DOCTOR OF PHILOSOPHY

Growth and survival of Penaeus monodon (Fabricius) larvae and postlarvae on natural and artificial diets.

Amjad, Shahid.

Award date:
1990

Awarding institution:
Bangor University

GROWTH AND SURVIVAL OF PENAEUS MONODON (FABRICIUS) LARVAE AND POSTLARVAE ON NATURAL AND ARTIFICIAL DIETS

A THESIS SUBMITTED TO THE UNIVERSITY OF WALES

by

SHAHID AMJAD

M.Sc (Karachi), Cand. Scient. (Oslo)

IN CANDIDATURE FOR THE DEGREE OF PHILOSOPHIAE DOCTOR

School of Ocean Sciences,
University College of North Wales,
Menai Bridge,
Gwynedd, LL59 5EY.
U.K.

May, 1990.

ACKNOWLEDGEMENTS

I am indebted to Dr D.A. Jones for his excellent supervision throughout the course of this study. I am also grateful to Dr A.B. Yule for his advice and encouragement, and similarly thank Drs D. Holland, G. Walker, D.J. Grove and I.A.N. Lucas for their useful advice. Assistance provided by Mr M.D. Budd, Mr M. Abubakr, Mr P. Intriago, Mr G. Novarino, Mr B.W. Roberts, Mr P. Dewes and Miss C. Boutle is gratefully acknowledged. I am also grateful to Dr M. Keith, Research Director, Frippak Feeds for providing larvae and Frippak diets.

The Ministry of Science and Technology, Islamabad and The National Institute of Oceanography, Karachi is acknowledged and thanked for the provision of a scholarship award.

I am also grateful to Mrs E. Jones for typing the thesis. I also thank my family for their continued support and encouragement during this period.

SUMMARY

GROWTH AND SURVIVAL OF *Penaeus monodon* (Fab.) LARVAE AND POSTLARVAE ON NATURAL AND ARTIFICIAL DIETS.

Larval development of *Penaeus monodon* from protozoea (PZ1) to postlarval stages is described on natural and commercially available artificial larval diets under controlled laboratory feed trials.

Five species of live microalgae (*Tetraselmis chuii, Chaetoceros calcitrans, Skeletonema costatum, Rhodomonas baltica* and *Pavlova lutheri*) were evaluated in feed trials at seven (5-60 cells μl^{-1}) cell concentrations with protozoeal larval stages. Performance of five animal diets (rotifers, *Mytilus* eggs, *Artemia* nauplii, barnacle nauplii and crab eggs) were examined in feed trials from Mysis (M1) to postlarvae (PL1). Best results for protozoeal stages were obtained on a combination diet *T. chuii* + *R. baltica* at 40 cells μl^{-1}, while results from mysis to postlarvae were best achieved on *Artemia* nauplii. Natural feeds are expensive, difficult to maintain in mass culture and often vary in nutritional value.

Larval performance on ten leading brands of artificial larval diet marketed using different process technologies were evaluated in replicated feed trials, together with factors causing variability in larval growth and survival of *P. monodon.* It was found that nutrient leach loss from unstable artificial feeds in culture conditions increases levels of ammonia and nitrite beyond safe limits. Live microalgal species at 1000 cells μl^{-1} added to culture water reduces these toxic levels. The role of microalgae in larval culture is also examined.

To further improve larval growth and survival on artificial diets, proteins from different sources, lipid, vitamin and mineral levels were incorporated in microencapsulated feeds. As a result improvement in larval development to postlarval stage was achieved with feeds containing 60% soya + mussel protein, 5% lipid and 13.9% vitamin and mineral mix. Algal homogenates supplemented to encapsulated feeds produced larval growth and survival comparable to live feed controls.

P. monodon larvae were grown to the juvenile prawn stage solely on Frippak feeds. Larval growth was comparable to growth achieved on live feed controls.

This study forms a basis for further investigations into nutritional requirements of penaeid prawns.

CONTENTS

GENERAL INTRODUCTION

INTRODUCTION

An intensification of the effort devoted to prawn farming worldwide produced 565,000 metric tons (live weight) in 1989, which was 26% of the total prawns harvested from the world oceans (2.2 million metric tons). These farm cultured prawns fetched 6 billion dollars at retail prices (Rosenberry, 1988; 1989). Whilst the demand for fish and shellfish continues to grow at a rapid rate, predicted by FAO to be 130 million metric tons by early next century, the traditional fishery resources from the world oceans fast approach the predicted maximum sustainable yield levels of 90 million metric tons (FAO 1982), any further exploitation of these resources by using modern fishery techniques would merely accelerate further depletion resulting in decreased commercial yields. Hence aquaculture is perhaps the only way to redress the imbalance between supply and demand. Although aquaculture will by no means replace traditional fisheries, it provides a means to augment natural productivity.

Prawn culture in recent years has evolved into a new industry and has attracted considerable interest worldwide. Currently there are in over forty countries 31,000 prawn farms, these are supported by 3,500 hatcheries and 765,000 hectares of pond production (Rosenberry, 1988). Production is centred around South and Central America and Asia, with Ecuador as the major producer (70,000 metric tons) in the former region, and Taiwan, China, Indonesia, Philippines, India and Thailand as the main Asian contributors (300,000 metric tons, Rosenberry,

1988). Since prawns fetch high prices in the world (U.S. $10. kg^{-1}) there is often a strong desire in many developing countries to rapidly develop ponds and cultivate prawns in captivity. Often initially profitable ventures become economically marginal due to lack of scientific knowledge. The success of prawn farming depends on the availability of essential inputs such as viable seed stocks, nutritionally balanced and readily available diet and proper management.

Prawn farms essentially require dependable post larval stocks either from the wild or from prawn hatcheries. Availability of penaeid prawn larvae for stocking in growout ponds from natural breeding grounds in the creeks is seasonal. Juveniles collected from the breeding grounds are often mixed with many unwanted pests and separating the desired species of penaeids from each other becomes a cumbersome and time consuming task. Further, the supply of post larvae from the wild can be extremely unpredictable, depending on environmental factors (Jones, 1988). On the other hand hatchery produced seed stock are selective and can be made available on year-round basis in substantial quantities for extensive, semi-intensive or intensive prawn farming. To keep pace with the demand for seed prawn hatchery construction has increased dramatically since 1975 with the result that there are now estimated to be some 1500 hatcheries in Taiwan, 1000 in Thailand, 400 in the Philippines, 300 in China, a 100 in Ecuador and 90 in Indonesia (Rosenberry, 1988). Production of post larvae in Ecuador alone produced 3-4 billion post larvae

in 1987 which ultimately produced 70,000 metric tons of marketable prawns (Rosenberry, 1988). Despite this larval production level Ecuador still cannot supply prawn farm post larvae requirements from hatcheries, and continues to depend upon wild seed for stocking of prawn farms (Wickins, 1986; Sandifer, 1988). In a number of countries in South-east Asia, Central and South America hatchery capacity is being increased to augment shortage of post larvae (Wickins, 1986). The full production potential of hatcheries depends not only on larval survival and nutrition, but also on disease and water quality. The lack of essential nutrients can result in specific deficiency diseases as well as general mortality and retardation of growth (Kanazawa et al., 1982). Larval nutrition is therefore one of the most important factors affecting larval development of penaeid prawns (Yufera et al., 1984).

Various aspects of the dietary requirements of penaeid species have been studied. New (1976, 1980) presented an extensive bibliography of research directed towards understanding the basic nutritional requirements of penaeids. Much of the work reviewed is concerned with defining nutritional requirements with the aim of preparing a formulated feed high in nutritional value but at a relatively low cost. Many of the dietary requirements for adult penaeids are known (Kanazawa et al., 1970, 1971; Kitabayashi et al., 1971; Deshimaru and Shigueno, 1972; Kittaka, 1976; Forster, 1976; Wickins, 1982). The influence of dietary protein and energy levels on growth of penaeid prawns have been studied by Andrew

et al., 1972; Sedgwick, 1979; Teshima et al., 1986; and the utilization of carbohydrates by penaeids in the form of polysaccharides by Sick and Andrews 1973; Alva and Pascul 1987. Fisher (1960) reviewed requirements of trace elements, vitamins and minerals of crustaceans. By supplementing vitamins and minerals in the diet of *P. merguiensis* Sedgwick (1980) showed improvement in the growth rates of the prawn. Despite the recent advances in understanding adult and juvenile prawn nutrition, much less emphasis has been given to determining specific metabolic requirements essential for penaeid larval growth. This has been limited to a few studies on lipid biosynthesis (Jones et al., 1976, 1979b, Kanazawa et al., 1985), energy budgets (Emmerson, 1980, 1984; Yufera et al., 1984; Kurmaly et al., 1989b) and digestive enzyme (MacDonald et al., 1989).

Currently, most prawn hatcheries rely on live foods such as microalgae (diatoms, flagellates), rotifers, *Artemia* and other planktonic crustaceans as feed sources (Bardach et al., 1972; Liao, 1983; Langdon et al., 1985). Acquisition and culture of live food on a commercial scale is frequently difficult to control, both seasonally and regionally, and costly in terms of space and manpower utilization. Also out door algal cultures, which are conventionally used in hatcheries, rapidly lead to collapse of the culture or are taken over by other species better adapted to prevailing outdoor conditions (James et al., 1988). This has often been considered to be the most serious impediment to the development of commercial aquaculture to its fullest potential (Bardach et

al., 1972; Kanazawa et al.,1977a; Scott and Middleton, 1979; Langdon and Waldock, 1981; Watanabe et al., 1983; Scura, 1984; Lei and Su, 1985). Algal monoculture for penaeid planktonic, suspension feeding larval stages are often difficult (Liao 1983), expensive to maintain (Helm and Laing, 1981; Fox, 1983) and unreliable in supply and nutritional value (Persoone and Claus, 1980; Langdon et al., 1985; McConaugha, 1985).

The penaeid larva changes from a herbivorous to an omnivorous mode of feeding during metamorphosis. Although several species of small motile animals could serve as food for penaeid larval stages (Watanabe et al., 1983; McConaugha, 1985) *Artemia* nauplii and the rotifer *Brachionus* are the only prey organisms available commercially for late protozoea, early mysis and post larval stages (Hudinaga and Kittaka, 1967; Cook and Murphy, 1969; Shigueno, 1975; Lumare et al., 1978; Liao et al., 1983; Sorgeloos et al., 1983; Yufera et al., 1984). Use of rotifers is restricted in hatcheries due to difficulties in mass culture (Schluter et al., 1987; Snell et al., 1987).

Artemia are expensive (U.S. $ 70 Kg^{-1}) and there is concern over availability and nutritional variability between strains (Wickins, 1972; Watanabe et al., 1980, 1983; McConaugha, 1985; Leger et al., 1985, 1986), however they are preferred over other prey organisms as *Artemia* cysts constitute the most practical food (Sorgeloos, 1980). Introduction of live feeds also provides a source of pollution (Bookhout and Costlow, 1970; Mock et al.,1980a) and of parasitic invasion in the culture system (Spotte, 1971). Contaminated *Artemia* cysts can also introduce pathogens into the hatchery (Scura et al.,

1984). Accumulation of ammonia and nitrites excreted by prawn and live crustacean prey organisms in prawn hatcheries also have detrimental effects on larvae, juvenile and adult prawns in closed culture systems (Wickins, 1976; Mevel and Chamroux, 1981; Chen et al., 1986; Chen and Chin, 1988; Jones et al., 1989). Therefore, exclusive use of live foods in a prawn hatchery and growout nursery ponds is not always desirable.

In order to obtain predictable production of high quality hatchery reared penaeid post larvae, feeding regimes must be improved (Wilkenfeld et al., 1981; Quinitio et al., 1983; Kuban et al., 1985). Hence pressure to develop artificially produced diets to replace cultured live food organisms has intensified in the recent years, to alleviate many of the problems currently limiting hatchery production. These inert feeds should be readily acceptable (Moller et al., 1979; Ache, 1983), neutrally buoyant (Meyers, 1971) right size range (Jones et al., 1974; Clark et al., 1986), water stable (Jones et al., 1979b). Their use will simplify hatchery design and capital cost requirement, thereby facilitating small scale hatchery development.

Various types of natural products and artificial particulates have also been used for larval rearing (table 1). Natural products are processed or prepared either by spray drying or freeze drying microalgae, yeast and shellfish crumbles (Sick and Beaty, 1975; Murai and Andrews, 1978; Mock et al., 1980b) but have had limited success in hatcheries due to cell lysis with subsequent bacterial fouling of larvae culture medium. Teshima and Kanazawa (1983) report that

P. japonicus larvae grow better on freeze-dried microgel food particles which were prepared simply by mixing dietary ingredient together with a gelling agent to encapsulate organic solvent e.g. calcium alginate, carageenan, Zein or agar (Kanazawa et al., 1982; Teshima et al., 1983 ; Langdon, 1983; Levine et al., 1983; Langdon et al., 1985). However these diets have poor water stability (Adron et al., 1974; Jones et al., 1979a) and accelerate culture collapse due to the high feeding densities required by filter feeders (Gatesoupe et al., 1977; Goldblatt et al., 1980).

Recognizing the importance of inert larval feeds in the development of aquaculture Jones et al., (1974) adapted and developed the microencapsulation techniques introduced by Chang et al., (1966) to produce nylon-protein microcapsules containing haemoglobin as artificial red blood cells. Jones et al., (1974) used nylon-protein microcapsules to deliver nutrients to aquatic filter feeders, and to study the nutritional requirement of crustaceans larvae. Jones et al., (1979a, 1979b) studied the nutritional requirement of *P. japonicus* larvae through metamorphosis using a microencapsulated diet. Microcapsules have since been used by numerous workers to study ingestion growth and survival amongst larval forms.

Attempts to culture a wide range of other crustacean larvae on nylon-protein encapsulated artificial diets have been less successful (Sasaki, 1981; Teshima et al., 1981, 1982). One of the problems in feeding larvae on these artificial diets was poor digestibility of the capsule wall. In addition

capsules tended to aggregate, and the permeable capsule allowed rapid nutrient leach into culture water which accelerated bacterial fouling of larval culture (Jones et al., 1976; Jones et al., 1979a; Langdon, 1980). Nylon-protein microcapsules were also shown to contain traces of toxic detergent (Tween 20) used to separate capsules from organic phase, which interfered with larval feeding behaviour (Moller et al., 1979).

Jones et al. (1984) modified the nylon-protein encapsulation method so as to eliminate the need for toxic chemicals in the preparation of microcapsule by using a stronger acid chloride and eliminating nylon and Tween 20 (British Patent nos. 79437454 and 2103568). This method produces protein-walled microcapsules which protect the diet from dissolution and resist bacterial contamination. The cross-linked protein microcapsule is available commercially in dehydrated form with a range of different capsule diameters (2.5 μ - 250 μ) and can be rehydrated for use as larval feeds. Jones et al, (1987) demonstrated complete replacement of all live foods in commercial hatcheries for *P. vannamei* *P. stylirostris* and *P. monodon* larvae. Microencapsulation has now become an acceptable tool in the study of nutritional requirement for crustacean larvae (Jones et al., 1979b; Sakamoto et al., 1982; Levine and Sulkin, 1984), bivalves (Langdon and Waldock, 1981; Chu et al., 1982), and larval and post larval fish (Gatesoupe et al., 1977; Jones et al., 1984).

Section one of the present study evaluates growth and survival of *P. monodon* larvae on different algal species and optimum algal cell concentrations which are required for larval

development. Microalgal species which support good larval growth in feed trials were ranked accordingly. Zooplankton species have also been evaluated in larval feed trials from mysis (M1) to post larvae (PL1) stages. Based on larval survival and growth achieved on each of the feeds, zooplankton species as prey organisms have also been ranked.

Section two examines a wide range of artificially produced diets currently available as substitutes for live feeds. These diets were evaluated to assess factors relating to culture performance using *P. monodon* larvae under controlled culture conditions, either as total replacement to live feeds, or with live microalgae at 10 cells μl^{-1} as a cofeed. To determine factors affecting performance, diet composition, nutrient leach loss, toxicant production, particle feed stability, bacterial production in culture water environment and feed efficiency of artificial diets were examined. Better larval growth and survival achieved by inclusion of live algae as supplemental cofeed with artificial diets prompted the work reported in the next section.

Section three looks at the role of algae in the culture medium, and examines the relationship between the addition of artificial larval feeds and the production of ammonia and nitrite in *P. monodon* culture, and their control by algae acting as a biofilter. Microalgae was also 'caged' in culture medium in a simple experiment to determine whether secreted algal metabolic products act as chemoattractants for larval stages of *P. monodon.*

Section four evaluated performance of microencapsulated

feeds, produced by different sources and levels of proteins, lipids, vitamins and trace metals. Comparatively better larval growth and survival were achieved in feed trials, however larval development was less than that achieved on live algal controls. Inclusion of homogenised algae added to microcapsules in larval feed trials gave significantly better larval growth and survival which were comparable to live feed controls.

Section five evaluates growth and survival of nursery stage prawns on commercially available artificial diets and flakes. In feed trial *P. monodon* larvae were also reared through metamorphosis from protozoea (PZ1) to juveniles on the same artificial diet.

The aim of this thesis is to evaluate live feeds and a wide range of artificially produced diets, currently available using different process technologies, to act as substitutes for live feeds used in prawn culture. Each process type was evaluated to assess factors relating to culture performance in larval feed trials. Since microalgae used as supplemental food gave better performance in feed trials, the role of microalgae in larviculture was examined. The aim of this study was also to compare *P. monodon* larval development on a series of nutritionally improved microencapsulated feeds as total replacements and with supplements of growth promoting substance taken from live feeds. Further, crustacean algal replacement (CAR) diet were used in the absence of all live conventional feeds to rear *P. monodon* larvae through metamorphosis from protozoea to juvenile prawn on a single artificial diet.

Table 1 : Artificial and processed natural diets used to culture mollusc and crustacean filter feeding larvae

i. Uncoated artificial diets

Species	Type of diet	Result	Reference
Mollusca.			
Crassostrea virginica	Microgel particles	75% growth of algal fed controls	Langdon & Siegfried (1984)
C. virginica	Microgel particles	52% growth of algal fed controls	Langdon & Bolton (1984)
Crustacea.			
Macrobrachium rosenbergii	Freeze dried catfish	11% survival to metamorphosis	Sick & Beaty (1975)
M. rosenbergii	Freeze dried crumbles of oyster and trout feed	2% survival to metamorphosis	Murai & Andrews (1978)
Palaemonetes pugio	Freeze dried squid	26.7% survival to post larval stage	Sandifer & Williams (1980)
P. vulgaris	Freeze dried plankton (euphausiid) particles	13.3% survival to post larval stage	Sandifer & Williams (1980)
Penaeus aztecus	Freeze dried and frozen diatoms	45% survival to mysis 1 stage	Brown (1972)
P. japonicus	Soyabean cake crumble	Poor growth and survival	Hirata et al., (1975)
P. japonicus	Microgel: diet B (Kanazawa et al., 1977)	67% survival to post larvae	Jones et al., (1979a)
P. japonicus	Microgel: diet B	94% survival to post larvae	Kanazawa et al., (1982)
P. setiferus	Frozen *skeletonema costatum* and *Artemia*	80% survival to post larvae	Mock et al., (1980a)
Eurypanopeus depressus	Microgel: calcium alginate rotifers and *Artemia*	50% survival to megalopa	Levine et al., (1983)

ii. Coated artificial diets

Mollusca.			
Crassostrea gigas	Nylon-protein, glycopeptide, microcapsule	Promoted growth in spat	Gabbott et al., (1975)
C. gigas	Gelatin-acacia microcapsules	Exhibited enhanced spat growth	Langdon & Waldock (1981)
C. virginica	Gelatin-acacia and lipid walled microcapsules	25% survival after 25 days	Chu et al., (1987)
Crustacea.			
Artemia	Cross-linked nylon-protein microcapsules	25% survived after 10 days	Jones et al., (1974)
Artemia	Nylon cross-linked protein-wall microcapsule	34.9% survival to adult	Sakamoto et al., (1982)
M. rosenbergii	Nylon-protein microcapsules	Larvae survived to 4th stage of development	Jones et al., (1975)
P. merguiensis	Nylon-protein microcapsules	Survival to mysis 1	Jones et al., (1975)
P. japonicus	Nylon-protein microcapsules	50% survival to post larval stage	Jones et al., (1979a)
P. japonicus	Nylon-protein microcapsules	78% survival to post larval stage	Kanazawa et al., (1982)
P. japonicus	microparticulate diet with carageenan binder	36% survival to PL1	Teshima & Kanazawa (1983)
P. monodon	Micro-encapsulated diet	47% survival to PL7	Jones et al., (1987)
P. monodon	Micro-encapsulated diet plus algae	Survival to post larvae ranged from 58 to 69%	Kurmaly et al., (1989a)
P. vannamei	70% X-linked protein micro-encapsulated diet	81% survival to PL5 stage	Jones et al., (1987)
P. stylirostris	Micro-encapsulated diet	65% survival to PL5	Jones et al., (1987)

Eurypanopeus depressus	Nylon-protein microcapsules	83% survival to magalopa	Levine et al., (1983)
Portunus trituberculatus	Nylon-protein microcapsules plus rotifers	16.1% survival to juvenile crabs	Kanazawa et al., (1983)

SECTION 1

Growth and survival of *Penaeus monodon* larval stages on live foods

INTRODUCTION

Currently, most prawn hatcheries are dependent upon live foods (Liao, 1984; Langdon et al., 1985) such as microalgae (diatoms, phytoflagellates), rotifer, *Artemia* and a variety of other small zooplankton as food sources for *P. monodon* larval stages which change during development from a herbivorous to an omnivorous mode of feeding. Algae are provided at the outset of the protozoeal stage, and zooplankton added usually at the beginning of the first mysis substage (Hudinaga and Kittaka, 1967; Cook and Murphy, 1969; Liao, 1984). Natural live feeds are generally considered to be nutritionally superior to inert diets (Depauw, 1988). It is not widely recognised, however, that many live feeds give inferior growth and survival. Certain species of algae are better than the others (Scott and Middleton, 1979), similarly, some live or frozen prey organisms used for mysis larval stages may not support growth and development to postlarval stages (Simpson et al., 1983).

There is therefore a continuous need to evaluate algal species and select those which promote rapid growth and high survival when fed to prawn larvae. To be acceptable as a food source algae must possess an acceptable size range, be non-toxic, have adequate nutrition and possess a digestible cell wall (Web and Chu, 1982). Several species of algae have been used successfully as food in the culture of penaeid protozoea (Simon, 1978). The centric diatoms *Skeletonema costatum* (Greville) and *Chaetoceros sp.* have been used extensively (Hudinaga, 1942; Hudinaga and Kittaka, 1967; Cook

and Murphy, 1969; Mock and Murphy, 1971; Liao and Huang, 1972; Mock et al., 1980a; Simon, 1981; Tobias-Quinito and Villegas, 1982; Kuban et al., 1985), together with *Thalassiosira sp.* (Cook and Murphy, 1966; Thomas et al., 1976; Emmerson, 1980). Amongst the flagellates, *Isochrysis sp.* (Bardach et al., 1972; Wilkenfeld et al., 1981; Kuban et al., 1983) and *Tetraselmis* sp. (Hudinaga, 1942; Mock et al., 1980b; Tobias-Quinito and Villegas, 1982) have also been successfully used to culture penaeid larvae.

Although zooplankter such as rotifers, *Brachionus plicatilis* (Müller) and brine shrimp *Artemia salina* (L.) have been widely employed as a food source for protozoea and mysis stage (Hudinaga and Kittaka 1967; Cook and Murphy, 1969; Liao, 1984; Yufera et al., 1984; Hirata et al., 1985; Chu and Shing 1986; Samocha et al., 1989), there are disadvantages in their use. *Artemia* has been shown to be inconsistent in hatchability (Bookhout and Costlow, 1970), vary in nutritional quality (Wickins, 1972) and may be expensive (U.S. $ 15-30 per 454g of cysts). Several other species of zooplankters have been used as food for larval stages of penaeids (Simpson et al., 1983; McConaugha, 1985; Beidenbach et al., 1989). As partial or complete replacement for *Artemia* nauplii.

The purpose of the present study was to identify potential natural diets and evaluate their performance on growth and survival of *P. monodon* larvae from protozoea stage (PZ1) to protozoea stage III (PZ3) using five species of unicellular algae (diatoms, phytoflagellates) selected on the basis of cell size, digestibility and nutritional quality. Some species,

either individually or in combination have not previously been fed to *P. monodon* larvae. Each algal species was evaluated at a range of feeding concentrations under controlled laboratory feed trials to determine minimum and maximum cell densities required for optimum larval growth and survival. The best algae were identified and ranked according to the growth and survival performance of larvae at protozoea III (PZ3)/ mysis substages.

Alternative zooplankton sources both live and frozen were also evaluated in terms of larval growth and survival from mysis I substage to postlarval (PL1) stages of development.

MATERIALS AND METHODS

MICROALGAL FEEDS

Live monospecific cultures of five phytoplankton species (*Tetraselmis chuii* [Butcher], *Chaetoceros calcitrans* [Paulsen], *Skeletonema costatum* [Greville], *Rhodomonas baltica* [Karsten], *Pavlova lutheri* [Droop]) were fed to *P. monodon* larvae from protozoea to mysis substage at cell densities ranging from 5 $c\mu l^{-1}$, 10 $c\mu l^{-1}$, 20 $c\mu l^{-1}$, 30 $c\mu l^{-1}$, 40 $c\mu l^{-1}$, 50 $c\mu l^{-1}$ and 60 $c\mu l^{-1}$, (table 1) to determine feeding levels which promote optimal growth and survival.

At the School of Ocean Sciences algal cultures were maintained in the exponential growth phase for larval feeding. Conway medium (Walne, 1966) is the source of nutrient for the algal cultures. Algal cell densities were estimated at the time of harvesting prior to feeding the larvae by Coulter Counter model ZB with an orifice of 100 μ and haemocytometer, cell densities from each subsample were counted five times for accuracy. Algal feeding rates were determined by :

$$\text{Vol. of A required (mls)} = \frac{\text{Vol. of B (mls) x Conc. algae req'd in B } (c\mu l^{-1})}{\text{Conc. of algae in A } (c\mu l^{-1})}$$

where A = algal culture

B = larval culture

ZOOPLANKTON FEEDS

Five animal food sources, both live (freshly hatched *Artemia salina* (L) nauplii, live barnacle larvae *Elminius modestus* Darwin, live rotifers *Brachionus plicatilis* [Müller]), and frozen (fertilized eggs of mussels *Mytilus edulis* [Linnaeus] and undeveloped frozen eggs of crab, *Hyas araneus* [L.]) were fed to *P. monodon* larvae to determine the optimum zooplankton food source required for rapid growth and high survival from mysis (M1) to post larvae (PL1). Food concentration levels are listed in table 2.

Artemia (Instar 1 San Francisco Bay Brand) were hatched in U/V-sterilized sea water at a salinity of 32‰ and water temperature of 28°C. Non-feeding *Artemia* nauplii were concentrated to a light source, three nauplii counts were made to assess the number of *Artemia* nauplii prior to feeding. Specimens of the barnacle *E. modestus* containing ripe egg masses within the mantle cavity were removed from the wooden piles of the Menai Bridge pier, placed in a bucket filled with seawater, broken up and stirred vigorously to release hatching substance (Crisp, 1956; Crisp and Spencer, 1958) to promote hatching of the ripe eggs. First stage nauplii were collected at the illuminated surface of the bucket and transferred by pipette to a beaker containing, irradiated (U.V.) and filtered seawater. Stage 1 nauplii were non-feeding and extremely active. Fertilized eggs of *Mytilus edulis* were obtained in the laboratory by induced spawning (Bayne, 1976) and frozen. Adult rotifers, *B. plicatilis* were obtained from continuous cultures

maintained in the laboratory on algal feeds. Berried females of *Hyas araneus* captured from the wild were frozen. Frozen eggs from both the crab and mussels were thawed prior to feeding. *P. monodon* larvae were previously fed on *Tetraselmis chuii* and *Rhodomonas baltica* in a ratio of 1:1 at 20 cμl^{-1} each from PZ1 to PZ3 larval stages.

FEED TRIAL PROCEDURE

Non-feeding stages of *P. monodon* nauplii (N4 - N6) subsisting entirely on their egg yolk, were flown in from Frippak Research Centre, Aberdeen, enclosed in styrofoam packing material. The larvae were acclimatised to the laboratory culture temperature and stocked in 10 litre round bottom glass containers at 100 l^{-1} in well aerated sea water maintained at a temperature of 28 ± 1°C and 32‰ salinity. At the protozoeal (PZ1) stage, characterised by dark eye spots, a well-rounded carapace, developing abdominal region and elongated antennae and caudal spine, the larvae were stocked in feed trial experiments.

Healthy PZ1 were concentrated by a portable light source (6 volts) and stocked in 2-litre round bottom flasks using U.V.-sterilised sea water filtered through 0.2 μm filters. The salinity of the culture water was 32‰ and was maintained at 28 ± 1°C. Gentle aeration was supplied through a glass tubing at the bottom of the flask, at a rate of 2 - 3 air bubbles sec^{-1}, which not only provided the culture water with oxygen but also ensured uniform distribution of larvae and food.

Prior to feeding 50% of the culture water was exchanged daily to renew water quality (McVey and Fox, 1983). Algal feed trials were terminated when larvae metamorphosed to PZ3/mysis (M1) stage. A similar procedure was employed when animal feeds were evaluated from mysis (M1) larval stage to post larvae (PL1).

Both algal and animal feed trials were replicated and larval growth and percentage survival were monitored every other day for diet performance. Growth measurements were taken as body lengths of ten randomly sampled larvae per flask, which were recorded from the base of the rostrum spine to the end of telson excluding caudal spines (Motoh, 1979). Individual larvae were measured carefully under a binocular microscope with a calibrated ocular micrometer and returned to the experimental flask as soon as possible as delays in measurement and rough handling caused stress to the larvae. To estimate percentage larval survival, individual counts of larvae per flask was taken. To facilitate counting, larvae together with their culture water were gently poured on to a glass trough and placed on top of a light box with frosted glass. Individual larvae were picked up and counted using a soft plastic pipette then returned to the experimental flask.

Statistical analysis for algal feeds was performed using 2-way ANOVA between algal species, cell densities, larval growth and survival, together with 1-way ANOVA to determine any significant difference on larval growth and survival when algal species were fed at 40 $c\mu l^{-1}$, 50 $c\mu l^{-1}$ and 60 $c\mu l^{-1}$. 1-way ANOVA was also performed to evaluate differences between the

various animal feeds offered. This was followed by Bartletts-Box test (Sokal and Rohlf, 1981) for homogenity of variance. Tukeys/Scheffe's multiple pairwise comparison were done to determine where the differences were at (P = 0.05) level of significance. All statistical analyses were performed using SPSSX statistical package on the VAX mainframe computer.

RESULTS

GROWTH AND SURVIVAL OF *P. monodon* LARVAE (PZ1 - PZ3/M1) ON MICROALGAL FEEDS

Larval growth and survival responses of *P. monodon* larvae (PZ1 - PZ3/M1), on five algal species fed as individual diets and two algal species mixed together as combination diets at seven levels of cell concentrations are given in tables 3 and 4.

Highest growth rates and percentage survival were obtained when larvae were fed on a combination diet of the phytoflagellates *T. chuii* and *R. baltica* fed at a ratio of 1:1 at 40 $c\mu l^{-1}$, 50 $c\mu l^{-1}$ and 60 $c\mu l^{-1}$ concentrations. With the exception of *S. costatum,* algal cell concentrations below 20 $c\mu l^{-1}$ did not support larval growth and survival. At feed levels of 20 and 30 $c\mu l^{-1}$ both larval growth and survival were relatively poor (fig. 1 and fig. 2).

