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(Indian Council of Agricultural Research)
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4

SOME ASPECTS OF PHYSIOLOGY OF PEARL OYSTER

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INTRODUCTION

Pearl oysters of the genus *Pinctada* occur in different environments, from the intertidal zone to shallow coastal waters as well as at depths of 12-23 m as on the pearl banks of Gulf of Mannar. A knowledge of the physiological characteristics of pearl oyster is necessary for successful rearing in the farm as well as for production of quality pearls. Realising this, several studies have been carried out on the physiology of *Pinctada fucata* of the Japanese seas. Itoh (1976) has studied the relation of oxygen consumption and ammonia nitrogen excreted in the adult oyster to body size and water temperature. Cahn (1949) has reported the role of oxygen during conditioning of the pearl oyster *P. martensii*. The influence of low saline water on growth and mortality of *P. martensii* and quality of cultured pearls has been studied by Katada (1959). In India there have been only a few studies on the physiological aspects of pearl oyster. Variation in the metabolic rate of *P. fucata* and *P. sugillata* collected from two different environments has been studied by Dharmaraj (1983). Alagarswami and Victor (1976) have reported the salinity tolerance and rate of water filtration in *P. fucata*. Seasonal variation of biochemical constituents of pearl oyster in the Gulf of Kutch has been reported by Desai *et al.* (1979). The results on oxygen consumption and salinity tolerance already published (Dharmaraj, 1983; Alagarswami and Victor, 1976) are briefly summarised. Other observations are reported for the first time in this paper.

MATERIAL AND METHODS

For the biochemical investigations pearl oysters (45-60 mm in dorso-ventral measurement) were collected from pearl banks of Gulf of Mannar. The soft parts were dried immediately in oven at 80°C to constant weight, and the water, glycogen, lipid and protein contents were determined. Glycogen was estimated by the method of Oser (1954) and followed by Umbreit *et al.* (1959), lipid by extraction with a mixture of methanol chloroform (Bligh and Dyer, 1959) and protein by semimicrokjeldahl method (Bock and Benedict, 1915). The biochemical constituents of the homogenised animal (without shell) and also of specific organs such as the gonads, hepatopancreas, adductor muscle and mantle in different phases of reproduction were estimated.

Pearl oysters for the experiments on salinity tolerance and rate of filtration were obtained from the natural beds off Tuticorin coast in the Gulf of Mannar (Alagarswami and Victor, 1976). Seawater of experimental salinities was prepared by adding freshwater to normal seawater for dilution and adding fresh common salt collected from the salt pans for increasing the concentration. In all, 16 experiments were carried out in the salinity range of 14.01‰ to 57.99‰ with larger intervals of about 4‰ to 5‰ nearer the normal salinity and smaller intervals of about 1‰ to 2‰ nearer the extremes. The neutral red technique of Cole and Hepper (1954) was employed to study the rate of filtration. The optical density of the solution was read out in a photocolorimeter (Alagarswami and Victor, 1976).

The rate of oxygen consumption was estimated by Winkler's method and expressed in terms of μl of O_2/hour. The post-exposure rate of oxygen consumption was estimated based on wet tissue weight in varying temperature from 26.0°C to 29.4°C (mean 27.9°C). *P. sugillata* from pearl banks (21.8-54.0 mm) and *P. fucata* from pearl culture farm (30.0-54.5 mm) were used for anaerobic study (Dharmaraj, 1983).

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Biochemical Changes in *Pinctada fucata* Associated with Reproduction

Water

The water content in the pearl oyster *P. fucata* was more than 70% in the total tissue as well as in individual organs like gonad, hepatopancreas, adductor muscle and mantle. The mantle had a high percentage of water, being 81.99% wet weight in immature stage, 86.39% in maturing stage, 85.37% in ripe stage, 84.16% in spent stage and 85.85% in resting stage (Table 1).

