STUDIES ON LARVAL NUTRITION IN THE PEARL OYSTER **PINCTADA FUCATA** (GOULD)

THESIS SUBMITTED
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BY
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APRIL 1987

Declaration

I hereby declare that this thesis entitled " STUDIES ON LARVAL NUTRITION IN THE PEARL OYSTER PINCTADA FUCATA (GOULD) " has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar titles or recognition.

Anuradha Krishnan

Cochin - 682 031,
April 1987.

[ANURADHA KRISHNAN]

Certificate

This is to certify that the thesis entitled " **STUDIES ON LARVAL NUTRITION IN THE PEARL OYSTER** PINCTADA FUCATA **(GOULD)** " is the bonafide record of the work carried out by **Miss ANURADHA KRISHNAN** under my guidance and supervision and that no part thereof has been presented for the award of any other Degree.

Cochin - 682 031,
April 1987.

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PREFACE

India has, in the recent years, made rapid strides in mariculture. Technologies have been developed for production of several species of finfish, crustaceans, molluscs and seaweeds. The Centre of Advanced Studies (CAS) in Mariculture (ICAR/UNDP/FAO) was established at the Central Marine Fisheries Research Institute (CMFRI) for carrying out research in the several disciplines concerned with mariculture and also as a centre for post-graduate education in this specialisation.

Kum. Anuradha Krishnan joined the CAS in Mariculture as a Senior Research Fellow in the second batch of the Ph.D programme in October, 1981. During the first semester, she underwent a comprehensive course work in mariculture and, during the second semester, she made an in-depth study of the optional subject 'Nutrition of Molluscs'. She has passed the Ph.D. qualifying examination of the University of Cochin.

The subject 'Studies on larval nutrition in the pearl oyster Pinctada fucata (Gould)' was chosen by the candidate for research based on practical considerations. 'Seed' availability is one of the most important

pre-requisites for any form of aquaculture. It is well known that natural production of seed is most undependable for commercial farming operations. Hatchery technologies have been developed to mitigate the problem of seed availability and for other advantages of producing improved strains. The CMFRI achieved a major breakthrough in developing the techniques of hatchery production of pearl oyster seed, late in 1981. Initially, the different aspects were developed on an empirical basis. But it became necessary to make further detailed investigations on each subsystem of the hatchery to standardise the larval rearing techniques. Knowledge on the nutritional requirements of pearl oyster larvae was considered essential for proper hatchery management. Since the hatchery technology for pearl oyster is adoptable for several other species of tropical marine bivalves, the results of this study would have a wider implication and usage.

The CMFRI has established the research projects on pearl culture and pearl oyster hatchery at the Tuticorin Research Centre with all facilities. The experimental work of the present thesis was therefore carried out at Tuticorin. Certain other aspects such as estimations of radioactive larval and algal sample and microanalysis of biochemical composition of pearl oyster larvae were carried out at the laboratories of the headquarters of CMFRI at Cochin.

The present work has yielded a great deal of information on nutrition of pearl oyster larvae. The results presented may be used effectively and with advantage in improving the larval rearing system with specific reference to nutritional aspects. It is also hoped that this first comprehensive study on pearl oyster larval nutrition would stimulate further detailed investigations on many of the finer aspects of tropical bivalve larval nutrition.

ACKNOWLEDGMENTS

I wish to express my deep gratitude to Dr. K. Alagarswami, Senior Scientist, Central Marine Fisheries Research Institute, my Supervising Teacher, whose unstinted guidance, sustained interest and constructive criticism enabled this work to materialise. To Dr. E.G. Silas, former Director, and Sub-Project Co-ordinator, CAS in Mariculture, CMFRI, I wish to express my sincere gratitude for extending excellent laboratory facilities and for his valuable advice and suggestions. It is my privilege to thank Dr. P.S.B.R. James, Director, CMFRI, for extending all facilities for completing this thesis.

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I wish to record my sincere thanks to Dr.C.P.Gopinathan, Scientist, CMFRI, for helping with the microalgal cultures and

in the culturing of the mixed phytoplankton. I acknowledge with thanks the help rendered by Shri D. Kandaswami, Scientist, CMFRI, in the preparation of the artificial diet and for his useful suggestions and Shri. M. Srinath who helped me in the statistical analysis of the data. My thanks are also due to scientists, Shri A. Chellam, Shri S. Dharmaraj, Shri T.S. Velayudhan and Shri M.E. Rajapandian and Staff of the Tuticorin Research Centre of Central Marine Fisheries Research Institute, for their timely help. To Ms. Ammini Joseph, and fellow scholars Mr. C. Gopal, Mr. Subhash Soni, Ms. Elizabeth Joseph and Mr. G.P. Mahobia, I express my sincere thanks for their valuable suggestions and their timely help.

I thank the Indian Council of Agricultural Research, New Delhi, for offering me the Senior Research Fellowship to carry out this work at the ICAR/UNDP/FAO Centre of Advanced Studies in Mariculture, Central Marine Fisheries Research Institute, Cochin.

STUDIES ON LARVAL NUTRITION IN THE PEARL OYSTER *PINCTADA FUCATA* (GOULD)

C O N T E N T S

CHAPTER 1

GENERAL INTRODUCTION

Dependence on the naturally occurring seed imposes severe restrictions, such as unpredictability of supply and heterogenity of stocks, on aquaculture production. In countries with advanced aquaculture systems, commercial hatcheries meet part of the total seed requirements for both finfish and shellfish production. Hatcheries for the molluscan shellfish such as oyster, clam, scallop and abalone function in U.S.A., Canada, U.K., France and Japan (Loosanoff and Davis, 1963a; Davis, 1969; Flassch *et al.*, 1975; Imai, 1977).

Hatchery technology for molluscs has been developed to the present level with the experience of over a period of about a century. Brooks (1880) worked on the development of eggs and early larval stages of the American oyster, *Crassostrea virginica*. Ryder (1883) and Winslow (1884) reared the larvae of the above species but could not obtain metamorphosis. Wells (1920) for the first time succeeded in rearing the American oyster larvae to metamorphosis, using 'clarified' seawater. His later works (1927) led to the development of the Wells-Glancy method of larval rearing. Simultaneous to the work of Wells, Prytherch(1924)

reared American oyster larvae up to spat stage using no supplementary food, but a running seawater system.

In tank culture experiments, Cole (1937, 1938) found that the essential factor for successful rearing of the European oyster, Ostrea edulis larvae was the character of the food organism. Larvae utilised as food only minute naked flagellates of the Chlamydomonadaceae, Cryptomonadaceae and Chrysomonadaceae, but were unable to utilise non-motile species with cellulose. For O. edulis, Bruce et al.(1940) were the first to develop satisfactory laboratory techniques for larval rearing. Greenish-yellow or golden-brown flagellates were found to give the most successful results in spat setting (Bruce et al., 1940). These flagellates were later identified as Isochrysis galbana and Pyramimonas grossi (Ukeles, 1971). The work of Bruce et al. (1940) ushered in an era of intensive work on microalgae as bivalve larval food in several laboratories in U.S.A., U.K. and Japan.

An important milestone in the development of hatchery technology was the success in achieving out-of-season spawning and delayed spawning of C. virginica by Loosanoff (1945) at the Milford laboratory. This greatly enhanced the scope of larval rearing work by making larvae available throughout the year. Besides, an effective method for mass culture of phytoplankton under virtually sterile conditions was developed

using cultures of M. Parke's collection, Plymouth. With the identification of Isochrysis galbana and Monochrysis lutheri as good foods for oyster larvae, successful rearing of oyster larvae was achieved. Techniques that were found suitable for C. virginica larvae were soon found applicable with suitable modifications for a host of other bivalve species (Loosanoff and Davis, 1963b).

In U.K., the initial work of Cole (1937, 1938) and Bruce et al. (1940) was continued by Walne (1956a, 1963, 1974). His studies were largely confined to observations on the food value of several species of phytoplankton to larvae of O. edulis.

In Japan, Hori and Kusakabe (1926) were among the first to rear C. gigas larvae up to metamorphosis using the non-motile alga Chlorella pacifica. Several species of oysters, clams, scallops and cockles were spawned and their larvae reared to metamorphosis (Imai et al., 1950, 1954; Imai, 1977). Initially the larvae were reared on the colourless flagellate Monas sp. cultured on bacteria (Imai and Hatanake, 1949). Later, mixtures of flagellates Isochrysis galbana, Pavlova lutheri and the diatom Chaetoceros calcitrans were routinely employed for larval rearing (Imai, 1977).

The recognition, isolation and mass culture of the naked flagellates, especially I. galbana and P. lutheri,

as consistently 'good' foods paved the way to successful larval rearing of a variety of bivalve species like the soft clam Mya arenaria (Stickney, 1964), quahog, Mercenaria mercenaria, oyster, C. virginica (Matthiessen and Toner, 1966), silverlip pearl oyster, Pinctada margaritifera (Minaur, 1969), bay scallop, Pecten maximus (Gruffydd and Beaumont, 1972), clam, Scapha subcrenata (Iwasaki et al., 1971), the Japanese pearl oyster, P. fucata (Wada, 1973) and the clam, Tapes japonica (Sunderlin et al., 1976) around the world. The majority of these studies have been qualitative and several species of microalgae were identified as having good, moderate or poor food value. Ukeles (1971, 1975), Ryther and Goldman (1975), Kinne (1977) and Sastry (1979) have reviewed works on bivalve larval nutrition. Some investigations were also carried out in making quantitative estimates of feeding (Ukeles and Sweeney, 1969; Rhodes and Landers, 1973; Walne, 1974; Riisgard et al., 1980, 1981; Gerdes, 1983).

These studies clearly demonstrated that algae differed in their nutritional value. Bivalve larvae were shown to exhibit both quantitative and qualitative regulatory mechanisms (Loosanoff and Davis, 1963b). As compared to single species, mixtures of microalgae yielded better results (Kanno, 1965; Matthiessen and Toner, 1966; Gruffydd and Beaumont, 1972; Ukeles, 1975).

The finding that adult oysters are capable of absorbing dissolved nutrients across the gill-surface (Collier et al., 1953; Stephens and Schinske, 1961; Gillespie et al., 1961) led to several investigations on the uptake of dissolved nutrients by bivalve larvae (Davis and Chanley, 1956; Chanley and Normandin, 1967; Rice et al., 1980; Manahan and Crisp, 1982, 1983). Alternate sources of nutrition, which would solve the problems that mass culture of algae present, have been sought after for many years. Loosanoff et al. (1951), Carriker (1956), Hidu and Ukeles (1962), Loosanoff and Davis (1963b) and Chanley and Normandin (1967) attempted to rear bivalve larvae on a variety of diets like yeast cells, pablum flakes, frozen algal cells, ground preparations of Ulva lactuca and dried and freeze dried algae but met with little success. Likewise, Walne (1974), experimenting with spray-dried Chlorella, vacuum-dried P. lutheri and freeze-dried I. galbana reported poor growth of oyster larvae.

With the advent of the technique of microencapsulation which is a method of supplying artificial diets to filter-feeding larvae, avoiding the problems of bacterial contamination and breakdown of the diet (Gabbott et al., 1975), several attempts have been made to culture shellfish larvae on microparticulate diets. Biphasic diets (Langdon, 1983), nylon-protein microcapsules (Chu et al., 1982) and micro-

binding diets (Teshima et al., 1982) have been investigated for their acceptability to bivalves.

However, microalgae remain the best known accepted diet for bivalve larvae. Several investigators have attempted to explain the difference in food value of different species of microalgae by analysing the gross chemical composition of the algae (Parsons et al., 1961; Riley and Roth, 1971; Walne, 1974). With the recognition of the role of lipids and fatty acids in bivalve larval development (Millar and Scott, 1967; Helm et al., 1973; Holland et al., 1975; Holland, 1978; Waldock and Nascimento, 1979), the fatty acid profile of several species of algae were investigated (Ackman et al., 1968; Chuecas and Riley, 1969). Differences in food value of microalgal species have also been related to their amino acid and carbohydrate profiles (Webb and Chu, 1983).

Other than food, an important requisite for standardising larval rearing is the identification of the optimum temperature and salinity range. Monofactorial (Walne, 1956a; Davis and Ansell, 1962; Bayne, 1965; Helm and Millican, 1977) and multivariate experiments (Davis and Calabrese, 1964; Lough and Gonor, 1973a, b; Siddall 1978a, b) have been carried out to determine these factors.

As hatchery techniques involve the rearing of large number of larvae in a closed system at high densities, the conflicting role of bacteria, both in causing diseases and as a source of nutrition, has been investigated (Davis, 1953; Carriker, 1956; Walne, 1956b; Hidu and Tubiash, 1963; Tubiash et al., 1965; Brown, 1973; Murchelano et al., 1975; Brown and Losee, 1978; Martin, 1979; Elston, 1979a, b; Elston et al., 1982).

The developments in bivalve larval rearing and establishment of hatcheries for commercial production of seed have taken place in temperate waters in countries such as U.S.A., Canada, U.K., France and Japan which have traditionally exploited the oyster, clam and abalone resources as an important item of food. In these areas, the shortfall in wild seed supply and large-scale mortality among cultivated stocks due to disease outbreaks have necessitated such developments in artificial propagation using hatchery techniques. There has been no parallel development in the tropics, as molluscs as food source were not so important in the dietary habits of the people and the little exploitation on subsistence scale did not form any threat to the resources. But with changes in food habits, development of widespread tourism and finding of markets for tropical seafood in the developed countries, the situation has changed leading to greater awareness of the importance of molluscan resources and their increased exploitation in the tropics.

The workshop on "Bivalve culture in Asia and Pacific" (Davy and Graham, 1982) highlighted this awareness and significance and discussed the recent experimental work on bivalve larval rearing and spat production in the region. In India, Rao et al. (1976) and Rao (1980) reared the larvae of the Indian mussel, Perna viridis under laboratory conditions. AQUACOP (1977) reported the mass production of the spat of C. gigas in a tropical environment. Later, AQUACOP (1979, 1983) reported success in the mass culture of the green mussel, Mytilus viridis in French Polynesia. In India, for the first time, pearl oyster larvae were successfully raised and reared to the spat stage and beyond under laboratory conditions (Alagarswami et al., 1983b,c) which paved the way for development of hatchery techniques for the production of molluscan seed.

Technical feasibility of culture of economically important species of molluscs has been established in India: for pearl culture (Alagarswami and Qasim, 1973; Alagarswami, 1974), oyster culture (Nayar and Mahadevan, 1983), mussel culture (Appukuttan, 1980; Kuriakose, 1980; Nayar and Mahadevan, 1980; Appukuttan and Nair, 1983) and clam culture (Narasimham, 1980, 1983). But, seed availability has been identified as one of the major constraints in establishing commercial operations. The breakthrough achieved in controlled breeding and spat production for the

pearl oyster, Pinctada fucata (Alagarswami et al., 1983a, b, c) has led to similar success in Crassostrea madrasensis (Nayar et al., 1982. 1984), Perna viridis (Rangarajan, 1983) and P. indica (Appukuttan et al., 1984).

Detailed investigations in bivalve larval nutrition have been felt essential to put this recently developed hatchery technology on a firm footing. The present work was taken up to fulfil this important need. The study on the larval nutrition of the pearl oyster, Pinctada fucata, included the nutritional evaluation of seven algal species both singly and in combination; dissolved nutrients and artificial diets; role of environmental parameters, effects of aeration, antibiotics and the mode of delivery of food; assimilation using C^{14} - labelled phytoplankton; and biochemical changes during larval development.

CHAPTER 2

EXPERIMENTAL LARVAL REARING SYSTEM

Studies on larval nutrition of the pearl oyster Pinctada fucata were carried out over a period of two years from October 1982 to September 1984, using several broods of larvae. The experiments were conducted at the Tuticorin Research Centre of the Central Marine Fisheries Research Institute. Certain aspects of biochemical investigations and food assimilation estimates were made using the facilities of the Institute at Cochin. The general experimental conditions of larval rearing are presented in this chapter. The detailed methodology of different studies are presented in the relevant chapters.

Broodstock

Pearl oyster broodstock was obtained either from the pearl banks of the Gulf of Mannar during October-April, being the traditional season of oyster collection, or from the pearl oyster farm of the Central Marine Fisheries Research Institute in the Tuticorin Harbour basin. Oysters in the fully mature stage were brought to the laboratory and maintained in 200-l fibreglass tanks holding seawater. Oysters used for spawning were in the size range of 40-60mm,

weighing between 10-30g. Water was changed daily and the broodstock was fed to saturation level with a mixed culture of phytoplankton.

Spawning

Larvae were obtained either through natural or induced spawning in the laboratory. Mature pearl oysters spawned spontaneously on many occasions. Oysters were held in batches of 10-15, with fewer males than females. Induced spawning through pH elevation was resorted to when natural spawning failed to occur. The pH of the medium was raised to 9 using TRIS (Hydroxymethyl) methylamine buffer (Morse et al., 1976; Alagarswami et al., 1983a). The oysters were transferred to fresh sterilised seawater of ambient pH (8.1-8.3) once spawning was initiated. The manner of ejection of gametes enabled distinguishing males from females (Alagarswami et al., 1983a) and presence of both sexes was ensured in the above transfer.

Fertilisation

When spawning was completed, the pearl oysters were removed and only the gametes remained in the spawning tanks. After 2 hours, when fertilisation was complete and the fertilised eggs had settled down, the supernatant water containing excess sperm suspension was decanted.

Adding fresh seawater, the process was repeated thrice to remove excess sperm. The remaining water was sieved through a 60 µm mesh screen to remove the debris. The fertilised eggs were maintained in sterile seawater without aeration. About 20-24 hours from fertilisation, straight-hinge veliger larvae appeared in the tanks. These free-swimming larvae were siphoned gently through a 30 µm mesh sieve and transferred to 5-l beakers containing sterile seawater. Larvae for experimental rearing were removed from this general stock.

Quality of seawater

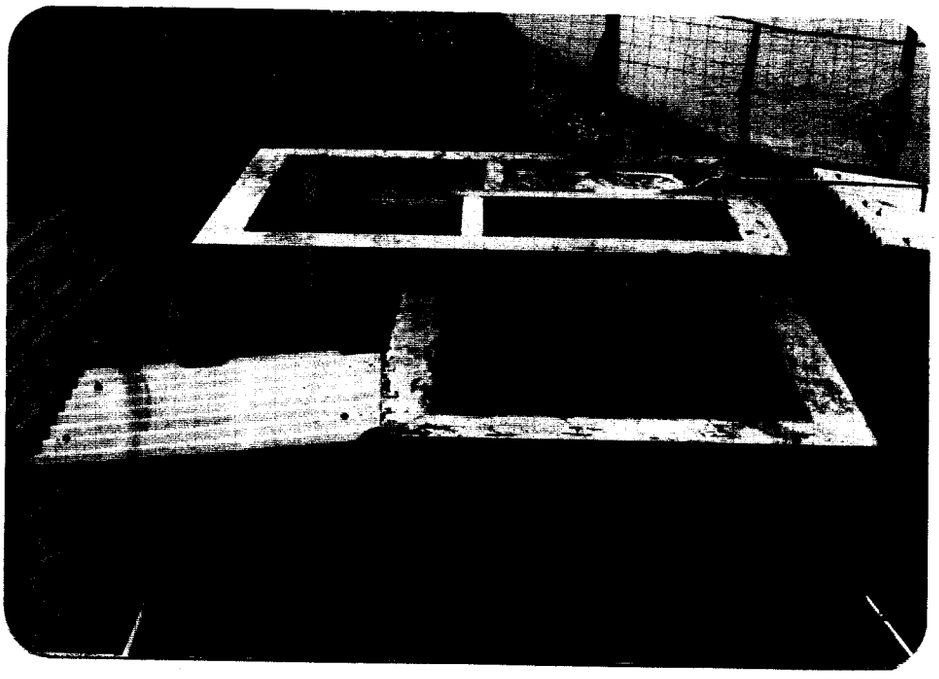
For larval rearing, seawater was collected from Tuticorin Bay (Gulf of Mannar) in a seawater well, pumped and passed through a biological filter and stored in a concrete sump (Pl. IA). From here, it was pumped via a PVC hose, the outlet of which was plugged with surgical cotton, into a one tonne fibreglass overhead tank. The water was replenished periodically depending on the quantity drawn for larval rearing. The seawater was passed by gravity through a graded series of cartridge filters of pore sizes 10 µ, 5 µ, and 1 µ. Seawater was then passed into a UV-sterilisation unit (Pl. IB) and collected in a 200-l fibreglass tank for immediate use. When the cartridge filters got frequently clogged and could not be replaced, they were dispensed with.

Plate I

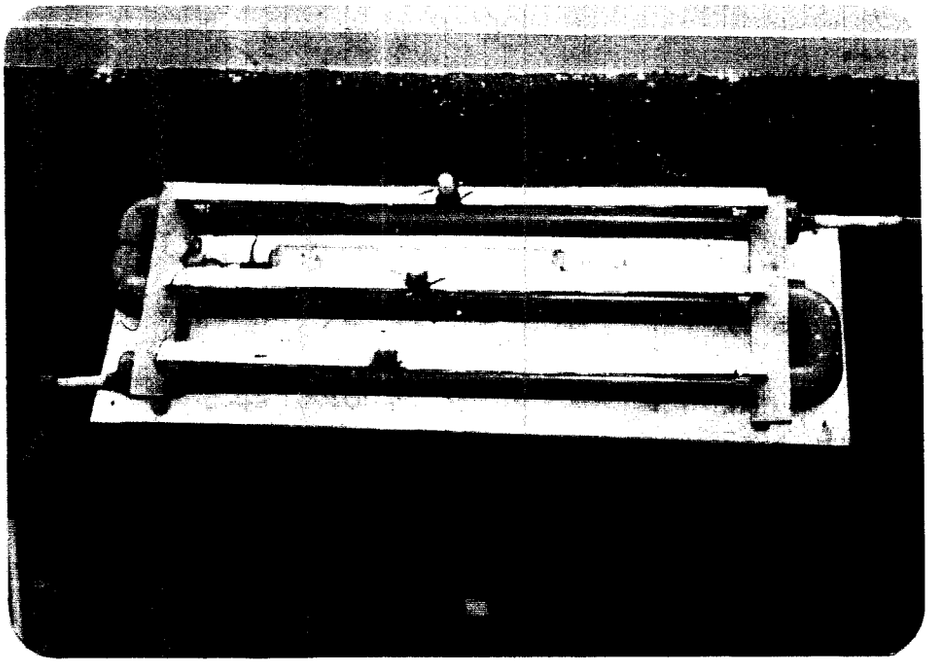
A. Biological filtration unit

B. UV filtration unit

PLATE I



A



B

Larval density estimation

Pearl oyster larvae collected in 5-l beakers were mixed thoroughly taking care to see that the larvae were not damaged and were evenly distributed. Three aliquots of 1 ml each were removed with a pipette into a larval counting chamber. The larval counting chamber has a capacity of 1 ml and is subdivided into 100 divisions. Larvae were fixed in 5% formalin and counted under the microscope. Estimates of larval density, in the 5-l beakers, were made from the above count. Calculated quantities of the general stock were drawn from these beakers and used to get the required experimental larval densities in the larval rearing beakers. These estimates were checked for their accuracy in the initial set of experiments. For this, the larvae introduced into the larval rearing beaker were killed by adding a few drops of 5% formalin. Three aliquots of 1 ml were removed after dispersing the larvae evenly in the medium and counted in a larval counting chamber. The error between the former and present estimates was found to be $\pm$ 3.3%. Since this error was considered negligible, this counter-check procedure was dispensed with in the later experiments.

Larval rearing

In all experiments, larvae in the required densities were reared in a volume of 2.5 l of sterile, filtered and UV

treated seawater. Glass beakers (Borosil make) of three-litre capacity were used throughout as experimental vessels. Larvae were maintained at ambient pH (8.10-8.30), temperature (23.9-31.5°C) and salinity (30.5-38.1‰), unless otherwise mentioned. The larval rearing beakers were kept half immersed in a waterbath to minimise temperature variation and were maintained without aeration (Pl. II.). Water was changed in the beakers on alternate days. During water changes, larvae from individual beakers were collected in an appropriately meshed nylobolt sieve, washed gently and released into fresh beakers containing 2.5 l of seawater.

Feeding

The microalgae used a food for pearl oyster larvae were drawn from 20-l glass carboys containing algal culture (Pl.IIIA). They were drawn from the carboys during the exponential phase between days 4 and 6. A sample of the algal species was removed into individual glass vials and fixed using a few drops of Lugol's iodine fixative. Since the algal cells are very small and in high concentrations, an estimate of the number of algal cells per ml of the culture was made using a blood counting chamber. Algal cell counts ranged 0.850-1.470 x 10^6 cells/ml for Isochrysis galbana; 1.245-2.888 x 10^6 cells/ml for Pavlova lutheri; 1.005 - 1.870 x 10^6 cells/ml for Chromulina freiburgensis; 1.960 - 2.890 x 10^6 cells/ml for Synechocystis salina;

Plate II

Larval rearing set up

0.223 - 0.513 x 10^6 cells/ml for Tetraselmis gracilis; 0.623 - 0.933 cells x 10^6 cells/ml for T. chui and 2.750 - 3.359 x 10^6 cells/ml for Chlorella salina. Depending on the experimental algal density chosen for larval rearing, the amount of culture to be added to the medium was calculated. This quantity was added to the larval rearing beaker in one lot, once a day. In all cases, the total volume of larval medium (seawater + algal culture) was maintained at 2.5 l.

Algal culture

Seven species of microalgae were evaluated for their food value to pearl oyster larvae, viz., Isochrysis galbana, Pavlova lutheri, Chromulina freiburgensis, Synechocystis salina, Tetraselmis gracilis, T. chui and Chlorella salina, either singly or in combinations. The taxonomic position and the size of the algae are given in Table 1.

The algal species Pavlova lutheri and Tetraselmis gracilis were isolated from the bay water of Tuticorin. The alga, Chromulina freiburgensis was isolated from the waters off the coast of Cochin (Joseph, 1983). All unialgal cultures were grown in filtered, heat sterilised, seawater contained in 20-l glass carboys (Pl.IIIA). The

Table 1. Taxonomic position of the microalgae used as pearl oyster larval food.

S.No.	Class	Order	Family	Genus	Species	Size
1.	Haptophyceae	Isochrysidales	Isochrysidaceae	Isochrysis	I. galbana Parke	7-8 µ
2.	Haptophyceae	Pavlovales	Pavlovaceae	Pavlova	P. lutheri Droop	8 µ
3.	Haptophyceae	Isochrysidales	Chromulinaceae	Chromulina	C. freiburgensis Green	8-9 µ
4.	Cyanophyceae	Chroococcales	Chroococcaceae	Synechocystis	S. salina Sauvageau	3 µ
5.	Chlorophyceae	Chlamydomonadales	Chlamydomonadaceae	Tetraselmis	T. gracilis Kylin	12 µ
6.	Chlorophyceae	Chlamydomonadales	Chlamydomonadaceae	Tetraselmis	T. chui West	15 µ
7.	Chlorophyceae	Chlorococcales	Chlorophyceae	Chlorella	C. salina Butcher	5-6 µ

water was enriched using Walne's enrichment medium (Walne, 1974). Algal cultures were maintained at a room temperature of 27°C and exposed to a light intensity of 2000 lux for 12 hours a day.

Mixed phytoplankton

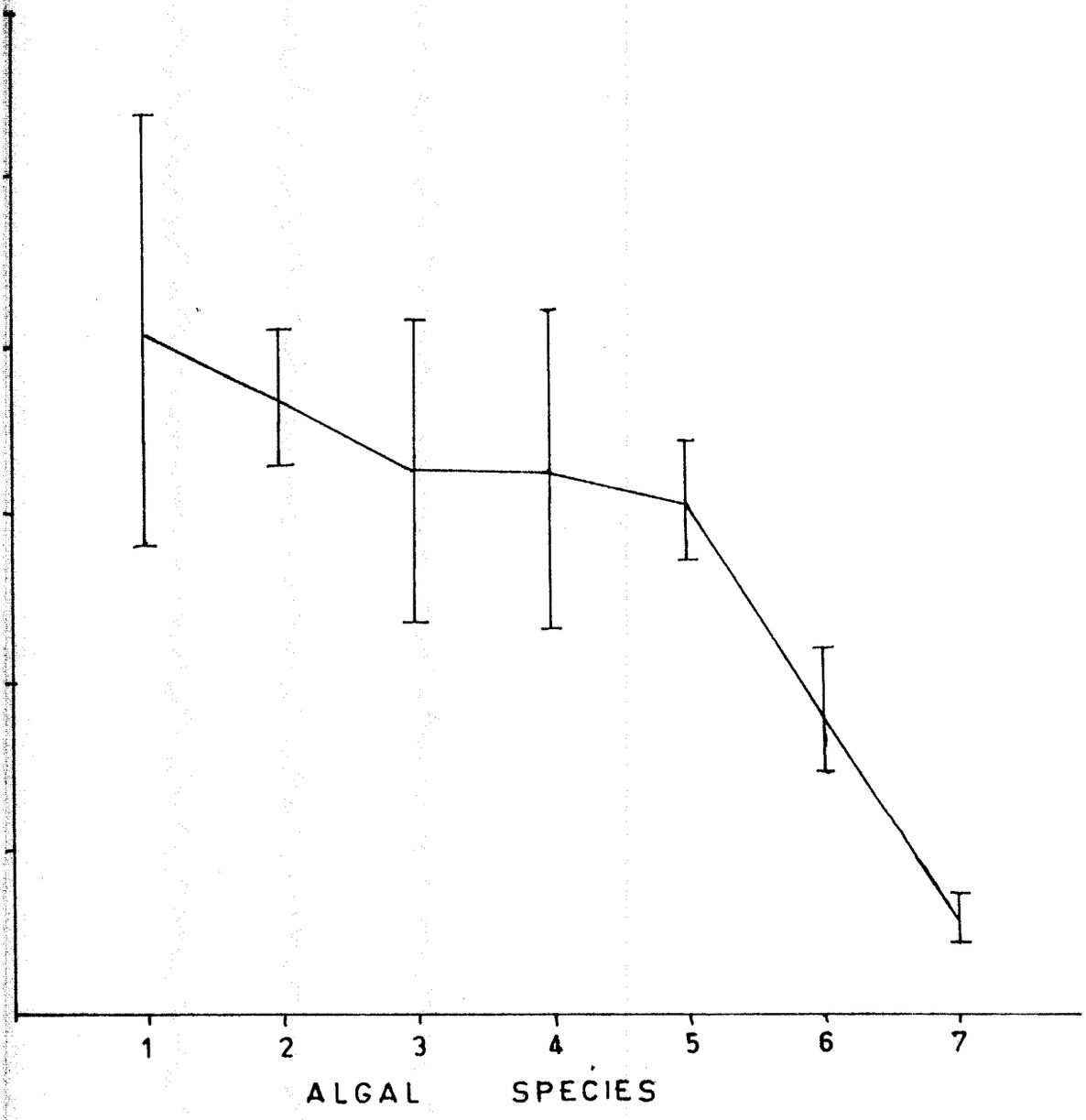
In addition to the unialgal species, phytoplankton collected from the pearl banks of Gulf of Mannar off Tuticorin was cultured in the laboratory and used for feeding the larvae. Seawater was filtered down to 25 µm through a series of sieves to remove the larger planktonic organisms and fertilised with the following chemicals.

	g/100 l seawater
Potassium nitrate	1.32
EDTA	0.66
Sodium phosphate	0.66
Sodium silicate	0.33

The tank of 200-l capacity was covered with a transparent polythene sheet and exposed to sunlight outdoors (Pl.IIIB). Two-day old blooms in the tank were examined microscopically for the presence of phytoplankton. The phytoplankton blooms were screened through a 10 µm nylobolt sieve to eliminate the larger plankton and subcultured in 5-l beakers. The resulting cultures

consisted mainly of phytoflagellates (10 µm), Chlorella sp. (3 µm), Synechocystis (3 µm) , Nannochloris (1 µm), Skeletonema (12-15 µm), Navicula (10-15 µm) and Chaetoceros (12-15 µm). The percentage composition of the differen t microalgae is shown in Fig. 1. The mixed culture was maintained by reculturing with fresh media until the end of the experiment.

FIG. 1. COMPOSITION OF MIXED PHYTOPLANKTON CULTURE



1. PHYTOFLAGELLATES; 2. NANNOCHLORIS Sp; 3. CHLORELLA Sp;
4. SKELETONEMA Sp; 5. SYNECHOCYSTIS Sp; 6. CHAETOCEROS Sp;
7. NAVICULA Sp;

CHAPTER 3

EVALUATION OF NUTRITIONAL VALUE OF MICROALGAE FOR LARVAL GROWTH AND SETTLEMENT

INTRODUCTION

The successful rearing of lamellibranch larvae depended upon the realisation that their natural foods were the small cells, the nanoplankton, and the development of procedures for maintaining cultures of nanoplankton organisms in the laboratory (Loosanoff and Davis, 1963b; Loosanoff, 1969; Kinne, 1977; Sastry, 1979). For larval rearing of Crassostrea virginica and Mercenaria mercenaria (Loosanoff and Davis, 1963b) and Ostrea edulis (Walne, 1974), several species of microalgae were tested for their food value and identified as good, mediocre or toxic foods.

Among the tridacnid clams, Gwyther and Munro (1981) and Beckvar (1981) reported on a symbiotic association of their larvae and zooxanthellae. Several species of diatoms which were thought to be too big and difficult to digest have also been used either as single food species or in combination with other algal species to feed larvae approaching metamorphosis (Mathiessen and Toner, 1966; Gruffydd and Beaumont, 1972; Koganezawa, 1975).

Instances of successful rearing of bivalve larvae in India are only a few. Rao et al. (1976) and Rao (1980) cultured the larvae of mussel Perna viridis and reported fairly good growth when fed a mixture of Synechocystis and Tetraselmis gracilis and a mixture of four species, Synechocystis, T. gracilis, T. chui and Chlorella and poor growth when fed with Synechocystis alone. Desai et al. (1980) could rear the larvae of different species of oyster Crassostrea rivularis, C. belcheri, C. gryphoides and O. cucullata from egg to straight hinge stage.

Alagarswami et al. (1983c) succeeded in rearing the larvae of the Indian pearl oyster Pinctada fucata to spat stage using the alga Isochrysis galbana as food. This was followed by the successful rearing of the edible oyster Crassostrea madrasensis larvae (Nayar et al., 1982, 1984) and with suitable modifications in the rearing of the larvae of the mussel Perna indica (Appukuttan et al., 1983) and the green mussel P. viridis (Rangarajan, 1983).

It is evident from the literature that using different species of microalgae as food for bivalve larvae there is not much consistency in rearing success. Whereas a few species such as Isochrysis galbana and Pavlova lutheri have given good results for most of the bivalve species in different regions of the world, the same is not true for

several other species of microalgae. With a view to elucidating the specific food requirements of the Indian pearl oyster larvae, seven species of microalgae were tested both individually and in combination. This is in keeping with the lines of investigations followed in the recent works on bivalve larval rearing using unialgal cultures (Loosanoff and Davis, 1963b; Sastry, 1979). It is a known fact that larvae in the wild are dependent largely on phytoplankton that is locally available. Therefore, the food value of algal cultures developed from the phytoplankters of the Gulf of Mannar for the larvae of the pearl oyster was also investigated.

MATERIALS AND METHODS

The experiments to evaluate the nutritional value of microalgae to the pearl oyster, Pinctada fucata (Gould) larvae were designed for meeting specific objectives of the investigations. The specific objectives were determination of:

Optimum larval density,

optimum algal cell concentration,

nutritional value of single species of microalgae,

nutritional value of combined species of microalgae,

efficacy of stratified feeding, and

nutritional value of mixed phytoplankton raised from natural stock of phytoplankton.

The general conditions of larval rearing have already been dealt with in Chapter 2. Since the haptophycean naked flagellate, Isochrysis galbana has already been successfully used as food for P. fucata larvae (Alagarswami et al., 1983c) and as it gave consistently good results in the preliminary trials, I. galbana was extensively used in this study and also as a standard for comparison of the food value of other species whereever required. The nutritional value of the microalgae was assessed based on

a) the growth of larvae,
b) survival rate,
c) the day and duration of spat setting, and
d) the number of spat produced.

All experiments were carried out in duplicates. The experimental protocol for the various determinations is given below.

I. Determination of optimum larval density

Five larval densities of 1/ml, 3/ml, 5/ml, 8/ml and 10/ml were tested while the feeding level was maintained uniformly at 25 cells/μl using Isochrysis galbana as food.

II. Determination of optimum algal cell concentration

The algae, I. galbana and Pavlova lutheri were tested at the concentrations of 10, 25, 50 and 100 cells/μl.

Larval density was maintained uniformly at 5/ml.

III. Determination of the nutritional value of single species of microalgae

Three species of microalgae, Chromulina freiburgensis, Synechocystis salina and Tetraselmis gracilis were used singly. All species were tested at the feeding level of 25 cells/μl.

IV. Determination of the nutritional value of combined species of microalgae

The algal species were combined in the following manner and tested for their efficacy:

A. I. galbana + P. lutheri
B. I. galbana + C. freiburgensis
C. I. galbana + S. salina
D. I. galbana + T. gracilis
E. S. salina + T. gracilis
F. I. galbana + S. salina + T. gracilis

In all experiments, the total algal cell concentration was maintained at 25 cells/μl and the algal combinations were given in equal proportions throughout the larval rearing.

V. Determination of efficacy of stratified feeding

Two experiments were conducted. In one, I. galbana was used. In the other, different algal species were

provided with increase in larval size. Three stages of pearl oyster larvae were identified for the present experiment - D shape (straight-hinge), early umbo and the eyed umbo stage. The algal cell concentration was increased by a step of 5 cells/μl with the onset of every stage, as given in the following table:

Larval Stage	*I. galbana* (cells/μl)			
	Treatment 1	Treatment 2	Treatment 3	Treatment 4
D shape	10	15	20	25
Early umbo	15	20	25	30
Eyed umbo	20	25	30	35

The second experiment incorporated different algal species while feeding density remained constant at 25 cells/μl as shown in the following table:

Larval Stage	Algal species			
	Treatment 1	Treatment 2	Treatment 3	Treatment 4
D shape	I	I	I + T	I + T
Early umbo	I	I + T	I + T	I + T + C
Eyed umbo	I + T	I + T + C	I + T + C	I + T + C

Abbreviations: I - *Isochrysis galbana*; T - *Tetraselmis chui*; C - *Chlorella salina*

Determination of the nutritional value of mixed phytoplankton raised from natural stock of phytoplankton

The mixed phytoplankton raised from the natural stock of phytoplankton as described in Chapter 2, was given as food to the larvae at different stages. I. galbana was used for comparison as per the following schedule:

Larval Stage	Treatment 1	Treatment 2	Treatment 3	Standard
D shape	M.P.	I.	I	I
Early umbo	M.P.	M.P.	I	I
Eyed umbo	M.P.	M.P.	M.P.	I

Abbreviations: M.P. - Mixed phytoplankton; I - I. galbana

The algal cell concentration of I. galbana was maintained at 25 cells/µl. Where the mixed phytoplankton was used, samples of 2 ml were withdrawn to make quantitative estimate of the number of algal cells in the larval rearing medium. Algal cell concentration was calculated to be 100 ± 21.32 cells/µl.

Estimation of parameters

Larval growth

Larval growth was monitored by measuring a sample of 50 larvae from each rearing beaker along the antero-posterior axis every fourth day. The larvae, randomly

picked from the larval rearing beakers were transferred along with a few drops of sea-water to cavity slides and measured under the microscope using an ocular micrometer. The larvae were then returned to their respective beakers.

To facilitate comparisons between individual treatments, the average larval measurements and the standard deviations were calculated for each day of sampling. Growth measurements thus calculated were tabulated against the age in days of the larvae. Actual growth rate was calculated for three different periods of larval growth, viz., day 1-9, when most of the larvae were in the D shape stage; day 9-17 in the early umbo stage and day 17-25 in the late umbo, eyed umbo and the pediveliger stages. Overall growth rate for the period day 1-25 was also calculated. It was seen from these tables that once spat setting commenced, there was no appreciable increase in the growth rate of the free-swimming larvae. In order to subject the data to statistical treatment, a day falling immediately after the commencement of spat setting was chosen and regression analysis was performed for larval growth up to this period. The regression of growth on age for individual treatments was calculated using the equation

$$\log y = a + bx$$

where, y = average antero-posterior length of the larvae
x = age in days
a = constant
b = growth coefficient.

The value of b is the instantaneous rate of growth or growth regression. The b value has been used in the present study to make comparisons between treatments in an experiment and is expressed as log/um/day. Larval growth was statistically tested for significance after performing the Analysis of Co-variance and calculating the F value (Snedecor and Cochran, 1967). When the F value was found to be statistically significant, the student's t-test was performed to find out significance between the growth regressions of two treatments within an experiment(Snedecor and Cochran, 1967).

Survival rate

Three aliquots of 1 ml were pipetted from the experimental beakers after thorough mixing. The number of live and dead larvae were counted under the microscope. Survival was expressed as the percentage of live larvae present in the whole population.

Spat setting and production

Once spat setting was initiated, the day of first setting was recorded and the number of spat was counted. The free-swimming larvae were transferred to a spare beaker and the newly settled spat were counted. Spat were counted with every change of water until no swimming larvae were observed. No spat collectors were used as the larvae

normally attached themselves with the byssal thread either to the bottom or the side of the beaker. Total enumeration of the spat was made by counting from the outside of the beaker, using a grid of netting with each mesh measuring 2 cm x 2 cm. The data on spat production were statistically treated for significance by using the normal deviate test and calculating the Z value (Snedecor and Cochran, 1967).

Algal consumption

Algal consumption was monitored 24 h after feeding. Samples of 2 ml water were removed from the rearing beakers, fixed in Lugol's iodine fixative and the algal cell counts taken using a blood counting chamber. These counts were taken to represent the level of algal cells present in the medium at the end of 24 h from feeding. Algal consumption was calculated by subtracting this from the initial cell concentration.

RESULTS

I. DETERMINATION OF OPTIMUM LARVAL DENSITY

The experiment to determine the optimum larval density was carried out during August-September, 1983. Larval densities chosen were (larvae/ml): 1, 3, 5, 8 and 10. *I. galbana* was used as larval food and supplied at a

cell concentration of 25 cells/µl throughout the larval rearing phase up to spat settlement. The environmental parameters in ambient conditions ranged as follows: Temperature : 29.0 - 31.5°C; salinity : 37.0 - 38.1‰; and pH : 8.15 - 8.30. Within the experiment, the larval rearing conditions were identical for the different larval densities.

1. Larval growth

a) Mean size of larvae (Table 2, Fig. 2): The mean larval sizes attained by the Pinctada fucata larvae at different experimental densities on the different days, along with their standard deviations are given in Table 2. The initial size of the D shape (straight hinge) larvae at 24 h from spawning was 69.0 ± 2.3 µm.

The sampling of larvae for measurements was restricted to those in the column water of the rearing beakers after gentle mixing. These larvae included the D shape, umbo, eyed umbo and pediveligers in the active velar phase of movement. The pediveligers which are in the creeping phase of movement, plantigrades which have finally settled at the sides and the bottom and the spat which have already metamorphosed and attached with byssus have been excluded. The mean values of larval sizes presented in Table 2 refer only to those stages sampled from the water column. The

Age of culture (days)	Mean size of larvae (μm)				
	1/ml	3/ml	5/ml	8/ml	10/ml
1	69.0 ± 2.3	69.0 ± 2.3	69.0 ± 2.3	69.0 ± 2.3	69.0 ± 2.3
5	79.8 ± 3.3	82.4 ± 3.6	77.8 ± 3.5	83.9 ± 4.0	83.0 ± 3.4
9	96.8 ± 5.9	102.2 ± 7.1	98.7 ± 6.0	101.9 ± 6.6	104.7 ± 10.4
13	115.5 ± 13.1	114.1 ± 11.1	131.8 ± 16.2	117.0 ± 11.0	122.6 ± 13.0
17	134.0 ± 16.5	133.0 ± 19.2	160.4 ± 22.2	144.7 ± 19.4	138.1 ± 17.0
21	154.0 ± 21.3	155.5 ± 22.8	186.0 ± 25.4	159.5 ± 19.6	159.5 ± 19.1
25	162.8 ± 22.3	165.0 ± 20.6	198.6 ± 23.2	168.2 ± 21.2	159.9 ± 21.9
29	172.6 ± 16.0	157.8 ± 18.3	202.2 ± 15.2	167.0 ± 15.7	168.8 ± 17.5
33	174.2 ± 20.1	159.3 ± 20.7	-	176.6 ± 19.0	178.0 ± 19.8

Day of first setting	25	22	20	22	22
Day of final setting	32	38	28	38	32
Total no. of spat produced	61	132	1035	489	57
Rate of spat production(%)	2.4	1.8	8.3	2.4	0.2

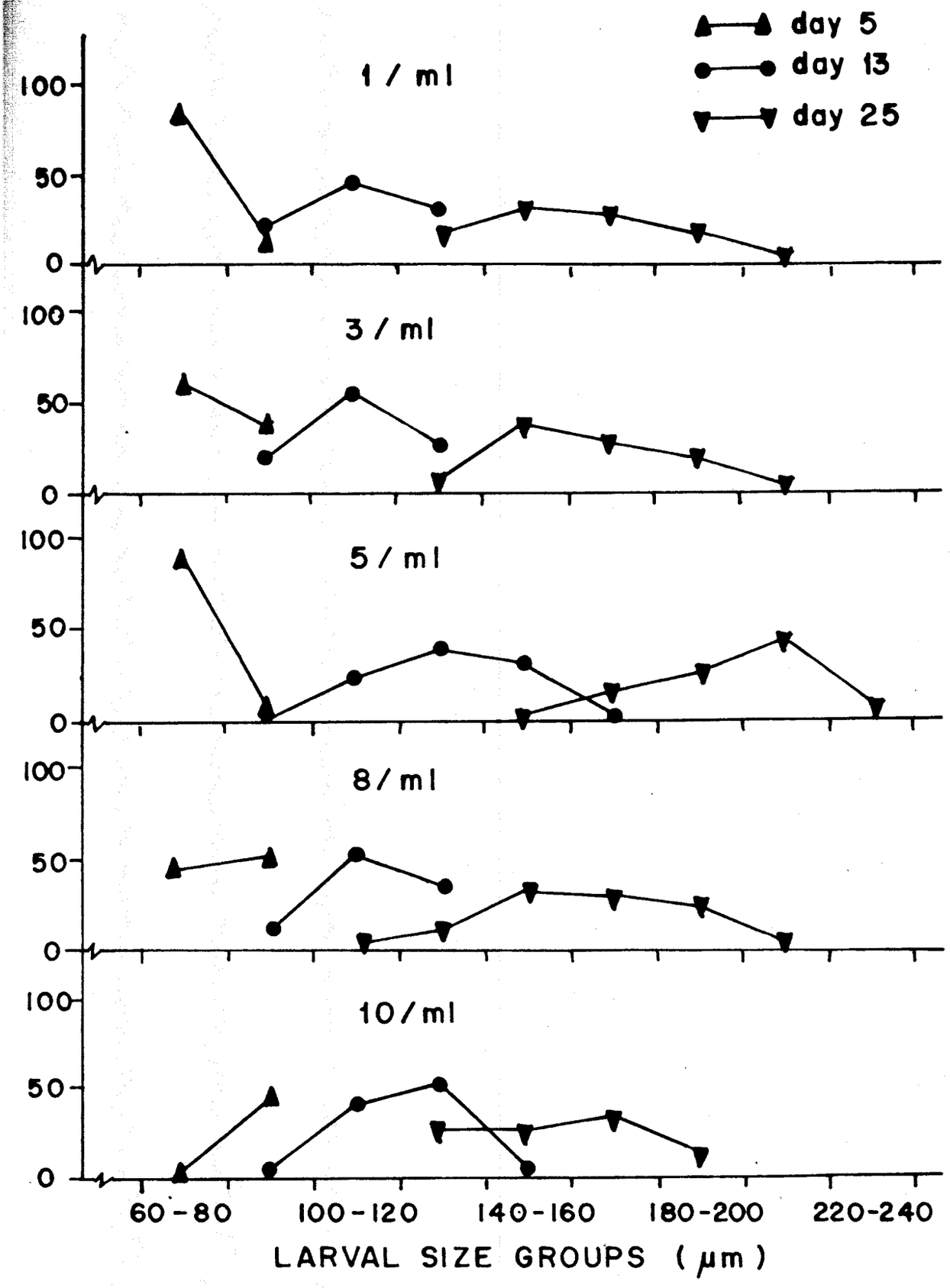
observed mean sizes of different larval stages were 103.5 µm for early umbo and 187.0 µm for eyed umbo.

It is seen from Table 2 that the only larval density which has consistently given better results is 5/ml. Although the larvae were smaller than all other densities up to day 9, they attained the maximum growth on every day of the subsequent observations. The final setting was observed on day 28, much ahead of all other densities.

Considering the larval growth data in densities less than 5/ml. i.e., 1/ml and 3/ml and greater than 5/ml, i.e., 8/ml and 10/ml, no definite trend is evident, except for the fact that at density 3/ml, the larvae have shown poor mean growth, remaining at the minimum on days 17, 29 and 33. The larvae in lower and higher densities have remained behind in growth.

b) Size frequency distribution (Fig. 2): The standard deviation (S.D.) of larval measurements are given in Table 2. To further amplify the data, the size frequency distribution of larvae (20 µm intervals) on days 5, 13, and 25 has been presented in Fig. 2. There is a progressive increase in the size spread of larvae with advance in time at all experimental densities. On day 5, the S.D. range of D shape larvae at different densities is 3.3 - 4.0 µm. There is a notable increase in S.D. when the larvae reach umbo stage. On day 13, the S.D. range is 11.0 - 16.2 µm.

Fig. 2. SIZE FREQUENCY DISTRIBUTION OF PEARL OYSTER LARVAE CULTURED AT DIFFERENT DENSITIES



The maximum S.D. is noticed on day 25, the range being 20.6 - 23.2 µm. The data for Fig. 2 have been limited to 3 observation days, namely, days 5, 13, and 25 and it is found that the size group of larvae present on day 5 is also present on day 13; so also the size group on day 13, on day 25. Growth is not uniform within the same larval population. The wider spread of larval size on day 25, is indicative of prolonged spat settlement. The modal larval size group on day 25 is at 140-160 µm at densities 1, 3 and 8/ml, at 160-180 µm at 10 ml and 200-220 µm for 5/ml. The modal group at higher larval size at density 5/ml explains the early completion of spat settlement compared to all other densities.

c) Growth rate (Tables 3 and 4): The average growth rate of larvae, between day 1-25, at densities (larvae/ml) of 1, 3, 5, 8 and 10 were, respectively, 3.91 µm/day, 4.00 µm/day, 5.40 µm/day, 4.13 µm/day and 3.79 µm/day (Table 3). The maximum growth rate was observed at density 5/ml. In all larval densities (except 10/ml), the maximum growth occurred during the period day 9-17, when the larvae were predominantly in the umbo and the eyed umbo stages. For this period the maximum growth of 7.71 µm/day was observed at the density 5/ml. Generally, the growth during the early phase (day 1-9) and later pelagic phase (after day 17) was less with a few exceptions. The slow growth recorded after day 17 may be the result of a large number of fast growing larvae having

reached pediveliger stage and, therefore, not included in the samples from column water.

Table 3. Larval growth rate at different larval densities.

Period (days)	Larval growth per day (/um/day)				
	1/ml	3/ml	5/ml	8/ml	10/ml
1-9	3.48	4.15	3.71	4.11	4.46
9-17	4.65	3.85	7.71	5.35	4.18
17-25	3.60	4.00	4.78	2.94	2.73
1-25	3.91	4.00	5.40	4.13	3.79

The larval growth data have been analysed and compared statistically (Table 4). Linear regression analysis of the data gave the growth regressions of 0.0163, 0.0161, 0.0234, 0.0166, and 0.0159 log/um/day for larval densities 1, 3, 5, 8 and 10/ml respectively. These differences were found to be highly significant ($P < 0.01$) when tested by the analysis of covariance (Table 4). The growth regression, 0.0234 log/um/day at the density of 5/ml was significantly greater than those at all other densities ($P < 0.01$).

d) Growth curve (Fig. 3): It is seen that the growth rate at 5/ml has been higher than at the other densities after day 9, and this trend becomes more dominant after day 17 (Fig. 3). While the growth curves for all

densities (data from Table 2).

Sl. No.	Treatment (larvae/ml)	d.f.	Ex^2	Exy	Ey^2	b	d f	c.s.s.	
1	1	6	448	7.3192	0.1202709	0.0163	5	0.00069347	
2	3	6	448	7.2072	0.1178334	0.0161	5	0.00188757	
3	5	5	280	6.5440	0.1543508	0.0234	4	0.00140817	
4	8	6	448	7.4804	0.1269213	0.0166	5	0.00201865	
5	10	6	448	7.1252	0.1170212	0.0159	5	0.00369871	
6							24	0.00970657	0.00040444
7		29	2072	35.6760	0.6363976	-	28	0.0221229	
8	Difference between slopes						4	0.0124164	0.00310412

$F = 7.675^*$ (d f, 4, 24)

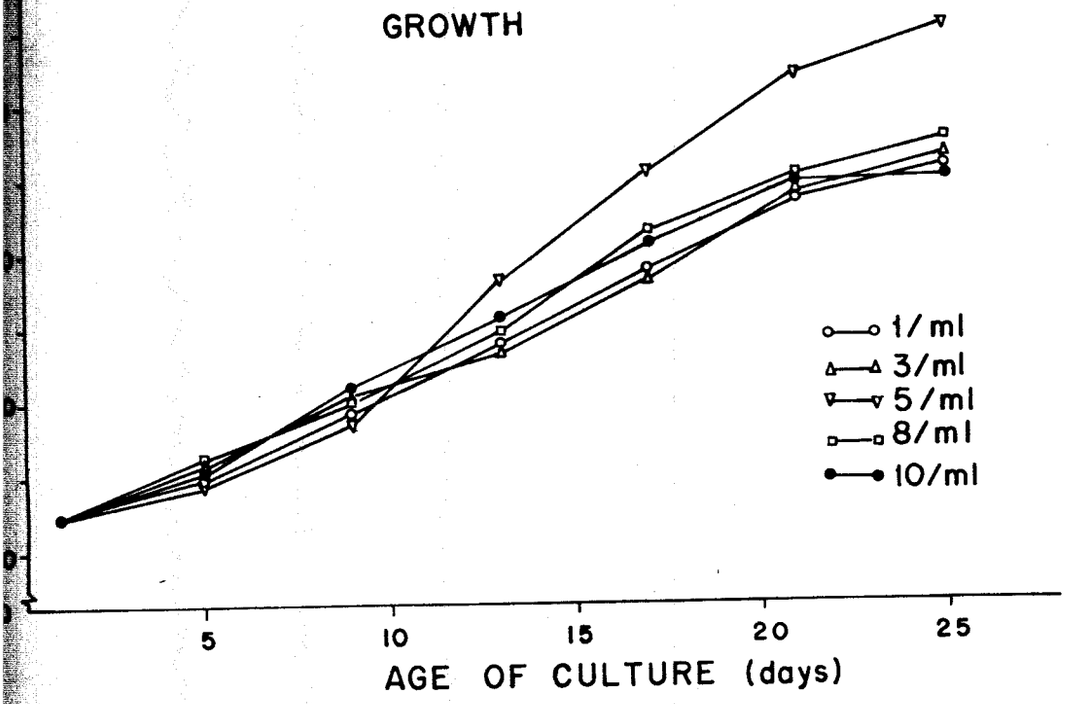
*P < 0.01 highly significant

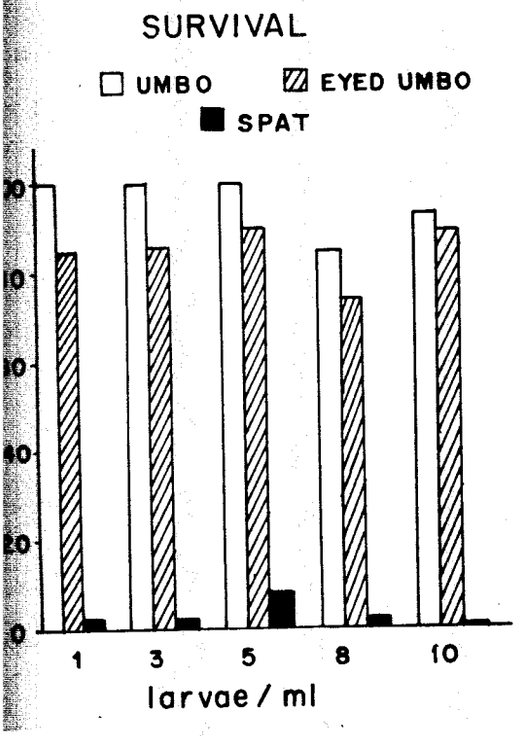
Abbreviations: d f - degrees of freedom; b - instantaneous growth rate; c.s.s. - Corrected sum of squares; m.s. - mean square.

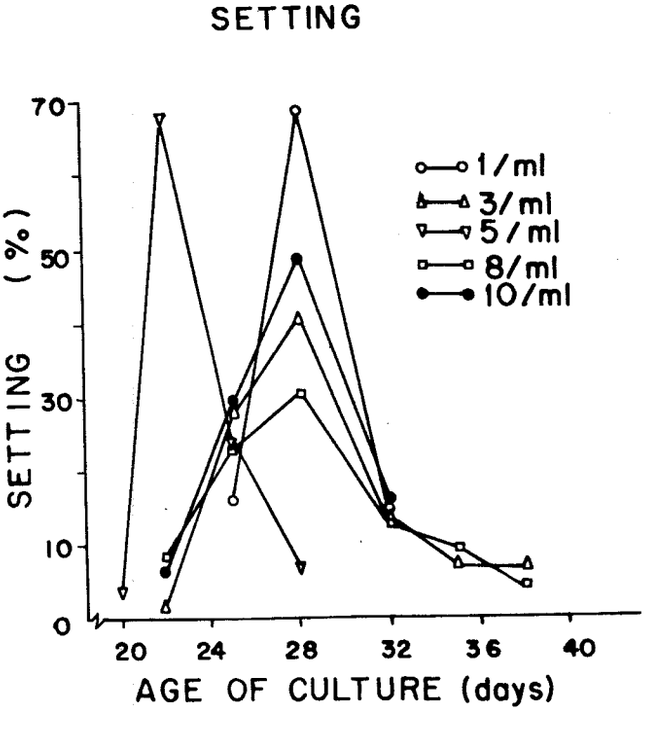
Foot Note:-

These abbreviations are common to all analyses of co-variance tables presented in the thesis.

Fig. 3. GROWTH, SURVIVAL AND SETTING OF PEARL OYSTER LARVAE AT DIFFERENT DENSITIES







other densities are more or less close to one another, that for 5/ml shows that the density has resulted in much faster growth rate.

Survival rate (Fig. 3)

From D shape to umbo, survival was 100% at the densities 1, 3 and 5/ml, 95.7% at 10/ml and 86.9% at 8/ml (Fig. 3). The progressive survival rate to eyed umbo stage was 91.5% at density 10/ml, followed by 90.0% at 5/ml, 86.6% at 3/ml, 85.0% at 1/ml and 77.3% at 8/ml. Survival to spat stage was 8.3% at density 5/ml, followed by 2.4% each at 1/ml and 8/ml, 1.8% at 3/ml and 0.2% at 10/ml. It is seen that survival rate is very high up to eyed umbo stage but, thereafter, there is considerable mortality at all larval densities resulting in relatively low spat production.

Spat setting and production (Tables 2 and 5, Fig. 3)

The earliest setting took place on day 20 at the larval density of 5/ml. At the densities 3/ml, 8/ml, and 10/ml, initial setting took place on day 22, and at 1/ml on day 25. The size frequency distribution of larvae on day 25 (Fig. 2), which shows a wide spread would indicate that spat setting would also be prolonged. Completion of setting took 7 days in the case of density 1/ml, 8 days at 5/ml, 10 days at 10/ml and 16 days at 3/ml and 8/ml. The

percentage of spat production to initial larval population was the highest at 8.3% at density 5/ml, followed by 2.4% at 1/ml and 8/ml, 1.8% at 3/ml and 0.2% at 10/ml. The percentage spat setting between the densities 3 and 5/ml, 5 and 8/ml, 8 and 10/ml were found to be statistically significant by applying the normal deviate test ($P < 0.01$).

Table 5. Normal deviate test (Z value): comparison of mean spat setting at different larval densities.

Larvae/ml	1	3	5	8	10
1	-	1.76**	15.08*	0	7.21*
3		-	22.56*	3.33*	10.59*
5			-	22.18*	33.20*
8				-	20.99*
10					-

* $P < 0.01$

** $P < 0.05$

Peak setting took place on day 22 at larval density 5/ml and on day 28 at all other experimental densities(Fig. 3).

Algal cell consumption (Table 6)

The data on algal cell consumption are presented in Table 6. The percentages were as observed in the rearing vessels and were not adjusted to larval survival. However, it may be seen from Fig. 3 that survival at the different

densities are comparable. Two trends are seen from the table: (1) there is an increase in algal consumption with increase in larval size and (2) there is also an increase in consumption with increase in larval density. Cent per cent consumption was recorded for densities 8/ml and 10/ml. At the algal cell concentration of 25 cells/µl, there appears to be a greater wastage of cells at the lower larval density and less or no wastage of cells at the higher density. However, relating the growth and setting parameters, it is seen that maximum growth was observed at neither the highest nor the lowest density, but at the medium density of 5/ml.

Table 6. Consumption of I. galbana cells by pearl oyster larvae at different larval densities (algal cell concentration 25 cells/µl).

S. No.	Larval density (larvae/ml)	Algal consumption on different days (%)					
		d-4	d-10	d-14	d-17	d-20	d-23
1.	1	25.0	30.0	35.0	35.0	40.0	40.0
2.	3	35.0	50.0	55.0	55.0	65.0	75.0
3.	5	55.0	55.0	60.0	70.0	75.0	80.0
4.	8	95.0	95.0	100.0	100.0	100.0	100.0
5.	10	100.0	100.0	100.0	100.0	100.0	100.0

- Optimum larval density (Table 7)

In terms of the four parameters considered for determination of optimum larval density, the density of 5/ml has given the best results as can be seen from the comparison of the salient features shown in Table 7. Therefore, the larval density of 5/ml was adopted as standard for determination of other aspects of this study on pearl oyster larval nutrition.

Table 7. Summary of results of larval rearing using different densities (data from Tables 2, 3 amd 4).

S.No.	Larval density	Mean size of larvae on day 25 (/um)	Larval growth per day (/um)	Growth regression (log /um/day)	Day of first setting	Duration of spat setting (days)	Spat production(%)
1.	1/ml	162.8	3.91	0.0163	25	7	2.4
2.	3/ml	165.0	4.00	0.0161	22	16	1.8
3.	5/ml	198.6	5.40	0.0234	20	8	8.3
4.	8/ml	168.2	4.13	0.0166	22	16	2.4
5.	10/ml	159.9	3.79	0.0159	22	10	0.2

II. DETERMINATION OF OPTIMUM ALGAL CELL CONCENTRATION

A. Isochrysis galbana

The experiment to determine the optimum algal cell concentration of I. galbana for pearl oyster larvae was carried out during November-December, 1983. The microalga was given at four cell concentrations of 10, 25, 50 and 100 cells/µl. The larvae were stocked at the uniform density of 5/ml. The range of environmental parameters in ambient conditions was as follows:
Temperature : 29.2 - 31.5°C; salinity : 35.5 - 36.5‰; and pH : 8.25 - 8.30. Within the experiment, larval rearing conditions were identical for the different algal cell concentrations.

- Larval growth

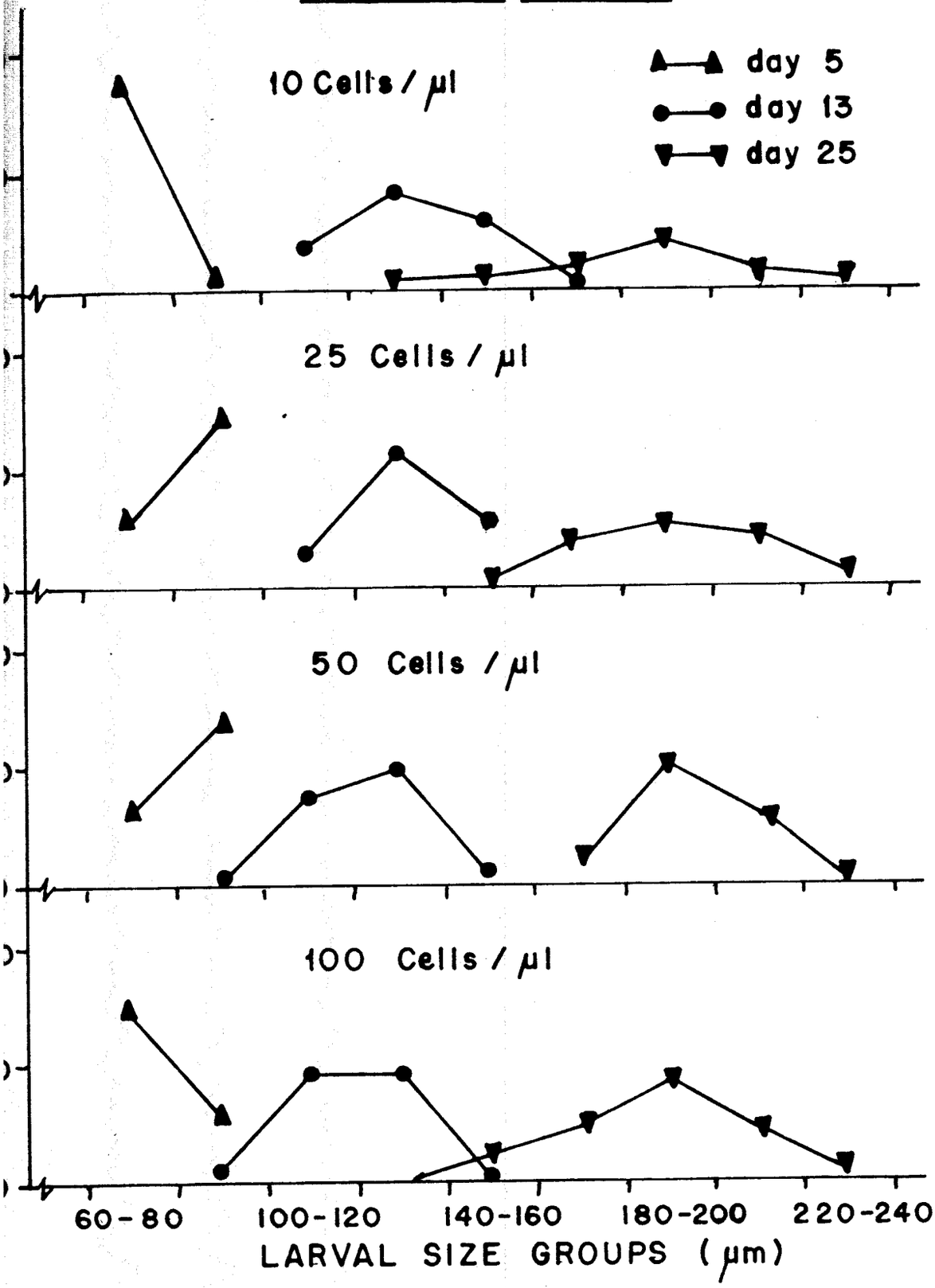
a) Mean size of larvae (Table 8, Fig. 5): It is seen from Table 8 that maximum mean larval size from day 5 up to day 33 has fluctuated between the cell concentrations of 25 and 50 cells/µl. For most days, (5, 9, 17, 21 and 29) minimum mean larval size is observed at the cell concentration of 10 cells/µl.

b) Size frequency distribution (Fig. 4): At all feeding levels there is an increase in the size ranges of the larvae with advance of time (Fig. 4). It is seen that

Table 8. Growth and setting of pearl oyster larvae fed at different d of *Isochrysis galbana* (Larval density : 5/ml)

Age of culture (days)	Mean size of larvae (μm)			
	10 cells/μl	25 cells/μl	50 cells/μl	100 cells/μl
1	71.1 ± 3.1	71.1 ± 3.1	71.1 ± 3.1	71.1 ± 3.1
5	80.0 ± 4.0	83.2 ± 2.8	81.6 ± 3.1	80.6 ± 3.3
9	99.2 ± 4.2	99.9 ± 3.7	103.0 ± 4.8	100.4 ± 4.1
13	123.7 ± 16.6	132.5 ± 11.9	129.2 ± 14.3	120.8 ± 10.0
17	143.0 ± 15.0	154.4 ± 5.1	160.3 ± 9.3	157.5 ± 11.8
21	170.0 ± 24.6	183.3 ± 20.1	185.1 ± 15.1	184.7 ± 15.6
25	185.8 ± 22.5	195.1 ± 17.2	185.0 ± 16.7	186.2 ± 20.4
29	184.3 ± 23.6	206.6 ± 9.4	191.6 ± 12.3	204.3 ± 13.9
33	194.1 ± 11.8	195.4 ± 9.1	192.9 ± 15.3	192.1 ± 14.1
Day of first setting	23	23	23	23
Day of final setting	44	41	41	41
Total no. of spat	3958	4343	3525	2969
Rate of spat production(%)	31.7	34.7	28.3	23.8

1. SIZE FREQUENCY DISTRIBUTION OF PEARL OYSTER LARVAE FED WITH DIFFERENT LEVELS OF ISOCHRYSIS GALBANA



similar modal groups appear on the same days at different algal densities, except on day 5. However, the range is narrower on day 13 at 25 cells/µl, and on day 25 at 50 cells/µl as compared to other densities. The smaller size groups of 120-140µm persist even on day 25 at densities 100 cells/µl. Comparing all 4 algal cell concentrations, 25 cells/µl and 50 cells/µl gave better results.

c) Growth rate (Tables 9 and 10): The average growth of larvae during day 1-25 was 4.78, 5.17, 4.75 and 4.80µm/day for the respective algal cell concentrations of 10, 25, 50 and 100 cells/µl(Table 9). The maximum growth rate of 5.17µm/day was obtained for the concentration of 25 cells/µl. The growth rate during day 9-17, when the larvae were predominantly in the umbo and eyed umbo stages, was higher than in the preceding and succeeding periods. For the duration day 9-17, the algal cell concentration of 50 cells/µl gave the highest growth rate of 7.16µm/day.