LARVAL GROWTH (PZ1 - PZ3/M1)

To study the effect of algal feeds and cell densities on larval growth, two-way ANOVA between variable algal feeds and cell concentrations at seven levels was done. Results of two-way ANOVA for larval growth by variable algae and feed levels are given in table 5a. Both algal species and feed levels produced a significant effect on larval growth ($P < 0.05$). Multiple pair wise comparison for larval growth by

algal species is given in table 5b and pair wise comparison for larval growth by seven levels of cell concentration is given in table 5c. Since the two-way interaction between algal diets and cell concentration on larval growth was significant ($P < 0.05$), (raw data shows that both larval survival and growth were dependent on ration size and algal species offered as diet). One-way ANOVA was performed to determine the effects of individual algal diet on larval growth at different levels of cell concentration.

Larval Growth at 20 $c\mu l^{-1}$.

Larval growth on each of the algal diets fed at 20 $c\mu l^{-1}$, either as a single feed or in combination were significantly different ($P < 0.05$ table 6a). Multiple pairwise comparison indicates that larval growth was significantly better on a combination algal diet of *R. baltica* and *T. chuii* fed at a ratio of 1:1 compared to single algal diets (*C. calcitrans*, *T. chuii*, *P. lutheri*, *R. baltica*) but growth was not significantly different to the diatom *S. costatum* and the algal combination of *S. costatum* and *R. baltica* fed in a ratio of 1:1. Summary of multiple pairwise comparisons are given in table 6b.

Larval Growth at 30 $c\mu l^{-1}$.

Larval growth on individual algae fed singly or as a mixture gave significantly different ($P < 0.05$) growth rates (table 7a). Multiple pairwise comparisons shows *S. costatum* fed singly performed significantly better than algal diets

T. chuii, *P. lutheri*, combinations of *S. costatum* and *R. baltica*, in a ratio of 1:1 and with *C. calcitrans*. Larval growth however was not significantly different from the algal diet *R. baltica* fed on its own and *R. baltica* and *T. chuii* fed as a mixture in a ratio of 1:1. Summary of multiple comparisons given in table 7b.

Larval Growth at 40 $c\mu l^{-1}$.

Larval Growth performance on algal feeds at 40 $c\mu l^{-1}$ were significantly different ($P < 0.05$ table 8a). Multiple pairwise comparison (table 8b) shows that the algal combination diet *R. baltica* and *T. chuii* fed at a ratio of 1:1 gave a significantly better growth rate compared to algal feeds of *P. lutheri*, *C. calcitrans*, *T. chuii*, *S. costatum* fed singly, but growth was not significantly different with *R. baltica* and with the feed combination of *S. costatum* and *R. baltica* fed in a ratio of 1:1.

Larval Growth at 50 $c\mu l^{-1}$.

Significant differences ($P < 0.05$) in larval growth were evaluated with algal diets fed at 50 $c\mu l^{-1}$ (table 9a). Multiple pairwise comparison (table 9b) shows that the algal combination diet *R. baltica* and *T. chuii* fed in a ratio of 1:1 at 50 $c\mu l^{-1}$ gave a significantly better growth performance when compared to *P. lutheri*, *C. calcitrans*, *R. baltica*, *T. chuii*, and *S. costatum* fed as a single diet. Growth performance was however not significantly different from the algal diet of *S. costatum* and *R. baltica* fed in a ratio of 1:1.

Larval Growth at 60 $c\mu l^{-1}$.

Larval growth were significantly different ($P < 0.05$) with different algal diets (table 10a). Multiple pairwise comparison (table 10b) shows that the algal diet *R. baltica* and *T. chuii* gave significantly better larval growth rates when fed in a combination (ratio of 1:1) compared to algal diets of *P. lutheri, C. calcitrans, T. chuii, S. costatum*, but growth was not significantly different compared to *R. baltica* and *R. baltica* and *S. costatum* fed in combination of 1:1.

LARVAL SURVIVAL (PZ1 - PZ3/M1)

The effects of algal diets and cell concentration levels on survival of *P. monodon* larval stages PZ1 - PZ3/M1 were evaluated by employing two-way ANOVA to test variability between algal feeds, cell concentration and their effect on larval survival. Results of two-way ANOVA for larval survival by different algae and feed levels are given in table 11a. The main effects of both algae and feed levels on larval survival were highly significant ($P < 0.05$). Multiple pairwise comparison of larval survival on algal species is given in table 11b, and pairwise comparison of larval survival at seven cell concentrations is given in table 11c. Since the two-way interaction between algal feeds and cell concentration on larval survival was significant ($P < 0.05$), one-way ANOVA was employed to determine the effects of individual algal diets on larval survival at different levels of cell concentration. Feed levels below a cell concentration of 20 $c\mu l^{-1}$ did not

support larval survival through to metamorphosis.

Larval Survival at 20 $c\mu l^{-1}$.

One-way ANOVA shows an overall significant ($P < 0.05$) difference in larval survival with individual algal species fed at 20 $c\mu l^{-1}$ table 12a. Multiple pairwise comparison between survival and algae species shows the combination diet *S. costatum* and *R. baltica* to be significantly different from single species algal diets *P. lutheri*, *S. costatum*, and *C. calcitrans*, but survivals were not significantly different from *R. baltica*, *T. chuii* or with a mixture of *T. chuii* and *R. baltica* in a ratio of 1:1. Summary of multiple comparisons are given in table 12b.

Larval Survival at 30 $c\mu l^{-1}$.

There was an overall significant ($P < 0.05$) difference in larval survival with differing algal species fed at 30 $c\mu l^{-1}$ table 13a. Multiple pairwise comparisons table 13b shows that algae fed in a combination of *R. baltica* and *T. chuii* in a ratio of 1:1 gave significantly ($P < 0.05$) better survival rates than with *P. lutheri* and *C. calcitrans* algae fed alone. However larval survival was not significantly different from survival rates obtained on algal feeds *S. costatum*, *R. baltica*, *T. chuii* fed as a single diet and with *S. costatum* and *R. baltica* fed in combination (ratio of 1:1).

Larval Survival at 40 $c\mu l^{-1}$.

Larval survival was overall significantly different

($P < 0.05$) with algal types fed at 40 $c\mu l^{-1}$ (table 14a). Multiple pairwise comparison (table 14b) shows that larval survival were significantly better on the algal combination of *R. baltica* and *T. chuii* fed in a ratio of 1:1 in comparison to *S. costatum, C. calcitrans* and *P. lutheri*. Larval survival was not significantly different from *T. chuii, R. baltica* fed as a single diet and with *S. costatum* and *R. baltica* when fed as a combination diet in a ratio of 1:1.

Larval Survival at 50 $c\mu l^{-1}$.

There was an overall significant difference ($P < 0.05$) in larval survival with different algal species at 50 $c\mu l^{-1}$ (table 15a). Multiple pairwise comparison shows that the algal combination diet of *R. baltica* and *T. chuii* fed in a ratio of 1:1 gave a significantly better survival when compared with algal diets *C. calcitrans, S. costatum,* and *P. lutheri* fed alone. Larval survival however was not significantly different from individual algal diets of *T. chuii, R. baltica* and combination algal diet *S. costatum* and *R. baltica* fed in a ratio of 1:1, table 15b.

Larval Survival at 60 $c\mu l^{-1}$.

An overall significant difference ($P < 0.05$) was recorded for larval survival when algal diets were fed at 60 $c\mu l^{-1}$, results for ANOVA are summarised in table 16a. Multiple pairwise comparisons between algal diets fed singly or as a combination and larval survival are given in table 16b. The algal combination diet *R. baltica* and *T. chuii* gave

significantly better survival than *C. calcitrans*, *S. costatum*, *P. lutheri*, *R. baltica* and *T. chuii* when fed as a single algal diet. Larval survival however was not significantly different from algal combination diet *S. costatum* and *R. baltica* fed to larvae at 60 cμl^{-1} in a ratio of 1:1.

GROWTH AND SURVIVAL OF *P. monodon* LARVAE (M1 - PL1) ON ZOOPLANKTON

Results for larval growth and percentage survival of mysis larval stages fed on five diets consisting of live/frozen crustacean/mollusc eggs and larval forms are ranked and summarised in table 17. Ration levels and diets fed are listed in table 2.

Highest larval growth rates (BL = 4.77 ± 0.133mm) and percentage survival (89%) were obtained for postlarvae (PL1) fed with *Artemia salina* nauplii, followed by larvae fed on fertilized eggs of *Mytilus edulis* (BL = 4.33 ± 0.109mm) when percentage survival to PL1 was 87%. Larval growth on frozen undeveloped crab eggs was 4.14 ± 0.185mm with a survival of 84% to PL1 stage. Growth rates on live rotifers were 3.88 ± 0.147mm and percentage survival to intermediate PL was 55%. Lowest growth (3.85 ± 0.101mm) was obtained for larvae fed live barnacle nauplii, where percentage survival was 61% to intermediate PL stage. Figures 4 and 5 give growth and survival of larvae to post larvae stages on different animal feeds.

Larval Growth (M1 - PL1)

To determine the effects of five animal diets on larval growth, one-way ANOVA analysis was employed, table 18a shows an overall significant response ($P < 0.05$) in terms of larval growth for larvae fed different animal diets. Tukeys multiple pairwise comparison (table 18b) indicates that freshly hatched *Artemia* nauplii supported significantly ($P < 0.05$) higher larval growth rates in comparison to larvae fed on live barnacle nauplii, rotifers, crab eggs and fertilized mussel eggs. Larval growth on fertilized mussel eggs was significantly better than larval growth achieved on diets of barnacle nauplii, live rotifers and crab eggs. Similarly, larval growth on a diet of undeveloped crab eggs was significantly ($P < 0.05$) different from growth obtained on a diet of barnacle nauplii and live rotifers. However larval growth on diets of live rotifers was not significantly different ($P < 0.05$) from larval growth achieved with a diet of barnacle nauplii.

Larval Survival (M1 - PL1)

Results of five animal feeds in terms of larval survival were determined by one-way ANOVA. Table 19a indicates an overall significant ($P < 0.05$) effect on larval survival with differing animal diets. Tukeys multiple pairwise comparison (table 19b) was employed to identify diets which gave significantly ($P < 0.05$) higher survivals. Larval survival on freshly hatched *Artemia* nauplii were significantly better then survivals obtained on diets of live rotifers and live barnacle

nauplii. Percentage larval survival of larvae fed *Artemia* nauplii was however not significantly different ($P < 0.05$) from survival obtained for larvae on diets of undeveloped crab eggs and fertilized mussel eggs.

DISCUSSION

Amongst the five unicellular algal species (table 1) tested in *P. monodon* larval feed trials (PZ1 - PZ3/M1), best growths were obtained with the combination diet of *R. baltica* and *T. chuii* mixed in a ratio of 1:1 at ration levels of 40 $c\mu l^{-1}$, 50 $c\mu l^{-1}$ and 60 $c\mu l^{-1}$. The algal combination of *S. costatum* and *R. baltica* mixed in a ratio of 1:1 also promoted good growth at 40 $c\mu l^{-1}$, 50 $c\mu l^{-1}$ and 60 $c\mu l^{-1}$ (table 3). Larval growth on *R. baltica* and *S. costatum* fed individually as single algae at 40 $c\mu l^{-1}$ performed moderately well and growth was not significantly different from the combination diets at 40 $c\mu l^{-1}$ and 60 $c\mu l^{-1}$ (tables 8b and 10b). Algal ration levels of 20 $c\mu l^{-1}$ and 30 $c\mu l^{-1}$ support larval growth but the larvae are less active, show poor growth rates and delayed metamorphosis compared to larvae fed at optimum cell densities between 40 $c\mu l^{-1}$ and 60 $c\mu l^{-1}$. Larval growth was poor when the flagellate *P. lutheri* was fed at all feeding levels. Partially digested *P. lutheri* cells were observed in larval faeces, and it appears that *P. monodon* larvae are unable to digest and break down cells of the species mechanically or enzymatically. *P. lutheri* was also shown to be of poor food for oyster spat (Langdon and Waldock, 1981). Larval survival

was also best on the algal combination diet *R. baltica* and *T. chuii* fed in a ratio of 1:1 at ration levels 40 $c\mu l^{-1}$, 50 $c\mu l^{-1}$ and 60 $c\mu l^{-1}$, followed by the combination diet *S. costatum* and *R. baltica* also fed at 1:1 ratio. Larval survival were not significantly different ($P < 0.05$) to diets comprising the single algal species *T. chuii* and *R. baltica* at 40 $c\mu l^{-1}$ and 50 $c\mu l^{-1}$ (table 14b and 15b). Algae cell concentrations below 20 $c\mu l^{-1}$ do not sustain survival. Percentage larval survival were relatively poor at algal densities of 20 $c\mu l^{-1}$ and 30 $c\mu l^{-1}$ compared to survival on algal diets at 40 $c\mu l^{-1}$, 50 $c\mu l^{-1}$ and 60 $c\mu l^{-1}$, table 4. *S. costatum, C. calcitrans* and *P. lutheri* gave comparatively lower survival when fed as single species.

Amongst the single algal species, the only previous reference to *R. baltica* as a *P. monodon* larval diet is by Kurmaly et al., (1989a) who obtained highest percentage survival and larval growth on this species. These results are also consistent with the present study in which highest growth and larval survival were obtained (table 3 and 4) with *R. baltica* fed as a single algal species at 40 cells μl^{-1} in comparison with other species (*T. chuii, C. calcitrans, S. costatum* and *P. lutheri*). *Rhodomonas* spp are known to contain high levels of both protein and lipids (Enright et al., 1986).

Macronutrient composition of algae and levels of polyunsaturated fatty acid (PUFA) have been tabulated from literature (table 20 and 21). When different algal species are used as diets for larvae, variability in larval growth and survival may be attributed to nutritive value, digestibility,

cell size or chemical composition of algal cells (Webb and Chu 1982; Volkman et al., 1989). Fernandez-Reiriz et al., (1989) have shown variation in the biochemical composition of marine microalgae when harvested at the exponential and stationary growth phases. It was shown that carbohydrate levels in *Rhodomonas sp., P. lutheri, Tetraselmis sp.* and *C. calcitrans* increases as the culture develops.

Protein levels in *P. lutheri, Rhodomonas* sp. and *C. calcitrans* increases in the stationary phase and a decreased level of proteins in *Tetraselmis* sp. and *P. lutheri* was shown to increase with the development of culture. Lipid levels for *Tetraselmis* sp. and *C. calcitrans* were observed to be high in the early stationary phase of growth. *Rhodomonas* sp. was shown to contain the highest values of polyunsaturated fatty acids (73.60%) in the exponential phase compared with *C. calcitrans* (33.7%), *P. lutheri* (42.0%) and *Tetraselmis* sp. (51.20%). A deficiency in some essential fatty acids in algae such as 20-carbon and 22-carbon fatty acids of the ω3 series seems to be the major reason for poor nutritional value. It has been shown that some PUFA's 20:5ω3 (eicosapentaenoic acid) and 22:6ω3 (decosahexaenoic acid) synthesized by algae are essential for normal growth and development of penaeid prawns (Colvin, 1976; Guary et al., 1976; Kanazawa et al., 1977a; Kanazawa et al., 1977b; Jones et al., 1979b).

Conventional larval rearing methods rely heavily on live *S. costatum* in prawn hatcheries as a single diet (Liao et al., 1988). Present laboratory algal feed trials with *P. monodon* larvae using *S. costatum* gave inferior growth and survival

compared to mixed algal diets. *S. costatum* is known to contain relatively small quantities of certain essential amino acids e.g. tyrosine (Chuecas and Riley, 1969). Kurmaly et al., (1989a) also suggest that mechanical fouling of cephalic appendages by *S. costatum* may occur inhibiting effective feeding and respiration. Jones et al., (1987) also observed low annual larval survival in a Taiwan prawn hatchery where *S. costatum* was used exclusively. Liao et al., (1983) reports *S. costatum* to be harmful for penaeid larvae if harvested in or after the stationary growth phase. Therefore, exclusive use of *S. costatum* as a single diet in prawn hatcheries is not recommended.

In general, best growth and survival is obtained on mixed algal diets (Fig. 3) which are more likely to contain the diversity of macro and micro nutrients to satisfy most nutritional requirements for larval growth and development. Two or more sources of protein are better then one as the amino acid spectra and levels of amino acids are likely to be greater (Rumsey and Ketola, 1975).

Optimum larval growth and survival obtained in the present algal feed trials were on the mixed diets of *T. chuii/R. baltica* and *S. costatum/R. baltica.* It is likely that mixed algal diet provides the larvae with a better balance of nutrients such as amino acids, fatty acids and carbohydrates, as well as micronutrients, e.g. vitamins and minerals. Thus any nutritional deficiency in one algal species may be compensated by its presence in another species. Changes can occur in the biochemical composition of microalgae. Species

considered as successful food species occasionally and as yet unpredictably, become unsuitable and even harmful to prawn larvae when cells are harvested in or after the stationary phase (Liao et al., 1983).

Algal food concentrations offered to the larvae is also an important factor in determining larval growth and survival. Estimates of energy budget calculated by Kurmaly et al., (1989b) for *P. monodon* larvae fed *T. chuii* at 60 cellsμl^{-1} showed that PZ1 larval stage required 0.746 Joules. larva^{-1}. day^{-1} for development. In the present feed trial *T. chuii* fed at cell concentrations below 20 cμl^{-1} did not support larval growth. Cell concentrations of 10, 20 and 30 cμl^{-1} provided 0.124, 0.248 and 0.373 J. larva^{-1}. day^{-1}, this amount of energy maintains larval growth but it is inadequate for optimum development, both larval growth and survival are inhibited (table 3 and 4). The demand for energy increases as the larvae metamorphose through successive developmental stages. Larval development in the present protozoeal feed trial was optimum between cell concentrations of 40, 50 and 60 cμl^{-1} which provides 0.500, 0.621 and 0.746 J. larva^{-1}.day^{-1} respectively. The assimilation efficiency of protozoeal stage fed at cell concentrations of 40 cμl^{-1} was calculated to be between 18 to 35%. Assimilation efficiency was lower for cell concentrations fed at 50 cμl^{-1} (15 to 19%) and 60 cμl^{-1} (12 to 16%). At high feed concentrations (60 cμl^{-1}) Kurmaly et al., (1989b) obtained assimilation efficiency at around 16% for protozoeal stages. Lower assimilation efficiency at high algal feed concentration was attributed to superfluous feeding by the larvae (Kurmaly et

al., 1989b). This author suggested that optimal energy gains may be best obtained by the larvae if food is shunted rapidly through the gut partially digested, but replenished quickly. *P. monodon* larvae can refill their guts 5-7 times h^{-1} when feeding on dense algal cultures (Jones and Kurmaly, 1987). It can be concluded that *P. monodon* larval growth and survival from PZ1 to PZ3 are highly dependent on both cell concentration and algal species offered as a diet.

When the planktonic filter feeding protozoeal stages of penaeid larvae metamorphose into mysis stage, the larvae assume an adult-like appearance and move to the next trophic level in the planktonic food chain. The herbivorous larvae become omnivorous and actively feed on both phytoplankton and zooplankton. During the mysis substages there is a gradual transition from herbivorous feeding to zooplankton as the preferred food, thus becoming first level predators. Ideal prey must therefore be of an appropriate size for easy capture and available for consumption in adequate concentrations and should have essential dietary nutrients.

The comparative macronutrient biochemical composition of zooplankton species offered as food in the present feed trial (M1 - PL1) have been extracted from the literature and are listed in table 22. Variation in the nutritional value of live diet may explain the variability obtained in larval survival and growth of *P. monodon* larvae on different diets. Simpson et al., (1983) suggest dietary level of protein requirement for prawn larvae to be in the range of 52-57%. Live freshly hatched nauplii of brine shrimp *Artemia* (Instar 1, San

Francisco Bay Brand) have a protein component of 69%. Highest larval growth (4.77mm) and percentage survival (89%) were obtained on *Artemia* in comparison to other diets tested (table 17). *Artemia* constitutes the best live substitute for wild zooplankton for shrimp larvae (Liao et al., 1983) owing to its high nutritive value (Claus et al., 1979). Sulkin and Epifanio, (1975) reported that *Artemia* nauplii generally contain two or three times more lipid per dry weight than rotifers. In present work fertilized eggs of *Mytilus edulis* ranked second in supporting metamorphoses to PL stage (larval growth 4.33mm and survival 87%). Mussel eggs also have a high dietary protein level (60%) and a lipid content of 12% (Holland, 1978). Eggs of the crab *Hyas araneus* also contain 56% of dietary protein and a high lipid (21.9%) content (Holland, 1978), larval growth 4.14mm and survival 84% on this diet ranked third in performance. McConaugha, (1985) suggested that most decapod larvae require long chain fatty acids, to complete metamorphosis. Jones et al., (1979a), using microcapsules containing ^{14}C- palmitic acid, showed that *P. Japonicus* larvae are able to synthesize 20:5ω3 and 22:6ω3 from 18:3ω3. Kanazawa et al., (1979a, b) also showed that addition of 20:5ω3 and 22:6ω3 fatty acid to diets of prawn *P. japonicus* increased survival and weight gains. Rotifers sustained development from M1 to PL1, but larval survival (55%) and growth (3.88mm) is ranked fourth due to their poor nutritive value. The dietary composition of rotifers largely depends on their food (Ben-Amotz, et al., 1987). Emmerson, (1984) studied the energetic requirement of *P. indius* and found that energy

intake from rotifers was consistently low throughout the rearing period. He concluded that rotifers can be completely dispensed with for practical culture purposes. Poor larval survival (61%) and growth (3.85mm) on live barnacle nauplii resulted from the inability of the mysis stage to capture the extremely active (mean linear velocity 2.5mm. sec^{-1}) stage 1 barnacle nauplii. Although barnacle nauplii contain all the essential dietary requirements in terms of protein (63.7%) and lipids (8.1%) (Holland, 1978), they are not a suitable prey item for *P. monodon* larvae. The M1 metamorphosed to PL on dead barnacle nauplii.

Feed size does not appear to be an important criterion in food preference of mysis larvae. With the exception of mussel eggs (30 - 40 μm) other zooplankton feeds compared favourably with *Artemia* nauplii (250 - 430 μm), table 2. However rotifers and barnacle nauplii may be less than adequate substitutes for *Artemia* in mysis and postlarval stages. Larval survival on diets of crab and mussel eggs at 30 $ml^{-1}.day^{-1}$ were not significantly different from survival obtained on *Artemia* nauplii (table 19b). Both crab and mussel eggs contain relatively high protein and lipid contents. Samocha et al., (1989), however, suggest that *Artemia* alone may be a superior food for the mass culture of penaeid larvae.

Although the use of *Artemia* in prawn hatcheries has advantages and disadvantages they are not a natural diet for crustaceans since it is a continental genus abundant only in environments with very high salinity. Nutritional deficiencies amongst *Artemia* strains have been reported (Wickins, 1972),

they are expensive (Beidenbach et al., 1989) and excrete poisonous metabolites, compounding water quality problems (Samocha et al., 1989). The shell surface of *Artemia* cysts is usually covered with bacteria and other contaminants (Wheeler et al., 1979) and can easily introduce detrimental organisms such as ciliates into prawn larval cultures (Sorgeloos, 1978). The digestive tract of the prawn larvae feeding on *Artemia* nauplii may also be clogged by the empty shells and unhatched cysts due to their indigestibility (Sorgeloos et al., 1977). High densities of *Artemia* also compete with prawn larvae in hatcheries for the same resource - dissolved oxygen and algal food (Wilkenfeld et al., 1981) which are often limited.

As exclusive use of live foods in prawn hatcheries is not desirable, alternative food sources, in the form of artificial larval feeds currently available worldwide require evaluation as partial or complete replacements for live feeds under controlled laboratory feed trials.

Table 1 : Microalgal species used in algal feed trial (PZ1 - M1)

Class	Genus	Species	Code	Description	Cell size µm	Ration levels
Prasinophyceae	*Tetraselmis*	*chuii* (Butcher)	Tc	Naked flagellate	10-15µm	5-60 $c\mu l^{-1}.d^{-1}$
Bacillariophyceae	*Chaetoceros*	*calcitrans* (Paulsen)	Cc	Centric diatom	4-6µm	5-60$c\mu l^{-1}.d^{-1}$
	Skeletonema	*costatum* (Greville)	Sc	Centric diatom	8-10µm	5-60$c\mu l^{-1}.d^{-1}$
Cryptophyceae	*Rhodomonas*	*baltica* (Karsten)	Rb	Naked flagellate	8-10µm	5-60$c\mu l^{-1}.d^{-1}$
Haptophyceae	*Pavlova*	*lutheri* (Droop)	Pl	Naked flagellate	3-5µm	5-60$c\mu l^{-1}.d^{-1}$
Algal combination diet :						
Bacillariophyceae/ Cryptophyceae	*S. costatum/R. baltica*		Sc/Rb	diatom/flagellate	8-10/8-10µm	1:1 5 to 60 $c\mu l^{-1}.d^{-1}$
Cryptophyceae/ Prasinophyceae	*R. baltica/T. chuii*		Rb/Tc	flagellate/flagellate	8-10/10-15µm	1:1 5 to 60 $c\mu l^{-1}.d^{-1}$

Table 2 : Animal feeds and ration size used in mysis feed trial (M1-PL1)

Class	Genus	Species	Code	Description	Feed size	Ration lev
Rotifera	*Brachionus*	*plicatilis* (Müller)	Bp	Live adults	100-400 µm	8. $ml^{-1}.d^{-1}$
Bivalvia	*Mytilus*	*edulis* (Linnaeus)	Me	Frozen fertilized eggs (polar bodies)	30-40 µm	30. $ml^{-1}.d^{-}$
Crustacea	*Artemia*	*salina L.*	As	Live freshly hatched nauplii	250-430 µm	8. $ml^{-1}.d^{-1}$
	Elminius	*modestus* (Darwin)	Em	Live freshly hatched nauplii	100-200 µm	8. $ml^{-1}.d^{-1}$
	Hyas	*araneus* (L)	Ha	Undeveloped eggs	200-250 µm	30. $ml^{-1}.d^{-}$

Table 3 : Algal diets ranked according to growth response obtained with *P. monodon* larvae PZ3/M1 at algal feed levels of 40 $c\mu l^{-1}$

		PZ3-M1 Larval Growth (mm) ± SD						
Rank	Algal species/Cell conc.	5 $c\mu l^{-1}$	10 $c\mu l^{-1}$	20 $c\mu l^{-1}$	30 $c\mu l^{-1}$	40 $c\mu l^{-1}$	50 $c\mu l^{-1}$	60
1.	*T. chuii/R.baltica* (1:1)	0	0	2.86 ±.004	2.86 ±0.038	3.09 ±.054	3.10 ±.058	3.1 ±.0
2.	*S.costatum/R.baltica* (1:1)	0	0	2.75 ±.034	2.75 ±0.0084	3.07 ±.050	3.08 ±.028	3.0 ±.0
3.	*R. baltica*	0	0	2.23 ±0.051	2.86 ±0.181	3.05 ±.043	2.88 ±.087	3.0 ±.0
4.	*S. costatum*	0	2.37 ±2.69	2.75 ±.013	3.00 ±0.0084	3.00 ±.053	3.00 ±.046	3.02 ±.028
5.	*T. chuii*	0	0	2.11 ±0.012	2.53 ±0.022	2.92 ±.078	2.90 ±.0764	2.93 ±.065
6.	*C. calcitrans*	0	0	2.10 ±.0042	2.81 ±0.030	2.84 ±.101	2.88 ±.087	2.90 ±.079
7.	*P. lutheri*	0	0	2.12 ±.043	2.57 ±0.0091	2.62 ±.054	2.62 ±.090	2.68 ±.063

Table 4 : Algal diets ranked according to percentage survival obtained with *P. monodon* larvae at algal feed levels of 40 $c\mu l^{-1}$

		Percentage survival of larvae (PZ3 - M1) ± SD						
Rank	Algal sp./ Cell conc.	5 $c\mu l^{-1}$	10 $c\mu l^{-1}$	20 $c\mu l^{-1}$	30 $c\mu l^{-1}$	40 $c\mu l^{-1}$	50 $c\mu l^{-1}$	60
1.	*T.chuii/R.baltica* (1:1)	0	0	49.00 ±9.899	73.50 ±2.121	91.00 ±1.41	89.00 ±1.41	89. ±3.
2.	*S.costatum/R.baltica* (1:1)	0	0	66.00 ±5.656	72.00 ±2.828	78.00 ±8.49	82.00 ±8.49	78. ±4.
3.	*R.baltica*	0	0	41.00 ±1.414	60.00 ±7.071	67.50 ±10.60	67.50 ±3.53	62. ±3.
4.	*T.chuii*	0	0	60.00 ±7.071	57.50 ±5.656	62.00 ±2.82	63.00 ±7.07	64. ±0.71
5.	*P.lutheri*	0	0	14.00 ±5.656	16.50 ±2.121	43.50 ±12.02	49.00 ±12.72	48.50 ±9.19
6.	*C.calcitrans*	0	0	28.50 ±4.949	32.00 ±2.828	40.50 ±17.67	40.00 ±8.48	39.50 ±6.36
7.	*S.costatum*	0	6.50 ±4.950	21.50 ±9.192	53.00 ±9.899	39.0 ±12.72	44.50 ±2.12	45.00 ±7.07

Table 5a : Two-way ANOVA and interaction between algal species and cell concentration on larval growth (PZ1-PZ3/M1)

Source of Variation	Sum of Squares	DF	Mean Square	F	Sig of F
Main effects	734.710	12	61.226	11185.376	.001
ALGAE	18.296	6	3.049	557.077	.001
CELLS	716.415	6	119.402	21813.674	.001
2-way interactions					
ALGAE CELLS	42.692	36	1.186	216.648	.001
Explained	777.402	48	16.196	2958.830	.001
Residual	2.414	441	.005		
Total	779.816	489	1.595		

Table 5b : Multiple pair wise comparison, tabular summary of significance, larval growths (PZ1 - PZ3/M1) by algal feeds.

Means	Algal Feed	Code	Algal Feeds Pl	Tc	Cc	Rb	Sc/Rb	Rb/Tc	Sc
1.80	*P. lutheri*	Pl							
1.92	*T. chuii*	Tc	ns						
1.94	*C. calcitrans*	Cc	ns	ns					
2.04	*R. baltica*	Rb	ns	ns	ns				
2.10	*S. costatum*/ *R. baltica*	Sc/ Rb	*	*	*	*			
2.13	*R. baltica*/ *T. chuii*	Rb/ Tc	*	*	*	*	*		
2.45	*S. costatum*	Sc	*	*	*	*	*	*	

(*) Denotes pairs of groups significantly different at 0.050 level

ns indicates a non-significant result

Table 5c : Multiple pair wise comparison, tabular summary of significance, larval growths (PZ1 - PZ3/M1) by algal feed concentrations $c\mu l^{-1}.d^{-1}$.