Table 1. *Percentage of water content in tissues of* Pinctada fucata

Organ	Water content (%)				
	Immature	maturing	ripe	Spent	resting
Total tissue ..	81.13	79.94	79.50	82.10	81.41
Gonad ..	75.41	76.05	77.79	77.69	75.95
Hepatopancreas ..	76.90	73.91	70.88	70.42	70.76
Adductor muscle ..	79.10	79.14	73.55	74.90	74.01
Mantle ..	81.99	86.39	85.37	84.16	85.85

Glycogen

The glycogen level increased sharply from 2.64% to 22.51% in the total tissue and from 6.99% to 22.79% in the adductor muscle from maturing to spent stage. It declined in the resting stage to 13.93% and 12.23% in the total tissue and adductor muscle respectively indicating low metabolic energy demand. During gametogenesis glycogen level rose in gonad from 2.63% to 11.65%, in hepatopancreas from 3.91% to 10.34%, in adductor muscle from 6.99% to 22.79% and in the mantle from 1.91% to 9.97%. In all the organs the glycogen content declined after spawning. The data show accumulation of glycogen reserves in all the organs during gametogenesis and utilization during spawning. Accumulation of glycogen and lipid showed similar trend in the gonad, hepatopancreas and mantle. Biochemical changes during reproductive cycle in *P. fucata* are shown in Fig. 1.

Lipid

In the case of lipid the individual organs did not show wide variation in different stages of reproduction. The level increased gradually in the organs till ripe stage. In the gonad it increased from 3.61% to 8.65%, in the hepatopancreas from 4.97% to 8.22% and in the mantle from 3.18% to 5.60%. In the adductor muscle the lipid level remained almost constant. There was a close relationship between the levels of glycogen and lipid during the reproductive cycle both showing similar trend except in the adductor muscle.

Protein

The protein content was over 50% in the individual organs during maturation of gonads. It declined sharply in the total tissue from 93.43% in immature stage to 66.81% in spent stage and increased in resting stage. In hepatopancreas the level came down to 51.22% from 79.65% between the immature and spent stages and increased to 78.02% in resting stage. In contrast, the protein content of the mantle increased steadily from 60.69% in immature to 82.26% in spent stage and decreased to 76.67% in the resting stage. In gonad and adductor muscle the pattern of protein level was similar showing an increase from 62.91% to 66.67% in the former and 68.99% to 79.19% in the latter during maturation and a decline when the gonad was in fully ripe condition (56.10% to 59.20%) in the respective organs.

Energy Storage and Utilisation

Requirements of reproduction

Growth results when energy gain is in excess of energy expenditure. When the energy acquisition is less, endogenous reserve is used up for maintenance metabolism of the body. Bivalve molluscs may regulate individual components of energy balance in order to maximise net energy gain. Food availability fluctuates in nature with peaks and troughs. Therefore, a common response is to lay down reserves like carbohydrate, lipid or protein during the periods when food is abundant for later utilization. In most marine bivalves glycogen is the major carbohydrate storage reserve. The adductor muscle is a storage organ for glycogen. The steady increase in glycogen level in adductor muscle from 6.99% in the maturing stage to 22.79% in the spent stage is related to maturation and spawning processes. A similar rise noticed in the gonad from 2.63% to 11.65% might be attributed to the requirements of high gamete output. During gametogenesis glycogen in the hepatopancreas also increases from 3.91% to 10.34% which may be mobilised to other organs. The glycogen cycle in the mantle is similar and ranged from 1.91% to 9.97% whicy may be utilised for maintenance metabolism.