Table 9. Larval growth rate at different concentrations of Isochrysis galbana.

Period (days)	Larval growth per day (µm/day)			
	10 cells/µl	25 cells/µl	50 cells/µl	100 cells/µl
1-9	3.51	3.60	3.99	3.66
9-17	5.45	6.81	7.16	7.14
17-25	5.35	5.09	3.09	3.59
1-25	4.78	5.17	4.75	4.80

Growth regressions of 0.0184, 0.0196, 0.0192 and 0.0194 log/um/day were obtained at the algal cell concentrations of 10, 25, 50 and 100 cells/μl, respectively (Table 10). However, these values were not found to be statistically significant from one another ($P > 0.05$).

d) Growth curve (Fig. 5): In the early period of larval life, the growth curves for different feeding levels are close together (Fig. 5). On day 13, they become divergent and the final order of merit becomes distinct on day 25. Further data on day 29 show that the algal cell concentration of 25 cells/μl gives the highest larval growth followed by 100, 50 and 10 cells/μl.

Survival rate (Fig. 5)

Survival was 100% at the umbo stage for all 4 algal densities (Fig. 5). Survival rate was 90% at 10 cells/μl, 83.3% at 25 cells/μl, 81.8% at 50 cells/μl and 80.0% at 100 cells/μl for the eyed umbo stage. Survival to spat stage was 31.7, 34.7, 28.3 and 23.8% at 10, 25, 50 and 100 cells/μl respectively, indicating that considerable mortality had occurred between eyed umbo stage and spat setting.

Spat setting and production (Tables 8 and 11, Fig. 5)

At all feeding levels, spat setting was observed first on day 23 (Table 8). Maximum duration of 21 days setting

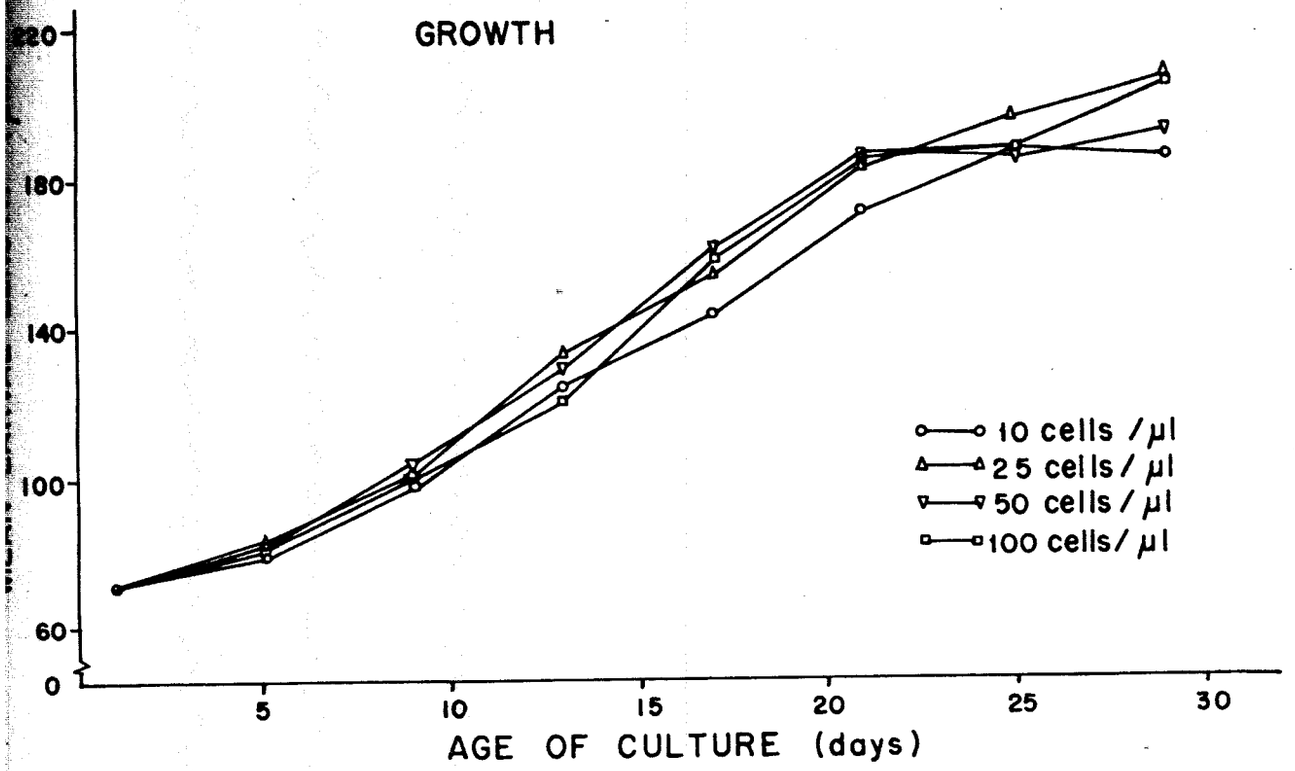
Table 10. Analysis of co-variance of pearl oyster larval growth data for different algal cell concentrations of *Isochrysis galbana* (data from Table 8).

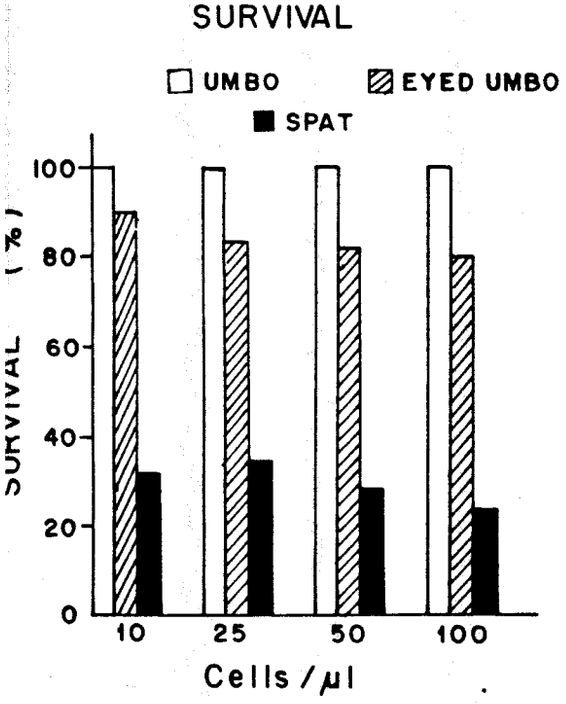
S.No.	*I. galbana* (cells/µl)	d f	Ex^2	Exy	Ey^2	b	d f	c.s.s.	m.s.
1.	10	6	448	8.2588	0.1539215	0.0184	5	0.00167190	
2.	25	6	448	8.7617	0.1750192	0.0196	5	0.00366342	
3.	50	6	448	8.5989	0.1710148	0.0192	5	0.00596774	
4.	100	6	448	8.6798	0.1724878	0.0194	5	0.00432055	
5.							20	0.01562361	0.00078117
6.		24	1792	34.2966	0.6724433		23	0.0159504	
7.	Difference between slopes						3	0.00032668	0.00010896

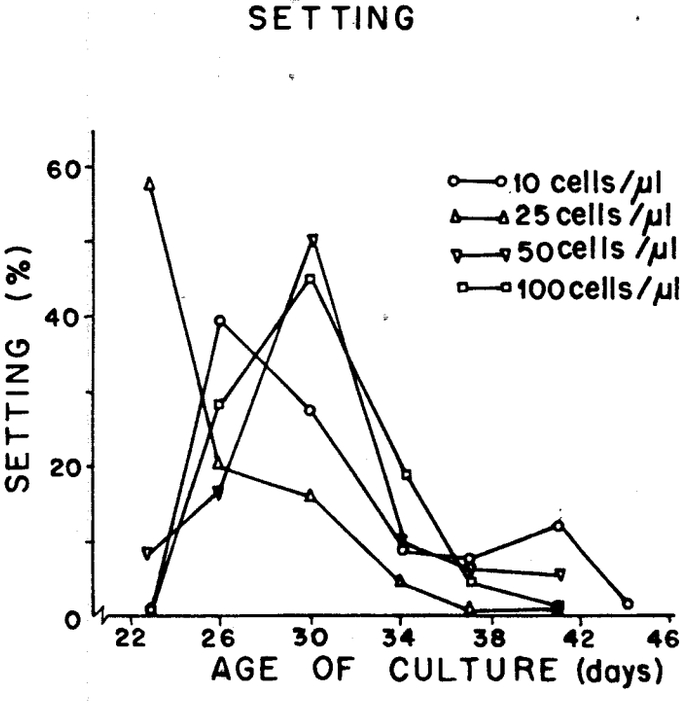
$F = 0.14^{*}$ (d f, 3, 20)

* ($P > 0.05$) No significance

Fig. 5. GROWTH, SURVIVAL AND SETTING OF PEARL OYSTER LARVAE FED WITH DIFFERENT LEVELS OF ISOCHRYSIS GALBANA







period was observed at 10 cells/µl (Fig. 5). At all other feeding levels, duration of spat setting was observed to be 18 days. The percentage of spat production was highest at 25 cells/µl, being 34.7%, followed by 31.7% at 10 cells/µl, 28.3% at 50 cells/µl and 23.8% at 100 cells/µl. The percentage spat setting between the treatments 10 and 25 cells/µl, 25 and 50 cells/µl, 50 and 100 cells/µl were found to be highly significant ($P < 0.01$).

Table 11. Normal deviate test (Z value): comparison of mean spat setting at different cell concentrations of Isochrysis galbana.

Cells/µl	10	25	50	100
10	-	5.08*	5.87*	14.01*
25		-	10.93*	19.09*
50			-	9.12*
100				-

*$P < 0.01$

Peak setting was seen to differ between treatments, occurring on day 23 at 25 cells/µl, on day 26 at 10 cells/µl and on day 30 at 50 and 100 cells/µl (Fig. 5).

Algal cell consumption (Table 12)

As in the case of larval density experiments two general trends of algal consumption are evident from the data: (1) there is an increase in algal consumption with increase in larval size (advance of time) in all experimental cell concentrations and (2) on a given day, there is decrease in algal cell consumption with increase in algal cell concentration (Table 12). For the larval density of 5/ml, the greatest utilisation efficiency is obtained at 10 cells/µl and the poorest and therefore, maximum wastage of algal cells, at 100 cells/µl. Although the lower concentration (10 cells/µl) gives up to 100% utilization, larval growth is not the best at this. Utilization of 35.0-55.0% at 50 cells/µl and 22.5-30.0% at 100 cells/µl leads to considerable wastage of food material.

Table 12. Consumption of *I. galbana* by pearl oyster larvae at different algal cell concentrations

Cell concentration (cells/µl)	Algal consumption on different days (%)					
	d-4	d-7	d-10	d-14	d-18	d-21
10	72.5	72.5	100.0	100.0	100.0	100.0
25	50.0	60.0	71.0	71.0	71.0	80.0
50	35.0	40.0	50.0	50.0	50.0	55.0
100	22.5	22.5	25.0	30.0	27.5	30.0

B. Pavlova lutheri

The species Pavlova lutheri is as important as I. galbana in bivalve larval rearing. The experiment to determine the optimum cell concentration of P. lutheri for P. fucata larvae was conducted during August-September, 1983. The following were the range of environmental parameters during the period of study:
Temperature : 29.2-31.5°C; Salinity : 27.0-38.1‰; and pH : 8.25-8.30.

Larval growth

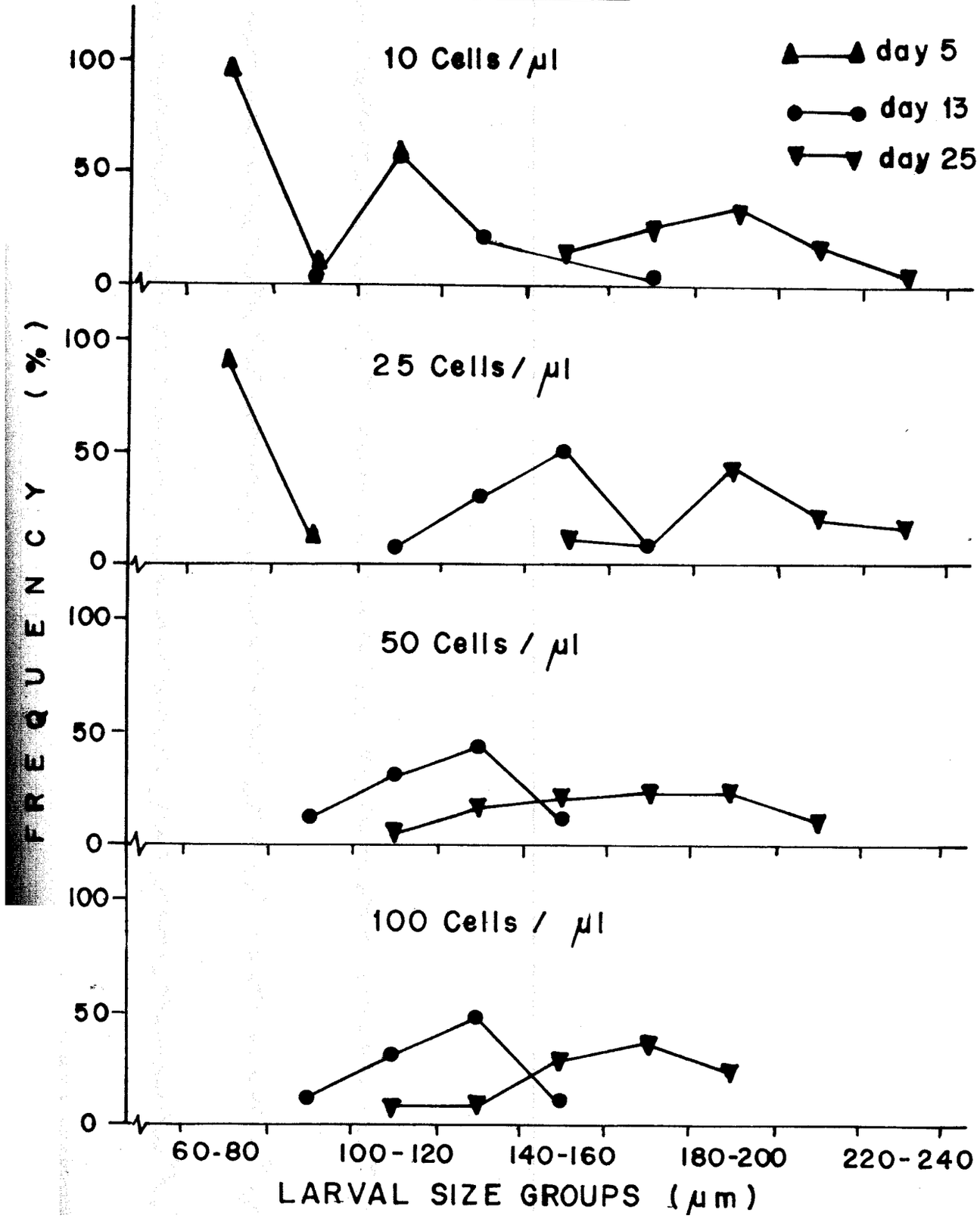
a) Mean size of larvae (Table 13, Fig. 7): The initial mean size of the straight-hinge larvae was 69.0 ± 2.3 µm (Table 13). It may be seen that larval growth at the lower concentrations of 10 and 25 cells/µl is better than growth of larvae at 50 and 100 cells/µl. The maximum mean larval size is observed consistently at 25 cells/µl. The minimum larval sizes have been recorded at the higher cell concentrations of 50 and 100 cells/µl, except on day 5.

b) Size frequency distribution (Fig. 6): A uniform modal group (60-80 µm) occurs on day 5 (Fig. 6). Thereafter, the range and the modal group differs at the four cell concentrations. On day 13, the widest size range is observed at 100 cells/µl and smaller size ranges for the other cell

Table 13. Growth and setting of pearl oyster larvae at different concentrations of Pavlova lutheri (Larval density; 5/ml).

Age of culture (days)	Mean size of larvae (µm)			
	10 cells/µl	25 cells/µl	50 cells/µl	100 cells/µl
1	69.0 ± 2.3	69.0 ± 2.3	69.0 ± 2.3	69.0 ± 2.3
5	76.6 ± 3.1	78.2 ± 3.4	77.0 ± 3.2	77.2 ± 2.5
9	98.0 ± 7.1	103.4 ± 9.4	96.8 ± 6.0	102.0 ± 7.9
13	122.4 ± 18.1	143.8 ± 15.0	104.0 ± 14.6	120.8 ± 13.8
17	182.0 ± 23.5	184.0 ± 22.6	161.0 ± 27.9	158.8 ± 21.6
21	196.4 ± 17.4	196.8 ± 22.2	192.4 ± 23.9	181.8 ± 22.0
25	185.6 ± 20.9	195.4 ± 20.0	169.2 ± 25.9	184.6 ± 22.4
29	215.0 ± 11.4	204.3 ± 15.0	196.1 ± 22.0	193.2 ± 17.8
Day of first Setting	18	18	18	20
Day of final setting	30	28	28	28
Total no. of spat	3620	2270	1668	656
Rate of spat Production(%)	29.0	18.2	13.3	5.2

Fig.6. SIZE FREQUENCY DISTRIBUTION OF PEARL OYSTER LARVAE FED WITH DIFFERENT LEVELS OF PAVLOVA LUTHERI



concentrations. The modal size group was 100-120 µm at 10 cells/µl, 140-160 µm at 25 cells/µl and 120-140 µm at 50 and 100 cells/µl. On day 25, the size range and modal size group (180-200 µm) were the same for both 10 and 25 cells/µl. A wide size range occurs at 50 and 100 cells/µl with a mode at 160-180 µm.

c) Growth rate (Tables 14 and 15): The actual larval growth rates for day 1-25 at the cell concentrations of 10, 25,50 and 100 cells/µl were respectively, 4.86 5.27, 4.18 and 4.82 µm/day (Table 14). The highest growth rate of 5.27 µm/day was obtained at 25 cells/µl. As in the case of the experiment with I. galbana, the larval growth rate during day 9-17 was much higher than during the preceding and succeeding periods. During day 9-17, the growth rate was the highest at 10.50 µm/day at the algal density of 10 cells/µl, followed by 10.08 µm/day in 25 cells/µl, 8.03 µm/day in 50 cells/µl and 7.10 µm/day in 100 cells/µl.

Table 14 Larval growth rate at different concentrations of Pavlova lutheri.

Period (days)	Larval growth per day (µm/day)			
	10 cells/µl	25 cells/µl	50 cells/µl	100 cells/µl
1-9	3.63	4.30	3.48	4.13
9-17	10.50	10.08	8.03	7.10
17-25	0.45	1.43	1.03	3.23
1-25	4.86	5.27	4.18	4.82

Linear regression analysis gave growth regressions of 0.0253, 0.0256, 0.0234 and 0.0226, log/um/day at 10, 25 50 and 100 cells/µl respectively (Table 15). Growth regression is seen to decrease with increasing cell concentrations from 25 to 100 cells/µl. The analysis of covariance, however, indicated that these values were not statistically significant from each other ($P > 0.05$).

d) Growth curve (Fig. 7): Up to day 9 growth in the different treatment is comparable, but subsequently, larval growth at 25 and 10 cells/µl is much better than growth at 50 and 100 cells/µl (Fig. 7).

Survival rate (Fig. 7)

In all treatments, survival was 100% at the umbo stage. Survival was reduced to 81.8% at 10 cells/µl, 70.6% at 25 cells/µl, 88.9% at 50 cells/µl and 80.0% at 100 cells/µl at the eyed umbo stage. Survival to spat production was 29.0% at 10 cells/µl, 18.2% at 25 cells/µl, 13.3% at 50 cells/µl and 5.2% at 100 cells/µl.

Spat setting and production (Tables 13 and 16, Fig. 7)

Setting was initiated on day 18 at 10, 25 and 50 cells/µl and on day 20 at 100 cells/µl (Table 13). Setting lasted for a maximum of 12 days at 10 cells/µl, 10 days each at 25 and 50 cells/µl and 8 days at 100

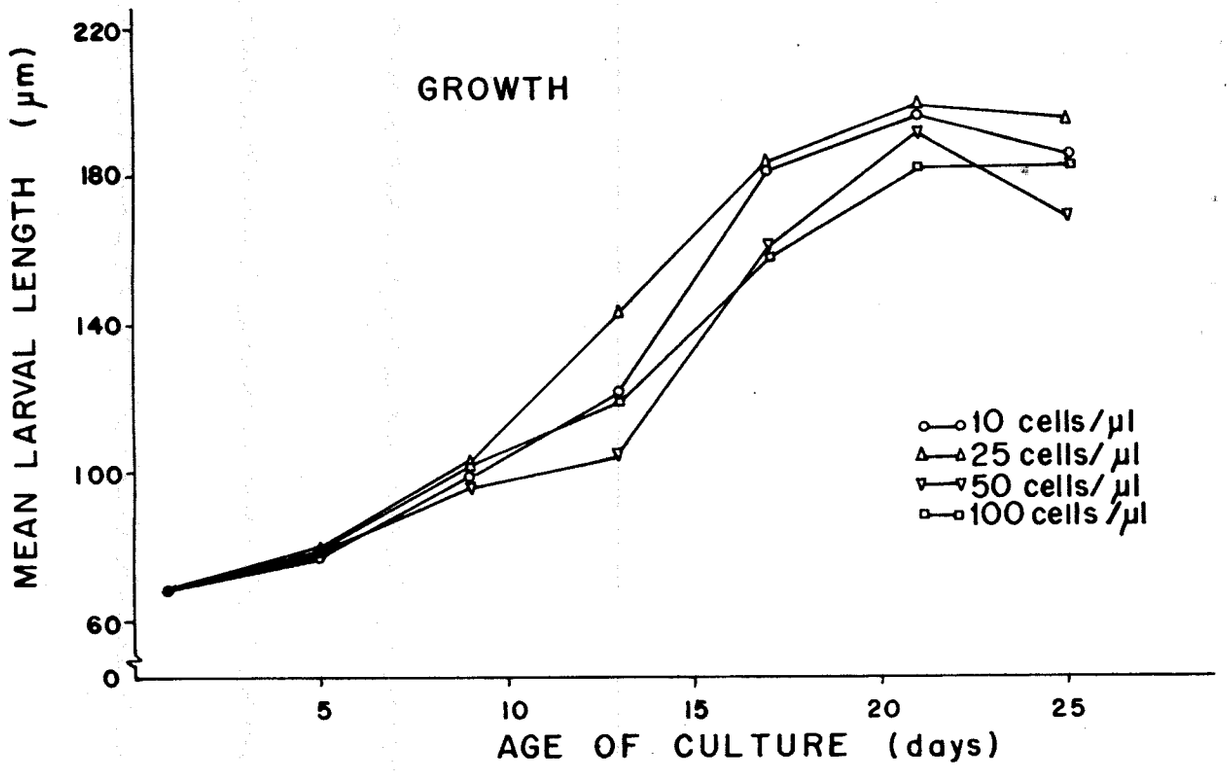
Table 15. Analysis of co-variance of pearl oyster larval growth data for different algal cell concentrations of Pavlova lutheri (data from Table 13).

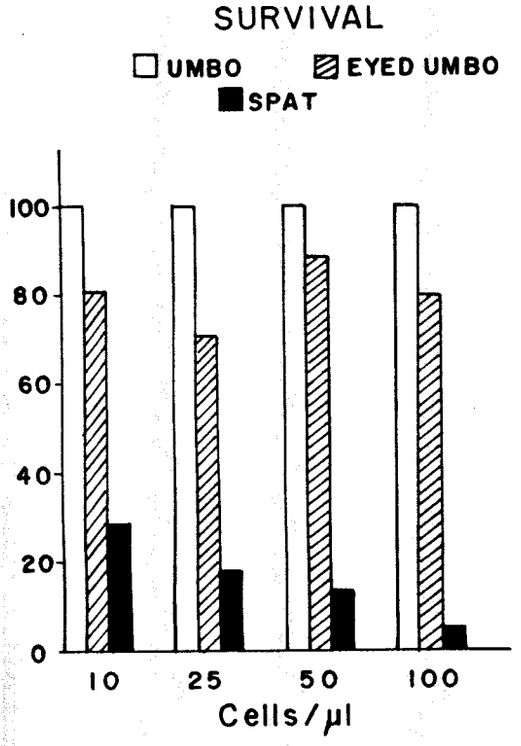
S.No.	P. lutheri (cells/µl)	d f	Ex^2	Exy	Ey^2	b	d f	c.s.s.	m.s.
1.	10	5	280	7.0906	0.1833625	0.0253	4	0.00380318	
2.	25	5	280	6.9989	0.1872136	0.0256	4	0.01226850	
3.	50	5	280	6.5376	0.1578625	0.0234	4	0.00521880	
4.	100	5	280	6.3328	0.1436918	0.0226	4	0.00046195	
5.							16	0.0217526	0.0013595
6.		20	1120	26.9599	0.6721304		19	0.0231695	
7.	Difference between slopes						3	0.0014169	0.0004723

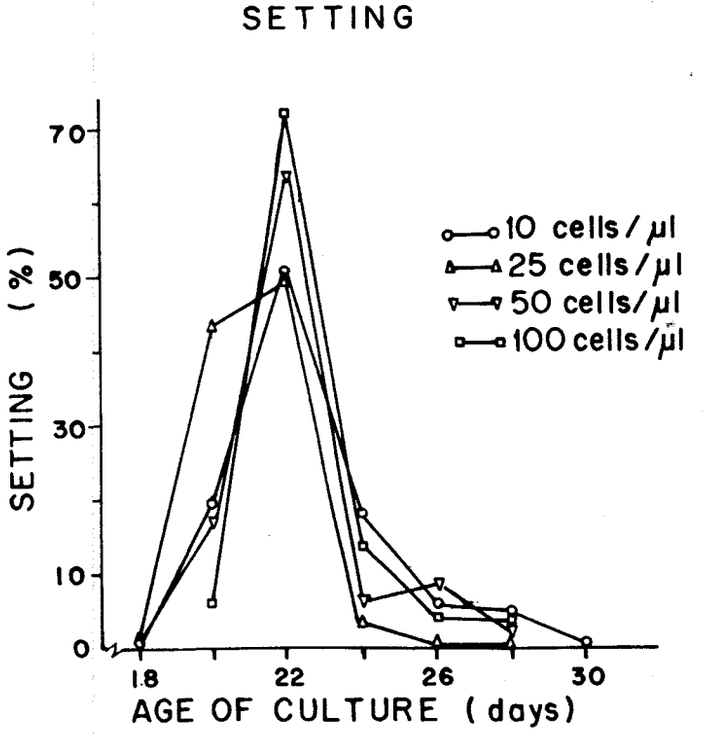
$F = 0.347^{*}$ (d f, 3, 16)

*($P > 0.05$) No significance

Fig. 7. GROWTH, SURVIVAL AND SETTING OF PEARL OYSTER LARVAE FED WITH DIFFERENT LEVELS OF PAVLOVA LUTHERI







cells/µl. Total spat production decreased progressively from 29.0% at 10 cells/µl, to 18.2% at 25 cells/µl, to 13.3% at 50 cells/µl and to 5.2% at 100 cells/µl.

The percentage spat production between 10 and 25 cells/µl, 25 and 50 cells/µl and 50 and 100 cells/µl were statistically different from each other ($P<0.01$).

Table 16. Normal deviate test (Z value): comparison of mean spat setting at different cell concentrations of Pavlova lutheri.

Cells/µl	10	25	50	100
10	-	20.30*	31.03*	52.69*
25		-	10.67*	32.71*
50			-	22.38*
100				-

* $P < 0.01$

A remarkably uniform peak setting was observed on day 22 in all algal cell concentrations (Fig. 7). After the peak setting on day 22, setting at all cell concentrations declined sharply.

Algal cell consumption (Table 17)

As observed for I. galbana, two trends are seen in algal consumption of P. lutheri: (1) there is an increase in algal cell consumption with increase in larval size (advance

of time) at all experimental cell concentrations, and (2) there is a decrease in algal consumption with increase in algal cell concentration for any given day.

Table 17. Consumption of P. lutheri cells by pearl oyster larvae at different cell concentrations

Cell Concentration (cells/µl)	Algal cell consumption on different days(%)				
	d-4	d-8	d-11	d-15	d-17
10	75.0	100.0	100.0	100.0	100.0
25	60.0	70.0	80.0	80.0	80.0
50	40.0	45.0	50.0	60.0	60.0
100	25.0	25.0	32.5	37.5	37.5

Although maximum (100%) utilisation of algal cells was observed at 10 cells/µl, larval growth was not optimum at this level. When studied in relation to the earlier data, the algal cell concentration of 25 cells/µl giving a consumption rate of 60-80% appears optimum for larval growth. The cell concentrations of 50 and 100 cells/µl where consumption was 40-60% and 25.0-37.5% lead to considerable wastage of algal cells.

C. Summary of results of larval rearing using different cell concentrations of I. galbana and P. lutheri

A comparison of the nutritive value of the two algal, species I. galbana and P. lutheri to the larvae of pearl oyster Pinctada fucata is attempted based on the results reported in the two earlier sections. As may be seen from Table 18, mean larval sizes on day 25 and actual growth rates for the period day 1-25 are very similar with different concentrations of I. galbana and P. lutheri, except at 50 cells/µl. The concentration of 25 cells/µl of both algal species has given higher growth rate. However, growth regressions of larvae fed with I. galbana (0.0184-0.0196 log µm/day) are generally lower than those of larvae fed with P. lutheri (0.0226-0.0256 log µm/day). This is due to the fact that larvae fed with P. lutheri reached the setting stage earlier (day 18) than larvae fed with I. galbana (day 23) and growth of larvae in the pelagic phase had thereafter stagnated. This may also be deduced from an examination of the actual larval growth rate for periods day 1-9, day 9-17 and day 17-25 (Tables 9 and 14). For the period day 9-17, much higher growth rates are recorded with P. lutheri as diet (7.10 to 10.50 µm/day) than with I. galbana as diet (5.45 to 7.14 µm/day). The poor larval growth rate with P. lutheri for the subsequent period, day 17-25 (0.45 - 3.23 µm/day) as compared to I. galbana

Table 18 Summary of results of larval rearing using different cell concentrations of *I. galbana* and *P. lutheri* (data from Tables 8, 9, 10, 13, 14 and 15).

S. No.	Algal species	Cell concentration (cells/µl)	Mean size of larvae on day 25 (µm)	Larval growth per day (µm)	Growth regression (log µm/day)	Day of first setting	Duration of setting (days)	Spat production (%)
1.	I	10	185.8	4.78	0.0184	23	18	31.7
	P	10	185.6	4.86	0.0253	18	12	29.0
2.	I	25	195.1	5.17	0.0196	23	18	34.7
	P	25	195.4	5.27	0.0256	18	10	18.2
3.	I	50	185.0	4.75	0.0192	23	18	28.3
	P	50	169.2	4.18	0.0234	18	10	13.3
4.	I	100	185.0	4.75	0.0192	23	18	23.8
	P	100	184.6	4.82	0.0226	18	8	5.2

Abbreviations : I - *Isochrysis galbana* ; P - *Pavlova lutheri*

(3.59 - 5.35 /um/day) reflects a stagnation in larval growth due to early commencement of setting. However, taken as a whole, the same trend in growth rate is observed.

Apart from the early initiation of setting using P. lutheri as diet, duration of setting was also short (8-12 days) as compared to larvae fed with I. galbana (18 days). Spat production was consistently higher with I. galbana was larval food as compared with P. lutheri at all cell concentrations.

Considering the more important parameters of growth and spat production, it is noted that while P. lutheri led to higher growth rates and rapid setting, spat setting was consistently higher with I. galbana (Table 18). The experiments with the two algal species having been conducted at different periods of time, the differences in results may also be partly due to differences in ambient conditions besides their nutritive value for pearl oyster larvae. For both algal species, pearl oyster larval growth has been maximum at the cell concentration of 25 cells/ µl. However, while maximum spat setting occurred at the cell concentration of 25 cells/ µl with I. galbana, maximum setting occurred at 10 cells/ µl with P. lutheri.

III. NUTRITIONAL VALUE OF SINGLE SPECIES OF MICROALGAE

A. Chromulina freiburgensis

Chromulina freiburgensis was used as experimental food for pearl oyster larvae at a larval density of 5/ml and algal cell concentration of 25 cells/µl. Concurrently, larval rearing was carried out with Isochrysis galbana as standard food and also with a combination of equal proportions of C. freiburgensis and I. galbana, at the same larval density and algal cell concentration. While the results of the above three types of larval rearing are presented in the accompanying tables and figures, only those of single species are considered here for treatment and discussion. The results of microalgal combination experiment are discussed in a later section.

The experiments were carried out during May, 1984, under the following ambient conditions:
Temperature : 27.9 - 29.1°C; Salinity : 33.5 - 35.0‰; and pH : 8.10 - 8.15.

Larval growth

a) Mean size of larvae (Table 19, Fig. 9): For days 5 and 9, larvae fed with C. freiburgensis measured 84.9 µm and 95.3 µm as compared to 84.7 µm and 92.3 µm attained by larvae fed with I. galbana (Table 19). Thereafter, growth

freiburgensis singly and in combination with *I. galbana*

Age of culture (days)	Mean size of larvae (μm)		
	C. freiburgensis	*C. freiburgensis* + *I. galbana*	*I. galbana*
1	67.5 ± 2.4	67.5 ± 2.4	67.5 ± 2.4
5	84.9 ± 4.5	86.2 ± 3.7	84.7 ± 4.3
9	95.3 ± 7.7	97.7 ± 7.8	92.3 ± 7.3
13	100.3 ± 4.7	103.3 ± 5.3	106.9 ± 13.1
17	115.4 ± 3.5	122.2 ± 14.6	145.7 ± 28.0
21	137.4 ± 20.6	158.9 ± 25.5	183.3 ± 34.1
25	163.8 ± 23.9	173.6 ± 24.3	185.5 ± 33.6
Day of first setting	21	21	21
Day of final setting	27	25	31
Total no. of spat	377	532	815
Rate of spat production (%)	3.0	4.3	6.5

of larvae in the standard food was found to be consistently greater. Larvae fed with I. galbana measured 106.9, 145.7, 183.3 and 185.5 µm for days, 13, 17, 21 and 25 as compared to mean sizes of 100.3, 115.4, 137.4, and 163.8 µm for C. freiburgensis-fed larvae for the same days.

b) Size frequency distribution (Fig. 8): Similar modal group (80-100 µm) for days 5 and 13 occurs for both C. freiburgensis-fed and I. galbana-fed larvae. However, on day 13, range of sizes is narrower for C. freiburgensis-fed larvae, and is restricted to the smaller size range (80-100 µm to 100-120 µm) as compared to that of standard (80-100 to 120-140 µm). On day 25, the range of larval sizes is wider in standard (100-120 to 220-240 µm) than for C. freiburgensis-fed larvae (120-140 to 200-220 µm).

c) Growth rate (Tables 20 and 21): The growth rate of pearl oyster larvae fed with C. freiburgensis is comparable to that of the standard up to day 9 but, thereafter, growth rate of the standard has remained consistently higher (Table 20). The actual larval growth rate for larvae fed with C. freiburgensis was 4.01 µm/day whereas it was 4.92 µm/day for standard for the duration day 1-25. It is also seen that for the period day 9-17 larval growth with C. freiburgensis was very poor at 2.52 µm/day, as compared to the maximum of 6.68 µm/day with I. galbana.

Fig. 8. SIZE FREQUENCY DISTRIBUTION OF PEARL OYSTER LARVAE FED WITH CHROMULINA FREIBURGENSIS SINGLY AND IN COMBINATION WITH ISOCHRYSIS GALBANA

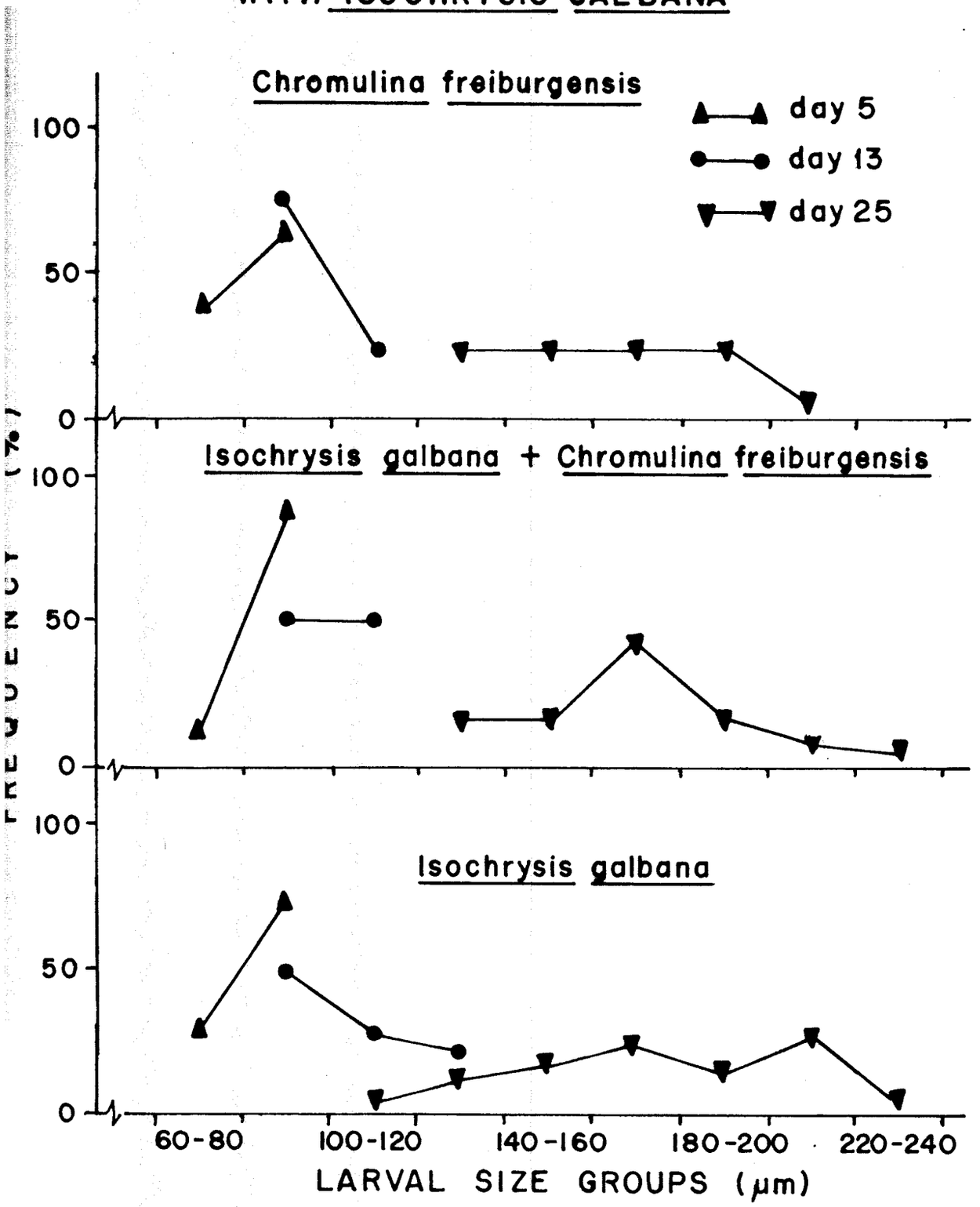


Table 20. Larval growth rate when fed with Chromulina freiburgensis singly and in combination with Isochrysis galbana.

Period (days)	Larval growth per day (µm/day)		
	C	I + C	I
1-9	3.48	3.78	3.10
9-17	2.52	3.06	6.68
17-25	6.05	6.43	4.98
1-25	4.01	4.42	4.92

Abbreviations : C - Chromulina freiburgensis;
I - Isochrysis galbana

Growth regressions were of 0.0196 log µm/day for I. galbana-fed larvae and 0.0148 log µm/day for C. freiburgensis-fed larvae (Table 21). The growth regression obtained on feeding with C. freiburgensis was statistically different from that with I. galbana ($P < 0.05$).

d) Growth curve (Fig. 9): Growth of larvae fed with I. galbana is seen to be far better than growth of larvae fed with C. freiburgensis (Fig. 9). The differences become marked from day 9 onwards.

Survival rate (Fig. 9)

Larval survival was 38.5% at the umbo stage when fed C. freiburgensis and 66.6% when fed I. galbana (Fig. 9). Survival was reduced to 17.5% during the eyed umbo stage

Table 21. Analysis of co-variance of pearl oyster larval growth [illegible] Chromulina freiburgensis singly and in combination with Isochrysis galbana (data from Table 19)

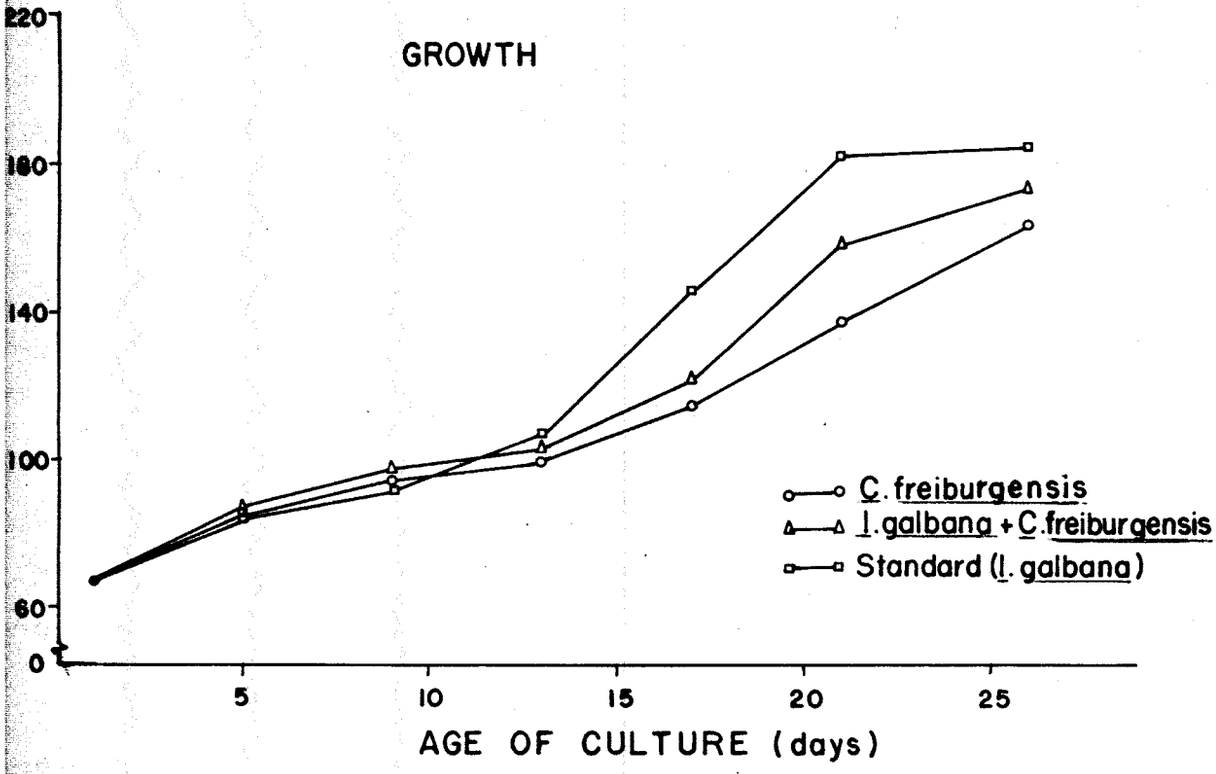
S.No.	Treatment	d f	Ex^2	Exy	Ey^2	b	d f	c.s.s.	m.s.
1.	C	6	448	6.6277	0.1006020	0.0148	5	0.00255198	
2.	I + C	6	448	7.4392	0.1263580	0.0166	5	0.00282742	
3.	I	6	448	8.7747	0.1753097	0.0196	5	0.00344505	
4.							15	0.00882446	0.000588297
5.		18	1344	22.8416	0.4022697		17	0.0140712	
6.	Difference between slopes						2	0.00524686	0.00262343

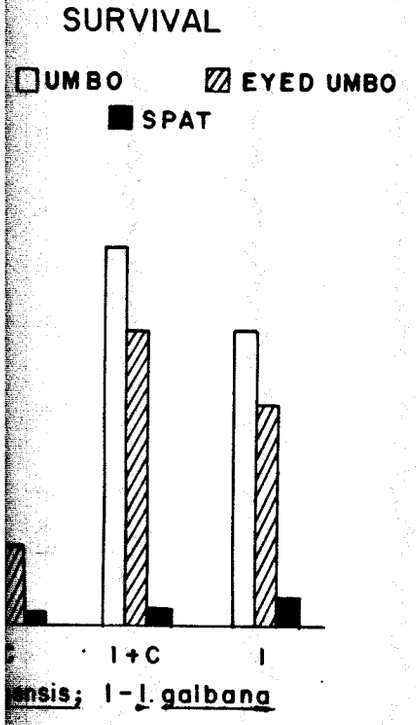
$F = 7.46^{*}$ (df, 2, 15)

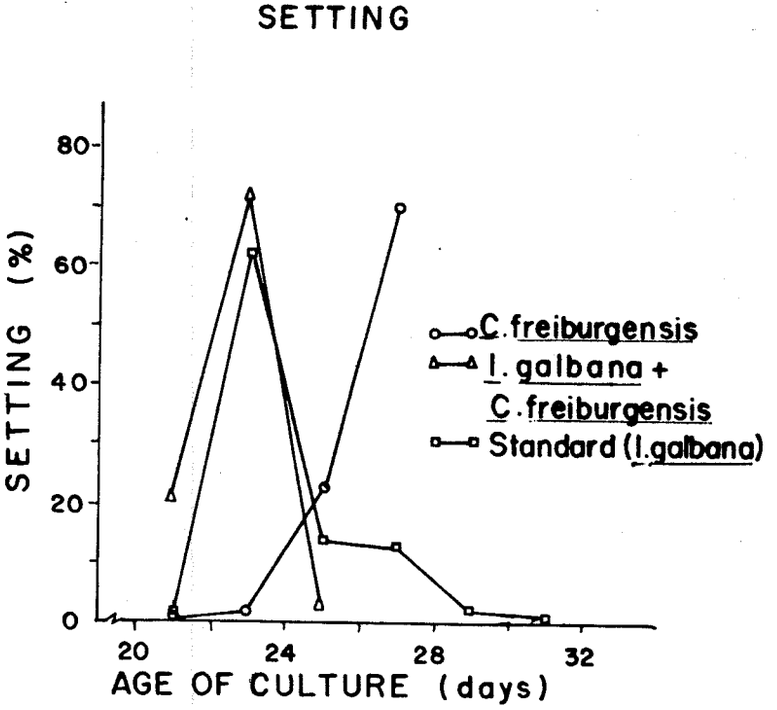
*($P < 0.05$) Significant

Abbreviations: C - Chromulina freiburgensis: I - Isochrysis galbana

9 ..GROWTH, SURVIVAL AND SETTING OF PEARL OYSTER LARVAE FED WITH CHROMULINA FREIBURGENSIS SINGLY AND IN COMBINATION WITH ISOCHRYSIS GALBANA







for larvae fed with C. freiburgensis and to 44.4% with I. galbana. Survival to spat stage was 3.0% for the former and 6.5% for the latter.

Spat setting and production (Tables 19 and 22, Fig. 9)

Setting was initiated on day 21 in both treatments but the setting duration was 6 days for C. freiburgensis-fed larvae and 8 days for larvae fed with I. galbana (Table 19).

Peak setting was observed on day 23 for I. galbana-fed larvae. (Fig. 9). Thereafter, there was a sharp decline in percentage spat setting but setting prolonged till day 31. On the other hand, larvae fed with C. freiburgensis set in small numbers initially and peak setting was observed on day 27. These results indicate that larvae fed on C. freiburgensis were slower in their growth rate and therefore took a longer time to set. Spat production was 3.0% for C. freiburgensis-fed larvae and 6.5% for I. galbana-fed larvae. Spat setting percentage of C. freiburgensis-fed larvae was found to be statistically significant from spat production of I. galbana-fed larvae ($P < 0.01$).

Table 22. Normal deviate test (Z value): comparison of mean spat setting with different algal diets.

Algal diet	C	I + C	I
C	-	5.49*	13.14*
I + C		-	7.73*
I			-

* $P < 0.01$
Abbreviations: C-Chromulina freiburgensis; I-Isochrysis galbana

Algal cell consumption (Table 23)

Consumption of C. freiburgensis cells is seen to be less than that of I. galbana cells. While consumption of I. galbana cells was seen to steadily increase with increase in larval size, consumption of C. freiburgensis was seen to decrease after day 11. An examination of the larval survival data shows that survival was reduced to 38.5% even at early umbo stage. The decreased consumption of C. freiburgensis may, therefore, be associated with the higher mortality observed for larvae fed with C. freiburgensis.

Table 23. Consumption of C. freiburgensis and I. galbana cells in single and combination diets by pearl oyster larvae at the concentration of 25 cells/µl.

Species	Algal cell consumption on different days (%)					
	d-4	d-8	d-11	d-15	d-17	d-20
C. freiburgensis	45.0	45.0	50.0	37.5	25.0	25.0
C. freiburgensis + I. galbana	40.0 70.0	50.0 70.0	50.0 80.0	60.0 80.0	60.0 90.0	60.0 90.0
I. galbana	62.5	65.0	70.0	75.0	75.0	75.0

B. *Synechocystis salina*

The experiment to test the nutritional value of *S. salina* was carried out during January - February, 1984 when environmental parameters in ambient conditions were as follows:
Temperature : 23.9 - 25.1°C; Salinity : 33.5-35.0‰; and pH : 8.10 - 8.20.

As in the case of *Chromulina freiburgensis*, standard larval rearing was done with *I. galbana* for comparison. Larval rearing to evaluate the nutritional value of *Tetraselmis gracilis* was also done concurrently. The data on the latter are presented in tables and figures along with those on *S. salina* but are discussed in the section under *T. gracilis*.

Larval growth

a) Mean size of larvae (Table 24, Fig. 11): As can be seen from Table 24, starting from day 5 up to day 41, the mean size of larvae fed on *I. galbana* has been consistently larger than that of larvae fed on *S. salina*. The larvae fed with *S. salina* remained in the D shape stage for a prolonged period, exhibiting a very slow rate of growth. On day 41, mean larval size was only 99.9 µm. In contrast, mean larval size on day 41 for larvae fed on *I. galbana* was 193.0 µm.

microalgae *Synechocystis salina* and *Tetraselmis gracilis*.

Age of culture (days)	Mean size of larvae (/um)		
	S. salina	*T. gracilis*	*T. galbana*
1	68.3 ± 2.4	68.3 ± 2.4	68.3 ± 2.4
5	76.3 ± 4.0	72.2 ± 3.2	81.1 ± 3.5
9	78.8 ± 4.0	75.7 ± 3.8	92.9 ± 5.2
13	84.0 ± 3.8	76.1 ± 3.7	97.6 ± 5.8
17	84.6 ± 4.2	76.2 ± 3.9	106.5 ± 5.5
21	86.0 ± 4.3	-	116.7 ± 10.7
25	89.0 ± 5.0	-	125.2 ± 18.1
29	88.5 ± 4.9	-	142.2 ± 20.2
33	92.8 ± 5.8	-	159.9 ± 25.0
37	96.5 ± 7.4	-	166.4 ± 16.5
41	99.9 ± 6.9	-	193.0 ± 17.1
45	102.9 ± 8.9	-	186.4 ± 12.3
49	101.4 ± 7.3	-	178.6 ± 6.4
Day of first setting	-	-	28
Day of final setting	-	-	52
Total no.of spat	-	-	1050
Rate of spat production(%)	-	-	8.8

b) Size frequency distribution (Fig. 10): While the larval size range was seen to increase in the standard, the size range of larvae fed on S. salina was very limited (Fig. 10). Although similar modal groups (60-80 µm and 80-100 µm) occurred for both treatments on days 5 and 13, the percentage of larger sized larvae in the 80-100 µm range on days 5 and 13 was greater in the standard. On day 25, larvae fed on S. salina had a modal group remaining at 80-100 µm and larvae of the standard at 100-120 µm. Larvae fed on I. galbana showed progressive growth up to 160-180 µm size range.

c) Growth rate (Tables 25 and 26): The growth rate of pearl oyster larvae fed with S. salina remained consistently lower than that of larvae fed with I. galbana cells (standard) (Table 25). The larval growth rate was seen to decline steadily with increase in age. For the duration day 1-25, the overall growth rate was seen to be 0.86 µm/day for larvae fed with S. salina was compared to 2.37 µm/day obtained with the standard. Even with the standard, the growth rate was relatively poor as compared to the results obtained in larval rearings with I. galbana at other times during this study. This may be due to the lower temperatures and salinity conditions that prevailed during this experiment.

g. 10. SIZE FREQUENCY DISTRIBUTION OF PEARL OYSTER LARVAE FED ON THE ALGAE, SYNECHOCYSTIS SALINA AND TETRASELMIS GRACILIS

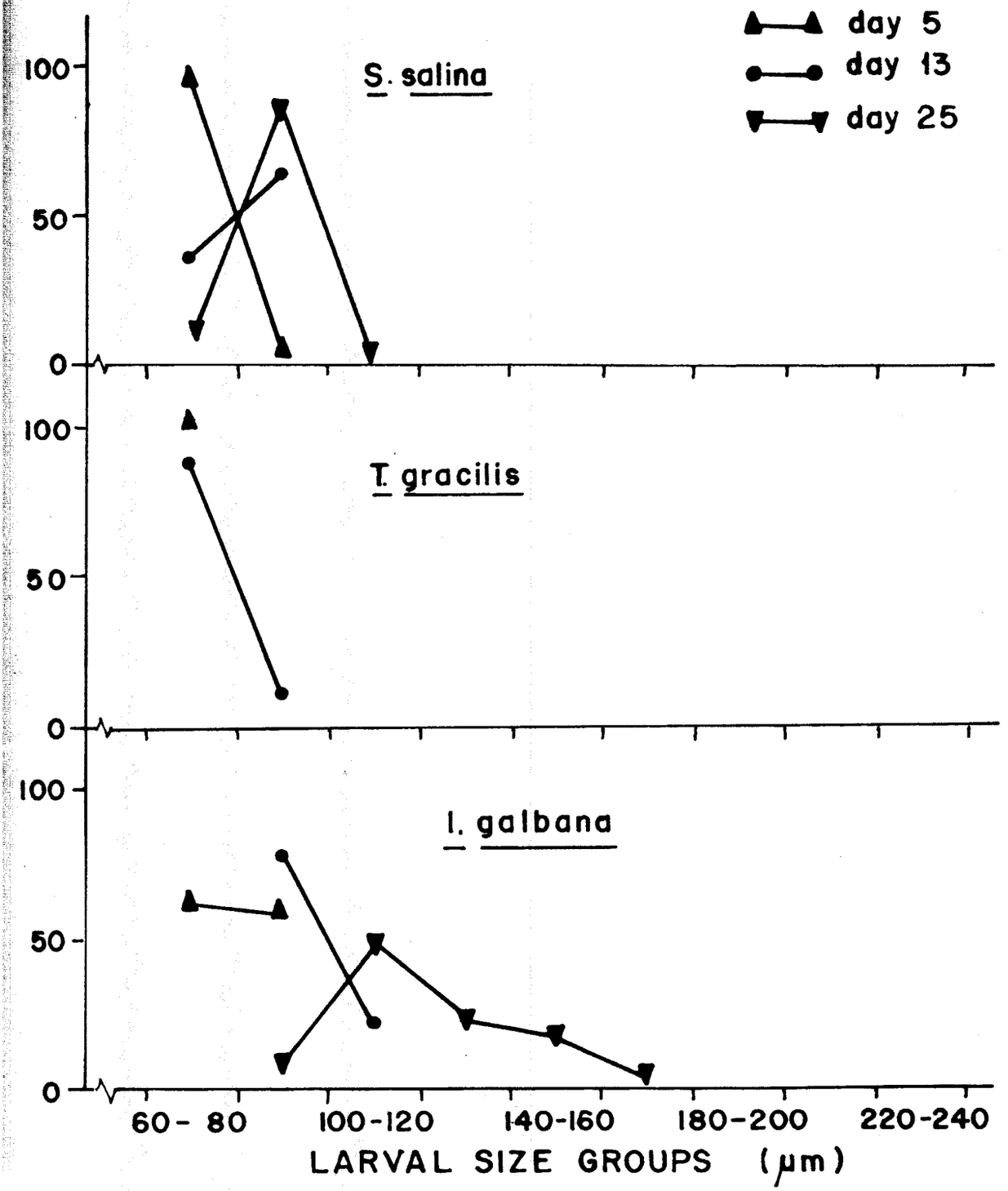


Table 25. Larval growth rate using the microalgae Synechocystis salina and Tetraselmis gracilis.

Period (days)	Larval growth per day (/um/day)		
	S	T	I
1-9	1.31	0.93	3.08
9-17	0.73	0.06	1.70
17-25	0.55	-	2.34
1-25	0.86	-	2.37

Abbreviations: S - Synechocystis salina; T - Tetraselmis gracilis; I - Isochrysis galbana

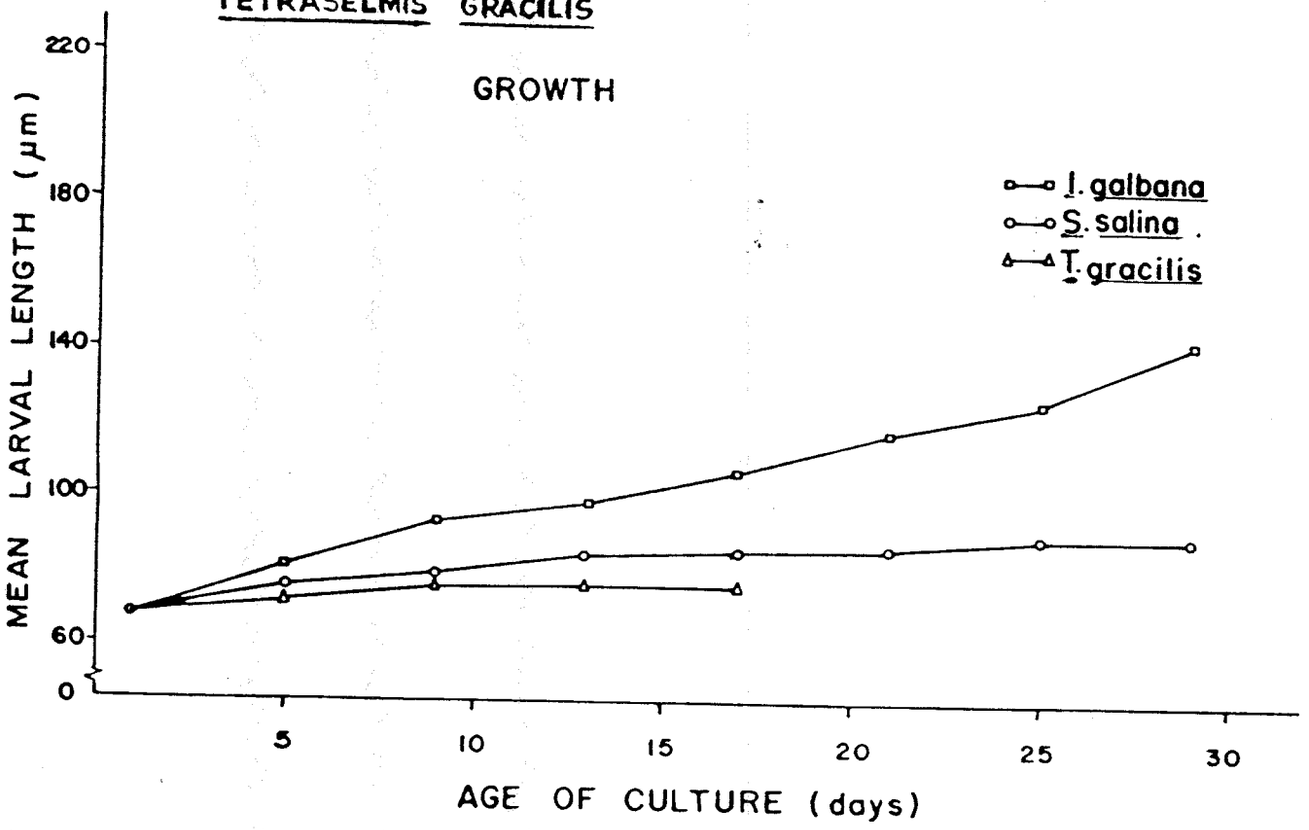
Growth regressions were 0.0105 log/um/day for I. galbana and 0.0034 log/um/day for S. salina (Table 26).

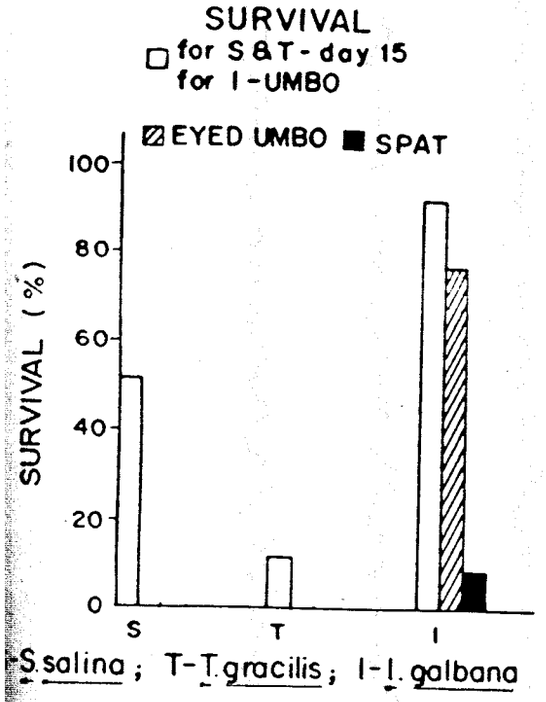
d) Growth curve(Fig. 11): The growth curve of larvae fed with S. salina is more or less a plateau from day 13 showing no perceptible increase in growth (Fig. 17).

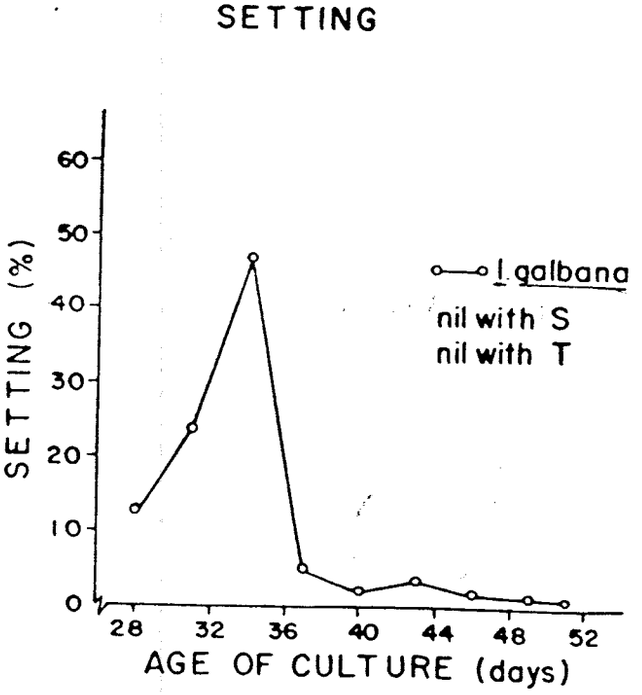
Survival rate (Fig. 11)

For larvae fed with S. salina survival at the onset of umbo stage was seen to be 52.4%. Total mortality occurred by day 53. In contrast, larvae fed on I. galbana showed a survival rate of 92.3% at the umbo stage and 76.8% at the eyed umbo stage.

Fig. 11. GROWTH, SURVIVAL AND SETTING OF PEARL OYSTER LARVAE FED WITH THE MICROALGAE SYNECHOCYSTIS SALINA AND TETRASELMIS GRACILIS







3. Spat setting and production (Table 24, Fig. 11)

There was no spat setting among larvae fed with S. salina. In larvae fed with I. galbana spat setting commenced on day 28 and lasted a period of 24 days yielding a total spat production of 8.8% (Table 24). Peak setting was seen to occur on day 34, although there was a prolonged setting phase up to day 52 (Fig. 11).

4. Algal cell consumption (Table 27)

Although S. salina cells were consumed by the larvae as confirmed by microscopic examination, the species appeared to have had very little nutritional value for the growth of P. fucata larvae.

Table 27. Consumption of S. salina and T. gracilis cells by pearl oyster larvae at the concentration of 25 cells/µl.

Species	Algal cell consumption on different days(%)					
	d-3	d-6	d-9	d-16	d-20	d-23
S. salina	65.0	70.0	80.0	80.0	85.0	85.0
T. gracilis	15.0	15.0	15.0	10.0	10.0	-
I. galbana	62.5	65.0	68.0	70.0	75.0	75.0

C. *Tetraselmis gracilis*

Nutritional value of the microalga *T. gracilis* for the pearl oyster larvae was evaluated in an experiment carried out during January-February, 1984. The environmental parameters were as described for *S. salina*.