Means	Cell Nos	Cell concentration $5c\mu l^{-1}$	$10c\mu l^{-1}$	$20c\mu l^{-1}$	$30c\mu l^{-1}$	$40c\mu l^{-1}$	$50c\mu l^{-1}$	$60c\mu l^{-1}$
0.00	$5c\mu l^{-1}$							
0.34	$10c\mu l^{-1}$	ns						
2.40	$20c\mu l^{-1}$	*	*					
2.77	$30c\mu l^{-1}$	*	*	*				
2.94	$40c\mu l^{-1}$	*	*	*	*			
2.95	$50c\mu l^{-1}$	*	*	*	*	*		
2.97	$60c\mu l^{-1}$	*	*	*	*	*	*	

(*) Denotes pairs of groups significantly different at 0.050 level.

ns indicates a non-significant result.

Table 6a : Analysis of variance on larval growths by algal feeds at cell densities of 20 $c\mu l^{-1}$.

SOURCE	D.F.	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
BETWEEN GROUPS	6	6.4872	1.0812	252.628	.001
WITHIN GROUPS	63	.2696	.0043		
TOTAL	69	6.7569			

Test for Homogeneity of Variance

Bartlett-Box F = 2.546, P = .018

Table 6b : Multiple pairwise comparison, tabular summary of significance, larval growth by algal cells at 20 $c\mu l^{-1}$.

Mean	Algal Feeds	Code	Algal Feeds Cc	Tc	Pl	Rb	Sc	Sc/Rb	Rb/Tc
2.1011	*C.calcitrans*	Cc							
2.1133	*T.chuii*	Tc	ns						
2.1225	*P.lutheri*	Pl	ns	ns					
2.2331	*R.baltica*	Rb	*	*	*				
2.7472	*S.costatum*	Sc	*	*	*	*			
2.7502	*S.costatum/* *R.baltica*	Sc/ Rb	*	*	*	*	ns		
2.7595	*R.baltica/* *T.chuii*	Rb/ Tc	*	*	*	*	ns	ns	

(*) Denotes pairs of groups significantly different at 0.050 level

ns indicates a non-significant result

Table 7a : Analysis of Variance on larval growth by algal feeds at cell densities of 30 $c\mu l^{-1}$.

SOURCE	D.F.	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
BETWEEN GROUPS	6	1.6950	.2825	22.183	.001
WITHIN GROUPS	63	.8023	.0127		
TOTAL	69	2.4974			

Test for Homogeneity of Variance

Bartlett-Box F = 4.492, P = .000

Table 7b : Multiple pairwise comparisons, tabular summary of significance, larval growth by algal cell at 30 $c\mu l^{-1}$.

Mean	Algal Feeds	Code	Algal Feeds Tc	Pl	Sc/Rb	Cc	Rb	Rb/Tc	Sc
2.5318	*T.chuii*	Tc							
2.5719	*P.lutheri*	Pl	ns						
2.7502	*S.costatum/ R.baltica*	Sc/ Rb	*	*					
2.8148	*C.calcitrans*	Cc	*	*	ns				
2.8609	*R.baltica*	Rb	*	*	ns	ns			
2.8641	*R.baltica/ T.chuii*	Rb/ Tc	*	*	ns	ns	ns		
3.0024	*S.costatum*	Sc	*	*	*	*	ns	ns	

(*) Denotes pairs of groups significantly different at 0.050 level

ns indicates a non-significant result

Table 8a : Analysis of Variance on larval growth by algal feeds at cell densities of 40 $c\mu l^{-1}$.

SOURCE	D.F.	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
BETWEEN GROUPS	6	1.7059	.2843	67.247	.001
WITHIN GROUPS	63	.2664	.0042		
TOTAL	69	1.9722			

Test for Homogeneity of Variance

Bartlett-Box F = 1.714, P = .113

Table 8b : Multiple pairwise comparisons, tabular summary of significance, larval growth by algal cells at 40 $c\mu l^{-1}$.

Means	Algal Feeds	Code	Algal Feeds Pl	Cc	Tc	Sc	Rb	Sc/Rb	Rb/Tc
2.6150	*P. lutheri*	Pl							
2.8425	*C. calcitrans*	Cc	*						
2.9225	*T. chuii*	Tc	*	ns					
3.0025	*S. costatum*	Sc	*	*	ns				
3.0454	*R. baltica*	Rb	*	*	*	ns			
3.0671	*S. costatum/ R. baltica*	Sc/ Rb	*	*	*	ns	ns		
3.0949	*R. baltica/ T. chuii*	Rb/ Tc	*	*	*	*	ns	ns	

(*) Denotes pairs of groups significantly different at 0.050 level

ns indicates a non-significant result

Table 9a : Analysis of Variance on larval growth by algal feeds at cell densities of 50 $c\mu l^{-1}$.

SOURCE	D.F.	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
BETWEEN GROUPS	6	1.5677	.2613	51.672	.001
WITHIN GROUPS	63	.3186	.0051		
TOTAL	69	1.8862			

Test for Homogeneity of Variance
Bartlett-Box F = 2.454, P = .023

Table 9b : Multiple pairwise comparisons, tabular summary of significance, larval growth by algal cells at 50 $c\mu l^{-1}$.

Means	Algal Feeds	Code	Algal Feeds Pl	Cc	Rb	Tc	Sc	Sc/Rb	Rb/Tc
2.6210	*P. lutheri*	Pl							
2.8824	*C. clacitrans*	Cc	*						
2.8824	*R. baltica*	Rb	*	ns					
2.9040	*T. chuii*	Tc	*	ns	ns				
2.9995	*S. costatum*	Sc	*	*	*	ns			
3.0825	*S. costatum/ R. baltica*	Sc/ Rb	*	*	*	*	ns		
3.1080	*R. baltica/ T. chuii*	Rb/ Tc	*	*	*	*	*	ns	

(*) Denotes pairs of groups significantly different at 0.050 level

ns indicates a non-significant result

Table 10a : Analysis of Variance on larval growth by algal feeds at cell densities of 60 $c\mu l^{-1}$.

SOURCE	D.F.	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
BETWEEN GROUPS	6	1.3420	.2237	76.613	.001
WITHIN GROUPS	63	.1839	.0029		
TOTAL	69	1.5260			

Test for Homogeneity of Variance
Bartlett-Box F = 3.399, P = .002

Table 10b : Multiple pairwise comparisons, tabular summary of significance, larval growth by algal cells at 60 $c\mu l^{-1}$.

Means	Algal Feeds	Code	Algal Feeds Pl	Cc	Tc	Sc	Rb	Sc/Rb	Rb/Tc
2.6824	*P. lutheri*	Pl							
2.8979	*C. calcitrans*	Cc	*						
2.9349	*T. chuii*	Tc	*	ns					
3.0211	*S. costatum*	Sc	*	*	*				
3.0641	*R. baltica*	Rb	*	*	*	ns			
3.0762	*S. costatum/ R. baltica*	Sc/ Rb	*	*	*	ns	ns		
3.1194	*R. baltica/ T. chuii*	Rb/ Tc	*	*	*	*	ns	ns	

(*) Denotes pairs of groups significantly different at 0.050 level

ns indicates a non-significant result

Table 11a : Two-way ANOVA and interaction between algal species and cell concentration on larval survival (PZ1-M1)

Source of Variation	Sum of Squares	DF	Mean Square	F	Sig. of F
Main effects	78769.286	12	6564.107	165.241	.001
ALGAE	14138.429	6	2356.405	59.319	.001
CELLS	64630.857	6	10771.810	271.163	.001
2-way Interactions					
ALGAE CELLS	8916.714	36	247.687	6.235	.001
Explained	87686.000	48	1826.792	45.987	.001
Residual	1946.500	49	39.724		
Total	89632.500	97	924.046		

Table 11b : Summary of multiple pairwise comparison for larval survival (PZ1-M1) with algal feeds.

Means	Algal Feeds	Code	Algal Feeds P1	Cc	Sc	Rb	Tc	Sc/Rb	Rb/Tc
24.50	*P. lutheri*	P1							
25.79	*C. calcitrans*	Cc	ns						
29.93	*S. costatum*	Sc	ns	ns					
42.64	*R. baltica*	Rb	*	*	*				
43.86	*T. chuii*	Tc	*	*	*	*			
53.79	*S. costatum/ R. baltica*	Sc/ Rb	*	*	*	*	*		
56.00	*R. baltica/ T. chuii*	Rb/ Tc	*	*	*	*	*	*	

(*) Denotes pairs of group significantly different at the 0.050 level

ns indicates a non-significant result

Table 11c : Summary of multiple pairwise comparison for larval survival (PZ1-M1) with cell concentrations fed.

Means	Cell Nos.	Cell concentration $5c\mu l^{-1}$	$10c\mu l^{-1}$	$20c\mu l^{-1}$	$30c\mu l^{-1}$	$40c\mu l^{-1}$	$60c\mu l^{-1}$	$50c\mu l^{-1}$
0.00	$5c\mu l^{-1}$							
0.93	$10c\mu l^{-1}$	ns						
40.00	$20c\mu l^{-1}$	*	*					
52.07	$30c\mu l^{-1}$	*	*	*				
60.21	$40c\mu l^{-1}$	*	*	*	*			
61.41	$60c\mu l^{-1}$	*	*	*	*	*		
62.14	$50c\mu l^{-1}$	*	*	*	*	*	*	

(*) Denotes pairs of groups significantly different at the 0.050 level

ns indicates a non-significant result

Table 12a : Analysis of Variance on larval survival by algal feeds at cell densities of 20 $c\mu l^{-1}$.

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
BETWEEN GROUPS	6	3884.8571	647.4762	15.8751	.0009
WITHIN GROUPS	7	285.5000	40.7857		
TOTAL	13	4170.3571			

Test for Homogeneity of Variance
Bartlett-Box = .409, P = .870

Table 12b : Multiple pairwise comparison, tabular summary of significance, larval survival by algal cells at 20 $c\mu l^{-1}$.

Means	Algal Feeds	Code	Algal Feeds Pl	Sc	Cc	Rb	Tc	Rb/Tc	Sc/Rb
14.00	*P. lutheri*	Pl							
21.50	*S. costatum*	Sc	ns						
28.50	*C. calcitrans*	Cc	ns	ns					
41.00	*R. baltica*	Rb	*	ns	ns				
47.50	*T. chuii*	Tc	*	*	ns	ns			
49.00	*R. baltica/ T. chuii*	Rb/ Tc	*	*	ns	ns	ns		
66.00	*S. costatum/ R. baltica*	Sc/ Rb	*	*	*	ns	ns	ns	

(*) Denotes pairs of groups significantly different at 0.050 level

ns indicates a non-significant result

Table 13a : Analysis of Variance on larval survival by algal feeds at cell densities of 30 $c\mu l^{-1}$.

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
BETWEEN GROUPS	6	5300.4286	883.4048	27.7302	.0002
WITHIN GROUPS	7	223.0000	31.8571		
TOTAL	13	5523.4286			

Test for Homogeneity of Variance
Bartlett-Box = .538, P = .777

Table 13b : Multiple pairwise comparison, tabular summary of significance, larval survival by algal cells at 30 $c\mu l^{-1}$.

Means	Algal Feeds	Code	Algal Feeds Pl	Cc	Sc	Rb	Tc	Sc/Rb	Rb/Tc
16.50	*P. lutheri*	Pl							
32.00	*C. calcitrans*	Cc	ns						
53.00	*S. costatum*	Sc	*	ns					
60.00	*R. baltica*	Rb	*	*	ns				
60.00	*T. chuii*	Tc	*	*	ns	ns			
72.00	*S. costatum*/ *R. baltica*	Sc/ Rb	*	*	ns	ns	ns		
73.00	*R. baltica*/ *T. chuii*	Rb/ Tc	*	*	ns	ns	ns	ns	

(*) Denotes pairs of groups significantly different at 0.050 level

ns indicates a non-significant result

Table 14a : Analysis of variance on larval survival by algal feeds at cell densities of 40 $c\mu l^{-1}$.

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
BETWEEN GROUPS	6	4876.8571	812.8095	6.9941	.0108
WITHIN GROUPS	7	813.5000	116.2143		
TOTAL	13	5690.3571			

Test for Homogeneity of Variance
Bartlett-Box = .669, P = .675

Table 14b : Multiple pairwise comparison, tabular summary of significance, larval survival by algal cells at 40 $c\mu l^{-1}$.

Means	Algal Feeds	Code	Algal Feeds Sc	Cc	Pl	Tc	Rb	Sc/Rb	Rb/Tc
39.00	*S.costatum*	Sc							
40.50	*C.calcitrans*	Cc	ns						
43.50	*P.lutheri*	Pl	ns	ns					
62.00	*T.chuii*	Tc	ns	ns	ns				
67.50	*R.baltica*	Rb	ns	ns	ns	ns			
78.00	*S.costatum/ R.baltica*	Sc/ Rb	ns	ns	ns	ns	ns		
91.00	*R.baltica/ T.chuii*	Rb/ Tc	*	*	*	ns	ns	ns	

(*) Denotes pairs of groups significantly different at 0.050 level

ns indicates a non-significant result

Table 15a : Analysis of Variance on larval survival by algal feeds at cell densities of 50 $c\mu l^{-1}$.

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
BETWEEN GROUPS	6	4238.7143	706.4524	13.1971	.0017
WITHIN GROUPS	7	375.0000	53.5714		
TOTAL	13	4613.7143			

Test for Homogeneity of Variance

Bartlett-Box = .654, P = .687

Table 15b : Multiple pairwise comparison, tabular summary of significance, larval survival by algal cells at 50 $c\mu l^{-1}$.

Means	Algal Feeds	Code	Algal Feeds Cc	Sc	Pl	Tc	Rb	Sc/Rb	Rb/Tc
40.00	*C.calcitrans*	Cc							
44.50	*S.costatum*	Sc	ns						
49.00	*P.lutheri*	Pl	ns	ns					
63.00	*T.chuii*	Tc	ns	ns	ns				
67.50	*R.baltica*	Rb	ns	ns	ns	ns			
82.00	*S.costatum/ R.baltica*	Sc/ Rb	*	*	*	ns	ns		
89.00	*R.baltica/ T.chuii*	Rb/ Tc	*	*	*	ns	ns	ns	

(*) Denotes pairs of groups significantly different at 0.050 level

ns indicates a non-significant result

Table 16a : Analysis of Variance on larval survival by algal feeds at cell densities of 60 $c\mu l^{-1}$.

SOURCE	D.F	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
BETWEEN GROUPS	6	4014.7143	669.1190	20.8170	.0004
WITHIN GROUPS	7	225.0000	32.1429		
TOTAL	13	4239.7143			

Test for Homogeneity of Variance

Bartlett-Box = .542, P = .774

Table 16b : Multiple pairwise comparison, tabular summary of significance, larval survival by algal cells at 60 $c\mu l^{-1}$.

Means	Algal Feeds	Code	Algal Feeds Cc	Sc	Pl	Rb	Tc	Sc/Rb	Rb/Tc
39.50	*C.calcitrans*	Cc							
45.00	*S.costatum*	Sc	ns						
48.50	*P.lutheri*	Pl	ns	ns					
62.50	*R.baltica*	Rb	*	ns	ns				
64.50	*T.chuii*	Tc	*	ns	ns	ns			
78.50	*S.costatum/ R.baltica*	Sc/ Rb	*	*	*	ns	ns		
89.50	*R.baltica/ T.chuii*	Rb/ Tc	*	*	*	*	*	ns	

(*) Denotes pairs of groups significantly different at 0.050 level

ns indicates a non-significant result

Table 17 : Animal diets ranked according to performance in terms of larval growth and percentage survival at PL1 ± S.D.

SPECIES	DIET TYPE	Initial Stocking size (mm)	Larval Growth BL (mm)	Larval Survival (%)
Artemia salina	Instar 1 nauplius	3.0 ±.050	4.775 ±0.133	89.00 ±3.536
Mytilus edulis	fertilized eggs	3.0 ±.050	4.335 ±0.109	87.00 ±4.243
Hyas araneus	undeveloped eggs	3.0 ±.050	4.144 ±0.185	84.00 ±5.657
Brachionus plicatilis	Live adults	3.0 ±.050	3.883 ±0.147	55.00 ±9.900
Elminius modestus	stage 1 nauplius	3.0 ±.050	3.855 ±0.101	61.00 ±4.243

Table 18a : Analysis of variance on larval growth (M1 - PL1) by animal feeds.

SOURCE	D.F.	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
BETWEEN GROUPS	4	5.7141	1.4285	74.5020	.00
WITHIN GROUPS	45	.8628	.0192		
TOTAL	49	6.5769			

Test for Homogeneity of Variance

Bartlett-Box = 1.009, P = .401

Table 18b : Multiple pairwise comparisons, tabular summary of significance, larval growth by animal feeds.

Means	Animal feeds	Code	Animal Feeds Em	Bp	Ha	Me	As
3.8549	*E.modestus* (Nauplii)	Em					
3.8826	*B.plicatilis* (Adults)	Bp	ns				
4.1440	*H.araneus* (eggs)	Ha	*	*			
4.3348	*M.edulis* (eggs)	Me	*	*	*		
4.7747	*A.salina* (Nauplii)	As	*	*	*	*	

(*) Denotes pairs of groups significantly different at 0.050 level

ns indicates a non-significant result

Table 19a : Analysis of variance on larval survival (M1 - PL1) by animal feeds.

SOURCE	D.F.	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
BETWEEN GROUPS	4	2061.6000	515.4000	14.4370	.0059
WITHIN GROUPS	5	178.5000	35.7000		
TOTAL	9	2240.1000			

Test for Homogeneity of Variance
Bartlett-Box = .255, P = .905

Table 19b : Multiple pairwise comparison, tabular summary of significance, larval survival by animal feeds.

Means	Animal Feeds	Code	Animal Feeds Bp	Em	Ha	Me	As
55.00	*B.plicatilis* (adults)	Bp					
61.00	*E.modestus* (nauplii)	Em	ns				
84.00	*H.araneus* (eggs)	Ha	*	ns			
87.00	*M.edulis* (eggs)	Me	*	*	ns		
89.50	*A.salina* (nauplii)	As	*	*	ns	ns	

(*) Denotes pairs of groups significantly different at 0.050 level

ns indicates a non-significant result

Table 20 : Percentage dry weight macronutrient composition of algae fed to *Penaeus monodon* larvae

Food	Protein %	Carbohydrate %	Lipid %	Ash %	Reference
Tetraselmis chuii	48.8	24.7	4.3	22.2	Kurmaly et al., (1989a)
Chaetoceros calcitrans	23.94	19.01	8.69	-	Tobias-Quinito & Villegas (1982)
Skeletonema costatum	33.3	22.6	8.1	36.0	Kurmaly et al., (1989a)
Rhodomonas baltica	52.0	33.7	4.3	4.3	Kurmaly et al., (1989a)
Pavlova lutheri	49.0	31.4	11.6	6.4	Parsons et al., (1961)

Table 21 : Percentage composition of fatty acids in flagellate and diatoms used as food for *P. monodon* larvae.

	Flagellates		Diatoms		
	T. chuii[a]	*R. baltica*[a]	*P. lutheri*[b]	*C. calcitrans*[b]	*S. costatum*[a]
Saturates					
12:0	-	-	0.3	TR	-
14:0	4.0	4.9	11.5	17.5	19.7
15:0	-	-	0.5	0.8	0.5
16:0	26.0	11.0	21.3	10.7	10.7
17:0	-	-	0.2	0.3	-
18:0	-	-	1.3	0.8	-
20:0	-	-	0.3	TR	-
22:0	-	-	0.3	TR	-
24:0	-	-	0.2	0.1	-
Sum %	30.0	15.9	35.9	30.2	30.9
Monosaturates					
16:1(ω-10)			-	-	-
16:1(ω-9)	0.7	0.5	-	-	-
16:1(ω-7)	0.6	1.3	16.8	30.3	32.6
16:1(ω-5)	-	-	TR	0.1	-
16:1(ω-13)t	-	-	-	0.7	-
18:1(ω-10)	-	-	0.3	-	-
18:1(ω-9)	11.5	3.9	1.7	2.8	1.7
18:1(ω-7)	4.3	3.2	1.4	0.2	1.2
20:1(ω-9)	-	-	0.2	-	-
Sum %	17.1	8.9	20.4	33.8	35.5
Polyunsaturates					
16:2(ω-7)	-	-	0.2	.3.5	-
16:2(ω-6)	-	-	-	-	-
16:2(ω-4)	-	-	0.2	1.6	-
16:3(ω-6)	-	-	-	-	-
16:3(ω-4)	-	-	0.4	.8.0	-
16:3(ω-3)	-	-	-	-	-
16:4(ω-3)	-	-	-	-	-
16:4(ω-1)	-	-	-	0.3	-
18:2(ω-9)	-	-	0.4	0.8	-
18:2(ω-6)	11.5	14.2	1.5	0.8	1.1
18:3(ω-6)	2.6	2.7	0.4	0.4	0.2
18:3(ω-3)	23.1	16.5	1.8	TR	0.9
18:4(ω-3)	9.5	17.3	6.0	0.5	4.4
20:1(ω-9)	0.7	0.4	-	-	-
20:4(ω-6)	0.4	3.0	TR	5.7	0.2
20:4(ω-3)	-	-	-	0.2	-
20:5(ω-3)	3.4	11.6	19.7	11.1	22.9
22:1(ω-11)	-	0.2	-	-	-
22:5(ω-3)	-	0.1	-	-	-
22:5(ω-6)	-	-	2.0	-	-
22:6(ω-3)	-	7.6	9.4	0.8	2.9
Sum %	51.2	73.6	42.0	33.7	32.6
Others	1.7	1.6	1.8	2.3	1.0
Total	100	100	100	100	100

a Kurmaly et al., 1989a b Volkman et al., 1989

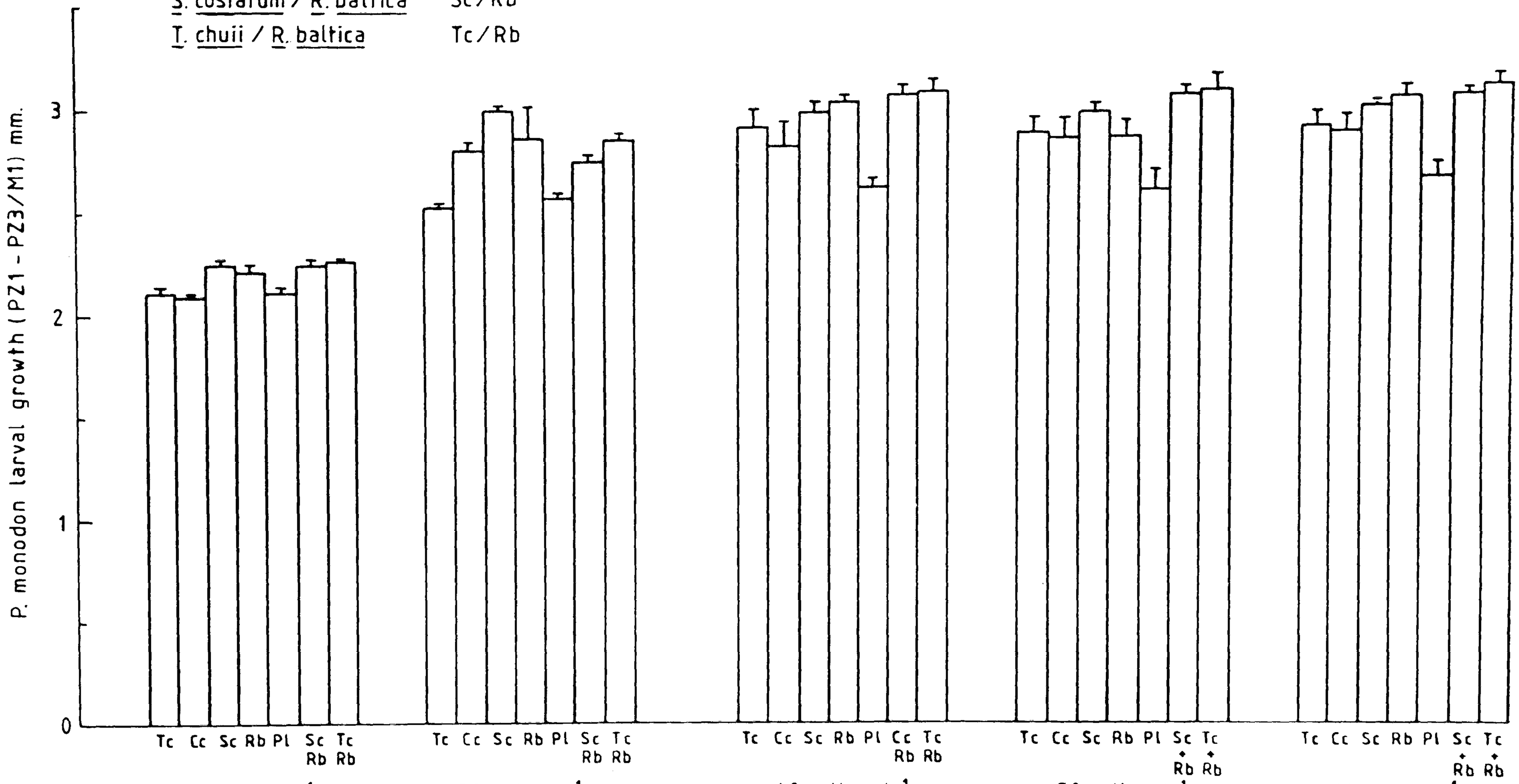
Table 22 : Biochemical composition of animal feeds expressed as percentage of total dry weight.

DIETS	PROTEINS %	LIPIDS %	CARBO-HYDRATES %	REFERENCE
ARTEMIA NAUPLII *(Artemia salina)*	69.02	12.84	9.25	Gabaudan et al., (1980)
MUSSEL EGGS *(Mytilus edulis)*	60.6	12.1	1.5	Holland, D.L., (1978)
CRAB EGGS *(Hyas araneus)*	56.5	21.9	0.8	Holland, D.L., (1978)
ROTIFERS *(B. plicatilis)*	32.0	20.1	44.9	Ben-Amotz et al., (1987)
BARNACLE NAUPLII *(E. modestus)*	63.7	8.1	2.0	Holland, D.L., (1978)

Figure 1. Larval growth response of P. monodon protozoeal larval stages to different algal species at cell concentrations of 20, 30, 40, 50 and 60 cells μl^{-1}. Vertical bars are standard deviation.

LEGEND

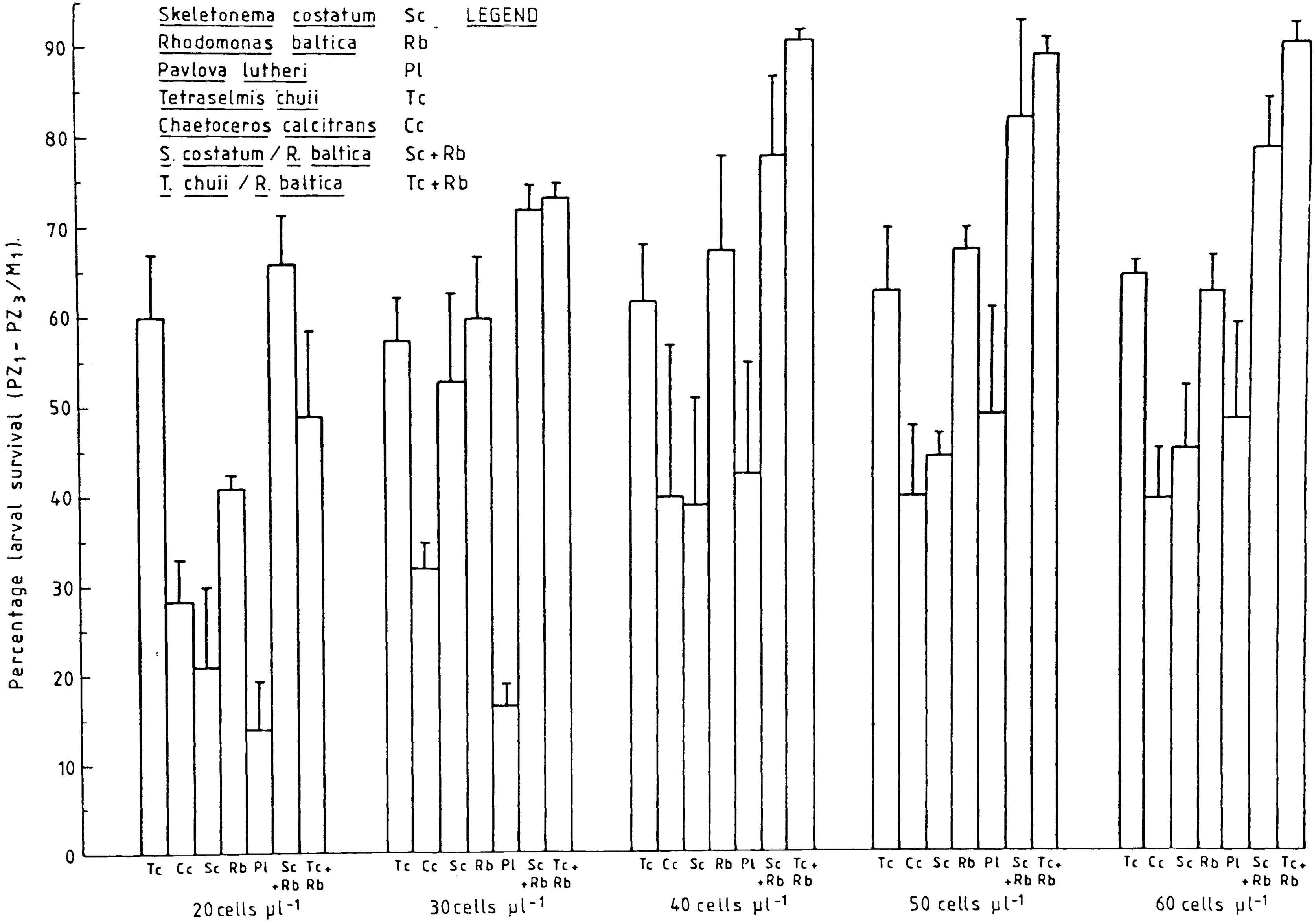
Skeletonema costatum	Sc
Rhodomonas baltica	Rb
Pavlova lutheri	Pl
Tetraselmis chuii	Tc
Chaetoceros calcitrans	Cc
S. costatum / R. baltica	Sc/Rb
T. chuii / R. baltica	Tc/Rb



Algal feeds and cell concentration (cells μl^{-1}).

Figure 2. Percentage larval survival of P. monodon protozoeal stages to different algal species at different cell concentrations of 20, 30, 40, 50 and 60 cells μl^{-1}.

Verticial bars are standard deviation.



Algal feeds and cell concentration (cells μl^{-1}).

Figure 3. Larval growth and percentage survival of P. monodon larvae to postlarval stages on a mixed algal diet of R. baltica and T. chuii on different cell concentrations.