Lipid is another source of energy available for various metabolic activities. In all the organs the variations in lipid level closely resemble those of glycogen levels. Among the organs, the gonad showed high percentage of lipid (3.61% to 8.65%) during maturation which is mostly utilised for gametogenesis. In the case of adductor muscle, as it had high glycogen for its activity, the lipid level remained unchanged throughout the reproductive cycle. Storage and utilisation of lipid in the mantle

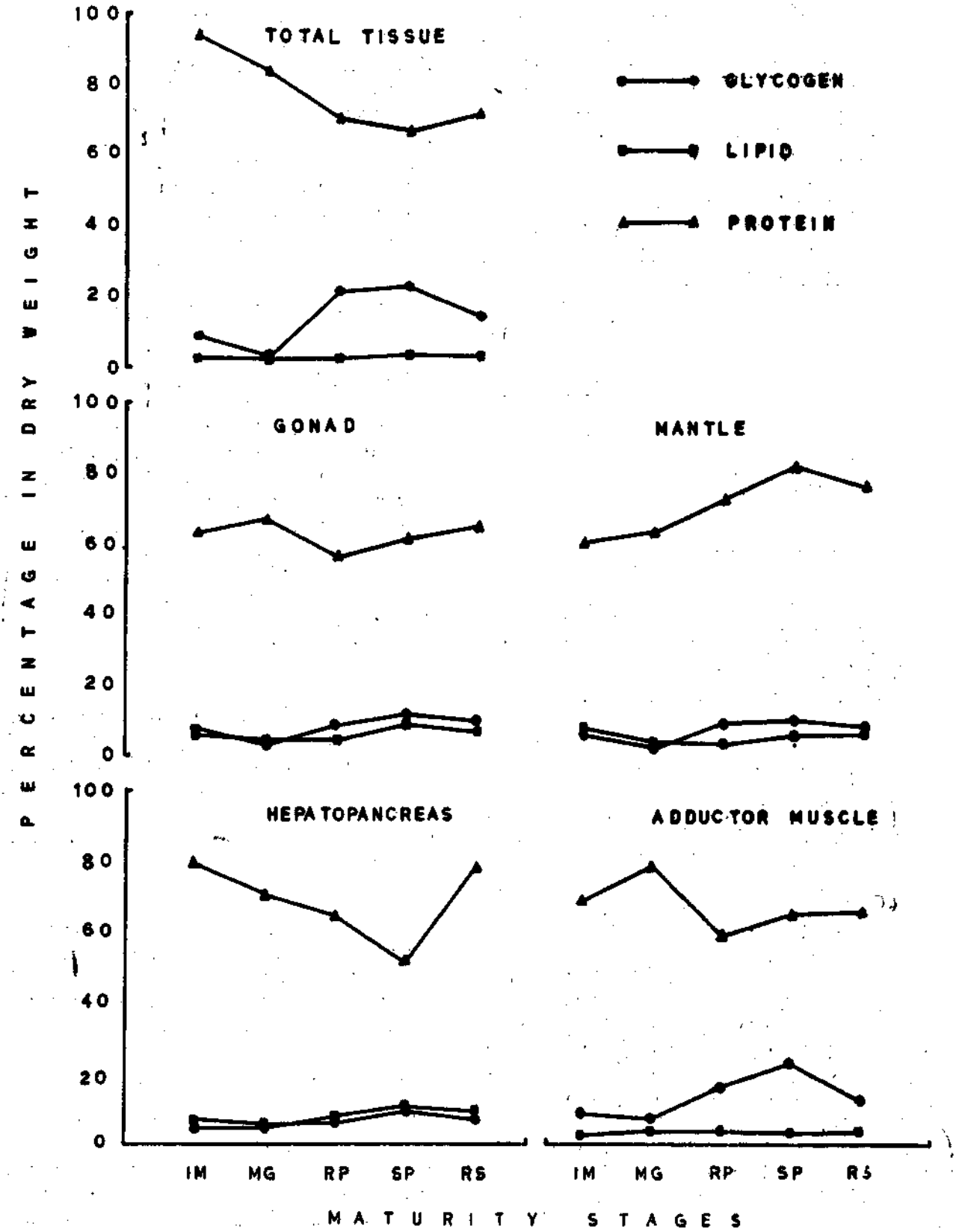

FIG. 1. Biochemical changes during different maturity stages in *Pinctada fucata*. IM—immature; MG—maturing, RP—ripe, SP—spent and RS—resting.

and hepatopancreas are associated with maturation of gonads. When the energy demands for gametogenesis are more, the maximum growth efficiency is less. In some molluscan species reproduction is not initiated until somatic growth ceases, while in others growth continues after the age of first maturity but an increasing proportion of surplus energy is utilised for gametogenesis (Bayne and Newell, 1983).