Larval growth

a) Mean size of larvae (Table 24, Fig. 11): Growth of larvae fed with *T. gracilis* cells has been very poor (Table 24). Mean larval sizes on days 5, 9, 13 and 17 were, respectively, 72.2, 75.7, 76.1 and 76.2 µm as compared to 81.1, 92.9, 97.6 and 106.5 µm attained by larvae fed with *I. galbana* for the same days. The larvae fed with *T. gracilis* did not grow beyond the D shape stage. All larvae had perished by day 20.

b) Size frequency distribution (Fig. 10): On day 5, all larvae fed with *T. gracilis* were in the size group 60-80 µm (Fig. 10). On day 13, while the majority of larvae remained in the same group, some had advanced to 80-100 µm. However, the larvae fed with *I. galbana* showed a progressive modal shift from 60-80 µm on day 5 to 80-100 µm on day 13 and to 100-120 µm on day 25.

c) Growth rate (Tables 25 and 26): The growth rate of pearl oyster larvae fed with *T. gracilis* cells has remained

consistently lower than that of larvae fed with I. galbana (Table 25). It was 0.93 µm/day during day 1-9 and 0.06 µm/day during day 9-17 as compared to 3.08 µm/day and 1.70 µm/day obtained with I. galbana for the same periods.

Linear regression analysis of growth data gave growth regressions of 0.0029 log µm/day for T. gracilis-fed larvae and 0.0105 log µm/day for I. galbana-fed larvae (Table 26). The growth regression of larvae fed with T. gracilis was statistically significant from that of larvae fed with I. galbana ($P < 0.01$).

d) Growth curve (Fig. 11): Very poor growth of larvae fed with T. gracilis is quite evident (Fig. 11).

- Survival rate (Fig. 11)

Since none of the larvae fed with T. gracilis were seen to reach the umbo stage, count on survival was made at the same time of sampling. Survival of D shape larvae on day 15 was 12.5% with T. gracilis as against 92.3% with I. galbana. Mortality of larvae fed with T. gracilis was complete by day 20 (Fig. 11).

- Spat setting and production (Table 24)

No spat setting of larvae fed with T. gracilis was observed (Table 24).

Algal cell consumption (Table 27)

It is seen that there is very poor consumption of T. gracilis (10-15%) as compared to that of the standard (62.5 - 75.0%) (Table 27).

D. Relative merits of C. freiburgensis, S. salina and T. gracilis as larval food of pearl oyster larvae

In the experiments described before, Chromulina freiburgensis had been tested as larval food for Pinctada fucata larvae. Isochrysis galbana was used as standard food for comparison. Similarly, Synechocystis salina and Tetraselmis gracilis were tested independently with I. galbana as the common standard. The salient features of merits and demerits of the test foods are presented in Table 28.

The mean size of the larvae from day 13 onwards was larger with I. galbana (185.5 µm on day 25) as compared to C. freiburgensis (163.8 µm on day 25). Growth regression was greater (0.0196 log µm/day) with I. galbana than with C. freiburgensis (0.0148 log µm/day). Larval survival rate was higher with I. galbana. Although spat setting commenced on the same day with both food species, spat production was higher (6.5%) in the case of I. galbana than with C. freibugensis (3.0%). The data indicate that

Table 28. Summary of results of larval rearing data to evaluate the nutritional value of single species of microalgae at the concentration of 25 cells/µl (data from Tables 19, 20, 21, 24, 25 and 26).

S.No.	Algal species	Mean size of larvae on day 25 (µm)	Larval growth per day (µm)	Growth regression (log µm/day)	Day of first setting	Duration of spat setting (days)	Spat production (%)
1.	C. freiburgensis	163.8	4.01	0.0148	21	6	3.0
2.	I. galbana	185.5	4.92	0.0196	21	10	6.5
3.	S. salina	89.0	0.86	0.0056	--	--	--
4.	T. gracilis	--	--	0.0029	--	--	--
5.	I. galbana	125.2	2.37	0.0105	28	24	8.8

IV. NUTRITIONAL VALUE OF COMBINED SPECIES OF ALGAE

In a series of experiments carried out during different periods of this study, six combinations of microalgal species were tested for their nutritional value for the pearl oyster larvae. Larval rearing with I. galbana as food was done concurrently for comparison of results.

A. Isochrysis galbana + Pavlova lutheri

The larval rearing with I. galbana + P. lutheri combination was carried out during June-July 1983, under the following ambient conditions:
Temperature : 29.2° - 31.5°C; Salinity : 36.0 - 38.0‰; and pH : 8.25 - 8.30.

1. Larval growth

a) Mean size of larvae (Table 29, Fig. 13): The initial size of the straight hinge larvae was 67.4 ± 2.5 µm (Table 29). On different days of observation, the larval sizes in the combination and standard are comparable, although minor differences are noticed. Such differences are random and do not indicate any particular trend. However, around the setting time, the larvae fed with combination diet were larger at 138.3 µm and 154.1 µm on days 21 and 25, as compared to those fed with I. galbana alone which are at 129.1 µm and 149.8 µm.

b) Size frequency distribution (Fig. 12): The pattern of size distribution is seen to be more or less

I. freiburgensis, although relatively lower in nutritional value as compared to I. galbana, is a useful food source for the pearl oyster larvae.

The microalgal species, S. salina and T. gracilis have been found to be very poor foods for pearl oyster larvae. Although the larvae survived for 53 days in S. salina, they did not grow beyond the eyed umbo stage. In the case of T. gracilis mortality of larvae was complete by day 20 and the larvae had not grown past the D shape stage. On the other hand, larval rearing with the standard I. galbana as food was normal. The growth regression of larvae for the period of rearing were 0.0035 log/um/day with S. salina and 0.0029 log/um/day with T. gracilis as compared to 0.0105 log/um/day for the standard. These two species have proved to be nutritionally inadequate for pearl oyster larvae.

Table 29. Growth and setting of pearl oyster larvae fed with a combination of *Isochrysis galbana* and *Pavlova lutheri*

Age of culture (days)	Mean size of larvae (μm)	
	I. galbana + *P. lutheri*	*I. galbana*
1	67.4 ± 2.5	67.4 ± 2.5
5	72.7 ± 2.7	71.5 ± 2.7
9	76.9 ± 3.9	76.6 ± 3.3
13	86.7 ± 7.8	89.8 ± 7.2
17	113.0 ± 16.9	118.2 ± 14.6
21	138.3 ± 29.3	129.1 ± 18.3
25	154.1 ± 27.6	149.8 ± 29.3
29	154.2 ± 22.3	158.8 ± 26.2
Day of first setting	25	25
Day of final setting	42	47
Total no. of spat	953	867
Rate of spat production(%)	7.6	6.9

uniform for larvae fed on combination diet as well as *I. galbana* diet (Fig. 12). Similar modal size groups are observed for days 5 (60-80 µm) and 13 (80-100 µm). On day 25, although the range of larval size is similar, the modes are different. With standard there is a single mode at 160-180 µm, whereas for larvae fed with combination diet there are two modes at 120-140 µm and 180-220 µm.

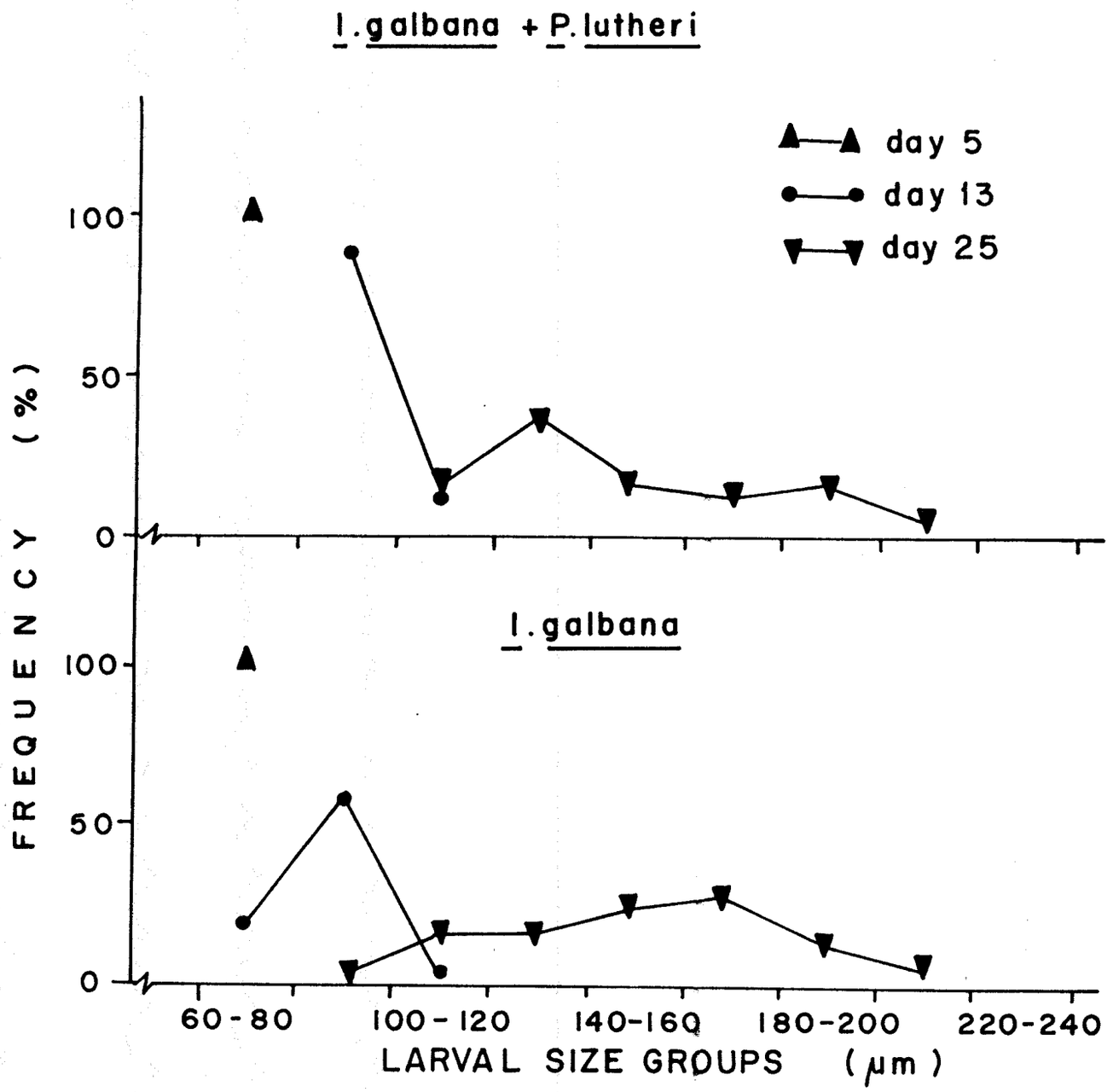
c) Growth rate (Tables 30 and 31): The overall growth rate for the period day 1-25 is marginally higher for larvae fed with the combination diet of *I. galbana* and *P. lutheri* (3.62 µm/day) as compared to that of the standard (3.43 µm/day; Table 30). Maximum growth rate was recorded during the period day 9-17 for larvae of the standard but during day 17-25 for larvae fed with the combination diet.

Table 30. Larval growth rate when fed with a combination diet of *I. galbana* and *P. lutheri*.

Period (days)	Larval growth rate per day (µm/day)	
	I + P	I
1-9	1.19	1.15
9-17	4.51	5.20
17-25	5.14	3.95
1-25	3.62	3.43

Abbreviations: P - *Pavlova lutheri*; I - *Isochrysis galbana*

Fig.12. SIZE FREQUENCY DISTRIBUTION OF PEARL OYSTER LARVAE FED ON A COMBINATION OF ISOCHRYSIS GALBANA AND PAVLOVA LUTHERI



Growth regressions of 0.0161 log$_{\mu}$m/day for larvae fed combination diet and 0.0155 log$_{\mu}$m/day for the standard (Table 31). The growth regression of larvae fed with the combination diet and standard were not found to be statistically different from each other ($P > 0.05$).

d) Growth curve (Fig. 13): Trend in larval growth is similar up to day 9 (Fig. 13). Till day 17, growth in standard is seen to be better. Thereafter, growth of larvae fed on the combination diet is seen to be better.

, Survival rate (Fig. 13)

Survival was 77.3% at the umbo stage and remained the same at the eyed umbo stage for larvae fed the combination diet. (Fig. 13). With I. galbana as diet, larval survival was 92.3% at umbo and 87.9% at eyed umbo stage. Survival to spat stage for larvae fed with the combination diet was 7.6% as compared to the 6.9% for the standard.

3. Spat setting and production (Table 29, Fig. 13)

Setting was first observed on day 25 in both larval populations but lasted for different periods of time(Table 29). Peak setting of larvae fed the combination diet was observed on day 30(Fig. 13). Thereafter, there was a gradual decline and setting was complete on day 42. For the standard, peak setting was observed on day 36, beyond which there was a

Table 31. Analysis of co-variance of pearl oyster larval growth data using a combination diet of I. galbana and P. lutheri (data from Table 29).

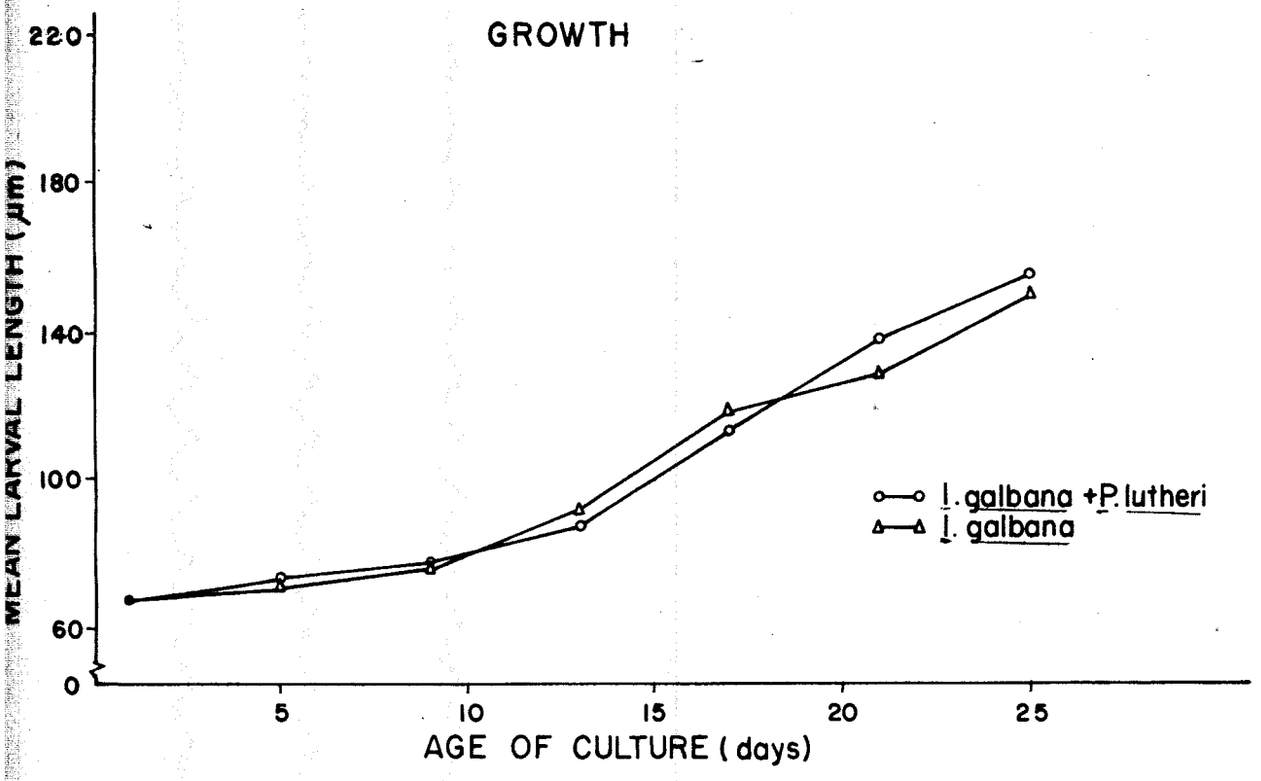
S.No.	Treatment	d f	Ex^2	Exy	Ey^2	b	d f	c.s.s.	m.s.
1.	I + P	6	448	7.2158	0.1226369	0.0161	5	0.00631420	
2.	I	6	448	6.9663	0.1121490	0.0155	5	0.00382459	
3.							10	0.0101387	0.00101387
4.		12	896	14.1821	0.2346859		11	0.0102082	
5.	Difference between slopes						1	0.0000694757	

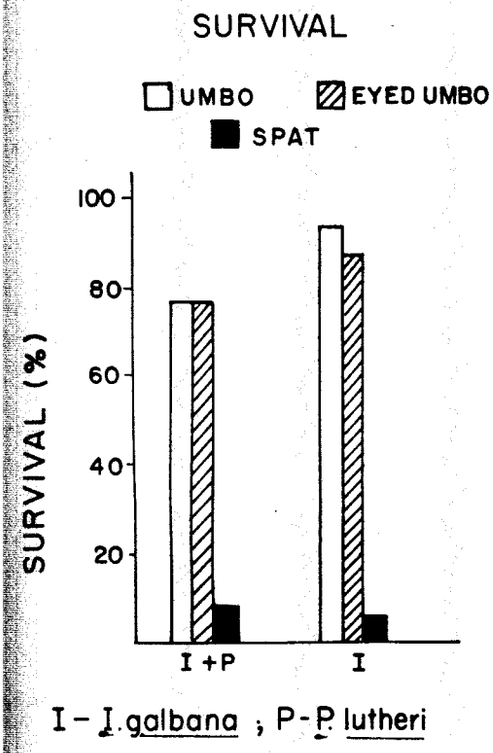
$F = 0.069^{*}(df\ 1, 10)$

*(P > 0.05) No significance.

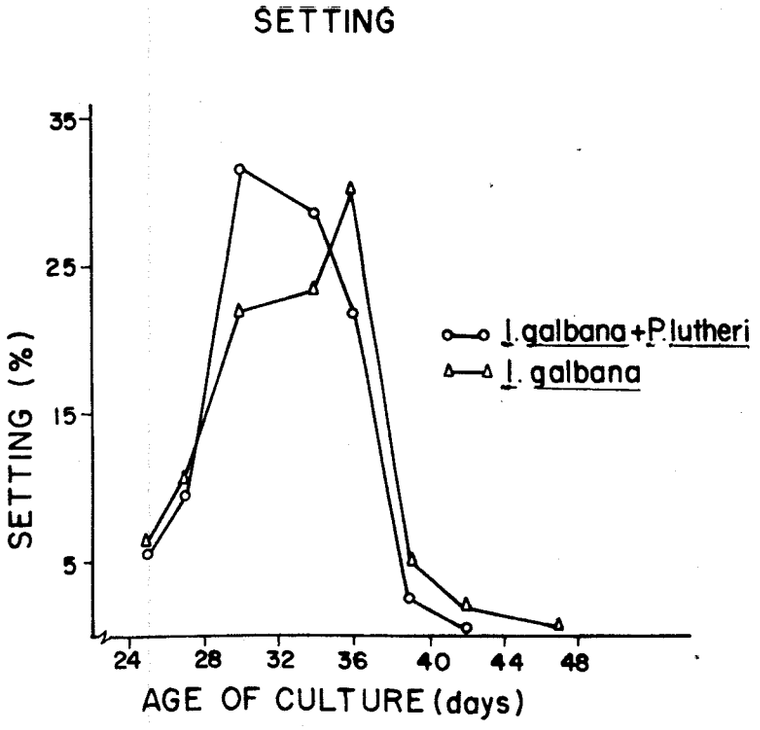
Abbreviations: I - Isochrysis galbana; P - Pavlova lutheri.

Fig. 13. GROWTH, SURVIVAL AND SETTING OF PEARL OYSTER LARVAE FED WITH A COMBINATION OF ISOCHRYSIS GALBANA AND PAVLOVA LUTHERI





I - I. galbana ; P - P. lutheri



sharp decline and setting lasted a longer period till day 47. Spat production was 6.9% in the standard and 7.6% in the combination diet. The normal deviate test showed a Z value of 2.14, indicating that spat setting in the 2 treatments was statistically significant from each other (P 0.01).

- Algal cell consumption (Table 32)

Consumption rates of P. lutheri and I. galbana species for larvae fed with the combination diet and standard I. galbana were similar (Table 32). Consumption of algal cells increased with the age of the larvae.

Table 32. Consumption of I. galbana and P. lutheri cells by pearl oyster larvae fed a combination diet

Species	Algal cell consumption on different days(%)					
	d-5	d-9	d-13	d-17	d-21	d-25
I. galbana + P. lutheri	60.0	60.0	70.0	70.0	80.0	80.0
	60.0	70.0	70.0	70.0	70.0	80.0
I. galbana	60.0	70.0	70.0	70.0	72.0	85.0

B. Isochrysis galbana + Chromulina freiburgensis

The larval rearing using the combination diet of I. galbana + C. freiburgensis was carried out during May-June

1984 when experimental conditions were as described in section III. A.

Larval growth

a) Mean size of larvae (Table 19, Fig. 9): On days 5 and 9, the mean sizes of larvae fed the combination diet were higher than those of the standard, measuring 86.2 µm and 97.7 µm as compared to 84.7 µm and 92.3 µm of the standard. Thereafter, mean sizes of larvae fed the standard diet of I. galbana were consistently greater.

b) Size frequency distribution (Fig. 8): On day 5, larvae of both treatments showed a similar size frequency distribution (Fig. 8). Thereafter, the larval size range in combination diet was narrower than for standard. While there was a single modal group at 160-180 µm on day 25 for the combination diet, there were two modal groups at 160-180 µm and 200-220 µm for the standard.

c) Growth rate (Tables 20 and 21): The overall larval growth rate for day 1-25 was higher for the standard (4.92 µm/day) than for larvae fed with the combination diet (4.42 µm/day; Table 20). The growth rate for day 1-9 was higher for larvae fed with the combination diet (3.78 µm/day) than for the standard (3.10 µm/day). Maximum growth rate was observed during day 17-25 for larvae fed with the combination diet and during day 9-17 for the standard.

Growth regressions obtained were 0.0166 log μm/day for the combination diet and 0.0196 log μm/day for standard (Table 21). The analysis of co-variance showed that the growth regressions of larvae fed combination diet and standard were statistically significant from each other ($P < 0.05$).

d) Growth curve (Fig. 9): It is seen that growth of larvae fed with the combination of I. galbana and C. freiburgensis was poorer than growth of larvae fed I. galbana alone (Fig. 9).

Survival rate (Fig.9)

Survival was 85.7% at the umbo stage in the combination diet was compared to 66.6% for the standard (Fig. 9). It was 66.6% at the eyed umbo stage on combination diet and to 44.4% in the standard. Survival to spat stage was 4.3% for the combination diet as compared to 6.5% for the standard. The data would show that although larval survival was slightly higher during the eyed umbo stage for the combination diet, spat production was relatively higher for the standard.

Spat setting and production (Tables 19 and 22, Fig. 9)

Setting was first observed on day 21 in both combination diet-fed larval rearing beakers and in the standard (Table 19). The duration of setting was seen to be only 4 days in the former and 10 days in the latter. For both standard as well as

larvae fed the combination diet, spat setting was initiated with small numbers and reached a peak on day 23 (Fig. 9). Setting was completed by day 25 in the combination diet and by day 31 in the standard diet. Survival to spat stage was 4.3% with the combination diet and 6.5% with the standard. The Z value of 7.73 (Table 22) showed that this difference was statistically significant ($P < 0.01$).

Algal cell consumption (Table 23)

Of the combination diet, relatively less C. freiburgensis cells (40-60%) was consumed I. galbana cells (70-90%) (Table 23). In the standard, larvae consumed 62.5-75.0% of I. galbana cells.

C. Isochrysis galbana + Synechocystis salina

The experiment to test the nutritional value of the combination diet of I. galbana and S. salina was carried out during January-February, 1984. I. galbana-based larval rearing was the control. Ambient conditions were as described in Section III. B.

Larval growth

a) Mean size of larvae (Table 33, Fig. 15): In both treatments, larval rearing was prolonged. While the

Synechocystis salina, *Tetraselmis gracilis* and *Isochrysis galbana* at the concentration of 25 cells/μl.

Age of culture (days)	Mean size of larvae (μm)				
	I. galbana + *S. salina*	*I. galbana* + *T. gracilis*	*S. salina* + *T. gracilis*	*I. galbana* + *S. salina* + *T. gracilis*	*I. galbana*
1	68.3 ± 2.4	68.3 ± 2.4	68.3 ± 2.4	68.3 ± 2.4	68.3 ± 2.4
5	79.2 ± 3.5	73.8 ± 6.7	73.4 ± 2.9	79.0 ± 4.0	81.1 ± 3.5
9	81.1 ± 4.9	77.5 ± 3.8	77.6 ± 4.8	82.2 ± 4.4	92.9 ± 5.2
13	100.8 ± 6.3	85.0 ± 6.4	78.5 ± 3.1	92.6 ± 6.6	97.6 ± 5.8
17	106.8 ± 8.6	92.0 ± 5.7	79.0 ± 3.4	96.1 ± 6.5	106.5 ± 5.5
21	116.7 ± 10.2	91.4 ± 5.0	79.9 ± 3.0	104.3 ± 7.9	116.7 ± 10.7
25	128.4 ± 17.6	91.9 ± 6.1	80.7 ± 3.4	106.0 ± 8.2	125.2 ± 18.1
29	129.1 ± 17.3	94.8 ± 4.7	82.4 ± 3.1	108.7 ± 8.5	142.2 ± 20.2
33	145.1 ± 2.6	-	-	106.4 ± 8.1	159.9 ± 25.0
37	150.9 ± 2.5	-	-	111.4 ± 9.7	166.4 ± 16.5
41	150.2 ± 22.5	-	-	-	193.0 ± 17.1
45	147.5 ± 24.6	-	-	-	186.4 ± 12.3
49	183.0 ± 25.0	-	-	-	178.6 ± 6.4
Day of first setting	38	-	-	-	28
Day of final setting	52	-	-	-	52
Total No. of spat	370	-	-	-	1050
Rate of spat production(%)	3.0	-	-	-	8.8

mean larval sizes were more or less comparable for both up to day 25, during the extended period, mean sizes were greater for the I. galbana-fed larvae than for the combination diet-fed larvae.

b) Size frequency distribution (Fig. 14): Similar modal size groups were seen to occur for both treatments on day 5 (60-80 µm), day 13 (80-100 µm) and day 25 (100-120 µm), while the range of larval size was seen to differ (Fig. 14).

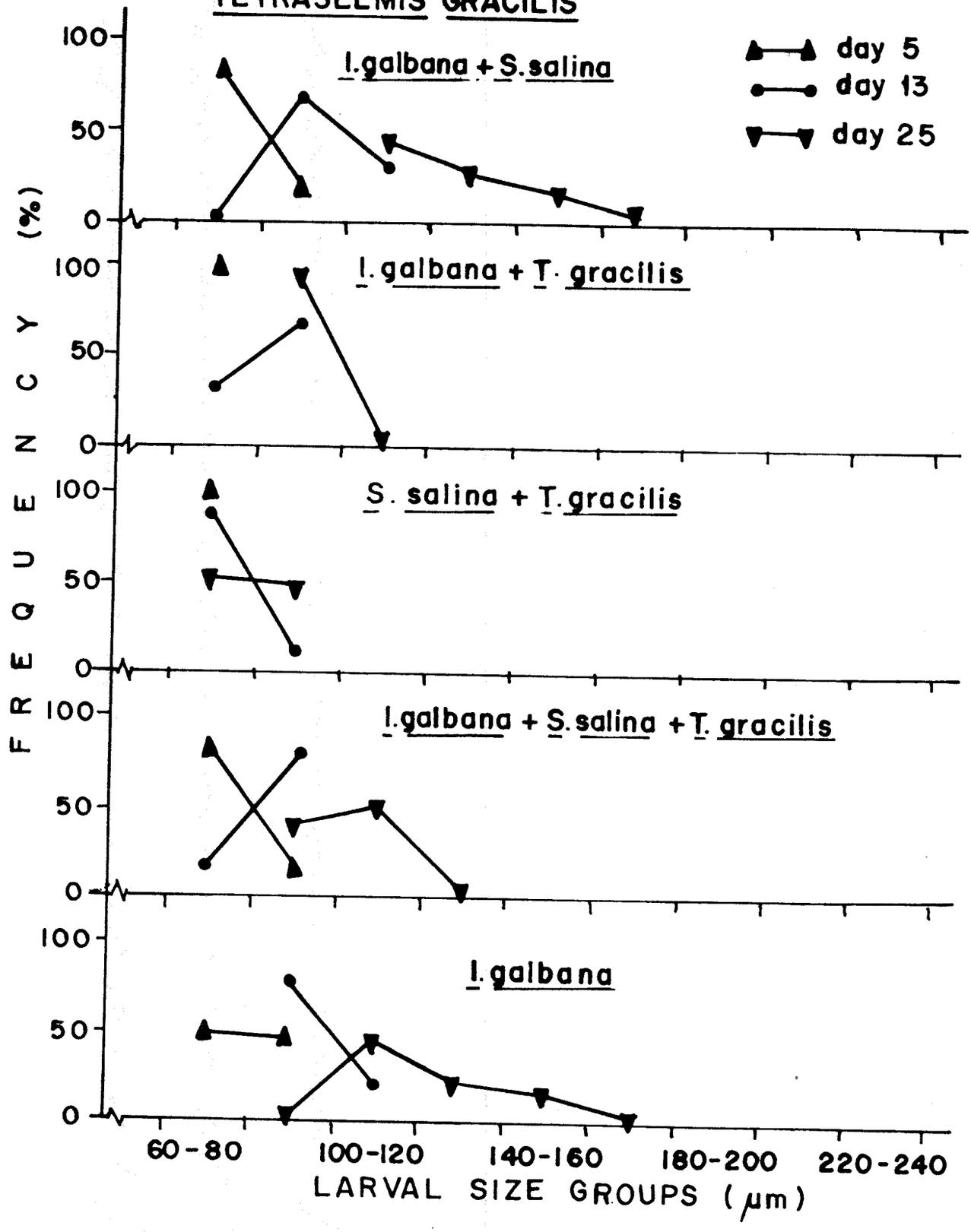
c) Growth rate (Tables 34 and 35): The overall growth rate of pearl oyster larvae fed on the combination diet of I. galbana and S. salina was marginally higher (2.50 µm/day) than that of the standard (2.37 µm/day) for day 1-25, except that the standard diet gave a higher growth rate (3.08 µm/day) than the combination diet (2.60 µm/day) during day 1-9 (Table 34).

Table 34. Larval growth rate using combinations of the microalgae S. salina, T. gracilis and I. galbana at the concentration of 25 cells/µl.

Period (days)	Larval growth per day (µm/day)				
	I + S	I + T	S + T	I+S+T	I
1-9	2.60	1.15	1.16	1.74	3.08
9-17	3.21	1.81	0.18	1.74	1.70
17-25	2.70	-	0.21	1.24	2.34
1-25	2.50	0.98	0.52	1.57	2.37

Abbreviations: I-Isochrysis galbana; S-Synechocystis salina; T-Tetraselmis gracilis.

Fig. 14. SIZE FREQUENCY DISTRIBUTION OF PEARL OYSTER LARVAE FED ON COMBINATIONS OF ISOCHRYSIS GALBANA, SYNECHOCYSTIS SALINA AND TETRASELMIS GRACILIS



Growth regressions obtained were 0.0105 log/um/day for the standard and 0.0085 log/um/day for larvae fed the combination diet of I. galbana and S. salina (Table 35). The growth regressions of the two treatments were statistically significant from each other ($P < 0.01$).

d) Growth curve (Fig. 15): Growth of larvae in both treatments is seen to follow the same trend, in spite of minor differences of mean size on different days (Fig.15). After day 25, however, I. galbana appears to support a higher growth of larvae than the combination diet.

2. Survival rate (Fig. 15)

Survival during the umbo stage was 92.3% for standard and 95.8% for larvae fed the combination diet (Fig. 15). Survival at the eyed umbo stage was reduced to 76.9% and 28.2% for the standard and larvae fed the combination diet respectively. Survival to spat stage was 3.0% and 8.8% respectively for the combination diet and the standard.

3. Spat setting and production (Table 33, Fig. 15)

Spat setting commenced on day 28 in the standard but was delayed to day 38 for larvae fed with the combination diet (Table 33). Setting lasted a period of 24 days in the standard and a period of 17 days for larvae fed with the combination diet. A total of 3.0% of the larvae reached

Table 35. Analysis of co-variance of pearl oyster larval growth data using combinations of *S. salina*, *T. gracilis* and *I. galbana* (data from Table 33).

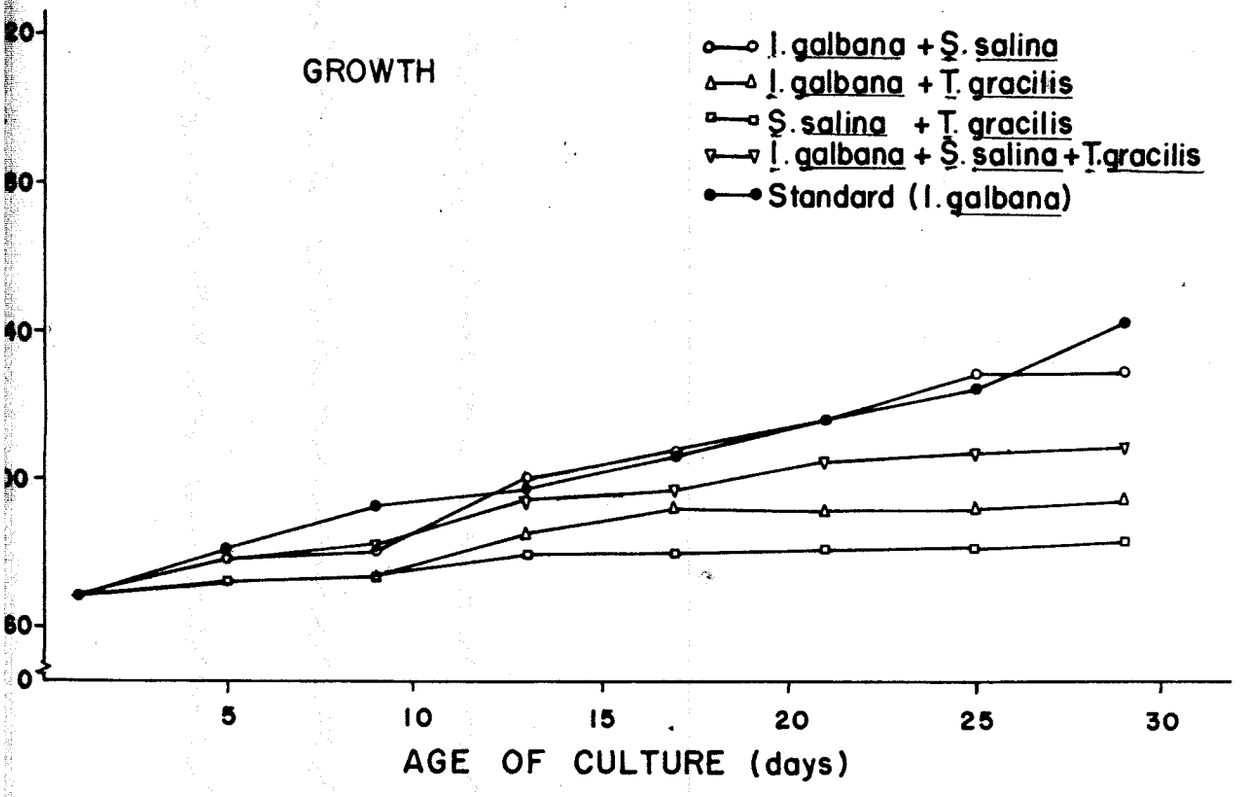
S.No.	Algal species	d f	Ex^2	Exy	Ey^2	b	d f	c.s.s.	m.s.
1.	I + S	10	1760	15.0464	0.1343880	0.0085	9	0.00575495	
2.	I + T	7	672	3.4422	0.0206573	0.0051	6	0.00302524	
3.	S + T	7	672	1.6468	0.0079648	0.0025	6	0.00392915	
4.	I + S + T	9	1320	7.3276	0.0439292	0.0056	8	0.00325213	
5.	I	10	1760	18.4255	0.1929562	0.0105	9	0.00005901	
6.							38	0.0160205	0.000421592
7.		43	6184	45.8885	0.3998956		42	0.059379	
8.	Difference between slopes						4	0.0433585	0.0108396

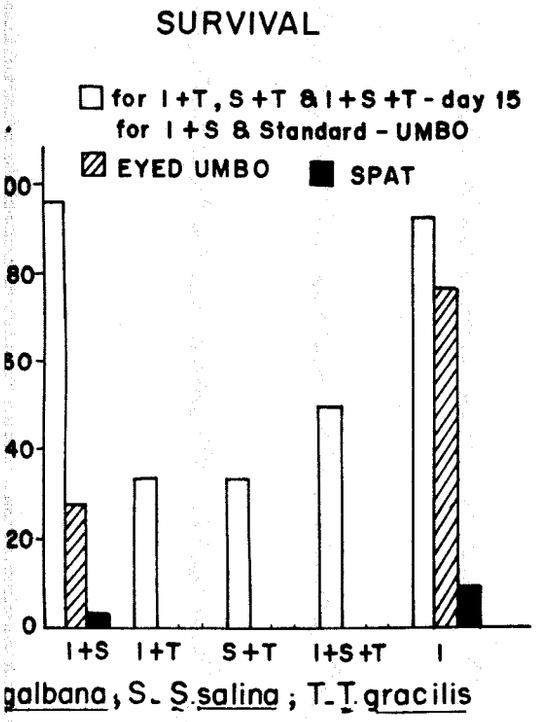
$F = 25.71$ (d f, 4, 38)*

*($P < 0.01$) Highly significant

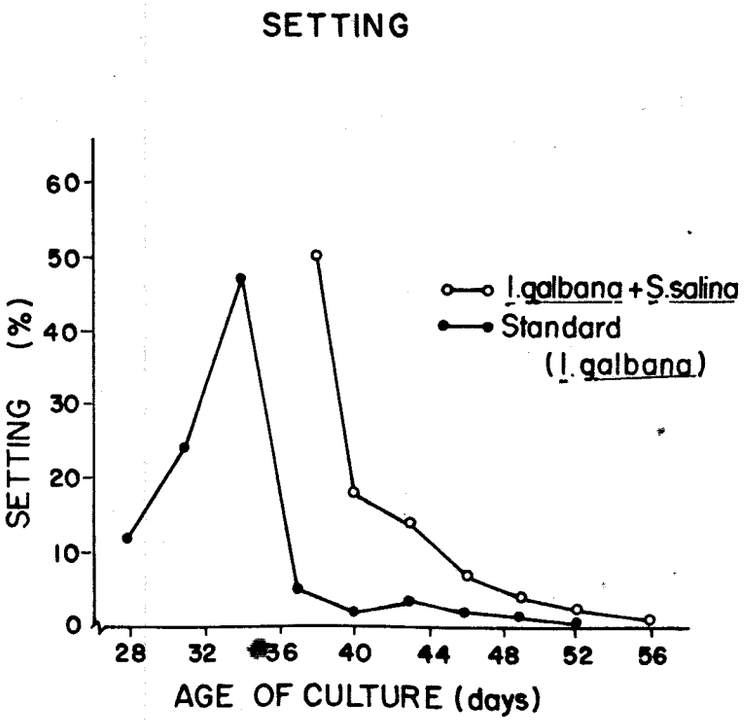
Abbreviations: I - *Isochrysis galbana*; S - *Synechocystis salina*; T - *Tetraselmis gracilis*

g.15. GROWTH SURVIVAL AND SETTING OF PEARL OYSTER LARVAE WHEN FED WITH COMBINATIONS OF SYNECHOCYSTIS SALINA, TETRASELMIS GRACILIS AND ISOCHRYSIS GALBANA





galbana ; S - S. salina ; T - T. gracilis



the spat stage on the combination diet as compared to the 8.8% of the standard. The Z value being 19.66, showed that percentage spat production of larvae fed with combination diet and standard were statistically significant from each other ($P < 0.01$).

Peak setting was observed on day 34 for I. galbana-fed larvae and on day 38 for larvae fed with the combination diet (Fig. 15).

- Algal cell consumption (Table 36)

Up to day 31, consumption has been steadily increasing with the combination diet (60-80% for S. salina and 70-90% for I. galbana). When fed with I. galbana alone, consumption was relatively lower (62.5-75.0%).

D. Isochrysis galbana + Tetraselmis gracilis

Evaluation of the combination diet of I. galbana and T. gracilis was carried out during January-February, 1984 when environmental parameters were as described for the combination diet of I. galbana + S. salina.

- Larval growth

a) Mean size of larvae (Table 33, Fig. 15): From Table 33, it is seen that on all days, growth of larvae fed with the

Table 36. Consumption of I. galbana, S. salina and T. gracilis cells by pearl oyster larvae when fed with combination diets.

Algal Species	Algal cell consumption on different days (%)					
	d-3	d-6	d-12	d-20	d-27	d-31
S. salina	60.0	70.0	70.0	70.0	80.0	80.0
+						
I. galbana	70.0	80.0	80.0	80.0	90.0	90.0
T. gracilis	10.0	20.0	20.0	20.0	25.0	-
+						
I. galbana	70.0	70.0	70.0	75.0	75.0	-
S. salina	70.0	70.0	70.0	75.0	85.0	-
+						
T. gracilis	10.0	20.0	20.0	20.0	10.0	--
I. galbana	90.0	90.0	100.0	100.0	100.0	100.0
+						
S. salina	45.0	45.0	60.0	60.0	75.0	90.0
+						
T. gracilis	15.00	15.0	30.00	30.0	30.0	
I. galbana	62.5	65.0	65.0	70.0	70.0	75.0

combination diet of I. galbana + T. gracilis has been consistently inferior to that of the standard. It is also seen that the majority of the larvae were still in the D shape stage even on day 25 in the combination diet, whereas for I. galbana diet, umbo stage had been reached on day 17.

b) Size frequency distribution (Fig. 14): The pattern of size distribution shows that larvae fed on combination diet has a restricted increase in size as compared to that of the standard (Fig. 14). None of the larvae fed the combination diet grew beyond 100-120 µm size, while larvae of the standard were observed at the 160-180 µm range.

c) Growth rate (Tables 34 and 35): The overall growth rate of pearl oyster larvae for day 1-25 has been poor at 0.98 µm/day (Table 34) for larvae fed with a combination of I. galbana and T. gracilis as compared to that of the standard (2.37 µm/day).

Linear regression analysis of the larval growth data yielded growth regressions of 0.0051 log µm/day for larvae fed the combination diet and 0.0105 log µm/day of the standard (Table 35). As expected, the analysis of covariance showed that these values were highly significant between each other ($P < 0.01$).

d) Growth curve (Fig. 15): It is seen that growth of the larvae fed the combination diet was far below that of the standard (Fig. 15).

Survival rate (Fig. 15)

Larvae fed on the combination diet of I. galbana + T. gracilis did not survive beyond day 29, with very few larvae having reached the umbo stage. However, when survival was monitored during the early umbo stage for the standard (day 15), a similar count was taken for larvae fed the combination diet (Fig. 15). Survival was seen to be 33.3% for larvae fed with the combination diet, while it was 92.3% for the standard. Thereafter, there was total mortality in the former case, none of the larvae surviving beyond day 29.

Spat setting and production (Table 33, Fig. 15)

As indicated earlier, the larvae did not grow beyond 100-120 µm size with the combination diet (Table 33). Spat setting was initiated on day 28 for the standard, lasting a period of 24 days and yielding a total spat production of 8.8%. Peak setting was observed on day 34 (Fig. 15).

Algal cell consumption (Table 36)

The algal consumption data presented below show that there was poor consumption of T. gracilis cells 10-25%. On

the other hand, consumption of I. galbana cells in the standard was 62.5-75.0%.

E. Synechocystis salina + Tetraselmis gracilis

Nutritional evaluation of the combination diet S. salina and T. gracilis was carried out during January-February 1984 when environmental parameters were as described III C.

- Larval growth

a) Mean size of larvae (Table 33, Fig.15): Table 33 shows that from day 5, the rate of growth of larvae fed with the combination diet of S. salina and T. gracilis was consistently poorer than that of the standard, and that larvae did not grow beyond the straight hinge stage.

b) Size frequency distribution (Fig. 14): The modal size group for days 5, 13 and 25 remained in the 60-80 µm size group indicating the very poor rate of growth of larvae fed on this combination diet (Fig. 14).

c) Growth rate (Tables 34 and 35): The overall growth rate for day 1-25 of pearl oyster larvae fed with S. salina and T. gracilis (0.52 µm/day) is far below that of the standard (2.37 µm/day). Growth rate declined from

1.16 µm/day for day 1-9 to 0.18 and 0.21 µm/day, respectively, for day 9-17 and day 17-25 (Table 34).

Growth regressions of 0.0025 log µm/day for larvae fed with the combination diet and 0.0105 log µm/day for standard were obtained. As expected the analysis of covariance (Table 35) showed that the growth regression of larvae fed on combination diet was statistically significant from that of the standard ($P < 0.01$).

d) Growth curve (Fig. 15): Growth of larvae fed on the combination diet has been consistently poor from day 5 onwards (Fig. 15).

Survival rate (Fig. 15)

Survival was 92.3% for larvae of the standard at the umbo stage. On the same day of observation, survival was seen to be only 33.3% for larvae fed with the combination diet (Fig. 15). Larvae fed on the combination diet did not survive beyond day 29.

Spat setting and production (Table 33)

As stated earlier, the larvae did not grow beyond D shape stage on the combination diet. The total spat production was 8.8% for the standard (Table 33).

Algal cell consumption (Table 36)

It is seen from Table 36 that consumption of S. salina cells was high (70-85%) while that of T. gracilis cells remained very low (10-20%). Consumption of I. galbana cells in the standard was in the range of 62.5-75.0%.

Isochrysis galbana + Synechocystis salina + Tetraselmis gracilis

A triple combination of I. galbana, S. salina and T. gracilis was tested for its nutritional value during January-February, 1984. Temperature ranged from 23.9 to 25.1°C, salinity from 33.5 to 35.0‰ and pH from 8.10 to 8.20.

Larval growth

a) Mean size of larvae (Table 33, Fig. 15): It is seen from Table 33 that, from day 5 growth of the pearl oyster larvae fed this triple combination diet has been slower than that of the standard.

The early umbo stage was observed by day 17 in the standard and by day 21 in the combination diet. Although the larvae survived for 37 days on the combination diet, they did not grow beyond the umbo stage.

b) Size frequency distribution (Fig. 14): The modes were at 60-80/um on day 5, 80-100/um on day 13 and

100-120 µm on day 25 in the combination diet (Fig. 14). Although the modes were the same as for standard, the size range was restricted to the lower size groups up to 120-140 µm in the combination diet.

c) Growth rate (Tables 34 and 35): The overall growth rate of pearl oyster larvae fed with the combination diet of I. galbana, S. salina and T. gracilis was 1.57 µm/day as compared to 2.37 µm/day for the standard (Table 34). Growth rate declined from 1.74 µm/day observed for day 1-9 and 9-17 to 1.24 µm/day for day 17-25 for larvae fed with combination diet.

Linear regression analysis of growth data yielded growth regression of 0.0056 log µm/day for larvae fed with the combination diet and 0.0105 log µm/day for standard. The analysis of co-variance (Table 35) showed that the growth regression of the two treatments were significant between each other ($P < 0.01$).

d) Growth curve (Fig. 15): It is seen from Fig. 15 that growth of larvae fed this combination diet was consistently poorer than that of larvae fed on I. galbana.

Survival rate (Fig. 15)

Larval survival was monitored for the umbo stage and calculated to be 47.4% for larvae fed the combination diet

as compared to the 92.3% for the standard (Fig. 15). Mortality was total beyond day 37, the larvae not progressing beyond the early umbo stage using the combination diet.

Spat setting and production (Table 33)

No spat setting occurred using the combination diet. In comparison, there was a total spat production of 8.8% for the standard.

Algal cell consumption (Table 36)

Consumption of T. gracilis cells was relatively poor (15-30%) while consumption of S. salina cells was higher (45- 90%) and that of I. galbana cells was maximum(Table 36).

G. Relative merits of combined algal diets as larval food of pearl oyster larvae

The salient data on the algal combinations diet, I. galbana + P. lutheri, I. galbana + C. freiburgensis, I. galbana + S. salina, S. salina + T. gracilis and I. galbana + S. salina + T. gracilis are presented in Table 37 for comparison.

In the combination I. galbana + P. lutheri, although mean larval size was seen to fluctuate between larvae fed

on this diet and standard I. galbana, growth regression was greater for the former (0.0161 log/um/day) (Table 37). Spat setting commenced on the same day (day 25), but duration of setting was shorter for larvae fed on combination diet (17 days) than for larvae of the standard diet (22 days). Total spat production was also higher for larvae fed on combination diet (7.6%) than for standard (6.9%).

Pearl oyster larvae reared on I. galbana + C. freiburgensis recorded higher mean values up to day 9, but thereafter larvae fed on standard I. galbana remained consistently bigger. The growth regression of larvae reared on combination diet (0.0166 log/um/day) was lower than that of the standard (0.0196 log/um/day). Survival of larvae reared on combination diet was seen to be higher at both umbo and eyed umbo stages, but total spat production of the standard (6.5%) was seen to be greater than that for the combination diet (4.3%). Again, although spat setting was initiated on day 21 in both treatments, setting lasted for a duration of 4 days for larvae fed on the combination diet, and for 10 days standard (Table 37).

Where the combined diet I. galbana + S. salina was used, mean size of larvae was 128.4/um as compared to 125.2/um of the standard on day 25. Thereafter, however, larval size of the standard remained greater. The growth regression of the standard rearing was 0.0105 log/um/day and that of the

Table 37. Summary of results of larval rearing data to evaluate the nutritional value of combined algal species at the uniform feeding level of 25 cells/µl (data from Tables 19, 20, 21, 29, 30, 31, 33, 34 and 35).

S.No.	Algal diet	Mean size of larvae on day 25 (µm)	Larval growth per day (µm/day)	Growth regression (log µm/day)	Day of first setting	Duration of spat setting (days)	Spat production (%)
1.	I + P	154.1	3.62	0.0161	25	17	7.6
2.	I	149.8	3.43	0.0155	25	22	6.8
3.	I + C	173.6	4.42	0.0166	21	4	4.3
4.	I	185.5	4.92	0.0196	21	10	6.5
5.	I + S	128.4	2.50	0.0085	38	17	3.0
6.	I + T	91.9	0.98	0.0051	--	--	Nil
7.	S + T	80.7	0.52	0.0025	--	--	Nil
8.	I + T + S	106.0	1.57	0.0056	--	--	Nil
9.	I	125.2	2.37	0.0105	28	24	8.8

Abbreviations : I - *Isochrysis galbana*; P - *Pavlova lutheri*; C - *Chromulina freiburgensis*; S - *Synechocystis salina*; T - *Tetraselmis gracilis*

larvae fed with combination diet was 0.0085 log/um/day. Survival of larvae fed on combination diet at the eyed umbo stage was very poor (28.2%) as compared to that of the standard (76.9%). Total spat production was also seen to be poor for larvae fed on combination diet (3.0%) as compared to the standard diet (8.8%).

The other combination diets, I. galbana + T. gracilis, S. salina + T. gracilis and I. galbana + S. salina + T.gracilis were seen to be relatively poor combinations for pearl oyster larvae. Mean size of larvae fed on I. galbana + T. gracilis showed that by day 25 the majority of the larvae were still in the D shape stage (91.9/um) while that of the standard were already in the umbo stage (125.2/um). None of the larvae here survived beyond day 29. On day 25, the larvae fed with S. salina + T. gracilis were in the straight-hinge stage (80.7/um) while those of the standard had reached the umbo stage(125.2/um). Pearl oyster larvae reared on a combination diet of I. galbana + S. salina + T. gracilis showed a comparatively longer survival period (37 days). The majority had reached the umbo stage (104.3/um) by day 21 as compared to the standard (106.5/um) on day 17. Growth beyond this period was very slow. The growth regressions were seen to be 0.0051 log/um/day for larvae reared on I. galbana + T. gracilis; 0.0025 log/um/day for larvae reared on

S. salina + T. gracilis and 0.0056 log µm/day for larvae reared on I. galbana + S. salina + T. gracilis. These algal diets were seen to be nutritionally inadequate for pearl oyster larvae.

H. Index of relative nutritional value of microalgal diets

Reviewing the experimental results of larval rearing on the five algal species I. galbana, P. lutheri, C. freiburgensis, S. salina and T. gracilis, either singly or in combination at 25 cells/µl, it is seen that these species had varying effects on pearl oyster larval growth and setting. In order to make comparisons between these experiments, an index of relative nutritional value has been calculated, based on the growth regressions of larvae in different rearings. The index is derived as follows:

$$\text{Index of relative nutritional value of the test algal species} = \frac{\text{Larval growth regression with the test algal species}}{\text{Larval growth regression with the standard algal species}}$$

Isochrysis galbana has been used as the standard food in all experiments. The indices of relative nutritional value of the experimental diets are presented in Table 38.

The only algal species that has given a relative nutritional value greater than 1 is Pavlova lutheri (1.07).

All other algal species, viz., C. freiburgensis, S. salina and T. gracilis has shown values less than 1. The five algal species with their relative indices may be written in order of merit as Pavlova lutheri (1.07), Isochrysis galbana (1.00), Chromulina freiburgensis (0.77), Synechocystis salina (0.32) and Tetraselmis gracilis(0.28).

It should be noted that with the exception of the two algal species S. salina and T. gracilis that were run concurrently, the other test algal species, P. lutheri and C. freiburgensis were evaluated for their food value at different periods of the year. Larval growth, survival and setting production of these experiments would have been subject to the prevailing ambient conditions and their parentage. This may particularly be noted by the growth regressions of the standard algal species I. galbana which varied from 0.0105 to 0.0233 log/um/day.

The combination diet I. galbana + P. lutheri, although conducted at a different time as P. lutheri alone, also yielded a relative nutritional value index greater than 1 (1.04). Combination of I. galbana + C. freiburgensis was conducted simultaneously along with the single species C. freiburgensis. Likewise, combinations of I. galbana with S. salina and T. gracilis were conducted simultaneously with experiments with S. salina and T. gracilis alone. In

Table 38. Index of relative nutritional value of the microalgal diets for pearl oyster larvae against the standard of I. galbana.

Sl No.	Algal diet.	Growth regression (log µm/day)		Relative Index
		Standard diet	Test diet	
1.	I	0.0233		-
2.	P		0.0250	1.07
3.	I	0.0196		-
4.	C		0.0150	0.77
5.	I+C		0.0166	0.85
6.	I	0.0105		-
7.	S		0.0034	0.32
8.	T		0.0029	0.28
9.	I+S		0.0085	0.81
10.	S+T		0.0051	0.49
11.	S+T		0.0025	0.24
12.	I+S+T		0.0056	0.53
13.	I	0.0155		-
14.	I+P		0.0161	1.04

Abbreviations: I - Isochrysis galbana; P - Pavlova lutheri
C - Chromulina freiburgensis; S - Synechocystis salina
T - Tetraselmis gracilis.

each of these instances the addition of the standard alga, I. galbana improved the food value of the test algal species. Thus, for C. freiburgensis, relative nutritional value index improved from 0.77 to 0.85; for S. salina, the index improved from 0.32 to 0.81 and for T. gracilis from 0.28 to 0.49. A triple combination diet of I. galbana + S. salina + T.gracilis had an index (0.53) that was less than that observed for I. galbana + S. salina (0.81) and greater than that of I. galbana * T. gracilis (0.49). Lowest index was recorded for the combination diet of S. salina and T. gracilis (0.24). This value was lower than that recorded for both species when fed alone (0.32 for S. salina and 0.28 for T. gracilis). By value of their relative index, the combination diets may be considered in the following order of merit: I. galbana + P. lutheri (1.04), I. galbana + C. freiburgensis (0.85) I. galbana + S. salina (0.81) I. galbana + S. salina + T. gracilis (0.53), I. galbana + T. gracilis (0.49) and S. salina + T. gracilis (0.24). Amongst these diets, only the first three resulted in spat production, while the latter three did not produce any spat.

V. EFFICACY OF STRATIFIED FEEDING

Two experiments were conducted to study the efficacy of stratified feeding on larval growth and spat setting. In the first, there was a stepwise quantitative increase in algal cell concentration with the onset of each larval stage. In the second, while the algal cell concentration was kept constant at 25 cells/µl qualitative changes were introduced using different algal species with the onset of each larval stage.

A. Stratified feeding schedule of Isochrysis galbana

The first experiment with Isochrysis galbana as food was conducted during June-July 1983, when the environmental parameters were as follows:
Temperature : 29.2 - 31.5°C; Salinity : 36.0 - 38.0‰; and pH : 8.25-8.30.

The feeding protocol of the four treatments numbered 1, 2, 3 and 4 was as follows:

Larval stage	Algal cell concentration (cells/µl)			
	Treatment 1	Treatment 2	Treatment 3	Treatment 4
D shape	10	15	20	25
Umbo	15	20	25	30
Eyed umbo	20	25	30	35

Larval growth

a) Mean size of larvae (Table 39, Fig. 17): Up to day 9, there has been very little difference in mean larval sizes between treatments (Table 39). On days 13, 17 and 21, growth was seen to be maximum in treatment 3 (89.6, 119.7, and 131.6 µm) and minimum in treatment 1 (84.6, 103.9 and 119.0 µm). On days 25 and 29, maximum mean larval size was observed in treatment 4 (158.3 and 163.6 µm) and minimum in treatment 1(138.7 and 141.4 µm).

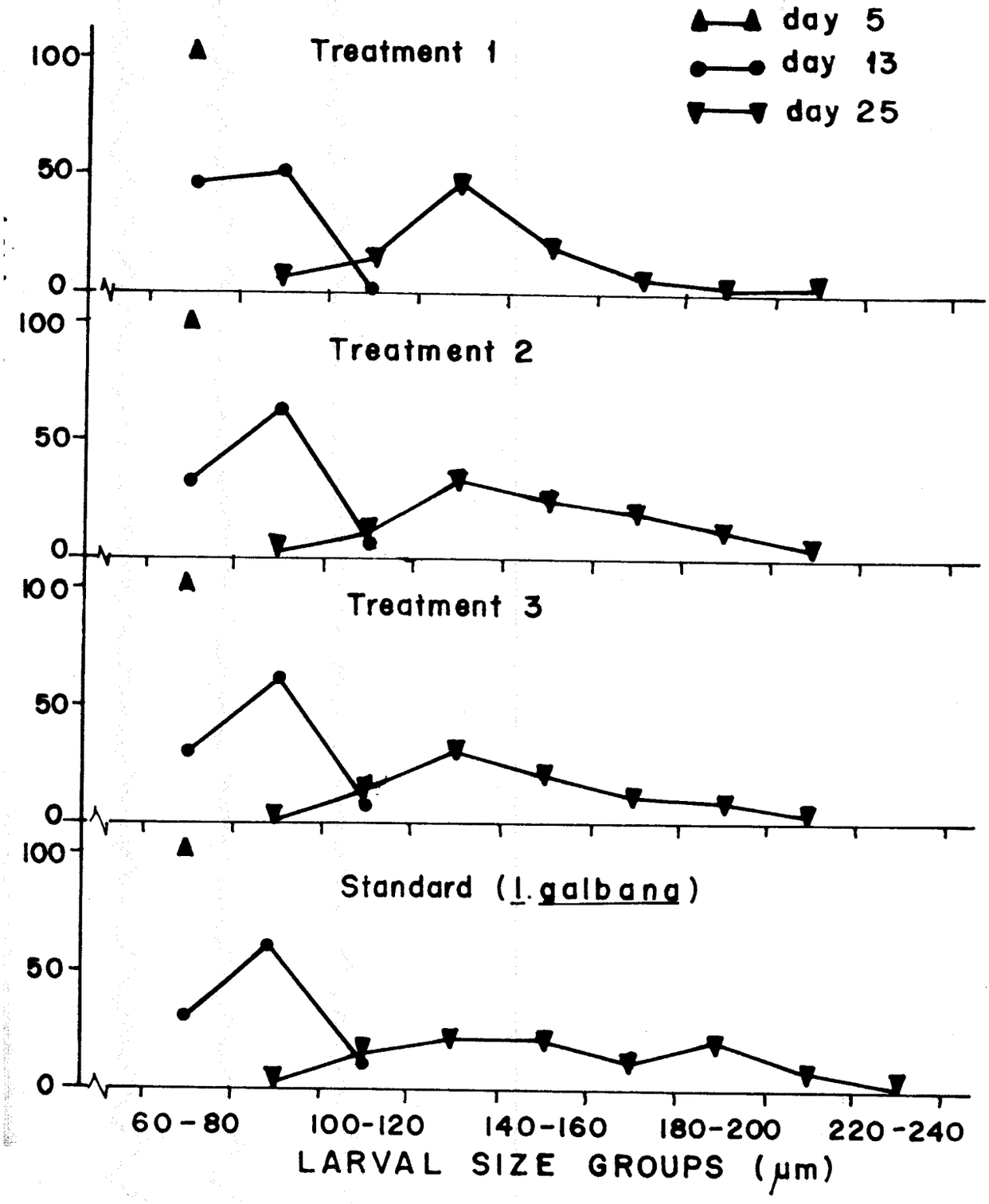
b) Size frequency distribution (Fig. 16): For days 5 and 13, the range of larval size groups as well as the modal groups are the same in all treatments (Fig. 16). On day 25, although the modal size group is the same for all treatments, the range is wider for treatment 4 (80-100 to 220-240 µm) than for treatments 1, 2 and 3 (80-100 to 200-220 µm).

c) Growth rate (Tables40 and 41): The growth rate shows progressive increase from treatment 1 through 4, indicating that, generally, the higher amounts of ration supplied as the larvae grew had resulted in higher rates of growth (Table 40). Initially during day 1-9, there was not much difference in growth rates (1.18-1.20 µm/day) among treatments 2-4 (15, 20 and 25 cells/µl), but were all higher than growth rate of larvae of treatment 1 (10 cells/µl)

schedule of *Isochrysis galbana*

Age of culture(days)	Mean size of larvae (μm)			
	Treatment 1	Treatment 2	Treatment 3	Treatment 4
1	67.4 ± 2.5	67.4 ± 2.5	67.4 ± 2.5	67.4 ± 2.5
5	72.2 ± 2.9	72.4 ± 2.7	72.0 ± 2.9	72.3 ± 3.1
9	74.9 ± 3.9	77.0 ± 3.5	76.8 ± 2.8	77.0 ± 3.4
13	84.6 ± 7.9	87.8 ± 7.6	89.6 ± 9.2	89.5 ± 9.7
17	103.9 ± 13.7	112.0 ± 15.6	119.7 ± 20.0	111.5 ± 14.5
21	119.0 ± 20.3	125.0 ± 19.1	131.6 ± 27.6	127.0 ± 22.7
25	138.7 ± 24.0	150.1 ± 19.5	157.9 ± 28.7	158.3 ± 32.6
29	141.4 ± 17.2	144.2 ± 26.4	159.0 ± 24.7	163.6 ± 23.2
33	161.9 ± 23.9		180.3 ± 19.1	173.2 ± 22.4
Day of first setting	25	25	25	25
Day of final setting	47	47	47	47
Total No. of spat	767	1515	2172	722
Rateof spat production (%)	6.1	12.1	17.4	5.8

Fig. 16. SIZE FREQUENCY DISTRIBUTION OF PEARL OYSTER LARVAE FED ON A STRATIFIED FEEDING SCHEDULE OF ISOCHRYSIS GALBANA



(0.94 µm/day). During the next phase day 9-17, growth rate in treatment 3 at 25 cells/µl was highest (5.36 µm/day), as compared to growth rates in treatment 1 at 15 cells/µl (3.63 µm/day), in treatment 2 at 20 cells/µl (4.38 µm/day) and in treatment 4 at 30 cells/µl (4.34 µm/day). For the period day 17-25, growth rate in treatment 4 at 35 cells/µl was maximum (5.85 µm/day).

Table 40. Larval growth rate when fed with a stratified feeding schedule of Isochrysis galbana.

Period (days)	Larval growth per day (µm/day)			
	Treatment 1	Treatment 2	Treatment 3	Treatment 4
1-9	0.94	1.20	1.18	1.20
9-17	3.63	4.38	5.36	4.34
17-25	4.35	4.76	4.78	5.85
1-25	2.97	3.45	3.77	3.79

Growth regressions of 0.0136, 0.0150, 0.0155 and 0.0157 log µm/day were obtained for treatments 1, 2, 3, and 4, respectively (Table 41). There was, however, no statistically significant difference in growth regressions between treatments ($P > 0.05$).

d) Growth curve (Fig. 17): Growth curves for all treatments are similar up to day 9(Fig. 17). Thereafter,

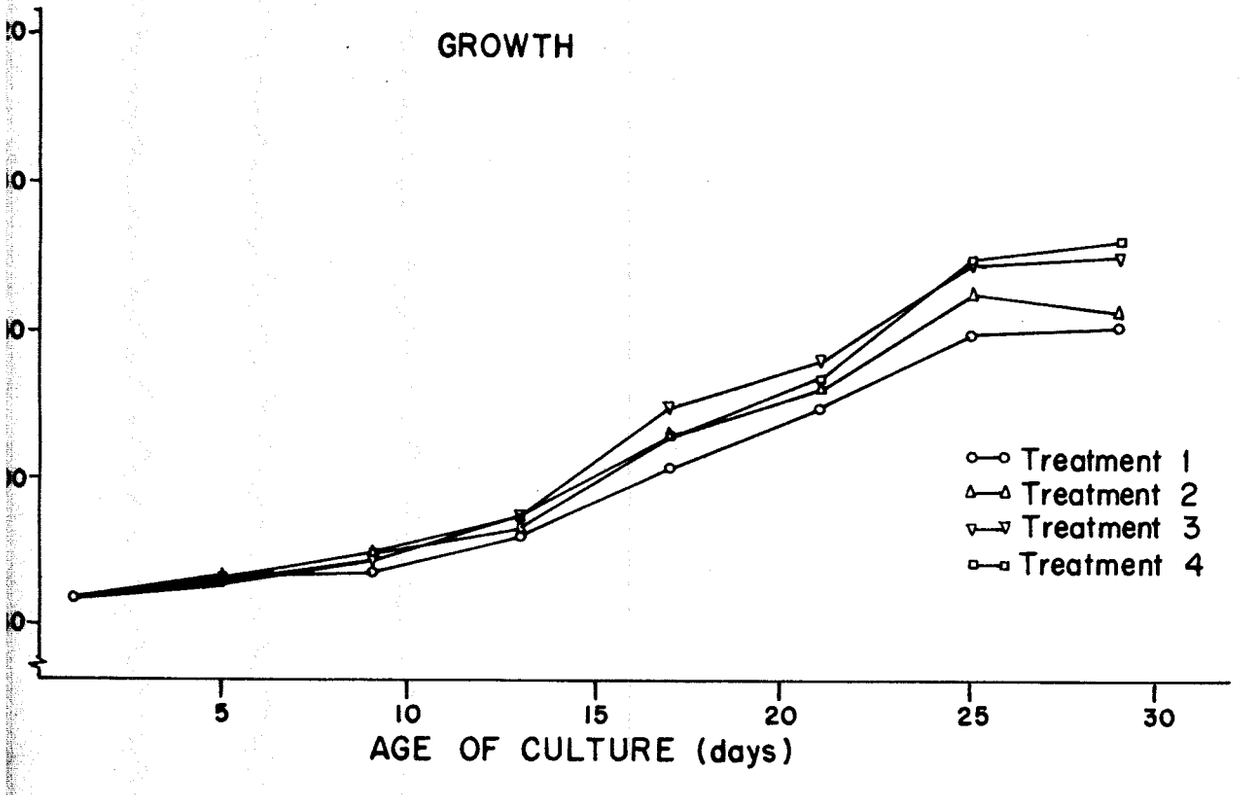
Table 41. Analysis of co-variance of pearl oyster larval growth data [illegible] stratified feeding schedule of Isochrysis galbana (data from Table 39).