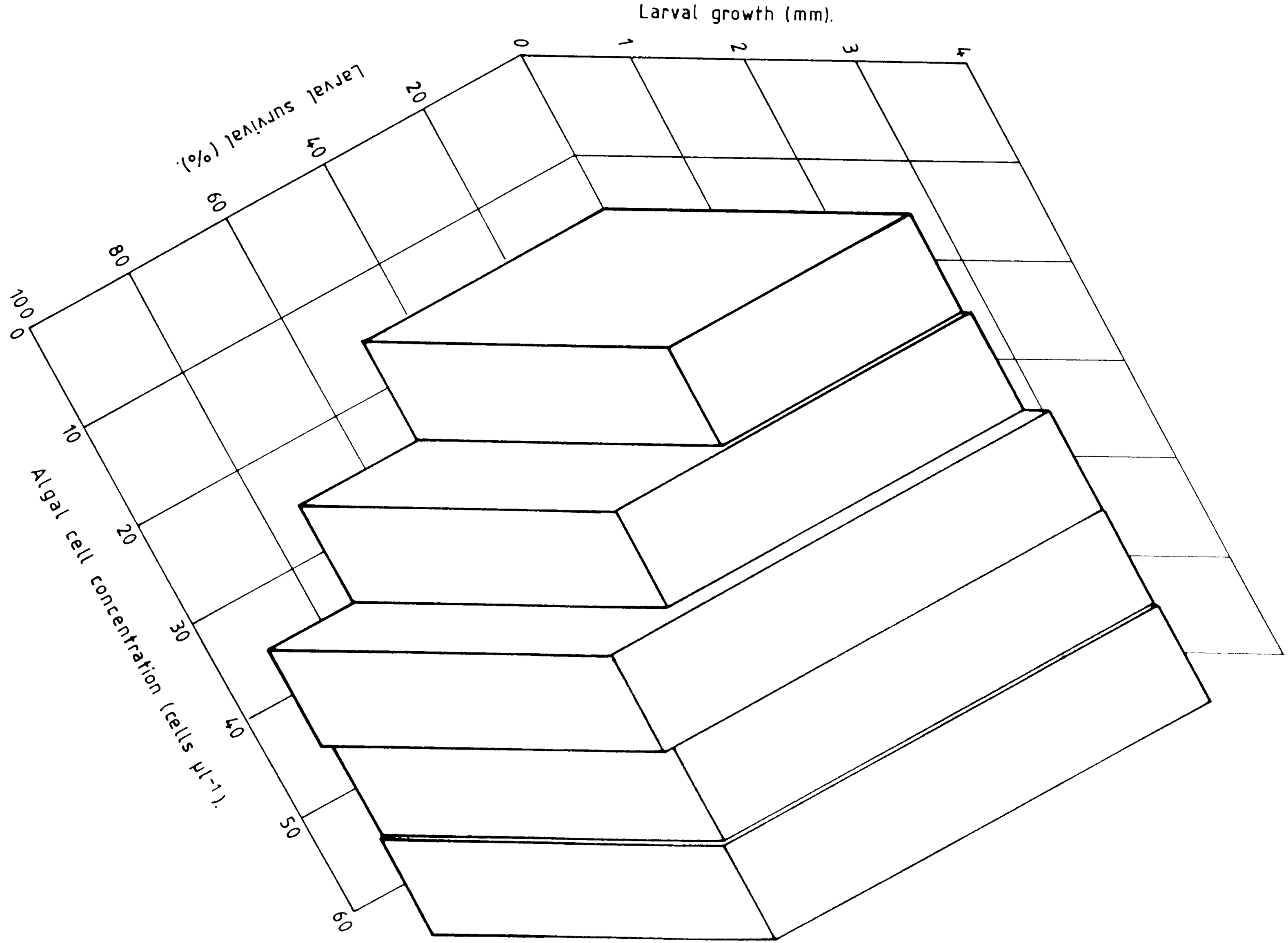
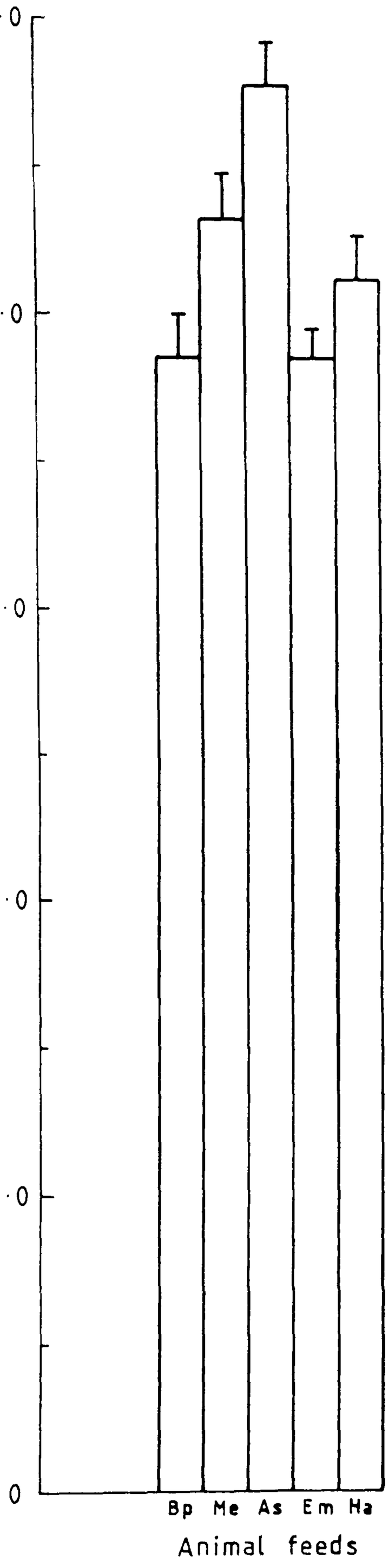

Larval growth (mm).
0
1
2
3
4
Larval survival (%).
0
20
40
60
80
100
Algal cell concentration (cells µl-1).
0
10
20
30
40
50
60

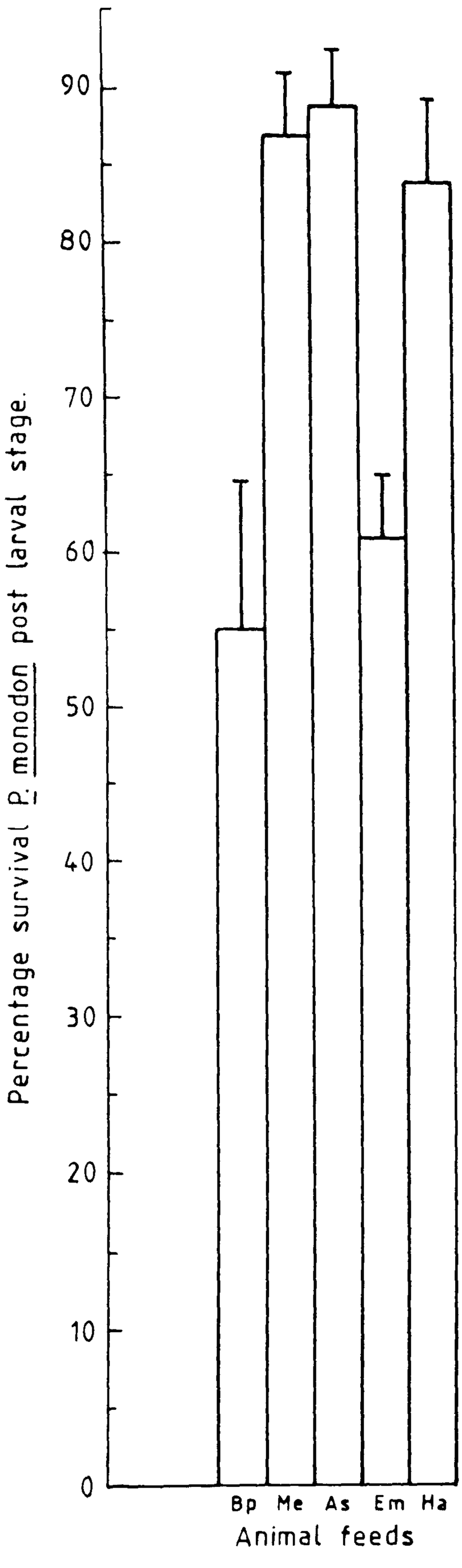
Figure 4. Larval growth of P. monodon (M1 - PL1) on animal diets in replicate feed trials. Vertical bars are standard deviation.



LEGEND

Bachionus plicatilis	Bp
Mytilus edulis	Me
Artemia salina	As
Elminius modestus	Em
Hyas araneus	Ha

Figure 5. Percentage survival of P. monodon larvae (M1 - PL1) on animal diets in replicate feed trials.Vertical bars are standard deviation.



LEGEND

Bachionus plicatilis	Bp
Mytilus edulis	Me
Artemia salina	As
Elminius modestus	Em
Hyas araneus	Ha

SECTION 2

Growth and survival of *Penaeus monodon* larval stages on artificial diets

This section was presented at the Third Egyptian - British Conference on Animal, Fish and Poultry Production, Alexandria, 7 - 10 October, 1989.

Title : **Comparison of Artificial Feeds used in Penaeid Shrimp Hatcheries.**

Authors : D.A. Jones, S. Amjad and K. Chitravadivelu.

Published in the Proceedings of the Third Egyptian - British Conference on Animal, Fish and Poultry Production. Faculty of Agriculture. Alexandria University, Alexandria, Egypt. 15 - 22.

INTRODUCTION

Increasingly a wide range of artificial larval diets have been developed in the laboratory and on a commercial scale using different process techniques to act as partial or complete substitutes for live feeds in fish, bivalves, crabs and prawn hatcheries.

Although artificial food particles have been shown to be acceptable to a wide range of crustacean larvae (Ling, 1967; Paffenhofer and Strickland, 1970; Jones et al., 1972; Teshima et al., 1982), only a few have been reported to support metamorphosis of prawn larvae (Jones et al., 1979a; Jones et al., 1979b; Kanazawa et al., 1982; Teshima and Kanazawa, 1983; Kanazawa, 1989; Kurmaly et al., 1989a; Kashio et al., 1989).

Currently the artificial larval diets marketed using different process technologies are in dehydrated form, either as spray dried natural algae or yeast, microparticulates, microspray dried formulation or microencapsulated feeds. The microencapsulated technique introduced by Chang et al., (1966) to produce nylon-protein microcapsules containing haemoglobin to simulate red blood cells was modified by Jones et al., (1974) to encapsulate and deliver nutrients within a nylon cross-linked protein membrane and used to successfully rear filter feeding *Artemia*. Further development of the diet formulation produced feeds which supported the laboratory culture of *P. japonicus* Bate from nauplius to postlarva in the absence of all conventional live foods (Jones et al., 1979a). Whilst the nylon-protein capsule was suitable for laboratory

scale experimentation, the wall membrane was too thin to with stand drying and thus impractical to use on a commercial scale (Jones et al., 1987). Recently, further refinement of the methodology by Jones et al., (1987) produced non-toxic protein-walled microcapsules of differing sizes which could be dried for storage and rehydrated for use as a larval feed. The commercial manufacturing process of artificial larval feeds is patented (British patent No.s 79437454 and 2103568). Microparticulate diets are normally prepared using presieved (appropriate sizes) dry ingredients which are mixed thoroughly with binders. The ingredient mixture is set by heating or mixing with alginates, oven or freeze dried, then ground and sieved through appropriate meshes (Kanazawa et al., 1985; Alava and Lim, 1988) for larval or post larval feeding purposes. Spray dried natural diets are sprayed forms of modified yeast/algae with elevated levels of HUFA and other essential ingredients, which are freeze dried (Artemia Systems sales literature). The spray formulation process involves spraying of homogenised mixture of ingredients into hot air.

The purpose of this study was to evaluate the performance of leading types of artificially produced diets of each process type, microparticulates, spray formulation, spray dried natural diets and microencapsulated feeds for *P. monodon* (Fab.) larvae under controlled laboratory conditions and to attempt to evaluate factors causing variability in larval growth and survival in replicated trials. Factors examined were gross nutritional contents of the feeds, particle sizes rehydrated over 24h, leach loss of dietary solubles which promotes

breakdown of feed particles and increases production of bacteria in culture water. This was measured as bacterial colonies per ml. grown on ZoBell's medium. Water quality factors such as levels of nitrite (NO_2.N), ionised ammonia (NH_4^+.N) unionised ammonia (NH_3.N) and pH were also examined in the larval culture medium.

Calculation of percent gain in growth by *P. monodon* larvae, and feed efficiency were also estimated.

MATERIALS AND METHODS

Ten leading brands of commercially available artificial larval diets (table 1) were used to rear *P. monodon* (Fab.) larvae from protozoea (PZ1) to post larval (PL1) stage under controlled conditions. *P. monodon* nauplii obtained from a single spawning were flown in from the Frippak Research Centre at Findon, Aberdeen, and groups were fed on each diet in replicated trials. Growth as body length (B.L.) was taken from the base of the rostrum to telson tip excluding caudal spines (Cook and Murphy, 1971), and percentage survival of larvae were recorded to assess diet performance.

The larva at PZ1 stage were stocked at 100 litre^{-1} in 2-litre round bottom flasks using U/V-irridated seawater filtered through 0.2 μm cartridge filter. Salinity of the culture water was 32‰ and temperature was maintained at 28±1°C. Gentle aeration was supplied through a glass tube at the bottom of the flask at the rate of 2-3 air bubbles sec.$^{-1}$, which provided not only dissolved oxygen but also kept the feed particles in suspension. Rehydrated artificial feeds (0.5g. in 100 ml. sea water) were given to larvae as equal rations 4 times a day (1000h, 1400h, 1800h and 2200h), the ration levels were 8 mg. litre^{-1}, from PZ1 to PZ3 and 16 mg. litre^{-1} from Mysis (M1) to PL1 stage. According to manufacturers' instructions larval feeds were also supplemented with microalgae as a co-feed at 5 cell μl^{-1}. of *Rhodomonas baltica* and 5 cells μl^{-1}. of *Tetraselmis chuii* in a ratio of 1:1. Rehydrated feeds were refrigerated at 4-5°C for subsequent use.

Controls were fed on live feeds of microalgae at 20 cells μl^{-1} *R. baltica* and 20 cells μl^{-1} of *T. chuii* in a ratio of 1:1 from PZ1 to PZ3, and newly hatched *Artemia* (San Francisco Bay Brand) nauplii at $5.ml.^{-1}$ from M1 to PL1. Percentage survival and growth of larvae was monitored from each treatment every other day.

50% of the water in the culture flask was exchanged daily to renew water quality which deteriorates with unstable feeds which leach soluble components, together with larval excretion. To determine factors affecting diet performance, ionised ammonia ($NH_4.N$) was measured by the Phenolhypochlorite method (Solarzano, 1969), and the unionised ammonia ($NH_3.N$) calculated from Bower-Bidwell's equation based on Whitfield's theory (Whitfield, 1974; Bower and Bidwell 1978). Nitrite ($NO_2.N$) was measured from procedures described by Bendschneider and Robinson (1952). Other parameters examined were proximate organic content of the diet, percentage loss of dietary solubles, particle stability and bacterial growth.

The proximate percentage organic content of artificial larval diets was estimated by the combustion process (Parson et al., 1985). 1 gm of the diet sample was placed in a preweighed crucible, the crucible containing the diet was placed in a muffle furnace at 450-500°C for 24h. The feed residue in the crucible was weighed and subtracted from the weight of the original diet sample, and percentage organic content was calculated. Organic content estimation was replicated for each of the artificial diets used in the feeding trial.

Percentage loss of dietary solubles was estimated by

rehydrating 0.5 gm dry weight of artificial larval diet in 100 ml filtered sea water in a conical flask at 28±1°C with 2-3 air bubbles per second to agitate and keep the feed particles in suspension. The contents of the flask were filtered through preweighed GF/C filter papers and placed in an oven at 60°C for 24h. The residue feed on the filter paper was weighed and percentage loss of dietary soluble was estimated for 4, 12, and 24h after rehydration. Estimates for dietary loss were replicated.

Feed particle sizes and the stability of the diets were measured under the microscope using a calibrated ocular micrometer. The feed particle sizes were measured as dry particles and rehydrated particles after 1h and 24h of immersion in water. The feed particles were evenly spread on a glass slide and approximately 100 particles were measured for their diameter randomly.

Bacterial growth was monitored in the culture water containing artificial feeds after 24h of feeding. Bacteria were grown on culture medium agar which contained sea water enriched by 0.5% peptone, 0.1% yeast extract and 0.01% ferric phosphate (Oppenheimer and ZoBell, 1952). Following autoclave sterilization the pH of the medium was adjusted to between 7.5 - 7.6. The culture plates innoculated with seawater containing feeds were incubated in an oven for 24h at 36°C. Bacterial colonies were counted and expressed as number of colonies per ml.

Calculation of percent gain in growth and feed efficiency were estimated according to Deshimaru (1982). Linear and

multiple stepwise regression were performed to evaluate larval growth and survival with dietary leach losses and the effects of ionised ammonia, unionised ammonia, nitrites and pH on larval growth and survival. In order to determine whether differences in larval growth and survival on each diet treatment were statistically significant, analysis of variance (ANOVA) one-way was used together with a test for homogeneity of variance (Bartlett's test), followed by Scheffe's multiple pairwise comparison to identify differences between individual treatment means at P = 0.05 level of significance.

RESULTS

DIETARY COMPOSITION OF FEEDS

Figure 1 shows the organic content of artificial larval diets used, together with proximate dietary composition of major nutrients as given by the feed manufacturers. These are compared to average values for phyto and zooplankton. Protein levels in the diets were between 40 to 50%, lipid ranged from 12 to 39% and carbohydrates were incorporated at levels ranging from 1 to 35%. No obvious correlation exists between organic content or composition and performance. However details of vitamins and other additives were not known for many of the diets and more importantly their availability for assimilation.

ARTIFICIAL DIET AS TOTAL REPLACEMENT

The performance of leading brands of artificial diets as total replacements in the absence of conventional live feeds was evaluated for larval growth and survival of *P. monodon* larvae. Amongst the ten artificial larval diets used the modified microencapsulated feed 'F'and spray dried formulation diet 'J' were able to support larval growth and survival to PL stages.

All other artificial larval feeds used as total replacements for *P. monodon* larvae were unable to support metamorphosis beyond PZ2 larval stage (Fig. 2). One-way ANOVA (table 2) shows that larval survival on microencapsulated diet 'F' and spray dried formulation diet 'J' was not significantly different ($P > .148$) from live feed controls. Larval growths

(table 3a) on the same diets however showed a significant difference ($P < .05$) compared to the live feed control. Multiple pairwise comparison (table 3b) shows that the live feed control (microalgae 40 cells μl^{-1} and 5. ml^{-1} *Artemia*) performed significantly better than the artificial diets. Amongst the artificial diets microencapsulated feed diet 'F' gave significantly better growth than larval growths obtained on all other formulations.

ARTIFICIAL LARVAL FEED TRIAL WITH MICROALGAE AS CO-FEED

Larval Growth.

Artificial larval diets produced from each manufacturing process were evaluated in terms of larval growth and survival, when the diets were supplemented by live microalgae *R. baltica* and *T. chuii* at 5 cells μl^{-1} each throughout the feed trial period in a ratio of 1:1. With the exception of diet 'G' (microparticulates) and diet 'E' (spray dried algae) all other diets were able to sustain larval growth and survival to PL stages (Fig. 3). One-way ANOVA (table 4a) shows larval growth on all diets to be significantly different ($P < 0.05$). Multiple pairwise comparison (table 4b) shows mean larval growth attained on control diet 'L' (20 cells μl^{-1} *R. baltica* and 20 cells μl^{-1} *T. chuii* and 5.ml^{-1} *Artemia*) were significantly better than all artificial diets tested. Amongst the artificial larval diets, growth achieved on microencapsulated diet 'F' was significantly better than growths achieved on diets E, G, A, H, D, I and C. Larval growth was not significantly different from spray dried

formulation diet 'J' and microbound particulate diet 'B'. Larval growth achieved on spray dried formulation diet 'J' were not significantly different ($P < 0.05$) from larval growths achieved on spray dried formulation diets I, C and microparticulate diet 'B'. Larval growth obtained with microparticulate diet 'B' was significantly better than mean growth obtained on diet 'E' (spray dried algae), diet 'G', 'A' (microparticulate), diet 'H' (spray dried formulation) and diet 'D' (spray dried yeast), but growth was not significantly different from spray dried formulations diet 'I' and 'C'. Larval growth on the spray dried formulation diet 'C' was significantly better than diets E, G, A, H and D, but growth was not significantly different from that achieved on diet 'I'. Larval growth on diet 'D' (spray dried yeast) was significantly better than mean growth obtained on diet 'E' (spray dried algae) and diet 'G' (microparticulates), but larval growth was not significantly different from that on microparticulate diet 'A' and spray dried formulation diet 'H'. Similarly larval growth on spray dried formulation diet 'H' was different from that on diet 'E' and 'G', but growth was not significantly different from that on microparticulate diet 'A'.

Larval growth on microparticulate diet 'A' was significantly different from larval growth achieved on the spray dried formulation 'H' and spray dried yeast diet 'D'. Growth performance on spray dried algae diet 'E' and microparticulate diet 'G' was poor, these two diets maintained larvae only to the PZ2 stage of development.

Percent Larval Survival

One-way ANOVA indicates that the performance of artificial larval diets in terms of larval survival were significantly ($P < 0.05$) different (table 5a). Mean survival to PL stages ranged between 0 to 89%.

Scheffes multiple pairwise comparison (table 5b) between different diets shows that larval survival on live feed control diet 'L' were not significantly different ($P < 0.05$) from mean percentage survivals obtained on the spray dried formulation diet 'I', 'J' and microencapsulated diet 'F'. Larval survival on spray dried formulation diet 'H' was significantly better than that on diets 'E' (spray dried algae), 'G' (microparticulates), 'D' (spray dried yeast), 'C', 'H' (spray dried formulation), 'B' and 'A' (microparticulates), but larval survival was not significantly different from that observed on diet 'I' and diet 'F'. Survival on microencapsulated diet 'F' was significantly ($P < 0.05$) better than survival obtained on diets 'E', 'G', 'D', 'C' 'H' and 'B'. Larval survival however was not significantly different from survival obtained on diet 'A' and 'I'. Larval survival performance on diet 'I' was not significantly different from survival obtained on diets 'D', 'C', 'H', 'B' and 'A'. Larval survival on diets 'A', 'B', 'H' and 'C' was significantly different from diet 'D', 'E' and 'G' but survival was not significantly different within these treatments. Larvae on diets 'E' and 'G' did not metamorphose beyond larval stage PZ2.

LEACH LOSS OF DIETARY SOLUBLES

Fig. 4 shows dietary leach loss from rehydrated artificial diets maintained at 28±1°C after 4, 12 and 24h of rehydration. This indicates that most diets lose over 25% of their contents into the soluble phase within 24h. The microencapsulated diet 'F' leached the least (18%). Microparticulate diet 'G' and spray dried algae diet 'E' have dietary losses of 26.6% and 34.4% respectively during the first 4h of rehydration. As there is no evidence that penaeid larvae are able to derive more than a small proportion of their nutrition from solubles, leached diet is lost to the larvae. There is a negative linear correlation (fig. 5) between percentage survival ($r = -.849$) and larval growth ($r = -.763$) and dietary leach loss.

PRODUCTION OF IONISED, UNIONISED AMMONIA AND NITRITE IN FEED TRIALS.

Production of NH_4^+.N (Fig. 6), NH_3.N (Fig. 7) and NO_2.N (Fig. 8) were measured for each of the artificial diets over a period of 4h, 12h and 24h incubated in aerated seawater. The ammonium products liberated from each of the artificial diets on their own remained below the toxic levels recommended by Wickins (1976) and more recently by Chin and Chen, (1987) and Chen and Chin, (1988). However, consistent nutrient loss from artificial diets in feed trials together with nitrogenous waste excreted by the larvae promotes the accumulation of toxic ammonia and nitrite. Despite water renewals of 50% .day^{-1} in the culture vessels during the feed trials, ammonia and nitrite levels did not decline. The levels of these toxicants in

cultures supplied with different feeds throughout the metamorphic stages of larval development are summarised in figures 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 together with percentage survival and growth of larvae. Accumulated levels of toxicants appear to produce a physiological stress on both larval growth and survival, constant exposure to high levels of NO_2.N (> 100 $\mu g.\ l^{-1}$) suppresses larval growth, the swimming activity becomes sluggish in early PZ stages and the larvae stop feeding. This is evident from the absence of the long faecal chains, produced by healthier larvae in culture water containing low levels of toxicants (controls fig. 9). Cultures fed diets 'G' and 'E' which showed a high leach loss of soluble nutrients (40 and 44% respectively), revealed high levels of NO_2.N, NH_4^+.N and NH_3.N measured on day 7 (Fig. 18 & 19). In these cultures larval mortality occurred on day 9 and day 13 of the feed trial at larval stages PZ3/M1 and M2 respectively.

Multiple regression of water quality variables NH_4^+.N, NH_3.N, NO_2.N and pH, measured during the feed trial, against larval survival demonstrated a significant effect ($P < 0.05$) ($r = 0.870$). Stepwise regression identified NO_2.N, NH_3.N, NH_4^+.N and pH (table 6a) as factors causing a significant ($P < 0.05$) effect upon larval survival.

Similarly, multiple regression of these variables against larval growth also produced a significant effect ($P < 0.05$) ($r = 0.748$). In this case NH_4^+.N, NH_3.N and NO_2.N (table 6b) also demonstrated a significant detrimental effect on larval growth.

STABILITY OF ARTIFICIAL DIETS

Fig. 20 details optimum particle sizes ingested by the larvae of *P. monodon* fed on carbon particles and latex beads. The smaller particle sizes ingested when carbon particles were used, are probably due to breakdown of carbon by the larval mouth parts. Fig. 21, plots the median particle size of leading feeds measured dry, after 1h rehydration and after 24h rehydration in seawater. Many feeds appear to have a large initial particle size which is rapidly broken down after contact with seawater.

BACTERIAL PRODUCTION

The breakdown and leaching of nutrient into the soluble phase by unstable diets placed in sterilized seawater at 28±1°C rapidly leads to bacterial growth. Bacterial growth was measured as number of bacterial colonies grown on ZoBells medium innoculated with samples of culture water taken from the feed trials. Fig. 22 shows that once again bacterial contamination appears to be generally related to leach loss. Microparticulate diet 'G' which has a high leach loss (40%) has a higher number of bacterial colonies ($93.ml^{-1}$). Diets which had low leach losses had relatively fewer bacterial colonies. Microencapsulated diet 'F' had a leach loss of 18% over a period of 24h and cultures produced bacterial colonies of $26.ml^{-1}$, spray dried formulation diet 'J' had a leach loss of 21% with bacterial colonies of $30.ml^{-1}$. Microparticulate diet 'B' had a 25% leach loss and $39.ml^{-1}$ bacterial colonies, spray dried formulation diet 'I' had leach loss of 29% with $33.ml^{-1}$

bacterial colonies. However great variability is present at intermediate levels. There is a weak correlation ($r = 0.274$) between number of bacterial colonies and leach loss of dietary solubles from artificial diets.

FEED EFFECTIVNESS

Calculation of percentage gain in growth and feed efficiency by *P. monodon* larvae, at postlarval stage was estimated for the feed trial period. A total of 112mg.litre^{-1} (dry weight) of artificial diet was provided to larvae through metamorphosis from PZ1 to PL1 stage (8mg.l^{-1}.day^{-1} from PZ1 to PZ3 and 16mg.l^{-1}.day^{-1} from M1 to PL1). Percentage growth gains and feed efficiencies obtained from each of the artificial diets used in the feed trial have been ranked (table 7), these estimates were calculated according to Deshimaru (1982).

Amongst the artificial larval diets, microencapsulated diet 'F' produced a high percent gain in growth of 418% in *P. monodon* larvae and a high feed efficiency of 323% was obtained. This was followed by larvae fed on spray dried formulation diet 'J' which showed growth gains of 367% from PZ1 to PL stages with a feed efficiency of 314%. Larvae fed on microparticulate diets 'B' and 'A' achieved gains in growth between 386 and 313% with feed efficiencies of 241 and 199% respectively. Larvae fed on spray dried formulation diet 'H' had comparatively lower growth gains (295%) and feed efficiency (193%), leach loss of dietary nutrient from diet 'H' was 36.6%. Generally, higher leach losses of soluble nutrient components

decreased feed efficiency and larval growth, larvae fed on diet 'G' and 'E' had high leach losses of 40 and 44% respectively and showed mortality.

DISCUSSION

The organic content of most artificial larval diets compares favourably with that of natural algae and zooplankton, but it is likely that some of the diets used in the feed trial were nutritionally inadequate. Differing ratios of protein-carbohydrate-lipid together with levels of micronutrients may have contributed to poor larval growth and survival through metamorphosis. Protein : Lipid ratios in artificial diets tested ranged from 1.6:1 to 4.1:1, few come close to that in natural diet (6.3:1). The inclusion of live microalgae at 10 cells μl^{-1} as a supplemental co-feed with artificial diets produced improved larval growth and survival for most of the feed types used in the feed trial. The algal feed combination (*R. baltica* and *T. chuii*) provides all the micronutrients essential for larval growth. Some of the essential nutrients such as amino acids, vitamins and trace minerals incorporated into artificial diets may be denatured during the feed manufacturing process (New, 1987), or these nutrients may have been lost as a result of leach loss into the soluble phase. Amongst the artificial feeds tested, microencapsulated diet 'F' had the lowest leach loss over 24h and supported the best growth. Jones et al., (1987) reported losses of only 18.9% protein from cross-linked protein microcapsule, Langdon, (1989) described microencapsulation to efficiently retain protein, with losses of less than 5% protein when suspended in seawater for 24h. In contrast microparticulated diet 'G' and spray dried algae diet 'E' had

comparatively high nutrient losses (40 and 44% respectively). These diets could not possibly meet the nutritional requirements of all larval stages and hence the larvae did not reach higher developmental stages. Goldblatt et al., (1979; 1980) reported losses of amino acids, water soluble vitamins and certain minerals from uncoated conventional compounded feeds. As there is no evidence that penaeid larvae are able to derive more than a small proportion of their nutrition from solubles, leached diet is lost to the larvae. A negative linear correlation was found between dietary leach loss and a decline in larval survival and growth (Fig. 5).

The breakdown and leaching of soluble components from rehydrated artificial diets not only decreases food availability for larval growth, but also pollutes culture water by increasing the production of bacteria and fouling larval cultures (Jones et al., 1974, 1975, 1987; Amjad and Jones 1989a). Despite water renewals of 50% $.day^{-1}$ in culture vessels, levels of toxic ammonia and nitrite do not decline. Accumulation of $NH_4^+.N$ and $NO_2.N$ result not only from the breakdown of unstable feed particles, but also by the nitrogenous waste products excreted by the larvae. Nitrite levels above 100 $\mu g.l^{-1}$ produce physiological stress on early protozoeal stages, observed as a loss of appetite revealed by the empty larval gut and the absence of faecal chain when food was abundantly available. Multiple stepwise regression of water quality variables $NH_4^+.N$, $NH_3.N$, $NO_2.N$ and pH measured in feed trials against larval growth and survival identifies $NH_4^+.N$, $NH_3.N$ and $NO_2.N$ as factors causing a significant

($P < 0.05$) detrimental effect both on larval growth and percentage survival. It is well documented that accumulated levels of ammonia and nitrite adversely effect growth and survival of penaeid larvae (Wickins, 1976). Chen et al., (1986) also observed larval survival to be significantly depressed by nitrite levels of 78 $\mu g.l^{-1}$ in a Taiwanese hatchery.

Many of the artificial diets appear to have a large initial particle size (Fig. 21) in view of the relatively small particle size (< 20 μm) ingested by early larval stages of *P. monodon* (Fig. 20), it is surprising that some diet formulations support any growth and survival. Although breakdown of microparticulate and spray dried formulation diets do rapidly produce a range of acceptable sized particles on contact with seawater, many large sized particles are excluded from ingestion and remain in the culture water to contribute to pollution.