Glycogen utilisation during starvation

During the periods of starvation the metabolic energy demands must be met from endogenous reserves. Weight loss indicates utilisation of energy reserves for maintaining body metabolism. In *P. fucata* the glycogen content decreased considerably during different stages of starvation as shown below :

Starvation duration	Glycogen %
Day 0 ..	25.23
Day 7 ..	21.66
Day 14 ..	8.07
Day 21 ..	1.66
Day 28 ..	1.32

The level of glycogen showed 14% reduction after 7 days of starvation, 68 % after 14 days, 76% after 21 days and 97.75% after 28 days of starvation. The study showed that *P. fucata* relies to a large extent on their glycogen reserves during starvation.

Oxygen Consumption

The results of experiments on oxygen consumption as reported by Dharmaraj (1983) are summarised here. The rate of oxygen uptake of pearl oyster varies in accordance with the size, physiological state and environmental conditions. Oxygen consumption of *P. fucata* collected from the pearl banks at a depth range of 12-23 m and of *P. sugillata* from the pearl banks as well as from the nearshore waters (depth 0.5-1.5 m) was estimated. In *P. fucata* oxygen consumption of the size group 40-50 mm was 1,339 μl/h, 50-60mm 1,650 μl/h, and 60-70 mm 1,810 μl/h. The rate of oxygen consumption of *P. sugillata* (from pearl banks) of the size groups 20-30, 30-40, 40-50 and 50-60 mm was 255, 345, 588 and 1,045 μl/h respectively and *P. sugillata* (from nearshore waters) of the size groups 10-20, 20-30, 30-40, 40-50, 50-60 and 60-70 mm was 618, 828, 510, 879, 1,170 and 1,361 μl/h respectively. It has been noted that *P. sugillata* of similar size groups collected from two ecologically different conditions exhibited differences in metabolic rate.

Post-exposure rate of oxygen consumption

P. fucata tested for oxygen consumption were kept out of water for different durations such as 6, 9, 12, 18, 21, 24 and 30 hours and the post-exposure rate of oxygen consumption was estimated on wet tissue weight basis in varying temperature from 26.0°C to 29.4°C (mean 27.9°C). Oysters exposed for 9, 12 and 18 hours showed higher rate of oxygen consumption during the first hour on reimmersion and normal rate of consumption was observed from second hour. *P. fucata* exposed to 6 and 21 hours showed less rate of consumption during the first hour itself on reimmersion. On return to seawater after 21 hours of exposure the oysters closed their valves partially throughout the experiment.

Conditioning time and shell activity

The conditioning time, i.e., the time taken by the shellfish to open valves on immersion, in the case of freshly collected pearl oysters ranged from 2 to 65 minutes, whereas the oysters exposed to air for 9 to 21 hours took 0 to 15 minutes. The amplitude of shell activity of nonexposed oysters was accelerated below ambient oxygen concentration. A minimum shell activity was recorded in normoxic conditions. At lower oxygen level the shell opened to a maximum extent, thus exposing the gills fully to the medium. The oysters in well aerated seawater kept their valves open only narrowly.

Tolerance limit in anaerobiosis

P. fucata was found to be less tolerant to anaerobic condition than *P. sugillata*. Mortality of the former in anaerobic medium set in early from 19th hour. In the case of *P. sugillata* from nearshore water the mortality began from 24th hour. *P. sugillata* from the pearl banks were found to tolerate upto 27 hours of anaerobiosis.

