3	40.8	
Feb	37.8	14.9	
Mar	112.2	91.2	84.8
Apr			43.4
May			31.0
Jun		52.3	63.0
Jul		125.3	133.6
Aug		147.9	110.3
Sep	450.0	325.0	
Oct	43.9	90.3	
Nov	232.0	332.0	
Dec	28.0	76.9	
Jan 85	162.0	186.7	
Feb	196.0	111.6	
Mar	134.0	217.4	
Apr	173.7	193.2	171.7
May			142.6
Jun		134.7	87.4

TABLE X MONTHLY VARIATION IN TISSUE CONCENTRATION OF COPPER ($\mu g\ g^{-1}$ DW) OF S. SCRIPTA

	MALE	FEMALE	INDIFFERENT
Jul 83			52.1
Aug			67.3
Sep	69.5	60.6	
Oct	63.2	9.5	
Nov	14.1	5.9	
Dec	6.2	3.9	
Jan 84	17.4	22.8	
Feb	8.0	41.4	
Mar	11.1	48.6	36.3
Apr			36.3
May			15.2
June		25.9	39.6
July		6.4	44.5
Aug		3.7	17.7
Sep	30.4	22.5	
Oct	7.3	2.8	
Nov	71.9	62.4	
Dec	2.6	12.1	
Jan 85	45.4	58.1	
Feb	70.3	56.8	
Mar	25.6	36.8	
Apr	48.1	53.8	52.2
May			38.0
Jun		56.2	30.0

TABLE XI MONTHLY VARIATION IN MEAN TISSUE CONCENTRATION OF CHROMIUM ($\mu g\ g^{-1}$ DW) OF S. SCRIPTA.

	MALE	FEMALE	INDIFFERENT
Jul 83			4.6
Aug			5.2
Sep	6.8	2.1	
Oct	8.1	5.7	
Nov	12.1	7.0	
Dec	2.8	4.1	
Jan 84	6.1	4.7	
Feb	4.2	8.3	
Mar	15.5	7.1	6.7
Apr			10.0
May			5.7
Jun		8.2	6.1
Jul		7.5	8.5
Aug		6.8	6.3
Sep	5.1	4.0	
Oct	2.8	4.6	
Nov	3.7	4.1	
Dec	4.9	7.9	
Jan 85	6.2	9.7	
Feb	8.9	7.8	
Mar	5.4	5.3	
Apr	9.2	8.3	8.5
May			4.6
Jun		7.3	6.8

TABLE XII MONTHLY VARIATION IN TISSUE CONCENTRATION OF ARSENIC $\mu g\ g^{-1}$ DW.

	MALE	FEMALE	INDIFFERENT
Jul 83			6.233×10^{-2}
Aug			5.501×10^{-2}
Sep	6.022×10^{-2}	8.666×10^{-2}	
Oct	6.958×10^{-2}	1.139×10^{-2}	
Nov	4.173×10^{-2}	1.002×10^{-2}	
Dec	6.557×10^{-2}	5.946×10^{-2}	
Jan 84	2.496×10^{-2}	1.024×10^{-2}	
Feb	N.D*	2.461×10^{-2}	
Mar	0.1998	0.1015	2.607×10^{-2}
Apr			1.838×10^{-2}
May			7.638×10^{-2}
Jun		2.916×10^{-2}	2.407×10^{-2}
Jul		1.575×10^{-2}	3.076×10^{-2}
Aug		1.463×10^{-2}	3.503×10^{-2}
Sep	N.D.	1.921×10^{-2}	
Oct	2.32×10^{-3}	8.330×10^{-3}	
Nov	1.117×10^{-2}	4.55×10^{-2}	
Dec	N.D.	4.639×10^{-2}	
Jan 85	$1.67\ 1 \times 10^{-2}$	N.D.	
Feb	4.108×10^{-2}	N.D.	
Mar	N.D.	3.496×10^{-2}	
Apr	N.D.	N.D.	N.D.
May			N.D.
Jun		3.538×10^{-2}	N.D.

*N.D = Not detected.

TABLE XIII METAL-ASH AND METAL-METAL CORRELATIONS IN TISSUES OF S. SCRIPTA.

	Copper	Chromium	Manganese	Arsenic	Ash
Copper	+1	+0.0269	+0.6342***	+0.1411	+0.2759
Chromium		+1	-0.0380	+0.4157*	+0.2263
Manganese			+1	+0.0374	+0.4367**
Arsenic				+1	+0.2212
Ash					+1

*Significant $P < 0.05$

**Highly significant $P < 0.005$

***Very highly significant $P < 0.001$

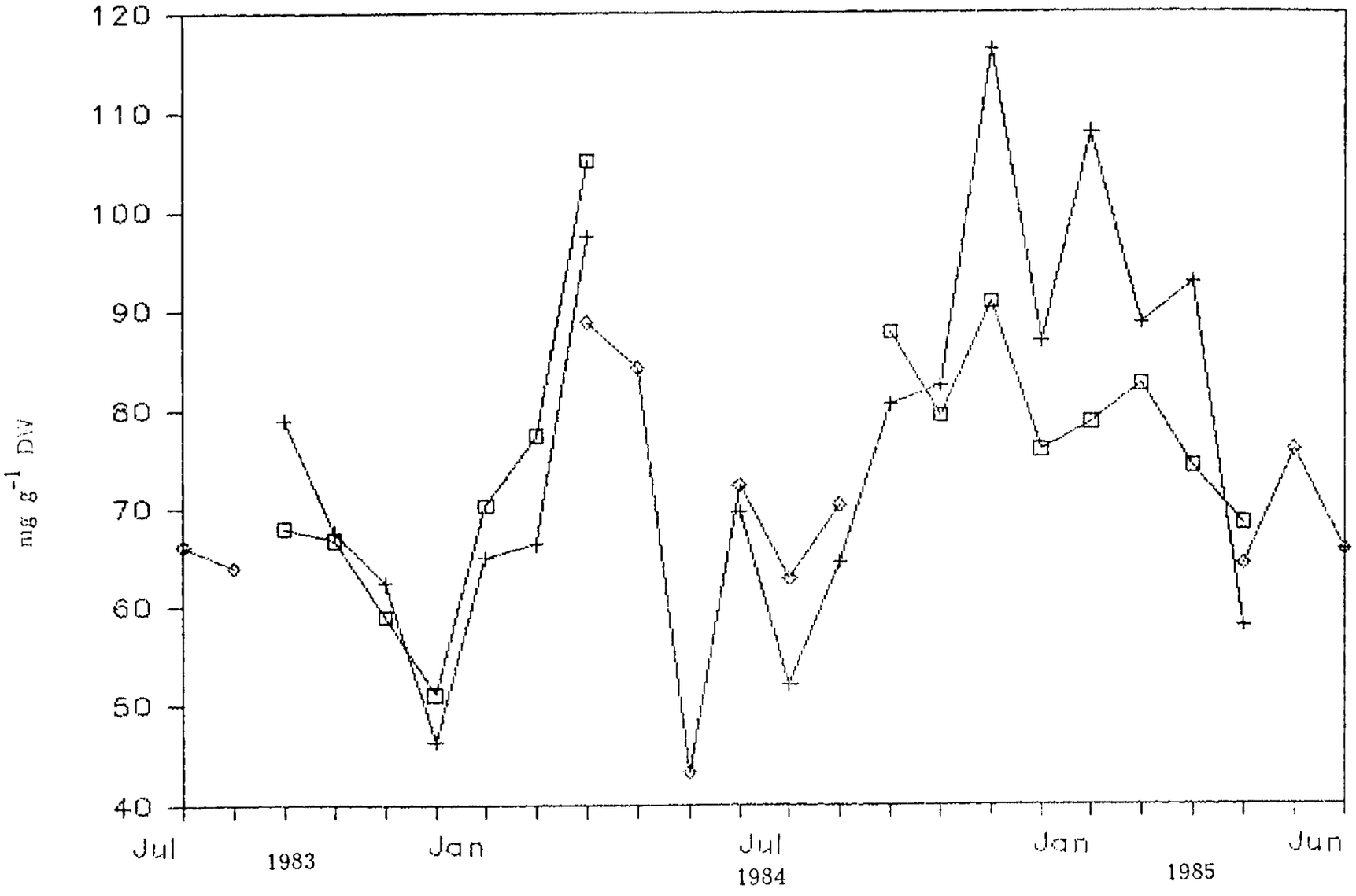

Figure 31. Monthly variations in ash levels of male (□), female (+) and indifferent (◇) S. scripta.

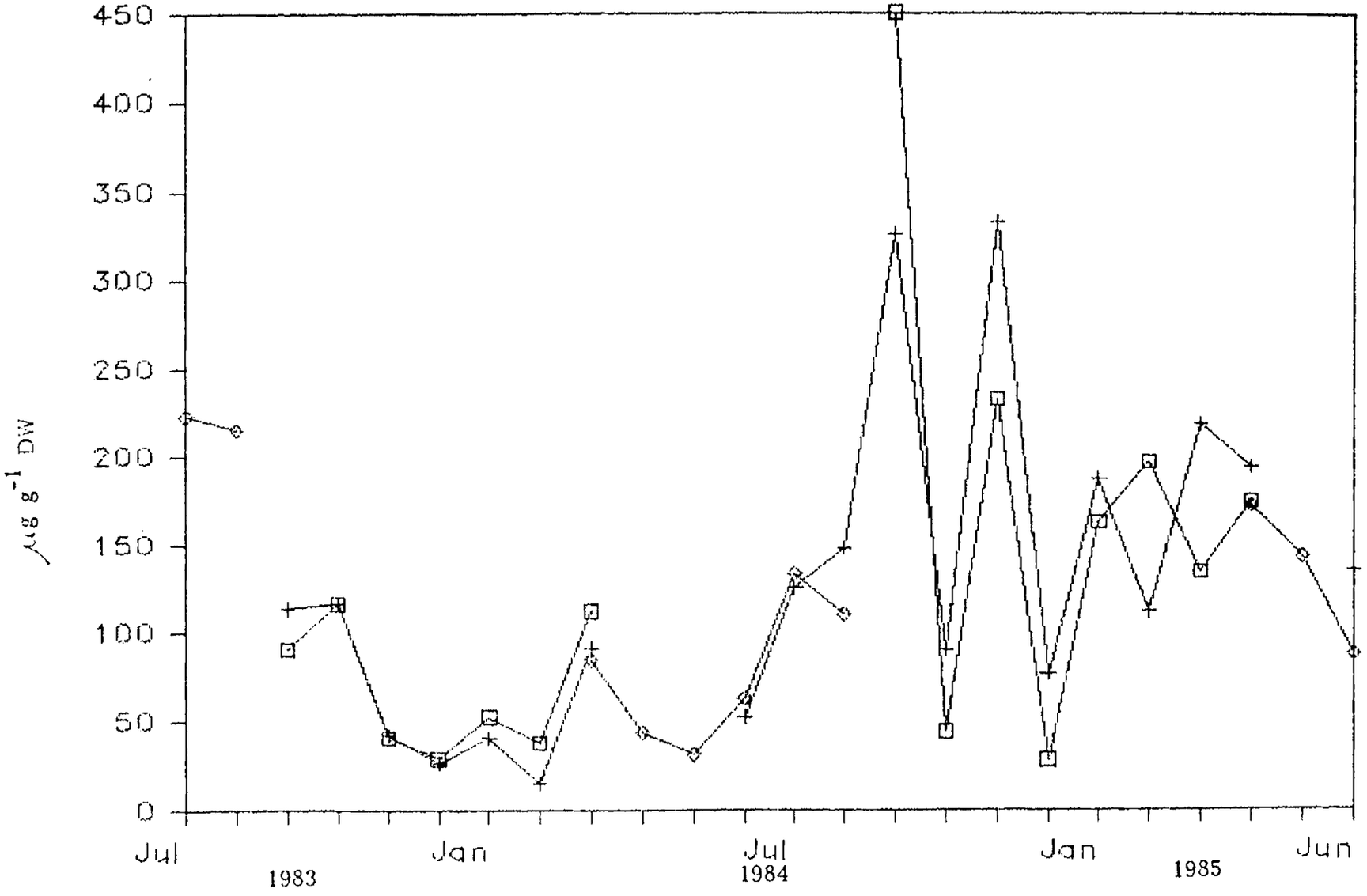

Figure 32. Monthly variations in manganese levels in male (□), female (+), and indifferent (◇), S. scripta.