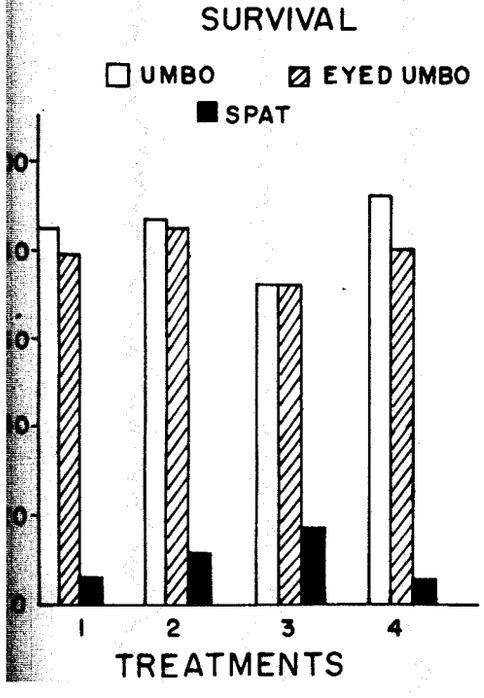
S. No.	Treatment	d f	Ex^2	Exy	Ey^2	b	d f	c.s.s.	m.s.
1.	1	6	448	6.0664	0.0859052	0.0136	5	0.00375937	
2.	2	6	448	6.7201	0.1030611	0.0150	5	0.0022581	
3.	3	6	448	6.9242	0.1111328	0.0155	5	0.00411372	
4.	4	6	448	7.0465	0.1138246	0.0157	5	0.00299164	
5.							20	0.0131462	0.00065731
6.		24	1792	26.7572	0.4139237		23	0.0143989	
7.	Difference between slopes						3	0.00125278	0.000417595

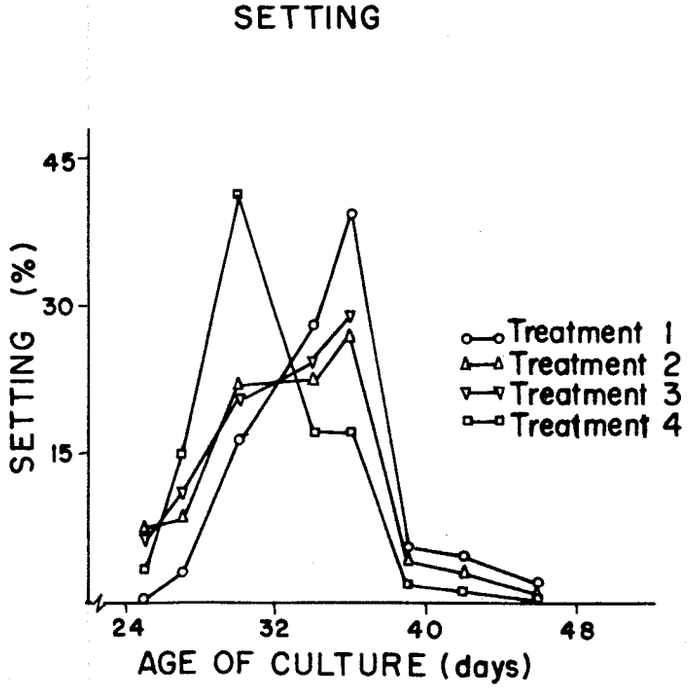
$F = 0.64^*$ (d f, 3, 20)

*($P > 0.05$) No significance

Fig. 17. GROWTH, SURVIVAL AND SETTING OF PEARL OYSTER LARVAE FED WITH A STRATIFIED FEEDING SCHEDULE OF ISOCHRYSIS GALBANA







differences in growth rates become distinct and the order on day 29 is treatment 4, 3, 2 and 1, the last being the lowest.

Survival rate (Fig. 17)

Larval survival during umbo stage was seen to be 84.2, 88.8, 72.4 and 90.0% for treatments 1, 2, 3 and 4, respectively (Fig. 17). Survival was 80.0, 84.6, 72.4 and 80.3% during the eyed umbo stage in the same order of treatments. While these values did not show any particular trend, final survival to spat increased from 6.1% in treatment 1, to 12.1% in treatment 2, and 17.4% in treatment 3 and declined to 5.8% in treatment 4.

Spat setting and production (Tables 39 and 42, Fig. 17)

Larval setting commenced uniformly on day 25 and lasted a total duration of 22 days in all treatments(Fig.17). However, while peak setting was observed on day 30 in treatment 4, it was delayed to day 36 in all other treatments. Total spat production was 6.1, 12.1, 17.4 and 5.8% for treatments 1, 2, 3 and 4 respectively (Table 39). There was statistically significant difference in percentage spat setting between treatments 1 and 2, 2 and 3 and 3 and 4 ($P < 0.01$).

Table 42. Normal deviate test (Z value): comparison of mean spat setting using a stratified feeding schedule of Isochrysis galbana.

Treatment	1	2	3	4
1	-	16.64*	28.25*	1.01**
2		-	11.85*	17.61*
3			-	28.00*
4				-

*P < 0.01

**P < 0.05

Summary of results of larval rearing using the stratified feeding schedule of I. galbana

The data show that the three growth related parameters, namely, mean larval size, daily growth rate and growth regression, show progressive increase from treatment 1 to treatment 4, although the differences between treatments 3 and 4 are only marginal. With regard to the two parameters on spat setting, namely initiation and duration, the four treatments have given identical results. Spat production increased from 6.1% in treatment 1 to 17.4% in treatment 3 but declined to 5.8% in treatment 4 (Table 43). In view of the fact that maximum growth rate for the three phases day 1-9, day 9-17 and day 17-25, occurred in treatments 2, 3 and 4 respectively, it appears that cell concentrations for the corresponding periods, i.e., 15, 25 and 35 cells/µl would be

Table 43. Comparison of the salient features of larval rearing data using a stratified feeding protocol (data from Tables 39, 40 and 41).

Treatment	Mean size of larvae on day 29 (µm)	Larval growth per day (µm/day)	Growth regression (log µm/day)	Day of first setting	Duration of spat setting (days)	Spat production (%)
1.	141.4	2.97	0.0136	25	22	6.1
2.	144.2	3.45	0.0150	25	22	12.1
3.	159.0	3.77	0.0155	25	22	17.4
4.	163.6	3.79	0.0157	25	22	5.8

the most appropriate stratification for feeding the pearl oyster larvae.

B. Stratified feeding protocol of mixed algal species

The experiment on stratified feeding, involving the use of three algal species, I. galbana, T. chui and Chlorella salina was conducted during August-September, 1983 when the ambient conditions were as follows: Temperature : 29.0-31.5°C; Salinity : 37.0-38.1‰; and pH : 8.15-8.20.

Larvae were fed at the uniform algal cell concentration of 25 cells/µl. I. galbana alone was used as food for comparison of results. The feeding protocol was as follows:

Larval Stage	Algal species used for feeding				
	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Standard
D Shape	I	I	I + T	I + T	I
Umbo	I	I + T	I + T	I + T + C	I
Eyed Umbo	I + T	I + T	I + T + C	I + T + C	I

Abbreviations: I - Isochrysis galbana; T - Tetraselmis chui; C - Chlorella salina

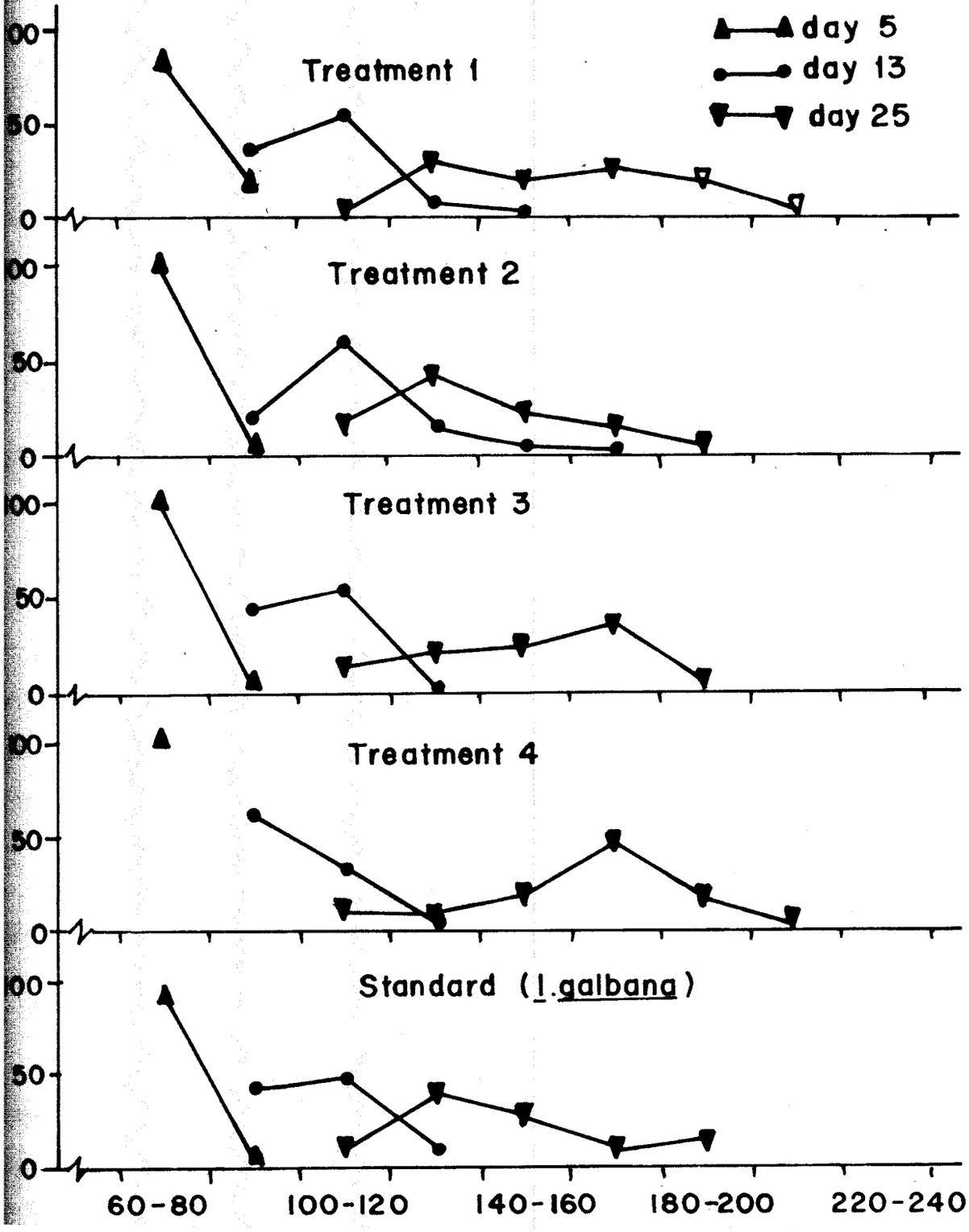
Larval growth

a) Mean size of larvae (Table 44, Fig. 19): In treatment 1, T. chui was introduced on day 20, causing no perceptible change in mean size (Table 44), over that of standard. In treatment 2, the same algae was introduced on day 14 after the onset of the umbo stage. As compared to the standard, average larval size was smaller from day 21. In treatment 3, T. chui was introduced from day 1. Larval size was similar to that of other treatments and standard till day 9, but thereafter, growth rate was poor. The alga , C. salina was introduced on day 20, and the growth rate improved as seen from the mean size on days 21 and 25 (133.6 and 153.3 µm). In treatment 4, where T. chui was provided from day 1 onwards, larval mean size on day 9 (95.8 µm) was higher than that of standard (87.2 µm). C. salina was introduced in the diet from day 15. Although larval mean size on day 17 (119.9 µm) was less than that of the standard (126.0 µm), maximum mean larval size was recorded in this treatment from day 21 to day 29.

b) Size frequency distribution (Fig. 18): On day 5, similar modal group is seen to occur for all treatments (Fig. 18). On day 13, similar size ranges and modal groups (100-120 µm) occur in treatments 1, 2, 3 and standard. Mode is at a smaller size group (80-100 µm) in treatment 4. On day 25, larvae of treatments 1 and 2, which are on a

Age of culture (days)	Mean size of larvae (μm)				
	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Standard
1	69.0 ± 2.3	69.0 ± 2.3	69.0 ± 2.3	69.0 ± 2.3	69.0 ± 2.3
5	74.0 ± 4.1	77.8 ± 2.9	78.0 ± 3.0	77.7 ± 3.1	77.8 ± 3.0
9	92.2 ± 5.7	92.8 ± 6.6	93.5 ± 6.7	95.8 ± 6.5	87.2 ± 4.8
13	107.9 ± 12.7	114.6 ± 15.1	103.4 ± 7.9	102.7 ± 8.0	106.6 ± 11.3
17	130.4 ± 17.9	131.6 ± 17.1	118.2 ± 11.8	119.9 ± 14.3	126.0 ± 26.9
21	145.2 ± 20.3	137.4 ± 25.1	133.6 ± 17.5	162.7 ± 23.0	152.1 ± 27.4
25	158.4 ± 25.8	139.7 ± 19.5	153.3 ± 22.5	164.1 ± 25.6	154.1 ± 18.9
29	170.1 ± 18.8	167.1 ± 18.3	159.4 ± 20.3	178.6 ± 16.7	175.8 ± 18.9
33	172.0 ± 18.1	165.3 ± 21.2	171.7 ± 21.0	175.6 ± 12.0	184.3 ± 17.6
Day of first Setting	22	22	22	22	22
Day of final setting	45	45	45	45	41
Total No. of spat	272	205	170	1083	324
Rate of spat production (%)	2.2	1.6	1.4	8.7	2.6

g. 18. SIZE FREQUENCY DISTRIBUTION OF PEARL OYSTER LARVAE FED ON A STRATIFIED FEEDING PROTOCOL OF MIXED ALGAL SPECIES



combination diet of I. galbana + T. chui show a wide range and a modal size group (120-140 µm) that is similar to that of standard. Larvae of treatments 3 and 4 which are on a combination diet of I. galbana + T. chui + C. salina show a modal size group (160-180 µm) which is greater than that of the standard (120-140 µm).

c) Growth rate (Tables 45 and 46): Growth rate was seen to fluctuate between treatments (Table 45). The overall growth rate for the period day 1-25 was maximum in treatment 4 (3.96 µm/day) and minimum in treatment 2 (2.95 µm/day). In treatment 1 where the combination diet of I. galbana and T. chu . was introduced from day 20, growth rate has more or less followed the same pattern as that of the standard (Table 45). Growth rate was maximum for the period 9-17 (4.78 µm/day) as compared to day 1-9 (2.90 µm/day) and day 17-25 (3.50 µm/day). In treatment 2, the combination of I. galbana and T. chui was introduced from day 14. Although the growth rate is comparable to that of the standard in the earlier two periods, a steep decline is observed for day 17-25 (1.01 µm/day as compared to 3.51 µm/day of the standard). In treatments 3 and 4, where the combination diet of I. galbana and T. chui was introduced from day 1 onwards, higher growth rate for the period from day 1-9 is seen for treatments 3(3.06 µm/day) and 4 (3.35 µm/day) than for standard (2.28 µm/day). In treatment 3, with the continuation

of the diet, growth rate has remained the same (3.09 /um/day) as compared to the higher growth rate observed for standard (4.85 /um/day). With the introduction of C. salina in the diet from day 20, growth rate of the larvae of treatment 3 has improved to 4.39 /um/day for the period day 17-25. The growth rate pattern of treatment 4 is also the same as that of the treatment 3, recording a decline in growth rate for the period day 9-17. The alga C. salina was introduced from day 15 onwards. Maximum growth rate of 5.53 /um/day was observed in this treatment from day 17-25.

Table 45. Larval growth rate when fed with a stratified feeding protocol.

Period (days)	Larval growth per day (/um/day)				
	Treatment 1	Treatment 2	Treatment 3	Treatment 4	I. galbana
1-9	2.90	2.98	3.06	3.35	2.28
9-17	4.78	4.85	3.09	3.01	4.85
17-25	3.50	1.01	4.39	5.53	3.51
1-25	3.73	2.95	3.51	3.96	3.55

Linear regression analysis of growth data gave growth regressions of 0.0162, 0.0139, 0.0144, 0.0167 and 0.0160 log /um/day for treatments 1, 2, 3, 4 and standard respectively (Table 46).

Table 46. Analysis of co-variance of pearl oyster larval growth data using a stratified feeding protocol. (data from Table 44).

S. No.	Treat-ment	d f	Ex^2	Exy	Ey^2	b	d f	c.s.s.	m.s.
1.	Std.	6	448	7.1529	0.1153487	0.0160	5	0.00114339	
2.	1	6	448	7.2740	0.119671	0.0162	5	0.00154201	
3.	2	6	448	6.2494	0.0912224	0.0139	5	0.004049	
4.	3	6	448	6.4380	0.009279	0.0144	5	0.00027249	
5.	4	6	448	7.4985	0.1280448	0.0167	5	0.00253698	
6.							25	0.0095436	0.000381744
7.		30	2240	34.6128	0.5470539		29	0.0122126	
8.	Difference between slopes						4	0.0026690	0.00066725

$F = 1.74^{*}$ (d f, 4,25)

*(P >0.05) No significance

d) Growth curve (Fig. 19): There is only a marginal difference in the growth of larvae till day 5.(Fig. 19). Thereafter, the growth curves become increasingly divergent. After day 17, the growth curves criss-cross one another with changes in the order of merit of different treatments. The growth curve of treatment 4 was generally higher than that of all other treatments during the later period.

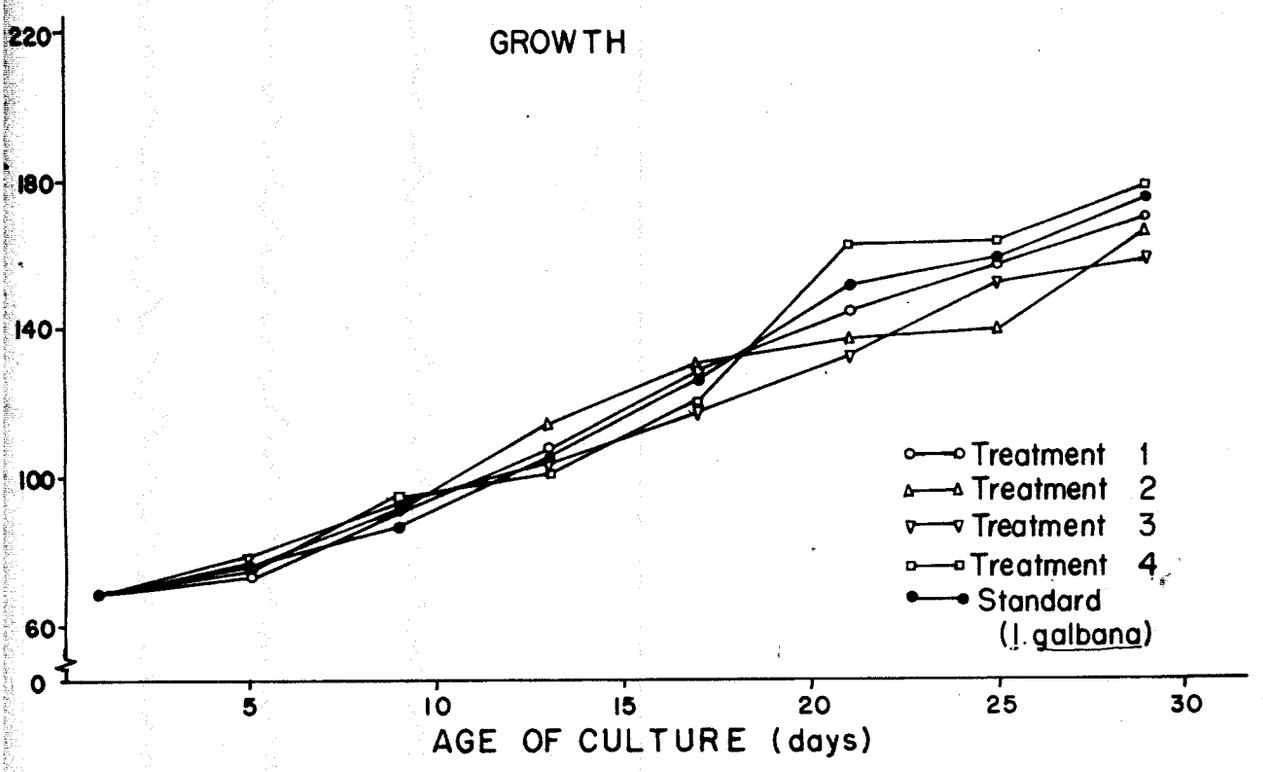
Survival rate (Fig. 19)

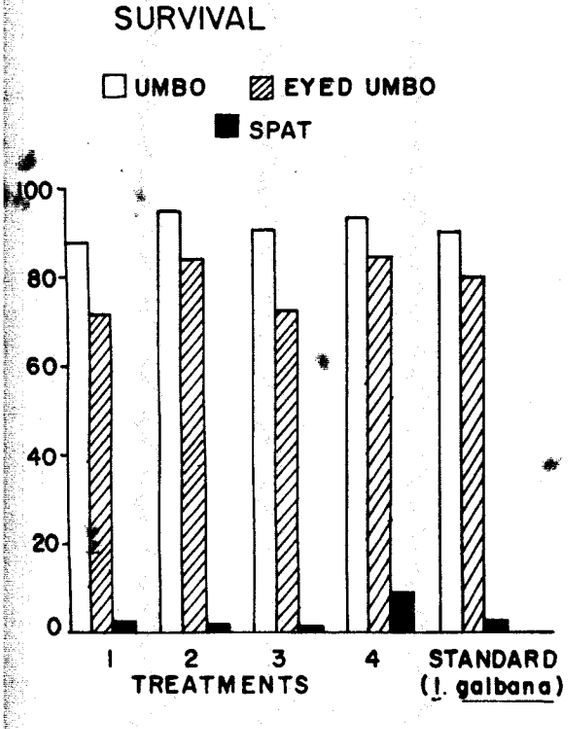
The larval survival rate monitored at the umbo stage was 88.0, 95.5, 91.3, 92.6 and 90.0% for treatments 1, 2, 3, 4 and standard respectively (Fig. 19). Percentage survival for the eyed umbo stage was 71.1, 84.0, 73.1, 82.8 and 80.0% for treatments 1, 2, 3, 4 and standard respectively, while total spat production was seen to be 2.2, 1.6, 1.4, 8.7 and 2.6% for the same order of treatments.

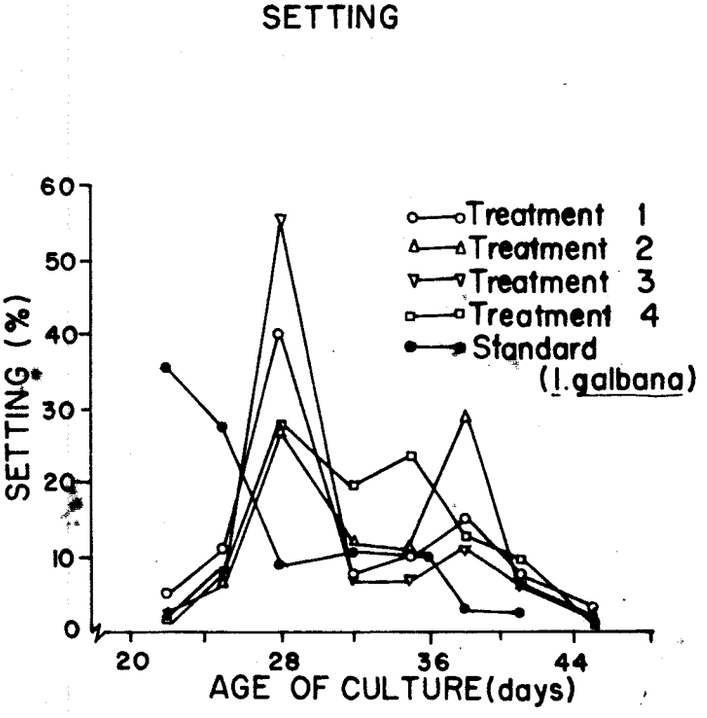
Spat setting and production (Tables 44 and 47, Fig. 19)

Setting was initiated on day 22 in all treatments and lasted till day 45 in treatments 1, 2, 3 and 4 (Table 44). Peak setting was observed on day 22 for standard and uniformly on day 28 for treatments 1, 2, 3 and 4 (Fig. 19). For all experimental conditions a smaller peak is observed on day 38, while setting in standard was seen to decline steadily. Total spat production was 2.2, 1.6, 1.4, 8.7 and 2.6% for treatments

Fig. 19.. GROWTH, SURVIVAL AND SETTING OF PEARL OYSTER LARVAE FED WITH A STRATIFIED FEEDING PROTOCOL







1, 2, 3, 4 and standard respectively. There was statistically significant difference between percentage spat production of treatments 1, 2, 3, 4 and standard ($P < 0.01$).

Table 47. Normal deviate test (Z value): comparison of mean spat setting using a stratified feeding protocol of mixed algae.

Treatment	1	2	3	4	Standard
1	-	3.52*	4.78*	22.98*	2.08*
2		-	1.32**	25.75*	5.59*
3			-	26.84*	6.82*
4				-	21.17*
Standard					-

*$P < 0.01$
**$P < 0.05$

Algal cell consumption (Table 48)

The consumption of I. galbana has remained high (45-70%) when introduced singly. When the proportion of I. galbana was reduced either to half or a third of 25 cells/µl, with combined feeding, consumption was seen to increase up to 100%. In comparison, consumption of T. chui cells has remained consistently low (6.9-20.0%). Consumption of C. salina cell was in the range of 48.6-85.0%.

Table 48. Consumption of I. galbana, T. chui and C. salina cells by pearl oyster larvae.

Treatment	Algal type	Algal cell consumption on different days(%)						
		d-4	d-7	d-10	d-14	d-17	d-20	d-23
1.	I. galbana	40.0	50.0	60.0	65.0	70.0	66.6	83.3
	T. chui						25.0	25.0
2.	I. galbana	50.0	55.0	60.0	70.0	80.0	80.0	90.0
	T. chui				10.0	20.0	10.0	10.0
3.	I. galbana	60.0	60.0	70.0	80.0	85.0	85.0	100.0
	T. chui	10.0	10.0	10.0	20.0	10.0	6.0	6.0
	C. salina						48.6	85.0
4.	I. galbana	50.0	60.0	60.0	70.0	85.0	85.0	100.0
	T. chui		10.0	20.0	10.0	6.9	17.4	6.9
	C. salina					48.6	85.0	85.0
Standard	I. galbana	55.0	55.0	60.0	60.0	75.0	75.0	80.0

Summary of results of larval rearing experiment using a stratified feeding protocol (Table 49)

The mean larval sizes and growth regressions do not follow any trend (Table 49). Although spat setting was initiated uniformly on day 22 in all treatments, duration of setting differed between treatments. The percentage spat setting did not show any trend. Treatment 4 gave the highest mean larval size (164.1 μm), maximum growth regression 0.0167 log μm/day and maximum spat setting (8.7%).

Table 49. Summary of results of larval rearing experiment using stratified feeding protocol.

S. No.	Treatment	Mean size of larvae on day 25 (µm)	Larval growth rate d 1-25 (µm day)	Growth regression (log µm day)	Day of first setting	Duration ofspat setting (days)	Spat production (%)
1.	1	158.4	3.73	0.0162	22	23	2.2
2.	2	139.7	2.95	0.0139	22	20	1.6
3.	3	153.3	3.51	0.0144	22	16	1.4
4.	4	164.1	3.96	0.0167	22	20	8.7
5.	Std	154.1	3.55	0.0160	22	20	2.6

VI. NUTRITIONAL VALUE OF MIXED PHYTOPLANKTON RAISED FROM A NATURAL STOCK OF PHYTOPLANKTON

The mixed phytoplankton bloom raised from a natural stock of phytoplankton was evaluated for its nutritional value for pearl oyster larvae during September-October, 1983. Environmental parameters of the larval rearing were as follows: Temperature : 26.3 - 28.6°C; salinity : 29.0 - 30.2‰; and pH : 8.15 - 8.20.

The experimental protocol was as given below:

Larval stage	Treatment 1	Treatment 2	Treatment 3	Standard
D shape	MP	I	I	I
Umbo	MP	MP	I	I
Eyed Umbo	MP	MP	MP	I

Abbreviations : I-*Isochrysis galbana*; MP-Mixed phytoplankton

Larval growth

a) Mean size of larvae (Table 50, Fig. 21): On day 5, the range of mean larval lengths was narrow between 77.5 - 77.9 µm in all treatments and the standard (Table 50). Treatment 3, where mixed phytoplankton was introduced during the eyed umbo stage, gave the highest larval sizes up to day 37. Treatment 1, where the mixed phytoplankton was introduced during the straight hinge stage, gave the least

feeding schedule of mixed phytoplankton.

Age of culture (days)	Mean size of larvae (μm)			
	Treatment 1	Treatment 2	Treatment 3	Standard
1	72.4 ± 3.0	72.4 ± 3.0	72.4 ± 3.0	72.4 ± 3.0
5	77.9 ± 3.0	77.5 ± 3.0	77.9 ± 3.5	77.6 ± 3.1
9	77.9 ± 2.8	86.2 ± 4.3	89.7 ± 5.7	88.2 ± 6.8
13	78.8 ± 3.6	92.2 ± 11.2	96.2 ± 10.3	93.6 ± 7.8
17	80.2 ± 4.9	99.6 ± 17.6	110.3 ± 11.0	110.5 ± 10.2
21	83.6 ± 4.8	118.9 ± 23.7	136.3 ± 17.3	133.8 ± 14.4
25	92.4 ± 9.6	144.0 ± 23.7	156.8 ± 21.2	143.4 ± 17.2
29	96.5 ± 11.7	128.6 ± 21.0	162.5 ± 22.4	150.1 ± 22.7
33	-	136.9 ± 18.9	185.8 ± 28.8	174.2 ± 20.3
37	-	165.8 ± 22.3	185.0 ± 24.1	174.4 ± 24.1
41	-	185.7 ± 27.2	179.2 ± 27.0	186.0 ± 8.6
45	-	-	178.2 ± 22.7	181.3 ± 16.7
49	-	-	175.7 ± 17.8	165.0 ± 14.7
Day of first setting	-	36	33	33
Day of final setting	-	48	53	51
Total no. of spat	-	9	964	494
Rate of spat production (%)	-	0.1	7.7	4.0

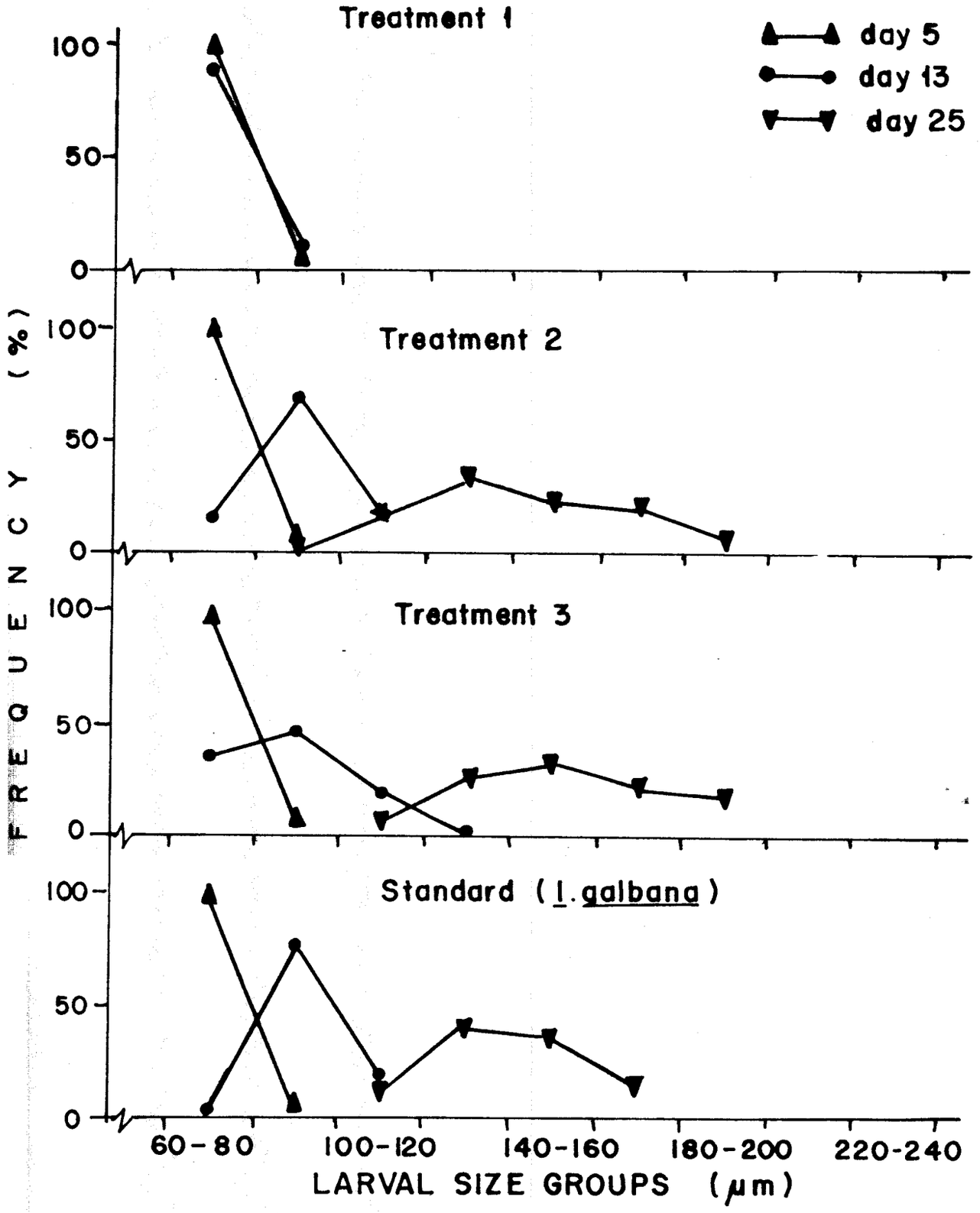
larval growth. By day 33, larvae of treatment 1 suffered total mortality.

Larvae in treatment 1 did not attain umbo stage. In treatment 2, where mixed phytoplankton was provided with the onset of the umbo stage, larval measurements were always less than those obtained in the standard and treatment 3.

b) Size frequency distribution (Fig. 20): Similar modal groups (60-80 µm) occur in all treatments for day 5 (Fig. 20). Larvae provided with mixed phytoplankton from the D shape remained in the same size range (60-80 µm to 80-100 µm) on day 5 as well as on day 13. For other treatments and standard, however, uniform modal groups (80-100 µm) occur on day 13. On day 25, range of larval sizes is narrower for treatment 3 and standard (100-120 to 160-180 µm) as compared to treatment 2 (80-100 to 180-200 µm) Modal size group of larvae of treatment 3 (140-160 µm) occurs at a higher range than for standard and treatment 2 (120-140 µm).

c) Growth rate (Tables 51 and 52): The overall growth rate of pearl oyster larvae for the period day 1-25 was minimum in treatment 1 (0.83 µm/day). and maximum in treatment 3 (3.52 µm/day), while growth rates of treatments 2 (2.98 µm/day) and standard (2.96 µm/day) were similar

Fig. 20. SIZE FREQUENCY DISTRIBUTION OF PEARL OYSTER LARVAE FED ON MIXED PHYTOPLANKTON



(Table 51). In treatment 1, where the mixed phytoplankton was introduced from the D shape stage, a decline in growth rate from day 1-9 (0.69 µm/day) to day 9-17 (0.29 µm/day) is observed. Maximum growth rate is observed for the period day 17-25 (1.53 µm/day). In treatment 2, where mixed phytoplankton was introduced from the umbo stage, there is a decline in growth rate for day 9-17 (1.68 µm/day) as compared to period day 1-9 (1.73 µm/day). Again, maximum growth rate for this treatment occurred during day 17-25 (5.55 µm/day). In treatment 3, where mixed phytoplankton was introduced after the onset of the eyed umbo stage, maximum growth rate (5.81 µm/day) was observed during day 17-25.

Table 51. Larval growth rate when fed with a stratified feeding schedule of mixed phytoplankton.

Period (days)	Larval growth per day (µm/day)			
	Treatment 1	Treatment 2	Treatment 3	Standard
1-9	0.69	1.73	2.16	1.98
9-17	0.29	1.68	2.58	2.79
17-25	1.53	5.55	5.81	4.11
1-25	0.83	2.98	3.52	2.96

Growth regressions were 0.0040, 0.0104, 0.0124 log µm/day for treatments, 1, 2, and 3 and 0.0116 log µm/day

for standard (Table 52). These were found to be statistically significant between each other ($P < 0.01$). The student's t test indicated that growth regression of treatment 3 was significant from all other values ($P < 0.01$). The growth regression of treatments 2 and standard and treatment 3 were not significantly different ($P > 0.05$). On the other hand, the growth regressions of treatment 2 and 3 were found to be significantly different ($P < 0.01$).

d) Growth curve (Fig. 21): The growth curve for larvae fed on mixed phytoplankton from D shape stage is almost flat up to day 21 and shows a slight rise thereafter (Fig. 21). Growth curve of larvae in treatment 2 was inferior to that of both standard and treatment 3 up to day 25. The growth curve for treatment 3 remained distinctly elevated throughout.

Survival rate (Fig. 21)

Survival at umbo stage was 85.7, 90.0 and 93.3% in treatments 2, 3 and standard respectively. Survival on the same day (day 15) for larvae of treatment 1 was 78.6% (Fig. 21). Survival was reduced to 36.7, 83.1 and 84.8% at the eyed umbo stage for the same order of treatments. Survival to spat stage was 0.1, 7.7 and 4.0% for treatments 2, 3 and standard respectively.

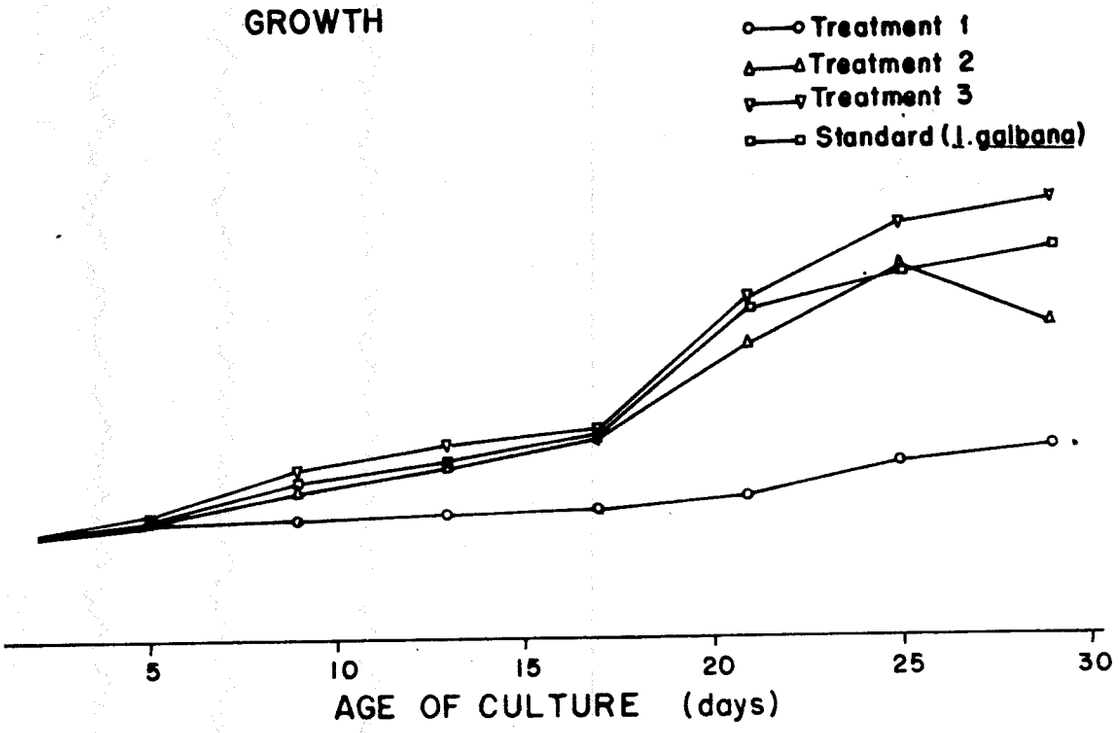
Table 52. Analysis of co-variance of pearl oyster larval growth data using a stratified feeding schedule of mixed phytoplankton (data from Table 50).

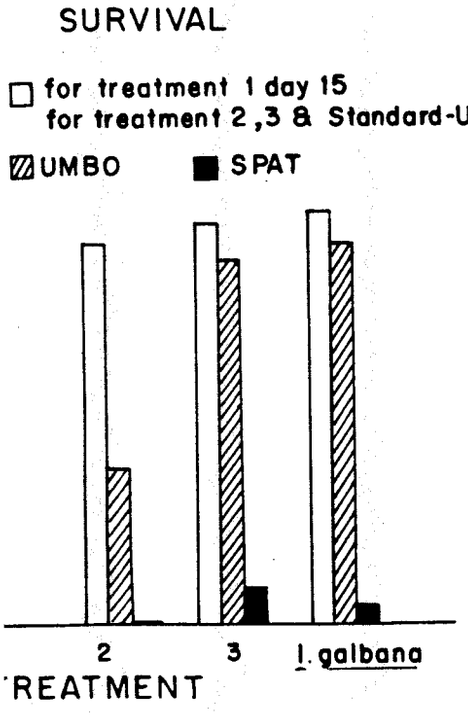
S. No.	Treat-ment	d f	Ex^2	Exy	Ey^2	b	d f	c.s.s	m.s
1.	1	7	672	2.6614	0.010594	0.0040	6	0.000054024	
2.	2	9	1320	13.7322	0.147608	0.0104	8	0.0038492	
3.	3	9	1320	16.3997	0.2071632	0.0124	8	0.00341307	
4.	Std.	9	1320	15.3681	0.1810287	0.0116	8	0.00210559	
5.							30	0.0094215	0.00031405
6.		34	4632	48.1614	0.5454939		33	0.0447346	
7.	Difference between slopes						3	0.0353131	0.011771

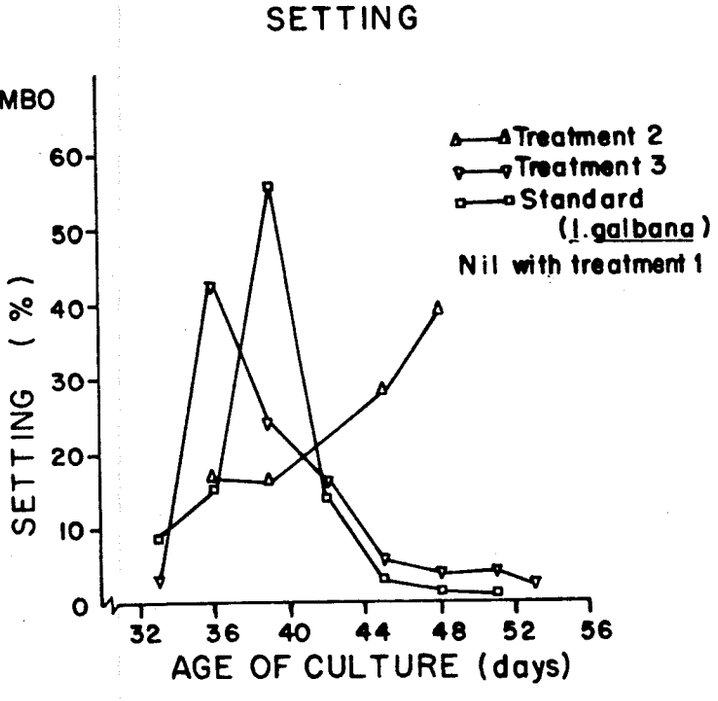
$F = 37.49^*$ (d f, 3, 30)

*($P < 0.01$) Highly significant

GROWTH, SURVIVAL AND SETTING OF PEARL OYSTER LARVAE FED WITH MIXED PHYTOPLANKTON







Spat setting and production (Tables 50 and 53, Fig.21)

In treatment 3 and standard, setting was initiated on day 33 (Table 50). It was delayed to day 36 in treatment 2. Setting lasted for different periods of time, being 18 days in standard, 20 days in treatment 3 and 12 days in standard. Total spat production was the least in treatment 2 at 0.1%. Spat production was 7.7% in treatment 3 and 4.0% in standard. There was statistically significant difference in percentage spat production between treatments 2, 3 and standard ($P < 0.01$).

Table 53. Normal deviate test (Z value):comparison of mean spat setting using mixed phytoplankton

Treatment	2	3	Standard
2	-	31.83*	22.15*
3		-	12.54*
Standard			-

*$P < 0.01$

Peak setting was observed on day 36 in treatment 3, on day 39 in standard and on day 48 in treatment 2(Fig. 21).

Summary of results of larval rearing experiment to evaluate the nutritional value of mixed phytoplankton (Table 54)

When mixed phytoplankton was given during the straight hinge stage, this resulted in mortality of all larvae. When

Table 54. Summary of results of larval rearing experiment to evaluate the nutritional value of mixed phytoplankton.

Treatment	Mean larval size on day 25 (μm)	Larval growth rate per day (μm day)	Growth regression (log μm day)	Day of first setting	Duration (days)	Spat production (%)
1	92.4	0.83	0.0040	-	-	-
2	144.0	2.98	0.0104	37	12	0.1
3	156.8	3.52	0.0124	33	20	7.7
Std	143.4	2.96	0.0116	33	18	4.0

introduced during the umbo stage both survival and growth was poor. But when introduced during the eyed umbo stage, it was seen that both growth and survival were enhanced. The highest mean larval size (156.8 µm), maximum growth regression (0.0124 log µm/day) and maximum spat setting (7.7%) was observed in treatment 3. These values are much higher than those obtained with the standard I. galbana feeding throughout the larval rearing.

DISCUSSION

It has been well recognised that one of the important factors in bringing large numbers of bivalve larvae to metamorphose is the type and the amount of food used for their rearing (Rhodes and Landers, 1973). Ukeles (1980) points out that larvae have very specific food requirements which, if not fulfilled, will result in cessation of normal development, disease and eventual mortality. The role of larval nutrition in seed production in a shellfish hatchery is, therefore, a critical one.

The nutritional value of diets for bivalve larvae may be determined by the percentage of larvae that successfully reach metamorphosis, or by the extent to which the food sources successfully support shell growth or whole

live weight (Ukeles, 1975). In most studies, larval growth characterised by increase in mean length (Rhodes and Landers, 1973) or by growth curves (Walne, 1956a; 1974; Bayne, 1965; Wada, 1973; Nascimento, 1980) has been used as the criterion for assessing the comparative merits of algal diets. In a few instances, the instantaneous relative growth rate, calculated from the initial and final lengths of the larvae has been used for comparison (Bayne, 1965). Setting percentage has rarely been used as a criterion in determining the nutritional value of diets (Windsor, 1977). In general, these experiments have been carried out until complete metamorphosis of larvae (Bayne, 1976). However, experiments lasting for shorter durations up to specific larval stages have also been conducted (Rhodes and Landers, 1973).

In the present study on larval nutrition in the pearl oyster Pinctada fucata (Gould), a very comprehensive set of factors has been considered in detail in evaluating the nutritional value of microalgae. Larval growth has been dealt with in terms of mean size and standard deviations, size frequency distribution, actual growth rate, growth regressions and growth curves. Calculation of growth regression has been improved by considering all the mean sizes on different days of larval life for regression analysis, as compared to the two-point

calculation done by earlier workers, for example, Bayne (1965) and Gerdes (1983). Larval survival, spat setting and algal cell consumption are other parameters investigated.

The relationship between the quantity of algae and the density of larvae in culture is of great importance to good survival and growth of the larvae (Ukeles, 1975). Lucas (1983) recommended strongly against both underfeeding and overfeeding. In the present study, this aspect was specifically investigated to study the effect of larval densities and algal cell concentrations. These results helped to standardise the larval density and the algal cell concentration level for carrying out further experiments on the evaluation of different microalgae.

A wide range of fixed larval densities has been used on bivalve larval rearing by various workers in evaluating different diets, ranging from 0.2/ml for the mussel Mytilus edulis (Jespersen and Olsen, 1982) to 10-50/ml for pearl oyster Pinctada maxima (Minaur, 1969). Few works relate to the effects of varying larval density on growth and survival. The larvae of Crassostrea virginica were reared in the range of 0.6 to 32.9/ml (Davis, 1953) and 1 to 10/ml (Windsor, 1977), of Mercenaria mercenaria in the range of 6 to 52/ml (Loosanoff and Davis, 1963 b), and of C. gigas in the range of 1 to 20/ml (Helm and

Millican, 1977).

Of the five larval densities (1, 3, 5, 8 and 10/ml) reared at the concentration of 25 cells/µl, the density of 5/ml has given both maximum growth and maximum setting percentage in the present study. Mean larval size on day 25 was 198.6 µm at the density of 5/ml as compared to 162.8 µm at 1/ml, 165.0 µm at 3/ml, 168.2 at 8/ml and 159.9 µm at 10/ml. The growth rate for the period day 1-25 was maximum at the density of 5/ml (5.40 µm/day). Growth rate calculated for the three phases, namely, day 1-9, day 9-17 and day 17-25, showed that it was during the second phase that major differences in growth rate were observed. Per-day growth rates for day 9-17 were 4.65, 3.85, 7.71, 5.35, and 4.18 µm/day at the respective larval densities of 1, 3, 5, 8 and 10/ml.

Davis (1953) recorded an inverse relationship between larval density and growth of C. virginica at densities of 0.6, 2.8, 18.5 and 32.9/ml using Chlorella at the cell concentration of 50 cells/µl. Loosanoff and Davis(1963 b) observed a similar relationship in the case of M. mercenaria larvae. At the densities of 6, 13, 26 and 52/ml, and using a uniform feeding level of 100 cells/µl of Chlorella, the above authors observed that 10-day old larvae measured respectively, 162, 156, 151 and 144 µm. In a second experiment, increasing the feeding levels to 100, 200, 400

and 800 cells/μl for the larval densities of 6, 13, 26 and 52/ml respectively, they found that 10-day old larvae measured 162 μm and 148 μm at the densities of 6 and 13/ml but observed complete mortality at the higher densities. For C. gigas larvae, Helm and Millican (1977) observed a 16% decrease in growth for D shape and a 60% decrease for the umbo larvae at the larval density of 5/ml, as compared to 1/ml. It may be seen that the inverse relationship observed by Loosanoff and Davis (1963 b) and Helm and Millican (1977) has not been noted for pearl oyster larvae in the present study.

Growth regressions of 0.0163, 0.0161, 0.0234, 0.0166 and 0.0159 log μm/day for densities 1, 3, 5, 8 and 10/ml were recorded in the present study. Growth rate of larvae reared at 5/ml was significantly different from all other densities. The differential rate of growth of larvae at different densities was reflected both in the initiation of spat setting and its duration. Earliest spat setting was observed at the density of 5/ml (day 20). Setting commenced on day 25 at 1/ml and on day 22 at all other densities. Duration of spat setting was maximum at the density of 1/ml (17 days) and minimum at the density of 5/ml (8 days). There was also a difference in the percentage of spat production, being maximum at the density of 5/ml (8.3%). The lack of residual algal cells observed

at the higher densities of 8/ml and 10/ml would seem to indicate that the ratio of algal cells to larvae was inadequate at these densities. This is supported by the poorer rate of growth (4.13 and 3.79 μm/day at the densities of 8 and 10/ml) and the lesser spat production (2.4 and 0.2% at the densities of 8 and 10/ml). The low algal cell consumption (25-40% and 35-75%) and poor growth rate (3.91 and 4.00 μm/day) and less spat production (2.4% and 1.8%) at the lower densities of 1/ml and 3/ml would imply that maintaining larvae in an environment of excess of algal cells does not produce optimum results.

Windsor (1977) recorded the instantaneous growth rate, size frequency distribution, growth and spat setting for C. virginica at the densities of 1, 3, 5, 7 and 10/ml. The density of 3/ml yielded maximum growth rate and maximum spat production. The slopes given as log μm/day (instantaneous growth rate) were 0.070, 0.081, 0.076, 0.063 and 0.052 at the increasing densities of 1, 3, 5, 7 and 10 larvae/ml. Total number of spat that metamorphosed was seen to decline from 87.3% at 3/ml to 86.1% at 1/ml, 70.5% at 5/ml, 23.2% at 7/ml and 3.8% at 10/ml.

Kinne (1977) pointed out that growth of bivalve larvae was affected by crowding. While some species can be reared at high densities up to 50 or 100 larvae/ml as in the case of M. mercenaria (Loosanoff and Davis, 1963 b) larvae of other species are sensitive to overcrowding.

In general, heavily crowded cultures are more susceptible to environmental stress and disease than less populated cultures and require more attention. Slower growth in crowded cultures has been attributed to reduced availability of food, frequent collisions among larvae and increased levels of metabolic end products. Imai (1977) points out that when larval density is high, the proportional increase in algal cell concentration causes larvae to lose their capacity to feed; instead they excrete mucus resulting in the settling of food. At low populations, the feed dosage has to be maintained higher than what is actually required to make food available to larvae in the swimming layers, resulting again in wastage of food.

It is evident from the results of the larval density experiment that densities not only influence larval growth, but also affect the production of spat. At densities below optimum, efficiency in utilisation of the available food cells in the rearing medium gets reduced, leading to a wastage of food cells. At densities higher than the optimum, poor larval growth and setting may be due to a possible competition for food, frequent collisions and accumulation of larval excretory products. The optimum density for P. fucata larvae, at the feeding level of 25 cells//ul, is seen to be 5 larvae/ml.

In attempts to work out optimum cell concentrations, authors have used different ranges of feeding levels for bivalve larvae, 5-30 cells/µl for the European oyster Ostrea edulis (Imai, 1977); 10-500 cells/µl (Newkirk et al., 1980), 25-100 cells/µl (Bayne, 1965) and 1-40 cells/µl for Mytilus edulis (Sprung, 1984a); 42-330 cells/µl (Cary et al., 1981) for the rock scallop Hinnites multirugosus; and 25-200 cells/µl for Crassostrea gigas (Wilson, 1978). A few authors have used 'packed cell volume' as a basis for increasing levels of cell concentration (Walne, 1963; Rhodes and Landers, 1973; Dupuy, 1975).

In the present study, P. fucata larvae, held at a density of 5/ml, were fed with two microalgal species Isochrysis galbana and Pavlova lutheri at the cell concentrations of 10, 25, 50 and 100 cells/µl. With I. galbana, maximum larval growth and spat setting was obtained at the cell concentration of 25 cells/µl. On day 25, the larval size was 195.1 µm at 25 cells/µl, as compared to 185.8, 185.0 and 186.2 µm at 10, 50 and 100 cells/µl, respectively. The highest growth rate (5.17 µm/day) and growth regression (0.0196 log µm/day) as also spat setting (34.7%) were observed at 25 cells/µl. With P. lutheri, the results were similar. Maximum larval size of 195.4 µm on day 25, growth rate of 5.27 µm/day,

growth regression of 0.0256 log µm/day were obtained at 5 cells/µl. However, maximum spat production (29.0%) was at the cell concentration of 10 cells/µl. Statistical tests showed that there was no significant difference between larval growth at the four cell concentrations of 10, 25, 50 and 100 cells/µl for both I. galbana and P. lutheri. There was, however, statistically significant difference in the percentage of spat that were recovered at each cell concentration for both algal species.

Results of larval growth studies at different cell concentrations have often been conflicting. Some of the investigations have shown that larval growth is enhanced with increasing cell concentrations. Davis and Guillard (1958) recorded mean sizes of 143, 159 and 180 µm for the hard clam, M. mercenaria larvae at the feeding levels of 31.25, 62.5, and 125 cells/µl of P. lutheri over an 8 day period. Imai (1977) recorded mean larval sizes of 220, 225, 229 and 233.5 µm with increasing cell concentrations of 5, 10, 20 and 30 cells/µl of M. lutheri from an initial mean size of 204.5 µm over a 3 day period for O. edulis larvae. For mussel M. edulis larvae, Bayne (1965) found an increase in the instantaneous growth rate (calculated on the basis of the final and initial larval measurements) with increasing cell concentrations of I. galbana at 20, 25, 40, 75 and 100 cells/µl over a period of 12-15 days.

Some studies have shown that increase in algal cell concentration does not improve larval growth or at least does not show any statistically significant difference. Davis and Guillard (1958) observed no difference in larval growth rates of the hard clam M. mercenaria reared at the I. galbana cell concentration levels of 25, 50, 100, 200 and 400 cells/µl. Using M. lutheri, they observed a difference in growth of the same larval species at the cell densities of 31.25, 62.5 and 125 cells/µl of M. lutheri, but there was no significance in larval growth at 125, 250 and 500 cells/µl (Davis and Guillard, 1958). Likewise, there was no improvement in larval growth of C. virginica at the algal densities (packed cell volume) of 690, 1380 and 2070 μ^3/l (Windsor, 1977), of C. gigas at the cell concentrations of 50, 75, 100 and 125 cells/µl and 10, 15 and 20 µl/l of I. galbana (Nascimento, 1980). Walne (1965) noted that at the density of 5-10 larvae/ml, increase in the algal concentration beyond 50-100 cells/µl did not improve larval growth. The absence of statistically significant difference between larval growth at the four cell concentrations of 10, 25, 50 and 100 cells/µl for both I. galbana and P. lutheri observed in the present study are in general agreement with the above works.

At the other extreme, a few authors have recorded decrease in larval growth rate with increasing cell

concentrations. Newkirk et al. (1980) observed a decrease in M. edulis larval growth from the cell concentration of 10/µl to 100/µl with both I. galbana and P. lutheri. Cary et al. (1981), likewise, observed that larval growth of the rock scallop Hinnites multirugosus was the least at the cell concentration of 230 cells/µl among 42, 84, 168 and 230 cells/µl of I. galbana and also the least at 240 cells/µl among 30, 60, 120 and 240 cells/µl of M. lutheri.

A few authors have examined the occurrence and the duration of spat setting as a criterion for assessing quality of the diet. In the present study, the percentage of spat ranged from 23.8 to 34.7% of the initial population with I. galbana as diet, and from 5.2 to 29.0% with P. lutheri as diet. Maximum spat production was observed at 25 cells/µl for I. galbana and at 10 cells/µl for P. lutheri. For both species minimum spat production occurred at 100 cells/µl. Loosanoff and Davis (1963 b) observed that both food and temperature influenced the duration of larval period. Even in healthy cultures of larvae, setting could last up to a period of 27 days. At 11°C, M. edulis larvae took 39 days to reach the pediveliger stage at the algal concentration of 100 cells/µl, and 70 days at 25 cells/µl. At 16°C, this was reduced to 16 days at 100 cells/µl and to 31 days at 25 cells/µl (Bayne, 1965).

A less perceptible difference in the onset of spat setting was observed for pearl oyster larvae reared at temperature of 29.0-31.5°C. With I. galbana as diet, setting began uniformly on day 23, at all cell concentrations. With P. lutheri, setting was delayed to day 20 at the concentration of 100 cells/µl as compared to day 18 at 10, 25 and 50 cells/µl.

Windsor (1977) recorded high spat production of 60.9, 56.4 and 57.1% at the three increasing cell densities of 690, 1380 and 2070 μ^3/l for larvae of C. virginica. For C. gigas, Wilson (1978) recorded very low spat production of 0.52, 0.29, 0.33 and 0.78% at the densities of 25, 50, 100 and 200 cells/µl. Windsor (1977) observed that larval growth was often not a good indicator of setting success. This has been noted in the experiment using P. lutheri as diet, where maximum spat setting was observed at 10 cells/µl, but maximum growth was at 25 cells/µl.

The concentration of algal cells in the medium is of obvious importance in larval rearing inasmuch as it influences the rate of filtration of larvae and consequent consumption. A normally feeding larva produces a continuous flow of mucus in which food particles are trapped and carried to the mouth to be ingested. In the presence of an excess number of cells, a large number of cells are rejected over the oral

palp as pseudofeces. A larva that is producing excessive pseudofeces is not only removing its food supply from suspension and making it unavailable, but is also producing and losing large amounts of mucus. Besides, strings of pseudofeces trail behind and often entangle and trap the swimming larvae (Yonge, 1926). For these reasons excessive algal cell concentrations in larval rearing are to be avoided.

Schulte (1975), working on mussels, put forward the concept of "critical cell concentration" which is one in which volumes are swept clear, all food particles ingested and no pseudofeces produced. At food concentrations higher than the critical cell concentration, there is a steady decrease in sweeping rates, with an increase in pseudofeces formation (Ukeles, 1975). In recent years, Cary et al.(1981) demonstrated the occurrence of mechanical interference and heavy pseudofeces production and severe packing of the gut at concentrations higher than 40 cells/µl through the technique of videofilming.

In the present study where pearl oyster larvae were reared at the concentration range of 10-100 cells/µl, growth was maximum at the lesser concentration of 25 cells/µl for both I. galbana and P. lutheri. Algal cell consumption data indicate that maximum consumption of cells (72.5-100%) occurred at the feeding level of 10 cells/µl and the least

(22.5-37.5%) at 100 cells/µl. As these values have been calculated with reference to the initial cell concentration in the rearing medium, it may also be noted that a greater number of cells have been removed from the medium with increasing cell concentration. Actual number of cells removed at the concentrations of 10, 25, 50 and 100 cells/µl would have been, respectively, 7.25 to 10 cells/µl, 12.5 to 20 cells/µl, 17.5 to 27.5 cells/µl and 22.5 to 37.5 cells/µl. When studied in relation to the works already described and the observed maximum growth at 25 cells/µl of the present study, it would appear that much of the cells cleared away from the rearing medium at 50 and 100 cells/µl were being rejected as pseudofeces.

Apart from mechanical interference, high cell concentrations may affect larvae chemically, via external metabolites (Kinne, 1977). Loosanoff et al. (1953) demonstrated that the algal filtrate containing external metabolites could cause mortality of hard clam, M. mercenaria larvae. On the other hand, Stephen and Manahan (1984) recording high concentrations of amino acids in algal filtrates, suggested that these organic nutrients would be of potential nutritional value to growing larvae. Retention time of algal cell within the gut was of longer duration at lower cell concentrations (Ukeles and Sweeney, 1969). At high cell concentrations, the shorter retention

time can lead to inadequate assimilation.

The above three factors, namely mechanical interference (Malouf and Breese, 1977; Cary et al., 1981), algal metabolites (Loosanoff et al., 1953; Newkirk et al., 1980) and shorter retention time at high cell concentrations (Ukeles and Sweeney, 1969) have been implicated as reasons for poor larval growth at densities higher than the optimum. Results obtained in the present study also seem to bear out these observations. In addition, it is noted that associated with an increase in the availability of cells in the rearing medium, there is a greater wastage of algal cells.

The optimum cell concentration is seen to vary from species to species and is also larval density dependent. Optimum concentration of I. galbana observed for other bivalve species are 300 cells/µl for Ostrea edulis at the larval density of 2/ml (Wilson, 1979); 50-400 cells/µl for O. edulis and M. mercenaria at the density of 10-15/ml (Davis and Guillard, 1958); 25-325 cells/µl for C. virginica at the density of 15/ml (Rhodes and Landers, 1973); 10 cells/µl for M. edulis at the density of 3-10/ml (Bayne, 1965); and 40-60 cells/µl of a mixture of I. galbana and P. lutheri for M. edulis at the density of 0.1-0.2/ml (Jespersen and Olsen, 1982). In the present study, an algal cell concentration of 25 cells/µl either with I. galbana or P. lutheri as food appears to be the

optimum for the rearing of Pinctada fucata larvae at the density of 5/ml.

Ever since Cole (1937) established that bivalve larvae could be reared in the laboratory using algal cultures, there has been a great deal of experimental work in isolating, identifying and culturing microalgal species and evaluating their food value for larvae. Perhaps, over 40 species of algae have been involved in these experimental works. Loosanoff and Davis (1963 b) used at least 22 species of algae and diatoms largely on the hard clam M. mercenaria and the oyster C. virginica and reared a further 17 species of bivalve larvae up to metamorphosis. Walne (1974) evaluated the nutritional value of 25 species of algae for Ostrea edulis. Davis and Guillard (1958) experimented with 10 algal species.

Many others have concentrated on experiments with fewer algal species as food for selected bivalves (Bayne, 1965; Wada, 1973; Xu and Li, 1980; Yiyao et al., 1985). These works have only highlighted the enormous variability among species and food. In India, larval rearing technology itself has been recent (Alagarswami et al., 1983 b, c) and the present study on pearl oyster larval rearing using Isochrysis galbana, Pavlova lutheri, Chromulina freiburgensis, Synechocystis salina and Tetraselmis gracilis is the first attempt in this challenging field of larval nutrition.

Growth is the primary physiological parameter of condition in the larval stages as most of the energy derived from nutrition is utilised for this purpose (Ukeles, 1975). Mean larval length, growth rate and growth curves have been used as criteria for comparing treatments (Bayne, 1965; Windsor, 1977; Coeroli et al., 1984; Sprung, 1984 a). Working with larvae of different size groups, Rhodes and Landers (1973) recorded 2.8 to 19.1 µm/day growth over 48 h for C. virginica. Growth rates of 6.72 µm/day for the larvae of oyster Saccostrea echinata for a period of 11 days (Coeroli et al., 1984) and 1.6 to 4.30 µm/day for the scallop Hinnites multirugosus (Cary et al., 1981) have been recorded.

Growth rate of the pearl oyster larvae in the present study ranged from 2.37 to 5.17 µm/day over the period day 1-25 at the algal concentration of 25 cells/µl. For the three phases, day 1-9, day 9-17 and day 17-25, it is seen that growth rate during the period day 1-9 is slow (1.18 to 3.10 µm/day), day 9-17 is greater (1.70 to 6.68 µm/day), and day 17-25 is again slow (2.34 to 4.98 µm/day). While these values generally agree with larval growth rates observed for pearl oyster by Wada (1973) and Alagarswami et al. (1983 c), these are lower than the maximum values recorded for the Crassostrea sp. (Rhodes and Landers, 1973; Breese and Malouf, 1975).

Bayne (1983) observed that molluscan larval growth curve from the earliest stage to metamorphosis may follow different trends: linear, asymptotic or sigmoidal. Different growth curves have been recorded for various bivalve species: exponential for O. edulis (Walne, 1974), C. gigas (His and Robert, 1982; Robert et al., 1982); sigmoidal for V. mercenaria (Loosanoff et al., 1951), O. edulis (Walne, 1956a), M. edulis (Bayne, 1965) and C. gigas (Gerdes, 1983); and linear for Venus striatula (Ansell, 1961), M. mercenaria (Carriker, 1961), O. edulis (Walne, 1965), Siliqua patula (Breese and Robinson, 1981), M. edulis (Jespersen and Olsen, 1982) and Arctica islandica (Lutz et al., 1982). The differences for the same larval species may be due to differences in experimental and/or sampling designs. Sprung (1984 a) recorded a linear fit for mussel larval growth data at 6°C at the cell concentrations of 10, 20 and 40 cells/µl but a sigmoidal fit at the lower cell concentrations of 1, 2 and 5 cells per µl. The growth curves in the present study are sigmoidal. Alagarswami et al. (1983 c) suggested that growth of P. fucata larvae may be a step function. Gerdes (1983) explains that the sigmoidal curve in C. gigas is caused by depressed feeding of the pediveliger when it starts reducing its velum. Linear curves have been recorded where selective sampling of the faster growing larvae has been resorted to (Sprung, 1984a).

Among the few authors who have studied setting success as a criterion in assessing nutritional value of diets, Windsor (1977) observed very low spat yields (< 1.0%) in C. virginica with I. galbana as diet. AQUACOP (1979) recorded spat yields of 50% for mussel larvae. In the present study, spat yield was in the range of 2.9% to 34.7%.