Exposure to air

P. fucata exposed to air for 21 hours showed 100% survival at the end of the period but at the end of 24 hours they showed very little shell movement and died. During exposure, oysters showed shell activity upto a period of 18 hours and it ceased on further exposure.

Salinity Tolerance and Rate of Filtration

The results of study on salinity tolerance and rate of filtration reported by Alagarswami and Victor (1976) are summarised here. The pearl oyster, a truly marine form, has been found to tolerate a wide range of salinity from 24‰ to 50‰ for short durations of 2-3 days under experimental conditions. The estuaries and adjoining marine realms along the Indian coast are

influenced by the enormous discharge of river water during the monsoons and are subject to salinity fluctuations.

Experiments on salinity tolerance were conducted in the following salinities (‰) 14.01, 15.03, 15.95, 16.99, 19.02, 24.03, 26.05, 29.03, 34.05, 38.05, 42.97, 45.00, 50.07, 52.08, 55.09 and 57.99. In normal seawater with 34.05‰ salinity all the oysters opened immediately on immersion. In dilutions the average conditioning time showed variations from 16 min. in 26.05‰ and 22 h 10 min in 14.01‰. The oysters opened within the 1st hour in the salinities of 29.03, 26.05 and 24.03‰, during the 6th hour in 19.02‰ and in 19th hour in 15.03 and 16.99‰. On the other hand, in higher concentrations the conditioning time showed a narrow variation from 12 min in 50.07‰ and 4 h 45 min in 57.99‰. In the lowest salinity of 14.01‰ the oysters did not open during the first day but remained open for considerable period during the second day. In dilutions of salinity from 15.03‰ to 16.99‰ the oysters remained open only for a short time during the first day, but for a longer duration on the second day. Between salinities of 19.02 and 29.03‰, the oysters remained open for 47 to 93% of the duration on the first day itself. In the immediate higher salinity concentration of 38.05 to 45.00‰ the oysters remained open for 58 to 99% of the time on the 1st day itself. In the salinity range 50.07-57.99‰ the oysters showed a decrease to 27.33% of the time during the first day and in 52.08‰ the duration was 72%. In this higher range of salinity the oysters remained open for 78 to 100% of the time during the second day. Shell activity was maximum in the normal seawater. In dilutions and in higher salinities the shell activity decreased gradually.

The rate of mortality was 100% among the oysters kept in the salinity of 57.99, 55.09 and 14.01‰, 67% in the salinity of 52.08‰, 50% in the sainity of 15.03‰ and 10% in the salinity of 15.95‰.

The rate of filtration was studied in the salinities of 13.98, 19.90, 24.10, 34.23, 43.97, 49.90 and 56.96‰. Filtration was maximum in normal seawater being 33.7% at the end of 1 hr, 52.1% at the end of 2 hrs, 76.1% at the end of 4 hrs and 92.6% at the end of 8 hrs. In the two salinities of 13.98 and 19.9‰ the rate of filtration was poor, being 13.7% and 22.9% respectively at the end of 4 hrs. In the salinity of 24.1‰ filtration was 51.3% at the end of 4 hrs. In the higher salinity of 56.96‰ the removal of neutral red was 41.8% at the end of 4 hrs and in 43.97‰ it was 49.5% for the same duration. At 49.9 ‰ the removal was 53.7% at the end of 4 hrs.

The mortality rate at the end of 48 hrs was 3 out of 5 oysters in the salinity of 19.90‰ and was total in the salinities of 13.98‰ and 56.96‰. In other salinities there was no mortality.

Byssus Formation

Byssus is secreted by the byssal gland and assembled by a specialised region of the foot. It consists of three parts: the root, stem and disc. The root is deeply embedded in the base of the foot and resembles an artist's paint brush. It consists of small fibres of flesh colour which emerge from different points and converge to form a single stem. The arrangement of small fibres facilitates better anchorage. Attached to the root is a tanned acellular stem which is thick green in colour and forms a thread. The disc at the end of the thread is meant for adhesion to the substratum.