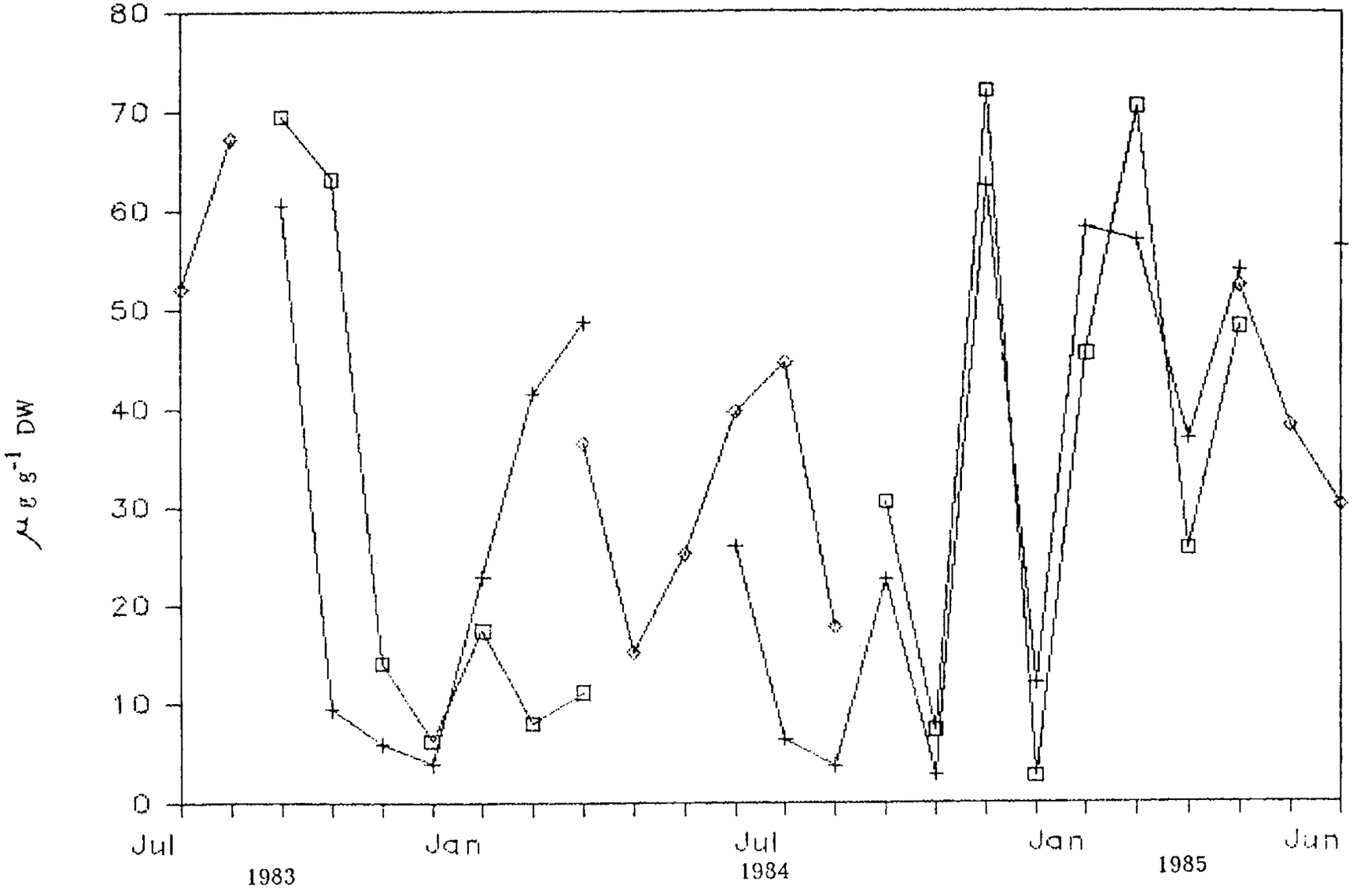

Figure 33. Monthly variations of copper levels in male (□), female (+) and indifferent (◊) S. scripta.

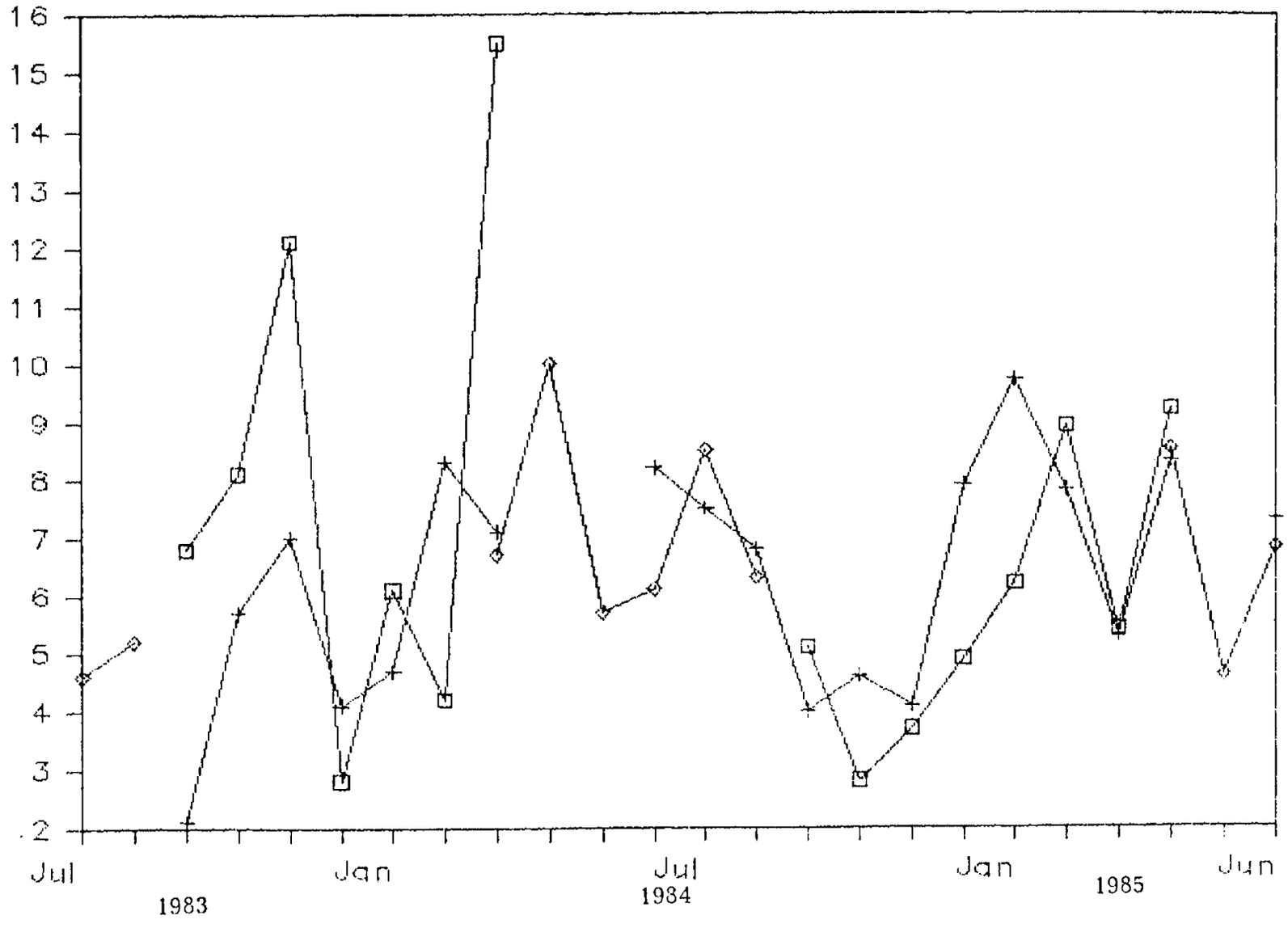

Figure 34. Monthly variations of chromium levels in male (▫), female (+) and indifferent (◇) S. scripta.

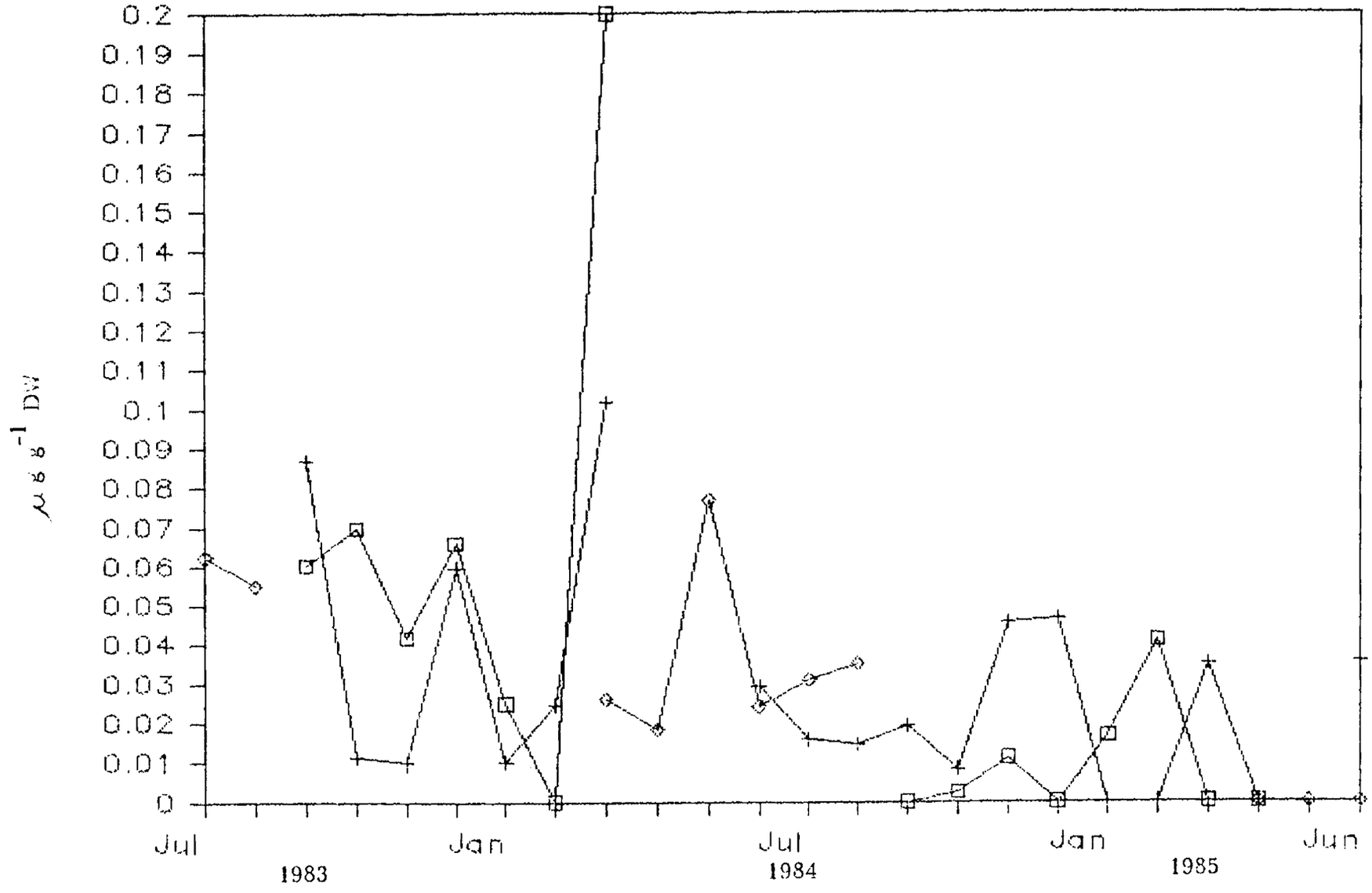

Figure 35. Monthly variations of arsenic levels in male (□), female (+) and indifferent (◊) S. scripta.

ash levels in male and female closely approximate each other, but in the second year values in female were higher. Ash level variations with manganese were found to be highly signficant. ($P < 0.005$, Table XIII). Ash values in clams of indifferent sex were generally low.

Manganese:- Manganese was one of the major fractions of the ash content of S. scripta. Compared to the second year of study manganese values showed little variation in the first year. In the second year differences between maxima and minima were greater than ten fold. Manganese levels were low in the indifferent phase but increased progressively towards the post-monsoon. The levels in males and females generally remained high till December when there was a fall in values. Manganese values showed highly significant correlation with copper. ($P < 0.001$; Table XII).

Copper:- Of all the elements examined, copper levels showed greatest fluctuations betwen maxima and minima, often greater than ten fold. Values for male clams were greater than in females but showed similar trends of variation. Whereas higher values were obtained during the monsoon period of the first year, peak values in the second year were observed in the post-monsoon.

Chromium:- Values for chromium showed random fluctuation and the peak and trough values observed in the first year showed temporal displacement during the second year of study. Levels for males were higher than in females during the first year but the values showed closer approximation in the subsequent year of study.

Arsenic:- Arsenic levels ranged from 0.1995 ppm to values below detectable limits. Arsenic values whenever present showed significant correlation with chromium ($P < 0.05$; Table XIII).

Nickel and Cobalt:- Values for Nickel and Cobalt were below detectable levels throughout the study period.

DISCUSSION

Metal concentrations in the tissues of the clam are dependent on availability of the metal in the immediate environment of the organism, i.e., in the sediment or ambient water; the hydro-climate (particularly, temperature and salinity); duration of exposure and physiological condition of the organism.

Manganese ranks highest in order of abundance of the metals analysed. Highest value (450.0 ppm DW) was obtained in male S. scripta in the post monsoon (Table IX, Fig. 32). Manganese metal is of low ionic toxicity but has high biological significance. Its role in enzyme activation is well established (Smith, 1951; Mounter and Chanutin, 1953). The metalloenzyme pyruvate kinase, in Crassostrea gigas was found to contain managanese (Hochachka and Mustafa, 1972). Manganese also influences the behaviour of other metals in the marine environment (Waldichuk, 1985). Precipitates of iron-manganese hydroxides in the water column may scavenge other metals such as copper, zinc, lead, cobalt and nickel, as they pass through the water column en route to the sediments and these metals are brought into solution when iron and manganese are converted to sulphides in reducing condition. Sankaranarayan and Stephen

(1978) reported approximately 75% of total manganese from Cochin in suspended particulate matter. Sanzgiri and Moraes (1979) found manganese to occur both as Mn^{2+} and Mn^{4+} in the Arabian Sea. Venugopal et al. (1982) found highest concentrations of manganese (208.7 ppm) in sediments of the barmouth region in the post-monsoon and lowest (168.5 ppm) in the monsoon periods. Bryan (1984) quoted typical oceanic concentration values for manganese as 0.08 ppb and Mn^{2+} as the major ion species. Levels of manganese in S. scripta are high but not unusual. It is generally acknowledged that bivalves collected near heavily populated and industrialised port areas exhibit higher manganese concentrations than specimens collected some distance from these areas (Fowler and Oregioni, 1976). Bryan (1973) reported values of manganese as high as 15,300 ppm in kidney of Pecten maximus. Shah et al. (1973) found high concentrations (441 ppm) in Sunetta donacina and moderately high concentrations (168 ppm) in Meretrix meretrix from Bombay. The other venerid clam, Katelysia marmorata, was a less efficient concentrator (22 ppm). Zingde et al. (1976) found low levels of manganese in Crassostrea cucullata from Goa (3.2 - 17.5 ppm). Sea weeds from the vicinity had higher concentrations and levels of manganese in the water were high (6-102 g l^{-1}). In Donax trunculus, (Orlando and Mauri, 1978) particulate manganese appeared to be more readily concentrated from the environment than soluble manganese species. In D. trunculus most of the manganese was concentrated in the kidney. In this study, higher concentrations of manganese were generally obtained in female clams of S. scripta which may be due to greater accumulation of storage products in female gametes. Mauri and Orlando (1983) explained the high values of manganese in D. trunculus in the spawning season

as possibly due to reproductive activity. They also suggest that higher values in females may stem from differential uptake, differential excretion and elimination of concretions or from both processes.