In addition to contributing to water pollution, high leach loss from microparticulate and some spray dried formulation diet (diet 'H', 'D', 'A', 'C' and 'B') correlates with lower feed efficiency resulting in reduced gains in larval growth (table 7). In contrast microencapsulated diet 'F' has a low leach loss, a stable acceptable particle size and a high organic content. As a result, this diet supports high larval survival in laboratory trials when used as a total replacement for live feeds, however, growth is usually less than that achieved on live algae and *Artemia* (Jones and Kurmaly, 1987). These authors have shown that growth, similar to that achieved

on live feeds may be obtained by the addition of only 10 live algal cells μl^{-1} to larval cultures fed in combination with microencapsulated feed. Perhaps the underdeveloped digestive system of newly hatched larvae is unable to cope efficiently with nutrients supplied in an encapsulated form? Alternatively algal diets supplied at low levels contain essential micronutrients missing from artificial diets. Another possibility is that algae may remove toxic ammonia and nitrite from the water, or even act to stimulate ingestion and assimilation of nutrients.

However for most artificial diets stability appears to be a key factor with a strong correlation between leach loss and survival and growth. Leach loss promotes breakdown of particles, increases production of bacteria and the release of toxicants such as ammonia and nitrites. Best larval performance requires a high energy diet which remains stable, has low nutrient leach losses and a constant supply of acceptable particle sizes. However it is clear that the inclusion of live algae as a supplemental co-feed gives better larval growth and survival. The next section investigates the role of microalgae in larval feed trials.

Table 1 : Artificial larval diets used in comparative larval rearing trials with *P. monodon* larvae.

ARTIFICIAL DIETS	FEED TYPE	RATION SIZE PZ_1-PZ_3	M_1-PL_1	CO-FEED
FEED A	MICROPARTICULATE	$8mg.l^{-1}.d^{-1}$	$16mg.l^{-1}.d^{-1}$	Microalgae $10\ c\mu l^{-1}$
FEED B	MICROPARTICULATE	$8mg.l^{-1}.d^{-1}$	$16mg.l^{-1}.d^{-1}$	Microalgae $10\ c\mu l^{-1}$
FEED C	SPRAY DRIED FORMULATION	$8mg.l^{-1}.d^{-1}$	$16mg.l^{-1}.d^{-1}$	Microalgae $10\ c\mu l^{-1}$
FEED D	SPRAY DRIED YEAST	$8mg.l^{-1}.d^{-1}$	$16mg.l^{-1}.d^{-1}$	Microalgae $10\ c\mu l^{-1}$
FEED E	SPRAY DRIED ALGAE	$8mg.l^{-1}.d^{-1}$	$16mg.l^{-1}.d^{-1}$	Microalgae $10\ c\mu l^{-1}$
FEED F	MICROENCAPSULATION	$8mg.l^{-1}.d^{-1}$	$16mg.l^{-1}.d^{-1}$	Microalgae $10\ c\mu l^{-1}$
FEED G	MICROPARTICULATE	$8mg.l^{-1}.d^{-1}$	$16mg.l^{-1}.d^{-1}$	Microalgae $10\ c\mu l^{-1}$
FEED H	SPRAY DRIED FORMULATION	$8mg.l^{-1}.d^{-1}$	$16mg.l^{-1}.d^{-1}$	Microalgae $10\ c\mu l^{-1}$
FEED I	SPRAY DRIED FORMULATION	$8mg.l^{-1}.d^{-1}$	$16mg.l^{-1}.d^{-1}$	Microalgae $10\ c\mu l^{-1}$
FEED J	SPRAY DRIED FORMULATION	$8mg.l^{-1}.d^{-1}$	$16mg.l^{-1}.d^{-1}$	Microalgae $10\ c\mu l^{-1}$
FEED L (CONTROL)	LIVE FEED MICRO-ALGAE / FRESHLY HATCHED ARTEMIA	40 cells μl^{-1}	$5.ml^{-1}.d^{-1}$	-

Table 2 : Statistical analysis of larval survival of *P. monodon* larvae (PL1) on artificial diet as total replacement

ANALYSIS OF VARIANCE

SOURCE	D.F.	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
BETWEEN GROUPS	2	1483.0833	741.5417	3.8480	.1485
WITHIN GROUPS	3	578.1250	192.7083		
TOTAL	5	2091.2083			

FEEDS	CODE	MEAN	STANDARD DEVIATION
DIET	F	70.5000	±10.6066
DIET	J	55.2500	±21.5668
DIET	L	93.5000	± .7071

Tests for Homogeneity of Variances
Cochrans C = Max. Variance/Sum (Variances) = .8045, P = .309
Bartlett-Box F = 2.242 , P = .131

Table 3a: Statistical analysis of larval growth of *P. monodon* larvae (PL1) on artificial diets as total replacement

ANALYSIS OF VARIANCE

SOURCE	D.F.	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
BETWEEN GROUPS	2	2.1550	1.0775	21.1855	.001
WITHIN GROUPS	57	2.8990	.0509		
TOTAL	59	5.0540			

FEEDS	CODE	MEAN	STANDARD DEVIATION
DIET	F	4.7749	± .2601
DIET	J	4.5293	± .2385
DIET	L	4.9932	± .1676

Tests for Homogeneity of Variances
Cochrans C = Max. Variance/Sum (Variances) = .4432, P = .328
Bartlett-Box F = 1.831 , P = .160

Table 3b: Summary of significance of pairwise comparison between artificial diets (total replacement)

FEED	CODE	MEAN	DIETS J	F	L
DIET	J	4.5293			
DIET	F	4.7749	*		
DIET	L	4.9932	*	*	

(*) Denotes pairs of groups significantly different at the 0.050 level

Table 4a: Statistical analysis of larval growth of *P. monodon* larvae (PL1) on artificial diets supplemented by algae at 10 cells $\mu l^{-1}.d^{-1}$.

ANALYSIS OF VARIANCE

SOURCE	D.F.	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
BETWEEN GROUPS	10	60.8548	6.0855	161.4625	.001
WITHIN GROUPS	81	3.0529	.0377		
TOTAL	91	63.9077			

FEEDS	CODE	MEAN	STANDARD DEVIATION
DIET	F	5.1003	± .2183
DIET	B	4.7890	± .2697
DIET	C	4.6394	± .1376
DIET	A	4.0677	± .2553
DIET	D	4.1595	± .1821
DIET	I	4.5557	± .2522
DIET	J	4.8496	± .1064
DIET	H	4.1152	± .1034
DIET	E	.0000	
DIET	G	.0000	
DIET	L	5.5059	± .1263

Tests for Homogeneity of Variance
Cochrans C = Max. Variance/Sum (Variances) = .2145, P = .297
Bartlett-Box F = 2.374 , P = .015

Table 4b: Summary of significance of pairwise comparison, larval growth and artificial diets supplemented by algae at 10 cell μl^{-1}. d^{-1}.

MEAN	FEEDS	DIETS E	G	A	H	D	I	C	B	J	F	L
.0000	DIET E											
.0000	DIET G	ns										
4.0677	DIET A	*	*									
4.1152	DIET H	*	*	ns								
4.1595	DIET D	*	*	ns	ns							
4.5557	DIET I	*	*	*	*	*						
4.6394	DIET C	*	*	*	*	*	ns					
4.7890	DIET B	*	*	*	*	*	ns	ns				
4.8496	DIET J	*	*	*	*	*	ns	ns	ns			
5.1003	DIET F	*	*	*	*	*	*	*	ns	ns		
5.5059	DIET L	*	*	*	*	*	*	*	*	*	*	

(*) Denotes pairs of groups significantly different at the 0.050 level

ns indicates a non-significant result

Table 5a: Statistical analysis of larval survival of *P. monodon* larvae (PL1) on artificial diets supplemented by algae at 10 cells $\mu l^{-1}.d^{-1}$.

ANALYSIS OF VARIANCE

SOURCE	D.F.	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
BETWEEN GROUPS	10	13224.2000	322.4200	40.2087	.001
WITHIN GROUPS	9	296.0000	32.8889		
TOTAL	19	13520.2000			

FEEDS	CODE	MEAN	STANDARD DEVIATION
DIET	F	76.0000	± 5.6569
DIET	B	42.0000	± 4.2426
DIET	C	40.0000	± 2.8284
DIET	A	45.0000	± 11.3137
DIET	D	39.0000	± .0000
DIET	I	71.0000	± 1.4142
DIET	J	84.0000	± 8.4853
DIET	H	41.0000	± 4.2426
DIET	E	.0000	
DIET	G	.0000	
DIET	L	89.0000	± 4.2426

Tests for Homogeneity of Variances
Cochrans C = Max. Variance/Sum (Variances) = .4324, P = .349
Bartlett-Box F = .521 , P = .817

Table 5b: Summary of significance of pairwise comparison, larval survival and artificial diets supplemented by algae at 10 cells $\mu l^{-1}.d^{-1}$.

MEAN	FEEDS	DIETS E	G	D	C	H	B	A	I	F	J	L
.0000	DIET E											
.0000	DIET G	ns										
39.0000	DIET D	ns	ns									
40.0000	DIET C	*	*	ns								
41.0000	DIET H	*	*	ns	ns							
42.0000	DIET B	*	*	ns	ns	ns						
45.0000	DIET A	*	*	ns	ns	ns	ns					
71.0000	DIET I	*	*	ns	ns	ns	ns	ns				
76.0000	DIET F	*	*	*	*	*	*	ns	ns			
84.0000	DIET J	*	*	*	*	*	*	*	ns	ns		
89.0000	DIET L	*	*	*	*	*	*	*	ns	ns	ns	

(*) Denotes pairs of groups significantly different at the 0.050 level
ns indicates a non-significant result.

Table 6a: ANOVA table for regression of water quality variables against larval survival

ANALYSIS OF VARIANCE

	D.F.	SUM OF SQUARES	MEAN SQUARE	F.	SIG.F
REGRESSION	4	45556.21515	11389.05379	56.3600	.0001
RESIDUAL	72	14549.51940	202.07666		

VARIABLES IN THE EQUATION

VARIABLE	B	SE B	BETA	T	SIG.T	
NH3	-.951973	.200917	-.861135	-4.738	.0001	S
PH	13.172468	1.307294	.600554	10.076	.0001	S
NO2	-.346271	.060848	-.533242	-5.691	.0001	S
NH4	.033270	.011105	.585780	2.996	.0038	S
(constant)	.018554	10.045798		.002	.9985	

Multiple R	.87059
R Square	.75793
Adjusted R Square	.74449
Standard Error	14.21537

Table 6b: ANOVA table for regression of water quality variables against larval growth

ANALYSIS OF VARIANCE

	D.F.	SUM OF SQUARES	MEAN SQUARE	F.	SIG.F
REGRESSION	4	90.02152	22.50538	22.9528	.0001
RESIDUAL	72	70.59634	.98050		

VARIABLES IN THE EQUATION

VARIABLE	B	SE B	BETA	T	SIG. T	
NH3	-.052449	.013995	-.917786	-3.748	.0004	S
PH	.175231	.091063	.154545	1.924	.0583	N
NO2	.010569	.004238	.314841	2.494	.0149	S
NH4	.003617	7.7357	1.231876	4.675	.0001	S
(Constant)	-.033282	.699763		-.048	.9622	

Multiple R	.74865
R Square	.56047
Adjusted R Square	.53605
Standard Error	.99020

Table 7: Growth and survival of *P. monodon* larvae ranked according to feed efficiency of artificial diet

	CONTROL	ARTIFICIAL LARVAL DIETS									
RANKING	1	2	3	4	5	6	7	8	9	10	11
	L	F	J	I	B	C	A	D	H	E	G
No. of larvae stocked at beginning of feed trial	200	200	200	200	200	200	200	200	200	200	200
Percentage Survival PL1	89	76	84	71	42	40	45	39	41	-	-
± S.D.	±3.53	±5.65	±8.48	±1.41	±3.53	±2.12	±7.74	-	±3.0	-	-
Mean growth (mm)											
Size at stocking (mm)	0.984	0.984	1.038	1.038	0.984	0.984	0.984	0.984	1.038	0.984	.984
Size at PL1 (mm)	5.51	5.10	4.85	4.55	4.79	4.64	4.07	4.16	4.11	-	-
± S.D.	±.109	±.184	±.075	±.252	±.221	±.113	±.221	±.148	±.100	-	-
Percent gain in growth (%) [a]	459.95	418.29	367.24	338.34	386.78	371.54	313.61	322.76	295.98	-	-
Feed efficiency (%) [b]	381.88	323.4	314.77	268.10	241.27	228.5	199.76	197.08	193.37	-	-

$$a = \left(\frac{G - Go}{Go} \right) \times 100$$

$$b = \frac{G - Go \left[\frac{N + No}{2} \right]}{F} \times 100$$

where F = Total amount of feed intake during feed trial
Go = mean growth of larvae (mm) at the beginning of feed trial
G = mean growth of larvae (mm) at the end of feed trial
No = number of larvae at the beginning of trial
N = number of larvae at the end of feed trial

Figure 1. Organic content (ash free dry weight) of leading artificial larval diets, together with protein, lipid and carbohydrate levels, compared to live feeds (Raymont, 1980). Vertical bars are standard deviation. Figures in bracket are growth (mm) of P. monodon postLarvae and percentage survival achieved in feed trials on each diet.

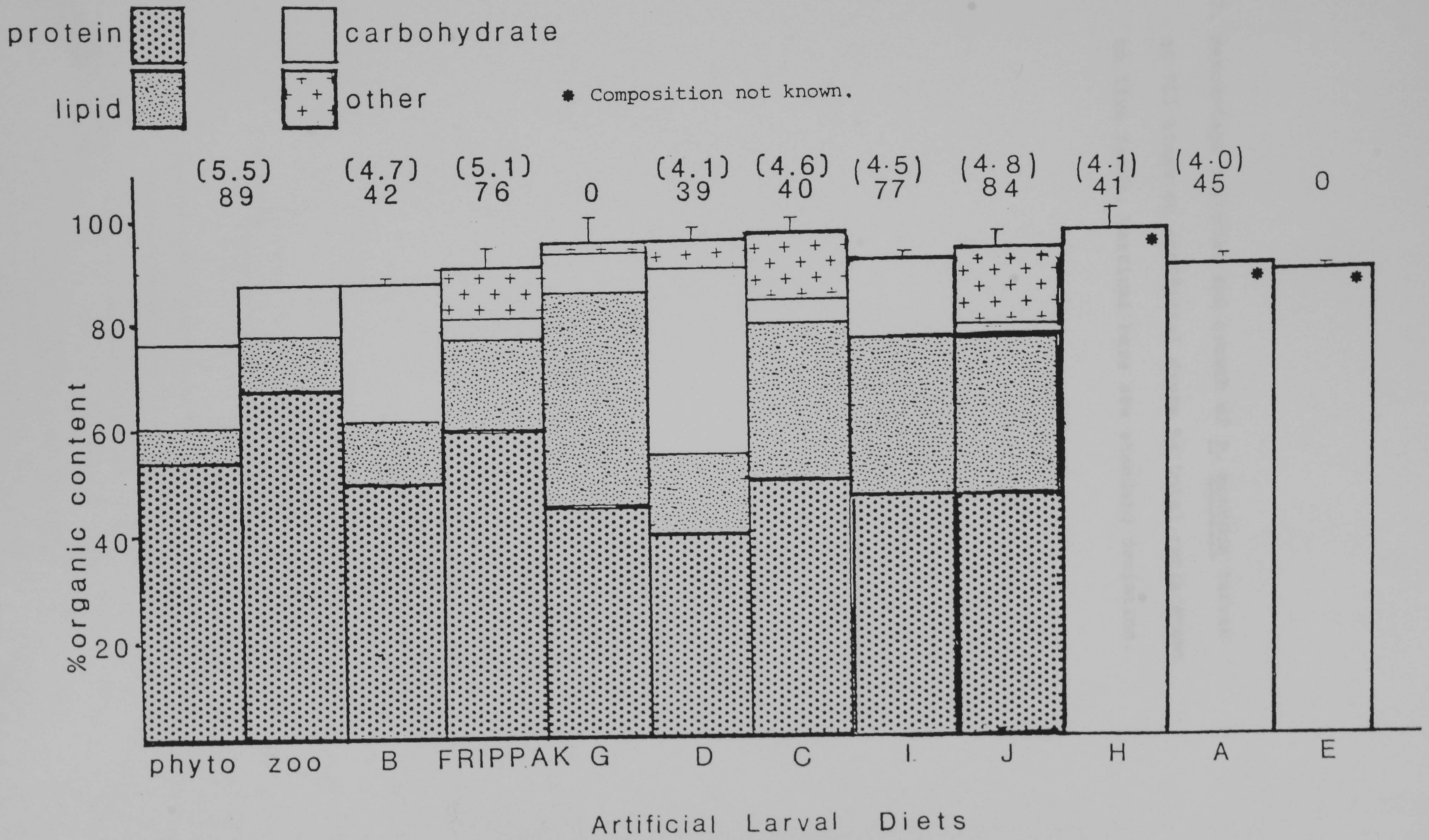

protein
carbohydrate
lipid
other
* Composition not known.
(5.5) 89
(4.7) 42
(5.1) 76
0
(4.1) 39
(4.6) 40
(4·5) 77
(4·8) 84
(4·1) 41
(4·0) 45
0
100
80
60
40
20
%organic content
phyto
zoo
B
FRIPPAK
G
D
C
I
J
H
A
E
Artificial Larval Diets

Figure 2. Percentage survival and growth of P. monodon larvae at PL1 stage on artificial diets as total replacement to live feeds. Vertical bars are standard deviation.

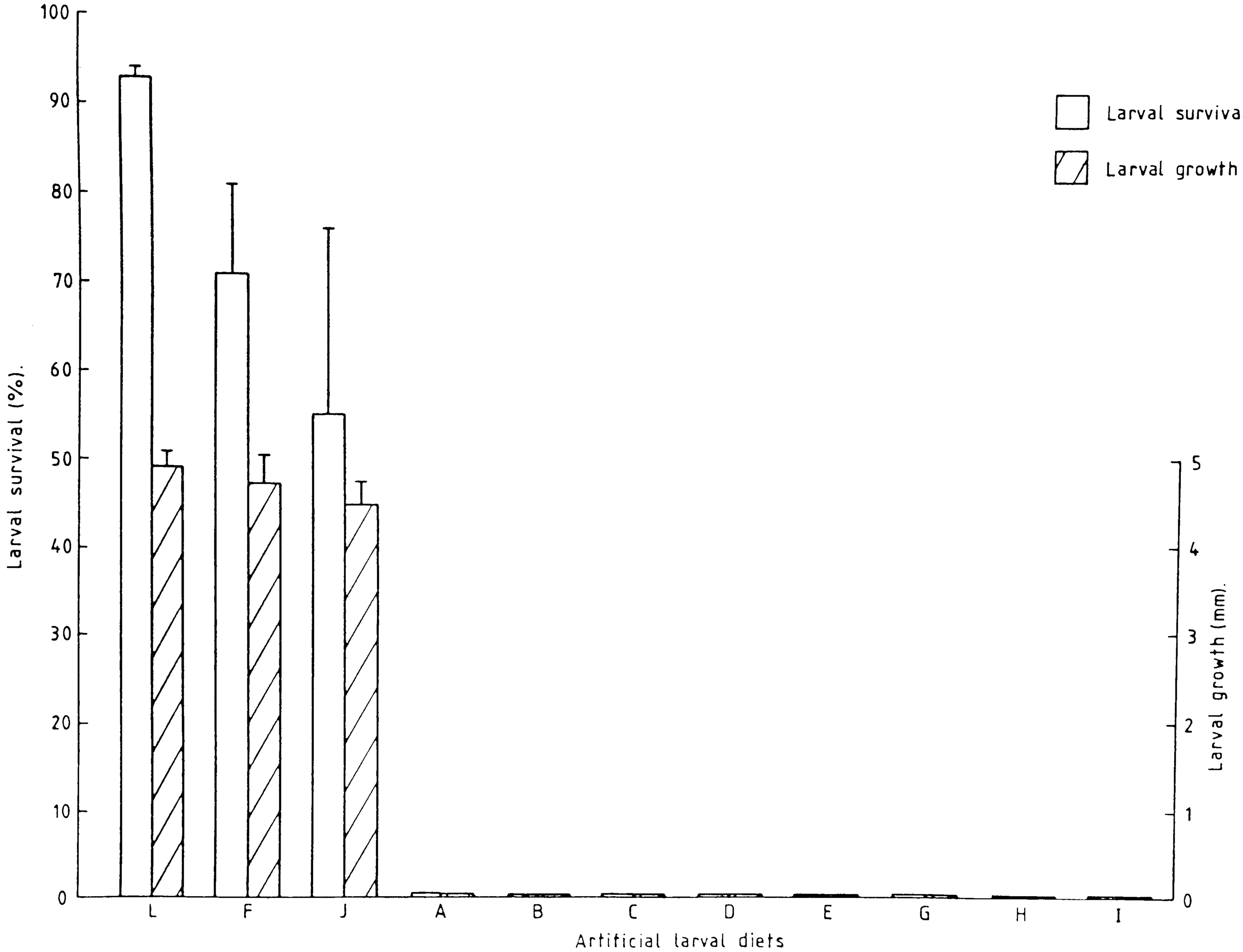

Larval survival
Larval growth
Larval survival (%).
Larval growth (mm).
100
90
80
70
60
50
40
30
20
10
0
5
4
3
2
1
0
L
F
J
A
B
C
D
E
G
H
I
Artificial larval diets

Figure 3. Larval survival and growth of P. monodon to PL1 stage on artificial diets supplemented by live microalgae at 10 cells μl^{-1} . Vertical bars are standard deviation. For diet codes see table 1.

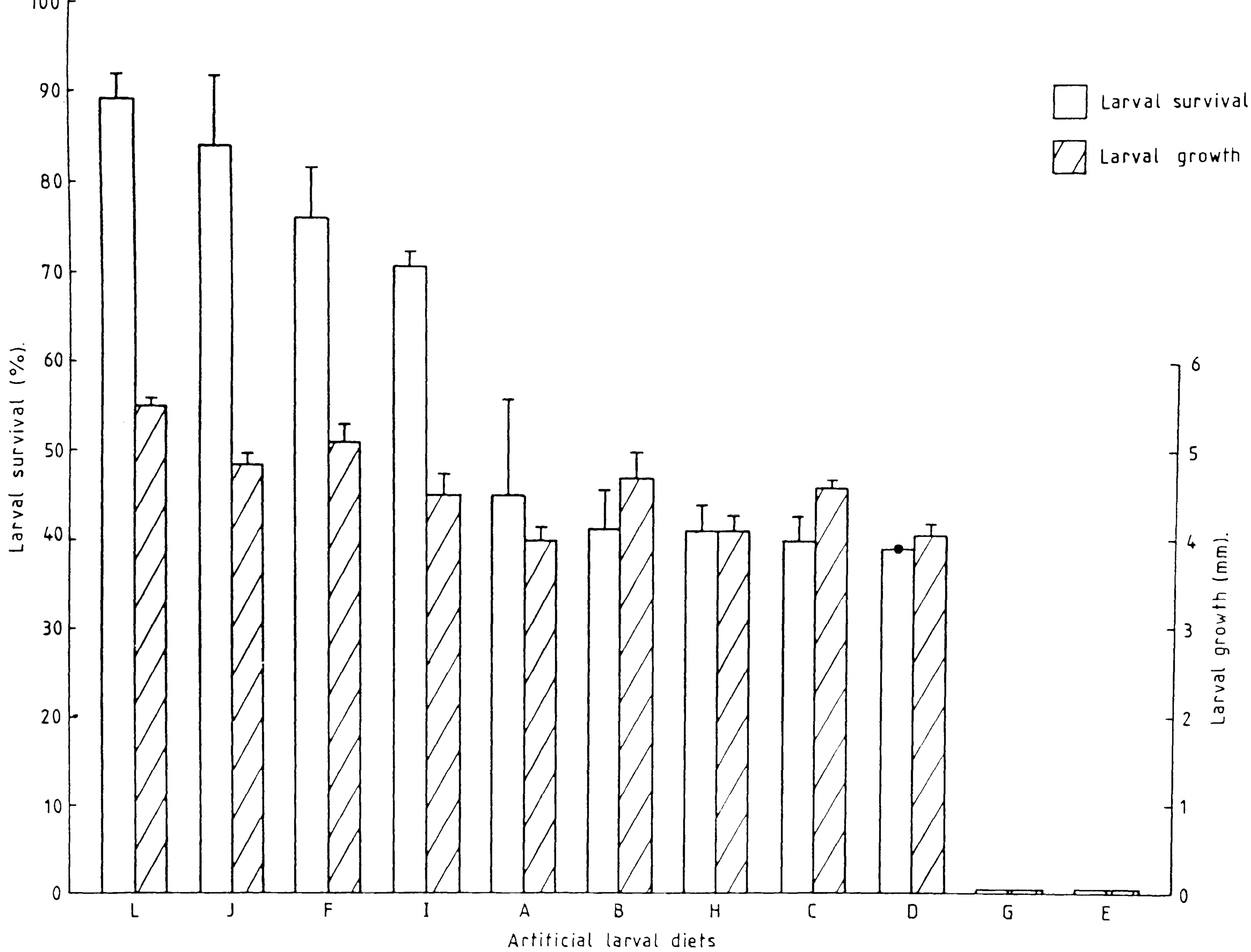

Larval survival
Larval growth
Larval survival (%).
Larval growth (mm).
100
90
80
70
60
50
40
30
20
10
0
6
5
4
3
2
1
0
L
J
F
I
A
B
H
C
D
G
E
Artificial larval diets

Figure 4. Percentage loss of soluble compounds from leading artificial larval diets over 24h in seawater at 28°C. For diet codes see table 1. Values are mean of two replicates.

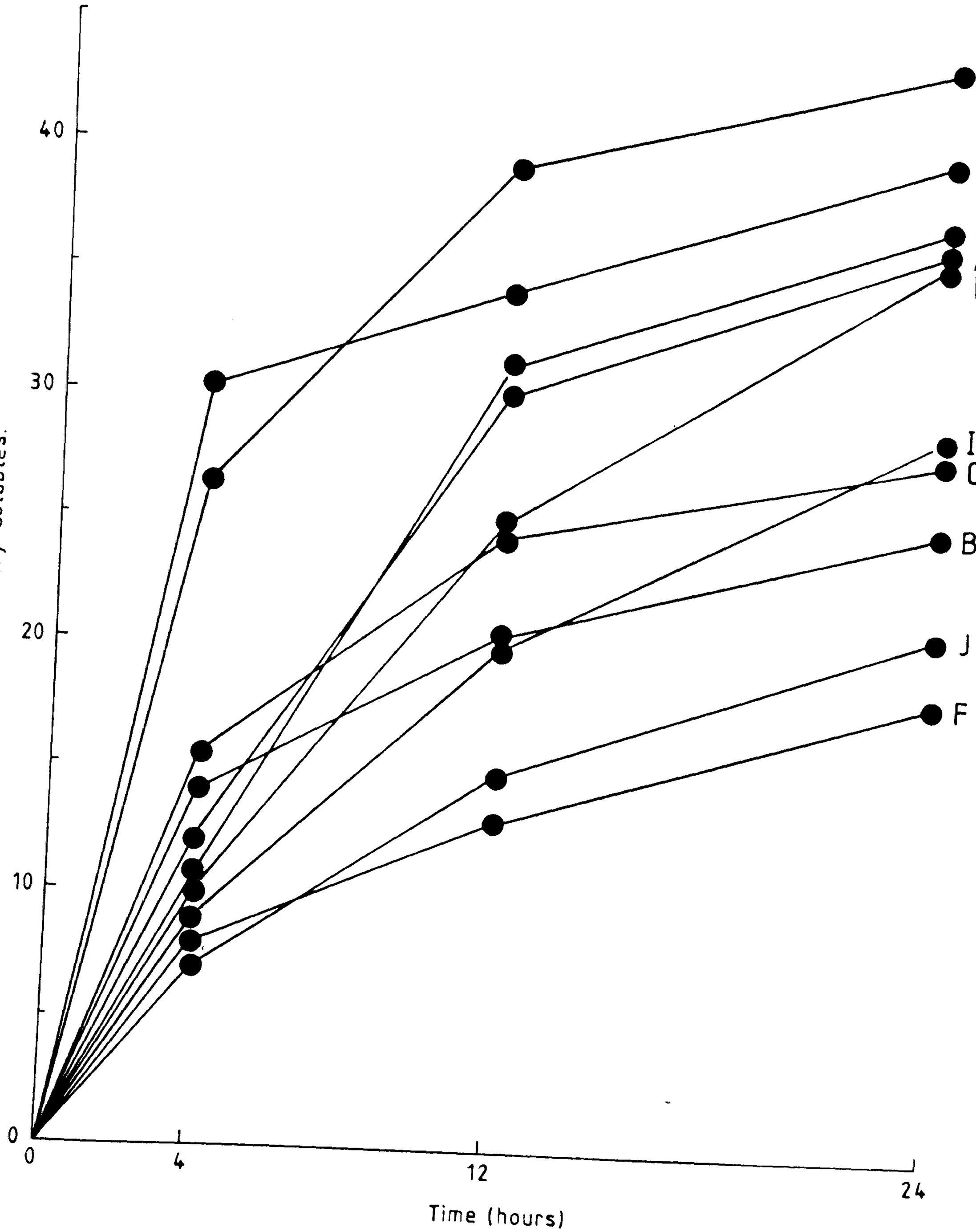

% Loss of dietary solubles.
40
30
20
10
0
0
4
12
24
Time (hours)
E
G
D
A
H
I
C
B
J
F

Figure 5a. Percentage leach loss of dietary solubles from artificial larval diets and its effect upon survival of P. monodon larvae in replicate feed trials.

Linear regression coefficient: Y = 43.083 + (-0.257xX).

Correlation coefficient: r = -0.849

Slope: -0.2774

DF = 9 , F = 20.52 , P = 0.000.

Figure 5b. Percent leach loss of dietary solubles from artificial larval diets and its effect upon larval growth of P. monodon in replicate feed trials.