When a new thread is formed, the foot is extended out of the shell with the tip touching the substratum. During this quiescent period, components of the thread are secreted into the byssal groove and moulded there in the form of a thread by muscular contractions. After a brief time in this position the animal withraws the foot allowing the new thread to remain attached.

Formation of byssus thread in the spat and adults upto 30 mm in size (DVM) was rapid. When the byssus threads were detached, immediate extrusion of foot was seen leading to the formation of new byssus thread, even during day time. In the oysters of 30 mm and above, though their threads were detached they did not extrude the foot during day time. Movement and attachment of these oysters took place mostly during night.

Some oysters with byssus threads *in situ* first ejected the existing threads and developed fresh threads. In other cases attachment was seen even before the old byssus threads were cast off. Byssal attachment takes place always with the right valve down to the substratum. Among oysters of 20.0-25.3 mm, 30.0-36.7% attached within 5 hrs during day time and the rest during night. But among those of 33.5-54.2 mm, none attached during day and 78.8-93.8% attached during night. The number of byssus threads in oysters ranged 10-28.

Discussion

In most marine bivalves the major biochemical components such as glycogen, lipid and protein are chiefly related to reproductive cycle. In *Mytilus edulis* the seasonal cycle of storage and utilisation of

glycogen is closely linked to reproduction (Bayne, 1976). The biochemical components of the pearl oyster *P. fucata* showed a definite pattern of changes in the individual organs during different reproductive phases. The trend in glycogen levels indicated a uniform pattern in the gonad, hepatopancreas, adductor muscle and mantle. The increase in the glycogen content during gametogenesis was kept up till spawning time. Vahl (1981) reported that mature individuals store more glycogen and this is done at the expense of rapid growth. Desai *et al.* (1979) reported that in *P. fucata* of Gulf of Kutch glycogen and lipids are stored during prespawning period and they decline in postspawning season. Presence of high glycogen content in the adductor muscle showed its glycolytic nature. According to Suryanarayanan and Alexander (1971) the slow muscle of *Lamellidens corrianus* has a low glycogen and high lipid content than the fast muscle and it may utilise lipids as the main fuel for oxidative metabolism.

Mane and Nagabhushanam (1975) attributed the low lipid content in several bivalves to their sedentary way of life and their capacity to survive anaerobic conditions. In the present study the lipid in the adductor muscle of *P. fucata* was low and was maintained almost constant. Probably the high glycogen content in the muscle may provide energy for oxidative metabolism during activity. Zandee *et al.* (1980) suggested that in *Mytilus edulis* lipids are the main source of energy production in growing mussels and may be utilised during gametogenesis. Voogt (1972) found that all bivalve species investigated by him are able to synthesise fatty acids from acetate. Teshima and Patterson (1981) showed that the American oyster, *Crassostrea virginica* is able to synthesise sterols from acetate. In *P. fucata* the trend of quantitative changes in glycogen and lipid was the same. It is probable that they are associated with the reproductive cycle.

Starvation experiments showed lipid to be the most important reserve in *C. gigas* (Riley, 1976). Nagabhushanam and Dhamne (1977) reported that in *Paphia laterisulca* lipid contents remained constant after starvation for twelve days, glycogen content decreased and water content increased. Small individuals with a relatively low glycogen reserve increase considerably their protein catabolism during starvation, whereas larger individuals rely to a greater extent on their relatively high glycogen stores (Bayne, 1976). In our study the glycogen content of *P. fucata* declined progressively during different stages of starvation. The reduction in the glycogen level has gone to the extent of 97.75% after starvation for 28 days.

The biochemical components of the byssus thread are derived from various exocrine glands in the foot. These glands are named as (1) white or collagen gland, (2) mucous gland, (3) phenol gland and (4) accessory gland (Brown, 1952; Allen *et al.*, 1976). From the functional point of view the collagen gland secretes the major fibrous components of the thread (Vitellaro-Zuccarello, 1980). The gland secretes a substance that may promote the function of a colloidal gel when mixed with other components (Tamarin *et al.*, 1976). The phenol gland produces the adhesive substance for the disc as reported by the same authors. The role of the accessory gland is not known.