Ukeles (1975) pointed out that there is an optimum food consumption at different stages in the life cycle of bivalves. Imai (1977) observed that optimum feed dosage increased with larval growth and this dosage differed greatly according to temperature, population density and type and mode of feeding. Some workers increased the food supply to provide for the greater nutritional needs of later stages (Wada, 1973; Dupuy, 1975; Windsor, 1977). In one of the experiments of the present study, cell density in the rearing medium was increased stepwise from the D shape to umbo and eyed umbo stages (from 10 cells/µl to 35 cells/µl). A progressive increase in larval growth was observed from treatment 1 to 4 (2.97, 3.45, 3.77 and 3.79 µm/day for the period day 1-25, Table 40). A closer examination of the data shows that maximum growth rate has occurred in treatment 2 for day 1-9, in treatment 3 for day 9-17 and in treatment 4 for day 17-25. This would suggest that cell densities of 15, 25 and 35 cells/µl may

be the food requirement for the D shape, umbo and the eyed umbo larvae, respectively.

Rhodes and Landers (1973), using I. galbana and working with seven different size groups of C. virginica larvae, showed that the food requirements increased 13-fold from 2.5 cells/µl for the D shape to 32.5 cells/µl for larvae of 255.0 µm length. Walne (1966) observed a 2.5 times increase in cell requirement for O. edulis larvae. Dupuy (1975) recommended a 3.15 times increase in cell volume for C. virginica larvae, while Windsor (1977) adopted a 2-fold increase in cell volume for the same species. On the basis of the present study, an increase in cell density from 15 cells/µl for the D shape to 35 cells/µl for the eyed umbo stage, i.e., a 2.33-fold increase in cell requirement in I. galbana can be projected. This is close to the findings of Dupuy (1975), Windsor (1977) and Walne (1974), but far below the increase recommended by Rhodes and Landers (1973). The latter, apart from working with a higher larval density of 15 larvae/ml, based their observations on short-term experiments lasting for 48 h. They did not consider the possible deleterious effects of continuous exposure to high concentrations of algal cells as has been reported for C. virginica larvae by Loosanoff and Engle (1947).

The haptophycean alga, Pavlova (= Monochrysis) lutheri, has been cited as one of the most widely accepted foods for bivalve larvae giving a nutritional value equal to or greater than that of I. galbana (Loosanoff and Davis, 1963 b; Ukeles, 1971, 1975; Walne, 1974; Ryther and Goldman, 1975; Imai, 1977; Kinne, 1977; Sastry, 1979).

Minaur (1969) identified Monochrysis lutheri as one of the better diets for larvae of pearl oyster P. maxima recording growth increments of greater than 10 µm/day over a period of 7 days. He failed to obtain larval metamorphosis, however. Davis and Guillard (1958) used P. lutheri successfully in the rearing of the oyster C. virginica and the hard clam M. mercenaria larvae in the cell concentration range of 31.25 to 500 cells/µl. Bayne (1965) reported results similar to those of I. galbana for larvae of the mussel M. edulis in the cell density range of 20-100 cells/µl. Iwasaki et al. (1971) reared larvae of the clam Scapharca subcrenata at two cell densities of 1/µl and 2/µl. They obtained growth (3.50 µm/day) at 2 cells/µl, but at 1/µl, there was complete mortality by day 22. Windsor (1977) reported relatively poor growth rates for larvae of C. virginica when fed with a pure diet of M. lutheri as compared to the regular feeding protocol of mixed algal species. Regression of growth was 0.0125

log µm/day (Windsor, 1977). Comparatively, regression of the present study was in the range 0.0192-0.0196 log µm/day. Newkirk et al. (1980) experimented with both young and old cultures of P. lutheri to larvae of M. edulis and noted a decrease in growth rate with increase in cell concentrations. Tanaka et al. (1970) found this algal superior and ingestion best when provided to pearl oyster P. margaritifera.

In the present study, setting occurred in 18-20 days, lasted for 8-12 days and yielded a total spat production of 5.2-29.0% at cell concentrations of 10-100 cells/µl and at the density of 5/ml. Flassch (1983) reports that in commercial hatcheries of Japan, rate of spat production of P. fucata, when fed with P. lutheri at 20 cells/µl and at 10 larvae/ml, was approximately 20%. Metamorphosis occurred in 23 days. AQUACOP (1979) obtained metamorphosis of Mytilus edulis between 10-17 days when P. lutheri was used as diet. Windsor (1977) observed very poor setting percentage (<0.05%) when fed with M. lutheri. Results of the present study show that P. lutheri has a nutritional value equal to that of I. galbana for the pearl oyster larvae.

In terms of larval growth, survival and setting, the nutritional value of the alga Chromulina freiburgensis may be inferior to that of I. galbana. Growth rates for

the periods day 1-9, day 9-17 and day 17-25 were 3.48, 2.52 and 6.05 µm/day and overall growth rate for the period day 1-25 was 4.01 µm/day. In comparison, growth rates for I. galbana for the same periods were, respectively, 3.10, 6.68 and 4.98 µm/day and overall growth rate was 4.92 µm/day.

Davis (1953) demonstrated that larvae of C. virginica could be reared to metamorphosis on pure cultures of Chromulina pleiades. Loosanoff and Davis (1963 b) described this species as of 'good' value for C. virginica larvae. However, Iwasaki et al. (1971), rearing larvae of Scapharca subcrenata on a diet of Chromulina sp. at the cell density of 2/µl, obtained very poor growth rate of 0.81 µm/day over a period of 11 days. By day 22, there was total mortality. More recently, Joseph (1983) demonstrated that the alga C. freiburgensis was an excellent diet for larvae of the Indian backwater oyster Crassostrea madrasensis. She obtained a growth of 17.4 µm/day over a 14-day period for C. freiburgensis as compared to 10.5 µm/day for I. galbana. The very high growth rates observed for C. madrasensis (Joseph, 1983) over that of P. fucata of this study may be attributed to the inclusion in the samples of the spat stages. Mean larval size on day 14 for C. madrasensis larvae fed with C. freiburgensis was 298.0 µm (Joseph, 1983). The low standard deviation recorded

would also suggest a selective sampling of larvae.

Setting commenced on day 21 in the present study and yielded a total of 3.0% spat as compared to 6.5% with I. galbana. Walne (1956a) recorded good larval growth and adequate setting of O. edulis larvae fed with C. pusilla and C. pleiades. He observed a total of 304 spat with C. pusilla and 684 spat with I. galbana out of an initial unknown but presumably equal number of larvae. In a second experiment, using C. pleiades, he recorded a total of 401 spat as compared to 55 spat when fed with I. galbana out of an initial 860 larvae. Joseph (1983) obtained a total of 2.9% spat production when C. madrasensis was fed with C. freiburgensis as compared to 0.24% when fed with I. galbana.

It would appear from the literature that C. freiburgensis has been used as food for the first time in pearl oyster larval rearing. Results indicate that C. freiburgensis is a nutritionally adequate diet but not superior in value to that of I. galbana. These results are in keeping with those observed for O. edulis larvae by Walne (1956a).

Growth and survival data of pearl oyster larvae fed with S. salina indicate that this species is a 'poor' food for the species. Overall larval growth for the period day 1-25 was 0.86 µm/day as compared to 2.37 µm/day

for larvae fed with I. galbana in simultaneously co-ordinated experiments. Although larvae survived for a total period of 53 days, growth was exceedingly poor. There was no spat setting (Table 24).

Very few authors have studied the nutritional value of the genus Synechocystis for growth and survival of bivalve larvae. Rao (1980) used this species as a diet for mussel Perna viridis larvae and recorded stunted growth. Walne (1956a) used another cyanophycean of the genus Synecococcus and recorded poor larval growth and total lack of metamorphosis.

The alga S. salina satisfied the physical characteristics required to be used as a food for bivalve larvae, by way of motility and the absence of cell wall. The fact that larvae survived for a long period of time rules out the possibility of toxicity associated with either the cell or as metabolites. The small size of the algal cell (3 µm) makes it easily consumable. The total volume of S. salina provided to the pearl oyster larvae would have been far less than that of I. galbana cells (7-8 µm) and, therefore, have not met the requirements of larval growth.

The species of Tetraselmis (previously called Platymonas) have been shown to have variable value for bivalve larvae (Loosanoff and Davis, 1963 b; Walne, 1974; Wilson, 1978; Zong-Qing and Mei-Fang 1980; Cary et al., 1981;

Yiyao et al., 1985). In the present study, pearl oyster larvae fed with T. gracilis exhibited very poor growth rate and failed to metamorphose. Mortality was total beyond day 17 (Table 24).

Cole (1937) demonstrated that for O. edulis larvae Tetraselmis sp. was variably successful. Davis and Guillard (1958) reported that it was an adequate but not reliable species for culturing of C. virginica larvae. Loosanoff and Davis (1963 b) classified this alga as "good" for clam M. mercenaria larvae but only of "medium" value for C. virginica. Walne (1965) observed that 10 cells/µl of T. suecica had the nutritional value of 50 cells/µl of I. galbana.

At a concentration of 50 cells/µl, Wilson (1978) recorded good growth of O. edulis and C. gigas larvae. In mussel, M. edulis, Zong-Qing and Mei-Fang (1980) reported that the Platymonas sp. was acceptable to M. edulis larvae 2 days after fertilisation and more easily after attaining the length of 110 µm. In large scale tank cultures at larval densities of 3-5/ml and cell concentration of 3.5-5.0 cells/µl setting was initiated in 15 days time.

Cary et al. (1981) reported that the nutritional value of Tetraselmis sp. to the larvae of the rock scallop Hinnites multirugosus was equivalent to that of unfed cultures. Unlike the results of Wilson (1978), Walne (1974) and Zong-Qing

and Mei-Fang (1980), growth of pearl oyster, P. fucata larvae fed with T. gracilis has been negligible.

Several authors have shown that combinations of algae have induced more rapid growth of bivalve larvae than a diet of a single species of algae (Loosanoff and Davis, 1963 b; Dupuy, 1975; Ukeles, 1975; Windsor, 1977). Combinations of I. galbana + P. lutheri, I. galbana + C. freiburgensis, I. galbana + S. salina, I. galbana + T. gracilis, S. salina + T. gracilis and I. galbana + S. salina + T. gracilis were tested in the present study for their nutritional value to pearl oyster larvae. In addition a stratified feeding schedule was adopted and tested.

The combination diet of I. galbana and P. lutheri has been shown to be a diet of high nutritional value for several species of bivalve larvae (Loosanoff and Davis, 1963 b; Windsor, 1977). In the present study, growth and setting of pearl oyster larvae fed with the same combination diet has shown marginally greater values than for I. galbana. growth rate for day 1-25 was 3.66 µm/day for the combination diet as compared to 3.42 µm/day for I. galbana alone. Likewise, spat setting was 7.8% as compared to 6.9% with I. galbana. All other combination diets were of less value to pearl oyster larvae than I. galbana.

The food value index calculated in the present study for the algal species used singly and in combination gives a fair idea of the nutritional value of each diet. The ratio between larval growth of an experimental diet to larval growth of a common standard had been used by Walne (1963) for assessing nutritional value of diets. It may be seen from the food value index (Table 38) that the only algal species equal to I. galbana has been P. lutheri used singly (1.07) or in combination with I. galbana(1.04). C. freiburgensis gave a food value of 0.77 when fed singly. This was improved to 0.85 by the addition of I. galbana. S. salina which had a low food value of 0.32 was seen to have an increased value by the addition of I. galbana (0.81). T. gracilis yielded the lowest index of 0.28. While larvae fed with combinations of I. galbana with P. lutheri, C. freiburgensis and S. salina grew to metamorphosis, there was complete failure to metamorphose when fed with combinations of I. galbana + T. gracilis, I. galbana + S. salina + T. gracilis and a combination of S. salina + T. gracilis. In order of merit, the algae used singly may be listed on the basis of their food value index as follows: P. lutheri, I. galbana, C. freiburgensis, S. salina and T. gracilis. The combination diets may be listed as follows: I. galbana + P. lutheri, I. galbana + C. freiburgensis, I. galbana + S. salina, I. galbana + S. salina + T. gracilis, I. galbana + T. gracilis and S. salina + T. gracilis.

It has been shown that several bivalve larvae can feed on a variety of species during the later larval stages (Loosanoff and Davis, 1963 b; Matthiessen and Toner, 1966; Yiyao *et al.*, 1985). Studying the results of the experiment using a stratified feeding schedule of *I. galbana*, *T. chui* and *C. salina*, it would seem that growth and setting of pearl oyster larvae were dependent on the proportion of utilisable algal cells in the rearing medium. When fed with *I. galbana* alone percentage spat setting was 2.6 but when *T. chui* was introduced during the eyed umbo stage spat production was reduced to 2.2%. Again, when *T. chui* was introduced during the straight hinge stage, spat setting was 1.6%. The addition of *C. salina* cells from the early umbo stage to larvae reared on a combination of *I. galbana* and *T. chui* from the D shape stage greatly enhanced spat production to 8.7%. Residual phytoplankton counts showed that there was relatively high consumption of *I. galbana* and *C. salina* cells but poor consumption of *T. chui* cells.

The food value of both *Tetraselmis* sp. and *Chlorella* sp. has been shown to vary for different species of bivalves. Cole (1938) was the first to report that larvae of *O. edulis* could not utilise *Chlorella* sp. Davis (1953), however, reported that larvae of *C. virginica* could make use of the alga during the later stages of larval development. Davis

and Guillard (1958) used Chlorella as an 'adequate' species for both C. virginica and M. mercenaria larvae. Loosanoff and Davis (1963 b) referred to the same as 'medium' for the same species. Wada (1973) on P. fucata, Walne (1974) on O. edulis and Zong-Qing and Mei-Fang (1980) on M. edulis reported failure to metamorphose and poor growth of larvae reared on a diet of Chlorella sp. Windsor (1977) pointed out that the amino acid tryptophan is found only in the Chlorella sp. So, although this species has been found to be a rather poor food by itself, it might be of some benefit in a mixed species algal diet.

The greater value of combination diets is based on the fact that no one algal species is a complete food source and mixtures of algae can better fulfil the dietary requirements (Ukeles, 1971, 1975; Kinne, 1977). In the present study optimum larval growth and spat production was observed for larvae reared on a combination of I. galbana and T. chui up to the early umbo stage and, thereafter, fed on I. galbana, T. chui and C. salina.

The food value of an algal diet to the bivalve larvae has been shown to be dependent on cell size, besides several other factors. The algae I. galbana (7-8 µm), P. lutheri (8 µm), C. freiburgensis (9 µm), S. salina (3 µm), T. gracilis (12 µm) T. chui (15 µm) and C. salina (3-4 µm) were used in the present study. Yonge (1926) and Millar (1955)

observed that there appeared to be no food sorting mechanism in the oyster larval gut other than the exclusion of large particles by the small diameter of the mouth and oesophagus. Ukeles and Sweeney (1969) approximated the size of the mouth of C. virginica larvae (78 x67 µm) at less than 10 µm. However, Mackie (1969) reported that larvae of C. virginica qualitatively selected algal cells ranging from 1 to 30 µm. Cell size has also been shown to affect filtration efficiency and ingestion (Hughes, 1969; Haven and Morales-Amo, 1970; Riisgard et al., 1980, 1981). Webb and Chu (1983) concluded that the suitable size range of food particles may differ between species. Non-motility of cells can cause settling of available food resulting in its poor food value to the larvae (Webb and Chu, 1983). However, Babinchak and Ukeles (1979), using epifluorescence microscopy, demonstrated the active intake of a non-motile species, Chlorella autotrophica. On the other hand, Cary et al. (1981) observed that a highly mobile species, Carteria pallida, displayed strong avoidance and at times was able to avoid entrainment by the larval feeding currents.

Algal food value is also dependent on the ability of the larvae to digest the cell membrane. Thick cell walls are often more resistant to digestion than the membranes (Kinne, 1977). Young C. virginica larvae were unable to utilise forms having cell walls but they were able to do so after reaching a size of approximately 110 µm (Loosanoff

and Davis, 1963 b). Poor growth of O. edulis larvae when fed with Chlorella and Coccomyxa has been attributed to the inability of larvae to digest the thick cell wall (Walne, 1974). These findings were supported by the works of Babinchak and Ukeles (1979) who observed the absence of lysis or digestion of Chlorella autotrophica cells in the larval gut of C. virginica, despite the active ingestion of these cells. The ability to digest Chlorella sp. is said to be dependent on temperature (Gastry, 1979). Larvae of Mercenaria mercenaria could utilise it at 25-30°C, but at 15°C, although they could ingest the algal cells, they could not assimilate it (Loosanoff and Davis, 1963 b). In the present study, the only species with cell wall was C. salina. At 29.0-31.5°C, the warm temperature might have aided its digestion.

Some organisms such as Prymnesium parvum and Stichococcus sp. have been repeatedly shown as toxic for bivalve larvae (Davis, 1953; Loosanoff and Davis, 1963 b; Webb and Chu, 1983). All algal species used in the present study are generally not known for the release of toxic substances, Droop (1968) found that Monochrysis released a vitamin B_{12} binding substance, thus deactivating vitamin B_{12}. The occurrence of tryptophan in the extracellular metabolites of Chlorella sp. has been cited as a reason for its food value in combined algal diets (Windsor, 1977). While

reporting on the occurrence of dissolved free amino acids in algal filtrates, Stephen and Manahan (1984) attributed a possible nutritive role to these algal metabolites.

The role of bacteria associated with algal cultures has been a contradictory one. However, it has generally been opined that while some bacterial species may enhance algal food value, a few others may be harmful to bivalve larvae (Martin and Mengus, 1977). Although a bacterial load of 41 + 12.3 colonies/ml has been associated with the present larval rearing, none of the larvae appeared diseased.

The retention time within the gut being short (Windsor, 1977), the better foods are those that are broken down faster. This would explain why the naked flagellates are better foods than species which have cell wall (Kinne, 1977). For this reason, the haptophyceans have been reported to be the best foods for bivalve larvae (Loosanoff and Davis, 1963b; Ukeles, 1975; Kinne, 1977) which is confirmed in the present study.

In contrast to the use of pure cultures as food for bivalve larvae, the Glancy method and the Hidu method have been used extensively and successfully in experimental as well as commercial hatcheries (Dupuy, 1975; Dupuy et al., 1977; Dexter et al., 1978; Bardach et al., 1972). These methods are based on utilisation of selectively filtered and centrifuged seawater allowing the smaller forms of

phytoplankton to be grazed upon by the larvae in the static cultures (Ryther and Goldman, 1975). The mixed phytoplankton developed for the present study was from the open sea. Pearl oyster larvae that were reared on the mixed culture from the straight hinge stage failed to metamorphose and grew very slowly. When introduced at the umbo stage, spat production was 0.1%, but introduced at the eyed umbo stage, there was high spat production. It is possible that as larvae grew in size, the ability to digest and utilise a greater variety of algae increases in association with an enhanced enzyme system. Davis and Guillard (1958) demonstrated that the ability to use different algal species increased with larval development. These results suggest that bivalve larvae can be reared on a restricted diet during the early stages and, thereafter, on a wider range of algae raised from general phytoplankton.

In marked contrast to the extensive literature on larval rearing of oysters, mussels and clams discussed here, there have been only a few published works on pearl oyster larval rearing (Minaur, 1969; Wada, 1973; Xu and Li, 1980; Alagarswami et al., 1983 b, c; Tanaka and Kumeta, 1981; Yiyao et al., 1985). Alagarswami et al., (1983 b, c) succeeded for the first time in rearing the pearl oyster, P. fucata under tropical conditions and pointed out the need for further investigations to determine

optimum larval density, critical cell concentration and the use of local microalgae to standardise hatchery rearing procedures. Minaur (1969) had studied the comparative merits of Dunaliella tertiolectum, I. galbana, M. lutheri, Nitzschia closterium and an algal isolate provisionally identified as Chlorella sp. for the larvae of the silver-lip pearl oyster P. maxima. Although the larvae did not grow up to metamorphosis, faster growth rate was observed with I. galbana or M. lutheri. Later, Xu and Li (1980) successfully reared larvae of P. maxima up to metamorphosis on Platymonas and yeast cells. Tanaka and Kumeta (1981) reared larvae of P. maxima up to juvenile stage using P. lutheri as diet. Larvae grew to a length of 234 µm in 19 days time.

Wada (1973) studied the relative values of M. lutheri, Chaeteceros calcitrans and Chlorella sp. for pearl oyster P. fucata both singly and in combination at the concentration of 10-20 cells/µl. M. lutheri was reported to induce best larval growth. C. calcitrans was identified as having excellent food value, while Chlorella sp. yielded very poor results. All combinations using M. lutheri also induced good growth of pearl oyster larvae.

Flassch (1983) reported that 20% of the pearl oyster for cultured pearl production was obtained by controlled reproduction in Japan. Three species of algae

P. lutheri, I. galbana and Chaetoceros sp. were used in larval rearing. Coeroli et al. (1984) failed in their attempts to rear the larvae of the blacklip pearl oyster P. margaritifera using techniques adopted from the classical methods of Loosanoff and Davis (1963 b), Walne (1966) and Dupuy (1975). Yiyao et al. (1985) reared larvae of the pearl oyster P. chemnitzii on yeast, sperm of P. fucata, a mixture of sperm of P. fucata and Dicrateria zhanjiangensis and D. zhanjiangensis alone. Yeast was not suitable for feeding D shape larvae, but sperm and a mixture of sperm and D. zhanjiangensis was most suitable. Larvae of the umbo stage were able to feed on Platymonas sp.

The present study has identified both I. galbana and P. lutheri as good larval diets and suggests an optimum concentration in the range 15-35 cells/µl at the larval density of 5 larvae/ml. C. freiburgensis has been observed as a nutritionally adequate diet for pearl oyster larvae. Loosanoff and Davis (1963 b) in their review, classified the bivalve larvae into three groups: (a) those which are able to utilise, during the early straight hinge stage, only a few of the many food algae offerred; (b) those which are able to utilise most of the algae tested, provided these are small enough to be ingested; and (c) those which are intermediate to the above two in their dietary requirements. In this generalised classification, Pinctada fucata appears to belong to the first group.

CHAPTER 4

FILTRATION RATE, UPTAKE AND RETENTION OF ALGAL CELLS IN PEARL OYSTER LARVAE

INTRODUCTION

Availability of algal cells in suitable concentrations is an important aspect of bivalve larval nutrition. Lucas (1983) points out that the quantitative evaluation of larval nutrition may be based on indirect methods like estimation of growth at different feeding levels (Davis and Guillard, 1958; Rhodes and Landers, 1973), rate of clearance of particles from the medium (Malouf and Breese, 1977; Wilson, 1979, 1980) or by direct methods like the use of radioactive labelled phytoplankton (Walne, 1965; Ukeles and Sweeney, 1969) or observations with flourescent microscopy (Babinchak and Ukeles, 1979; Lucas and Rangel, 1983).

Since bivalve larvae feed by filtration, an estimate of their filtration or clearance rates determines how much and what kind of food can be obtained by them (Malouf and Breese, 1977; Gerdes, 1983). In the present study, an indirect method to estimate filtration rate by measuring decrease in cell concentration over time in a static water system and a direct method using radioactive labelled algal

cells were adopted to make quantitative estimates of pearl oyster larval nutritional requirements.

MATERIALS AND METHODS

Filtration rate of pearl oyster larvae

The filtration rates of pearl oyster larvae were determined at four different algal cell concentrations. Larvae from the same brood of oysters were reared according to the methods described in Chapter 2.

Larvae belonging to four size groups (D shape at 79.5 ± 6.6 µm; Umbo 1 at 122.5 ± 8.4 µm; Umbo 2 at 144.0 ± 14.5 µm; and Umbo 3 at 187.0 ± 10.6 µm) were stocked in one-l beakers independently at the uniform density of 5 larvae/ml. Filtration rate was estimated at four cell concentrations of 10, 25, 50 and 100 cells/µl of *I. galbana*. For each larval size and each cell concentration, filtration rate was calculated from quadruplicate experiments. In a fifth, only the algal cell concentration under experimentation was set up without larvae, in order to test the possible multiplication of algal cells in the medium during the experiment. The changes were not found to be important to influence the experimental results. Filtration rates were determined by measuring the decrease in algal cell concentration at 20 minutes for an initial period of 3 h and

subsequently at 22 h and 24 h. For this purpose, two 2-ml samples were removed and fixed with Lugol's fixative and the cell count taken using a blood counting chamber. The residual cell count was averaged and filtration rate (F.R.) was calculated according to a modification of Gerdes (1983), using the formula

$$F.R. = \frac{\log_e C_1 - \log_e C_2}{t} \times \frac{V}{D}$$

where, C_1 - initial cell concentration in the medium

C_2 - final cell concentration in the medium

V - volume of the medium (ml)

t - duration of the experiment (h)

D - larval density.

For the results presented in Table 55, filtration rates have been calculated for a time period of 24 h for each algal cell concentration (10, 25, 50 and 100 cells/µl) clearance rate has been calculated as the total number of algal cells that were removed by the larvae from each ml of the medium at the end of 24 h. Clearance rate has also been expressed as percentage of the initial cell concentration of cells per ml of the medium. Mean clearance per larva has been expressed as the average of the clearance per larva at all the four larval size groups (D shape, Umbo - 1, 2, 3).

2. The uptake and retention of C^{14}-labelled algal cells

i) Uptake: Three species of microalgae, namely, Isochrysis galbana, Pavlova lutheri and Chromulina freiburgensis were used to study the uptake and retention of algal cells by the pearl oyster larvae. The experimental procedure was adopted from Ukeles and Sweeney (1969).

One hundred ml of the algal stock culture in the exponential phase of growth was inoculated with 1 ml of C^{14}-labelled sodium bicarbonate having a radioactivity of 5 μCi. This was incubated overnight under fluorescent lights. After 12 h exposure, the microalgae were centrifuged and the supernatant containing excess of labelled bicarbonate was discarded. The residual labelled algal cells were resuspended in sterile seawater. From this, calculated quantities of labelled algae as required for the experimental cell concentrations (25 or 50 cells/μl) were used for feeding the larvae. For each larval stage and for each time interval, the experiment was conducted in duplicate and the average of the two was taken.

At intervals of 1, 3, 20 and 24 h, the larvae in each beaker was sieved through a 30 μm mesh nylobolt and resuspended in sterile seawater, the residual algae having been discharged through the sieve. The larvae were filtered through a 0.32 μm membrane filter under vacuum. A drop of 5% formalin was added to preserve the larvae. The membrane

filters were stored in special planchet holders until further analysis. Quantities of algal cells equivalent to those used in feeding experiments were also filtered through the membrane filter and preserved as standard sample, in like manner. The larval and algal samples were subsequently analysed at the C^{14} laboratory of CMFRI, Cochin. The membrane filters were taken in glass vials and 1 ml of acetone added to each to dissolve the filter. 15 ml of a cocktail fluid was added and the vials were screwed tightly.

The radioactivity in algal and larval samples preserved as above were estimated using a Liquid Scintillation Counter (ECIL). The counter was set to give readings as counts/minute (cpm). The cocktail fluid used as the scintillation medium was prepared by dissolving 7 g of POPOP (phenylozazole) and 100 g of naphthalene in 1 l of dioxan.

The uptake of algal cells by the larvae under different experimental algal densities was calculated from the radioactivity of algal and larval samples as follows:

$$\text{Radioactivity/algal cell} = \frac{\text{Radioactivity of standard (cpm)}}{\text{Total number of algal cells in standard algal sample}}$$

$$\text{Total uptake of algal cells by larval stock} = \frac{\text{Radioactivity of larval sample (cpm)}}{\text{Radioactivity per algal cell (cpm)}}$$

Uptake of algae/larvae was calculated by dividing the total uptake by the number of larvae.

ii) Retention: In order to check the number of algal cells retained by larvae at the end of 48 h, a set of experimental beakers originally planned for the study on 24 h uptake, were kept for a further period of 24 h, feeding them at the same algal cell concentration but from an unlabelled stock culture used as a 'chaser'. After this total 48 h duration, the larvae were filtered, preserved and radioactivity counted in the same manner as for the uptake experiment. Retention has been expressed as the percentage of the total number of cells that have been taken up by pearl oyster larvae at the end of 24 h to the initial algal cell concentration.

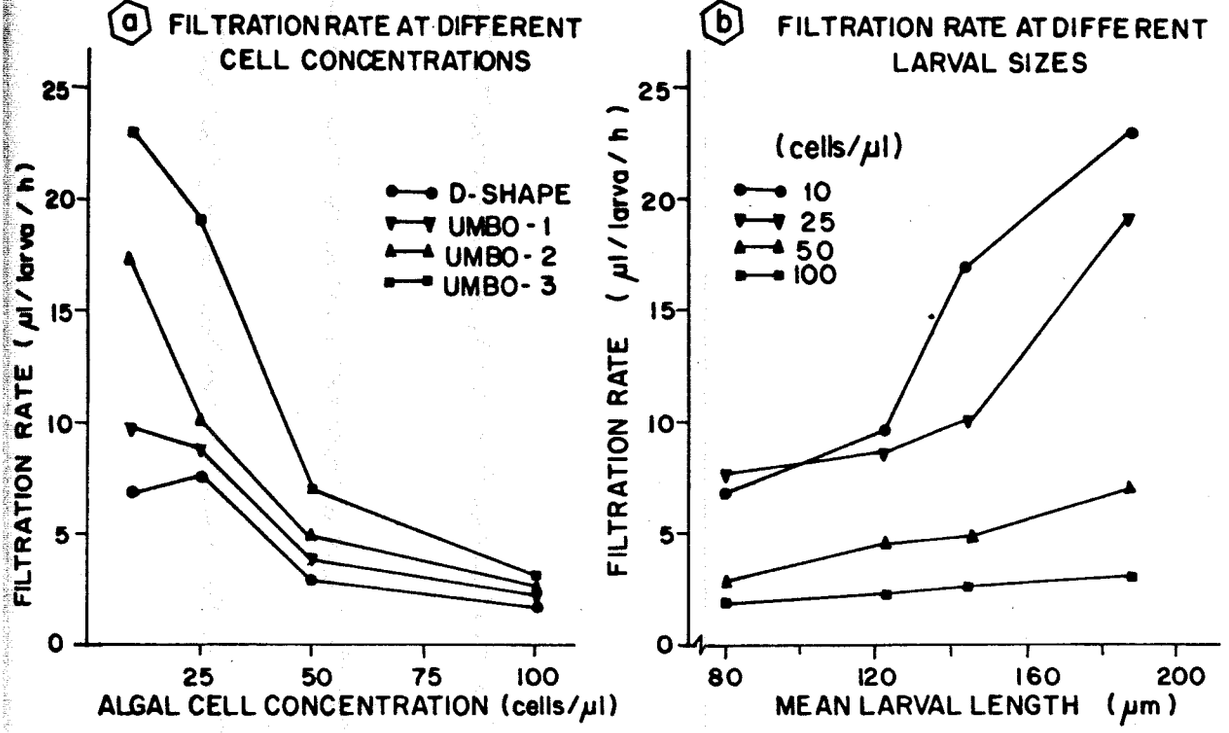
RESULTS

- Filtration rate

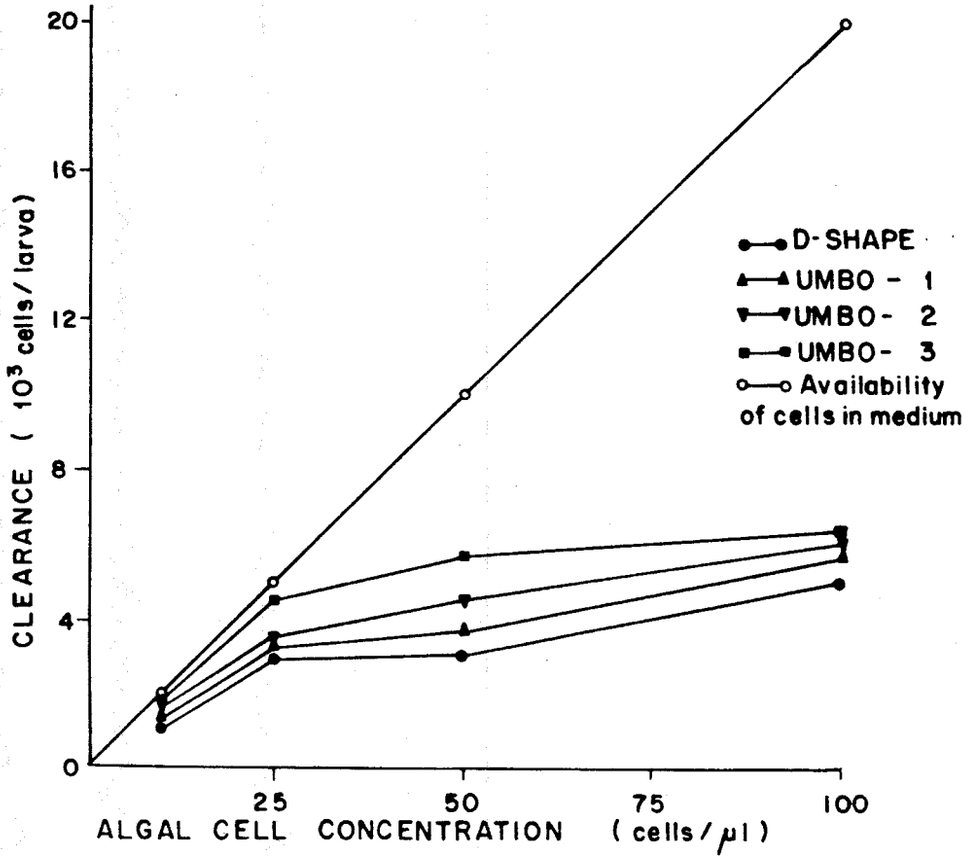
Filtration rates were seen to decrease with increasing cell concentrations (Table 55). At the mean larval size of 79.5 µm, filtration rates were 6.89, 7.64, 2.97 and 2.39 µl/larva/h at the concentrations of 10, 25, 50 and 100 cells/µl. Similar decrease in filtration rate with increasing cell concentration was also observed for the umbo larvae (122.5, 144.0 and 187.0 µm; Fig. 22 a).

No.	Mean larval size (µm)	Algal concentration (cells/µl)	Filtration rate (µl/larva/h)	Clearance at 24 h (10^3 cells/ml)		Clearance per larva (cells)	Clearance (%)
1.	79.5	10	6.89	5.63		1126	56.3
2.	122.5	10	9.69	6.88		1376	68.8
3.	144.0	10	17.33	8.75		1750	87.5
4.	187.0	10	23.10	9.38		1876	93.8
					Mean:	1532	76.6
5.	79.5	25	7.64	15.00		3000	60.0
6.	122.5	25	8.75	16.25		3250	65.0
7.	144.0	25	10.03	17.50		3500	70.0
8.	187.0	25	19.18	22.50		4500	90.0
					Mean:	3563	71.1
9.	79.5	50	2.97	15.00		3000	30.0
10.	122.5	50	3.92	18.75		3750	37.5
11.	144.0	50	4.98	22.50		4500	45.0
12.	187.0	50	7.13	28.75		5750	57.5
					Mean:	4250	42.5
13.	79.5	100	2.39	25.00		5000	25.0
14.	122.5	100	2.82	28.75		5750	28.8
15.	144.0	100	2.97	30.00		6000	30.0
16.	187.0	100	3.20	31.88		6376	31.9
					Mean:	5782	28.9

ig. 22. FILTRATION RATE AND ALGAL CELL CLEARANCE OF PEARL OYSTER LARVAE



(c) AVAILABILITY AND CLEARANCE OF ALGAL CELLS BY PEARL OYSTER LARVAE



Filtration rates were seen to increase with increasing larval size at all cell concentrations, from 6.89 to 23.10 µl/larva/h at 10 cells/µl; from 7.64 to 19.18 µl/larva/h at 25 cells/µl; from 2.97 to 7.13 µl/larva/h at 50 cells/µl; and from 2.39 to 3.20 µl/larva/h at 100 cells/µl. This corresponds to an increased clearance of algal cells (Table 55). At 10 cells/µl, percentage of algal cells cleared from the medium increased from 56.3% for larvae of 79.5 µm to 93.8% for larvae of 187.0 µm. A similar increase is also noted for larvae reared at the cell concentrations of 25, 50 and 100 cells/µl. Studied in relation to the initial cell concentration, however, it is seen that the range of percentage clearance has declined gradually from 56.3-93.8% at 10 cells/µl to 60.0-90.0% at 25 cells/µl, 30.0-57.5% at 50 cells/µl and 25-31.9% at 100 cells/µl.

Fig. 22c depicts the relation between the availability of cells in the rearing medium and clearance of algal cells per larva. While availability of cells increased from 2000/larva at 10 cells/µl to 20,000 cells/larva at 100 cells/µl, it may be seen that at the higher cell concentrations of 50 and 100 cells/µl, a greater proportion of algal cells is being wasted. At 10 cells/µl, with increase in size from 79.5 to 187.0 µm, clearance increased from 1126 to 1876 cells/larva, i.e., 56.3-93.8% of the available cells had been cleared from the medium. At 25 cells/µl, for a

similar increase in size, 3000 to 4500 cells were removed from the medium. At 50 and 100 cells/µl, number of cells removed from the medium ranged from 3000 to 5750 and 5000 to 6367 cells, respectively. Taking mean values of clearance (Table 55) it may be observed that percentage clearance decreased gradually with increasing cell concentrations (76.6% at 10 cells/µl, 71.1% at 25 cells/µl; 42.5% at 50 cells/µl and 28.9% at 100 cells/µl).

Fig. 23 shows the clearance of algal cells by pearl oyster larvae observed over 24 h at intervals. Initially, clearance of cells has been rapid. By 24 h clearance rate has slowed down.

The uptake and retention of C^{14}-labelled algal cells

The uptake and retention of C^{14}-labelled I. galbana, P. lutheri and C. freiburgensis cells by the D shape, umbo and the eyed umbo larvae are presented in Table 56.

i) Uptake: For all three algal species, the uptake of cells is seen to increase with time. Considering the uptake during 24 h period, about 50% of this amount is consumed by 3 h, e.g., 1077 cells of I. galbana/larva at 3 h and 2129 cells/larva at 24 h for the D shape larvae. By inference uptake of algal cells during the next 24 h is very slow.

FIG. 23. ALGAL CELL CLEARANCE BY PEARL OYSTER LARVAE OVER 24 HOURS AT DIFFERENT CELL CONCENTRATIONS.

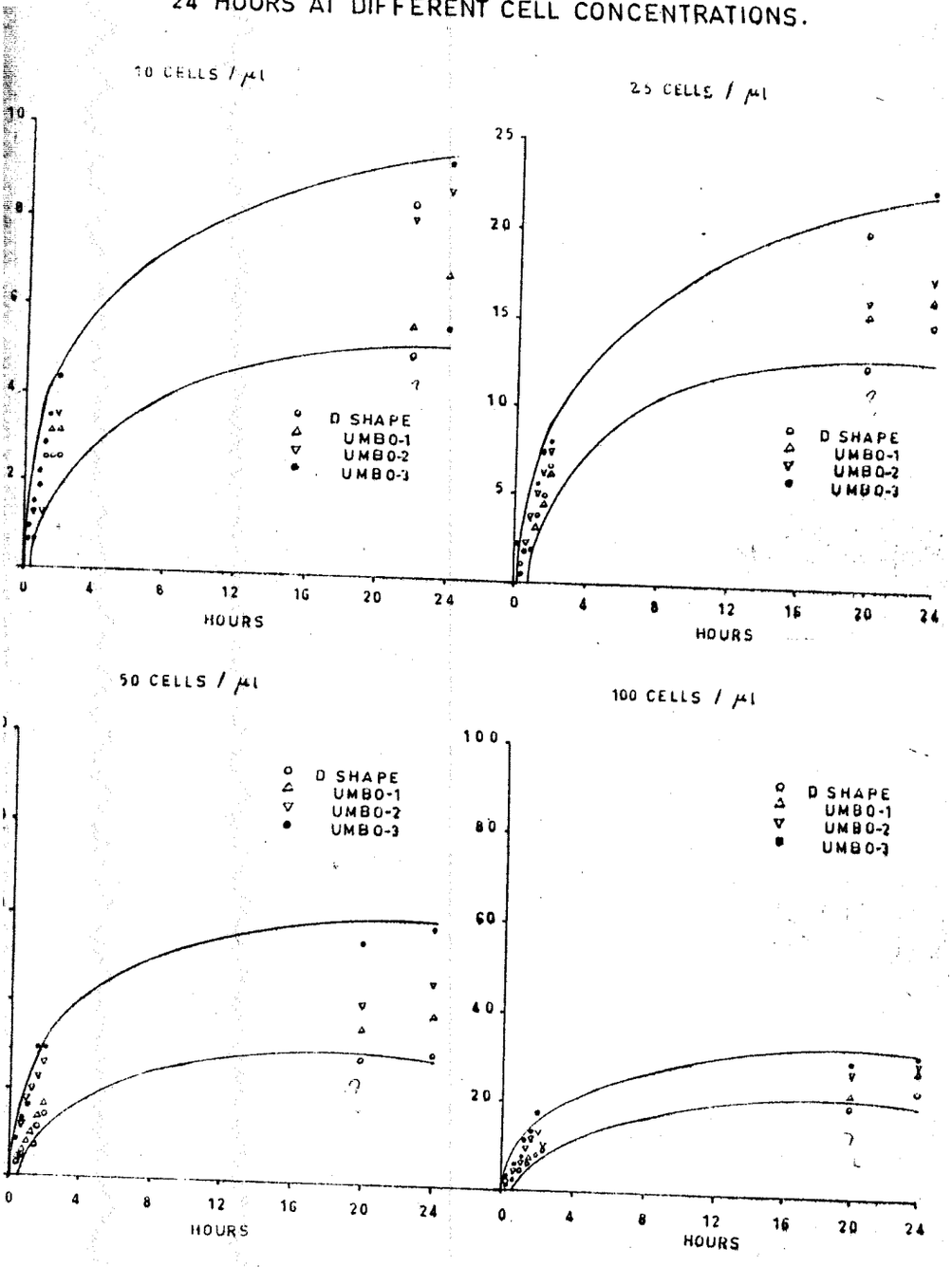


Table 56. The uptake and retention of C^{14}-labelled microalgae by pearl oyster larva.

S.No.	Algal species	Time (h)	D shape stage		Umbo stage		Eyed umbo stage	
			25 cells/µl	50 cells/µl	25 cells/µl	50 cells/µl	25 cells/µl	50 cells/µl
1.	I. galbana	1	686	543	403	677	808	946
2.		3	1077	1119	947	1110	1582	1393
3.		20	1762	1634	1652	2643	2037	3453
4.		24	2129	2763	2335	2710	3015	3742
5.	Retention	48	1703	2028	1786	2135	2041	2810
6.	% Retention		80.0	73.4	76.5	78.8	67.1	75.1
7.	P. lutheri	1	538	602	496	491	503	562
8.		3	725	727	544	684	656	631
9.		20	2116	2252	2188	2094	2342	2039
10.		24	2151	2329	2804	2307	3205	3341
11.	Retention	48	1721	1843	1947	1758	2346	2479
12.	% Retention		80.0	79.4	69.4	76.2	73.2	74.2
13.	C. freiburgensis	1	454	436	481	436	676	652
14.		3	559	558	558	498	1215	712
15.		20	1319	1423	1477	1605	1938	1738
16.		24	1531	1562	1617	1661	2083	2326
17.	Retention	48	1203	1251	1285	1308	1642	1871
18.	% Retention		78.6	80.1	79.5	78.7	78.8	80.4

Algal uptake at the end of 24 h was seen to increase progressively with increase in larval size. At 25 cells/μl, uptake of P. lutheri increased from 2151 cells/larva at the D shape to 3205 cells/larva at the eyed umbo stage. Similar increase in uptake was observed for both I. galbana and C. freiburgensis at 25 and 50 cells/μl.

Doubling the algal cell concentration from 25 to 50 cells/μl leads only to a marginal increase in uptake. At 24 h, uptake by the D shape, umbo and eyed umbo stages have been, respectively, 2129, 2335 and 3015 cells/larva at 25 cells/larva, while it was 2763, 2710 and 3742 cells/larva at 50 cells/μl, using I. galbana as diet. Similar results are observed for both P. lutheri and C. freiburgensis, except for umbo larvae fed with P. lutheri, where there has been a decrease in uptake at 50 cells/μl.

ii) Retention: The retention of labelled algal cells at the end of 48 h has been in the range of 67.1-80.0% for I. galbana, 69.4-80.0% for P. lutheri and 78.6-80.4% for C. freiburgensis.

DISCUSSION

Fretter and Montgammery (1968) and Strathman et al. (1972) observed that in suspension feeders, the clearance

mechanism determines the quantum and the kind of food obtained in a given environment. In this context, the role of the velum in feeding and filtration of particles is important. Its structure has been described in great detail (Yonge, 1926; Elston, 1980).

Quantitative estimates of filtration rates are few and far between. Some of the published results on different species are given in Table 57. It may be noted that filtration rates have been calculated under different temperature and feeding regimes. The range of filtration rate values observed in the present study (2.39 to 23.10 µl/larva/h) show fairly good agreement with those of other species. Certain general comparisons may be made.

There is a reduction in the filtration rate of pearl oyster larvae with increasing cell concentration (Table 55). This has also been observed for the mussel Mytilus edulis larvae by Bayne (1965) and Sprung (1984 b). Bayne (1965) calculated the filtration rate of mussel larvae at 18°C to be in the range of 14.6-23.3 µl/larva /h at the cell concentration (I. galbana) of 250-270 cells/µl and 19.2-34.6 µl/larva/h at the lower cell concentration of 40-75 cells/µl. Sprung (1984 b), studying the clearance rates at the three temperatures of 18°, 12° and 6°C, recorded likewise, a decrease in filtration rates of mussel M. edulis larvae with increasing I. galbana cell concentration levels.

S. No.	Species	Larval size range (µm)	Algal species	Concentration (10^6 cells per l)	Temperature (°C)	Filtration rate (µl/larva/h)	Authors
1.	Ostrea edulis	200	Flagellates	15- 26	20-22	27.08	Jorgensen (1952) (calculated from Bruce, Knight and Parke, 1940)
2.	Ostrea edulis	218-280	I. galbana	39- 44	19-25	17.9 -20.4	Walne (1956a)
3.	Ostrea edulis	161.0-178.2	I. galbana	85-117	24	2.6 - 9.8	Walne (1965a)
		203.0	I. galbana	100	23	10.5	
		219.3-230.3	I. galbana	123	23	16.7 -27.5	
4.	Crassostrea gigas	78.6-177.0	I. galbana	100	25	0.92-10.34	Gerdes (1983)
		76.0-133.0	I. galbana	25	25	1.98-12.35	
		76.8-106.8	I. galbana		25	0.49- 3.62	
		117.5-154.6			25	2.44-21.34	
		165.3-198.6			25	17.38-47.83	
5.	Mytilus edulis	170	I. galbana	110-200	18	3.6 - 5.4	Bayne (1965)
		210	I. galbana	25	18	8.75	
		210	I. galbana	60	18	10.0	
		210	I. galbana	350-330	18	3.8 - 7.1	
		240	I. galbana	250-270	18	14.6 -23.3	
		260	I. galbana	40-75	18	19.2 -34.6	
		260	I. galbana	290	18	27.5	
		260	I. galbana	64	18	13.4	
		260	I. galbana	60	18	2.38	

No.	Species	range (/um)	species	ion (10⁴ cells/ l)	ature (°C)	rate (/h, larvae/ h)	
6.	Mytilus edulis		I. galbana	1.5-5.5	12	11.4	Riisgard et al., (1980)
7.	Mytilus edulis		I. galbana or M. lutheri	3-6	15	10.0	Riisgard et al., (1981)
			I. galbana or M. lutheri	-	15	100.0	
			I. galbana or M. lutheri	3-6	12	11.0	
			I. galbana or M. lutheri	3-6	15	31.0	
8.	Mytilus edulis	139.0-261.0	I. galbana	1-5	6	3.6-24.0	Sprung (1984b)
		139.0-261.0	I. galbana	10	6	2.3- 8.3	
		139.0-261.0	I. galbana	20	6	1.6- 5.1	
		139.0-261.0	I. galbana	40	6	0.8- 3.4	
		139.0-261.0	I. galbana	1-5	12	12.4-85.0	
		139.0-261.0	I. galbana	10	12	8.2-42.2	
		139.0-261.0	I. galbana	20	12	4.4-19.9	
		139.0-261.0	I. galbana	40	12	2.1- 6.9	
		139.0-261.0	I. galbana	1-5	18	5.0-42.0	
		139.0-261.0	I. galbana	10	18	11.1-34.0	
		139.0-261.0	I. galbana	20	18	6.1-21.5	
		139.0-261.0	I. galbana	40	18	3.2-11.0	
9.	Pinctada fucata	79.5-187.0	I. galbana	10	-	6.89-23.10	Present study
		79.5-187.0	I. galbana	25	-	7.64-19.18	
		79.5-187.0	I. galbana	50	-	2.97- 7.13	
		79.5-187.0	I. galbana	100	-	2.39- 3.20	

Gerdes (1983) recorded values of 0.92-10.34 µl/larva/h at the concentration of 100 cells/µl and 1.98-12.35 µl/larva/h at the concentration of 25 cells/µl for the D shape larvae of Crassostrea gigas. This has also been observed in the present study with pearl oyster larvae.

Increase in filtration rates with increasing larval size as observed in the present study has been demonstrated for larvae of C. gigas by Gerdes (1983) who recorded filtration rate values of 0.49-3.62 µl/larva/h for the D shape larvae (76.8-106.8 µm), 2.44-21.34 µl/larvae/h for the umbo stage (117.5-154.6 µm) and 17.38-47.83 µl/larva/h for the late umbo stage (165.3-198.6 µm).

Fretter and Montgommery (1968), studying the treatment of food by 19 species of monocardium veligers belonging to 8 different superfamilies, observed that there was uniformity in the functioning of the gut. In the high concentrations of digestible food, a larva, previously starved will fill the stomach in a few minutes and then stop feeding, until digestion of the meal is under way. In low concentrations of food, only the occasional cell can be gathered and feeding is more or less continuous. The particles are passed rapidly to the intestine for excretion. In the present study, a steady decrease in algal cell concentration during a 24 h period at all four cell concentrations in the medium suggests that there is continuous feeding. It is,

however, possible that a large portion of the cells being cleared from the medium is being rejected over the oral palp and only a small percentage of these cells is entering the stomach. This has been seen to occur in larvae of C. gigas (Malouf and Breese, 1977). They observed that the removal of 2600 cells/h did not result in appreciably greater growth than did the removal of 1300 cells/h. Malouf and Breese (1977) suggested that many of the cells that were removed by the larvae at the higher feeding rates were not ingested and were incompletely assimilated or were simply cleared and rejected as pseudofeces. In addition, Malouf and Breese (1977) reported that, when 'batch'-fed, there is a rapid removal of algal cells from the medium. As the number of remaining cells in the medium decreases, there is corresponding decrease in removal of cells. The same is also true of pearl oyster larvae. Clearance was rapid for the first few hours (Table 55). By examining results of clearance at the later hours, it may be inferred that there has been a decrease in clearance thereafter.

The results of the present study on uptake and retention showed that, related with an increase in larval size, there was a corresponding increase in the uptake and retention of all three species, I. galbana, P. lutheri and C. freiburgensis. The uptake of labelled cells was quantitatively very similar for I. galbana (2129-3742 cells per

larva) and P. lutheri (2151-3341 cells/larva) but less for C. freiburgensis (1531-2326 cells/larva). Despite the doubling of the cell concentration from 25 to 50 cells/µl, there was only a marginal increase in the uptake of labelled cells at 50 cells/µl than at 25 cells/µl. This was observed for all three algal species, I. galbana, P. lutheri and C. freiburgensis.

Lucas and Rangel (1983) using epifluorescence microscopy observed the occurrence of filled and empty stomachs of C. gigas larvae, and suggested that an initial ration of 750 cells (P. lutheri and I. galbana) per larva was adequate at the temperature of 24°C. At 21°C, the daily requirement was estimated to be 400 cells/day.

Ukeles and Sweeney (1969) used labelled M. lutheri to study assimilation in larvae of C. virginica. They observed that the number of flagellates ingested becomes greater as the available number of cells increases (25-1500 cells/µl). However, retention or utilisation of flagellates did not increase concurrently (Ukeles and Sweeney, 1969). Incorporation of labelled flagellates increased rapidly in the first few hours of incubation, but more slowly thereafter. Although availability of cells increased from less than 1667 cells/larvae to 100,000 cells/larva, retention of cells remained steady beyond the cell concentration of 13,333 cells/larva. Maximum uptake of the label in 48 h was

greater at 13,333 cells/larva than at 700 cells/larva. Walne (1974) calculated the average daily consumption of O. edulis larvae from the density of algal cells and larvae in the rearing bins, and estimated that it increases from 20,000 cells/day at the time of first liberation to 60,000 cells/day as the larvae approach metamorphosis. Using p^{32}-labelled I. galbana, he estimated that small larvae will assimilate 5000-10,000 cells of Isochrysis per day. In comparison, they assimilate 16,000-23,000 cells of a small species such as Micromonas, but only 400-1200 cells of a large species like Dunaliella tertiolecta (Walne, 1974).

In pearl oyster larvae, it was noted that 3000 to 4500 cells/larva was removed from the medium at 25 cells/μl. Using C^{14}-labelled I. galbana, daily consumption at the same cell concentration was 2129 to 3015 cells per larvae. Assimilation is not as high as observed for edible oyster O. edulis by Walne (1974), but is higher than that observed for C. gigas by Ukeles and Sweeney (1969) and Lucas and Rangel (1983). As in the case of O. edulis, the uptake of a larger sized species, i.e., C. freiburgensis is less than that of the smaller species I. galbana and P. lutheri in the present study.

Considering the values of clearance, uptake and retention of I. galbana at the concentrations of 25 and 50 cells/μl, it is observed that there is a steady reduction

in these values. Although the results are from two independent experiments, the data lend themselves for a sequential treatment of the three steps of clearance (data from Table 55), uptake and retention (data from Table 56). At 25 cells/μl, the mean clearance of *I. galbana* cells was 3563 cells/larva, mean uptake was 2493 cells/larva and retention was only 1843 cells per larva. At 50 cells/μl, the figures were, respectively, 4250, 3077, and 2329 cells per larva. These results suggest that pearl oyster larvae filter from the medium, a greater number of cells than are actually required. At the first instance, many of the cells removed by the larvae may be cleared and rejected as pseudofeces. Even after entry into the stomach, material may be rejected along with the feces without being assimilated. Larvae exposed to very high concentrations of algae could ingest algal cells and pass them through the digestive system without gaining any nutritional benefit from them (Millar, 1955). Chellam (1983) had pointed out that the adult pearl oyster *P. fucata* of the Gulf of Mannar is a wasteful feeder. A similar wastage of cells is also observed in the case of its larvae.

Bayne (1976), quoting the work of Walne (1965), observed that an average of 3-4 times the number of cells were removed than were actually assimilated. Comparing the

results of algal cell clearance (3563 and 4250 cells at 25 and 50 cells/µl) and the equivalent of labelled cells that were retained in the larvae (1843 and 2324 cells at 25 and 50 cells/µl), it is seen that approximately 1.9 and 1.8 times the actual intake have been cleared from the rearing medium.

In laboratory cultures, with relatively high concentrations of food cells being made available, the bivalve larvae remove from suspension, more cells than they can ingest and assimilate ingested cells with an efficiency of 40-70%(Bayne, 1976). The calculated assimilation efficiency of pearl oyster larvae using the data of algal clearance and retention is 51.7% at 25 cells/µl and 54.9% at 50 cells/µl. At lower concentrations of food cells, the larvae may respond by an increase in filtration and or assimilation efficiency (Bayne, 1976).

CHAPTER 5

PRELIMINARY STUDIES ON THE NUTRITIONAL VALUE OF ARTIFICIAL DIETS AND DISSOLVED NUTRIENTS ON PEARL OYSTER LARVAL GROWTH

INTRODUCTION

Mass culture of microalgae to meet the heavy and timely demands of larval rearing is expensive, requiring technical manpower, costly equipment and systems, and is also subject to quantitative and qualitative fluctuations depending on its culture conditions. Attempts have therefore been made to rear bivalve larvae on a variety of non-living diets (Davis, 1950; Loosanoff et al., 1951; Carriker, 1956; Hidu and Ukeles, 1962; Chanley and Normandin, 1967; Walne, 1974; Chu et al., 1982; Langdon, 1983). Such trials were aimed at providing a diet that was consistent in its nutritional quality and was readily available.

Authors experimented with a variety of materials like yeast (Davis, 1950), detritus (Loosanoff et al., 1951), pablum flakes (Carriker, 1956), non-living organic materials (Chanley and Normandin, 1967), dried and freeze-dried algae (Hidu and Ukeles, 1962; Walne, 1974) and biphasic diets consisting of both particulate matter and dissolved nutrients (Langdon, 1983).

In recent years, several types of microparticulate diets suitable for consumption by marine invertebrate larvae have been developed and tested (Chu et al., 1982; Teshima et al., 1982; Langdon, 1983). These include the microencapsulated diets (MED), the microbinding diets (MBD) and the microcoating diets (Teshima et al., 1982).

Manahan and Crisp (1982) postulated that since the majority of larvae have ciliated epithelium normally associated with nutrient absorption, dissolved nutrients may well form one of the energy sources during larval development. Investigations have been carried out on the role of dissolved nutrients in bivalve larval growth (Davis and Chanley, 1956; Crane et al., 1957; Chanley and Normandin, 1967; Stephen, 1968; Gustafson, 1980; Bayne, 1983). Manahan and Crisp (1982, 1983) established the uptake of aminoacids from seawater by veligers of C. gigas, O. edulis, M. edulis and newly settled larvae of C. gigas and Pecten maximus.

Realising that the field is open for baseline studies and no conclusive results are available on the merits of non-living diets as bivalve larval food, investigations were taken up to study the effect of freeze-dried algae and carrageenan bound microparticulate diet as well as dissolved nutrients, on the growth and survival of Pinctada fucata larvae.

MATERIALS AND METHODS

Two types of non-living diets were evaluated for their food value, viz.,

a) Freeze-dried I. galbana cells,
b) Carrageenan bound microparticulate diet (CBMD).

Dissolved nutrients in the form of water soluble vitamins and amino acids were added to the larval rearing medium both individually and in combination as shown below:

a) Amino acids mixture,
b) Vitamin mixture,
c) Amino acid + Vitamin mixture.

Preparation of the diets

- Freeze-dried algal cultures of I. galbana: Large volumes of algal cultures of I. galbana were centrifuged and stored in a refrigerator. The collected algal cells were then freeze-dried in a laboratory model udder type freeze-drier (Pl. IV). The freeze-dried material in the form of fine powder was stored in the deep-freeze under nitrogen gas atmosphere until further use.

- Carrageenan-bound microparticulate diet: The preparation of the diet was according to Kanazawa (1982). The diet composition is given in Tables 58, 59, 60, 61 and 62.

Twenty grams of the diet was mixed with 80 ml of water and heated to 80°C in a water bath. One gram of carrageenan

Table 58. Gross composition of the microparticulate diet (CEMD)

	Ingredient	Weight(g)
1.	Casein	8.0
2.	Starch	2.0
3.	Dextrin	4.0
4.	Lipid (cod liver oil)	3.0
5.	Mineral mix	2.0
6.	Vitamins*	0.6
7.	Amino acids*	0.4
	Total	20.0

Table 59. Composition of amino acid mixture*

	Amino acid	mg/l
1.	L-alanine	97
2.	2 amino-n-butyric acid	18
3.	L-arginine	57
4.	Aspartic acid	99
5.	L-cysteine	5
6.	Glutamic acid	84
7.	Glycine	63
8.	L-histidine	19
9.	L-iso leucine	33
10.	Leucine	102
11.	L-lysine	73
12.	Ornithine	4
13.	Methionine	32
14.	Phenylalanine	44
15.	L-proline	67
16.	Serine	60
17.	Threonine	50
18.	Tryptophan	4
19.	Tyrosine	21
20.	Valine	68
	Total	1000

* according to Langdon (1983)

Table 60. Composition of water soluble vitamins*

	Vitamin	mg/l
1.	Thiamine	10
2.	Nicotinic acid	25
3.	Nicotinamide	25
4.	Calcium pantothenate	10
5.	Riboflavine	10
6.	Pyridoxal (HCl)	50
7.	p- aminobenzoic acid	50
8.	Biotin	10
9.	Choline chloride	500
10.	Inositol	50
11.	Folic acid	10
12.	Ascorbic acid	50
	Total	800

Table 61. Composition of fat soluble vitamins*

	Vitamin	mg/l
1.	Calciferol	10
2.	Menadione	1
3.	Vitamin	14
4.	α-tocopherol	1
5.	Cholesterol	20
	Total	46

Table 62. Composition of mineral mixture

	Mineral	mg/l
1.	K_2HPO_4	234
2.	$Ca_3(PO_4)_2$	318
3.	$Mg\ SO_4.7H_2O$	356
4.	$NaH_2PO_4.\ 2H_2O$	92
	Total	1000

* according to Langdon (1983)

was added to the diet with constant mixing. Potassium chloride (0.5g) was gradually added with constant mixing. This was then cooled in a refrigerator for half an hour. When the diet had solidified, it was cut into small cubes and freeze-dried. The cubes were homogenised in a blender and then passed through a graded series of sieves of 150, 53 and 20 µm. The diet in the form of fine powder was stored in a plastic bag inflated with nitrogen gas, in a deep-freeze and then used as and when required. The particle size of the diet obtained ranged from 2 µm to 20 µm.

Experimental design

Non-living diet

Pearl oyster larvae were obtained and stocked in the manner described in Chapter 2. Larval cultures were given mild aeration through sterilised hypodermic syringe whenever a prepared diet was given to the larvae. The antibiotic streptomycin sulphate was added to the rearing medium with each water change at the rate of 8mg/l. The diet was introduced successively at the D shape, umbo and the eyed umbo stages of larvae. The feeding protocol was as follows

Stages	Treatment 1	Treatment 2	Treatment 3	Standard
D shape	A.D.	I	I	I
Umbo	A.D.	A.D.	I	I
Eyed umbo	A.D.	A.D.	A.D.	I

Abbreviations: A.D. - Artificial diet (Freeze-dried algal cells/carrageenan-bound microparticulate diet);
I - Isochrysis galbana (live culture).

Wherever I. galbana was used as standard its concentration was maintained uniformly at 25 cells/µl. The artificial diet was also provided at the concentration of 25 particles/µl. For this purpose, the diet in the form of fine powder was suspended uniformly in 100 ml of sterile seawater. Counts of the particle concentration were made using the blood counting chamber. Calculated values to yield a concentration of 25 particles/µl were added to the larval rearing medium. The dosage of diet was renewed with each water change.

Dissolved nutrients

The amino acid mixture and the vitamin mixture used were prepared according to Langdon (1981). The concentration of amino acids was 0.004 mg/ml and that of vitamins was 0.0032 mg/ml in the rearing medium. Dissolved nutrients were used both singly and in combination with algal cultures as shown below.

Treatment No.	Diet
1	I. galbana + Amino acids
2	I. galbana + Vitamins
3	I. galbana + Amino acids + Vitamins
4	Amino acids
5	Vitamins
6	Amino acids + Vitamins

The diet was renewed with each water change.

Two controls were kept in the experiments:

1. Larvae fed with live I. galbana culture and
2. Starved larvae

Bacterial load

Bacterial load was estimated using the standard pour plate method and using the nutrient agar medium. Bacterial load is expressed as number of colonies/ml (Cappucino and Sherman, 1983)

RESULTS

A. Evaluation of freeze dried I. galbana for pearl oyster larval growth

The experiment to study the nutritional value of freeze dried I. galbana cells for pearl oyster larval growth and setting was carried out during May-June, 1984, when the ranges of temperature, salinity and pH were 27.9-29.0°C, 34.0-35.0‰ and 8.10-8.15, respectively.

Larval growth (Tables 63 and 64; Figs. 24 and 25)

From the mean larval sizes presented in Table 63, it may be observed that the least growth was observed in treatment 1 where freeze dried I. galbana cells were introduced from the D shape stage. There is progressive increase in

Table 63. Growth and setting of pearl oyster larvae fed with freeze dried *I. galbana* cells.

Age of larvae (days)	Mean size of larvae (μm)			
	Treatment 1	Treatment 2	Treatment 3	Standard
1	67.5 ± 2.4	67.5 ± 2.4	67.5 ± 2.4	67.5 ± 2.4
5	79.9 ± 3.4	84.0 ± 4.7	84.1 ± 4.6	84.7 ± 4.3
9	78.3 ± 4.1	90.6 ± 6.7	91.9 ± 5.3	90.4 ± 6.9
13	80.3 ± 4.8	102.6 ± 12.5	99.4 ± 6.2	106.9 ± 13.1
17	-	125.6 ± 21.7	139.3 ± 24.3	145.7 ± 28.9
21	-	165.6 ± 34.1	172.1 ± 33.4	183.3 ± 34.1
25	-	168.3 ± 24.2	167.6 ± 28.2	185.5 ± 33.6
29	-	185.0 ± 12.6	183.8 ± 25.9	198.6 ± 14.6
33	-	-	-	-
Day of first spat setting	-	21	21	21
Day of final spat setting	-	31	33	31
Total no. of spat	-	127	351	815
Rate of spat production (%)	-	1.0	2.8	6.5

growth values from treatment 1 to treatments 2 and 3, while maximum growth was observed for standard fed with the live algae (Table 63). This is further substantiated by the larval size frequency distribution on days 5, 13 and 25 (Fig. 24). The growth curve of larvae fed with freeze dried I. galbana cells from the straight hinge stage is almost flat indicating very poor larval growth (Fig. 25). Beyond day 13 growth of standard has remained consistently greater than all other treatments.

Further, the overall growth rate for the period day 1-25 is similar for treatments 2 and 4 (4.20 and 4.17 µm/day) and maximum for larvae fed I. galbana cells (4.83 µm/day). In treatment 2, growth rate for the period day 17-25 is maximum (5.34 µm/day), while in treatment 3, growth rate for the period day 9-17 is maximum (5.93 µm/day).

Table 64. Larval growth rate using freeze dried I. galbana as diet.