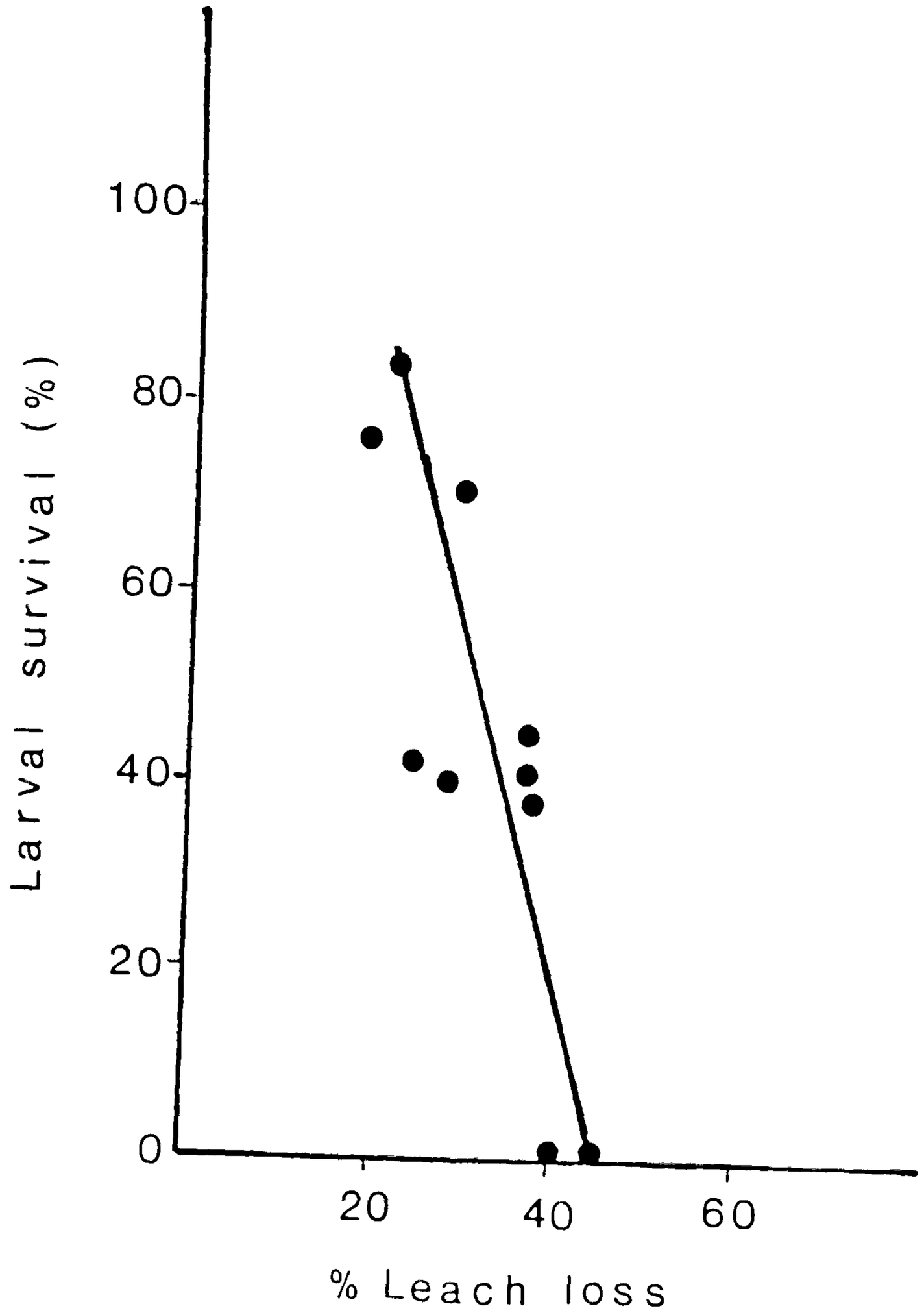
Linear regression coefficient: Y = 43.995 + (-3.400xX).

Correlation coefficient: r = -0.763

Slope = -4.8041

DF = 9 , F = 11.16 , P = 0.010.

a


Larval survival (%)
100
80
60
40
20
0
20
40
60
% Leach loss

b

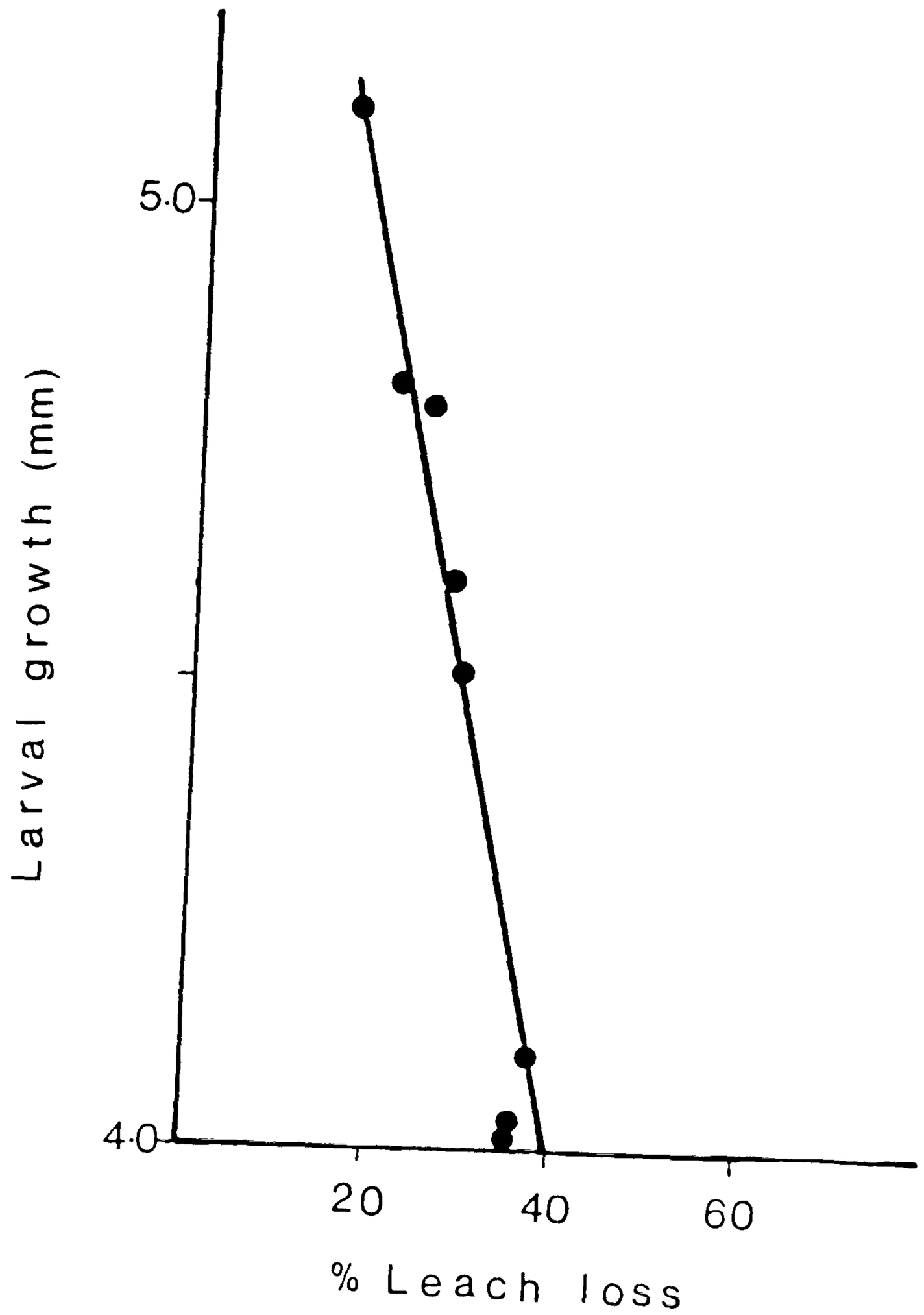

Larval growth (mm)
5·0
4·0
20
40
60
% Leach loss

Figure 6. Production of ionised ammonia (NH_4^+N) by leading brands of artificial larval diets over 24h incubated in seawater at 28°C. Values are mean of two replicates. Diet codes are listed in table 1.

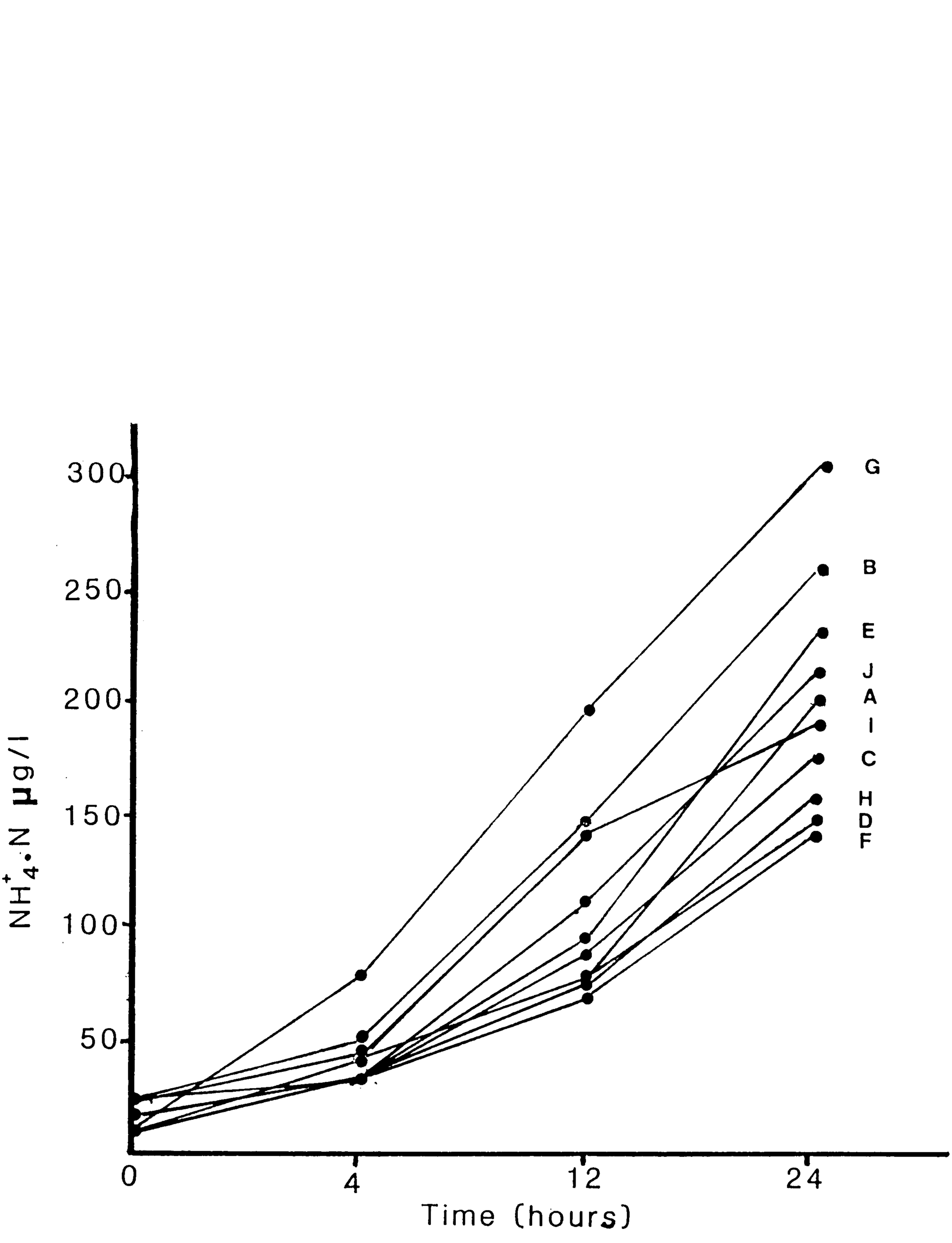
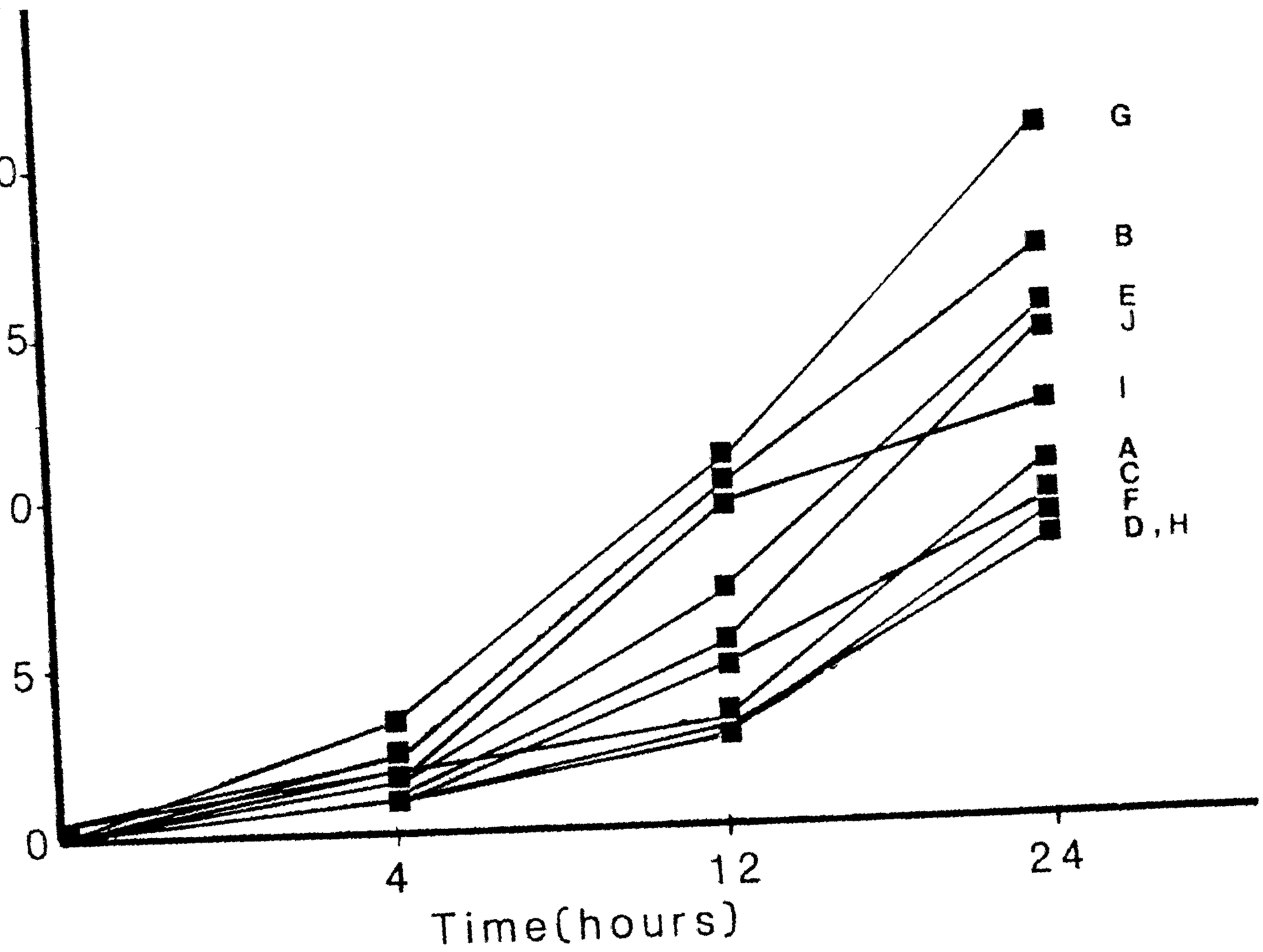

300
250
200
150
100
50
NH_4^+.N µg/l
0
4
12
24
Time (hours)
G
B
E
J
A
I
C
H
D
F

Figure 7. Production of unionised ammonia (NH_3 .N) by leading brands of artificial larval diets over 24h incubated in seawater at 28°C. Values are mean of two replicates. Diet codes are listed in table 1.

Figure 8. Production of nitrite (NO_2.N) by leading brands of artificial larval diets over 24h incubated in seawater at 28°C. Values are mean of two replicates. Diet codes are listed in table 1.


NH_3.N µg./l
20
15
10
5
0
4
12
24
Time(hours)
G
B
E
J
I
A
C
F
D, H

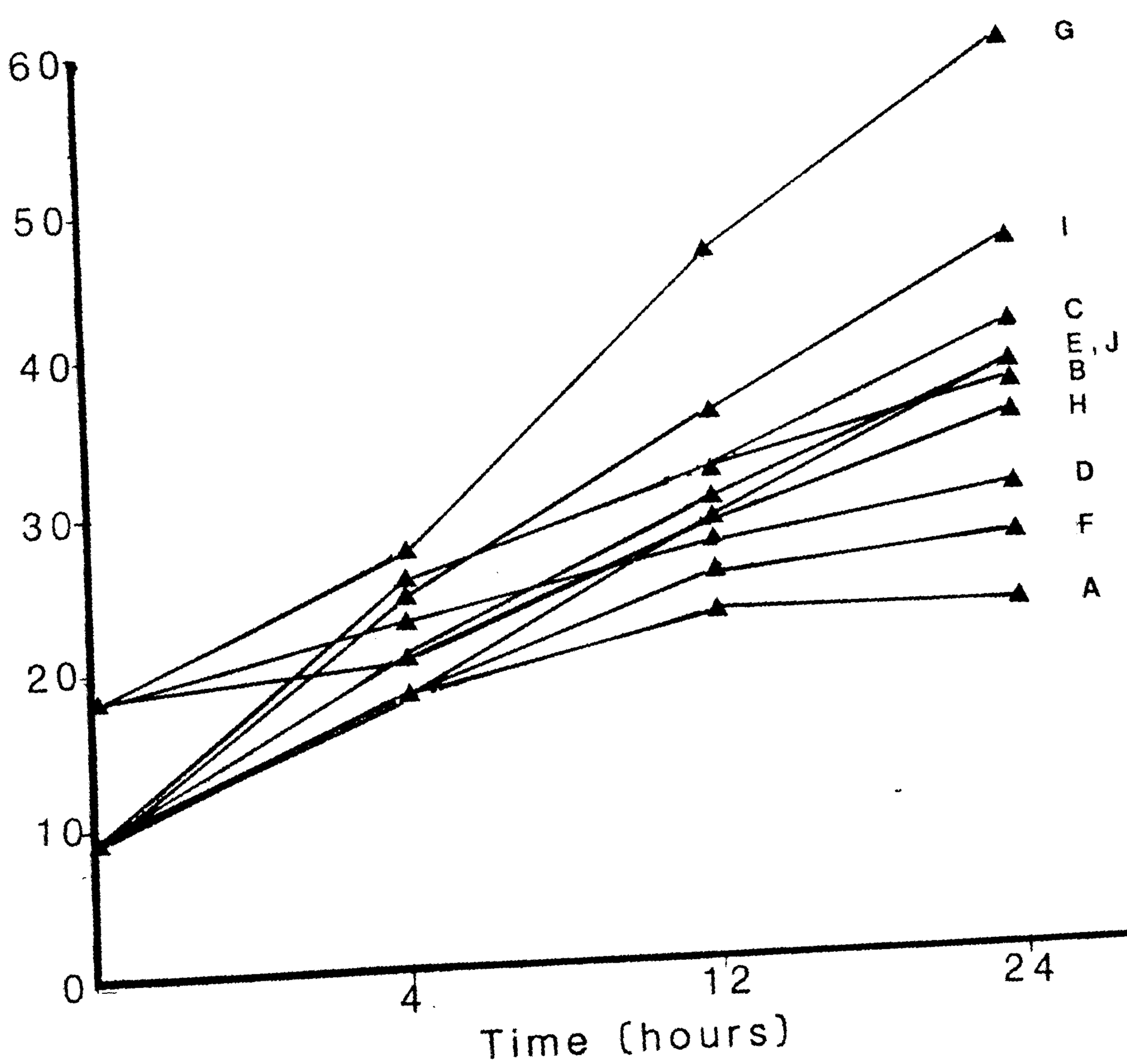
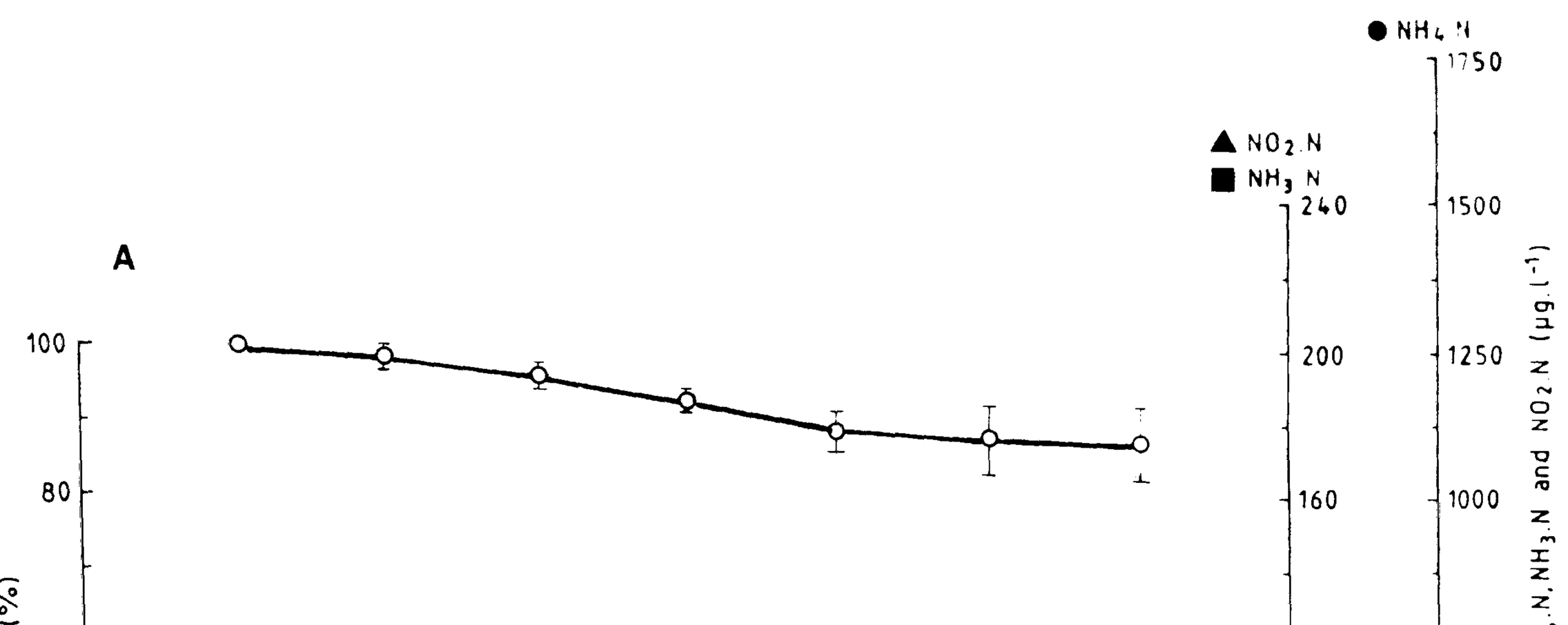

NO_2.N µg./l
60
50
40
30
20
10
0
4
12
24
Time (hours)
G
I
C
E, J
B
H
D
F
A

Figure 9a. P.monodon larval survival (○) on control diet of live feeds, microalgae at 40 cells μl^{-1} from PZ1 to PZ3 and Artemia fed at 5. ml^{-1} from M1 to PL1 stages, plotted together with NO_2.N (▲), NH_3.N (■) and NH_4.N (●) levels in replicated feed trials. Vertical bars are standard deviation.

Figure 9b. Larval growth (Δ) and development of P. monodon larvae on control diets, plotted together with NO_2.N, NH_3.N and NH_4.N levels in replicated feed trials.

A


● NH4.N
▲ NO2.N
■ NH3.N
Larval survival (%)
Production of NH4.N, NH3.N and NO2.N (µg.l-1)
100
80
60
40
20
0
240
200
160
120
80
40
1750
1500
1250
1000
750
500
250
0
1
3
5
7
9
11
13
Period (days)
Pl1
Pl2
Pl3-M1
M2
M3-Pl1
Pl2
Pl4
Larval stages

B

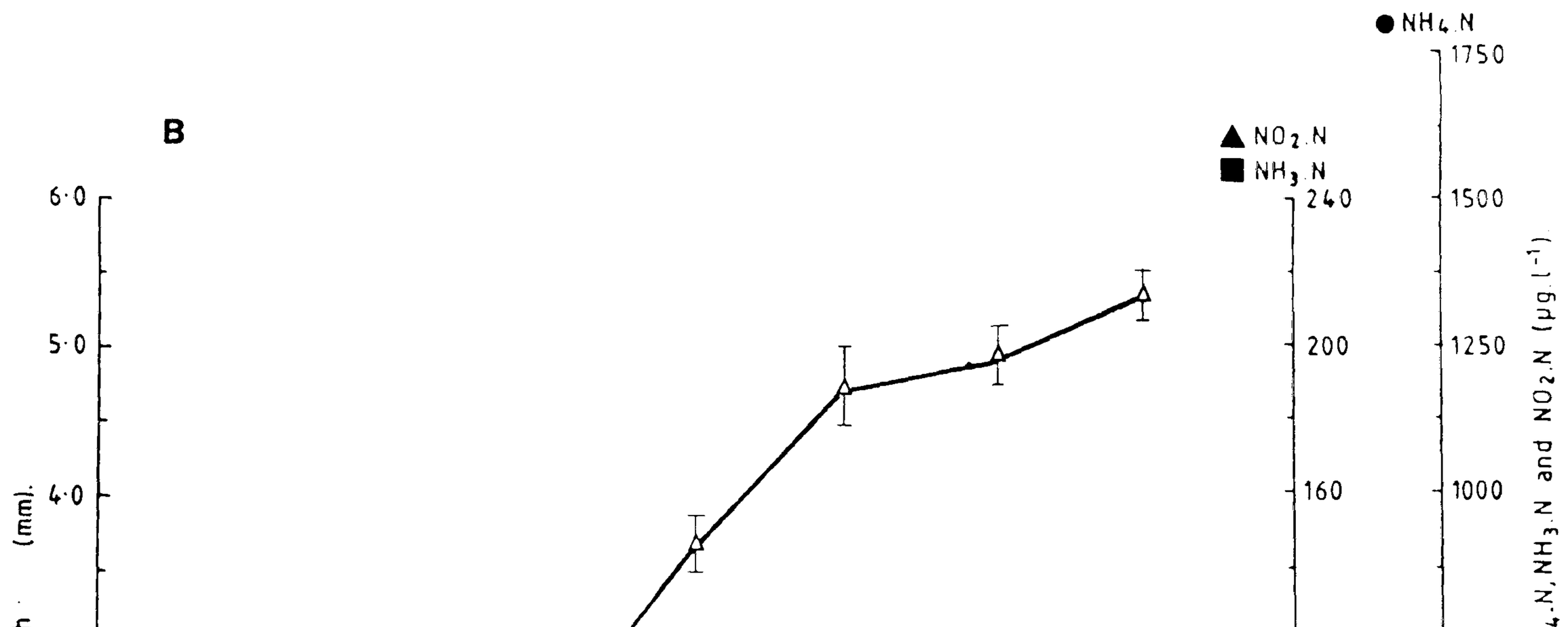
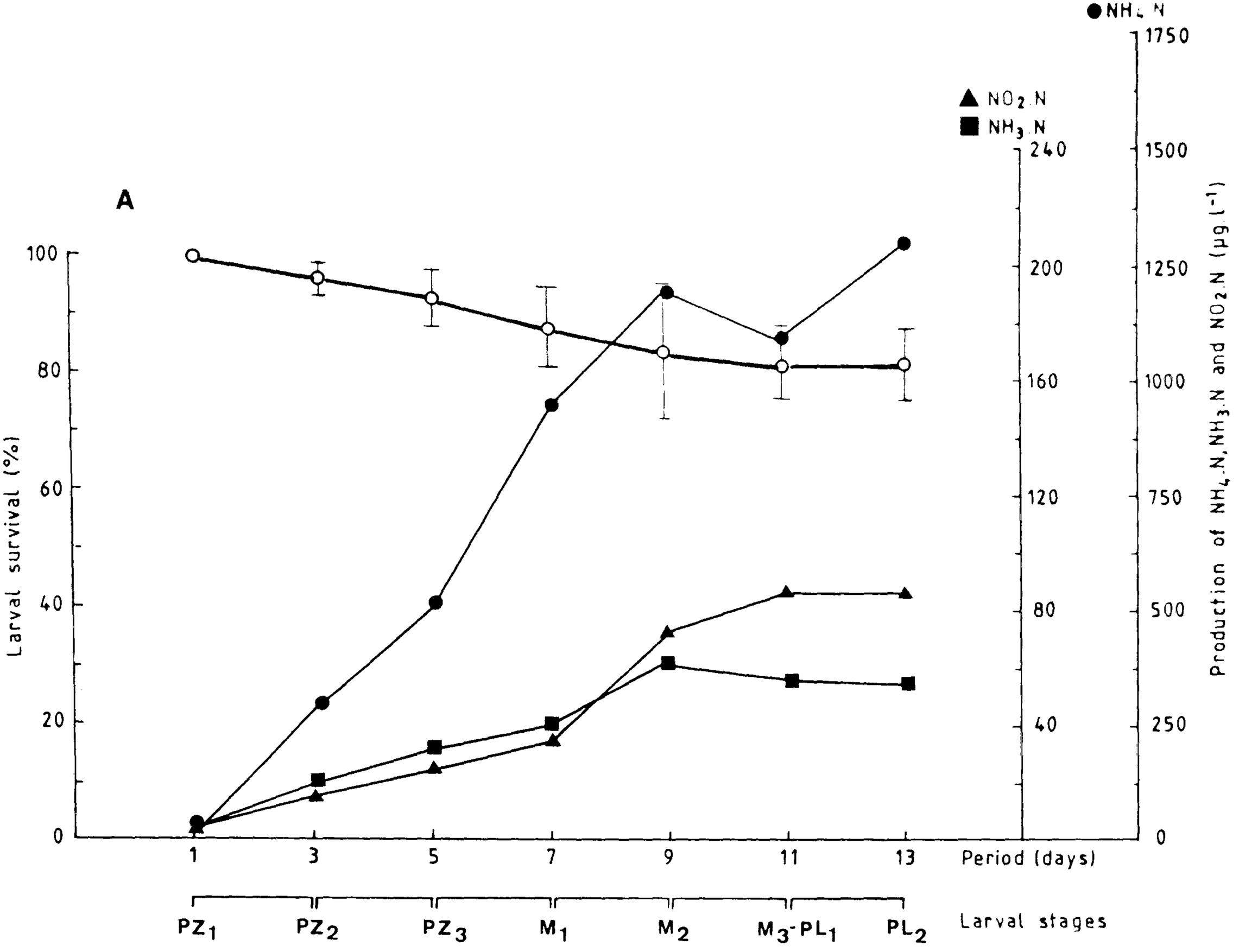

● NH4.N
▲ NO2.N
■ NH3.N
Larval growth (mm).
Production of NH4.N, NH3.N and NO2.N (µg.l-1)
6·0
5·0
4·0
3·0
2·0
1·0
0
240
200
160
120
80
40
1750
1500
1250
1000
750
500
250
0
1
3
5
7
9
11
13
Period (days)
Pl1
Pl2
Pl3-M1
M2
M3-Pl1
Pl2
Pl4
Larval stages

Figure 10a. P. monodon larval survival (○) on spray dried formulation diet 'J' supplemented by 10 cells μl^{-1} microalgae, plotted together with NO_2.N (▲), NH_3.N (■) and NH_4.N (●) levels in replicated feed trials. Vertical bars are standard deviation.

Figure 10b. Larval growth (Δ) and development of P. monodon larvae on diet 'J', plotted together with NO_2.N, NH_3.N and NH_4.N levels in replicated feed trials.