Copper in S. scripta was next in abundance only to manganese (Table X ; Fig. 33). Levels of copper ranged from 2.6 to 71.9 ppm DW. Among molluscs highest accumulation of copper were seen in cephalopods and in ostreid and crassostreid oysters; blood, digestive gland and kidney appeared to contain the highest concentrations of the element (Eisler, 1981).

Although copper is considered an essential element (Bryan, 1971), it is required by living organisms in trace amounts and any increments above the required level are highly toxic (Scott and Major, 1972). Copper forms part of the oxygen binding pigment in crustaceans and molluscs, though it is absent in most bivalves (Morton, 1958). Copper represents the metal fraction in the metalloenzyme, cytochrome oxidase of Crassostrea virginica (Chambers et al., 1975) and luciferase of Pholas dactylus (Henry et al., 1975). Copper containing cytochrome systems have been reported from the mitochondria of bivalves (Kawai, 1959). By its sheer abundance in the vicinity of urban settlements, ports and harbours, copper has come to be recognised as one of the most toxic metals in the marine environment. In full strength seawater, copper exists as inorganic species, principally $Cu(OH)_2^0$ and $CuCO_3^0$, though some organic complexes remain (Bruland et al., 1979). Venugopal et al. (1982) reported highest concentrations of copper from sediments in the post-monsoon (36.3 ppm) and lowest

in the pre-monsoon periods in the barmouth. Concentration of copper reported by Rajendran and Kurien (1986) from water was 0.17 to 0.42 ppm and from the sediment was 0.60 to 4.10 ppm. Average concentration of copper in the world oceans was 0.092 to 0.24 ppb (Bryan, 1984). Thampuran (1986) reports high copper tolerance in S. scripta with 100% survival at 6 ppm concentration. The great flux rates and narrow peaks in this study indicate rapid turnover of copper in S. scripta (Table X; Fig.. 33) Shah et al., (1973) reported concentration of 91 ppm in Sunetta donacina from Bombay. Of all the bivalves examined concentration of copper in S. donacina was second only to Crassostrea madrasensis. Concentrations of copper in adult C. madrasensis (Sankaranarayanan, et al., 1978) from Cochin Harbour area varied from 70 to 205 $\mu g\ g^{-1}$ and from a less polluted site at Quilon, average concentration was 67 $\mu g\ g^{-1}$. Rajendran and Kurian (1986) found concentrations of copper in C. madrasensis from Cochin to vary from 1.8 to 7.67 ppm DW. The difference in the two studies may be due to differences of location or temporal differences. Copper levels in the male clam were higher than levels in the female, but the difference was not significant and may have been due to artefacts of sampling. Nambisan et al. (1977) reported highest uptake in gills of Meretrix casta indicating direct uptake from solution. Though ionic species of copper are more biologically active it is possible that the metal concentration in S. scripta is derived from particulate sources, because concentrations of manganese and copper show highly significant positive correlation. Variations in copper do not show a consistent seasonal pattern and therefore it is possible that levels

in the clam are a reflection of environmental availability rather than the effect of physiological changes.

Chromium levels in S. scripta showed random fluctuations in values throughout the study (Table XI , Fig. 34). The levels in male clams were higher than in females in the first year of the study. The highest level was 15.5 ppm DW in male clams in the pre-monsoon.

The role of chromium in biological tissues has been stressed by Brooks and Rumsby (1965). Chromium was shown to restore activity to metal-free carboxypeptidases (Valle et al., 1958) and Strickland (1949) showed involvement of chromium in phospho-glucomutase activity. Jenkins (1982) discussing thermodynamics of chromium in sea water found Cr (III) to be predominent in shallow coastal areas receiving effluents high in organic matter and Cr (IV) in the open ocean. According to Cranston and Murray (1978) quoted in Bryan (1984), average concentration of chromium was 0.13 ppb in seawater. Chromium together with lead, zinc and copper are important constituents of bottom primers of ships and hence higher concentration can be expected in the vicinity of harbours. Young et al. (1979) found high concentrations of chromium in the tissue of Mytilus edulis in areas of high vessel activity from the California harbour. Brooks and Rumsby (1965) found concentration factor of chromium to be 200,000 to 320,000 in Pecten novae - zelandiae and Mytilus edulis aoteanus from New Zealand. Concentrations were highest in the gills, visceral mass and intestine and was attributed mainly to ingestion of sedimentary material. Preston (1971) concluded that in Crasostrea virginica from natural condition, though food supply may be the primary source, accumulation occurs more

readily by direct absorption. Fairly high chromium levels in S. scripta indicates some accumulation. No deleterious health effects have been reported among consumers of molluscs with occasional high chromium residues (Eisler, 1981).

The highest concentration of arsenic in S. scripta was 0.1998 ppm DW and levels were not detected in some of the samples (Table XII; Fig. 35). Arsenic has been indicated as a micronutrient for certain marine organisms (Le Blanc and Jackson, 1973). Industrialisation and modernisation of farming techniques have increased the influx of arsenic into coastal waters through rivers and land discharge. Fondekar and Reddy (1974 and 1977) found concentrations of arsenic in water between 3 and 67 μ g/l and 3.7 and 10.1 ppm in sediments off Goa and Bombay. These authors found it quite likely that arsenic in solution is carried to the bottom by absorption on ferric hydroxide and partly by flocculation, thus being locked up in sediments. Sediments with high clay concentrations retained maximum arsenic. Arsenic in the ocean is predominantly in the form of arsenate and is taken up indiscriminately by phytoplankton and other marine organisms. It has been suggested (Zingde et al., 1976) that as arsenate is chemically similar to phosphate, it may be transported into the cell along with phosphate. Arsenite formed from arsenate in the cell, might diffuse back again more easily than would arsenate, avoiding excessive accumulation of arsenic concentration in the cell. When cells containing arsenic are ingested by higher trophic levels the organo-arsenic compounds are rapidly excreted (Penrose, 1975; Fowler and Unlu, 1978). Arsonium phospholipids synthesised by plankton (Cooney et. al., 1978)

are readily passed up the food chain and are found in molluscan lipids (George, 1982). Concentrations of upto 500 mg g^{-1} DW have been reported in some fish species (Bohr, 1975) but the formation of soluble arsenoorganic compounds acts as a detoxicant mechanism which prevents toxicity of the As (III) species. Monier-Williams (1949) cites evidence that the arsenic present in marine organisms is in the form of an organic complex which is readily excreted by humans and is of low toxicity. Levels of arsenic in S. scripta were low and in some cases non-detectable. It appears likely that arsenic levels recorded in this survey do not constitute a health hazard, although further work will be required to establish clinical implications of arsenic in the clam.

Concentrations of nickel and cobalt were below detectable limits of the methods used, throughout the period of investigation. Venugopal et al. (1982) reported highest concentrations of cobalt and nickel in the sediment during the post-monsoon period. Dissolved cobalt and nickel are scavenged by hydrous oxides of manganese and iron on their way to the sediments. Concentrations of disolved nickel and cobalt in the Arabian Sea ranged from 1.0 - 115.0 $\mu g\ l^{-1}$ and 0.8 - 3.3 $\mu g\ l^{-1}$ respectively (Sankaranarayanan et al., 1978). Sanzgiri and Moraes (1979) found cobalt in most stations from the Arabian Sea to be below detectable limits. Average concentrations of cobalt and nickel in the world oceans were 0.01 ppb (Danielsson, 1980) and 0.228 - 0.693 ppb (Bruland et al., 1979) respectively. Pillai et al. (1986) found cobalt levels in Villorita cyprinoides from Cochin to be below analytical detection limits and levels of nickel

varying from 1.0 - 6.5 ppm DW. Considering the sediment feeding habit of S. scripta the absence of nickel and cobalt is surprising. Non-detection of these metals may also be due to their rapid, turnover and very small biological half life in the clam tissues.

From the close proximity to the sediment and the detritus feeding habit of the clam it can be safely presumed that the metal load in the organism is obtained from the sediment. This is supported by the close positive coupling observed between manganese and copper and chromium and arsenic levels in S. scripta. The absence of correlation between the other metals, may be due to differential elimination/excretion rates from the animal.

Metals are taken into molluscs by at least three independent pathways i.e. diffusion across lipid membranes as metallochlorine complexes (as for $CdCl_2$; Carpene and George, 1981); as chelated complexes (Coombs and George, 1978) or they may parasitize other ion pumps (Roesijadi, 1982).

The presence of high concentration of manganese, copper and chromium makes it apparent that these clams possess very efficient methods of preventing toxic metal interaction with essential enzymes of their cells.

Methods of detoxification in marine organisms include binding to high molecular weight proteins or polysaccharides, low molecular weight proteins (metallo-thioneins), demethylation (in case of mercury), conversion

to less toxic organic forms (arsenic); immobilisation in intracellular inclusions and immobilisation by incorporation into shell, skeletal material, etc. (Bryan, 1984). Metals accumulate in molluscs mainly in the hepatopancreas and kidney (Simkiss et al., 1982). These organs are also characterised by large accumulations of phosphates (George et al., 1980) pyrophosphates (Howard et al., 1981) and oxalate (Overnell, 1981) salts together with metallothionein-type protein with large thiol content (Roesijadi, 1981).

Processes of removal of metals from the body includes loss from the general body surface as granules, secretions or diapedesis of amoebocytes, urinary excretion and loss from the alimentary tract and incorporation in eggs (Bryan 1984).

Whether tolerance to metals is genetically determined is unknown, although general adaptability of estuarine organisms suggests that it is. The great resistance to copper in the polychaete Nereis diversicolor (Bryan and Hummerstone, 1971) and the brown fouling alga Ectocarpus siliculoses (Russel and Morris, 1972) from high copper situations was shown to be genetically determined. It has been suggested that accumulation of one metal may also offer some sort of protection against another as pointed out for the co-accumulation of selenium and mercury in tuna (Ganther and Sunde, 1974) and copper and lead tolerance in the isopod Asellus meridianus (Brown, 1976). Thus high levels of a relatively harmless metal such as manganese in S. scripta may be responsible for tolerance of the more toxic metals in the organism.

While detoxification processes apparently protects to some degree the organism ingesting a given metal, it does not protect the predator including man from an excessive intake of the metal. Trace metal levels in S. scripta though high are still within concentration limits recommended for human consumption.

From bioaccumulation studies of copper, Thampuran (1986) recommended the use of S. scripta for monitoring pollutant levels in the environment. In another experiment described in Chapter V, the clam showed normal shell valve and siphonal activity at 0.5 ppm copper concentration in 30 x 10^{-3} salinity - a concentration rarely exceeded in field conditions. It is probable therefore that S. scripta is a good biological monitor of copper, given sub-lethal levels of the metal which do not trigger the closure response. The variation in copper levels in S. scripta suggests a relationship with environmental levels, with modifications resulting from physiological changes at a minimum. Thampuran (1986) reported a slight but insignificant difference in uptake rates between different size groups. This can be eliminated by using specimens of a similar mature size which are available throughout the year. Concentration differences due to sex found in this study were insignificant and can also be minimized by using random collection procedures since the male:female ratio in S. scripta remains constant. Mackay et al., (1975) have suggested that metal correlation in the organism may, from chemical analysis for one metal, allow statistical estimation of other metals within a single estuary. Such estimates may be of considerable value in monitoring

of the clams for toxic metal levels. Cunningham and Tripp (1975) suggested the use of cultured bivalves of known age from clean environment, for biological monitoring of areas of suspected pollution. This method of monitoring under natural conditions would be far superior to poorly simulated experiments in laboratories.

CHAPTER V

COPPER INDUCED METABOLIC CHANGES IN SUNETTA SCRIPTA - OXYGEN UPTAKE AND LACTIC ACID PRODUCTION

Indian contributions relative to the effect of trace metal pollution in bivalves have dealt mainly with acute toxicity and uptake kinetics (Nambisan et al., 1977; Kumaraguru and Ramamoorthi, 1978; Lakshmanan, 1982). Thampuran (1986) has demonstrated high tolerance to ambient copper concentrations in S. scripta. The mechanisms working to protect aquatic organisms are still unknown but it should be recognised that before death as such is manifested, the biochemical and physiological systems would be affected even at significantly lower doses, well below or near threshold levels.

Physiological parameters such as oxygen consumption, filtration rate, ciliary activity etc., have been recognised as measures of metabolic change (Scott and Major, 1972; Manley, 1983; Mathew and Menon, 1984; Thampuran, 1986; Prabhudeva and Menon, 1986) but such measurements may be incomplete in themselves in that bivalves respond to stress by complete/partial closure of shell valves and temporary shifts to anaerobic metabolic pathways (de Zwaan, 1983). Anaerobic capacities of some molluscs have been recognised for a long time (Moore, 1931). During aerobic respiration bivalves metabolise glycogen to pyruvate and thus, via the citric acid cycle, to carbondioxide and water. During anaerobiosis, however, the metabolic pathway shifts to form succinate and alanine. The switching of these pathways occurs at phosphoenolpyruvate (PEP), which is broken down to pyruvate by pyruvate kinase in aerobic, but

to oxaloacetate by PEP carboxykinase, during anaerobic metabolism, (Livingstone and Bayne, 1977; Collicut and Hochachka, 1977; Ebberink et al. 1979). Volatile fatty acids such as propionate and acetate also accumulate in bivalves during air exposure and anoxia (Kluytmans et al., 1978; Schulz et al., 1982). An entirely new class of end products, alanopine [meso - N - (1 - carboxyethyl) - alanine] and strombine [N -(Carboxymethyl)-D - alanine] have also been shown to accumulate (de Zwaan and Zurburg, 1981; Nicchitta and Ellington, 1983) during anoxia. These pathways are characterised by a much reduced Pasteur effect and, when compared with the "classical lactate pathway", energy yield is increased, but the rate of energy production is decreased.

It is generally acknowledged that anaerobic energy metabolism in bivalve molluscs does not lead to a substantial accumulation of lactate (de Zwaan, 1977). However Meinardus and Gäde (1981), reported the major end product in Cardium edule to be D-lactate though other metabolites such as succinate and alanine were also produced. Lakshmanan and Nambisan (1985) observed that in whole tissues of Perna viridis and Villorita cyprinoides the lactic acid levels increased but glycogen levels decreased with increasing concentration of trace metals. Suresh and Mohandas (1987) also reported significant lactic acid accumulation in hemolymph of Sunetta scripta under prevailing anoxic/hypoxic condition subsequent to complete or partial valve closure induced by exposure to metal.