Period (days)	Larval growth per day (µm/day)			
	Treatment 1	Treatment 2	Treatment 3	Standard
1-9	1.35	2.89	3.05	2.86
9-17	-	4.38	5.93	6.91
17-25	-	5.34	3.54	4.70
1-25	-	4.20	4.17	4.83

Fig. 24. SIZE FREQUENCY DISTRIBUTION OF PEARL OYSTER LARVAE FED WITH FREEZE-DRIED *I. GALBANA*

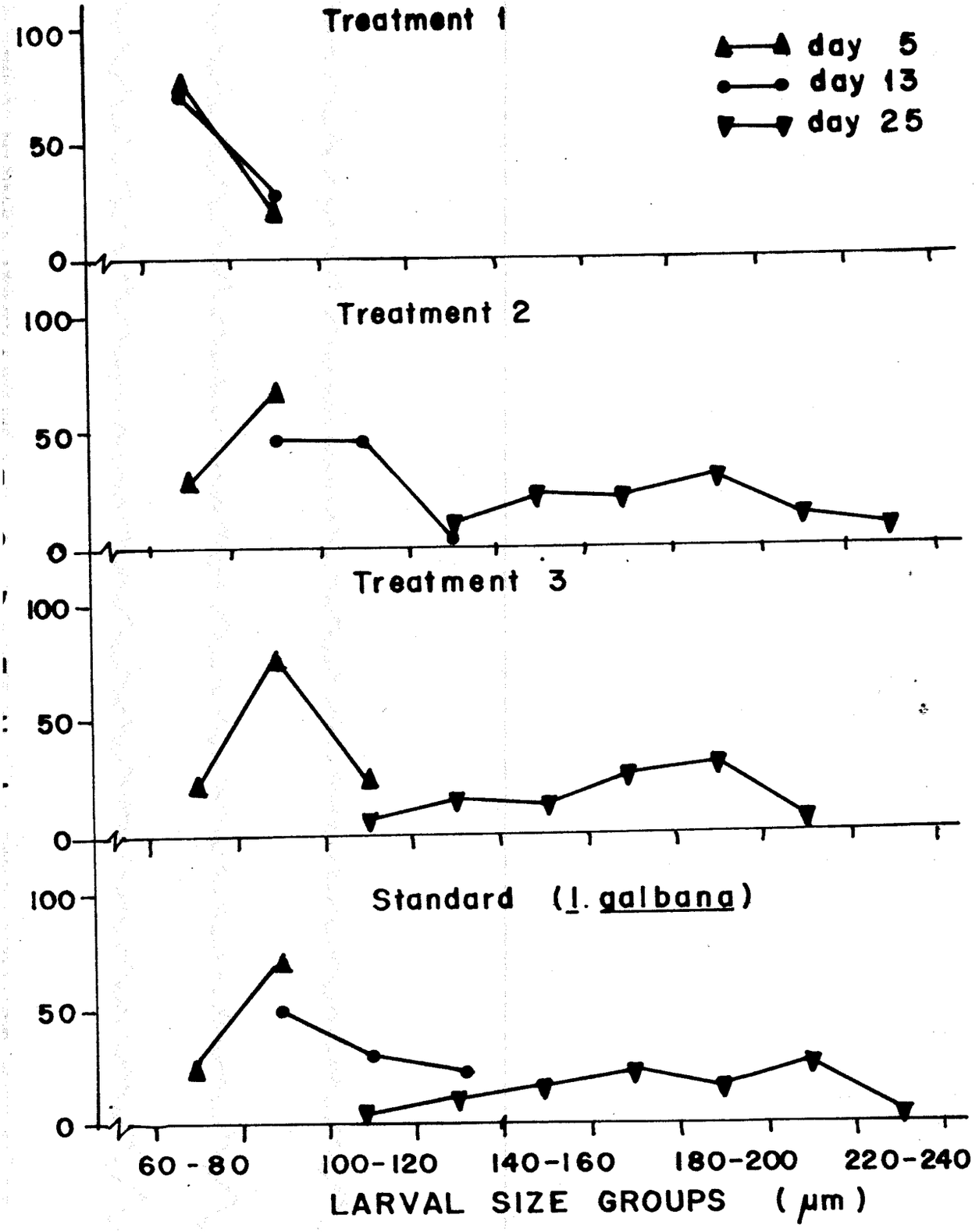
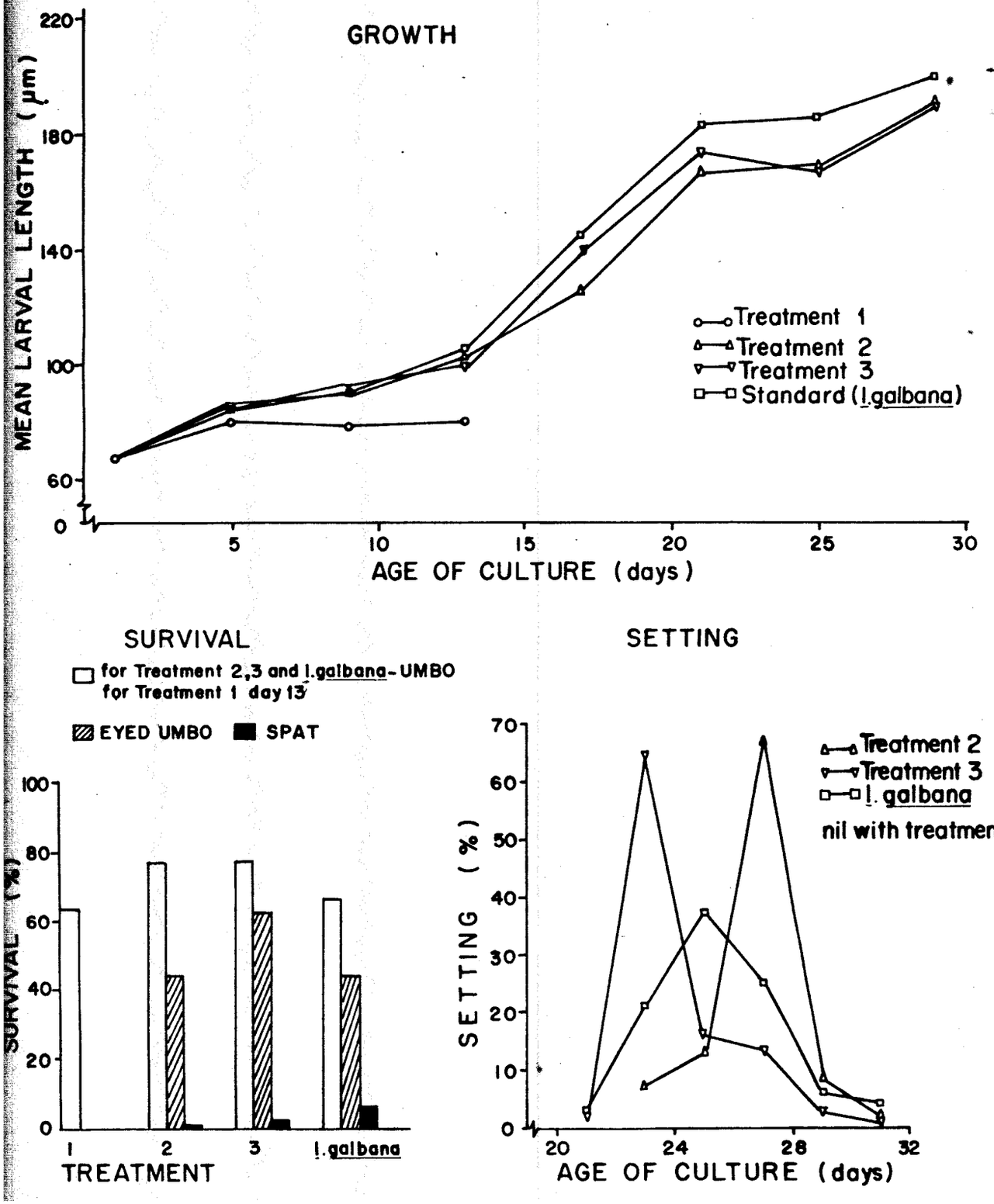


Fig. 25. GROWTH, SURVIVAL AND SETTING OF PEARL OYSTER LARVAE FED WITH FREEZE-DRIED I. GALBANA



Linear regression analysis yielded growth regressions of 0.0053, 0.0180, 0.0195 and 0.0211 log/um/day for treatments 1, 2, 3 and standard respectively. The analysis of co-variance showed that there was highly significant difference in the growth regressions between treatments ($P < 0.01$). The growth regressions of treatments 2, 3 and standard were not significant from each other ($P > 0.05$) but that of treatment 1 was significant from all others ($P < 0.01$).

2. Survival rate (Fig. 25)

Survival at the umbo stage was 77.7, 76.9 and 66.6% for treatments 2, 3 and standard, respectively. For the same day (13), survival in treatment 1 was 63.8%. Survival at the eyed umbo stage was 43.2, 62.9 and 44.4% for treatment 2, 3 and standard, respectively. Survival to spat stage was 1.0, 2.8 and 6.5% for treatments 2, 3 and standard, respectively (Fig. 25).

3. Spat setting and production (Table 63, Fig. 25)

Spat setting was initiated uniformly on day 21 in treatment 2, 3 and standard. Peak setting was observed on day 23 in standard, on day 25 in treatment 3 and on day 27 in treatment 2 (Fig. 25). Total spat production was maximum for standard (6.5%) and less in treatments 2 (1.0%) and treatment 3 (2.8%). There was highly significant difference

in percentage spat production between treatments 2, 3 and standard ($P < 0.01$).

Algal cell consumption

In treatment 1, where the freeze dried algal cells were provided at the D shape stage, consumption was in the range of 4.2-9.2%. In treatment 2, where freeze dried algal cells were given during the umbo stage, consumption of live algae up to umbo stage was in the range of 55.0-70.0%, while it was reduced to 5 to 10% with the introduction of freeze dried algal cells. In treatment 3, consumption of live algae was in the range of 50-75% while consumption of live algae in the standard was in the range of 60-75% of the total number of cells provided.

Bacterial load

The bacterial load was 116 ± 30, 106 ± 32, 93 ± 24 and 41 ± 14 colonies/ml in treatments 1, 2, 3 and standard, respectively.

Summary of results of larval rearing experiment using freeze-dried I. galbana.

A summary of the results of the experiment is presented in Table 65. Larvae of the standard have shown better larval growth (183.3 µm) and better spat setting (6.5%) than larvae fed on freeze dried algal cells. With

Table 65. Summary of results of larval rearing experiment using freeze dried *I. galbana*

Treatment	Mean larval size on day 25 (µm)	Larval growth rate per day (µm/day)	Growth regression (log µm/day)	Day of first setting	Duration (days)	Spat Production (%)
1.	-	-	0.0053	-	-	-
2.	165.6	4.20	0.0180	21	31	1.0
3.	172.1	4.17	0.0195	21	31	2.8
Std.	183.3	4.82	0.0211	21	31	6.5

delay in the feeding of freeze dried algal cells, there is both better growth and better setting of pearl oyster larvae.

B. Evaluation of CBMD for pearl oyster larval growth

The experiment to study the effect of CBMD on pearl oyster larval growth was carried out during the months of September-October 1983, when the ranges of temperature, salinity and pH were 26.3-28.6°C, 29.0-30.2‰ and 8.15-8.20 respectively.

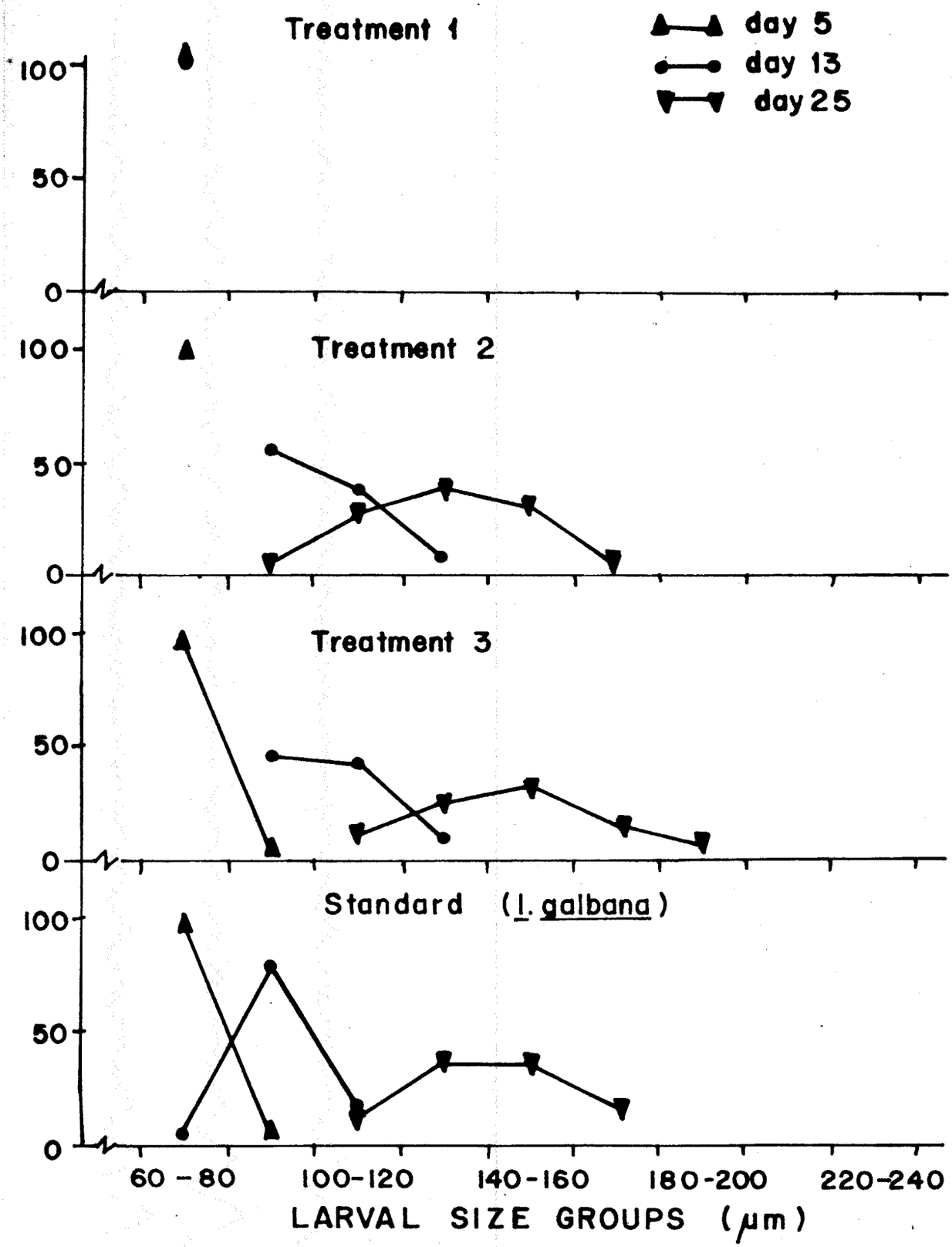
Larval growth (Tables 66 and 67, Figs. 26 and 27)

The CBMD was introduced from day 1, day 22 and day 27 to larvae of treatments 1, 2 and 3, respectively. While least growth was observed in treatment 2 where none of the larvae grew beyond the D shape stage (78.2 µm on days 13 and 17), it may be observed that larval growth in treatments 2 and 3 after the introduction of the CBMD has also been poor (Table 66). The size frequency distribution which demonstrates the larval size ranges on days 5, 13 and 25 is presented in Fig. 26. The growth curves presented in Fig.27 also substantiate the negligible growth of larvae fed with CBMD from the D shape stage onwards.

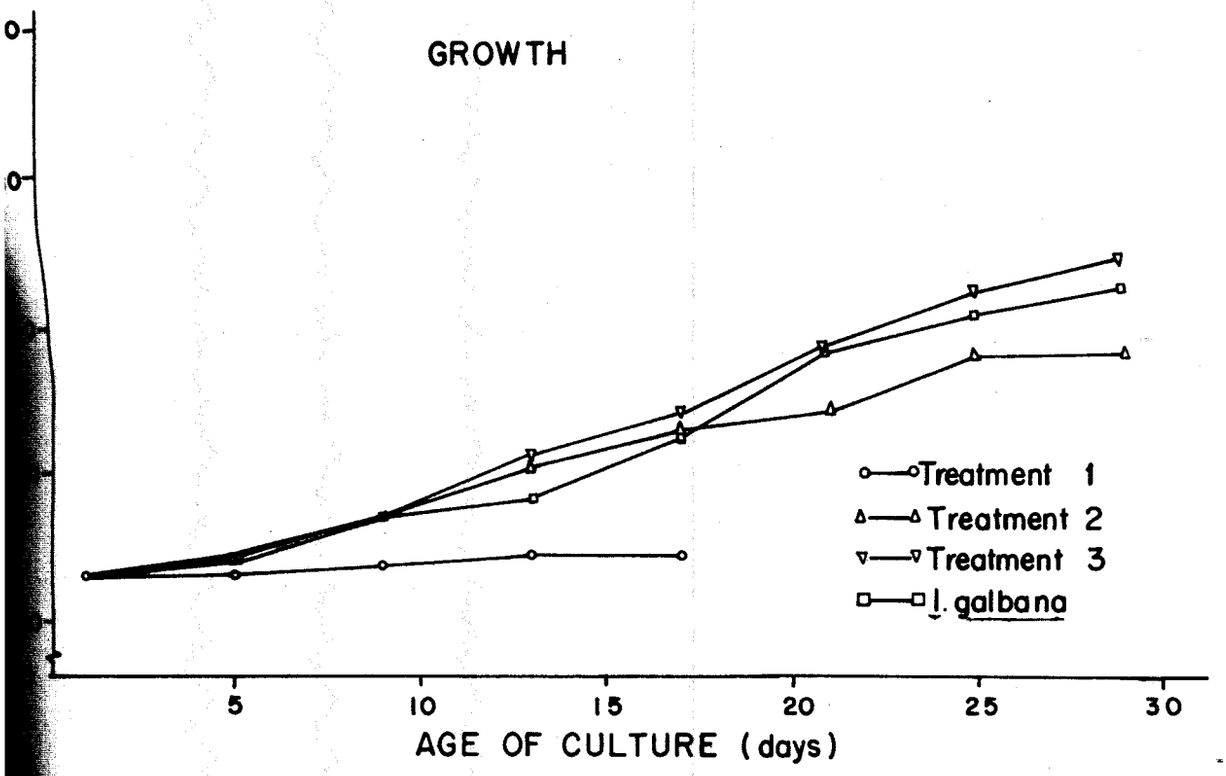
Least larval growth rate per day was observed in treatment 1 (Table 67). Introducing CBMD on day 22 in

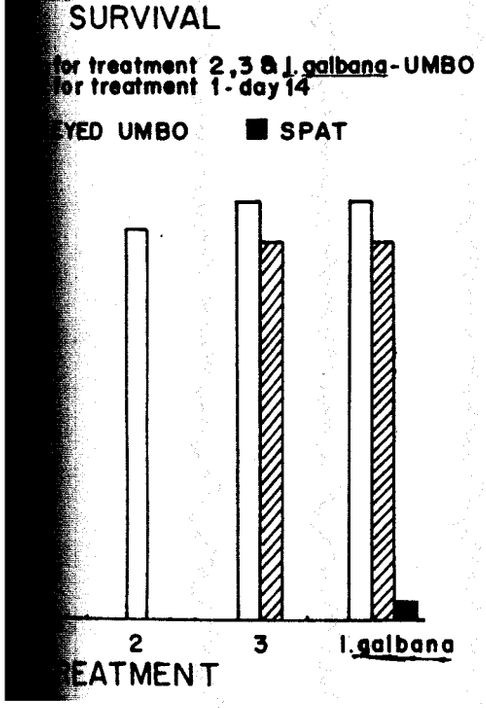
Age of larvae (days)	Mean size of larvae (μm)			
	Treatment 1	Treatment 2	Treatment3	Standard
1	72.2 ± 3.5	72.2 ± 3.5	72.2 ± 3.5	72.2 ± 3.5
5	72.4 ± 3.5	77.4 ± 2.9	77.8 ± 3.5	77.6 ± 3.1
9	74.8 ± 2.8	89.0 ± 6.2	88.8 ± 5.7	88.2 ± 6.8
13	78.2 ± 2.5	102.3 ± 12.5	104.1 ± 10.4	93.6 ± 7.8
17	78.2 ± 2.8	111.7 ± 15.6	116.2 ± 11.4	110.5 ± 10.2
21	-	117.3 ± 13.5	135.1 ± 16.6	133.8 ± 14.4
25	-	132.2 ± 16.9	148.3 ± 20.7	143.4 ± 17.2
29	-	133.1 ± 18.5	156.1 ± 21.3	150.1 ± 22.7
33	-	-	148.0 ± 16.4	174.2 ± 20.3
37	-	-	-	174.4 ± 24.1
41	-	-	-	186.0 ± 8.6
45	-	-	-	181.3 ± 16.7
49	-	-	-	165.0 ± 14.7
Day of first setting	-	-	-	33
Day of final setting	-	-	-	51
Total No. of spat	-	-	-	494
Rate of spat production (%)	-	-	-	4.0

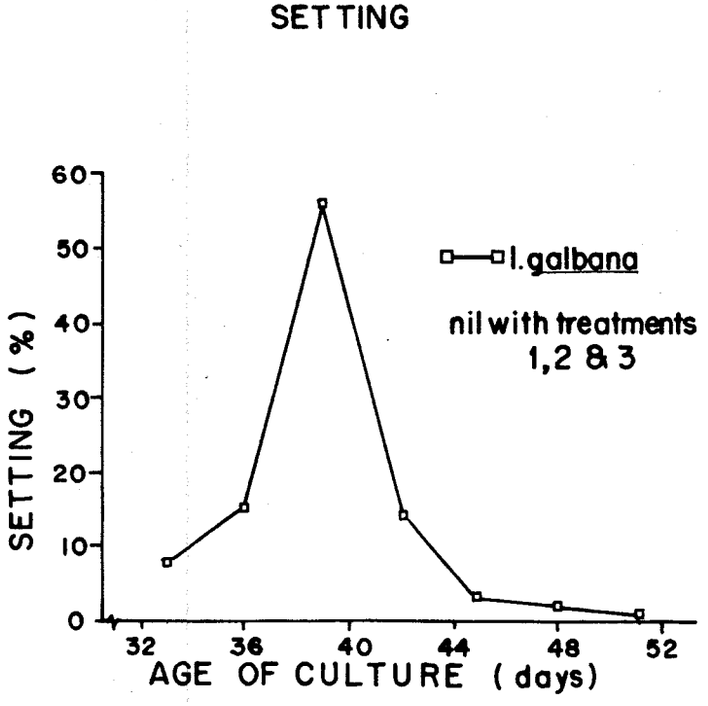
26. SIZE FREQUENCY DISTRIBUTION OF LARVAE FED WITH ARTIFICIAL DIET (C B M D)



g. 27. GROWTH, SURVIVAL AND SETTING OF PEARL OYSTER LARVAE FED WITH CARRAGEENAN BOUND MICROPARTICULATE DIET







treatment 2, least growth for the period day 17-25 is seen in treatment 2. Comparable rates of growth for treatments 3 and standard are recorded since the CBMD was introduced in treatment 3 only on day 27.

Table 67. Larval growth rate using CBMD as diet.

Period (days)	Larval growth per day (μm/day)			
	Treatment 1	Treatment 2	Treatment 3	Standard
1-9	0.32	2.10	2.08	2.00
9-17	0.43	2.84	3.43	2.79
17-25	-	2.56	4.01	4.11
1-25	-	2.50	3.17	2.97

Growth regressions obtained were 0.0024, 0.0102, 0.0113 and 0.0123 log μm/day for treatments, 1, 2, 3 and standard respectively. The analysis of covariance showed that the growth regressions between treatments were statistically significant ($P < 0.01$). The students t test showed that the growth regressions of treatments 2, 3 and standard were not significant from one another ($P > 0.05$), while that of treatment 1 was highly significant from the growth regressions of all the other treatments ($P < 0.01$).

!. Survival rate (Fig. 27)

Larval survival was 88.2, 93.8 and 93.3% for treatments 2, 3 and standard respectively. For the same day

larval survival in treatment 1 was 36.4% (Fig.27). By day 13, mortality was total in treatment 1. Larvae survived for 29 days in treatment 2 and for 33 days in treatment 3. Survival at the eyed umbo stage was 84.4 and 84.8% for treatment 2 and standard respectively. Setting was observed only in standard, yielding 4.0% spat.

Spat setting and production (Table 66, Fig. 27)

There was no larval metamorphosis in all treatments except standard. Spat setting was initiated on day 33 in the standard, yielding 4.0% spat.

Consumption

Particle counts were taken as for algal cells. It should be noted, however, that clumping of particles was observed. Microscopic examination of larvae fed artificial diet indicated that in at least a few larvae, ciliary activity of the stomach region showed consumption of particles. Generally, however, larvae presented a starved appearance.

Bacterial load

The bacterial load in the larval rearing medium was seen to be 168.2 $\pm$ 51.5; 192.5 $\pm$ 27.8 and 184.0 $\pm$ 38.6 colonies/ml of the standard.

Table 68. Summary of results of larval rearing experiment using CBMD as a diet.

Treatment	Mean larval size on day 25 (μm)	Larval growth rate per day (μm/day)	Growth regression (log μm/day)	Day of first setting	Duration (days)	Spat Production (%)
1.	-	-	0.0024	-	-	-
2.	132.2	2.50	0.0102	-	-	-
3.	148.3	3.17	0.0113	-	-	-
Std.	143.4	2.97	0.0123	33	18	4.0

Summary of results of larval rearing experiment using artificial diet (CBMD)

Table 68 gives the salient features of the experiment using CBMD. The response of pearl oyster larvae to the diet has been very poor.

C. Evaluation of the role of dissolved nutrients in pearl oyster larval growth

The experiment to evaluate the role of dissolved nutrients in pearl oyster larval nutrition was carried out during August-September, 1984. Range of temperature, salinity and pH were 29.0-31.5°C, 37.1-38.0‰ and 8.15-8.30 respectively.

Larval growth (Tables 69 and 70, Figs. 28 and 29)

When dissolved nutrients were supplemented along with live I. galbana cells (treatments 1, 2 and 3), growth comparable to that of standard was seen only in amino acid supplemented diet (treatment 1). From the maximum mean larval sizes recorded in these treatments, it is evident that larvae had reached the umbo stage but did not grow beyond (Table 69). Dissolved nutrients, alone(treatments 4, 5 and 6), caused a slight, but not substantial increase in mean larval size over that of control (starved larvae). The very limited increase in larval size in the absence of

6. Summary of results of larval rearing experiment using artificial diet (CBMD)

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Table 69. Growth and setting of pearl oyster larvae reared in dissolved nutrients.

Age of larvae (days)	Mean size of larvae (μm)							
	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5	Treatment 6	Standard	Starved Control
1	69.0 ± 2.3	69.0 ± 2.3	69.0 ± 2.3	69.0 ± 2.3	69.0 ± 2.3	69.0 ± 2.3	69.0 ± 2.3	69.0 ± 2.3
5	80.4 ± 4.7	82.6 ± 3.5	76.6 ± 3.7	75.6 ± 3.3	76.6 ± 3.7	75.4 ± 3.4	77.4 ± 3.5	72.2 ± 3.8
9	95.8 ± 3.7	104.2 ± 9.8	93.6 ± 4.8	76.8 ± 3.1	75.2 ± 3.0	81.4 ± 4.4	99.4 ± 6.2	75.4 ± 4.9
13	124.6 ± 11.4	123.3 ± 28.9	97.0 ± 4.8	84.2 ± 4.1	83.6 ± 4.4	85.2 ± 3.6	132.0 ± 14.5	77.4 ± 3.9
17	160.0 ± 18.9	115.0 ± 12.2	100.8 ± 6.6	85.4 ± 3.4	85.8 ± 3.4	82.6 ± 3.2	160.8 ± 22.2	78.8 ± 3.3
21	191.8 ± 22.9	-	-	-	83.4 ± 4.6	-	186.0 ± 25.7	78.4 ± 3.7
25	-	-	-	-	-	-	198.6 ± 13.7	-
Day of first setting	20	-	-	-	-	-	20	-
Day of final setting	22	-	-	-	-	-	28	-
Total No. of spat	119	-	-	-	-	-	1038	-
Rate of spat production(%)	1.0	-	-	-	-	-	8.3	-

an algal diet is also observed in their size frequency distribution as compared to algae-fed larvae (Fig. 28). This may also be observed in their growth curves (Fig.29). None of the larvae survived up to day 25 except in standard.

Among the treatments that were provided with I. galbana supplemented with dissolved nutrients (treatments 1, 2 and 3), only treatment 1 (supplemented with amino acids) yielded growth rate that was comparable to that of standard fed with I. galbana (Table 70). Larvae that were provided with dissolved nutrients alone recorded greater growth rate values than observed for larvae that were starved but far less than observed for larvae that were fed with I. galbana.

Table 70. Larval growth rate using dissolved nutrients.

Period (days)	Larval growth per day (µm/day)							
	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5	Treatment 6	Standard	Starved Control
1-9	3.35	4.40	3.08	0.98	0.78	1.55	3.80	0.80
9-17	8.03	1.35	0.90	1.08	1.33	0.15	7.68	0.43
17-25	-	-	-	-	-	-	4.73	-
1-25	-	-	-	-	-	-	5.40	-

Linear regression analysis of growth curves yielded growth regressions of 0.0234, 0.0159 and 0.0113 log µm/day for treatments 1, 2 and 3; 0.0063, 0.0061 and 0.0057 log µm/day

SIZE FREQUENCY DISTRIBUTION OF PEARL OYSTER LARVAE REARED WITH DISSOLVED NUTRIENTS ALONE AND WHEN SUPPLEMENTED WITH I. GALBANA

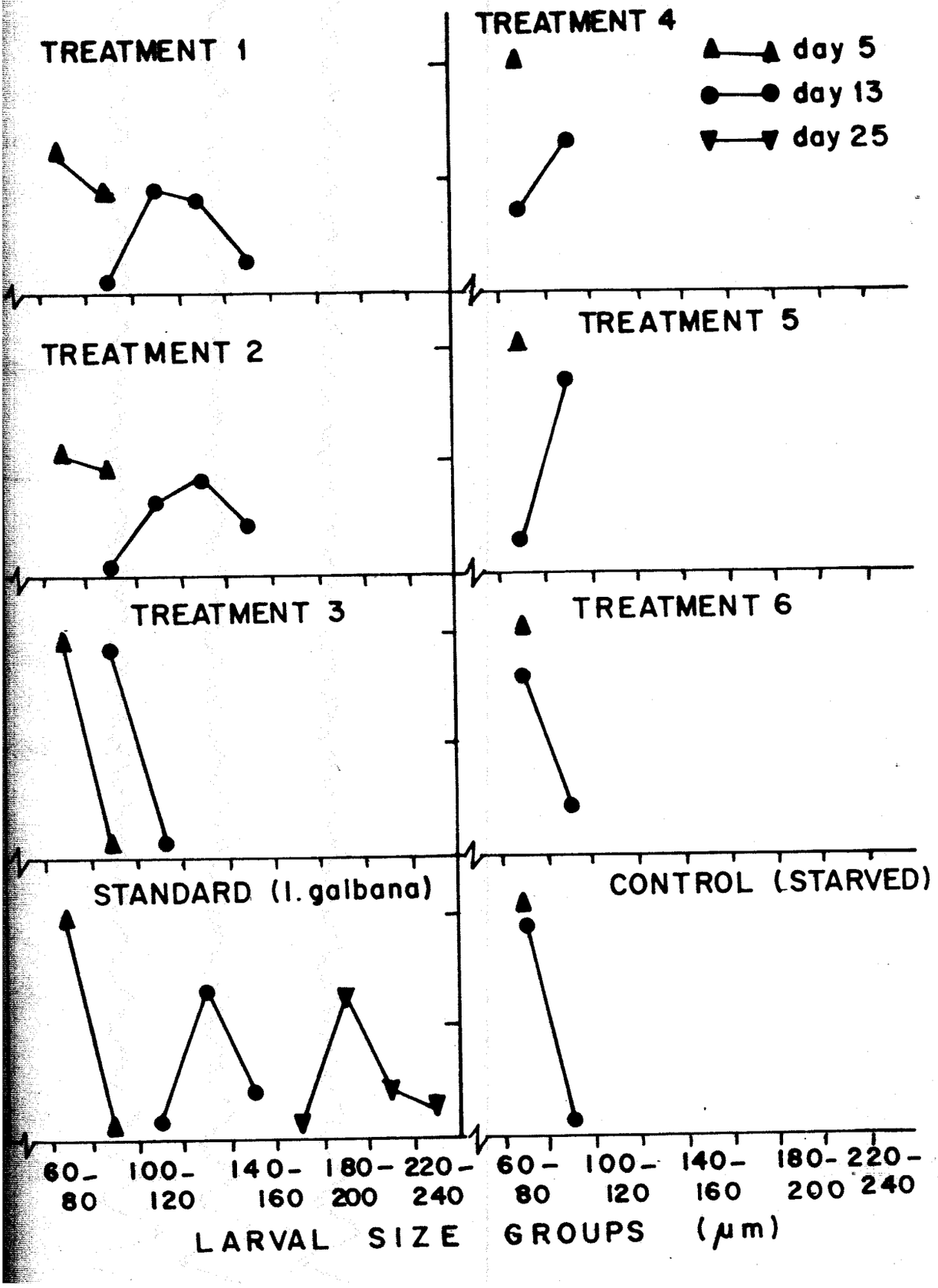
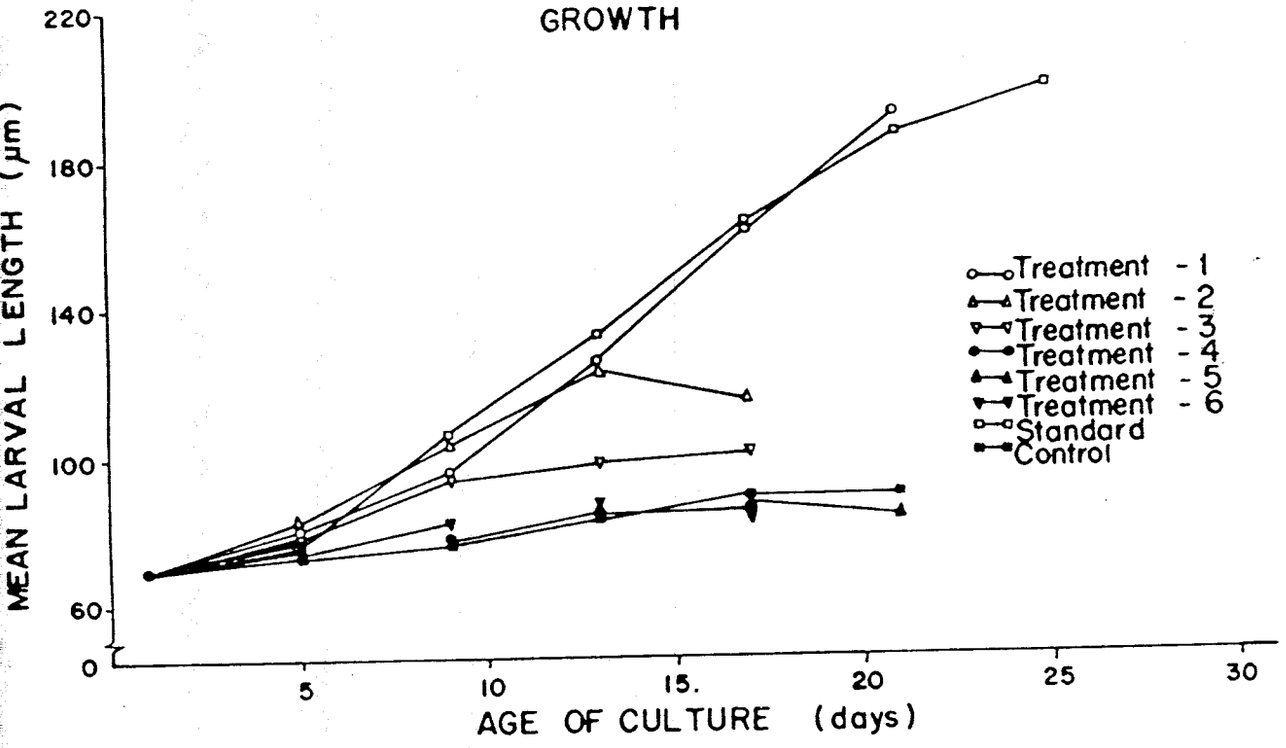
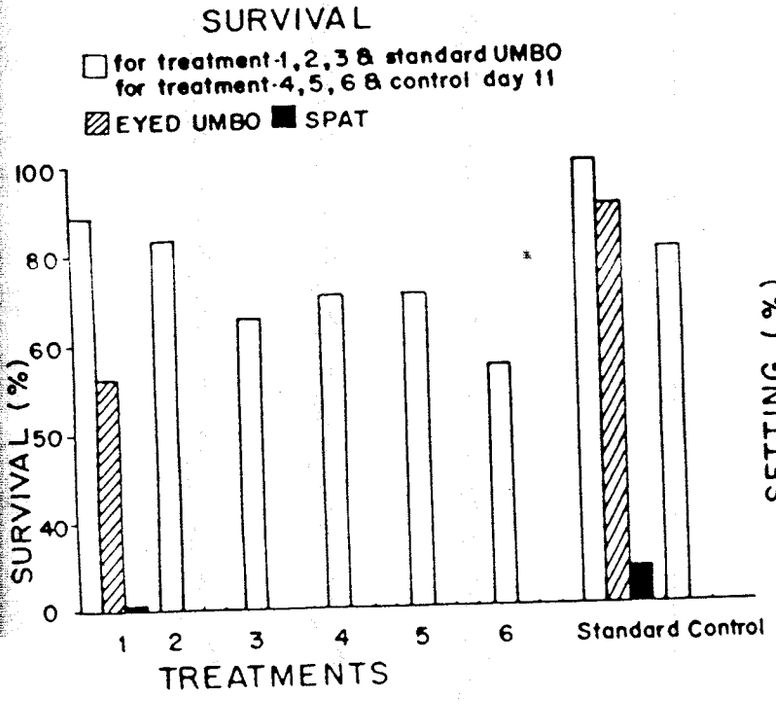
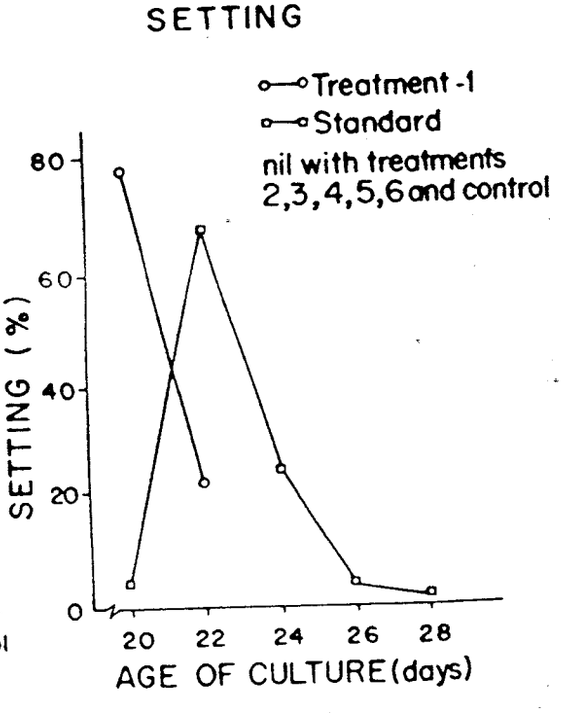


Fig. 29. GROWTH, SURVIVAL AND SETTING OF PEARL OYSTER LARVAE REARED WITH DISSOLVED NUTRIENTS ALONE AND WHEN SUPPLEMENTED WITH I. GALBANA







for treatments, 4, 5 and 6 and 0.0233 and 0.0036 log μm/day for standard and control respectively. The analysis of covariance indicated that there was statistically significant difference between growth regressions of all treatments ($P < 0.01$).

Survival rate (Fig. 29)

Survival was estimated to be 88.8, 83.3, 66.6 in treatments 1, 2 and 3 for the early umbo stage. On the same day (day 11), survival in treatments 4, 5 and 6 were 75.0, 75.0 and 54.5%, and was 100% and 80% for standard and control, respectively. Larval metamorphosis was observed only in treatment 1 and standard. Larvae survived over a period of 17 days in treatments 2 and 3 after attaining the umbo stage; 17 days in treatment 4 and 13 days in treatments 5 and 6, while remaining in the straight hinge stage. Survival was reduced to 51.8% in treatment 1 and 90.0% in standard. Survival to spat stage was 1.0% in treatment 1 and 8.3% in standard.

Spat setting and production (Table 69, Fig. 29)

Larval metamorphosis resulting in spat production was observed only in treatment 1 and in standard. Setting was initiated on day 20 in standard and treatment 1; and lasted for 8 days and 2 days in standard and treatment 1 respectively. (Fig.29). Total spat production was 1.0% in treatment 1 and

8.3% in standard. The percentage spat production of treatment 1 was significantly different from that of standard ($P < 0.01$).

Bacterial Load

Bacterial load was 202.6 ± 58.0, 170.0 ± 18.7, 300.0 ± 20.4, 176.6 ± 24.1, 208.3 ± 31.2, 243.3 ± 22.5 for treatments 1, 2, 3, 4, 5 and 6 respectively and 88.3 ± 10.5 and 24.6 ± 8.5 colonies/ml in standards 1 and 2.

Summary of results of larval rearing experiment using dissolved nutrients.

Pearl oyster larvae whose algal diet was supplemented with dissolved nutrients individually and in combination showed a growth rate that was generally less than of standard. Only larvae whose diet was supplemented with amino acids showed growth rate (growth regression 0.0234 log μm/day) that was comparable to standard (growth regression, 0.0233 log μm/day). Only larvae of treatment 1 yielded spat (1.0%). Larvae reared with dissolved nutrients alone have shown better growth than that of the fully starved larvae. Percentage of spat produced from a diet of *I. galbana* cells, however, was greater than that produced when the algal diet was supplemented with amino acids (Table 71).

Table 71. Summary of results of larval rearing experiment using dissolved nutrients.

Sl. No.	Treatment	Mean larval size on day 17 (μm)	Growth rate per day (μm/day)	Growth regression (log μm per day)	Day of first setting	Duration of setting	Spat production (%)
1.	1	160.0	-	0.0234	20	2	1.0
2.	2	115.0	-	0.0159	-	-	-
3.	3	100.8	-	0.0113	-	-	-
4.	4	85.4	-	0.0063	-	-	-
5.	5	85.8	-	0.0061	-	-	-
6.	6	82.6	-	0.0057	-	-	-
7.	Std.	160.4	5.40	0.0233	20	8	8.3
8.	Starved control	78.8	-	0.0036	-	-	-

DISCUSSION

A major constraint in the commercialisation of molluscan culture systems is the dependence of both adult and larval forms on live food (Chu et al., 1982). Over the years, several alternate forms of diets have been investigated for their nutritional value, both for bivalve adults and their larvae (Carriker, 1956; Hidu and Ukeles, 1962; Chanley and Normandin, 1967; Walne 1974; Chu et al., 1982; Teshima et al., 1982). These have ranged from organic detritus (Stickney, 1964; Zong Qing and Mei-Fang, 1980), dried and powdered macroalgae (Hidu and Ukeles, 1962), dried, spray dried, vacuum dried or freeze dried preparations of microalgae (Hidu and Ukeles, 1962; Chanley and Normandin, 1967; Walne, 1974), to artificial formulations (Chanley and Normandin, 1967; Claus and Adler, 1970; Langdon, 1983), including microparticulate diets (Chu et al., 1982; Teshima et al., 1982; Langdon, 1983).

Results of these experiments have generally shown that for juveniles and adults, growth has been poor as compared to the results with cultures of live algae (Langdon and Waldock, 1981; Langdon, 1983). For larval forms, the dried algal preparations and a few of the microparticulate diets have produced varying results (Teshima et al., 1982). Among the microparticulate diets, the nylon protein microencapsulated

diet has been found very difficult to digest, taking as much as 72 h for their complete breakdown; the gelatin acacia capsules were found more suitable for providing lipid fractions (Langdon, 1981) but were more susceptible to bacterial attack (Chu et al., 1982).

In the present study, larvae fed with freeze dried I. galbana from D shape stage showed a poor growth response. However, those fed with the freeze dried cells from the umbo and the eyed umbo stages onward, grew to metamorphosis. Growth regressions were 0.0053, 0.0180, 0.0195 and 0.0211 log μm/day for larvae fed with freeze dried algal cells from the D shape, umbo, eyed umbo stage and the standard, respectively. Spat production was 1% when the freeze dried diet was introduced during the umbo stage and 2.8% when introduced during the eyed umbo. It was 6.5% with live algal diet. It would seem that although pearl oyster larvae could utilize the freeze dried algal cells for growth, its nutritional value is more when introduced to larvae of later stages (eyed umbo onwards). However, it is a poor substitute for live algal cells of I. galbana.

Chanley and Normandin (1967) observed that larvae of M. mercenaria fed with dried foods grew less than those receiving live unicellular algae but generally more than unfed larvae. They also observed that larvae fed with dried

foods metamorphosed at larger sizes. Results that are very similar to those observed in the present study were also recorded by Hidu and Ukeles (1962). Hidu and Ukeles (1962) reared larvae of the clam M. mercenaria on three dried unicellular algae, Dunaliella euchlora, Scenedesmes obliquus and Isochrysis galbana. Larvae reared on S. obliquus grew to a mean size of 163.6 µm with the addition of antibiotic sulmet and to 132.6 µm without its addition. Larvae fed with the flagellate mixture grew to 197.2 µm as compared to 129.4 µm of starved larvae, over a period of 12 days. Walne (1974) reported the failure of O. edulis larvae to grow on spray dried Chlorella sp., Pavlova vacuum dried over mannitol or freeze dried cultures of Isochrysis galbana.

In the present study, the CBMD-fed pearl oyster larvae suffered total mortality when introduced at the D shape, umbo and the eyed umbo stages. When introduced to the D shape stage, the larvae did not grow beyond the straight hinge stage. Growth regressions were 0.0102 and 0.0113 log µm/day for larvae fed with artificial diet at the umbo and the eyed umbo stages as compared to 0.0123 log µm/day of larvae fed with the live diet I. galbana.

Teshima et al. (1982) used the carrageenan micro-binding diet and a nylon protein diet for larvae of the noble scallop Mimachlamys nobilis and recorded mean size of 875 µm when fed with a 1:1 mixture of Chlorella and CBMD and a survival of 4.8% over a period of 49 days. In

comparison, larvae of the standard fed with a 1:1 mixture of Chlorella and Chaetoceros recorded a mean size of 995 µm and a survival of 19.2% over a period of 47 days. Larvae fed with nylon protein microencapsulated diet gave a poor growth response comparable to that of feeding with Chlorella alone, and died in 23 days. It must be noted, however, that Teshima et al. (1982) used combinations of artificial diet and live algal cells and the observed good growth of the larvae might have been due to the included live algal diet. Chu et al. (1982), studying the acceptability and digestibility of microcapsules in C. virginica larvae, reported that larvae fed with microcapsules grew as rapidly as those fed with the algal Pseudoisochrysis paradoxa until about day 11.

Langdon (1981) evaluated a biphasic diet consisting of both dissolved nutrients and particulate matter for larvae of the oyster Crassostrea gigas. Most of the larvae fed with artificial diet reached the umbo stage within 6 days, but little further growth occurred. He commented on the inability of larvae to meet their requirements for vitamins by absorption from the culture medium. In separate experiments individual evaluation of the dissolved and particulate phases of the diet showed that C. gigas larvae failed to utilise the particulate matter (Langdon, 1983). The increased mean larval size observed for C. gigas reared on the biphasic diet

M. mercenaria larvae (Chanley and Normandin, 1967). Gustafson (1980) reported a significant improvement in larval survival over fully starved larvae when reared in low concentrations (5-8 μM) of dissolved free amino acids (DFAA). He also reported that larvae exposed to DFAA maintained higher protein and lipid resource than starved larvae. Zong-Qing and Mei-Fang (1980) successfully reared larvae of *M. edulis* up to metamorphosis on soyabean milk.

Several authors have demonstrated the uptake of dissolved monosaccharides from sea water (Johanes *et al.*, 1969; Efford and Tsumera, 1973; Schulte *et al.*, 1973; Schulte and Lawrence, 1977, 1978a, b; Fankboner and Burgh, 1978). Autoradiographic studies firmly established that bivalve larvae are capable of absorbing dissolved free amino acids (DFAA) from the water (Manahan and Crisp, 1982, 1983). Those authors suggested that this ability to absorb DFAA would give the larvae an enhanced chance of surviving in nature. In the present study, both larval growth and setting were inhibited by the presence of dissolved nutrients.

Chanley and Normandin (1967) observed that the addition of any food to larval cultures may enrich the water and result in substantial increase in the bacterial populations. In the present study, levels of bacteria increased in the larval

rearing medium whenever a non-living diet or dissolved nutrients were used despite the addition of antibiotic streptomycin sulphate. The role of bacteria in bivalve larval nutrition is highly speculative. Most investigators have found that bacteria have a detrimental rather than a beneficial effect on larvae (Davis, 1958; Guillard, 1958; 1959; Loosanoff and Davis 1963 b; Walne, 1964; Tubiash et al., 1965). In a few instances, bacteria have been implicated as a source of larval nutrition, (Carriker, 1956; Hidu and Tubiash, 1963; Prieur, 1983). Martin (1979) concluded that few bacterial species have noxious effects, others are inoffensive and a few can enhance growth. He speculated that larval mortality and growth can be related to the equilibrium which may exist between the different bacterial strains.

Murchelano et al. (1975) found no significant difference in the bacterial load of the larval rearing medium treated with the antibiotic sulphamethazine at 60 mg/l from that of normal seawater. UV irradiation of seawater, however, reduced the achromobacters, flavobacteria and the vibrios. The reduction in vibrios is particularly relevant as they cause extensive mortalities in cultures of oyster larvae.

In the present study, UV irradiated water was used continuously. In addition, the antibiotic streptomycin

sulphate was added to the larval rearing medium at 8 mg/l as recommended for hard clam larvae (Castagna and Krauter, 1981). Counts showed an increased bacterial population in the larval rearing medium. In the experiment using freeze dried I. galbana cells, the increase in spat setting observed in relation to the delay in introducing the freeze dried algal cells suggest that continuous exposure to microbes may have a debilitating effect on pearl oyster larval growth and survival.

The present study suggests that freeze dried algal cells may be a potential source of nutrition for pearl oyster larvae. Research attention paid to this aspect has been fractional as compared to the experimental and applied work done on live food organisms at different laboratories. With directed research in future, the freeze dried algal foods may play an important role in meeting the nutritional requirements of larval rearing in shellfish hatcheries.

CHAPTER 6

BIOCHEMICAL COMPOSITION OF MICROALGAE AND PEARL OYSTER LARVAE

INTRODUCTION

Investigations on bivalve larval nutrition have shown that there is considerable variation in the nutritional value of different algal species (Loosanoff and Davis, 1963b; Ukeles, 1975; Ryther and Goldman, 1975; Sastry, 1979; Kinne, 1977; Bayne, 1983). In an attempt to understand these differences, several authors have studied the biochemical composition of different algae (Spoehr et al., 1949; Parsons et al., 1961; Saddler and Taub, 1972; Walne, 1974; Chu and Dupuy, 1980; Langdon and Waldock, 1981; Wikfors et al., 1984). Webb and Chu (1983) have reviewed and discussed the amino acid, fatty acid and carbohydrate composition of algal species used as food for oyster larvae.

Apart from algal biochemical studies, few authors have also attempted to understand the biochemical changes during larval development (Millar and Scott, 1967; Holland and Spencer, 1973; Helm et al., 1973; Holland et al., 1975; Bartlett, 1979; Waldock and Nascimento, 1979).

MATERIALS AND METHODS

Biochemical composition of algae

The seven species of microalgae used as diets for pearl oyster larvae, namely, *Isochrysis galbana*, *Pavlova lutheri*, *Chromulina freiburgensis*, *Synechocystis salina*, *Tetraselmis gracilis*, *T. chui* and *Chlorella salina* were analysed for their biochemical composition. They were examined for moisture, total polysaccharides, total protein and total lipid.

Algae for the analysis was collected during the exponential phase of growth between days 4 and 6. Ten litres of algal culture were centrifuged and the packed cells washed with 0.9% ammonium formate, isotonic with seawater, to remove traces of salt, prior to analysis.

Moisture

The packed cells were weighed on a microbalance (0.001 g accuracy) immediately after centrifugation to obtain wet weight. The cells were dried to constant weight at 60°C for 10 to 12 h in a hot air oven and weighed (dry weight) after cooling in a dessicator over silica gel. The percentage of moisture content was calculated from these data.

Analysis of protein, polysaccharides and total lipid was carried out on wet samples.

Total lipid

The gravimetric method of Bligh and Dyer (1959) was used to estimate total lipid. Wet samples of algal cells were homogenized in 10 ml of 2:1 (V/V) chloroform: methanol mixture (analar grade). Extraction was done repeatedly with small volumes of the chloroform-methanol mixture. The lipid extract and washings were transferred to a separating flask and shaken with distilled water. Total lipid was isolated and its weight determined on a microbalance (0.001 g accuracy) after evaporating the solvent and drying the residue over silica gel.

Polysaccharides

Fat-extracted algal samples were used for analysis of polysaccharides. After fat extraction with chloroform-methanol mixture, the precipitate was air-dried and the carbohydrate extracted in 10% trichloroacetic acid (TCA). Aliquots of the supernatant were treated by the method of Dubois *et al.* (1956) using glucose as the standard. Optical density readings were taken at 490 nm on a spectrophotometer.

Total protein

Total protein was estimated by Lowry's method (Lowry *et al.*, 1951). Fat-extracted algal samples were dissolved in 1 N sodium hydroxide and the amount of protein determined using Folin-Ciocalteu reagent. Readings were calibrated on

a spectrophotometer against the standard bovine serum albumen at the wavelength of 540 nm.

The level of lipid, carbohydrate and protein in the microalgae was calculated as percent dry weight.

Biochemical composition of larvae

As large numbers of larvae were necessary for getting sufficient weight for analysis, pearl oyster larvae were reared in 200-l fibreglass tanks holding 100 l seawater. *I. galbana* was used as larval food. Samples for biochemical analysis were removed at five stages, namely, the early D shape, the late D shape, the early umbo, the eyed umbo and the plantigrade stages, by using appropriate nylobolt screens. All larval samples were washed with 0.9% ammonium formate, isotonic with seawater, to remove traces of salt.

The excess moisture that was present in the larval sample collected in the nylobolt screen was blotted out on filter paper, transferred to an aluminium foil and dried in the oven at 60°C for 12 h. The dried larval material was then stored in glass vials in a dessicator until further analysis. Larval samples with shell on were analysed for ash, protein, total lipid, phospholipid and total carbohydrate.

Ash

Inorganic matter was estimated gravimetrically after

ashing 20 mg larval sample in a muffle furnace at 700°C for 14 h. All weighings were done on an electric balance (0.01 mg accuracy).

For further analysis of protein, total lipid, phospholipid and carbohydrate, 20 mg of pulverised, dried larval material was weighed accurately on an electrobalance (0.01 mg accuracy) and homogenized in 5 ml of distilled water in a small ground tissue homogenizer. The methods recommended by Holland and Gabbott (1971) for marine invertebrate larvae were then used.

Total lipid

Lipid was extracted using a 1:2 (V/V) chloroform: methanol mixture. After standing at 4°C for 10 minutes, the lipid dissolved in chloroform was extracted by centrifugation at 10,000 rpm. Aliquots of the lipid extract were quantitatively estimated by the method of Marsh and Weinstein (1966) using tripalmitin as standard. After drying aliquots of lipid sample at 37°C, sulphuric acid was added and heated for 15 min at 200°C. On cooling, distilled water was added and the absorbance read at 375nm on a spectrophotometer.

Phospholipid

Lipid phosphorus was estimated as inorganic phosphate after acid digestion using potassium-di-hydrogen phosphate as standard. After drying aliquots of chloroform extracted lipid at 37°C for 15 min, samples were digested with

phosphorus digestion mixture (50:50 20 N H_2SO_4 and 8 N perchloric acid) successively at 120°C for 1 h, 200°C for 15 min and 320°C for 2 h 30 min. On cooling, ammonium molybdate, aminonaphthosulphonic acid reagent and distilled water were added successively. After heating the reaction mixture and cooling, absorbance was read at 850 nm on a spectrophotometer. The values of inorganic phosphorus were raised by a factor of 25 to give values of phospholipid (Holland and Gabbott, 1971).

Neutral lipid

The values of neutral lipid were calculated by subtracting mean values of phospholipid from total lipid.

Total carbohydrate

From the larval homogenate, total carbohydrate was dissolved in cold 15% TCA. A modification of the method of Folin and Malmros (1929) specified by Holland and Gabbott (1971) was used with glucose as a standard. Absorbance was read at 420 nm on a spectrophotometer.

Total protein

The protein precipitated from the 15% TCA for analysis of carbohydrate was dissolved in warm sodium hydroxide by heating for 30 min at 56°C. Samples were digested in sulphuric acid and the resulting ammonium nitrogen determined spectrophotometrically by the phenol

hypochlorite method (Solorzano, 1969). Ammonium sulphate was used as standard. The absorbance was determined at 635 nm. The protein nitrogen values thus obtained were multiplied by 6.25.

Lipid staining of larvae

A technique of lipid staining of bivalve larvae developed by Gallager and Mann (1981 a, b) was used in pearl oyster larvae. At particular stages (D shape, umbo and eyed umbo stages) of larval development, duplicate larval subsamples were removed. While one subsample was narcotized, fixed and stained specifically for lipid, the other subsample was placed in filtered seawater with no food for a period of three days and thereafter narcotized, fixed and stained. Magnesium chloride, formalin and Sudan black were used respectively for narcotizing, fixing and staining.

RESULTS

Biochemical composition of the microalgae

Moisture content was maximum for *S. salina* (95.1%). It was 90.5% for *C. salina*, 84.7% for *I. galbana*, 86.5% for *P. lutheri*, 90.0% for *C. freiburgensis*, 83.1% for *T. gracilis* and 84.1% for *T. chui* (Table 72).

Table 12. Proximate composition of algal species evaluated for their food value for pearl oyster larvae.

S.No.	Algal species	Moisture	% dry weight		
			Protein	Carbohydrate	Lipid
1.	*Isochrysis galbana*	84.7 ± 0.2	40.6 ± 3.4	21.2 ± 1.1	15.3 ± 2.9
2.	*Pavlova lutheri*	86.5 ± 2.4	37.3 ± 3.2	28.8 ± 1.2	13.8 ± 2.7
3.	*Chromulina freiburgensis*	90.0 ± 0.1	50.2 ± 3.5	19.5 ± 1.1	11.9 ± 2.1
4.	*Synechocystis salina*	95.1 ± 1.9	43.6 ± 4.8	22.4 ± 2.2	13.6 ± 3.1
5.	*Tetraselmis gracilis*	83.1 ± 2.7	50.5 ± 3.0	19.4 ± 2.3	7.3 ± 1.7
6.	*Tetraselmis chui*	84.1 ± 4.6	49.9 ± 3.5	15.6 ± 1.6	9.9 ± 2.4
7.	*Chlorella salina*	90.5 ± 1.9	53.8 ± 3.6	19.9 ± 1.9	8.5 ± 3.3

The protein content expressed as percentage dry weight was 40.6%, 37.3%, 50.2%, 43.6%, 50.5%, 49.9% and 53.8% for the algae I. galbana, P. lutheri, C. freiburgensis, S. salina, T. gracilis, T. chui and C. salina respectively.

Carbohydrate content was 21.2%, 28.8%, 19.5%, 22.4%, 19.4%, 15.6% and 19.9%, respectively for I. galbana, P. lutheri, C. freiburgensis, S. salina, T. gracilis, T. chui, and C. salina.

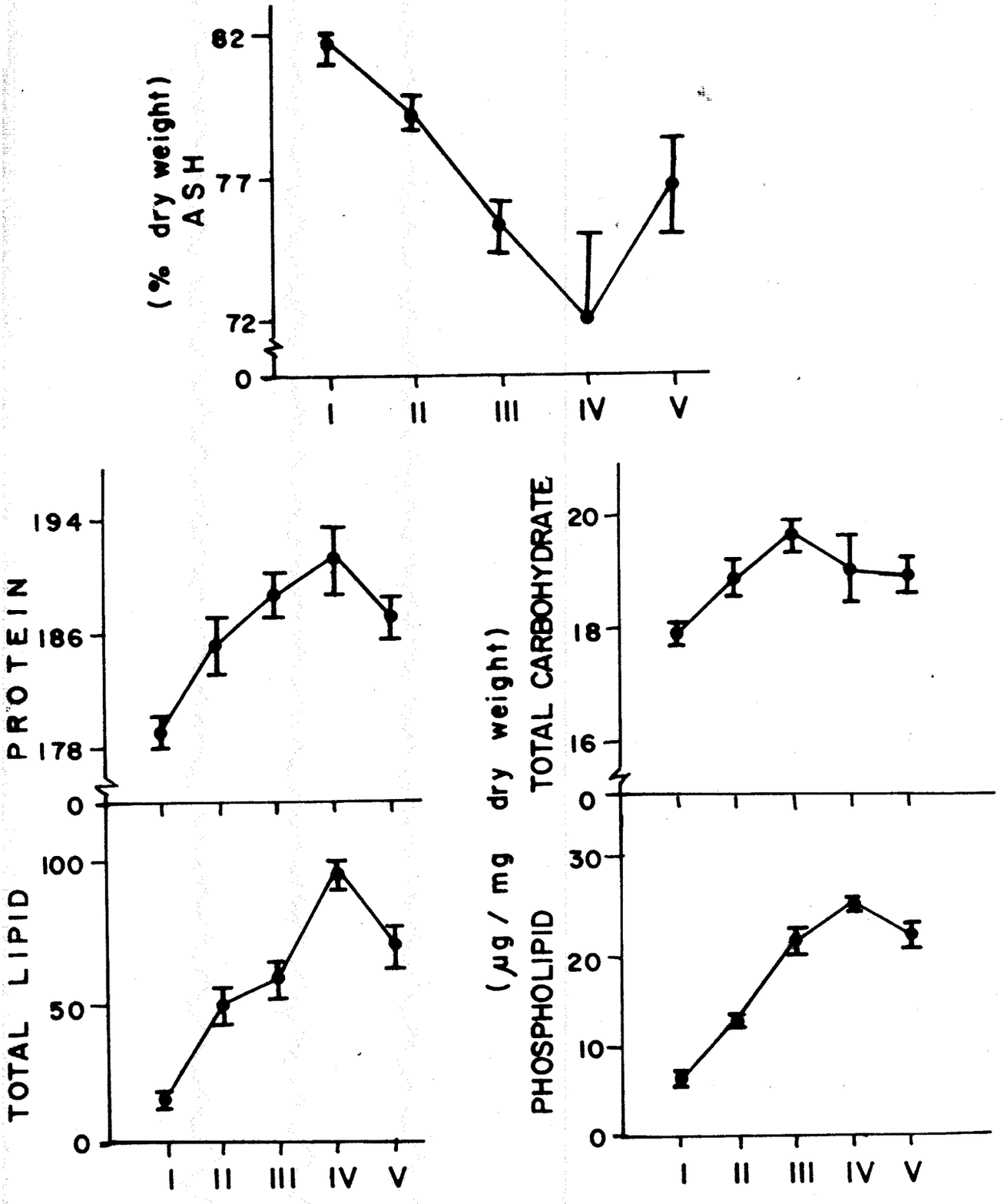
Lipid levels ranged from 7.3% for T. gracilis to 15.3% for I. galbana. For P. lutheri, C. freiburgensis, S. salina, T. chui and C. salina, lipid levels were 13.8%, 11.9%, 13.6%, 9.9% and 8.5% respectively.

Biochemical changes in larvae

Percentage ash was seen to decrease progressively from the early D shape larvae to the eyed umbo stage, being 81.7% in the early D shape larvae, 79.3% for the late D shape larvae, 75.3% for the umbo, and 71.9% for the eyed umbo larvae. Plantigrade larvae showed an increased ash content up to 76.7% of the total dry weight (Fig. 30).

Total protein was seen to increase steadily from the D shape to the eyed umbo stage and to decline thereafter in the plantigrade stage. Protein levels were 179.3, 185.4, 188.8, 191.3 and 186.9 µg/mg for the early D shape,

g. 30. BIOCHEMICAL CHANGES DURING PEARL OYSTER LARVAL DEVELOPMENT



I-D-SHAPE ; II-LATE D-SHAPE ; III-UMBO ; IV - EYED UMBO ;
V - PLANTIGRADE

late D shape, early umbo, eyed umbo and the plantigrade stages (Fig. 30).

Carbohydrate levels were, respectively, 17.8, 18.9, 19.6, 19.0 and 18.9 µg/mg for the same stages.

Total lipid levels showed a significant increase from 17.5 µg/mg for early D shape, 49.8 µg/mg for late D shape larvae, 58.9 µg/mg for umbo larvae, 94.8 µg/mg for the eyed umbo larvae and thereafter decreased to 69.8 µg/mg for plantigrade larvae. A large part of the increase in total lipid levels came from increase in neutral lipid levels. This was seen to increase from 9.6 µg/mg for early D shape larvae to 33.9 µg/mg for late D shape larvae, to 35.3 µg/mg for early umbo larvae and 69.6 µg/mg for eyed umbo larvae. Neutral lipid declined to 45.6 µg/mg for the plantigrade larvae.

Lipid staining of larvae

It was seen from the intensity of the stain taken up by the larvae that those which were starved for a period of 3 days showed a lesser amount of lipid. This was more clearly demarcated for the umbo and eyed umbo stages than for the D shape larvae indicating that lipid is stored with increasing larval growth (Pl. V).

DISCUSSION

Apart from the biological approach, there has been very few quantitative and qualitative analysis of the major chemical components (protein, lipid and carbohydrate) of algal species used as larval oyster foods (Chu and Dupuy, 1980). Likewise, in comparison with the morphological and behavioural studies, very little work has been done on the biochemistry of molluscan larvae (Gabbott, 1983). Knowledge concerning the nutritional requirements of bivalve larvae is, therefore, still limited.

In the present study, where the proximate composition of pearl oyster, P. fucata larvae was studied at five stages of larval development. It was seen that ash forms the major component of the total dry weight at all stages (71.9% to 81.7%). This is evidently due to the inclusion of the shell. Total organic matter was seen to increase from 18.3% for the early D shape larvae to 28.1% of the total dry weight for the eyed umbo stage, corresponding to a decrease in ash content for the same stages. Similar increase in organic matter has also been reported for Ostrea edulis larvae (Holland and Spencer, 1973) and for Mytilus edulis larvae (Sprung, 1984a). An increase in total organic matter from 17% to 26.5% of the total dry weight was observed for O. edulis larvae (Holland and Spencer, 1973). Sprung (1984a) recorded an increase in organic matter from

37.3% at 120 µm to 48.2% at 298 µm. Millar and Scott (1967) had earlier shown that total organic matter varied from 14.5 to 28.6% of the total dry weight in different batches of O. edulis larvae. For the same species, Helm et al. (1973) recorded values ranging from 17% to 24%. Bayne (1976) reported 15.9% organic matter for the veliger of M. edulis.

The values of total organic matter observed in the present study are in general agreement with those observed for the other species O. edulis and M. edulis. Much of this increase in total organic matter, has been largely in the form of total lipid and protein and to a lesser extent in carbohydrate (Fig. 30). Total lipid was seen to increase from 1.75% at the D shape to 9.48% at the eyed umbo stage. A major increase in total lipid was seen to be due to increase in neutral lipid levels from 9.6 µg/mg to 68.6 µg/mg for the eyed umbo stage. A similar increase in total lipid from 17.3% to 29.9% of the total organic matter with neutral lipid increasing from 8.8% to 23.2% for the same period was reported by Holland and Spencer (1973). Bartlett (1979), however, reported a decrease in neutral lipid from 21% to 10% and an increase from 6 to 10% in phospho-lipid of the total organic matter for the oyster Crassostrea gigas.

Results of the present study also indicate increasing levels of protein. Protein increased from 179.3 µg/mg at the D shape stage to 191.3 µg/mg at the eyed umbo stage.

Bartlett (1979) observed an increase in protein from 65% to 85% of the total organic matter for C. gigas larvae prior to metamorphosis. On the other hand, Holland and Spencer (1973) reported a decrease in protein levels from 68.7% to 59.5% from one-day to 12-day old larvae of O. edulis. In the present study, carbohydrate level was also seen to increase to a lesser extent from 17.8 µg/mg at the D shape stage to 19.0 µg/mg at the eyed umbo stage.

Relating the changes in larval biochemistry to the anatomical changes during pearl oyster larval development as described by Alagarswami et al. (1983 b, c), the results would suggest that a large part of the increase in protein, lipid and carbohydrate is utilized in building up tissues. At the eyed umbo stage, larvae are seen to have a foot primordium and a ctenidial ridge. Metamorphosis marks a crucial phase in the development of the larvae. Correlated with the transition from the eyed umbo (free-swimming larvae) to the plantigrade (creeping stage) stage, several changes occur. During this stage, the velum is resorbed, and organs such as the foot, gill filaments are elaborated and other organs such as labial palps appear (Alagarswami et al., 1983 b,c). Bartlett (1979) suggests that associated with this transition, there is a cessation of feeding, while a new mode of feeding using the labial palps and the gills is being established. A large part of the stored energy is diverted to maintenance and tissue build up during this change. Gabbott (1983) observed that the main loss of energy reserves occurred during

metamorphosis. Chu and Webb (1985) suggested that much of this energy was derived from neutral lipid.