A


● NH4.N
▲ NO2.N
■ NH3.N
Larval survival (%)
Production of NH4.N, NH3.N and NO2.N (µg.l-1)
Period (days)
1 3 5 7 9 11 13
PZ1 PZ2 PZ3 M1 M2 M3-PL1 PL2
Larval stages

B

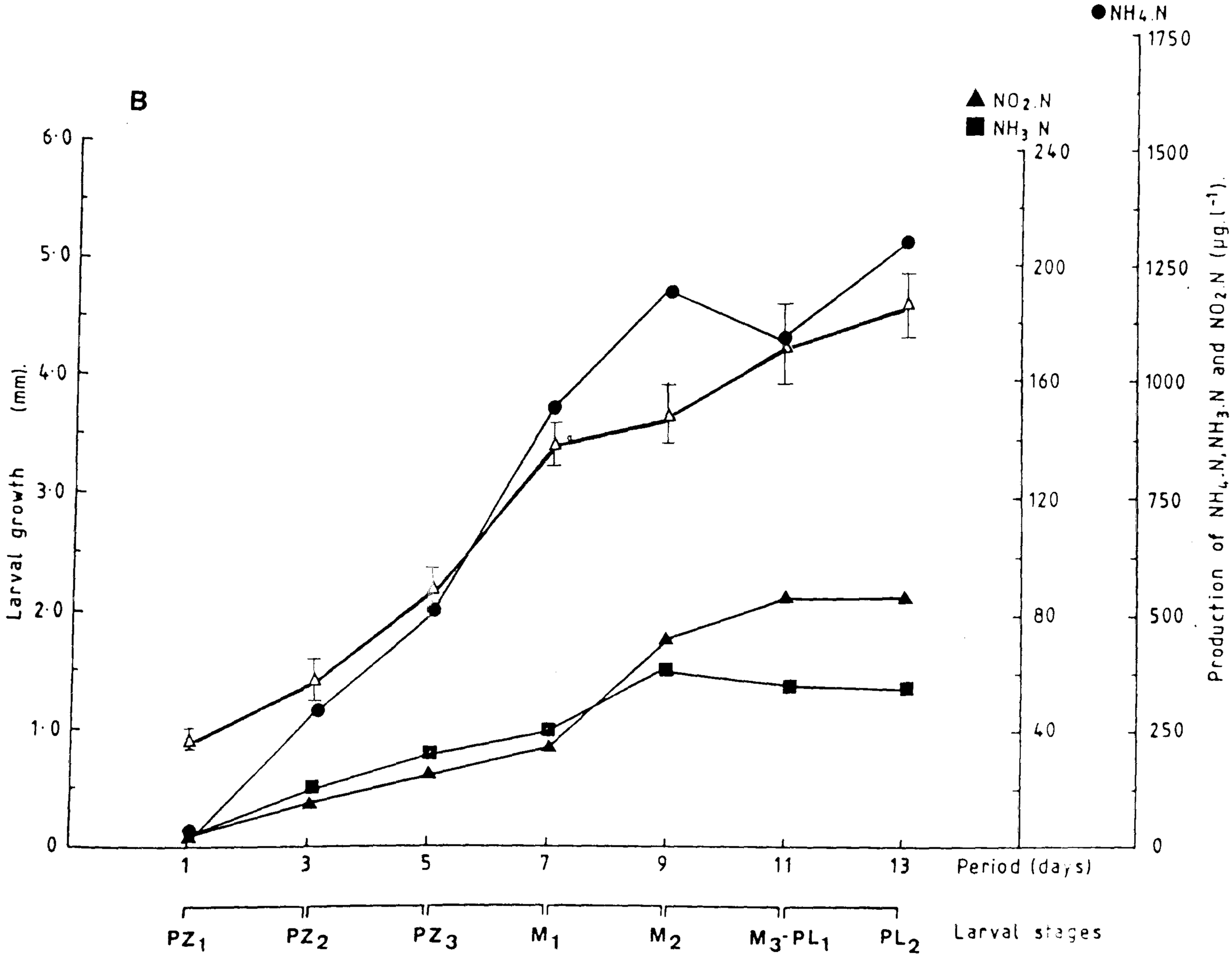
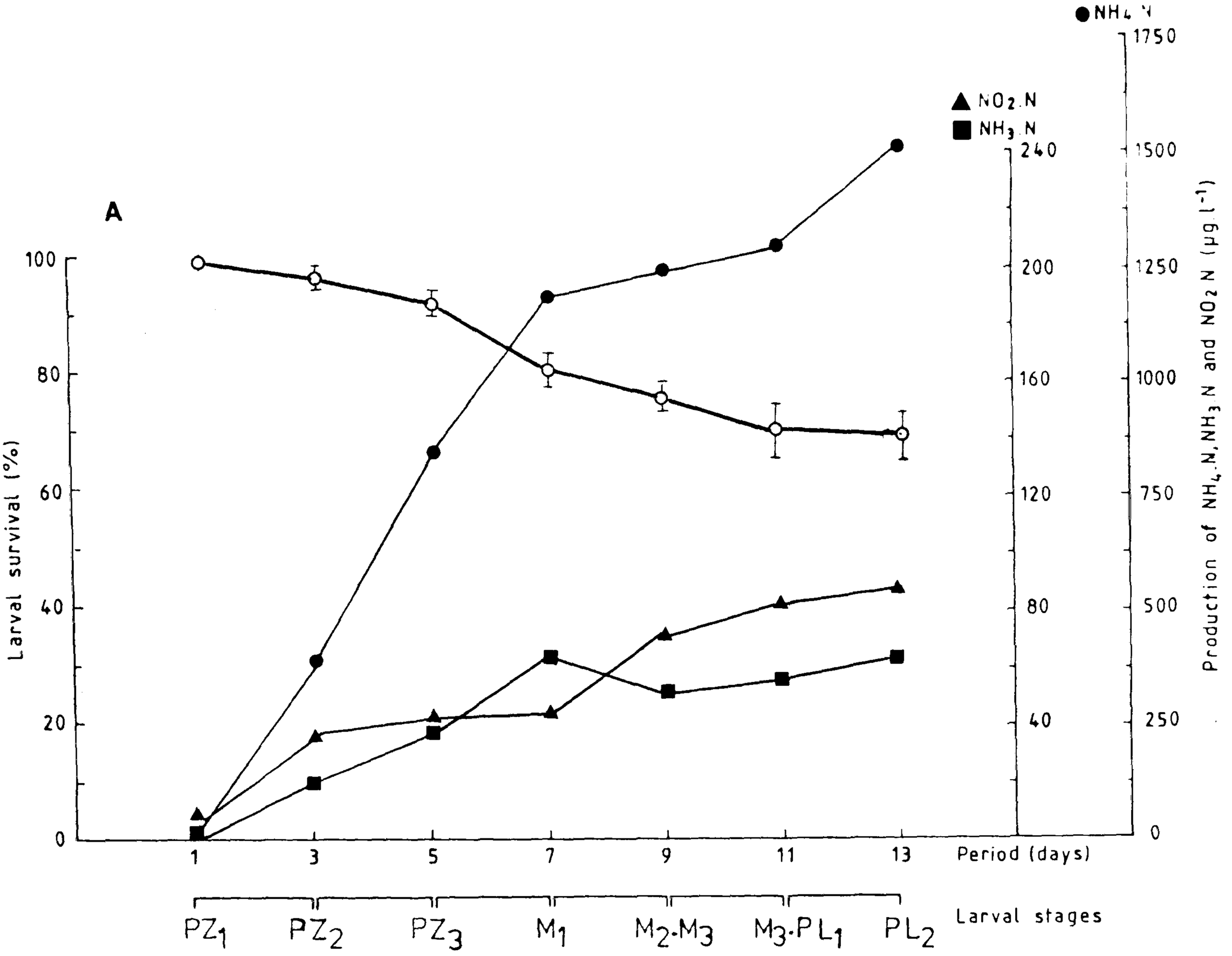

● NH4.N
▲ NO2.N
■ NH3.N
Larval growth (mm).
Production of NH4.N, NH3.N and NO2.N (µg.l-1)
Period (days)
1 3 5 7 9 11 13
PZ1 PZ2 PZ3 M1 M2 M3-PL1 PL2
Larval stages

Figure 11a. P. monodon larval survival (○) on microencapsulated diet 'F' supplemented by 10 cells μl^{-1} microalgae, plotted together with NO_2.N (▲), NH_3.N (■) and NH_4.N (●) levels in replicated feed trials. Vertical bars are standard deviation.

Figure 11b. Larval growth (Δ) and development of P. monodon larvae on diet 'F', plotted together with NO_2.N, NH_3.N and NH_4.N levels in replicated feed trials.

A


● NH4.N
▲ NO2.N
■ NH3.N
Larval survival (%)
Production of NH4.N, NH3.N and NO2.N (µg.l-1).
Period (days)
Larval stages
PZ1
PZ2
PZ3
M1
M2-M3
M3-PL1
PL2

B

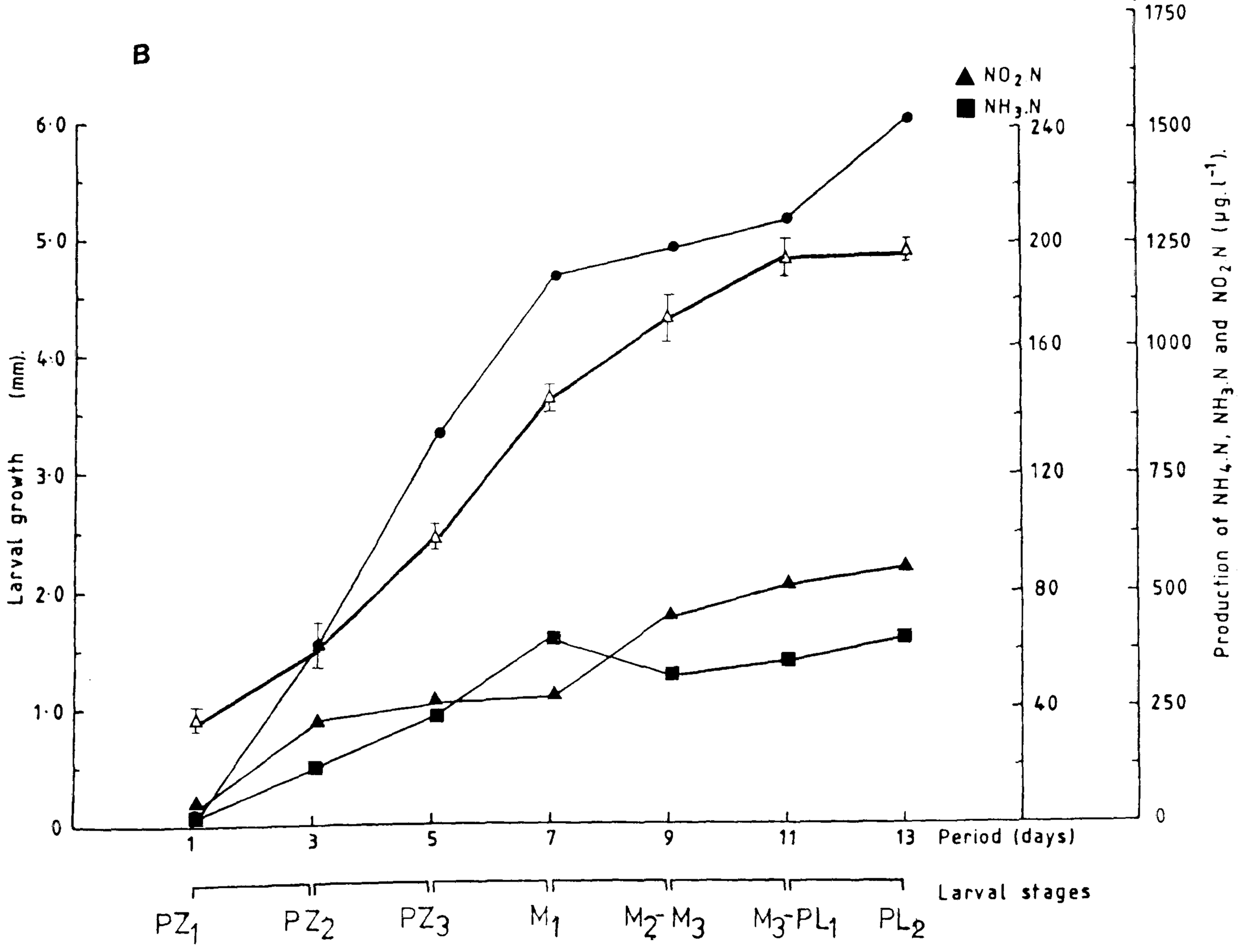
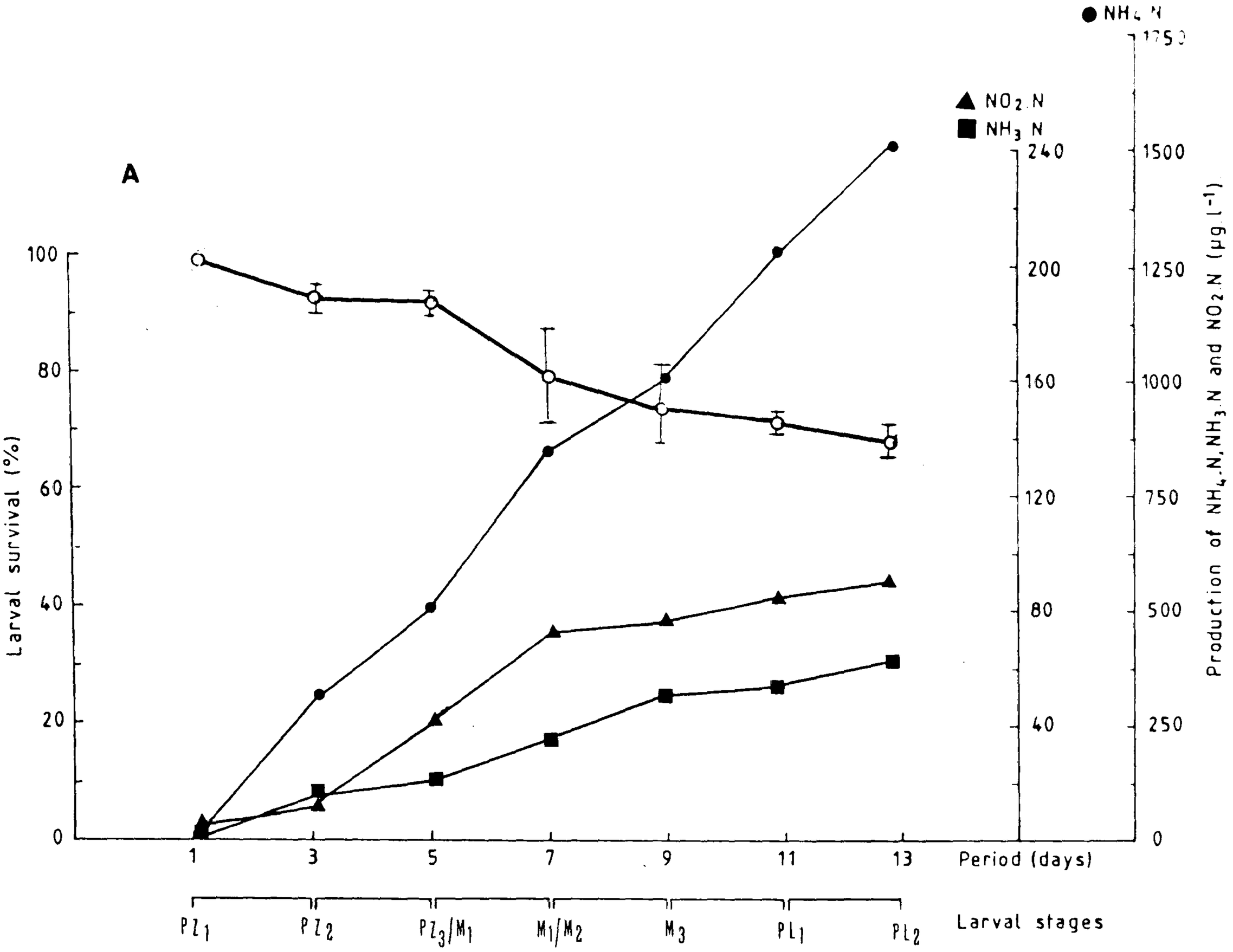

● NH4.N
▲ NO2.N
■ NH3.N
Larval growth (mm).
Production of NH4.N, NH3.N and NO2.N (µg.l-1).
Period (days)
Larval stages
PZ1
PZ2
PZ3
M1
M2-M3
M3-PL1
PL2

Figure 12a. P. monodon larval survival (○) on spray dried formulation diet 'I' supplemented by 10 cells μl^{-1}microalgae, plotted together with NO_2.N (▲), NH_3.N (■) and NH_4.N (●) levels in replicated feed trials. Vertical bars are standard deviation.

Figure 12b. Larval growth (Δ) and development of P. monodon larvae on diet 'I', plotted together with NO_2.N, NH_3.N and NH_4.N levels in replicated feed trials.

A


Larval survival (%)
100
80
60
40
20
0
1
3
5
7
9
11
13
Period (days)
NO_2 N
NH_3 N
NH_4 N
240
200
160
120
80
40
0
1750
1500
1250
1000
750
500
250
0
Production of NH_4.N, NH_3.N and NO_2.N ($\mu g.l^{-1}$)
PZ_1
PZ_2
PZ_3/M_1
M_1/M_2
M_3
PL_1
PL_2
Larval stages

B

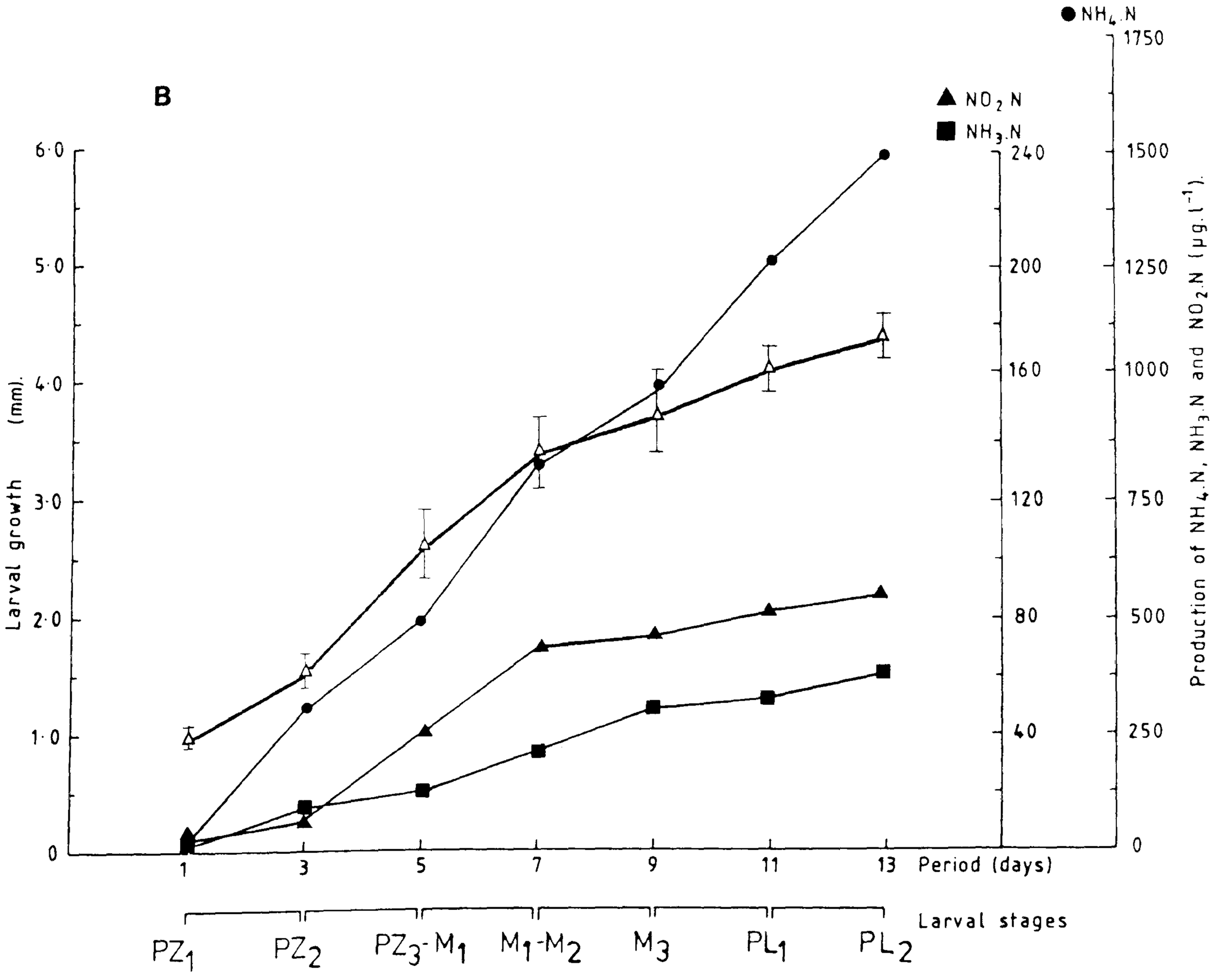
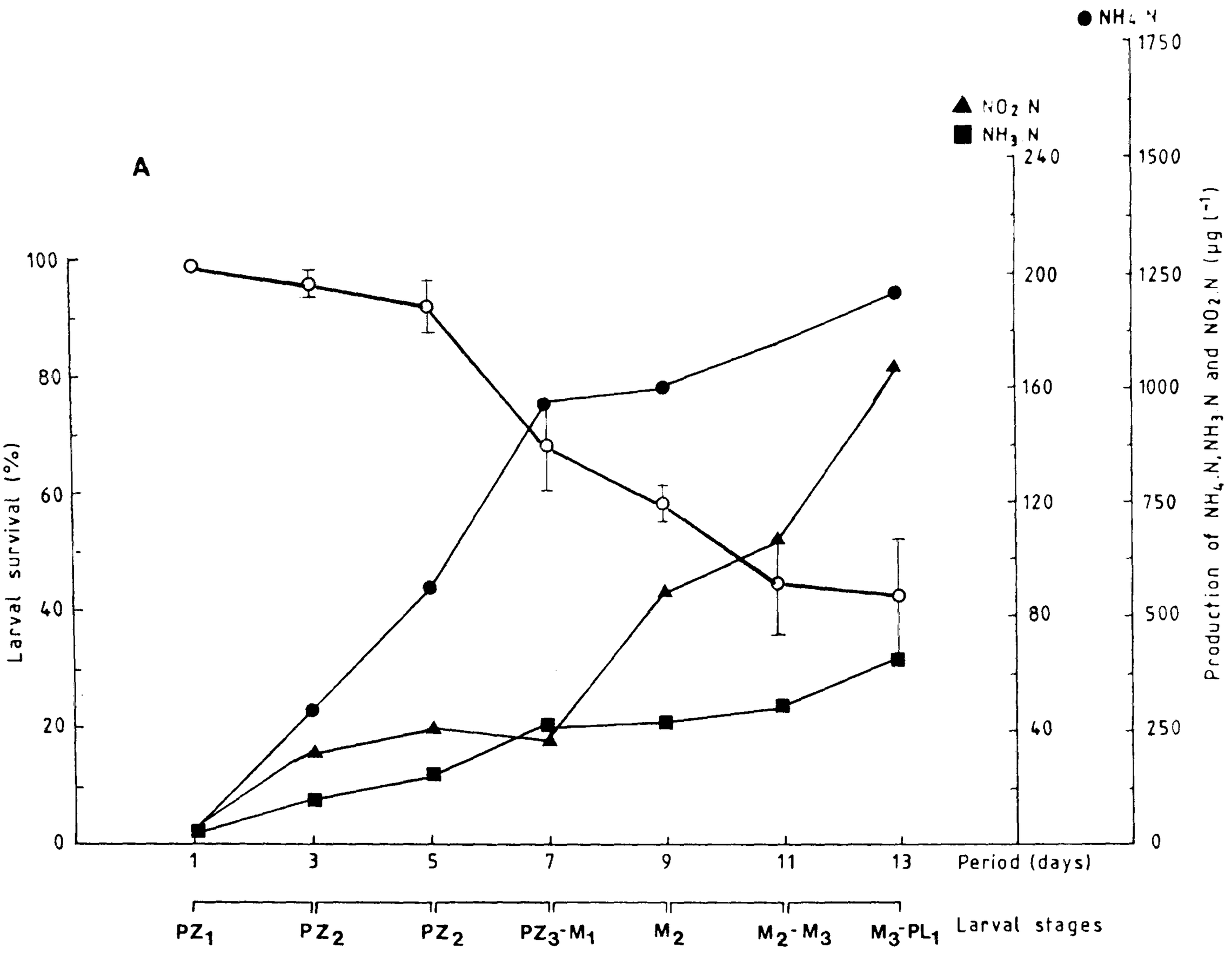

Larval growth (mm).
6·0
5·0
4·0
3·0
2·0
1·0
0
1
3
5
7
9
11
13
Period (days)
NO_2 N
NH_3.N
NH_4.N
240
200
160
120
80
40
0
1750
1500
1250
1000
750
500
250
0
Production of NH_4.N, NH_3.N and NO_2.N ($\mu g.l^{-1}$).
PZ_1
PZ_2
PZ_3-M_1
M_1-M_2
M_3
PL_1
PL_2
Larval stages

Figure 13a. P.monodon larval survival (○) on micropaticulate diet 'A' supplemented by 10 cells μl^{-1} microalgae, plotted together with NO_2.N (▲), NH_3.N (■) and NH_4.N (●) levels in replicated feed trials. Vertical bars are standard deviation.

Figure 13b. Larval growth (Δ) and development of P. monodon larvae on diet 'A', plotted together with NO_2.N, NH_3.N and NH_4.N levels in replicated feed trials.

A


● NH4 N
▲ NO2 N
■ NH3 N
Larval survival (%)
Production of NH4.N, NH3.N and NO2.N (µg l-1)
Period (days)
Larval stages
PZ1
PZ2
PZ2
PZ3-M1
M2
M2-M3
M3-PL1

B

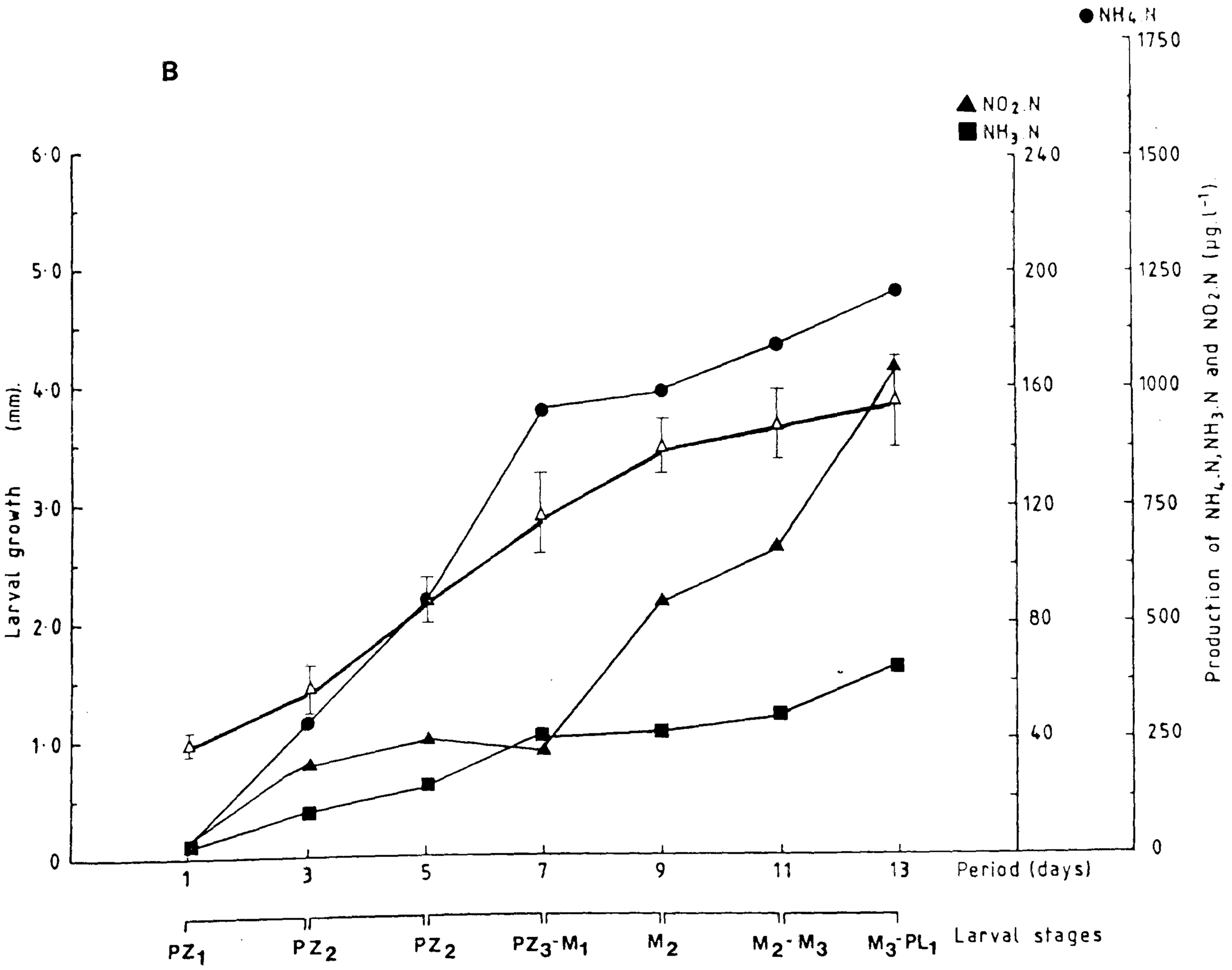
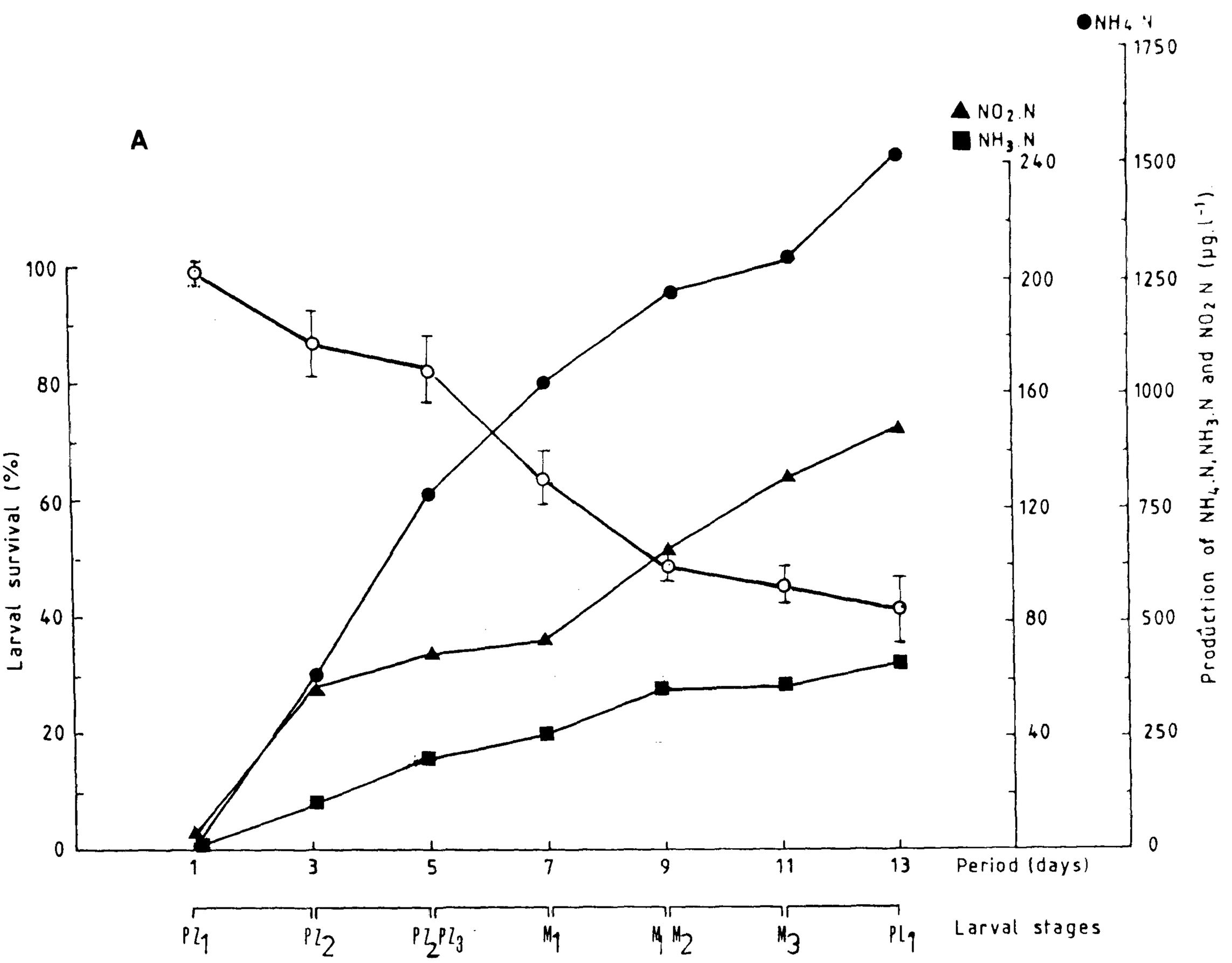

● NH4 N
▲ NO2 N
■ NH3 N
Larval growth (mm).
Production of NH4.N, NH3.N and NO2.N (µg l-1)
Period (days)
Larval stages
PZ1
PZ2
PZ2
PZ3-M1
M2
M2-M3
M3-PL1

Figure 14a. P. monodon larval survival (○) on microparticulate diet 'B' supplemented by 10 cells μl^{-1} microalgae, plotted together with NO_2.N (▲), NH_3.N (■) and NH_4.N (●) levels in replicated feed trials. Vertical bars are standard deviation.