The objective of this investigation was to study, 1) the extent of artificial anoxia/hypoxia experienced by S. scripta on exposure to sub-lethal copper concentration and 2) to relate oxygen consumption rates

to lactic acid production in selected tissues of the clam through 8 days of exposure and subsequently 7 days of depuration.

MATERIALS AND METHODS

The specimens of S. scripta for experimental use were collected from the clam bed, off Fort Cochin, transported to the laboratory, and acclimated for 4 days in 60 litre recirculating sea-water tanks fitted with biological filters (salinity, 30 x 10^{-3}S; temperature, 28 ± 1°C). Clams of 35-40 mm shell length were selected for the experiment. On the 5th day, 55 clams were transferred to each of eight 15 liter tanks. The tanks were filled with filtered sea-water and two tanks each were dosed with 0.3, 0.5 and 1.0 ppm copper. The salt used was Analar grade copper sulphate (Glaxo Labs, Bombay). The concentrations selected were such that they were close to realistic levels as possible and yet able to elicit measurable responses in the clam. These 6 tanks served as the experimental group. The last 2 tanks were the controls and contained clean filtered sea-water. The water was renewed every day, during the 15 days of the experiment, and the concentrations in the experimental tanks were maintained at their respective level throughout the exposure period of eight days. Feeding was stopped during the experimental period and the tanks were monitored continuously to ensure that non-experimental parameters viz., temperature, salinity, oxygen etc., were at or near optimal levels.

Six clams each, pre-exposed to the respective copper concentrations for periods of 24, 72, 120 and 168 h and subsequently 24, 72, 120 and 168 h after transfer to clean sea-water, were used to record oxygen

consumption. Each animal was placed in a 250 ml beaker containing 200 ml of the test solution, along with a control group. To avoid gaseous exchange with the atmosphere, the water column of the respiratory chamber was sealed with inert liquid paraffin. A 10 ml glass syringe, was used to draw an initial water sample 15 minutes after the introduction of the clam, and a final sample was drawn from the chamber 30 min to 3 h later, as the case may be. Oxygen content of the water was determined using the Winkler method (Welsh and Smith, 1953). After the experiment the animals were dissected and the fresh weight of the soft tissues determined. Oxygen consumption was expresed as $\mu l\ O_2\ g^{-1}$ FW h^{-1}.

In a parallel experiment, at the same time intervals, adductor muscle and digestive gland from 6 clams of each experimental group and control were dissected out, weighed and homogenised in 3 ml of 5% cold Trichloroacetic acid. The homogenate was centrifuged at 1040g for 15 min and the supernatant was removed for lactic acid determination following the method of Barker (1957) and expressed as μg lactic acid g^{-1} FW of tissue.

To 1.5 ml of the supernatant, 1 ml of 20% $CuSO_4$ and 2.5 ml of distilled water were added. Approximately 1 g of powdered $Ca(OH)_2$ was also added and the contents of the tubes were shaken well. The mixture was allowed to stand at room temperature for 30 mins, with occasional shaking and was then centrifuged. Duplicate aliquots of 1.0 ml of the supernatant fluid were carefully withdrawn from beneath any surface particles and transferred to a 20 ml test tube. 0.05 ml of 4% $CuSO_4$ solution was added and the tube was chilled in an ice and water bath. Exactly 6.0 ml of concentrated H_2SO_4 was added slowly from

a pipette and at the same time the contents of the tube were mixed. The rack of tubes was then placed in a boiling water bath for 5 minutes, removed and cooled to below 20°C. To each test tube 0.1 ml of p-hydroxy diphenyl solution was added and the precipitated reagent was dispersed throughout the acid as quickly and uniformly as possible. The tubes were then allowed to stand at room temperature for 30 minutes. The precipitated reagent was redispersed by shaking at least once during the incubation period. Excess reagent was dissolved by heating tubes in boiling water for 90 seconds, then cooled to room temperature. The developed colour was read against the reagent blank at 560nm. The average of the duplicate reading was used to calculate the lactate content of the aliquot by referring to a calibration curve prepared by using lithium lactate.

Analysis of variance test (Zar, 1974), was employed to compare all mean values of the experimental batches and of the control group, and the 'two-tailed t test' (Zar, 1974) was employed to determine statistical significance of difference in values of lactic acid in tissues and oxygen consumption under normal and experimental conditions.

RESULTS

In 1.0 ppm exposed clams siphonal activity and shell valve movements were terminated almost immediately on introduction to the copper dosed sea-water in the experimental tank. The 0.3 and 0.5 ppm exposed clams showed normal activity with extended siphons, valve movements and protrusion of siphons beyond the shell edge. At 120 h, the 0.5 ppm exposed clams showed a reduction of activity and at 168 h most of the clams appeared quiescent.

TABLE XIV CHANGES IN OXYGEN UPTAKE OF THE CLAM S. SCRIPTA DURING SUBLETHAL EXPOSURE TO COPPER AND RECOVERY FOR VARYING LENGTHS OF TIME

Conc. of Metal ions	Oxygen uptake $\mu l\ O_2 g^{-1}(FW)\ h^{-1}$**							
	Exposure Time				Recovery time			
	24 h	72 h	120 h	168 h	24 h	72 h	120 h	168 h
0.3 ppm	53.25 ±29.16	74.76 ±32.17	61.54 ±32.38	52.17 ±21.36	59.47 ±26.19	49.45 ±21.32	57.62 ±23.70	59.76 ±27.30
0.5 ppm	49.47 ±25.28	57.24 ±23.19	10.91* ±8.70	7.43* ±2.11	55.29 ±34.84	55.43 ±26.37	57.24 ±27.66	62.58 ±30.59
1.0 ppm	7.21* ±2.77	6.94* ±2.16	6.37* ±1.82	6.91* ±2.29	74.24* ±31.16	64.91 ±29.36	55.32 ±21.15	53.26 ±27.64
Control	55.33 ±23.35	63.52 ±27.26	57.31 ±35.23	48.43 ±37.16	57.72 ±29.41	51.73 ±32.77	62.85 ±19.29	54.38 ±24.11

**Each value is a mean ± 1 S.D. (n=3)

*All values significantly different from the control ($P<0.05$).

TABLE XV CHANGES IN LACTIC ACID LEVEL OF THE DIGESTIVE GLAND OF THE CLAM S. SCRIPTA DURING SUBLETHAL EXPOSURE TO COPPER AND RECOVERY FOR VARYING LENGTHS OF TIME.

Conc. of Metal ions	Lactic acid μg g^{-1} F.W.							
	Exposure Time				Recovery time			
	24 h	72 h	120 h	168 h	24 h	72 h	120 h	168 h
0.3 ppm	32.17	27.64	29.38	31.74	28.38	30.69	33.16	26.51
	±4.97	±2.69	±2.65	±4.16	±3.55	±4.82	±3.23	±2.37
0.5 ppm	31.39	30.74	33.54	29.26	26.49	27.15	30.48	32.76
	±2.11	±4.83	±3.91	±2.84	±2.36	±3.04	±4.16	±3.71
1.0 ppm	30.16	29.26	29.13	31.43	37.34**	29.23	30.08	29.44
	±4.23	±3.26	±3.38	±3.97	±3.97	±3.22	±2.10	±1.54
Control	33.26	29.52	32.42	30.21	30.62	27.81	31.74	28.93
	±3.58	±4.79	±3.26	±4.47	±3.65	±3.92	±4.57	±4.19

*Each value in a mean ±1 S.D. (n = 6).

**All values significantly different from the control ($P < 0.05$).

TABLE XVI CHANGES IN LACTIC ACID LEVEL OF THE ADDUCTOR MUSCLE OF THE CLAM S. SCRIPTA DURING SUBLETHAL EXPOSURE TO COPPER AND RECOVERY FOR VARYING LENGTHS OF TIME*

Conc. of Metal ions	Lactic acid $\mu g\ g^{-1}$ F.W.							
	Exposure Time				Recovery time			
	24 h	72 h	120 h	168 h	24 h	72 h	120 h	168 h
0.3 ppm	20.31 ±2.92	21.37 ±3.11	20.65 ±4.13	24.58 ±3.26	23.01 ±4.97	19.17 ±3.77	20.71 ±2.31	21.25 ±3.03
0.5 ppm	27.29** ±4.31	20.09 ±2.79	22.81 ±4.27	22.93 ±2.19	24.67 ±2.39	23.15 ±2.46	21.35 ±3.75	21.43 ±2.37
1.0 ppm	24.43 ±2.32	22.13 ±2.38	22.63 ±1.53	20.26 ±3.84	29.16** ±2.41	22.34 ±4.26	22.39 ±1.73	20.67 ±2.53
Control	21.59 ±3.25	23.23 ±2.78	24.83 ±4.69	22.91 ±1.22	22.74 ±3.96	20.38 ±2.34	25.19 ±2.57	23.67 ±3.76

*Each value is a mean ± 1 S.D. (n = 6)

**All values significantly different from the control ($P < 0.05$).

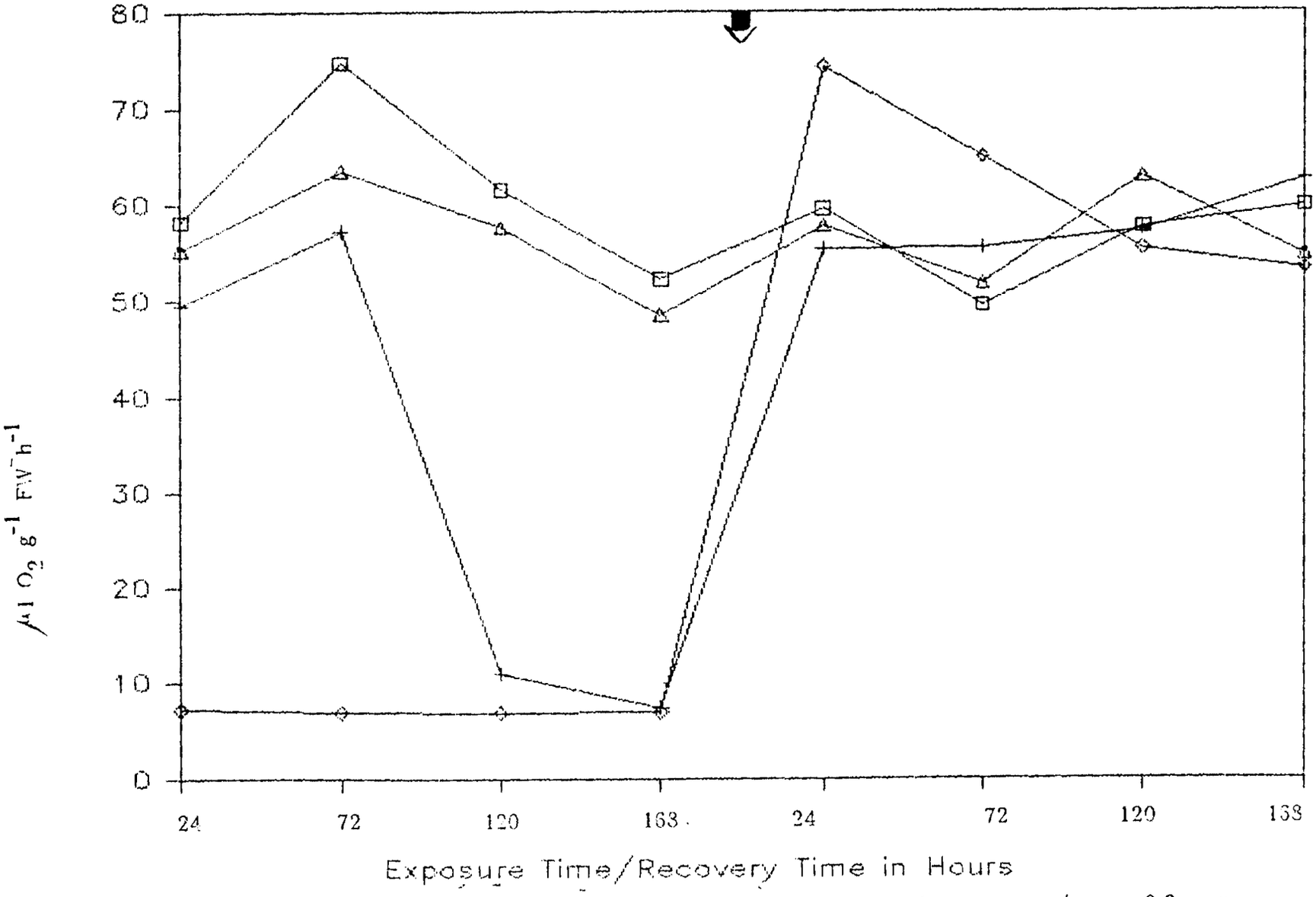

Figure 36. Oxygen uptake of S. scripta on exposure to copper and recovery. (□ = 0.3 ppm, + = 0.5ppm, ◊ = 1.0 ppm, △ = control). Arrow mark indicates onset of recovery.