Larval biochemistry of the pearl oyster reveals that associated with this change, there has been a decrease in the levels of the total lipid (composed to a great extent of neutral lipid), protein and to a lesser extent in carbohydrate. Levels of lipid, protein and carbohydrate decreased to 69.8, 186.9 and 18.9 µg/mg of the total organic matter. Among the lipid, the decrease in neutral lipid has been greater (from 68.6 to 45.6 µg/mg). The increase in ash content observed in the plantigrade larvae may have been caused relatively due to the decrease in the total organic matter or due to formation of new shell for the dissoconch shell (Alagarswami et al., 1983c).

The present study shows a significant loss in the neutral lipid and to a lesser extent in the protein levels in the plantigrade stage. Holland (1978), while reviewing the role of lipids in the energy metabolism of marine invertebrates, pointed out that both lipids and protein form the main energy reserves of marine bivalve larvae. Several other authors, however, stressing the importance of lipid have shown that neutral lipid in O. edulis (Millar and Scott, 1967) phospholipid and neutral lipid in M. edulis (Bayne et al., 1975) and phospholipid in O. edulis larvae(Holland and Spencer, 1973) are the main sources of energy.

Bayne (1976) pointed out that a striking feature of the gross biochemistry of marine bivalve larvae was the high proportion of lipid and a relatively low proportion of carbohydrate. This is in contrast to the majority of adult bivalves, where both lipid and carbohydrate form energy reserves (Watanebe and Ackman, 1974; Holland et al. 1975; Riley, 1976; Desai and Hirani, 1979). Holland et al.(1975), relating lipid levels in species of periwinkles to different reproductive strategies, suggested that neutral lipid is the major energy reserve for larvae of Littorina littorea and L. neritoides having a free swimming pelagic phase, while larvae of L. saxatilis and L. littoralis that are released at the crawling stage have a body chemistry closely parallel to the adult littorinids.

Starvation experiments in O. edulis larvae by Millar and Scott (1967) and Holland and Spencer (1973), and in M. edulis by Bayne et al. (1975) have shown that the large lipid stores in lamellibranch larvae are used as an energy reserve during planktonic life. Holland and Spencer (1973) showed that neutral lipid accounted for 41% of the total organic matter lost during starvation, whereas protein accounted for 34% and carbohydrate 25%. Bayne et al. (1975) recorded losses of both lipid and protein, during starvation. Gallager and Mann (1981 a,b), using a simple lipid specific staining technique, observed a decrease in larval bivalve condition index on starvation of C. virginica, O. edulis and Teredo navalis. Similar results were also observed in pearl

oyster larvae when starved (Pl. V). Crisp (1976) pointed out that lipid provides nearly twice as much energy when catabolised than does protein or carbohydrate and also confers buoyancy on the larvae. Viability, vigour, set and successful metamorphosis are also known to be related to egg or larval lipid content (Helm et al., 1973; Creekman, 1977). Gallager (personal communication) has suggested that a "critical threshold" level of lipid is necessary in the egg for successful larval growth.

Carbohydrate reserves seem to be unimportant in the metabolism of pearl oyster larvae as in other cases. Collyer (1957) had concluded that there was no correlation between larval viability and their glycogen content in O. edulis.

Investigations have shown that algal biochemistry is highly influenced by its environmental conditions, being affected by several factors like temperature, light intensity, photoperiod and levels of nutrients in the medium (Antia, et al., 1963; Holm-Hansen et al., 1968; Hobson and Pariser, 1971; Myklestad and Hang, 1972; Saddler and Taub, 1972; Terry et al., 1983; Redalje and Laws, 1983; Wikfors et al., 1984). In separate investigations, differences in nutritional values of different algal species have been attributed to provision of essential micronutrients (Pechenik and Fisher, 1979), absence of the amino acid tryptophan (Epifanio et al., 1981), higher concentrations of total protein (Webb and Chu, 1983) and to triacylglycerol content of the

algae Dicrateria inornata and Isochrysis galbana was species oriented rather than dependent on the diet.

Results of the study on biochemical changes during larval development indicate that in pearl oyster larvae there is a gradual building up of organic material in the form of protein, lipid and to a lesser extent of carbohydrate up to the premetamorphic stage. Part of this build up is channelised into tissue formation while another part is stored. At the transition from the free-swimming stage to the creeping stage, the stored energy is utilised for body maintenance and in the formation of new tissues. During this phase there is a large contribution of lipid in the form of neutral lipid and to a lesser extent of protein and carbohydrate. This is evidenced by the decrease in these levels at the plantigrade stage.

Analysis of the biochemical components of algal species used in the present study, showed no major differences between species. Lipid content of the three algae that were individually identified as good, I. galbana, P. lutheri, and C. freiburgensis has been slightly higher than that of S. salina, T. gracilis, T. chui and C. salina. It is suggested that the nutritional value of a diet may be related to lipid and amino acid profile than to gross composition.

CHAPTER 7

EFFECT OF ENVIRONMENTAL PARAMETERS ON PEARL OYSTER LARVAL GROWTH AND SETTING

INTRODUCTION

Besides diet, the rearing environment determines the success or failure of larval culture. Hancock (1973) and Bayne (1976) noted in particular the role of environmental conditions such as temperature and salinity in bivalve larval setting under natural conditions. Several investigations have been carried out to elucidate the role of these parameters in larval rearing (Walne, 1956a; Davis and Ansell, 1962; Davis and Calabrese, 1964; Hrs Brenko and Calabrese, 1969; Bourne and Smith, 1972; Lough and Gonor, 1971; Kingston, 1974; Helm and Millican, 1977; Siddall, 1978a, b). Generally, these studies have either been carried out as monofactorial experiments examining the effects of only one parameter at a time (Walne, 1956a; Bayne, 1965; Coeroli et al., 1984; Sprung, 1984a) or as multivariate experiments studying the effects of more than one factor simultaneously (Davis and Calabrese, 1964; Helm and Millican, 1977). Results of these experiments have been used to identify ideal rearing conditions.

The present investigation was carried out in monofactorial form and was aimed at studying the effects of salinity, temperature and pH individually on pearl oyster larval growth and setting.

MATERIALS AND METHODS

The effects of temperature, salinity and pH on pearl oyster larval growth and setting were studied individually and with different broods of larvae. Pearl oyster larvae were stocked at the uniform larval density of 5/ml and fed with Isochrysis galbana at the concentration of 25 cells/µl. The general larval rearing conditions have already been explained in Chapter 2.

1. Temperature

During the experimental period January-February, 1984, the range in ambient temperature, salinity and pH levels were 23.9-25.1°C, 33.5-35.0‰ and 8.1-8.2, respectively. Larvae were reared at four temperatures of 32°, 28° and 20°C and at the ambient water temperature. Rearing temperatures were obtained by immersing the beakers in waterbaths of the desired temperature. Temperatures higher than the ambient (32°C and 28°C) were obtained in the waterbath using a Joum heating apparatus having a silicon coated heating rod(Pl.V).

By aerating the waterbath, a uniform temperature throughout the bath was ensured. Larvae reared at the temperature of 20°C were maintained in the BOD incubator.

Salinity

Two separate experiments were conducted to study the effects of salinity on pearl oyster larval rearing. The first experiment was carried out during August-September, 1983, when range in ambient temperature, salinity and pH was 29.2-30.2°C, 37.0-38.1‰ and 8.15-8.30. Larvae were reared at the salinity levels of 30, 32, 34, 36‰ and at a mean ambient salinity of 37.7‰ (37.0-38.1‰).

The second experiment was carried out during November-December, 1983, when range in ambient temperature, salinity and pH were 25.1-27.5°C, 32.6-35.2‰ and 8.20-8.25. Larvae were reared at the salinity levels of 26, 28‰ and at a mean ambient salinity of 34.1‰ (32.6-35.2‰). For both experiments, seawater of the desired salinity was prepared prior to water change. UV sterilised seawater was taken in a vessel and diluted with distilled water until the desired salinity was obtained. The salinity was monitored using a salinometer and then estimated argentometrically (Strickland and Parsons, 1976). Larvae were acclimated to the lower salinities gradually by a step decrease of 2‰ per day.

pH

The experiment was conducted during March-April, 1984, when ambient temperature, salinity and pH were 24.7-26.3°C, 30.5-34.5‰ and 8.10-8.15. Larvae were maintained at four pH levels of 7.5, 8.5, 9.0 and mean ambient pH 8.12 (8.10-8.15). For raising the pH level of the medium to above that of the ambient, Tris (hydroxymethyl methylamine) buffer of pH 7-9 was used; while for bringing pH of the medium to below that of the ambient, solution of citric acid was used. Seawater of the desired pH level was prepared just prior to water change. UV sterilised seawater was taken in a vessel and solution of either Tris buffer or citric acid was added and mixed thoroughly until the pH meter reading was at the desired level. This seawater was used for larval rearing. Later, samples were again monitored. Range in pH values in the rearing vessels was 7.28-7.60 (pH 7.5), 8.37-8.51 (pH 8.5) and 8.84-9.22 (pH 9.0).

RESULTS

A. Effect of different temperatures on growth and survival of pearl oyster larvae

Larval growth (Tables, 73 and 74, Fig. 31 and 32)

A distinct trend in growth rate was observed with the maximum at the highest temperature of 32°C, declining steadily

Table 73. Growth and setting of pearl oyster larvae reared at different temperatures.

Age of culture (days)	Mean size of larvae (μm)			
	32°C	28°C	AMBIENT	20°C
1	68.3 ± 2.4	68.3 ± 2.4	68.3 ± 2.4	68.3 ± 2.4
5	92.5 ± 7.2	87.5 ± 4.7	81.1 ± 3.5	77.6 ± 5.3
9	103.5 ± 6.0	94.5 ± 8.1	92.9 ± 5.2	79.6 ± 4.6
13	128.5 ± 19.3	109.9 ± 18.3	97.6 ± 5.8	85.0 ± 4.7
17	157.1 ± 15.0	148.4 ± 14.6	106.5 ± 5.5	83.5 ± 4.7
21	178.3 ± 15.7	165.0 ± 22.7	116.7 ± 10.7	84.0 ± 4.2
25	177.3 ± 24.2	168.1 ± 30.1	125.2 ± 18.1	85.0 ± 4.4
29	196.5 ± 16.0	187.5 ± 23.7	142.2 ± 20.2	-
33	195.0 ± 15.8	176.7 ± 23.2	159.9 ± 25.0	-
37	192.0 ± 12.9	194.4 ± 14.7	166.4 ± 16.5	-
41	184.5 ± 15.9	185.3 ± 19.4	193.0 ± 17.1	-
45	201.9 ± 17.6	184.0 ± 16.2	186.4 ± 12.3	-
49	-	180.0 ± 6.3	178.6 ± 6.4	-
Day of first setting	22	22	28	-
Day of final setting	46	55	52	-
Total No. of spat	5889	4441	1100	-
Rate of spat production (%)	47.1	35.5	8.8	-

Fig. 31. SIZE FREQUENCY DISTRIBUTION OF PEARL OYSTER LARVAE REARED AT DIFFERENT TEMPERATURES

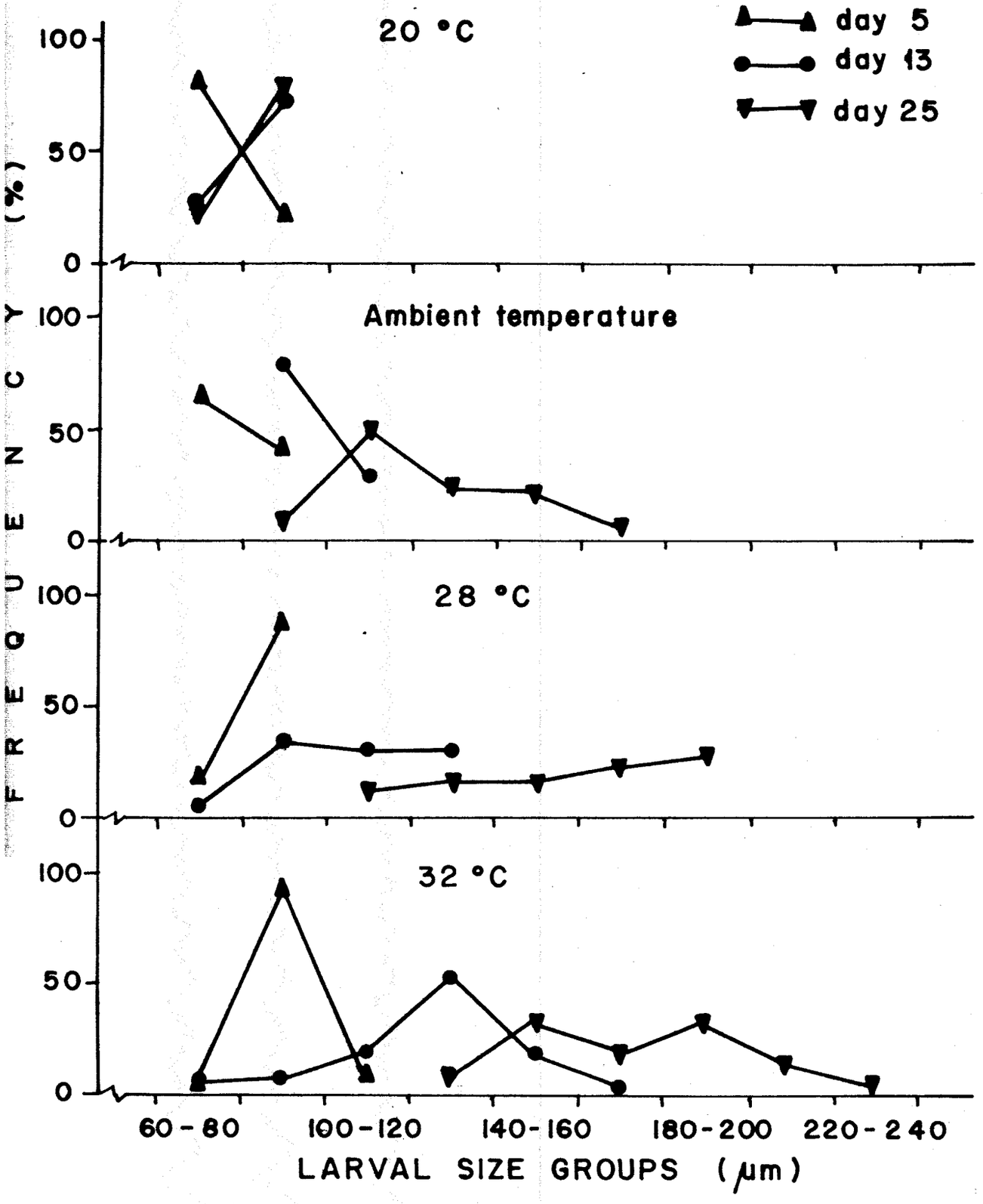
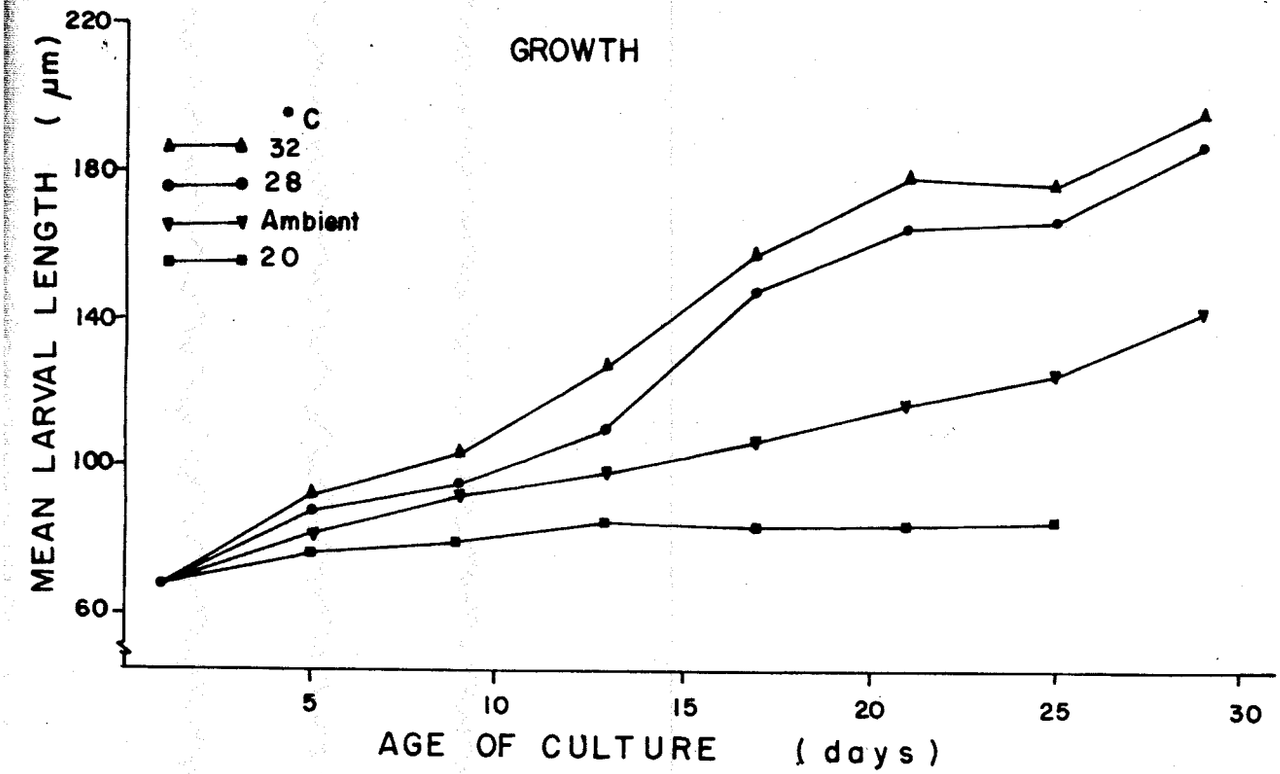
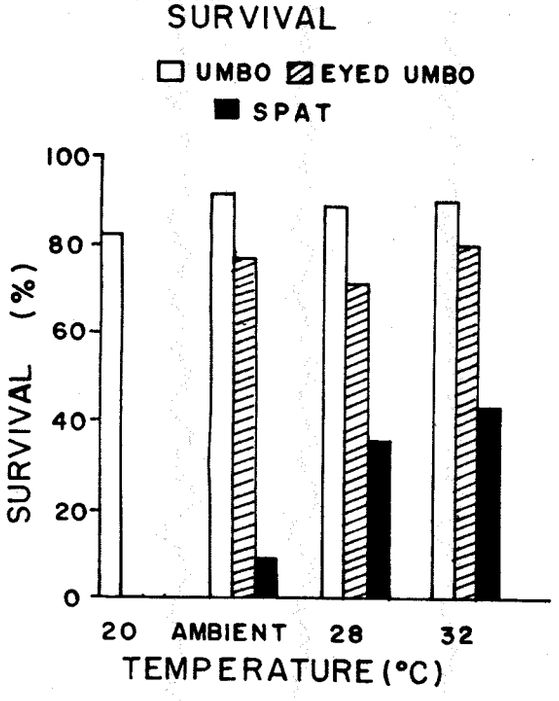
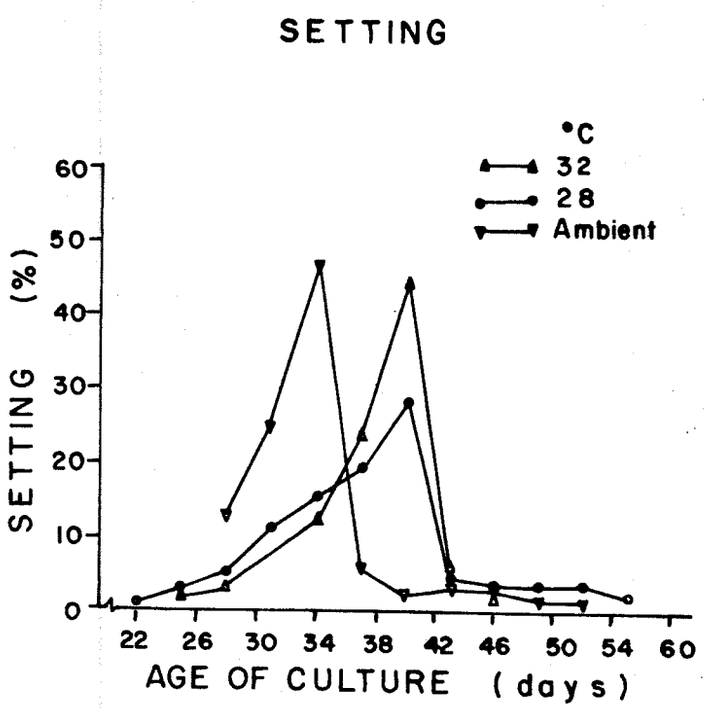


Fig. 32. GROWTH, SURVIVAL AND SETTING OF PEARL OYSTER LARVAE CULTURED AT DIFFERENT TEMPERATURES







to be the lowest at the least temperature tested, 20°C (Table 73, Fig. 31). This trend in growth is consistently maintained from day 5 up to the time of setting (day 22) and even thereafter up to day 33. At 20°C, none of the larvae survived beyond day 25.

The overall rate of growth for the period day 1-25 is seen to decrease with decrease in temperature (Table 74). The same trend has been maintained for the phases day 1-9 and day 17-25. Growth rate for the period day 9-17 is seen to be comparable for larvae reared at the temperatures of 28 and 32°C (6.70 μm/day at 32°C and 6.74 μm/day at 28°C).

Table 74. Larval growth rate at different temperatures.

Period (days)	Larval growth rate per day (μm/day)			
	32°C	28°C	Ambient	20°C
1-9	4.40	3.28	3.08	1.41
9-17	6.70	6.74	1.70	0.49
17-75	2.53	2.46	2.34	0.19
1-25	4.54	4.16	2.37	0.70

From linear regression analysis of the data, the growth regressions were calculated to be 0.0161, 0.0159, 0.0105 and 0.0033 log μm/day. Analysis of co-variance indicated highly significant difference between growth rates at the temperatures of 32°C, 28°C ambient and 20°C ($P < 0.01$). Whereas growth rate

at 32 and 28°C was found to be not significant from each other ($P > 0.05$), growth rate at 20°C and at ambient temperature was found significant from growth rate at all other temperatures ($P < 0.01$).

Survival rate (Fig. 32)

Survival at the umbo stage was seen to be 90.0, 88.8, 92.3 and 81.8% for larvae maintained at the temperatures of 32, 28, ambient and 20°C respectively (Fig. 32). Although none of the larvae at 20°C grew up to the umbo stage, survival count here was taken along with the others on day 11. For the eyed umbo stage survival was 80.0, 71.1 and 76.9% for larvae maintained at 32°, 28° and ambient temperature. Larvae maintained at 20°C, did not survive beyond day 25. Total spat production was seen to be 47.1, 35.5 and 8.8% for larvae maintained at 32°C, 28°C and ambient temperature respectively.

Spat setting and production (Table 73, Fig. 32)

There was a decrease in the total number of spat produced, with decrease in temperature (Table 73). Onset of metamorphosis occurred in 22 days at 32° and 28°C and in 28 days at ambient temperature. Setting lasted for 22 days at 32°C, 24 days at ambient temperature and 33 days at 28°C.

Peak spat setting was observed on day 40 for larvae reared at 32°C and 28°C, and on day 34 at ambient temperature (Fig. 32). Total spat production was 47.1, 35.5 and 8.8% at

the temperatures of 32°C, 28°C and ambient respectively. There was highly significant difference between the percentage of spat production at temperatures 32°C, 28°C and ambient ($P < 0.01$).

Algal cell consumption (Table 75)

Algal consumption was seen to increase with increase in temperature being the least at the temperature of 20°C (8.5-15.0%) and the maximum (65.0-80.0%) at the temperature of 32°C. Since algal cell concentration was maintained uniformly at 25 cells/µl at all temperatures, this would mean that fewer cells were being consumed at lower temperatures than at the higher temperatures.

Table 75. Algal cell consumption by larvae reared at different temperatures

S. No.	Temperature (°C)	Algal cell consumption (%)					
		d-2	d-7	d-10	d-13	d-18	d-21
1.	32	65.0	70.0	75.0	80.0	80.0	80.0
2.	28	62.5	65.0	70.0	70.0	75.0	75.0
3.	Ambient	62.5	62.5	62.5	65.0	70.0	70.0
4.	20	8.5	8.5	12.5	15.0	12.5	-

Summary of results of larval rearing experiment at different temperatures (Table 76).

There is a progressive increase in larval growth rates with increase in temperature (Table 76). Maximum growth in

Table 76. Summary of results of larval growth data at different temperatures (data from Tables 73 and 74)

S. No.	Temp (°C)	Mean larval size on day 25 (μm)	Growth rate per day (μm/day)	Growth regression (Log μm/day)	Day of first setting	Duration of setting (days)	Spat production (%)
1.	32	177.3	4.54	0.0161	22	22	47.1
2.	28	168.1	4.16	0.0159	22	32	35.5
3.	Ambient	125.2	2.37	0.0105	28	24	8.8
4.	20	85.0	0.70	0.0033	-	-	-

terms of mean larval size (177.3 µm on day 25), actual growth rate (4.54 µm/day for day 1-25) and growth regression (0.0161 log µm/day) was observed at the temperature of 32°C, while the least size (85.0 µm/on day 25), growth rate (0.70 µm/day) and growth regression (0.0033log µm/day) was observed at 20°C. This difference was also reflected in the spat setting data. Shortest time for initiation of setting (22 days) and maximum spat production (47.1%) was observed at the highest temperature of 32°C.

B. Effect of different salinities on pearl oyster larval growth and settlement

EXPERIMENT 1: Sal. 30, 32, 34, 36 and ambient 37.0-38.1‰

1. Larval growth (Tables 77 and 78, Figs. 33 and 34)

Mean larval size is seen to decrease with increasing salinity. Upto day 21, the minimum size is observed consistently at ambient salinity. Maximum mean larval size up to day 17 is observed at 30‰ (Table 77). Taken as a whole, larval growth rates at 30, 32 and 34‰ have been comparable, while it is the least at 36‰ and ambient salinity.

The difference in larval size range between treatments is also observed in their size frequency distribution (Fig.33), and in their growth curves (Fig. 34). The overall rate of growth for the period day 1-25 is seen to decrease steadily

Table 77. Growth and setting of pearl oyster larvae reared at different salinities (Experiment 1)

Age of culture (days)	Mean size of larvae (μm)				
	30‰	32‰	34‰	36‰	Ambient
1	69.0 ± 2.3	69.0 ± 2.3	69.0 ± 2.3	69.0 ± 2.3	69.9 ± 2.3
5	81.9 ± 3.3	80.3 ± 2.5	81.4 ± 3.5	79.4 ± 2.6	77.8 ± 3.0
9	94.1 ± 6.5	93.2 ± 3.7	94.0 ± 6.9	89.8 ± 4.4	87.2 ± 4.8
13	141.2 ± 21.2	143.1 ± 20.4	135.7 ± 14.6	120.1 ± 16.7	106.6 ± 11.3
17	172.5 ± 23.6	169.0 ± 20.6	163.6 ± 27.7	153.1 ± 21.1	126.0 ± 16.9
21	172.8 ± 22.2	170.5 ± 22.2	177.2 ± 24.3	153.3 ± 26.7	152.1 ± 27.4
25	170.8 ± 20.4	166.1 ± 23.0	164.7 ± 25.6	138.1 ± 24.4	147.8 ± 22.6
29	-	-	173.5 ± 21.3	-	154.1 ± 18.9
33	-	-	185.0 ± 4.1	-	175.8 ± 18.6
Day of first setting	18	18	18	20	22
Day of final setting	28	28	35	28	42
Total no. of spat	1506	1170	1061	458	324
Rate of spat production (%)	12.0	9.4	8.5	3.7	2.6

SIZE FREQUENCY DISTRIBUTION OF PEARL OYSTER LARVAE REARED AT DIFFERENT SALINITIES (EXPT. 1)

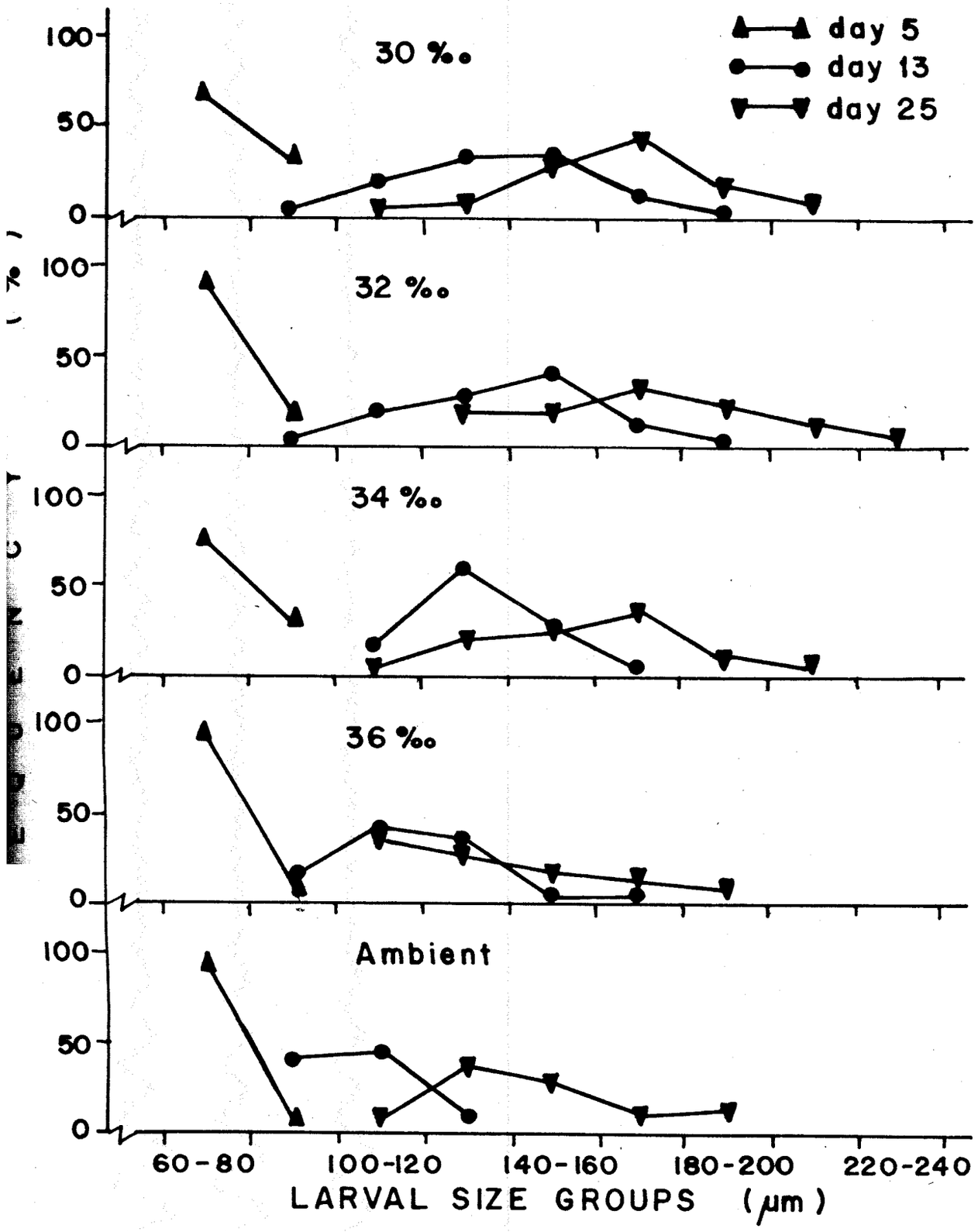
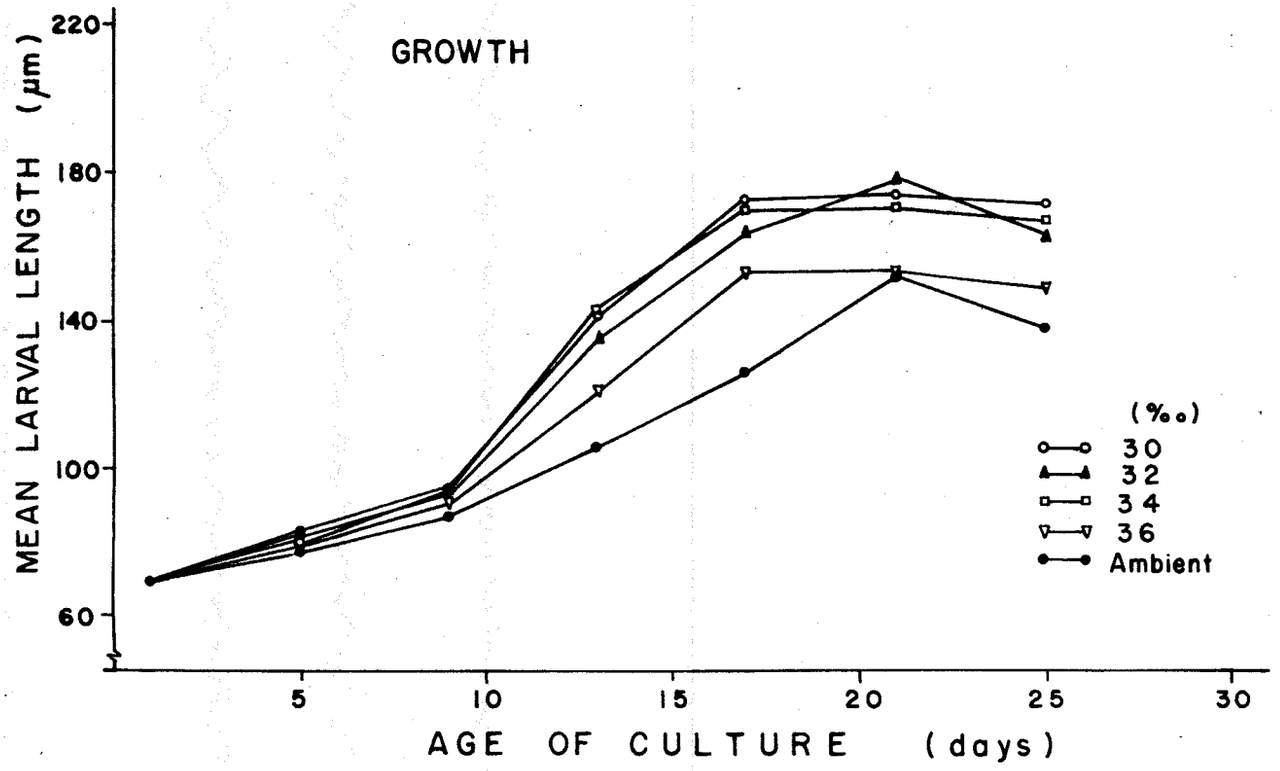
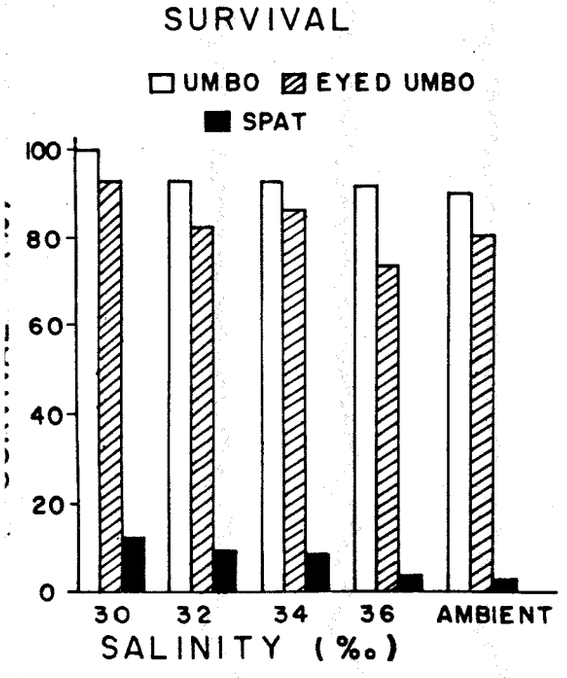
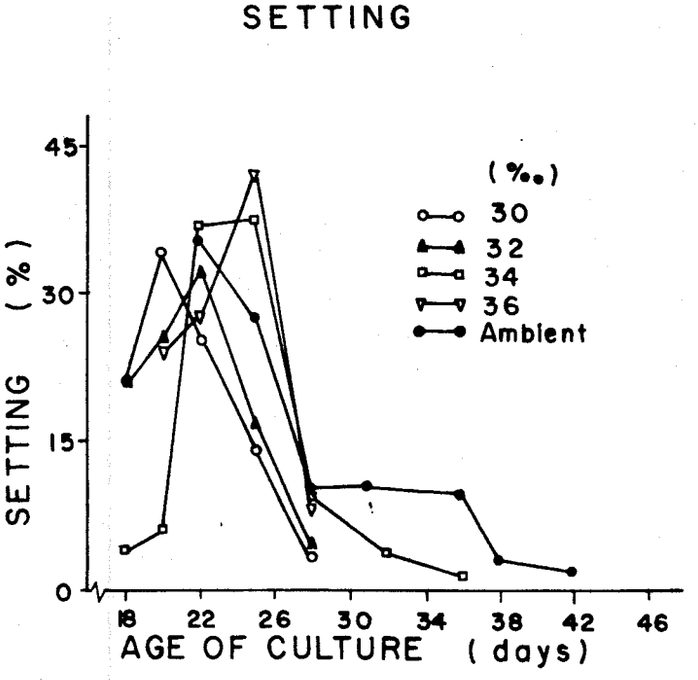


Fig.34. GROWTH, SURVIVAL AND SETTING OF PEARL OYSTER LARVAE CULTURED AT DIFFERENT SALINITIES (EXPT. 1)







with increase in salinities recording the maximum at 30‰ and the least at ambient salinity. The difference in growth rates between larvae reared at 30 to 34‰ for the period day 1-9 and between larvae at 30 and 32‰ for the period day 9-17, however, are not as sharply defined as the difference between larvae at 34 and 36‰.

Table 78 Larval growth rate at different salinities (Experiment 1)

Period (days)	Larval growth per day (μm/day)				
	30‰	32‰	34‰	36‰	Ambient
1-9	3.14	3.03	3.13	2.60	2.28
9-17	9.80	9.48	8.70	7.91	4.85
17-25	-	-	-	-	2.73
1-25	4.24	4.05	3.99	2.88	3.28

The growth regression was calculated to be 0.0224, 0.0223, 0.0222, 0.0194 and 0.0174 log μm/day for larvae maintained at salinities of 30, 32, 34, 36‰ and ambient salinity, respectively. The analysis of co-variance, however, showed that there was statistically no significant difference between these values ($P > 0.05$).

2. Survival rate (Fig. 34)

Survival at the umbo stage was 100.0, 94.1, 94.1, 92.3 and 90.0% for larvae reared at 30, 32, 34, 36‰ and ambient salinity, respectively. Survival at the eyed umbo

stage was 94.1, 84.2, 85.7, 73.8 and 79.8% and total spat production was 12.0, 9.4, 8.5, 3.7 and 2.6%, for the same order of treatments (Fig. 34).

Spat setting and production (Table 77, Fig. 34)

Spat setting was initiated on day 18 at salinities 30, 32 and 34‰, on day 20 at 36‰ and on day 22 at ambient salinity. Setting lasted for 10 days each at 30 and 32‰, 17 days at 34‰, 8 days at 36‰ and a maximum of 20 days at ambient salinity (Table 77).

Peak setting was observed on day 20 at 30‰ day 22 at 32‰, day 25 at 34 and 36‰ and day 22 at ambient salinity. Setting was prolonged at 34‰ and at ambient salinity to 17 and 20 days respectively. Total spat setting reduced progressively from that at 30‰. There was highly significant difference in percentage spat production between all treatments ($P < 0.01$).

Algal cell consumption (Table 79)

It is seen that there is a marginal increase in algal cell consumption with decreasing salinities (Table 79).

Table 79. Algal cell consumption at different salinities (Experiment 1)

Salinity (‰)	Algal cell consumption (%)					
	d-4	d-10	d-14	d-18	d-20	d-23
30	50.0	60.0	70.0	75.0	75.0	80.0
32	45.0	55.0	70.0	70.0	75.0	80.0
34	45.0	50.0	50.0	65.0	70.0	70.0
36	40.0	55.0	60.0	60.0	60.0	65.0
Ambient	45.0	50.0	55.0	55.0	60.0	65.0

EXPERIMENT 2: 26, 28 and ambient 32.6-35.2‰

1. Larval growth (Tables 80 and 81, Figs.35 and 36)

There are only small differences between mean larval sizes at 28 and 26‰. Throughout the larval rearing, mean size of larvae reared at ambient salinity remained the least recorded (Table 80). Similar trends in larval size ranges when reared at 26 and 28‰ are also observed in their size frequency distribution (Fig. 35) and in their growth curves (Fig. 36). Growth of larvae reared at ambient salinity remained less than that of larvae reared at 26 and 28‰.

The overall growth rate of larvae at 26‰ and 28‰ are very similar (5.18 and 5.15 µm/day respectively) and the least at ambient salinity (4.35 µm/day). This trend has been maintained for all three phases days 1-9 9-17 and 17-25 (Table 81).

Growth regression values of 0.0196, 0.0197, and 0.0176 log µm/day were calculated for the salinities of 26, 28‰ and ambient salinity respectively. Results of the analysis of co-variance, however, indicated that there was no statistically significant difference between these regression values ($P > 0.05$).

Table 80. Growth and setting of pearl oyster larvae reared at different salinities (Experiment 2).

Age of culture (days)	Mean size of larvae (μm)		
	28‰	26‰	Ambient
1	71.1 ± 3.1	71.1 ± 3.1	71.1 ± 3.1
5	83.3 ± 2.9	82.8 ± 2.9	79.4 ± 3.1
9	102.0 ± 4.2	100.7 ± 4.0	97.2 ± 3.6
13	137.4 ± 14.7	126.4 ± 10.8	125.8 ± 10.5
17	155.9 ± 12.9	155.9 ± 11.8	142.9 ± 15.8
21	188.8 ± 26.5	181.9 ± 20.2	163.8 ± 23.7
25	194.8 ± 24.0	195.5 ± 18.5	175.4 ± 35.0
29	200.9 ± 13.0	203.6 ± 13.8	180.1 ± 21.6
33	190.8 ± 40.6	202.2 ± 17.7	193.9 ± 18.2
37	207.2 ± 10.2	207.6 ± 10.8	205.0 ± 16.2
Day of first setting	23	23	23
Day of final setting	41	41	41
Total No. of spat	4893	3512	2268
Rate of spat production(%)	39.1	28.1	18.1

g.35. SIZE FREQUENCY DISTRIBUTION OF PEARL OYSTER LARVAE REARED AT DIFFERENT SALINITIES (EXPT. 2)

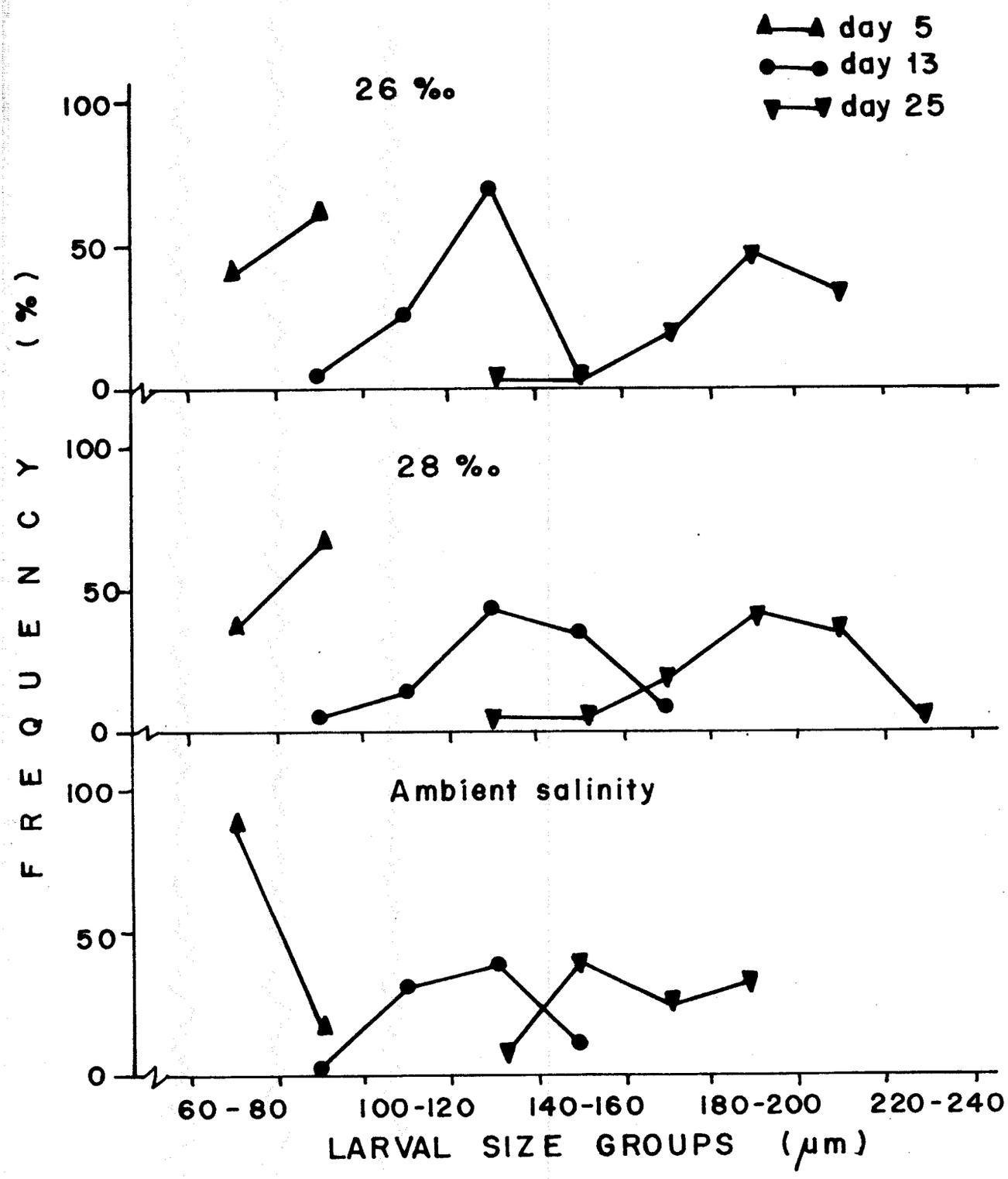
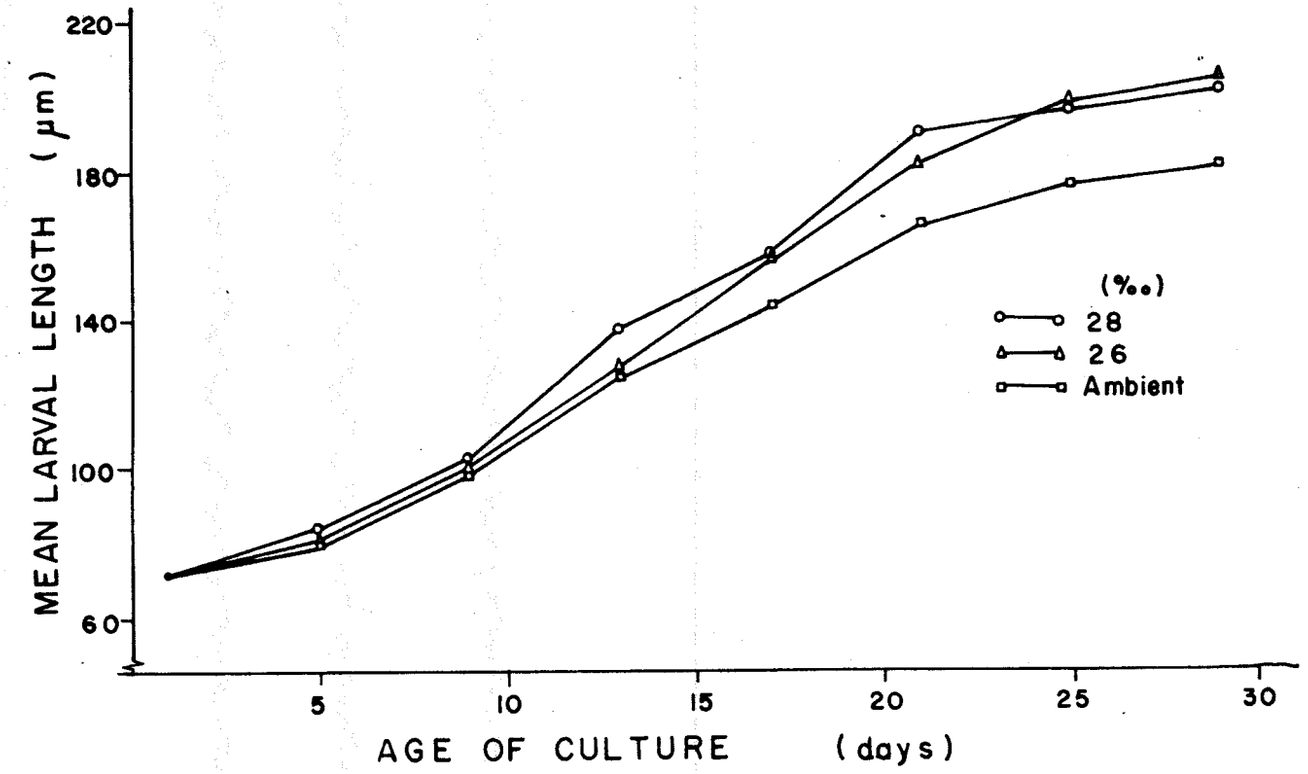
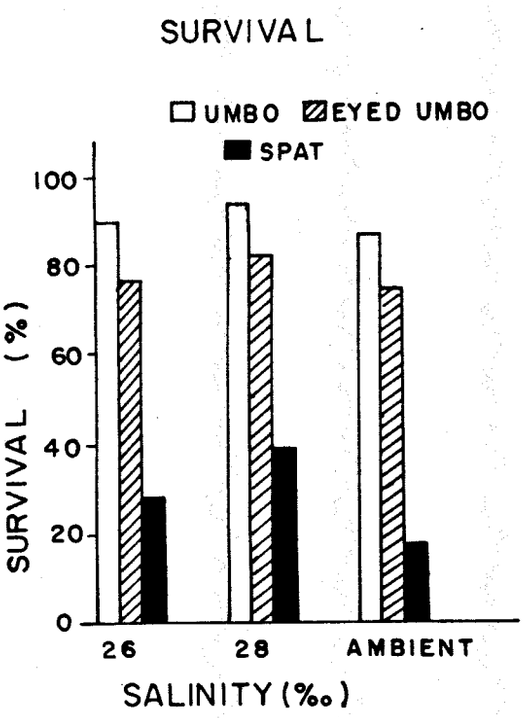


Fig. 36. GROWTH, SURVIVAL AND SETTING OF PEARL OYSTER LARVAE CULTURED AT DIFFERENT SALINITIES (EXPT. 2)





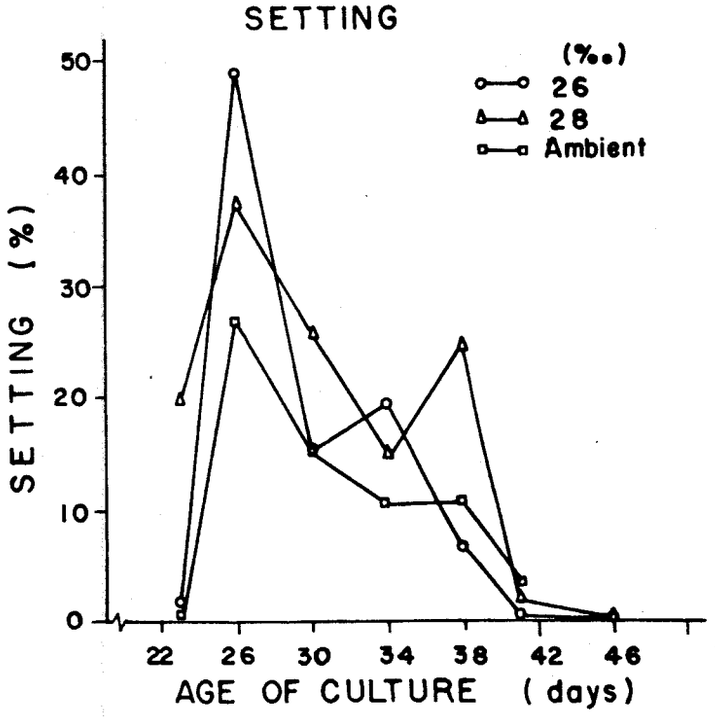


Table 81. Larval growth rate when reared at different salinities (Experiment 2)

Period (days)	Larval growth per day (µm/day)		
	26‰	28‰	Ambient
1-9	2.93	3.05	2.08
9-17	6.90	6.74	5.71
17-25	4.95	4.86	4.06
1-25	5.18	5.15	4.35

Survival rate (Fig. 36)

Survival was 90.4, 94.4 and 86.6% for salinities of 26, 28‰ and ambient, respectively, for the umbo stage (Fig. 36). During the eyed umbo stage, survival was 76.2, 82.6 and 75.1% for 26, 28‰ and ambient salinity respectively. Total spat production on completion of the experiment was 28.1, 39.1 and 18.1% for 26, 28% and ambient salinity respectively.

Spat setting and total production (Table 80, Fig. 36)

Spat setting was initiated uniformly on day 23 and lasted for a total period of 18 days at all salinity levels (Table 80). Peak spat setting was observed at all salinity levels on day 26 (Fig. 36). This was followed by a gradual decline, but prolonged setting period upto day 41. Total spat production was 28.1, 39.1 and 18.1%. There was highly significant statistical difference in percentage spat setting between all treatments ($P < 0.01$).

Algal cell consumption (Table 82).

It is seen that with advance in time and with increase in larval size there is an increase in the consumption of algal cells. However, there is no difference in percentage consumption between treatments.

Table 82. Percentage algal cell consumption at different salinities (Experiment 2)

S. No.	Salinity	Algal cell consumption (%)					
		d-4	d-8	d-11	d-15	d-17	d-20
1.	26‰	50	50	60	70	70	70
2.	28‰	50	50	60	80	80	
3.	Ambient	50	50	60	70	70	70

Summary of results of larval rearing experiments using different salinities (Table 83)

From the details of mean larval size, growth rate and growth regressions of experiment 1 (Table 83), it is seen that larval growth at salinities 30-34‰ was comparable. Shortest time for initiation of metamorphosis (18 days) and greater percentage of setting (8.5-12.0%) was observed again for the salinities 30-34‰.

In the second experiment, maximum mean larval size (188.8 µm) and growth regression (0.0197 log µm/day) was observed at the salinity of 28‰.

Table 83. Summary of results of larval rearing experiments using different salinity levels(Experiments 1 and 2, data from Tables 77,78,80 and 81)

S. No.	Treatment (‰)	Mean larval size on day 25 (µm)	Growth rate per day (µm/day)	Growth regression (log µm/day)	Day of first setting	Duration of setting(days)	Spat production (%)
Experiment 1:							
1.	30	170.8	4.24	0.0224	18	10	12.0
2.	32	166.1	4.05	0.0223	18	10	9.4
3.	34	164.7	3.99	0.0222	18	17	8.5
4.	36	138.1	2.88	0.0194	20	8	3.7
5.	Ambient	147.8	3.28	0.0174	22	20	2.6
Experiment 2:							
6.	26	195.5	5.18	0.0196	23	18	18.1
7.	28	194.8	5.15	0.0197	23	18	39.1
8.	Ambient	175.4	4.35	0.0176	23	18	18.1

while no difference in spat setting, initiation and duration was recorded between salinities, maximum spat setting was observed at 28‰.

C. Effect of different pH levels on pearl oyster larval growth and survival

Larval growth (Tables 84 and 85, Figs. 37 and 38)

Taken as a whole, growth of pearl oyster larvae at the pH levels of 7.5 and 9.0 is seen to be poor as compared to growth at pH 8.1 and 8.5. None of the larvae at pH 7.5 and 9.0 survived beyond day 21 and grew beyond the umbo stage (Table 84). The poor larval growth at pH 7.5 and pH 9.0 is also reflected in the size frequency distribution (Fig. 37) and the growth curves (Fig. 38). Maximum growth rate for all the phases day 1-9, day 9-17 and day 17-25 is observed consistently at ambient pH (Table 85). Larval growth rate at pH 8.5 is marginally less than at ambient pH. Least growth rate was recorded at pH 9.0.

Table 84. Growth and setting of pearl oyster larvae reared at different pH levels.

Age of culture (days)	Mean size of larvae (μm)			
	7.5	8.1	8.5	9.0
1	69.2 ± 2.3	69.2 ± 2.3	69.2 ± 2.3	69.2 ± 2.3
5	91.9 ± 3.6	92.4 ± 4.0	93.9 ± 3.8	92.9 ± 4.9
9	104.2 ± 6.2	107.9 ± 7.1	106.3 ± 5.0	102.0 ± 5.8
13	120.0 ± 7.7	159.0 ± 18.6	147.8 ± 17.0	114.3 ± 7.7
17	126.6 ± 11.1	185.0 ± 24.5	178.1 ± 14.2	122.9 ± 9.5
21	125.5 ± 12.6	204.8 ± 13.2	192.4 ± 20.9	111.2 ± 10.3
25	-	208.0 ± 12.4	196.4 ± 19.9	-
29	-	-	202.4 ± 12.5	-
Day of first setting	-	17	20	-
Day of final setting	-	29	29	-
Total No. of spat	-	848	529	-
Rate of spat production(%)	-	6.8	4.2	-

Fig. 37. SIZE FREQUENCY DISTRIBUTION OF PEARL OYSTER LARVAE MAINTAINED AT DIFFERENT *pH* LEVELS

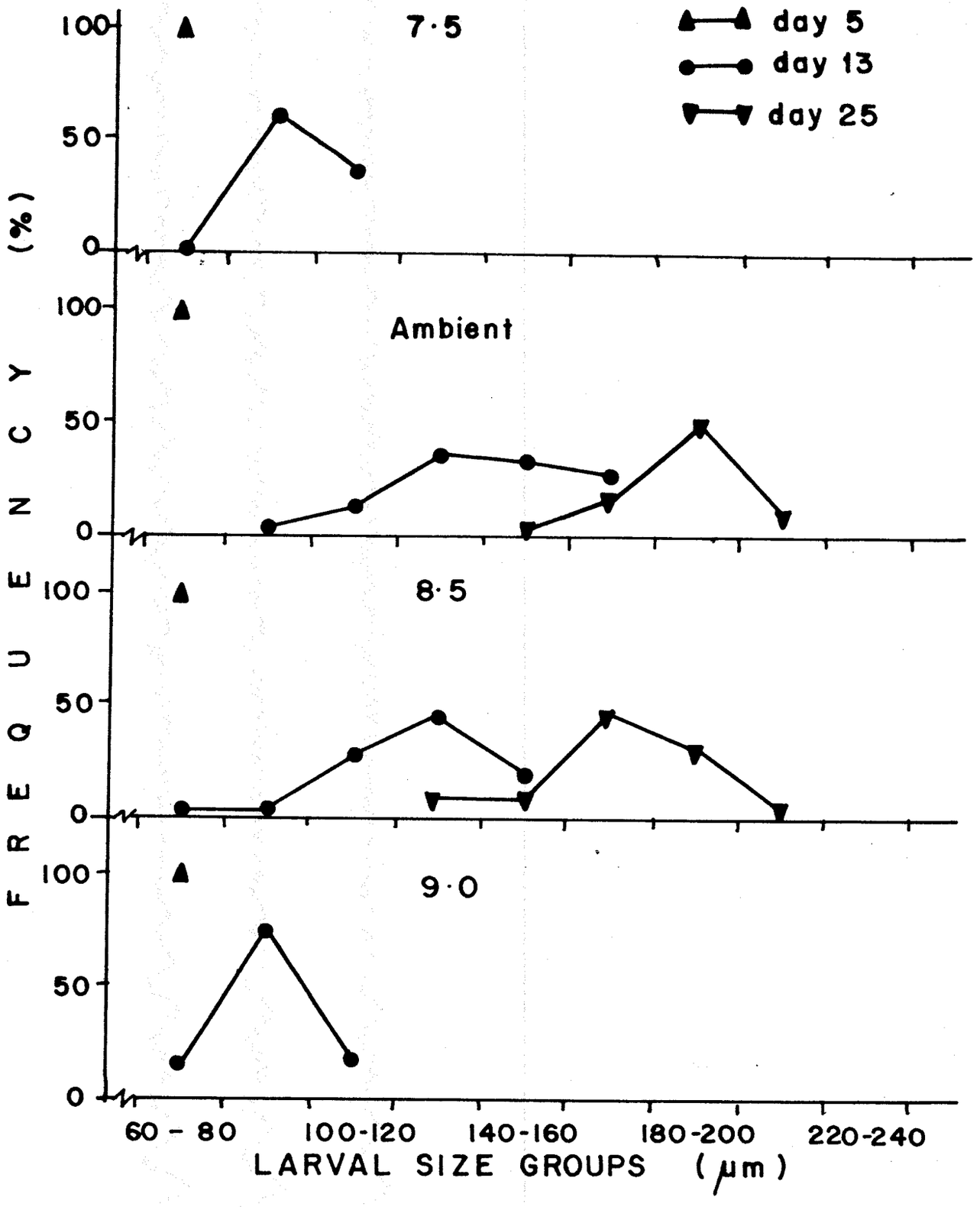
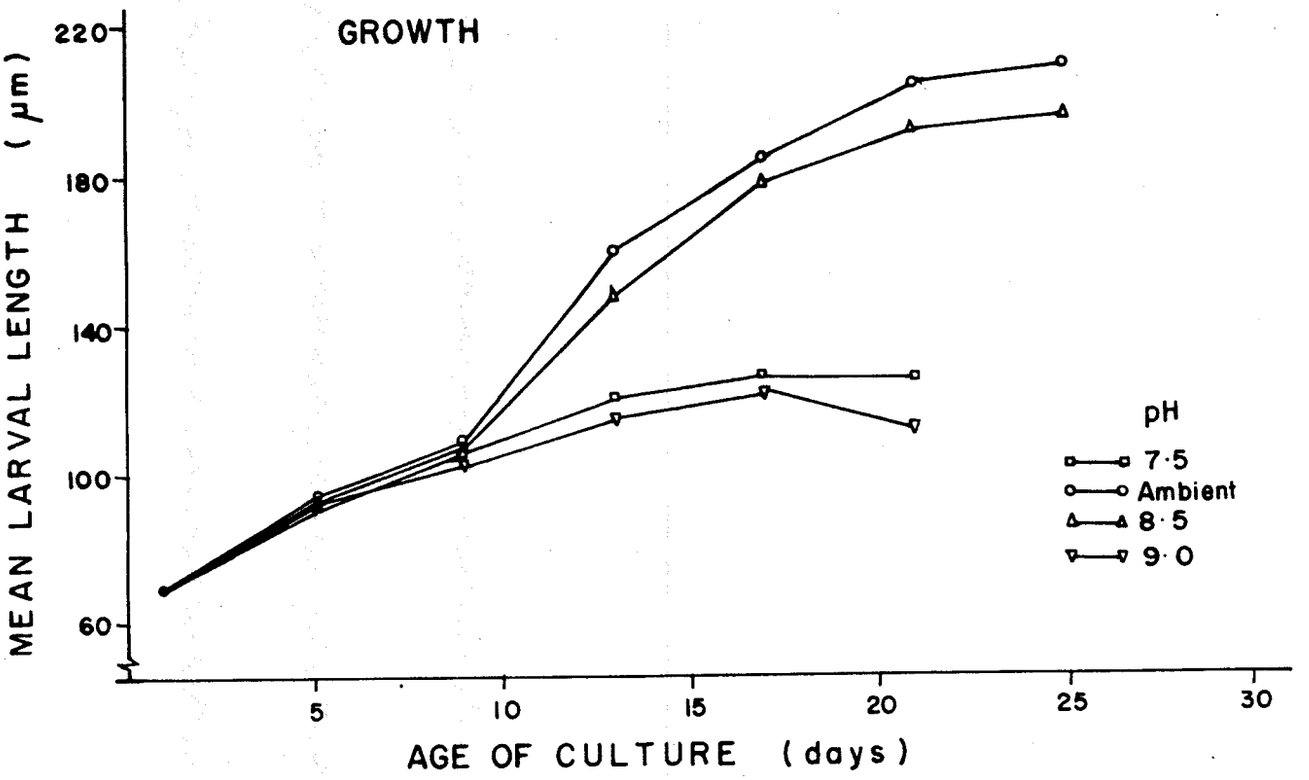
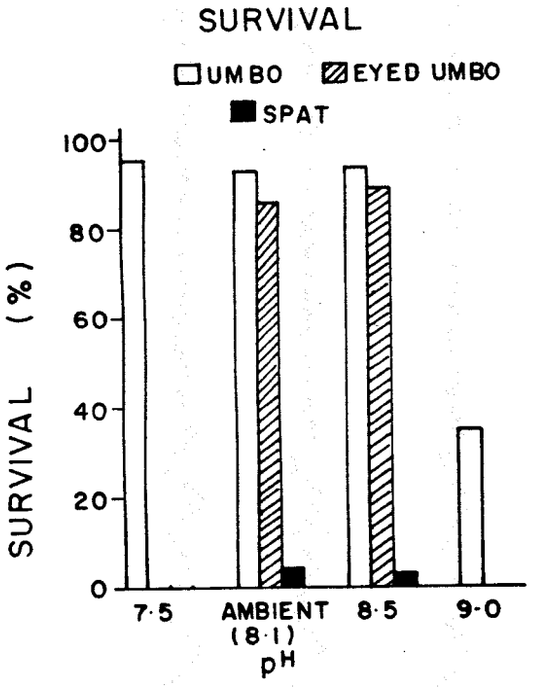


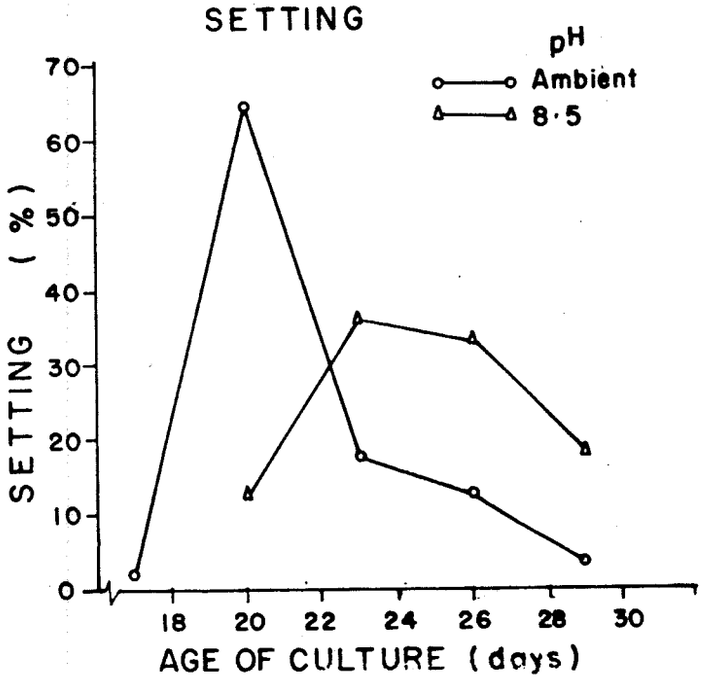
Table 85. Larval growth rate at different pH levels

Period (days)	Larval growth per day (µm/day)			
	pH 7.5	Ambient pH	pH 8.5	pH 9.0
1-9	4.38	4.84	4.64	4.10
9-17	2.80	9.64	8.98	2.61
17-25	-	2.88	2.29	-
1-25	-	5.78	5.30	-

Fig. 38. GROWTH, SURVIVAL AND SETTING OF PEARL OYSTER LARVAE CULTURED AT DIFFEREN $_{p}$H LEVELS







Growth regressions of 0.0126, 0.0228 and 0.0103 log μm/day were obtained for larvae reared at pH 7.5, 8.1, 8.5 and 9.0 respectively. Results of the analysis of covariance showed that these values were statistically significant ($P < 0.01$). The students t test further showed that growth regressions of larvae maintained at pH 7.5 and pH 9.0, were both statistically significant from that of pH 8.1 and 8.5 ($P < 0.01$). There was no significance between growth regressions of larvae maintained at pH 8.1 and 8.5 ($P > 0.05$).