The byssus of *Mytilus* and *Modiolus* appears to contain collagen as suggested by the high glycine content and presence of hydroxyproline. As the pearl oyster *Pinctada alba* has high glycine 242 residues per 1000, the presence of collagen in the fibres is indicated. Stary and Andratschke (1925) showed that the material in *Mytilus* and *Pinna* byssus threads was a sclerotised protein.

The average dissolved oxygen content in the bottom water of pearl banks was 4.37 ml/l and that of the pearl farm in harbour 4.77 ml/l. *P. sugillata* collected from these two areas showed variation in the rate of oxygen consumption. The oysters from the pearl banks exhibited low metabolic rate characteristic of benthic species. Further the oysters from the pearl banks withstood anaerobic condition for 27 hrs whereas the same species from nearshore waters could tolerate only for 24 hrs. *P. fucata* survived upto 19th hrs. in anaerobic medium. The species withstood 21 hrs of exposure to air. It is probable that in aqueous medium the metabolic end products might readily be released which in turn caused deleterious effect on the oysters. Korringa (1952) reported similar feature in *Gryphaea virginica*. In a practical situation, *P. sugillata* were transported to a distance of 1,896 km from Tuticorin to Dhauli in a duration of 43 h without mortality by covering them with wet jute piece but occasionally immersing them in seawater. The survival time of wedge clam *Donax cuneatus* exposed to air at room temperature (30° C) was found to be 69 h and that of *D. faba* in the same conditions 94 hrs (Rao and Kutty, 1968).

In *P. fucata* the oxygen consumption of the size group 40-50 mm was 1339 µl/hr of 50-60 mm 1650 µl/hr and of 60-70 mm 1810 µl/hr. Oxygen tension of the medium should be ascertained while conditioning the oysters before nucleus implantation for pearl production. The information on the relationship between the shell gaping and oxygen tension in the medium has much

value in the controlled culture of oysters in the laboratory. The measure of shell gape has been recognised as an indicator of the level of oxygen in the medium and it is inversely proportional to oxygen level.

Experimental results on salinity tolerance and the low saline conditions observed in Veppalodai farm showed the range of salinity which the pearl oyster could survive (Alagarswami and Victor, 1976). The tolerance limit of *P. fucata* appears to extend over a wide range of 24 and 50‰ at least for 2-3 days. Katada (1959) found that the unoperated oysters remained unaffected in the density ranging from 6.53 to 21.90 during the first 24 hrs, whereas the operated oysters could remain so only for the first 12 hrs. He concluded that the density of 15.00 which is nearly equal to a salinity of 20.65‰ was the safe limit for the Japanese pearl oyster. According to Alagarswami and Victor (1976) a salinity of about 24‰ appears to be the safe limit in dilutions for *P. fucata* of Gulf of Mannar. The European mussel *Mytilus edulis* occurs in salinities ranging from 30 to 10‰ and even as low as 4‰ in some areas and the American oyster *Crassostrea virginica* lives for weeks at salinities just above freshwater (Gunter *et al.*, 1973).

In *C. virginica* a sharp reduction in salinity from 27 to 20, 15, 10 and 5‰ decreased the pumping rate to 24, 89, 91 and 99.6‰ respectively for approximately 6 hrs after transfer (Loosanoff, 1953). Decreased filtration rate has been observed in the pearl oyster in lower salinities and it is less than 25% in salinities of 14‰ and 20‰ even after 4 hrs. In *Meretrix casta* the rate of filtration was adversely affected both at low and high salinities (Durve, 1963). In the clam the filtration rate remained fairly high even at salinities of 45 and 56‰ and became erratic at 64‰. In *P. fucata* the rate of filtration was high in the higher salinity range.

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