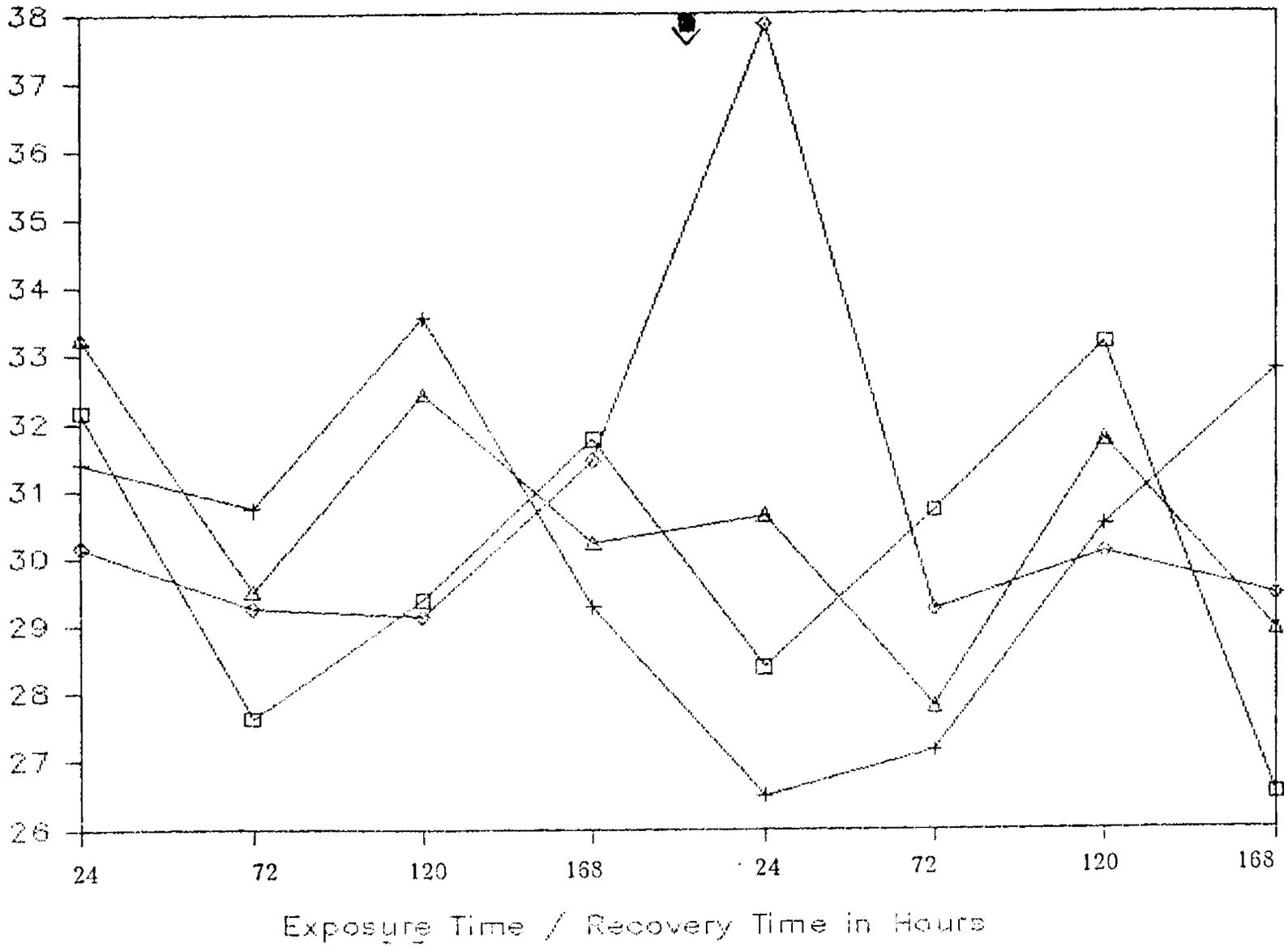

Figure 37. Lactic acid levels in digestive gland of S. scripta on exposure to copper and recovery. (Symbols as in

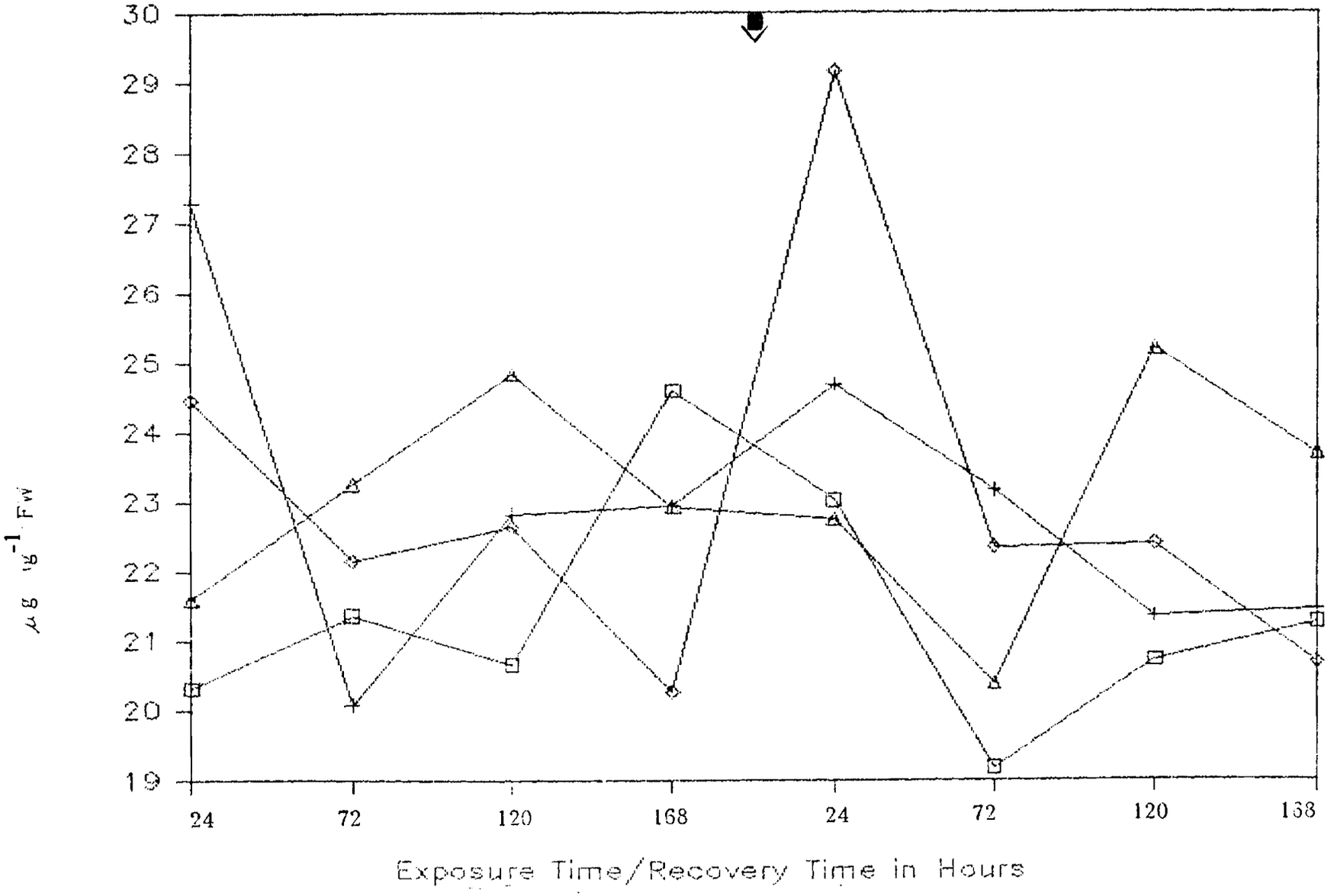

Figure 33. Lactic acid levels in adductor muscle of S. scripta on exposure to copper and recovery. (Symbols as

The clams in 1.0 ppm copper dosed sea-water showed significant reduction in oxygen consumption (Table XIV, Fig. 36) compared to clams in the control experiment and clams exposed to 0.3 ppm and 0.5 ppm of copper. The clams exposed to 0.5 ppm showed a significant reduction of oxygen consumption at 120 h and 168 h as compared to the control and 0.3 ppm exposed clams. At 24 h after the start of depuration, clams previously exposed to 1.0 ppm of copper showed a slightly significant increase in oxygen consumption as compared to clams in the control experiment and those previously exposed to 0.3 and 0.5 ppm concentrations. Thereafter at 72, 120 and 168 h of depuration, oxygen consumption of control and experimental clams showed no significant differences.

A slight but statistically significant increase (Table XV, XVI Fig.37, Fig. 38) in lactic acid levels was noticed in the adductor muscle and digestive gland of 1.0 ppm dosed clams 24 h after start of depuration. Lactic acid level in adductor muscle of 0.5 ppm dosed clams (Table XVI, Fig. 38) showed a significantly higher value over control clams after 24 h of exposure. Lactic acid levels otherwise showed no significant difference between control and experimental animals throughout the study.

DISCUSSION

S. scripta displays a remarkable tolerance to added copper in experimental studies (Thampuran, 1986). In the present investigation at concentrations of 0.3 ppm added copper, the animal showed near normal activity with no significant reduction in oxygen uptake. At 0.5 ppm added copper concentrations, oxygen uptake is normal at 24 h and 72 h of exposure but is subsequently depressed at 120 h and 168 h (Table

XIV, Fig. 36). Behavioral changes included a decrease in valve movement and siphonal activity. At 1.0 ppm added copper, depression of valve movement was noticed almost immediately upon introduction to the test solution. Shell valve adduction to overcome stress is ubiquitous among the bivalves (Bayne et al., 1976; Manley, 1983). Copper ions are also known to cause respiratory and cardiovascular depression (Scott and Major, 1972; Brown and Newell, 1972; Thampuran, 1986). At the lowest concentration used in this experiment, copper entering into the tissue appears to be easily metabolised and detoxified. At 0.5 ppm added copper, the detoxification mechanisms are probably saturated after prolonged exposure and hence a depression in ventilatory activity at the longer exposure times, effectively prevents toxic influx of copper ions into the tissue. At 1.0 ppm added copper, the rate of influx is often greater than the detoxifying capacity of the clam and hence shell closure is the immediate response. Mclusky and Phillips (1975) from studies on Phyllodace maculata suggested that the rate of uptake of a toxicant rather than the actual amount accumulated may be the deciding factor determining toxicity of a solution. It is probable that the shell valve closure at the higher copper concentrations is not total and that the animal can still sample the ambient water, because they quickly resume normal activity on restoration of environmental quality. Any oxygen consumption at the higher concentrations may be ascribed to this "testing behaviour" when shell valves show intermittent movement (Manley, 1983).

At 24 h after depuration in a copper free medium, oxygen uptake in 1.0 ppm-exposed animals showed a significantly higher value

in comparison with the control and 0.3 and 0.5 ppm pre-exposed animals. Prolonged shell valve adduction in the animal at the highest concentration results in the inflation of oxygen consumption rates. This inflation probably facilitates the repayment of the "oxygen debt" incurred by the animal during shell valve closure. There are considerable references in the literature to substantiate this explanation (Bayne et al., 1975; Moon and Pritchard, 1977; de Zwaan, 1977). Oxygen consumption was restored to normal levels at 72 h post-exposure and remained at this level thereafter till the termination of the experiment. In the 0.5 ppm pre-exposed clams the initial period of elevated oxygen consumption rates may not have been detected, the "oxygen debt" being compensated within 24 h of recovery.

In addition to the metabolic demands of the influx of copper ions, the closure response of the shell results in metabolic stress through inability to fed, a limit to gas exchange and accumulation of toxic metabolic end-products. A reduction in oxygen availability would necessitate a conversion, to anaerobic pathways of energy metabolism. Investigations by Zebe et al. (1980) have resulted in the distinction of two types of anaerobic metabolism. Oxygen deficiency which is brought about by a depletion in the medium (ecological anoxia), leads to a gradual shift towards the fumarate-succinate pathway for energy supply, with succinate and propionate as major end-products. A lack of oxygen caused by high muscular activity leads to a glycolytic energy supply by one of the pyruvate pathways with lactate and/or opines as end products (functional anoxia).

Time dependant changes in the accumulation of anaerobic fermentation products have been demonstrated for the sea mussel, Mytilus edulis (de Zwaan and van Marrewijk, 1973; Kluytmans, et al., 1977) for the freshwater bivalve Anodonta cygnea (Gäde et al., 1975) for the common

cockle, Cardium edule (Meinardus and Gäde, 1981) and for the polychaete worms, Arenicíola marina and Nereis diversicolor (Schöttler, 1978). Although complete oxidative breakdown of energy substrates via the tricarboxylic acid cycle is not fully blocked during anaerobiosis (e.g. Mytilus edulis; de Zwaan, 1977) it is nevertheless severely restricted and necessarily accompanied by the activation of the phosphoenolpyruvate pathway to form succinate or propionate (Kluytmans et al., 1975). Other routes employed, either to maintain redox balance or to gain ATP by substrate level phosphorylations, may lead to a variety of other intermediates such as lactate, alanine, acetate, octopine etc.

In this study, metal induced hypoxia did not result in a significant increase in lactic acid levels in digestive gland or adductor muscle of S. scripta at all exposure concentrations except at 24 h of exposure in the adductor muscle of 0.5 ppm exposed clams (Table XVI, Fig.38). The reason for this exceptional value is not clear. It may have been due to muscular activity on introduction to the copper solution though oxygen consumption did not vary significantly with the control. Analytical error also cannot be ruled out though care was taken to adhere to analytical recommendations as much as posible.

The absence of lactate accumulation and the capacity for long term anaerobiosis supports the theory of the alternative glycolytic pathway in S. scripta. Protein can be metabolised by both aerobic and anerobic pathway but complete oxidation of lipids can only take place aerobically as there is no experimental evidence of a functional glyoxylate cycle in bivalve moluscs (Gabbot, 1983).

Glycogen may be used together with aspartate from the free amino acid pool during initial anoxia to produce succinate and alanine. During prolonged anoxia carbohydrate is the main metabolic substrate. High concentrations of carbohydrate reserves in S. scripta (see chapter IV) may be an adaptation for anaerobiosis. Glycolysis proceeds to phosphoenolpyruvate (PEP) which acts as the branch-point for pyruvate kinase (PK) and phosphoenolpyruvate - carboxykinase (PEP-CK) pathways. de Vooys (1980) suggested the ratio of PK to PEP - CK activity as an indicator of anaerobic capacity. The lower the ratio the greater the adaptation to anaerobic bio-chemistry. In molluscs PK is under the control of FDP, ATP and L-alanine and shows co-operative kinetics with PEP (de Zwaan, 1977). PK is also regulated by a phosphorylation dephosphorylation process, the non-phosphorylated form being less active. The H^+ ion concentration also regulates PK activity. A drop in pH together with alanine accumulation effectively inhibits PK activity and results in a switch to the PEP-CK pathway. de Zwaan et al. (1975) reported a pH of 7.6 corresponding to an aerobic condition and pH 6.2 corresponding to anaerobiosis. Mn^{2+} and Mg^{2+} are essential for full activity of PK while Cu^{2+} inhibits PK activity in Scapharca inaequivalvis (Cortesi et. al., 1985). Inhibitory activity would depend upon binding of copper to NH_2 or - SH ligands at or near the active site or direct competition with Mg^{++} or Mn^{++} for ADP (Cortesi et al., 1985). The funneling of carbon through the PEP-CK pathway may be a dose dependent response to Cu^{++} by PK.