2. Survival rate (Fig. 38)

Survival during the umbo stage was 96.2, 94.1, 95.2, and 42.8% for larvae reared at pH 7.5, 8.1, 8.5, and 9.0 respectively. Survival at the eyed umbo stage at pH 8.1 and 8.5 was 88.8 and 89.9%, respectively. Total spat production was 6.8% at pH 8.1 and 4.2% at 8.5.

3. Spat setting and production (Table 84, Fig. 38)

Spat setting was initiated on day 17 at pH 8.1 but was delayed to day 20 at pH 8.5 (Table 84). There was no setting at pH 7.5 and 9.0. Setting lasted for a period of 12 days in pH 8.1 and 9 days at pH 8.5. Peak spat setting was observed on day 20 for larvae reared at pH 8.1, after which there was a sharp decline (Fig. 38). At pH 8.5, peak setting was observed on day 23, gradually declining up to day 29. Total spat production was 6.8 and 4.2% at pH levels

of 8.1 and 8.5 respectively. There was highly significant difference in percentage spat setting at these pH levels ($P < 0.01$).

4. Algal cell consumption (Table 86)

Consumption is seen to increase with larval size only at 2 pH levels of 8.1 and 8.5. At the other two pH levels of 7.5 and 9.0, algal cell consumption is seen to decrease after day 9. Also, the consumption percentage is seen to be far below that of larvae maintained at pH 8.1 and 8.5.

Table 86. Algal cell consumption of larvae reared at different pH levels.

S. No.	pH	Algal cell consumption on different days (%)						
		d-3	d-6	d-9	d-13	d-16	d-19	d-23
1	7.5	35.0	40.0	35.0	25.0	25.0	25.0	-
2	8.1	60.0	65.0	65.0	70.0	70.0	70.0	-
3	8.5	55.0	55.0	60.0	60.0	65.0	65.0	65.0
4	9.0	30.0	40.0	40.0	25.0	10.0	10.0	-

5. Summary of results of larval rearing experiment using different pH levels (Table 87)

Larvae reared at the ambient pH showed the highest mean larval size (204.8 µm), growth regression (0.0244 log µm/day) and maximum spat production (6.8%). Onset of metamorphosis was also the earliest at 17 days (Table 87) at ambient pH.

Table 87. Summary of results of the larval rearing experiment at different pH levels (data from Tables 84 and 85).

S. No.	Treatment (pH)	Mean larval size on day 21 (μm)	Larval growth rate (μm/day)	Growth regression (log μm/day)	Day of first setting	Duration of setting (days)	Spat production (%)
1.	7.5	125.5	-	0.0126	-	-	-
2.	8.1	204.8	5.78	0.0244	17	12	6.8
3.	8.5	192.4	5.30	0.0228	20	9	4.2
4.	9.0	111.2	-	0.0103	-	-	-

DISCUSSION

Pillay (1962a, b), studying the physicochemical factors prevailing in the pearl oyster banks of Gulf of Mannar, reported the minimum and maximum temperature of 26.5° and 30.5°C, salinity of 32.2‰ and 35.9‰ and pH of 8.1 and 8.6, respectively. These reflect the prevalence of fairly stable conditions throughout the year. In pearl oyster farms at Veppalodai, Victor (1983) recorded minimum and maximum values of 25.5° and 32.1°C for temperature, 28.5 and 35.6‰ for salinity and 7.96 and 8.42 for pH. Alagarswami and Victor (1977) recorded the lowest salinity level of 15.6‰ for a short duration in the same area. Bayne (1983) noted that tolerance limits of larvae are normally wide and encompass the variations that may be experienced in nature. Keeping this in mind, and extending the natural limits, pearl oyster larvae were reared at a temperature range of 20-32°C, salinity range of 26-37.7‰ and a pH range of 7.5-9.0.

Results of the experimental larval rearing at the four different temperatures of 20°, 24.9° (ambient), 28° and 32°C indicate a direct relationship between temperature and larval growth and spat production. Growth rates were 0.70, 2.37, 4.16, and 4.54 µm/day and the growth regressions were 0.0033, 0.0105, 0.0159 and 0.0161 log µm/day for the same

order of temperatures. There was total mortality of larvae reared at 20°C. Progressive increase in total spat production was observed with increase in temperature (8.8% at 24.9°C, 35.5% at 28°C and 47.1% at 32°C). Setting was earliest at 22 days at 28° and 32°C and at 28 days at mean ambient temperature of 24.9°C.

Bayne (1983) observed that rates of growth of larvae increase with rise in temperature to an optimum and then decline with further temperature increase. Within a range of 5-30°C, Hrs-Brenko and Calabrese (1969) observed progressive increase in larval growth rate of Mytilus edulis from 5° to 20°C and, thereafter, a decline at 25°C. There was complete mortality at 30°C. Bourne and Smith (1972) observed increasing growth rates of 5.4 µm/day, 7.2 µm/day and 7.9 µm/day at temperatures of 5°, 10° and 15°C and total mortality at 20°C for larvae of the horse clam Tresinx capax. Within a temperature range of 10-35°C, normal growth was observed at 20-25°C for larvae of the cockle Cardium edule and at 30°C for larvae of C. glaucum (Kingston, 1974). Loosanoff and Davis (1963 b) observed normal development of Crassostrea virginica at 17.5-30°C, and of Mercenaria mercenaria at the maximum temperature of 25-30°C. In the tropical oyster Saccostrea echinata larvae measured 136 and 203 µm at temperatures of 25° and 27°C on day 17, and 212 µm at 29°C on day 16 (Coeroli et al., 1984). Larvae reared at 25°C, however, suffered complete mortality.

The influence of temperature on spat production has been reported for a few bivalve species. Loosanoff et al. (1951) observed setting of M. mercenaria larvae in 7 days at 30°C and in 16 days at 18°C. M. edulis larvae took 31 days at 16°C and 70 days at 11°C at the feeding level identical to the present study (Bayne, 1965). For O. edulis larvae, setting occurred in 26 days at 17.5°C, 14 days at 20°C, 8-12 days at 25-30°C (Davis and Calabreae, 1969). For the cockle, C. edule, setting occurred in 26-30 days at 15°C, and 20-24 days at 20° and 25°C. Hou-Cheng Chen (1984) noted setting in 17 days at 23°C while no setting was observed at 18°C in clams and abalones. In S. echinata setting was initiated in 18 days at 29°C and 22 days at 27°C (Coeroli et al., 1984). In the present experiment, initiation of setting occurred in 22 days time at the temperatures of 28°C and 32°C, while it was delayed to 28 days at the mean ambient temperature of 24.9°C.

Very few accounts of total spat production at different temperature regimes have been cited in literature. Davis and Calabrese (1969) observed an inverse relation between total spat production of O. edulis and temperature. With increasing temperatures of 20°, 22.5°, 25°, 27.5°and 30°C, total spat produced from an initial population of 6000-8000 D shape larvae were, respectively, 4964, 4863, 3790, 602 and 14 spat. In the present study, however, a direct relationship between

temperature and total spat production within the temperature range of 20-32°C is seen. Total spat produced increased from 8.8% at mean ambient temperature of 24.9°C to 35.1% at 28°C and to 47.1% at 32°C.

The following optimum temperature for larval rearing have been identified for several temperate and a few tropical species: 17.2-23.2°C for Mya arenaria (Stickney, 1964); 15-20°C for Mytilus edulis (Hrs-Brenko and Calabrese, 1969); 18°C for Pecten maximus (Gruffydd and Beaumont, 1972); 15°C for Tresinx capax (Bourne and Smith, 1972); 10-15°C for Adula californiensis (Lough and Gonor, 1973 b); 22°C for Ostrea edulis (Walne, 1974); 20-25°C for Cardium edule (Kingston, 1974) and 20-24°C for Haliotis feulgens (Leighton et al., 1981). Higher temperatures of 30°C for oyster Crassostrea virginica (Loosanoff and Davis, 1963 a; Davis, 1969) 30°C for Mercenaria mercenaria (Loosanoff et al., 1951; Loosanoff and Davis, 1963 a), 28°C for Crassostrea gigas (Helm and Millican, 1977), 29°C for Saccostrea echinata (Coeroli et al., 1984) and 27.5°C for Mulinia lateralis (Calabrese, 1969) have been found optimum for larval rearing. In the present study, within the range of 20-32°C, 32°C yielded the best results by way of growth and spat production.

Davis and Calabrese (1969) noted that temperatures above and below a given range caused weakening of the oyster larvae and made them more susceptible to toxins and diseases. Bayne (1965) and Davis and Calabrese (1964) suggested that the

failure of bivalve larvae to grow at low temperatures appeared to be caused by their inability to digest food although food was ingested. It is suggested that the very insignificant growth of pearl oyster larvae at 20°C might have been primarily due to insufficient consumption and digestion of cells.

Ukeles (1961) demonstrated that I. galbana and P. lutheri were destroyed at temperatures above 27°C. Bayne (1965) attributed mortality of mussel larvae at 30°C to the destruction of algal cells which could have caused a bacterial build up in the larval rearing system. However, the present study demonstrates that the maximum growth rate and setting was obtained at the temperature of 32°C. Although the algal cultures were maintained at 27°C, they were fed to larvae at experimental temperatures. Algal cells were found normal. Joseph (1983) reported maximum photosynthetic activity of I. galbana at 30°C. This would confirm that microalgae of the same species in different geographical locations may have different temperature responses.

With regard to salinity, while there were no sharp differences in larval growth rates as observed with temperature changes, a decreasing trend with increasing salinity was noticed. Growth regressions decreased progressively from 0.0224 log μm/day to 0.0174 log μm/day with increasing

salinity levels from 30‰ to 37.7‰. Likewise, in the second experiment growth rate of pearl oyster larvae reared at 26‰ and 28‰ were higher than that at 34.1‰. Setting was also affected by changes in salinity. At 30-34‰, spat setting was the earliest at 18 days. At 36‰ and ambient salinity, setting was delayed to 20 and 22 days respectively. Total spat production was seen to decline progressively with increase in salinities from 30‰ to 37.7‰. In the second experiment, although setting was initiated on day 23 and lasted for 18 days in all cases, spat production was maximum at 28‰ and least at 34.1‰.

Hrs-Brenko and Calabrese (1969) observed maximum increase in mean length at the salinity range of 25-35‰. Between 5-60‰, Kingston (1974) observed maximum growth of Cardium edule and C. glaucum larvae at 30-35‰. Within the range of 15 to 34‰, Helm and Millican (1977) observed maximum larval growth at 25‰. Bayne (1965) observed sluggish growth of M. edulis larvae at 15‰, slow growth at 24‰ and quickest growth at 30-32‰. For the tropical oyster Saccostrea echinata, best growth occurred at 20-30‰. Davis and Ansell (1962) studying the effects of lowered salinities on larvae of O. edulis reported normal growth of larvae down to 22‰ from the ambient 27‰.

Walne (1956a) obtained 3.2 and 0.9% spat at the salinities of 25.9-30.3‰ and 27.9-34.1‰. Davis and Ansell

(1962) recorded 2.6, 8.4, 2.0, 1.9 and 0.3% spat at 26-27‰ 25‰ , 22.5‰, 20‰ and 17.5‰ respectively. Further decrease in salinity did not yield any spat.

Calabrese and Davis (1966) in their study on pH tolerance of embryos and larvae of M. mercenaria and C. virginica reported normal development of clams in the pH range of 7 to 8.75. Above pH 9.0, development was affected. The range of normal growth was narrower than for survival. Rapid growth occurred at pH 8.25-8.50 in clams (Davis and Calabrese, 1966). In the present study, very slow growth rate was observed at the two pH levels, 7.0 and 9.0. Mean size of larvae reared at ambient pH (8.12) was greater than that of larvae reared at pH 8.5. Setting was observed only at pH 8.5 and at ambient pH (8.12).

Davis and Calabrese (1966) ruled out the possible unacceptability of the live algal cells to the larvae after reporting that algal cells remained unaffected by pH change.

Joseph (1983) reported that flagellates grew best at pH 8.0, while acidic pH was unsuitable. However, marine algal species are reported to tolerate pH levels of 6.8-9.6 with an optimum at pH 8.0 (Kain and Fogg, 1958). Davis and Calabrese (1966) have also reported the toxicity to Tris Buffer to bivalve larvae above pH 8.6. In the present study, Tris Buffer was used for maintaining pH at 9.0 and 8.5. While

larvae at pH 8.5 grew and metamorphosed, larvae at pH 9.0 suffered mortality. Working with the scallop, Argopecten irradians irradians larvae, Tettlebach and Rhodes (1981) observed that while both temperature and salinity affected development and larval growth, temperature was the more dominant factor. Calabrese and Davis (1969) had stressed the importance of maintaining optimum temperature in hatcheries, as growth below 70% of the optimum could increase cost of labour and reduce efficiency.

The results of the present study would indicate that, apart from the genetic diversity of the parents used for spawning, the differences in ambient rearing conditions might be responsible for the differences in larval growth and setting. From the results of the monofactorial experiments conducted in the present study, it is suggested that temperature of 30-32°C, salinity of 28-30‰ and ambient pH (8.10-8.15) would be optimum for pearl oyster larval rearing in the hatchery.

CHAPTER 8

SOME ASPECTS OF WATER QUALITY MANAGEMENT DURING THE PRESENT STUDY

INTRODUCTION

Most investigations on bivalve larval rearing have resorted to routine addition of antibiotics in the culture medium as a precautionary control measure against disease (Stickney, 1964; Bayne, 1965; Gruffydd and Beaumont, 1972; Lucain and Martin, 1974; Martin and Vicente, 1975). Le Pennec and Prieur (1977) have reviewed antibiotic usage in bivalve larval culture. Castagna and Krauter (1974) recommended occasional antibiotic washes during water changes, whenever the occurrence of disease was imminent.

While Walne (1974) considered that aeration for larval cultures was essential for growth and vigour of the bivalve species, Loosanoff and Davis (1963 b) did not consider this necessary if water was changed regularly. Nascimento (1980) observed that aeration was injurious to good larval growth. Helm and Spencer (1972) recommended the use of aeration only after reaching a particular larval size.

Ukeles (1975) stressed the importance of avoiding static water conditions in larval rearing. Most studies, however,

have successfully adopted static water system for larval rearing (Bayne, 1965; Wada, 1973; Sprung 1984 a ,b). A few attempts have been made in recent years to rear larvae in continuous flow systems (Flasch et al., 1975; Malouf and Breese, 1977; Wilson 1978).

In the light of the above observations, experiments on antibiotic treatment of larvae, provision of aeration and continuous flow system were carried out in an attempt to elucidate the role of these factors in pearl oyster larval rearing.

MATERIALS AND METHODS

The effects of antibiotics, aeration and a continuous-flow system on pearl oyster larval growth and setting were studied individually. Pearl oyster larvae were reared at the larval density of 5/ml and fed with I. galbana at the concentration of 25 cells/µl.

A. Antibiotic treatment

Streptomycin sulphate was used at a single dosage level of 8 mg/l, as recommended for the hard clam Mercenaria mercenaria larvae by Castagna and Krauter (1974). The antibiotic was either used as "washes" or added to the rearing medium. In the "wash" treatment pearl oyster larvae

were collected on a sieve and kept immersed in a litre of seawater treated with streptomycin sulphate for a period of one and a half hours. Larvae were then washed thoroughly with sterilised seawater and released back into the rearing beakers. In the second treatment, the antibiotic was directly added to the larval rearing beakers after each water change. In a third treatment, larvae were reared without the addition of antibiotics as a control.

B. Aeration

Mild aeration was provided to the pearl oyster larvae through sterilised hypodermic syringes. The culture medium was aerated from the D shape stage in treatment 1, umbo stage in treatment 2 and eyed umbo stage in treatment 3. The standard rearing without aeration was kept as control.

C. Continuous flow system

A continuous flow system was established experimentally for maintaining pearl oyster larvae of the umbo stage. An 80 µm nylobolt sieve was attached to a rectangular wooden frame and kept floating in a tank of seawater. The level of water inside the frame was maintained at a constant head. Flow rate was maintained at 9.1-l/h by continuously letting in water from an overhead tank. The standard rearing in a beaker was kept as control.

RESULTS

A. Antibiotic treatment

The experiment to study the effect of streptomycin sulphate was carried out during March-April 1984, when ambient temperature, salinity and pH ranges were 24.7-26.3°C 30.5-34.5‰ and 8.10-8.15, respectively.

1. Larval growth (Tables 88 and 89, Figs. 39 and 40)

Data in Table 88 show no particular trend of growth. The maximum sizes on different days either occur in treatment 1 or in standard. An almost similar pattern of size-frequency distribution is observed for larvae of both treatments and for standard (Fig. 39). The modal sizes are identical on different days. Larval growth curve is presented in Fig. 40. The growth rate for the period day 1-25 was maximum for larvae of the standard (5.78 µm/day) while it was 5.47 µm/day in treatment 1 and 5.55 µm/day in treatment 2 (Table 89). The highest growth rates for the periods day 1-9 and day 17-25 were observed in standard, but in treatment 1 for day 9-17. However the differences do not appear to be significant.

Table 88. Growth and setting of pearl oyster larvae treated with the antibiotic streptomycin sulphate.

Age of culture (days)	Mean size of larvae (μm)		
	Treatment 1	Treatment 2	Standard
1	69.2 ± 2.3	69.2 ± 2.3	69.2 ± 2.3
5	93.5 ± 3.9	92.3 ± 5.5	92.4 ± 4.0
9	106.3 ± 7.2	106.3 ± 5.0	107.9 ± 7.1
13	162.7 ± 14.7	154.7 ± 14.0	159.0 ± 18.6
17	184.4 ± 23.2	181.9 ± 28.0	185.0 ± 24.5
21	214.9 ± 25.4	200.4 ± 21.5	204.8 ± 13.2
25	200.4 ± 28.5	202.3 ± 13.6	208.0 ± 12.4
29	208.8 ± 13.0	182.2 ± 25.6	-
Day of first setting	17	17	17
Day of final setting	32	32	29
Total No. of spat	7559	2854	848
Rate of spat production (%)	60.5	22.9	6.8

1.39. SIZE FREQUENCY DISTRIBUTION OF PEARL OYSTER LARVAE CULTURED WITH STREPTOMYCIN SULPHATE

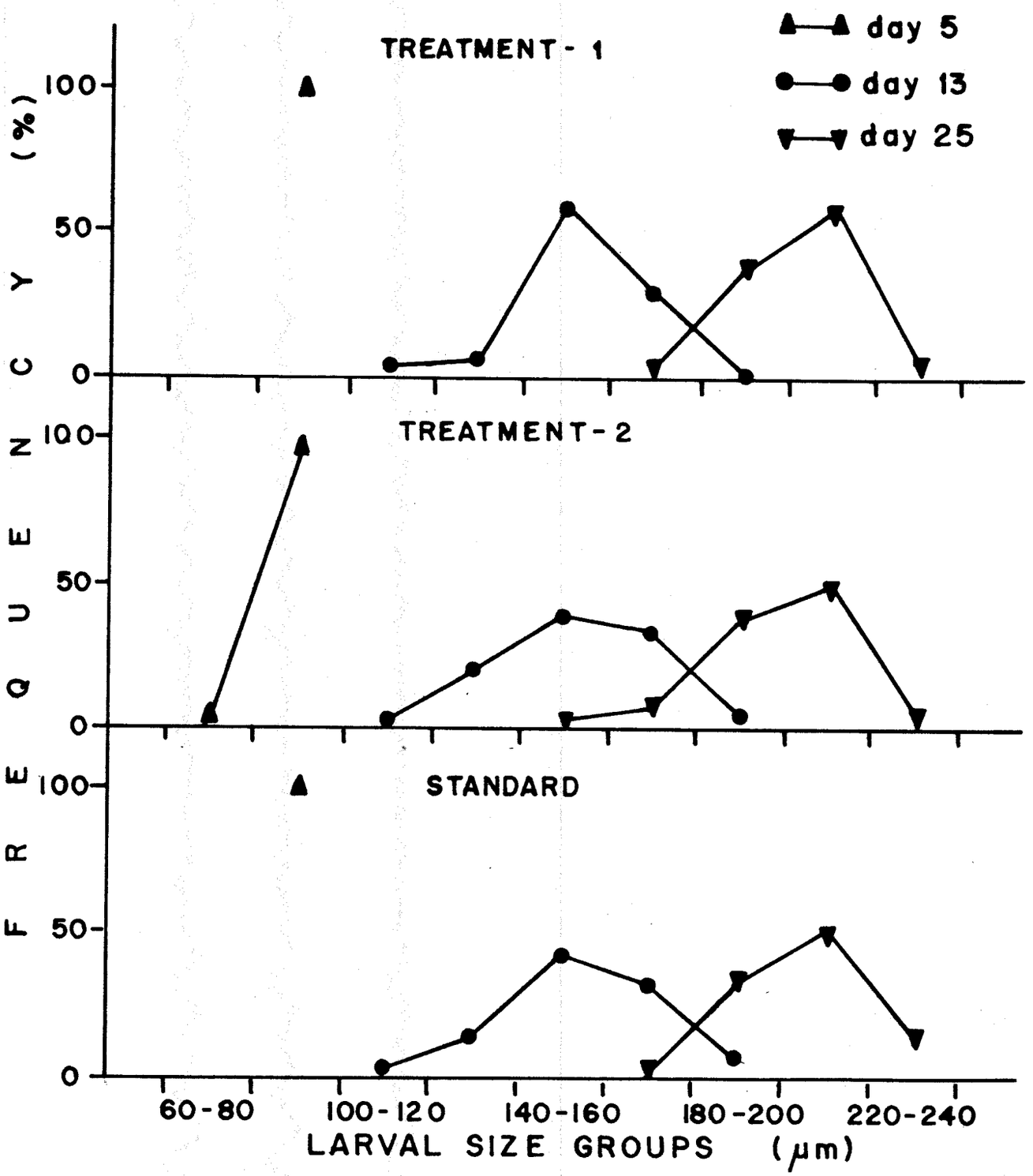
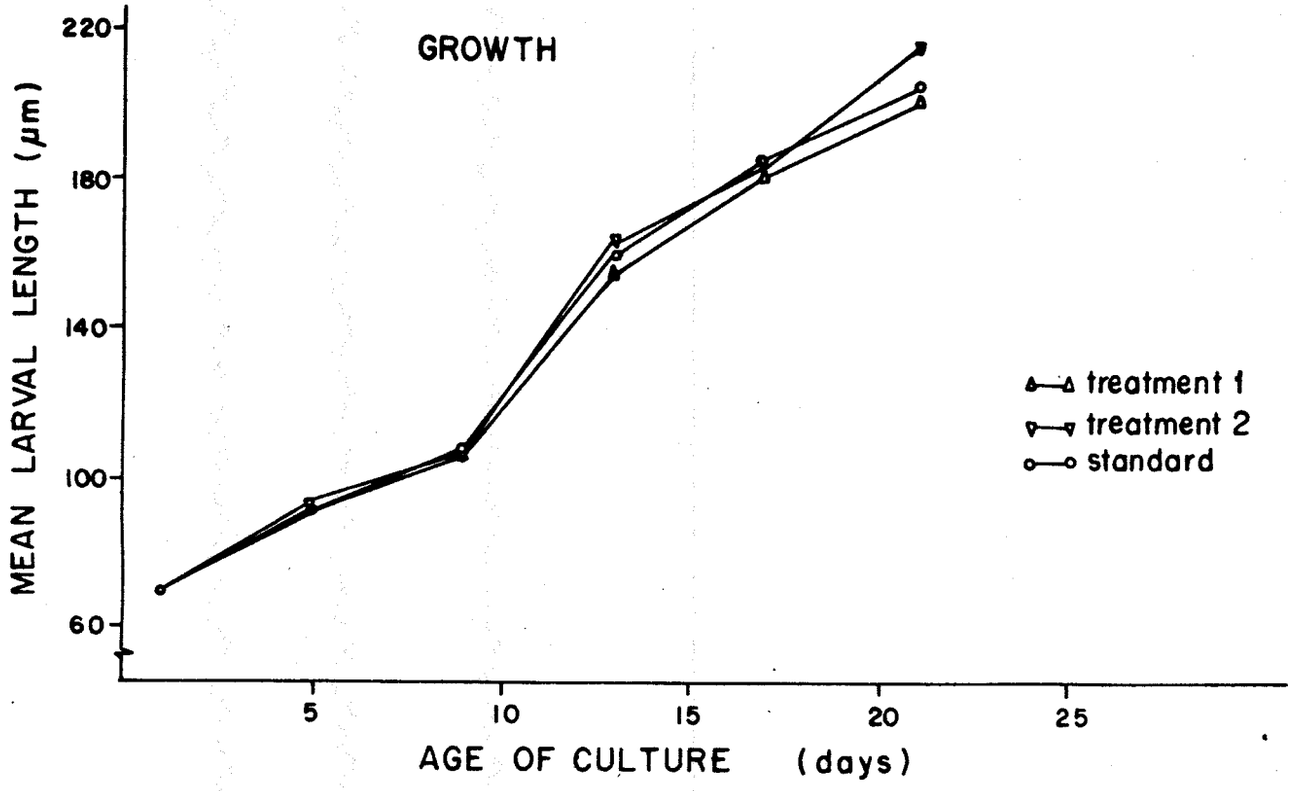
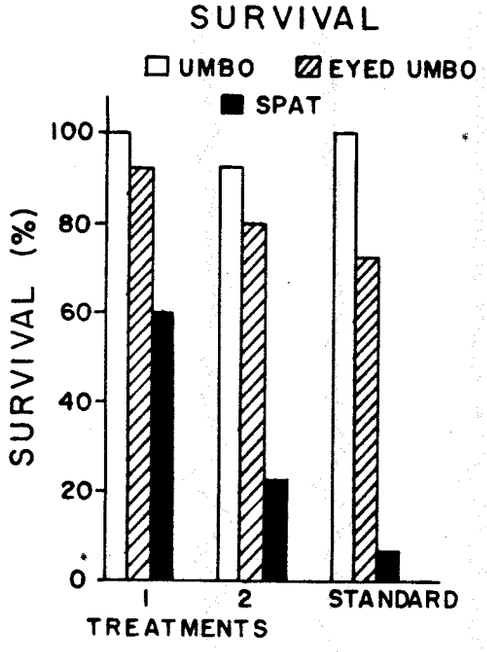


Fig. 40. GROWTH, SURVIVAL AND SETTING OF PEARL OYSTER LARVAE CULTURED WITH STREPTOMYCIN SULPHATE





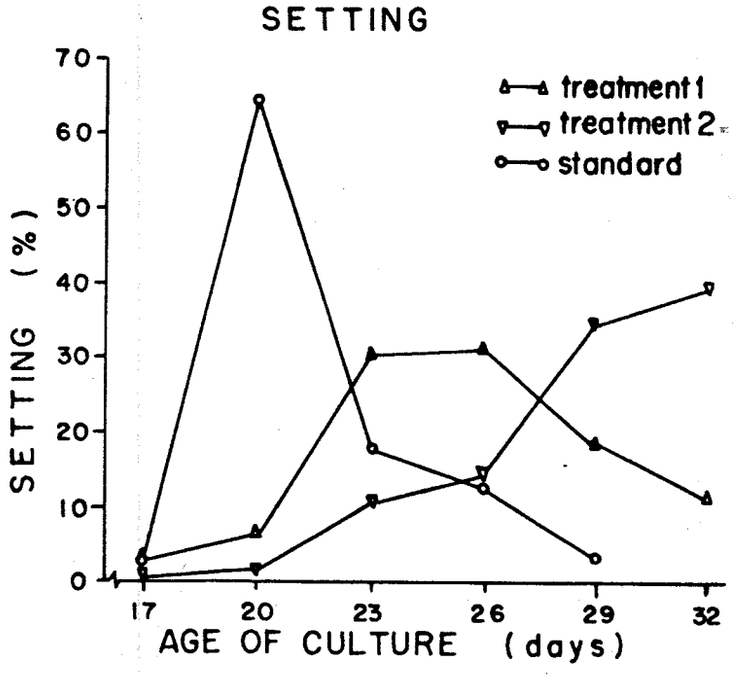


Table 89. Growth rate of larvae treated with streptomycin sulphate.

Period (days)	Larval growth per day (µm/day)		
	Treatment 1	Treatment 2	Standard
1-9	4.64	4.64	4.84
9-17	9.76	9.45	9.64
17-25	2.00	2.55	2.88
1-25	5.47	5.55	5.78

The growth regression values were 0.0250 log µm/day for treatment 1, 0.0242 log µm/day for treatment 2 and 0.0244 log µm/day for the standard. But the growth regressions were not statistically significant ($P > 0.05$).

2. Survival (Fig. 40)

Survival during the umbo stage was 100% for larvae of treatment 1 and standard and 92.0% for larvae of treatment 2. At the onset of the eyed umbo stage, survival was 92.0, 80.0 and 72.7% for larvae of treatments 1, 2 and standard respectively. Total spat production was 60.5% for treatment 1, 22.3% for treatment 2 and 6.8% for standard (Fig. 40).

3. Spat setting and spat production (Table 88, Fig. 40)

Setting began uniformly on day 17 and lasted the shortest duration of 12 days in the standard. Setting lasted for 15 days in treatments 1 and 2. Peak setting was observed on day 20 in standard, on days 22 and 26 for larvae of

treatment 1 and on day 32 for larvae of treatment 2. Total spat production between treatments and standard varied markedly. Percentage of setting was 60.5 in treatment 1, 22.3 in treatment 2 and only 6.8 in standard. There was highly significant statistical difference between percentage spat production of treatments and standard ($P < 0.01$).

B. Aeration

The experiment to study the effect of aeration at different larval stages was carried out during the month of November 1983, when range in ambient temperature, salinity and pH was 25.1-27.5°C, 32.6-35.2‰ and 8.10-8.15, respectively.

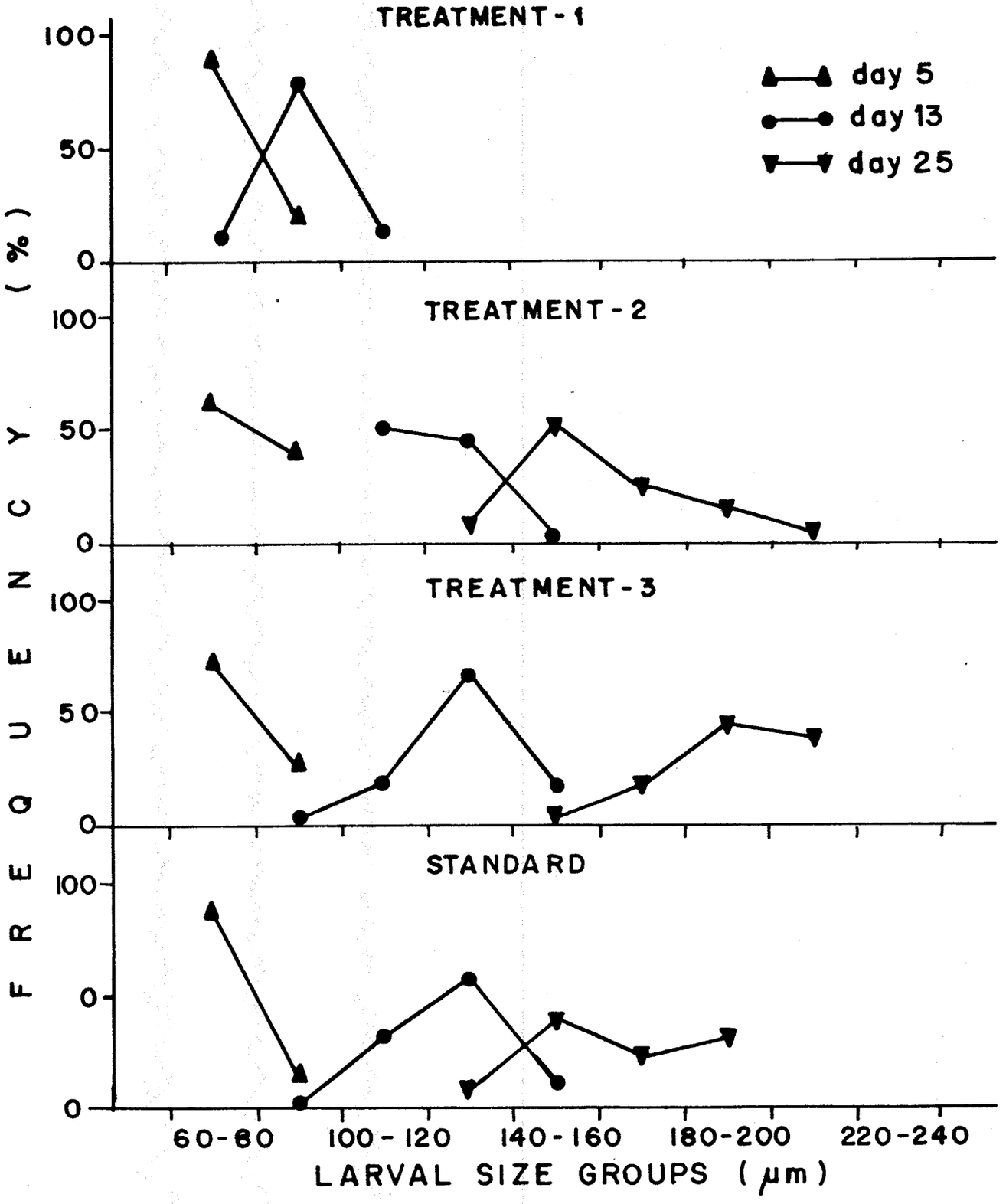
1. Larval growth (Tables 90 and 91, Figs. 41 and 42)

Larvae of treatment 2, where aeration was introduced from the D shape stage, have consistently recorded the least mean size up to day 21, beyond which none of them survived. Aeration was introduced in treatment 2 from day 12 onwards and the larvae have shown less mean size than that of the standard with no aeration. In treatment 3, aeration was introduced from day 19 and the highest mean larval size was recorded in this. From a uniform modal size group on day 5, size frequency distribution of larvae has differed subsequently on days 13 and 25 (Fig. 41). Growth rate for the

Table 90. Growth and setting of pearl oyster larvae cultured with aeration.

Age of culture (days)	Mean size of larvae (μm)			
	Treatment 1	Treatment 2	Treatment 3	Standard
1	71.1 ± 3.1	71.1 ± 3.1	71.1 ± 3.1	71.1 ± 3.1
5	77.0 ± 4.9	82.2 ± 3.8	80.9 ± 3.1	79.4 ± 3.1
9	86.6 ± 4.9	98.9 ± 4.9	98.6 ± 5.5	97.2 ± 3.7
13	94.7 ± 9.0	122.3 ± 11.1	128.7 ± 11.9	125.8 ± 10.6
17	108.7 ± 13.3	138.7 ± 14.4	145.5 ± 15.2	142.9 ± 15.9
21	114.5 ± 29.5	152.2 ± 15.6	175.1 ± 20.3	163.8 ± 24.0
25	-	169.3 ± 20.1	196.3 ± 16.9	175.4 ± 35.0
29	-	159.1 ± 27.7	191.9 ± 19.0	180.1 ± 21.6
33	-	151.2 ± 14.6	187.2 ± 16.1	193.9 ± 18.2
37	-	168.8 ± 22.8	192.1 ± 17.0	205.0 ± 16.2
Day of first setting	-	30	23	23
Day of final setting	-	41	38	41
Total No. of spat	-	225	2847	2268
Rate of spat production(%)	-	1.8	22.8	18.1

Fig.41. SIZE FREQUENCY DISTRIBUTION OF PEARL OYSTER LARVAE PROVIDED WITH AERATION



period day 1-25 was maximum in treatment 3 and the least in treatment 1 (Table 91).

Table 91. Growth rate of larvae cultured with aeration.

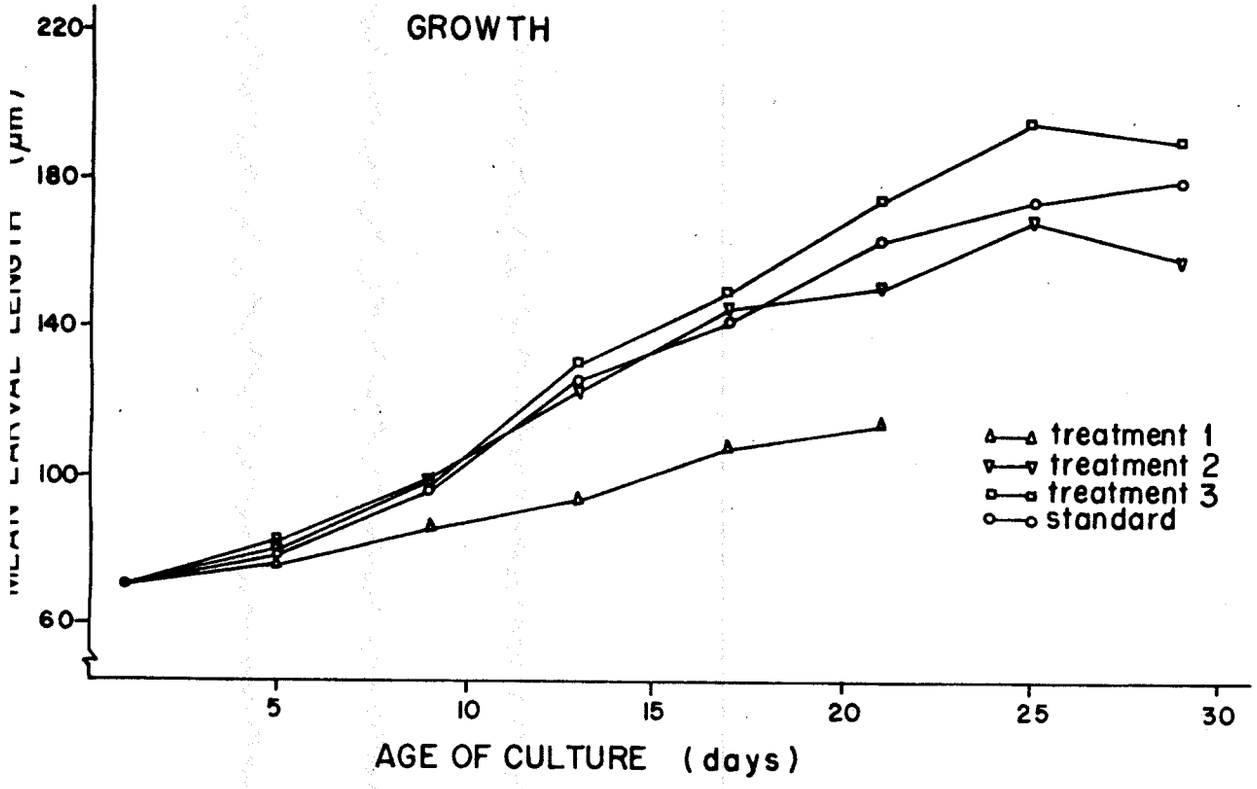
Period (days)	Larval growth per day (µm/day)			
	Treatment 1	Treatment 2	Treatment 3	Standard
1-9	1.94	3.48	3.44	3.22
9-17	2.76	4.90	5.86	5.71
17-25	-	3.90	6.35	4.06
1-25	-	4.09	5.22	4.35

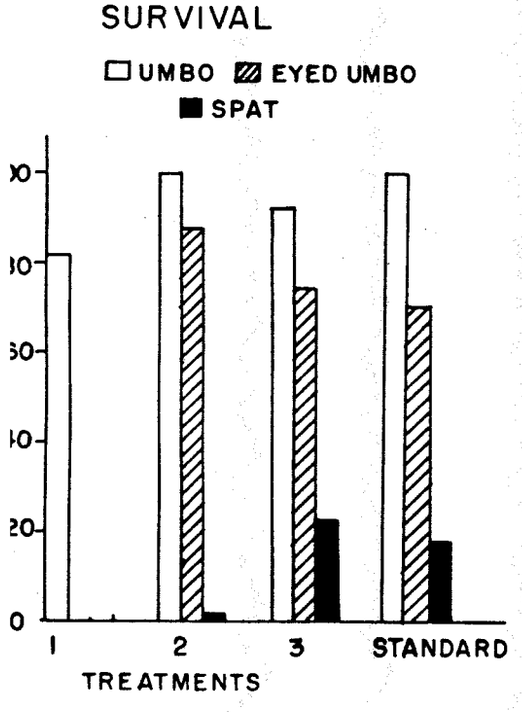
Linear regression analysis of the larval growth data yielded growth regressions of 0.0109 log µm/day for larvae of treatment 1, 0.0162 log µm/day for larvae of treatment 2, 0.0193 log µm/day for larvae of treatment 3 and 0.0184 µm/day for standard. The analysis of co-variance showed that there was statistically significant difference in growth regression of the treatments and standard ($P < 0.01$). The growth regression of treatment 1 was found to be statistically significant from that of all other treatments ($P < 0.01$).

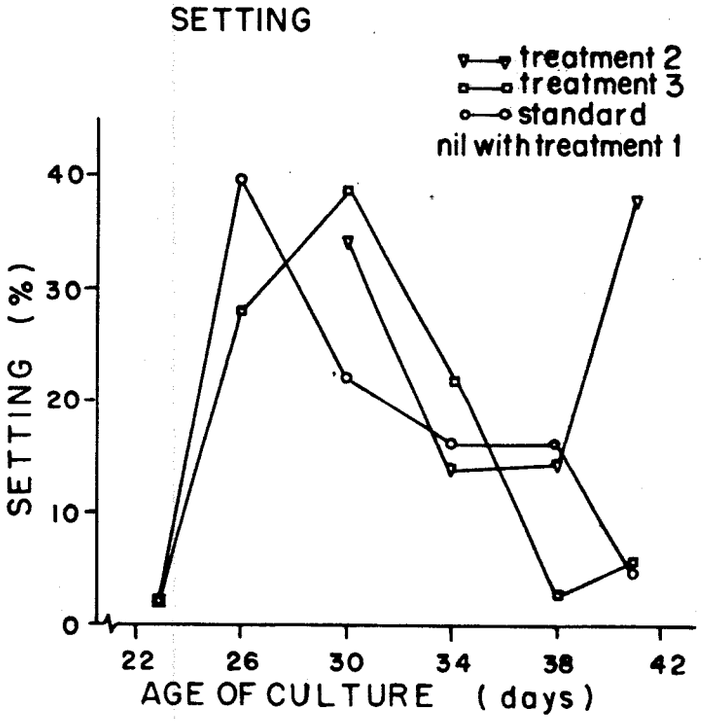
2. Survival (Fig. 42)

Survival during the umbo stage was 82.0, 100, 92.5 and 100% for treatments 1, 2, 3 and standard respectively (Fig.42).

Fig.42. GROWTH, SURVIVAL AND SETTING OF PEARL OYSTER LARVAE PROVIDED WITH AERATION







In treatment 1, none of the larvae survived beyond day 17, dying before they had reached the eyed umbo stage. For the eyed umbo stage, survival was 86.5, 73 and 75% for treatments, 2, 3 and standard respectively. Total spat production was 1.8, 22.8 and 18.1% for treatments 2, 3 and standard respectively.

3. Spat setting and production (Table 90, Fig. 42)

Setting began on day 23 in treatment 3 and standard and on day 30 in treatment 2. Setting lasted a period of 11 days in treatment 2, 15 days in treatment 3 and maximum of 18 days in standard (Table 90). Peak spat setting was observed on day 41 in treatment 2, day 30 in treatment 3 and on day 26 in standard. There was no spat setting in treatment 1. Percentage of spat setting was least in treatment 2 (1.8) and maximum in treatment 3 (22.8). Setting was 18.1% in standard with no aeration. Percentage of spat setting was statistically significant between all treatments ($P < 0.01$).

C. Continuous flow system

The efficacy of rearing pearl oyster larvae in continuous flow system after the onset of the umbo stage was tested in an experiment carried out during the month of March, 1984. Rangesin ambient conditions of temperature,

salinity and pH were 24.7-26.3°C, 30.5-34.5‰ and 8.10-8.15, respectively.

Larval growth (Tables 92 and 93, Figs. 43 and 44)

Mean size of pearl oyster larvae up to day 13 has been comparable (Table 92). From day 17 onwards, mean size of larvae cultured in the continuous flow system has been greater than mean size of larvae in a static water system. Similar modal size groups occur on day 5, day 13 and on day 25 (Fig.43) for both treatments. Overall growth rate for the period day 1-25 has been greater for larvae maintained in a continuous flow system as compared to the static water system (Table 93).

Linear regression analysis of larval growth data yielded growth regression of 0.0252 log μm/day for larvae of the continuous flow system as compared to 0.0244 log μm/day for larvae of the static system. There was no significant difference in the growth regressions ($P>0.05$).

Table 92. Growth and setting of pearl oyster larvae in a continuous-flow system.

Age of culture (days)	Mean size of larvae (μm)	
	Continuous-flow system	Static water system
1	69.2 + 2.3	69.2 + 2.3
5	93.8 + 3.9	92.4 + 4.0
9	105.8 + 5.1	107.9 + 7.1
13	158.0 + 15.1	159.0 + 18.5
17	194.0 18.0	185.0 + 24.5
21	209.0 10.1	204.8 + 13.2
25	215.6 15.2	208.0 + 12.4
Day of first setting	17	17
Day of final setting	29	29
Total no. of spat	4877	848
Rate of spat production (%)	39.0	6.8

Fig. 43. SIZE FREQUENCY DISTRIBUTION OF PEARL OYSTER LARVAE CULTURED IN A CONTINUOUS FLOW SYSTEM

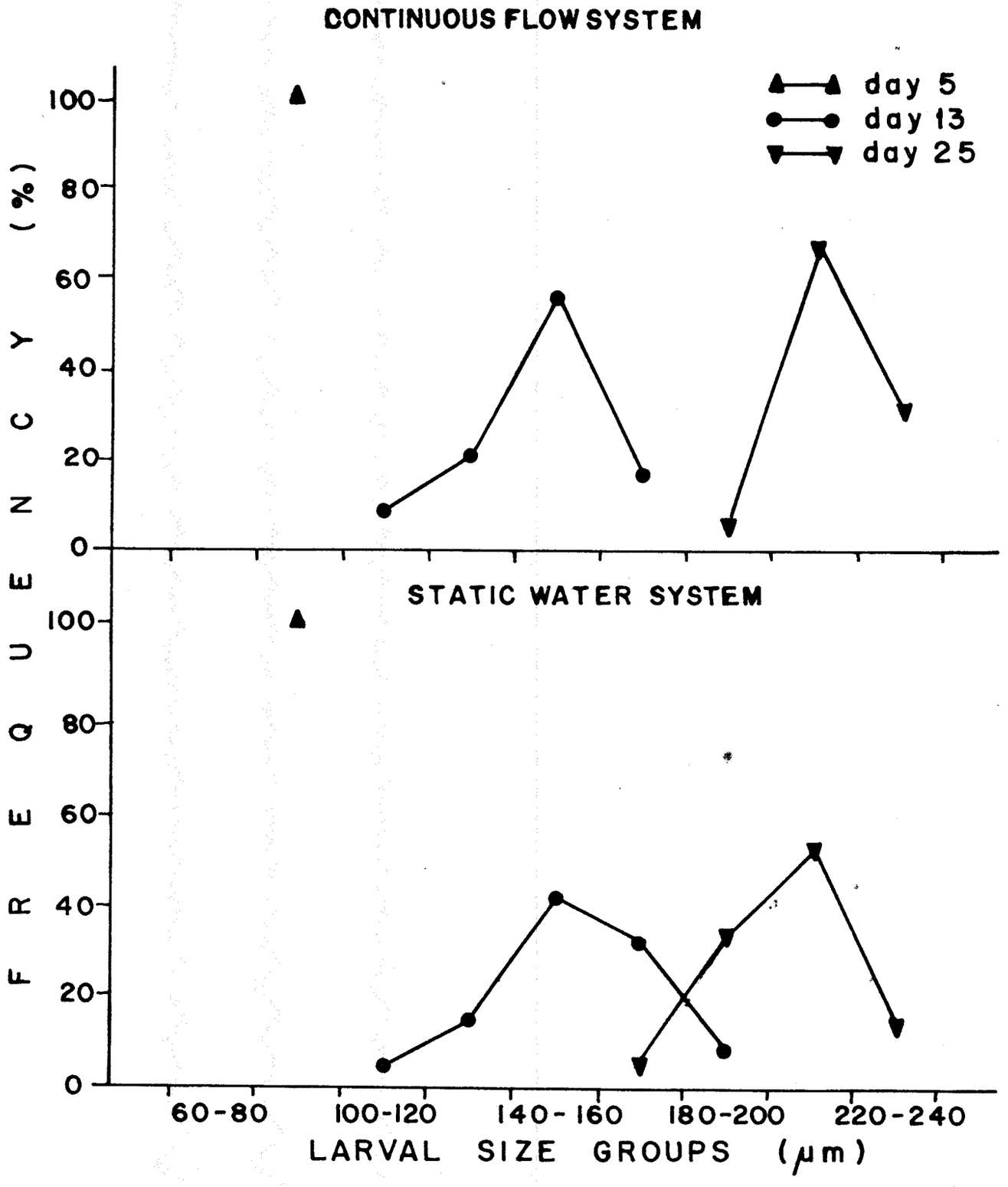


Table 93. Growth rate of larvae cultured in a continuous flow system.

Period (days)	Larval growth per day (μm/day)	
	Continuous flow system	Static Water System
1-9	4.58	4.84
9-17	11.03	9.64
17-25	2.70	2.88
1-25	6.10	5.78

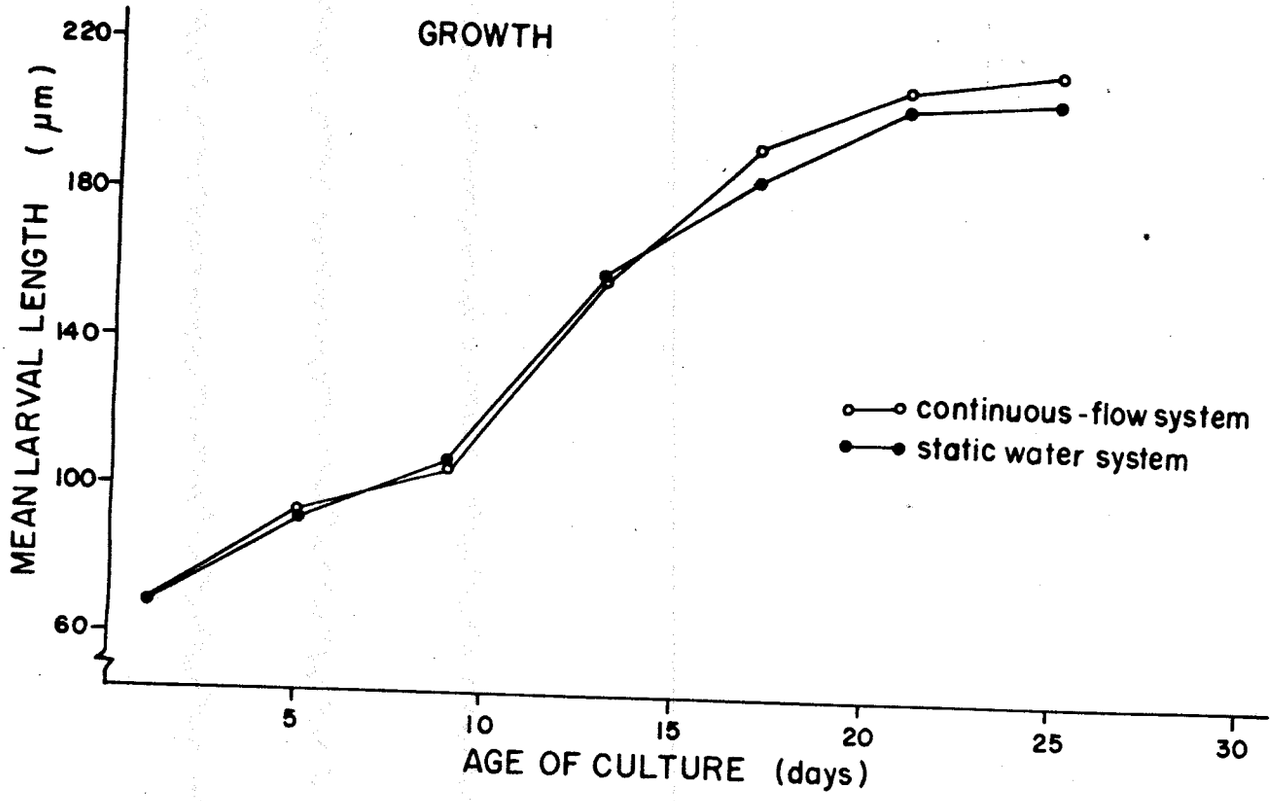
2. Survival (Fig.44)

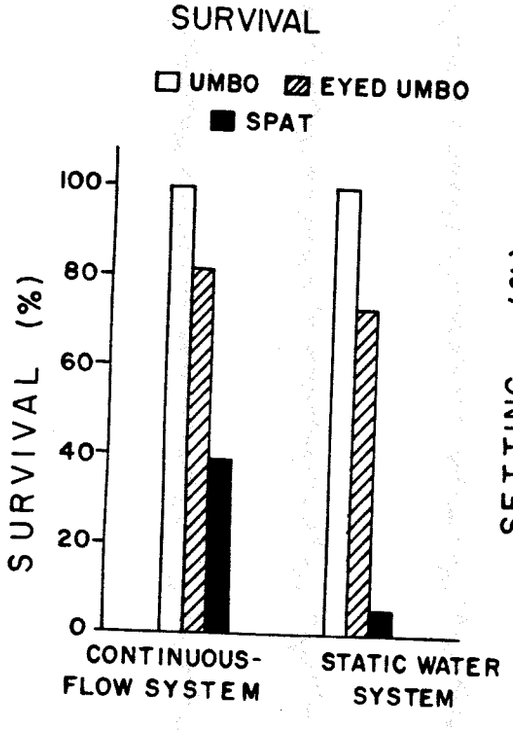
Percentage larval survival at the onset of the umbo stage was 100% for both treatments (Fig.44). At the eyed umbo stage, survival was 81.8% for larvae reared in the continuous flow system as compared to 72.7% of larvae of the standard. Total spat production was 39.0% in the continuous flow system and 6.8% in the static water system.

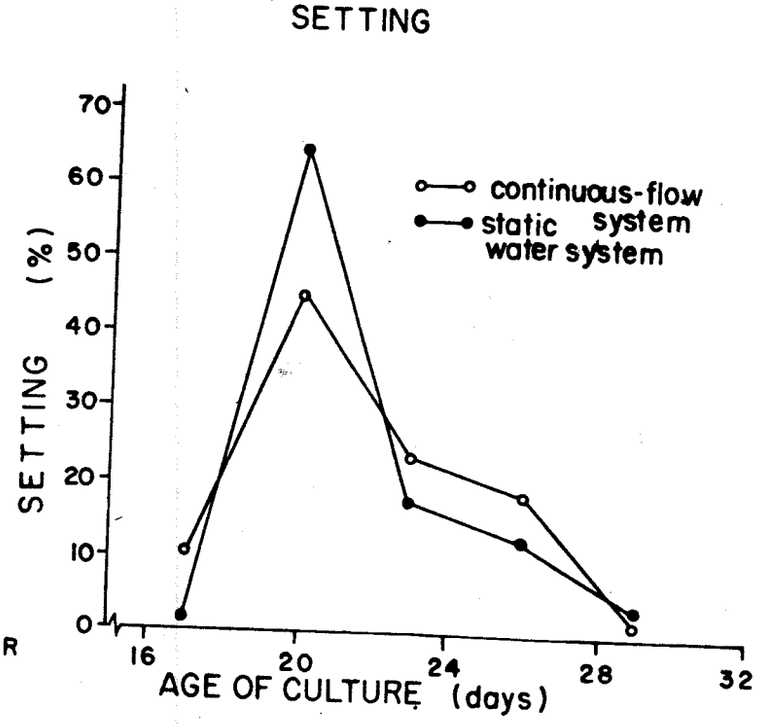
3. Spat setting and production (Table 92, Fig.44)

Spat setting was seen to follow the same trend for both treatments. Setting was initiated on day 17 and lasted a total of 12 days up to day 29 in both treatments. Peak setting was observed on day 20 (Fig.44). Total spat production in the continuous flow system was significantly greater than that of standard ($P < 0.01$). Percentage spat production was 39.0% for the former, as compared to 6.8% in the standard.

Fig.44. GROWTH, SURVIVAL AND SETTING OF PEARL OYSTER LARVAE CULTURED IN A CONTINUOUS-FLOW SYSTEM







DISCUSSION

In the present study, streptomycin sulphate was used at the single dosage level of 8 mg/1 either as additions to the rearing medium as commonly practised in most hatcheries or in the form of "washes" with every water change. Although there was relatively no difference in larval growth rates between the two treatments, there was a remarkable difference in the total number of spat that reached setting stage. A total of 60.5% of the initial population reached setting stage for the antibiotic wash treatment as compared to 22.9% for antibiotic addition. In the standard with no antibiotic, spat production was only 6.8%. These results would show that antibiotic wash of larvae is more desirable than its addition to the rearing medium.

Ukeles (1975) listed a number of reasons for not recommending the routine use of antibiotics to the larval rearing medium. These included the prohibitive cost for a commercial venture, the development of resistant strains of bacteria, unexpected side effects on larvae by the fillers used in technical grade antibiotics, stimulation of growth of moulds by inhibition of bacterial populations and the formation of larval anomalies. Antibiotics were, therefore, recommended only for use in emergencies. Antibiotic "washes" appears to provide a safe alternative to the above. However, administration of antibiotics would depend on situations which

can vary from vessel to vessel, particularly in static rearing conditions.

Besides Loosanoff and Davis (1963 b), most studies on larval nutrition where water was changed on alternate days have not provided aeration during rearing (Bayne, 1965; Minaur, 1969; Wada, 1973; Rhodes and Landers, 1973; AQUACOP, 1979; Ewart and Epifanio, 1981; Coeroli et al., 1984). Some workers have provided aeration routinely during larval culture (Walne, 1974; Dupuy, 1975; Wilson, 1978; Cary et al., 1981). Helm and Spencer (1972) advocated the use of aeration for C.gigas only after the larvae attained a length of 120 /um. Nascimento (1980) observed depressed growth of C.gigas larvae in aerated cultures given the diets Chaetoceros calcitrans or Pyramimonas virginica. Using I. galbana and Pseudoisochrysis paradoxa as diets, however he did not find any significant differences between larval growth rates of aerated and non-aerated cultures.

The results of the present study indicate the effects of aeration on pearl oyster larval growth and setting. When introduced at the D shape stage growth rate was poor (growth regression, 0.0109 log /um/day) and none of the larvae survived beyond day 25. Larvae that were given aeration from the umbo stage, exhibited growth rate that was less than that of control. On the other hand, introduction of aeration at the eyed umbo stage, enhanced larval growth rate. Total spat

production amounted to 1.8, 22.8 and 18.1% in the treatments where aeration was introduced at umbo and eyed umbo stages and in the control. These results would show that for the pearl oyster larvae, aeration is not desirable at the early stages of larvae but, given at later stages, it can not only enhance growth but also improve spat production. This follows closely the observation of Helm and Spencer (1972) on C.gigas larval rearing.

Ukeles (1975) stressed the importance of avoiding static water conditions in larval rearing. Most studies, however, have adopted static water systems for rearing (Rhodes and Landers, 1973; Wada, 1973; Walne, 1974). Loosanoff (1969) reported that the initial efforts at the Milford laboratory in rearing oyster larvae in slowly flowing water had consistently met with failure. Very few attempts have been made to rear larvae in a continuous flow system (Malouf and Breese, 1977; Wilson, 1978). In the present study, a continuous-flow system has yielded better results on larval growth and spat production. Larval growth rate was 6.10 µm/day in the flow system as compared to 5.78 µm/day for the static system. For the period day 9-17, the growth rate of 11.03 µm/day was the highest recorded during the entire study. There was also considerable difference in total spat production, being 39.0% in the continuous flow system and 6.8% in the static water system. The continuous flow system has the distinct advantage

offering an alternative to frequent handling of larvae, thereby reducing stress, and in avoiding the build up of metabolities in the medium. Malouf and Breese (1977) pointed out that the continuous flow system could give the maximum return in terms of larval growth.

SUMMARY

1. 'Studies on larval nutrition in the pearl oyster Pinctada fucata (Gould)' comprises a critical evaluation of the nutritional value of microalgal diets for larval growth and spat setting; quantitative estimations of filtration rate and uptake and retention of algal cells; evaluation of a few non-living diets; and biochemical investigations on pearl oyster larvae and the tested microalgal diets. Investigations on the role of environmental parameters and some aspects of water quality management were also taken up in the present study. Several sets of experiments, each with a specific objective, with standard and control for comparison, were carried out in the experimental shellfish hatchery laboratory at Tuticorin.

2. A comprehensive set of factors, larval growth, survival spat setting and production and algal cell consumption was used to assess the nutritional value of all tested diets in promoting larval growth and spat settlement. Appropriate statistical tests were employed to compare the results within each set of experiments to understand the significance of difference, if any.

3. Rearing pearl oyster larvae in the density range of 1-10 larvae/ml, it was evident that larval densities influence both growth and setting. At the lower densities, efficiency in utilisation of the available algal cells in the rearing medium is reduced, leading to a wastage of food cells. At the higher larval densities, poor growth and setting were observed. This may be due to a possible competition for food, frequent collisions and accumulation of excretory products. The optimum larval density for rearing pearl oyster larvae at the cell concentration of 25 cells/μl was seen to be 5 larvae/ml.

4. The concentration of algal cells in the medium is of importance in larval rearing inasmuch as it influences the rate of filtration of larvae and consequent consumption. Pearl oyster larvae reared at the algal cell concentration range of 10-100 cells/μl of both Isochrysis galbana and Pavlova lutheri showed maximum growth at 25 cells/μl for both algal species. Setting was maximum at 25 cells/μl for I. galbana and at 10 cells/μl for P. lutheri. The poor growth of pearl oyster larvae at the higher algal cell concentration of 50 and 100 cells/μl has been attributed to mechanical interference to the filtering apparatus, heavy pseudofeces formation, increased algal metabolites and poor retention time within the gut.

5. The microalgae Chromulina freiburgensis, Synechocystis salina and Tetraselmis gracilis were tested for their food value at the uniform cell density of 25 cells/µl. Results indicated that C. freiburgensis is a nutritionally adequate diet, but not superior in value to that of I. galbana. Growth and survival of pearl oyster larvae fed with S. salina indicate that the species is of 'poor' food value. Although larvae survived for a total period of 53 days, growth was exceedingly poor. There was no spat setting. Pearl oyster larvae fed with T. gracilis exhibited very poor growth rate and a total failure to metamorphose. Mortality was total by day 17.

6. An examination of the algal cell consumption data of the tested microalgal species at 25 cells/µl revealed high consumption of I. galbana (50-80%), P. lutheri (60-80%), and S. salina (65-85%), marginally lower consumption of C. freiburgensis (25-45%) and poor consumption of T. gracilis (10-15%). The difference in their cell consumption may possibly be due to their cell size, the larger cells (T. gracilis and C. freiburgensis) being consumed to a lesser extent than the smaller cells (I. galbana, P. lutheri and S. salina).

7. The combination diets of I. galbana + P. lutheri, I. galbana + C. freiburgensis and I. galbana + S. salina were seen to be nutritionally adequate foods for pearl oyster

larvae. On the other hand, the combination diets of I. galbana + T. gracilis, I. galbana + S. salina + T. gracilis and S. salina + T. gracilis were nutritionally inadequate foods for pearl oyster larvae. Differences in nutritional value of different algal species have been discussed in relation to their physical characteristics.

8. Based on the larval growth regressions (log μm/day) obtained for different algal species, a food value index was prepared to rank the diets for nutritional value. In order of merit, these algal diets may be written as: P. lutheri; I. galbana + P. lutheri; I. galbana; I. galbana + C. freiburgensis; I. galbana + S. salina; C. freiburgensis; S. salina; I. galbana + S. salina + T. gracilis; I. galbana + T. gracilis and S. salina + T. gracilis.

9. Larval growth and setting results obtained on a stratified feeding schedule with I. galbana showed that quantitatively, algal cell requirements would increase from 15 cells/μl of I. galbana at the D shape stage to 35 cells/μl at the eyed umbo stage. The stratified feeding protocol using I. galbana, T. chui and C. salina, however, showed variable results, the larval growth data and the setting percentages showing no particular trend.

10. The mixed phytoplankton culture that was developed from a stock of natural plankton collected from the open

sea proved to be of good food value to the larvae when fed from the eyed umbo stage onwards. The earlier stages of D shape and umbo larvae met with mortality when fed on a diet of mixed phytoplankton.

11. Quantitative estimates of filtration rates at four increasing cell concentrations from 10 to 100 cells/µl, showed two distinct trends: an increase in filtration rate with increase in larval size; and a decrease in filtration rate with increase in cell concentration. When studying the actual number of cells removed from the rearing medium, it was seen that greater numbers of cells were being removed from the medium at the higher cell concentration.

12. The use of radioactive C^{14}-labelled algal cells to make quantitative estimates of the actual number of cells taken in by the larvae showed that 2129 to 2729 I. galbana cells, 2151 to 3341 of P. lutheri and slightly lower values of 1531 to 2326 of C. freiburgensis cells were actually consumed by the larvae. Retention was approximately 67.1% to 80.4% of the total number of cells consumed. The increase in cell concentration from 25 to 50 cells/µl did not increase the uptake of algal cells substantially. The uptake of C. freiburgensis cells was quantitatively lower than that of I. galbana.

13. Relating the values obtained from clearance and those obtained while using labelled microalgae, it was seen

that pearl oyster larvae is a wasteful feeder. It is suggested that at high densities of 50 cells/μl, a major part of cells cleared from the medium may either be rejected as pseudofeces or poorly assimilated.

14. Two types of non-living diets, freeze dried *I. galbana* cells and a carrageenan bound microparticulate diet (CBMD) were evaluated for their food value. Results indicated that although pearl oyster larvae could utilise the freeze dried algal cells for growth, its nutritional value is more when introduced to larvae of later stages than the earlier D shape stage. However, it is a poor substitute for live algal cells of *I. galbana*. With CBMD, there was complete mortality of larvae at each stage. Using dissolved nutrients it was seen that although growth was better than that of starved larvae, there was no larval metamorphosis.

15. With increase in larval size, the total organic matter was seen to increase from the D shape stage to the eyed umbo stage. This increase in organic matter was largely in the form of protein and lipid and to a lesser extent in carbohydrate. With the onset of metamorphosis, levels of lipid, protein and carbohydrate were seen to decrease. It is suggested that a major part of this energy is diverted for use during metamorphosis. The role of neutral lipid seems to be more important. Biochemical

analysis of the tested algal species did not reveal any gross differences in composition of different species.

16. Monofactorial experiments were conducted to study the effects of temperature, salinity and pH on larval growth and setting. There was a direct relationship between growth and temperature within the temperature range of 20-32°C. Larvae reared at 20°C, did not grow beyond the early straight hinge stage and none survived beyond day 21. Growth and setting was influenced to a lesser extent by salinity. Within the pH range of 7.5-9.0, maximum growth and setting was observed at ambient pH(8.1).

17. Studying some aspects of water quality management, it was seen that antibiotic streptomycin sulphate greatly enhanced total spat production when provided as 'washes'. When added to the rearing medium, spat production was increased slightly. Aeration depressed growth rate when introduced at the D shape stage, but enhanced growth and setting when introduced during the eyed umbo stage. The continuous-flow system may be used effectively after the umbo stage. Spat production was greatly enhanced by such a system.

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