Propionate production results in the formation of 6.71 moles of ATP from each mole of glucose (de Zwaan, 1983). Thus efficiency

of molluscan anaerobic metabolism is between lactate fermentation (2 moles of ATP) and aerobic respiration (37 moles of ATP). Despite the lower energetic efficiency there was no clear indication of increased carbohydrate utilisation in a companion experiment. A possible explanation is that bivalves may reduce their energy demand under oxygen deficiency (de Zwaan and Wijsman, 1976; Ebberink et al., 1979). During anaerobiosis the rate of ATP utilisation is greatly reduced in bivalves and may be as low as 5% of the aerobic level of metabolism (de Zwaan, 1983).

At 24 h after the start of depuration a significant increase in lactic acid levels was observed in the adductor muscle and digestive gland of S. scripta corresponding to an increase in oxygen consumption. During recovery from anoxia there is a recharging of the high energy phosphate pools and resynthesis of aspartate (Nichitta, 1983). de Zwaan et al. (1983), suggested that elevated energy demands during recovery outstrip the aerobic capacity of the tissue, consequently there is elevated glycolytic flux and strombine accumulation to maintain energy balance. Zebe et al. (1980) found that the lack of oxygen caused by increased muscular activity leads to a glycolytic energy supply by one of the pyruvate pathways with lactate and octopines as end products. Coupling of pyruvate reductases with glyceraldehyde - 3 - phosphate dehydrogenase results in NAD being constantly replenished for the dehydrogenation of glyceraldehyde - 3 - phosphate while lactate or opines accumulate (Gäde and Grieshaber, 1986). In some species the importance of anaerobic glycolysis for both functional and environmental hypoxia is reflected in the presence of at least 2 dehydrogenases catalysing the terminal reaction of this pathway within the same species (Gäde and Grieshaber, 1986).

Increased ventilation and muscular activity, on removal of the stress factor can be considered as a possible explanation for the significant lactic acid level in the adductor muscle of the 1.0 ppm pre-exposed clams. The increased lactic acid concentration in the digestive gland may be due to transport of lactic acid from other sites for reutilisation, as reported in Helix pormatia (Wijsman et al., 1985).

There is only limited uniformity in the biochemical pathways of energy metabolism that are employed in bivalves. Differences are not only observed between various bivalve species, but also intraspecific, intra-organ and seasonal variations are also reported (Kluytmans et al., 1980; Zurburg and Kluytmans, 1980). This may explain the significant accumulation of lactic acid in the haemolymph of S. scripta during copper stress (Suresh and Mohandas, 1987).

Capacity for mobilisation of carbon via aerobic and anaerobic pathways depending upon the tissue oxygen supply, may represent an evolutionary process of adaptation, enabling the animal to occupy unfavourable environments with variable oxygen content. Muley et al. (1987) found the wedge clam Donax cuneatus to respond initially to pesticide exposure by valve closure, but later the valves were opened leading to mortality. Thus a reduction in anaerobic capacity incapacities the animal during long term stress. S. scripta seems to be efficiently adapted to anaerobiosis for moderately long periods. Such adaptive mechanisms ensure its survival in unstable conditions in the habitat. The onset of the summer monsoon considerably lowers the salinity of the clam bed from the optimum at

30 x 10^{-3}S. Larger clams show less survival at the lowered salinities than the smaller animals. It is interesting to speculate that the smaller clams may have a more efficient mechanism to survive anaerobiosis. Such a surmise is well worth further investigation.

CHAPTER VI

COPPER INDUCED METABOLIC ALTERATIONS IN SUNETTA SCRIPTA - TOTAL CARBOHYDRATE AND TOTAL PROTEIN LEVELS

Protein and carbohydrate are "older forms" of energy predominant in facultative anaerobes with low levels of metabolism (Shul'man, 1974). Complete oxidation of lipids can only take place aerobically but protein and carbohydrate can be metabolised by aerobic and anaerobic pathways. Furthermore, carbohydrates have the advantage of great mobility and capacity for storage as inert deposits. Metabolism in bivalves depends on a glycogen economy. Carbohydrate is the main energy store in adult bivalves, constituting about 58% of the dry weight in Tivela sp. (Giese, 1969). Curiously there are differences in energy utilisation, in bivalves, depending on the seasons as well as stage in the life cycle. In summer carbohydrate accounts for all of the energy loss in Mytilus edulis (Gabbot and Bayne, 1973). In autumn there is a pronounced increase in lipid utilisation, (Bayne, 1973) and in winter there is a shift to protein as the main respiratory substrate (Gabbot and Bayne, 1973; Zandee et al., 1980 a and b). In bivalve larvae with high metabolic levels protein and lipid are the main energy reserve, and in marked contrast to the adult, carbohydrates are the least important (Gabbot, 1976; Holland and Spencer, 1973).

In addition to their role as metabolic substrates, proteins have also been implicated in a variety of functions. Most biological reactions are created by the chemical properties of proteins. In bivalves, proteins have an important role in the maintenance of homeostasis, osmolality, transport and detoxification of ions and in the immune system. In metal-

exposed individuals, a large proportion of the incorporated metals are bound to low molecular weight binding proteins. The functions of the proteins are still not clearly understood but a heavy metal storage and detoxification function has been postulated for some time (Kagi and Vallee, 1961; Webb, 1971). There is now a general consensus that metals are protein bound as soon as they enter the vascular system so that a concentration gradient inward is maintained (Simkiss and Mason, 1983). This is supported by evidence from George and Coombs (1977) showing that ligand binding of cadmium, iron and lead increases the rate at which they enter M. edulis. There is increasing support to the theory that storage of metal ions in association with metal binding proteins is responsible for the high metal accumulation potential and persistence observed in many species (Langston and Zhou, 1987a).

This experiment was designed as a follow up of Chapter V. In similar experimental conditions variations in carbohydrate and protein were studied during exposure to copper and recovery.

MATERIALS AND METHODS

Methods of collection of the clam, rearing, acclimation, selection of size groups and statistical analysis of data were the same as described in Chapter V. Concentration of copper ions used, number of animals, sampled, periodicity of sampling and length of exposure and recovery period were also the same as in the previous experiement.

Samples of digestive gland and adductor muscle tissues were excised from the experimental and control animals at 24, 72, 120 and 168 h after exposure and recovery. The tissues were homogenised in

chilled 5% Trichloroacetic acid. The homogenate was centrifuged and the precipitate dissolved in 3 ml of 30% KOH.

Estimation of total protein was done by the method of Lowry et. al., (1951). For analysis 0.1 ml of the KOH solution was used. Five ml of alkaline copper reagent was then added and shaken well. After 10 minutes, 0.5 ml of Folin's Phenol reagent was added and shaken well. The optical density was read at 500 nm after 45 minutes. From the optical density the corresponding concentrations were obtained from a concentration curve employing bovine serum albumin as the standard.

Estimation of total carbohydrate was carried out following the method of Dubois et. al. (1956). A 0.1 ml sample of the 5% Trichloroacetic acid solution was pipetted into a large test tube containing 0.1 ml of 80% phenol. To this 1.9 ml of distilled water was added bringing the total volume to 2.1 ml. To the sample in the test tube 5.0 ml of concentrated sulphuric acid was added forcefully, to facilitate through mixing. The test tubes were left at room temperature for 30 minutes. After cooling the optical density was determined at 490 nm. From the optical density, the concentration of total carbohydrate in the sample was found out from a standard graph using glucose as the standard. The total carbohydrate was expressed as glucose equivalents.

RESULTS

Total carbohydrate and total protein levels in 0.3, 0.5 and 1.0 ppm exposed clam during exposure to copper and recovery are presented in Table XVIII-XX and Figs. 39-42.

Total Carbohydrate

Clams exposed to 0.3 and 0.5 ppm copper showed a significant

TABLE XVII CHANGES IN TOTAL CARBOHYDRATE LEVEL IN THE DIGESTIVE GLAND OF THE CLAM S. SCRIPTA DURING SUBLETHAL EXPOSURE TO COPPER AND RECOVERY FOR VARYING LENGTHS OF TIME.*

Conc. of Metal ions	Total carbohydrate mg g^{-1} FW							
	Exposure Time				Recovery time			
	24 h	72 h	120 h	168 h	24 h	72 h	120 h	168 h
0.3 ppm	70.27**	73.26	67.19	76.41	81.84	78.26	77.39	75.24
	±3.26	±8.16	±10.57	±6.25	±11.55	±14.01	±9.23	±6.59
0.5 ppm	70.59**	75.82	73.91	79.30	72.54	79.56	84.31	70.23
	±5.17	±9.76	±12.58	±10.35	±7.61	±8.59	±11.54	±10.57
1.0 ppm	73.25	72.87	70.37	70.84**	63.73**	74.26	73.57	77.27
	±7.92	±12.16	±9.11	±5.83	±8.27	±16.96	±7.23	±12.82
Control	77.36	69.75	73.28	81.54	76.30	75.82	79.65	80.56
	±5.27	±11.32	±13.53	±7.64	±5.16	±13.25	±8.93	±12.11

*Each values in a mean ± 1 S.D. (n = 6)

**All values significantly different from the control ($P < 0.05$)

TABLE XVIII CHANGES IN THE LEVEL OF TOTAL CARBOHYDRATE IN THE ABDUCTOR MUSCLE OF THE CLAM S. SCRIPTA DURING SUBLETHAL EXPOSURE TO COPPER AND RECOVERY FOR VARYING LENGTHS OF TIME.

Conc. of Metal ions	Total carbohydrate mg g^{-1} F W							
	Exposure Time				Recovery time			
	24 h	72 h	120 h	168 h	24 h	72 h	120 h	168 h
0.3 ppm	49.27 ±8.31	53.26 ±12.99	41.92** ±6.73	51.26 ±12.19	53.27 ±6.41	58.43 ±2.80	51.19 ±12.33	55.47 ±6.17
0.5 ppm	51.86 ±9.27	46.43 ±10.38	53.57 ±6.25	48.12 ±7.38	55.96 ±12.14	47.29 ±8.95	58.71 ±8.03	47.86 ±16.94
1.0 ppm	39.38 ±7.20	51.44 ±8.07	49.71 ±9.13	47.26 ±10.61	43.82** ±4.26	55.40 ±8.69	52.81 ±12.66	53.35 ±9.41
Control	43.08 ±5.73	48.69 ±6.57	56.41 ±8.59	49.15 ±7.35	54.73 ±6.21	50.84 ±11.65	49.73 ±8.16	53.47 ±13.25

*Each value in a mean ±1 S.D. (n = 6)

**All values significantly different from the controls ($P < 0.05$).

TABLE XIX CHANGES IN TOTAL PROTEIN LEVELS OF THE DIGESTIVE GLAND OF THE CLAM S. SCRIPTA DURING SUBLETHAL EXPOSURE TO COPPER AND RECOVERY FOR VARYING LENGTHS OF TIME.*

Conc. of Metal ions	Total protein mg g^{-1} F.W.							
	Exposure Time				Recovery time			
	24 h	72 h	120 h	168 h	24 h	72 h	120 h	168 h
0.3 ppm	112.31**	117.67**	117.58**	118.47**	118.26**	113.26**	97.36	94.57
	±6.13	±9.27	±17.26	±13.02	±6.14	±9.37	±8.22	±14.17
0.5 ppm	119.27**	115.81**	113.43	101.97	113.33	99.72	95.28	107.69
	±11.30	±11.53	±22.26	±12.17	±9.75	±7.63	±12.17	±9.95
1.0 ppm	109.26	104.79	97.96	103.25	98.72	114.26**	91.76	96.81
	±9.26	±18.23	±13.26	±8.48	±14.79	±6.51	±14.67	±15.81
Control	94.31	97.67	92.63	99.26	104.42	95.65	89.13	102.07
	±13.26	±11.27	±7.23	±7.59	±8.44	±5.29	±14.75	±12.26

*Each values in a mean ± 1 S.D. (n=6).

**All values signficantly different from control ($P < 0.05$)

TABLE XX CHANGES IN TOTAL PROTEIN LEVELS OF THE ADDUCTOR MUSCLE OF THE CLAM S. SCRIPTA DURING SUBLETHAL EXPOSURE TO COPPER AND RECOVERY FOR VARYING LENGTHS OF TIME.*

Conc. of Metal ions	Total protein mg g^{-1} F.W.							
	Exposure Time				Recovery time			
	24 h	72 h	120 h	168 h	24 h	72 h	120 h	168 h
0.3 ppm	94.72 ±14.81	108.53 ±8.26	101.19 ±12.35	110.63** ±6.27	108.74 ±14.19	103.75 ±8.72	96.24 ±12.03	104.26 ±12.58
0.5 ppm	107.56 ±12.57	104.78 ±7.53	97.51 ±10.64	99.06 ±16.18	115.23 ±13.80	98.31 ±17.12	101.17 ±8.48	102.08 ±12.23
1.0 ppm	118.91 ±21.73	98.32 ±12.31	95.17 ±15.84	104.96 ±11.28	93.55 ±16.27	83.23 ±12.58	96.36 ±10.09	97.63 ±14.87
Control	105.37 ±11.53	97.19 ±12.31	92.66 ±9.71	100.29 ±8.12	112.46 ±10.97	98.28 ±6.63	95.92 ±8.27	106.73 ±16.05

*Each value is a mean ± 1 S.D. (n=6).

**All values significantly different from controls ($P < 0.05$)

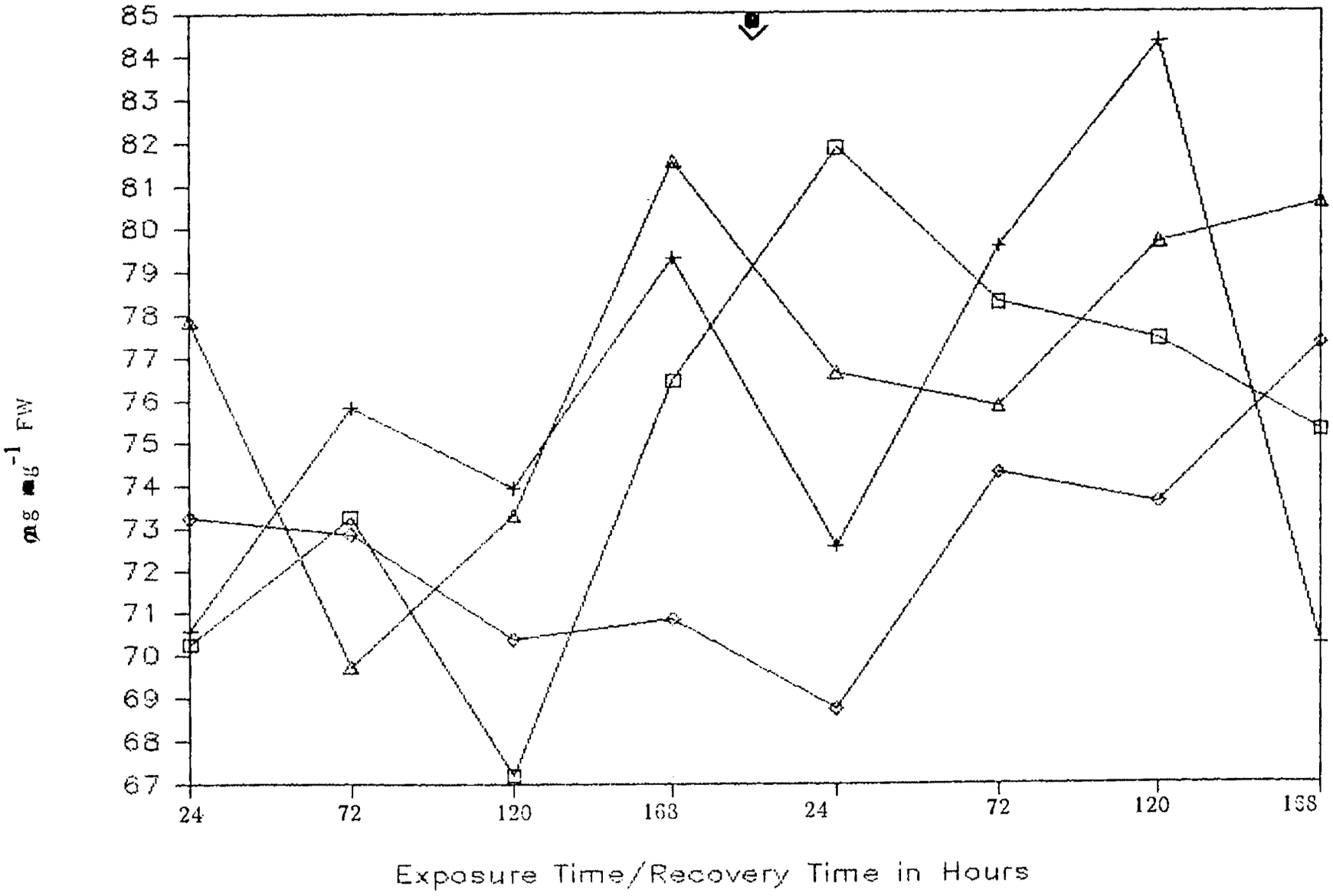

Figure 39. Total carbohydrate levels in digestive gland of S. scripta on exposure to copper and recovery. (Symbols as in Figure 36).

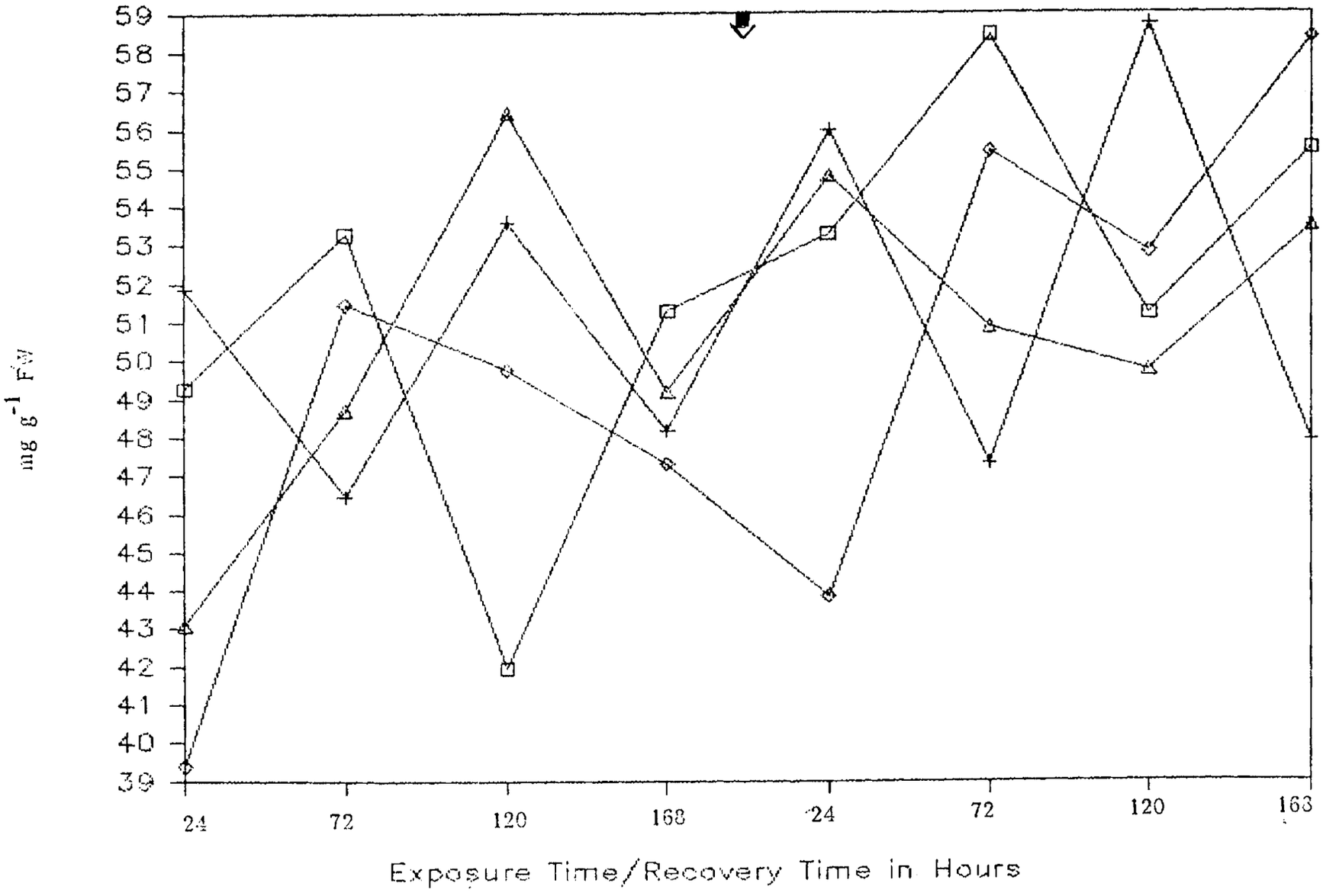

Figure 40. Total carbohydrate levels in adductor muscle of S. scripta on exposure to copper and recovery. (Symbols as in Figure 36).

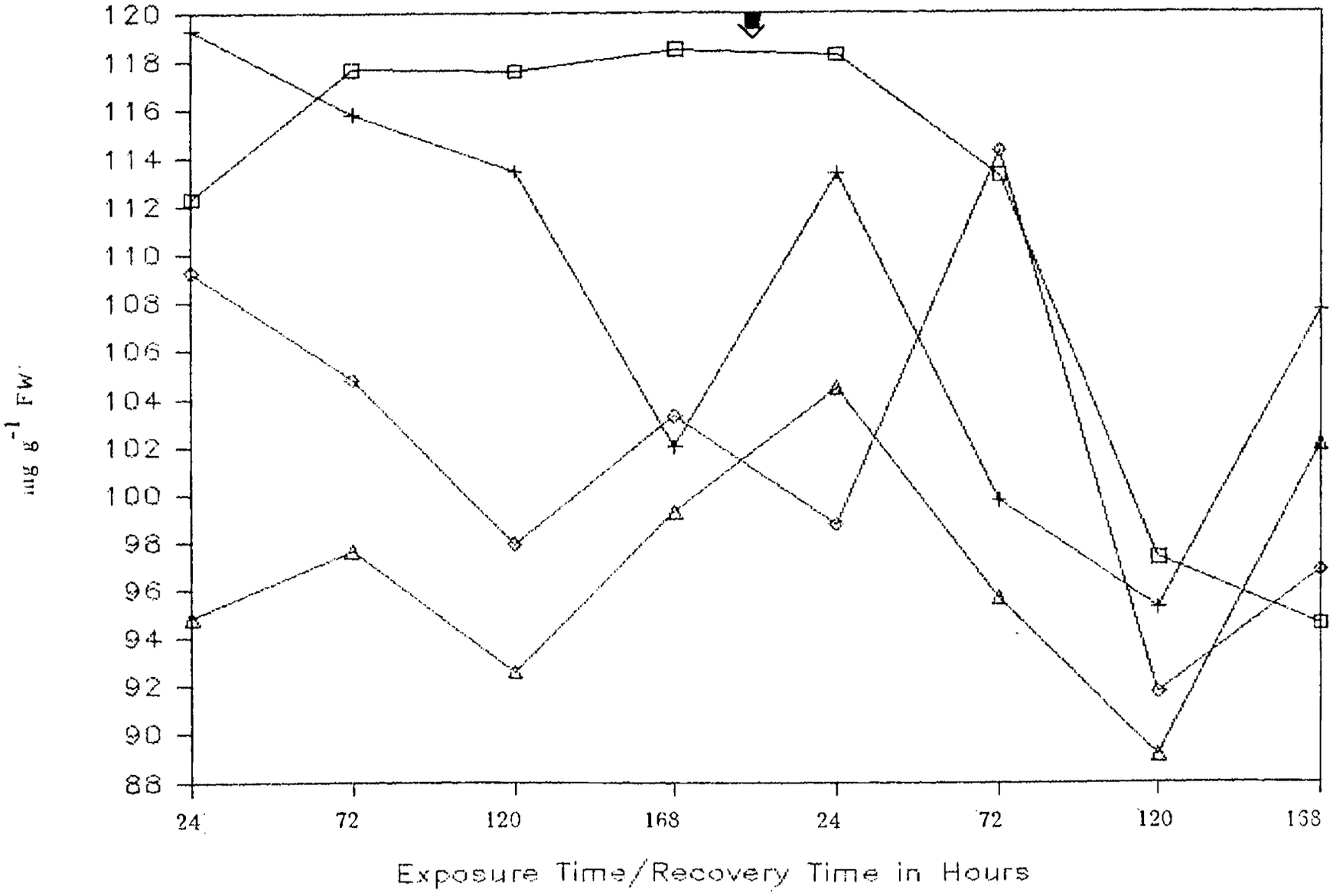

Figure 41. Total protein levels in digestive gland of S. scripta on exposure to copper and recovery. (Symbols

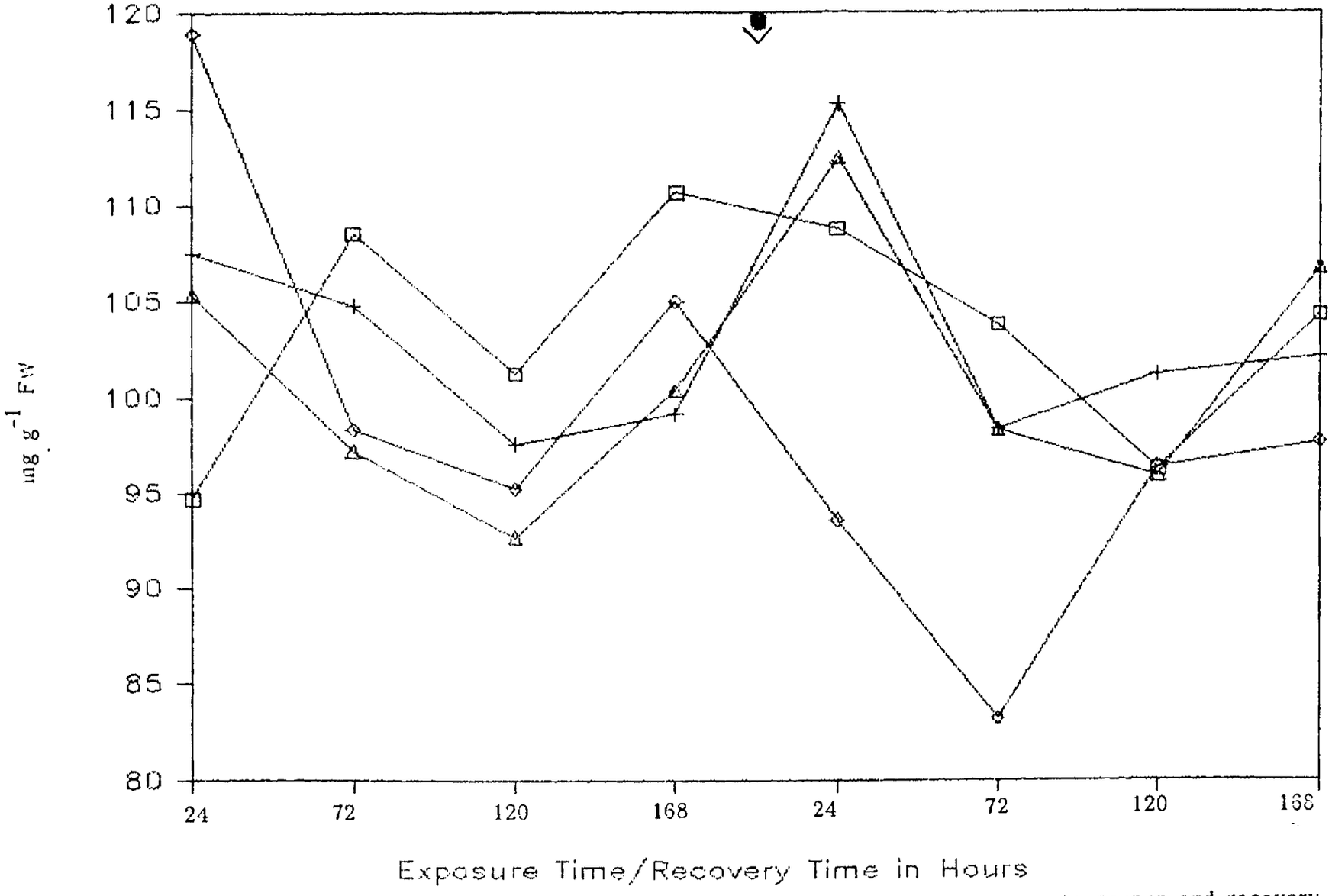

Figure 42. Total protein levels in adductor muscle of S. scripta on exposure to copper and recovery. (Symbols

$P < 0.05$) reduction in total carbohydrate in the digestive gland at 24 h of exposure. Thereafter there was no significant difference from control levels. In 1.0 ppm exposed clams, levels of total carbohydrate showed a decreasing trend from 24 h onwards but was significantly lower ($P < 0.05$) than controls only at 168 h of exposure. Clams exposed to 1.0 ppm showed significantly lower level ($P < 0.05$) of total carbohydrate at 24 h of recovery. Subsequently, levels appeared to increase but were not significantly different from the controls.

Total carbohydrate levels of the adductor muscle of 0.3 ppm exposed clams were significantly lower ($P < 0.05$) than controls at 120 h, showed a marginal increase at 168 h of exposure and continued to increase during recovery. Total carbohydrate levels of 0.5 ppm exposed clams did not vary significantly with control throughout the experiment. Levels of total carbohydrate in 1.0 ppm exposed clams were significantly lower ($P < 0.05$) than the control only at 24 h of recovery, but otherwise showed little variation with control.

Total Protein

Total protein in digestive gland of 0.3 ppm clams showed significantly higher ($P < 0.05$) levels than the control at 24, 72, 120 and 168 h of exposure, and 24 and 72 h of recovery. Clams exposed to 0.5 ppm showed significantly higher ($P < 0.05$) levels than the controls at 24 and 72 h of exposure. Clams exposed to 1.0 ppm of copper did not vary significantly with the control at any time throughout the experiment except at 72 h of recovery.

In the adductor muscle significantly higher ($P < 0.05$) levels of total protein were obtained in 0.3 ppm exposed clam at 168 h of exposure.

At all other sampling times and at all exposure concentrations levels of total protein in the adductor muscle were not significantly different from the control.

DISCUSSION

Bivalves respond to stress by closure of the shell valves thus isolating tissues from the stress factor (Bayne et al., 1976; Manley, 1983). Copper ions are also known to cause respiratory and cardiovascular depression (Scott and Major, 1972; Brown and Newell, 1972; Thampuran, 1986). From studies on oxygen uptake (Chapter V, Table XIV Fig. 36.), it appears that the 1.0 ppm copper exposed clams at 24, 72, 120, and 168 h, and 0.5 ppm exposed clams at 72 and 168 h of exposure show effects of stress. "Self induced anoxia" (Famme and Knudsen, 1983) caused by shell valve closure calls for a shift to anaerobic metabolism leading not to lactic acid accumulation but to succinate, alanine and other metabolites which are less toxic and also generate more energy (de Zwaan, 1983). But there is no Pasteur effect in most bivalves, instead valve closure may signal an overall reduction in metabolic rate to about 5% of the aerobic level (de Zwaan, 1983) Kluytmans et al. (1983) reported levels as low as 2% of aerobic values in Glycymeris pilosa. Shell valve closure also leads to a reduction in pH (Wijsman, 1975), a pronounced fall in the affinity of phosphofructokinase for fructose-6-phosphate, and a general inhibition of catalytic rate at all substrate levels (Ebberink, 1980). Phosphofructokinase is one of the rate limiting enzymes in the glycolytic pathway and depression in the activity of the enzyme contributes

to the reduction of carbohydrate catabolism in anoxia. This would explain the absence of a significant decrease in total carbohydrate levels in the 0.5 ppm and 1.0 ppm copper exposed animals (Table XVII and XVIII; Fig. 39 and 40). A decrease in the total carbohydrate levels of the 1.0 ppm copper exposed clam was evident only at 168 h of exposure in the digestive gland.

At 24 h of recovery after exposure, there is a significant decrease of carbohydrate levels from the control in adductor muscle as well as digestive gland. This may be explained as due to the increased glycolytic flux to meet the high energy demands of the restoration of aerobic conditions (Nichitta, 1983; de Zwaan et al., 1983). In 0.3 ppm exposed clams, where no shell valve adduction or depression in oxygen consumption was noticed there was a significant decrease in carbohydrate levels at 24 h of exposure in the digestive gland and 120 h of exposure in the adductor muscle. In 0.5 ppm exposed clams also a lowering of carbohydrate levels was noticed in the digestive gland at 24 h of exposure. In these groups it can be assumed that with normal filtration activity there is uptake of copper from solution at a rate which does not cause toxicity in the animal (Mclusky and Phillips, 1975). The carbohydrate decrease may have been used to fuel detoxification mechanisms operating within the animal.

An analysis of protein values showed significantly higher than control valves in digestive gland (Table XIX , Fig. 41) at 24, 72, 120 and 168 h of exposure and 24 and 72 h of recovery of the 0.3 ppm exposed clams and 24 and 72 h of exposure of the 0.5 ppm exposed clams. In the adductor muscle (Table XX , Fig.42) values higher than control were found in the 0.3 ppm exposed clam at 168 h of exposure.

In recent years much attention has been focussed on the role of metallothionien-like and other low molecular weight metal-binding proteins in marine organisms. The presence of such protein has been variously suggested as indicating involvement in uptake, storage, transport and elimination of metals. (Engel and Brouwer, 1982; Roesijadi 1982, Viarengo et al., 1985) detoxification of non-essential or excess essential metal (Engel and Brouwer, 1982; Brown et al., 1983) or in routine metabolism. It is probable that the sginificantly higher protein levels in the 0.3 ppm and 0.5 ppm dosed clams may indicate the synthesis of metal binding protein. The digestive gland being the seat of intense biosynthetic activity elevated protein levels in this tissue may indicate its importance in the detoxification of toxic metals. There does not seem to be any significant difference between the protein levels of 24 and 168 h of exposure. Two explanations may be considered. There may be rapid turnover of metal-binding protein from this organ and redistribution to other tissues. Levels of adductor muscle protein are higher than control at 168 h of exposure. It is also possible that there may be a limit to the synthesis of metal binding protein and spillover of metal on saturation of these proteins may be bound on other ligans.

At 120 h of recovery after exposure values of the previously copper exposed clams show no significant difference with controls. Rather than loss of metal from the body, Langston and Zhou (1987a) suggest that for cadmium binding proteins in Littorina sp. there may be a redistribution of the metal through the body.

In the 1.0 ppm copper exposed animals there is a significant increase of protein values at 72 h of recovery in the digestive gland.

It is unlikely that this unexpectedly high value is due to ligand synthesis. However it is possible that it may be due the resumption of normal synthetic activity after anaerobiosis.

Metal binding proteins have been reported from a number of mollusca (Neol-Lambot, 1976; Howard and Nickless, 1977; George et al., 1979; Viarengo et al., 1981). Heavy metal storage and detoxification functions have been postulated for sometime (Kagi and Vallee 1961; Webb, 1971). Metal toxicity occurs due to saturation of the metal binding capacity of the protein followed by spillage (Winge et al., 1973; Brown and Parsons, 1978) ofthe metals from the "detoxification" proteins to sensitive subcellular compartments such as other proteins, membranes and nucleic acids. Binding of metals to such proteins accounts for the apparent indifference of the organism to high metal concentrations in the tissues although there may be a threshold above which animals cannot metabolically control excess metal (Brown et al., 1983; Langston and Zhou, 1987a).

S. scripta has been found to accumulate consistently high levels of copper under a wide a variety of environmental conditions (Thampuran, 1986). High levels of copper (70 ppm DW) were also found by this author in clams from natural populations. The function performed by such high concentrations of this metal is obscure. Roesijadi (1980) suggested that Protothaca staminea maintained a ready supply of freely exchangeable copper to ensure that copper metalloenzymes did not become desaturated. It may also be that high concentrations of the metal may confer some protection against predators by reducing the palatability of the tissues in the same way that copper appears to be utilised by the polychaete Melinna palmata (Gibbs et al., 1981).

The ability to synthesise a pool of metal binding proteins determines the efficiency of the animal as an accumulator as in limpets (Noel Lambot et al,1978; Langston and Zhou, 1987a) with inducible ligand systems. The production of such metal binding proteins in response to an influx of metal seems to be the driving force behind accumulation in such species. Macoma balthica with a poorly developed metal binding capacity was found not to be an accurate indicator of cadmium (Langston and Zhou, 1987b). Ligands that are very specific for particular metals may be considered to be part of a precise physiological pathway. Multipurpose ligands that bind a wide variety of metals are more likely to be part detoxification systems (Simkiss et al., 1982).

A rapidly induced heavy metal-binding protein may confer substantial selective advantages on organisms in possesion of such a system; for survival in polluted environments.

SUMMARY

SUMMARY

Sunetta scripta, occuring in subtidal clam beds of 2.5 sq.km area on the northern side of the entrance to the barmouth, supports a moderately lucrative local fishery. The description of the species conforms to that of Satyamurti (1956) from Madras. A preliminary study indicates a population density of about 420 clams/m^2 but this was during the active southwest monsoon period when mortality is high. Densities are likely to be higher earlier in the year. The substratum is composed of sand, silt, mud and shell fragments; with silt predominating during the south-west monsoon and sand during the rest of the year. Salinity in the clam bed varied from 1.3 x 10^{-3}S during the south-west monsoon to 36.46 x 10^{-3}S during the pre-monsoon. Temperature showed less extreme fluctuations between 24.10 and 31.40°C.

Reproductive patterns in S. scripta were studied over two breeding cycles. The duration of each cycle being approximately 1 year. Sexes are separate and where they can be distinguished a 1:1 sex ratio is maintained. The clams first attain sexual maturity at aproximately 20.6 mm shell length. Gametogenesis in S. scripta generally follows the sequences described by Loosanoff (1937 a and b) and Tranter (1958). Gonad development appears to be adapted to ambient salinity fluctuations with (1) a recovery and slow early gametogenic phase occuring during the low salinity period, (2) a gametogenically active phase associated with the period of rising salinity and (3) spawning activity associated with high and relatively stable salinity. A relationship between important stages in the gametogenic cycle and phytoplankton production in the

clam bed also seems to be indicated. The role of endogenous control by the neurosecretory cycle, genetic variations between population and spawning stimuli such as the presence of conspecific gametes cannot be discredited.

Seasonal metabolic changes in S. scripta are influenced by nutrient levels and gonadal cycles which are in turn modified by hydrographic factors. Generally high metabolite levels are observed during gametogenesis and spawning and lower levels in the spent phase. High productivity in the clam beds obviates the necessity for elaborate nutrient stores in the animal. Thus only the gonad and the digestive gland of all the organs analysed show storage cycles. Very high levels of total carbohydrate occur in the gonad in the gametogenic and early maturing phase. A decline in carbohydrates corresponds to an increase in protein and lipid. In the digestive gland lipid is the predominant reserve store. Utilisation of resources in the digestive gland for gametogenesis and growth is indicated. In the other somatic tissues analysed, protein is the dominant organic constituent, but no well defined cycle of storage and utilisation is evident. Moisture levels in tissues are high in the pre-spawning period and declines during spawning. Calorific values show little fluctation through out the study. Because of the peculiar nature of the nutrient storage in bivalves it is suggested that an estimate of calorific value of a standard animal may have been more indicative of variations. Clam meat is generally consumed only during the monsoon season when availability of sea fish is low. In the post monsoon season the clams are fished as raw material for the lime industry and the meat is usually discarded. Consumption

of meat in the post-monsoon spawning period is recommended because of the high nutritive value of the clam at this time.

Manganese appears to be one of the chief constituents of the ash of S. scripta and shows significant correlations with ash levels. High manganese levels in the clam indicate a deposit feeding habit and metals are probably derived from the sediment. Copper in S. scripta of the metals analysed is next to abundance to manganese. It is suggested that the great variations of copper levels in S. scripta may reflect variations of copper in the environment. Highly significant correlations, between manganese and copper are indicated in S. scripta. Significant correlation also exists between chromium and arsenic. Levels of chromium and arsenic in clam tissues are too low to be harmful to consumers. Nickel and Cobalt were not detected in the samples throughout the study.

S. scripta appears to be highly tolerant to high environmental copper concentrations but biochemical and physiological systems may be affected at concentrations well below threshold levels. In a study of oxygen uptake and lactic acid accumulation in relation to sublethal copper stress, S. scripta shows shell valve adduction at the higher copper concentrations. But anaerobiosis does not lead to a build up of lactic acid in the digestive gland and adductor muscle of exposed clams even after prolonged exposure. During anaerobic fermentation in bivalves, phosphoenolpyruvate-carboxykinase is activated in favour of pyruvate-

kinase and succinate, alanine, propionate etc., are the end-products of glycolytic fermentation. Cu^{++} ions may also act to inhibit the activity of pyruvate kinase. Recovery from anaerobiosis, on transfer of the clam to clean water results in significant lactic acid accumulation. It is suggested that this production of lactic acid may be due to increased metabolic activity to compensate for the "Oxygen debt" and to recharge energy substrates in the tissues. The clams exposed to lower copper concentration show no response to influx of the metal

As an extension of the previous study and under identical experimental conditions, the utilisation of protein and carbohydrate was studied. At the higher concentrations of copper exposure a decline in carbohydrate level is noticed only after prolonged exposure. Absence of a Pasteur effect in bivalves is widely accepted. Lowering of glycolytic flux is effected by reducing metabolic rates of the animal to a fraction of aerobic levels and depression of phosphofructokinase activity by lowering of pH of the internal milieau In the lower concentrations when there is influx of metal into the animal, increase in levels of protein were found. It is suggested that protein synthesis occurs in response to metal influx. An inducible metal binding ligand system may augment metal accmulation in the animal. Metal binding proteins protect the organism from the toxic effects of metals and their presence may explain the high concentrations noticed in S. scripta.

S. scripta appears to be ideally suited for survival in the stressing environmental conditions of the barmouth. Spawning is timed so that larvae hatch in high but stable salinities and have access to a large food supply. High tolerance to extreme salinity variations (Thampuran, 1986)

especially in the younger clams allow the juveniles to tide over the unstable salinity conditions of the early monsoon period. The barmouth represents a highly polluted eco-system receiving industrial, agricultural, domestic, urban and harbour effluents. The tolerance exhibited by S. scripta to high pollutant levels and its ability to overcome stress by prolonged shell closure and anaerobiosis would certainly enhance its chances of survival in situations where a change of environment, may be of a transient nature.

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