Figure 14b. Larval growth (Δ) and development of P. monodon larvae on diet 'B', plotted together with NO_2.N, NH_3.N and NH_4.N in replicated feed trial.

A


●NH4.N
▲NO2.N
■NH3.N
Larval survival (%)
100
80
60
40
20
0
240
200
160
120
80
40
1750
1500
1250
1000
750
500
250
0
Production of NH4.N, NH3.N and NO2.N (µg.l-1)
1
3
5
7
9
11
13
Period (days)
Pl1
Pl2
Pl2Pl3
M1
M1M2
M3
Pl1
Larval stages

B

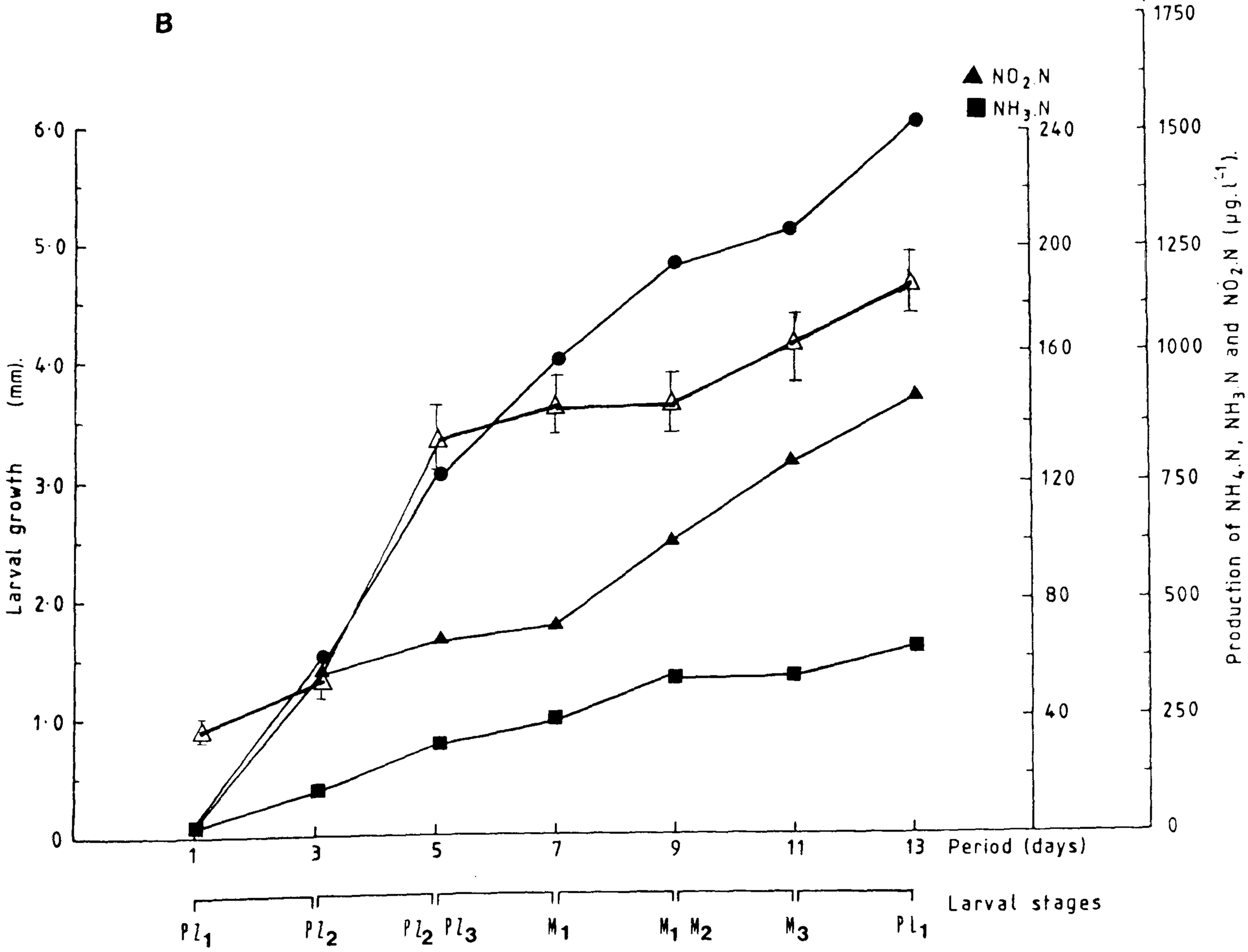
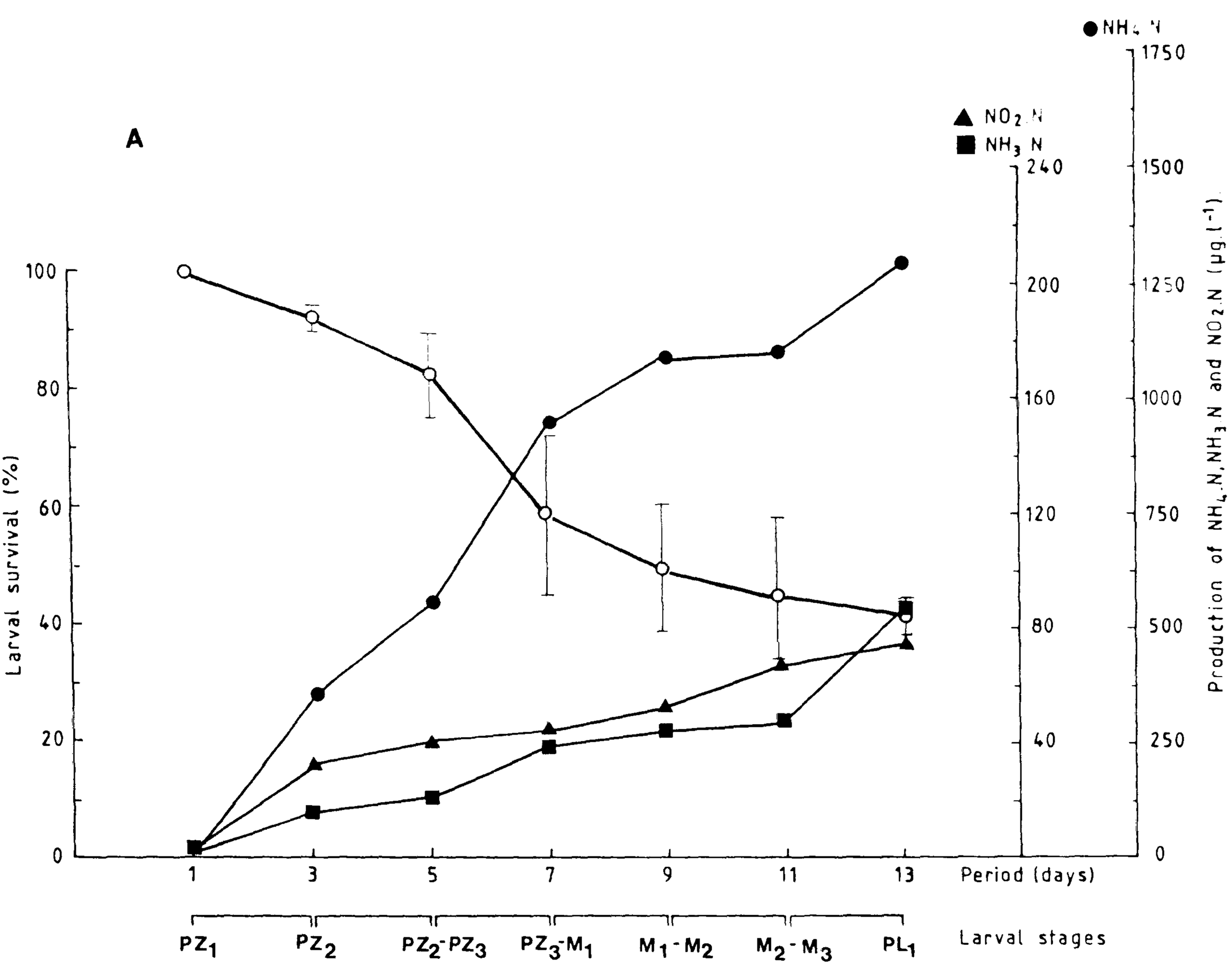

●NH4.N
▲NO2.N
■NH3.N
Larval growth (mm).
6·0
5·0
4·0
3·0
2·0
1·0
0
240
200
160
120
80
40
1750
1500
1250
1000
750
500
250
0
Production of NH4.N, NH3.N and NO2.N (µg.l-1).
1
3
5
7
9
11
13
Period (days)
Pl1
Pl2
Pl2Pl3
M1
M1M2
M3
Pl1
Larval stages

Figure 15a. P. monodon larval survival (○) on spray dried formulation diet 'H' supplemented by 10 cells μl^{-1} microalgae, plotted together with NO_2.N (▲), NH_3.H (■) and NH_4.N (●) levels in replicated feed trials. Vertical bars are standard deviation.

Figure 15b. Larval growth (Δ) and development of P. monodon larvae on diet 'H', plotted together with NO_2.N, NH_3.N and NH_4.N levels in replicated feed trials.

A


● NH4.N
▲ NO2.N
■ NH3.N
Larval survival (%)
Production of NH4.N, NH3.N and NO2.N (µg.l-1)
Period (days)
Larval stages
PZ1
PZ2
PZ2-PZ3
PZ3-M1
M1-M2
M2-M3
PL1

B

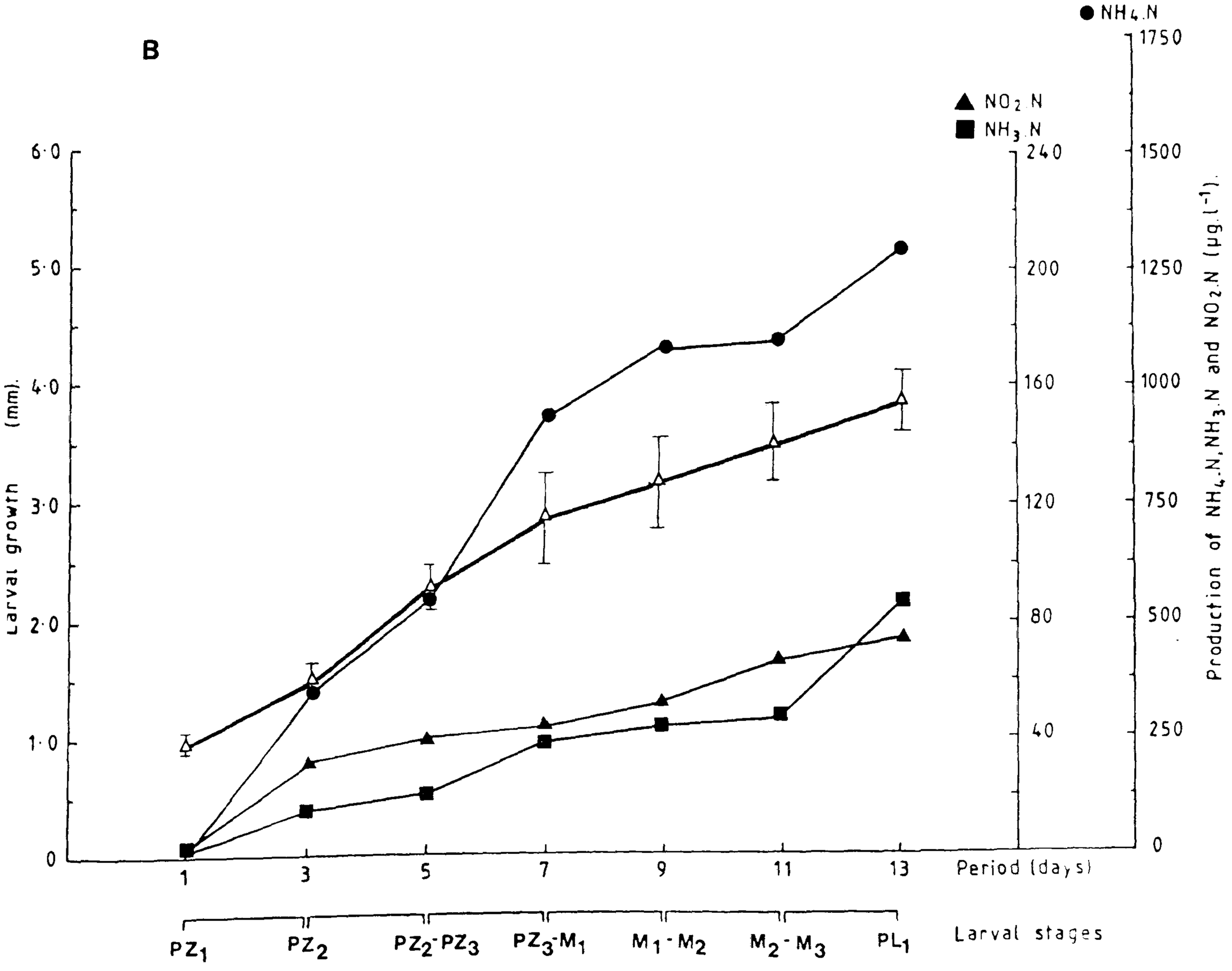
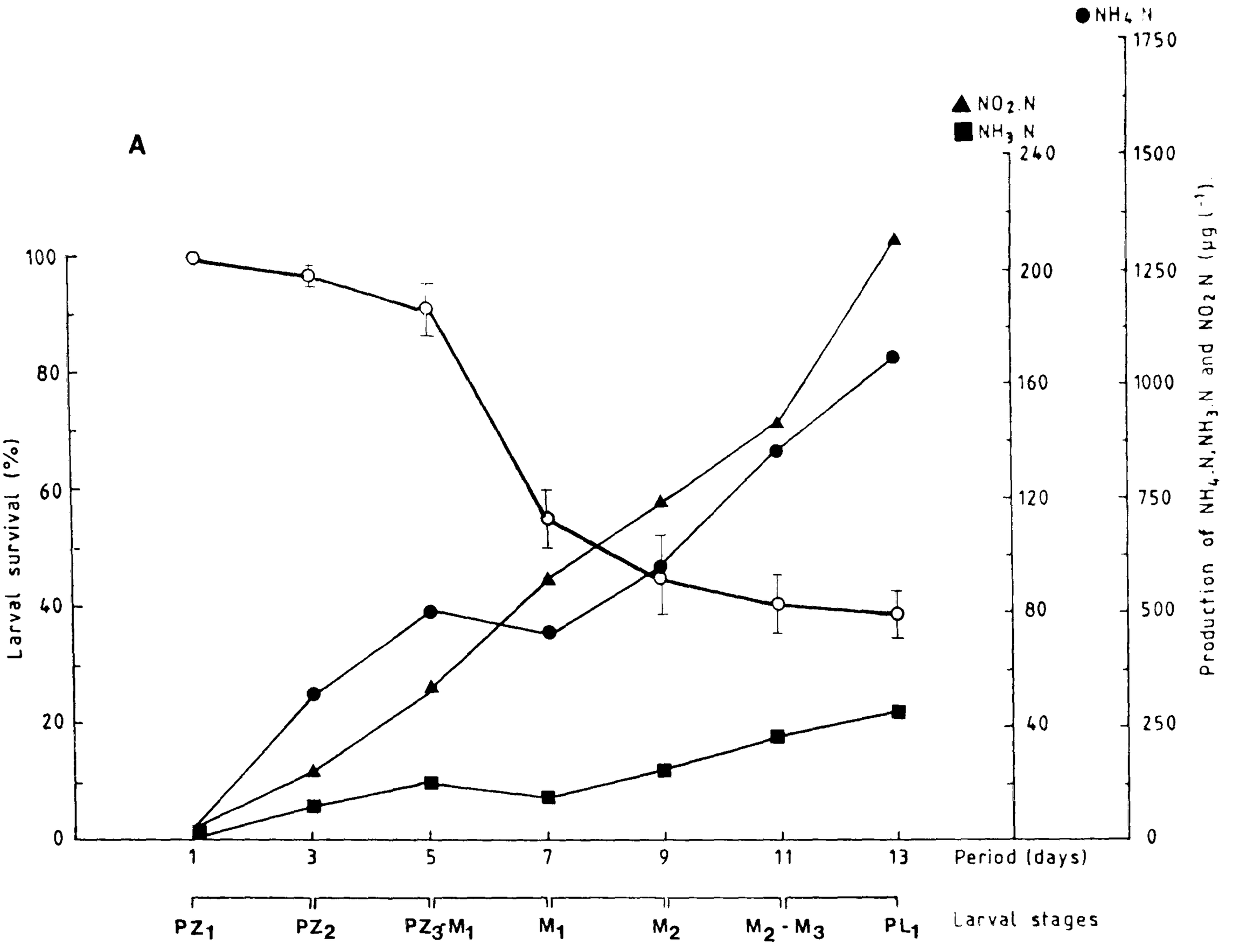

● NH4.N
▲ NO2.N
■ NH3.N
Larval growth (mm).
Production of NH4.N, NH3.N and NO2.N (µg.l-1).
Period (days)
Larval stages
PZ1
PZ2
PZ2-PZ3
PZ3-M1
M1-M2
M2-M3
PL1

Figure 16a. P. monodon larval survival (○) on spray dried formulation diet 'C' supplemented by 10 cells μl^{-1} microalgae, plotted together with NO_2.N (▲), NH_3.H (■) and NH_4.N (●) levels in replicated feed trials. Vertical bars are standard deviation.

Figure 16b. Larval growth (Δ) and development of P. monodon larvae on diet 'C', plotted together with NO_2.N, NH_3.N and NH_4.N levels in replicated feed trials.

A


Larval survival (%)
100
80
60
40
20
0
1
3
5
7
9
11
13
Period (days)
PZ1
PZ2
PZ3M1
M1
M2
M2 - M3
PL1
Larval stages
NO2.N
NH3 N
NH4 N
240
200
160
120
80
40
1750
1500
1250
1000
750
500
250
0
Production of NH4.N, NH3.N and NO2.N (µg.l-1)

B

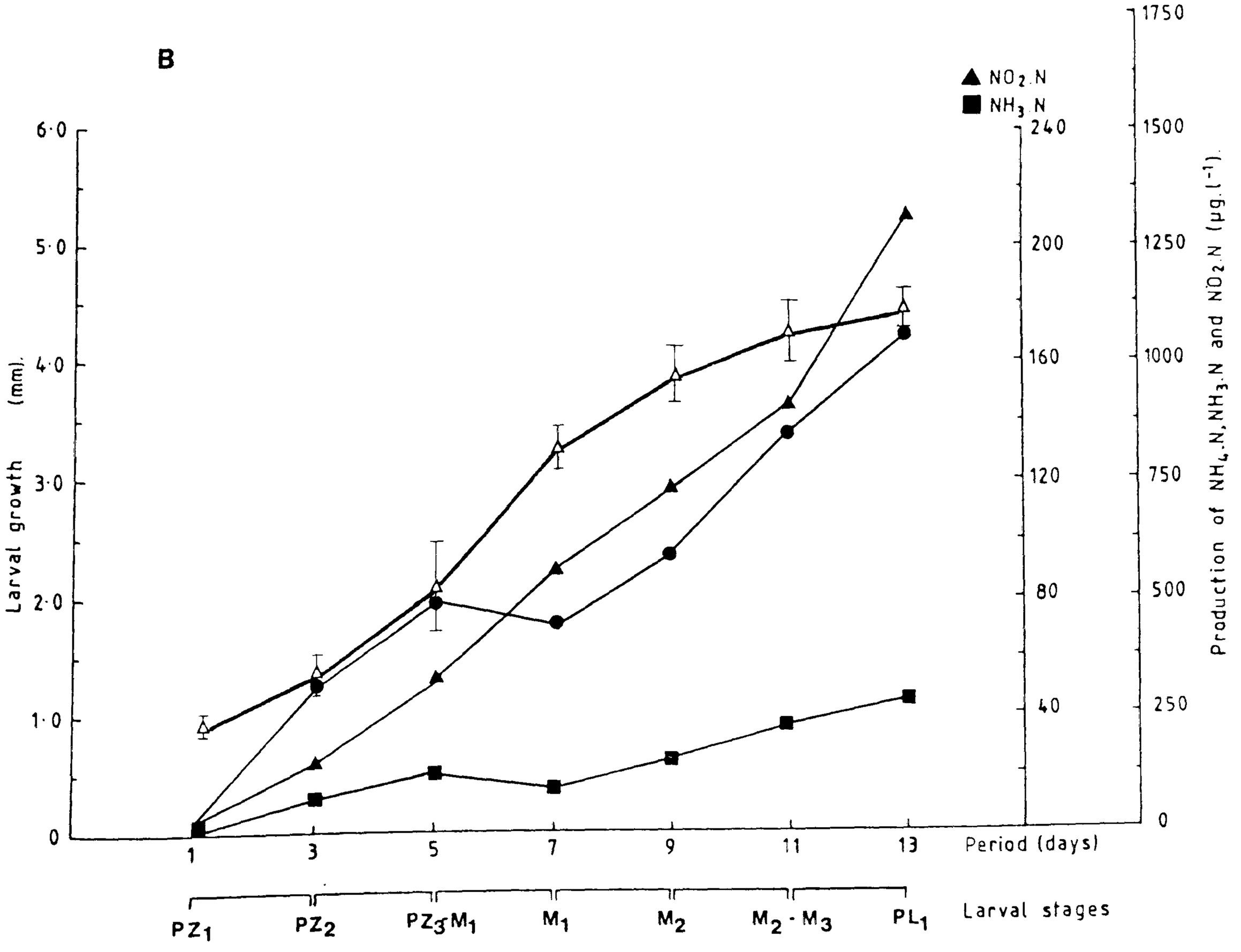
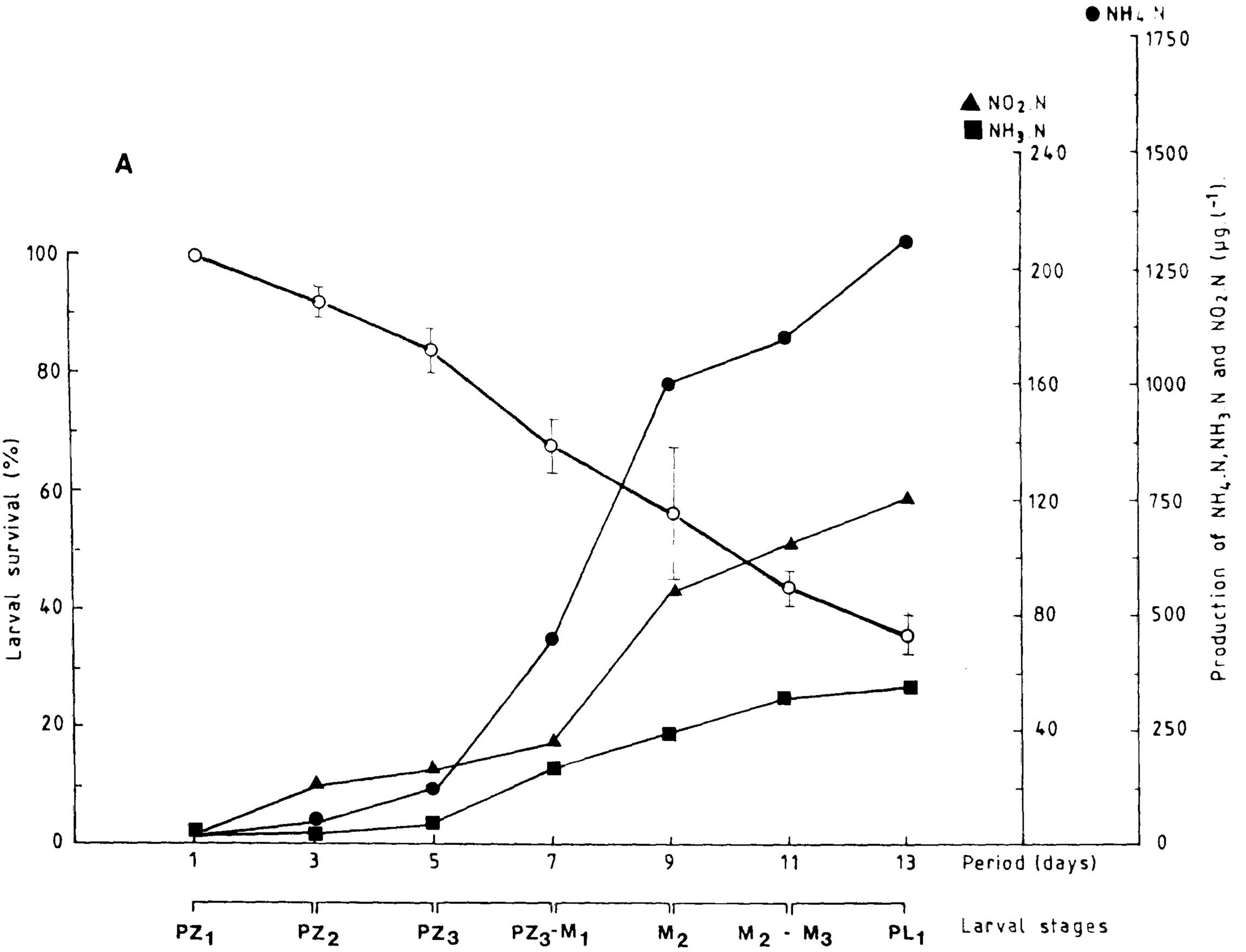

Larval growth (mm).
6·0
5·0
4·0
3·0
2·0
1·0
0
1
3
5
7
9
11
13
Period (days)
PZ1
PZ2
PZ3M1
M1
M2
M2 - M3
PL1
Larval stages
NO2.N
NH3 N
NH4 N
240
200
160
120
80
40
1750
1500
1250
1000
750
500
250
0
Production of NH4.N, NH3.N and NO2.N (µg.l-1)

Figure 17a. P. monodon larval survival (○) on spray dried yeast diet 'D' supplemented by 10 cells μl^{-1} microalgae, plotted together with NO_2.N (▲), NH_3.N (■) and NH_4.N (●) levels in replicated feed trials. Vertical bars are standard deviation.

Figure 17b. Larval growth (Δ) and development on spray dried yeast diet 'D', plotted together with NO_2.N, NH_3.N and NH_4.N levels in replicated feed trials.

A


● NH4.N
▲ NO2.N
■ NH3.N
Larval survival (%)
Production of NH4.N, NH3.N and NO2.N (µg.l-1)
Period (days)
1
3
5
7
9
11
13
0
20
40
60
80
100
120
160
200
240
250
500
750
1000
1250
1500
PZ1
PZ2
PZ3
PZ3-M1
M2
M2 - M3
PL1
Larval stages

B

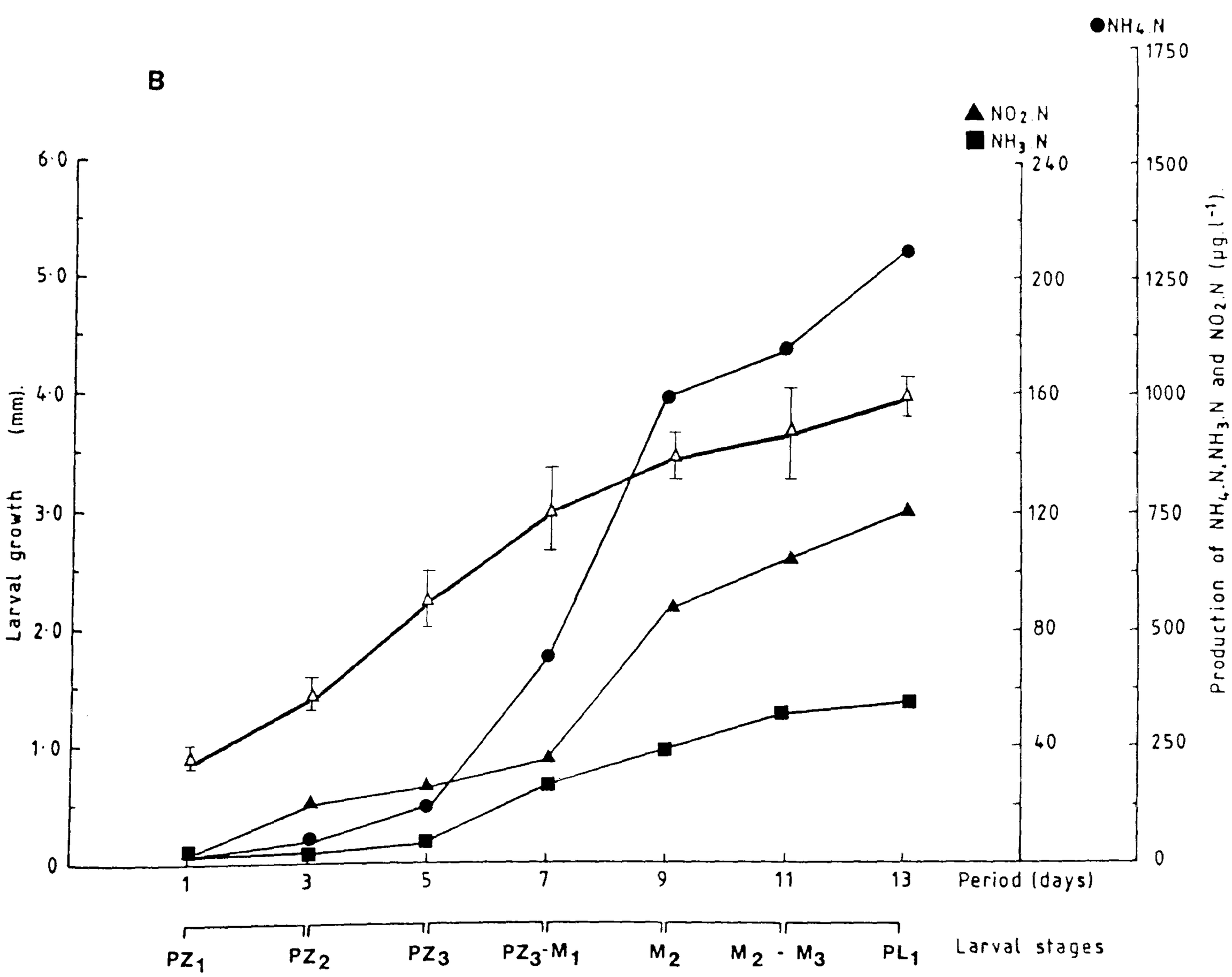
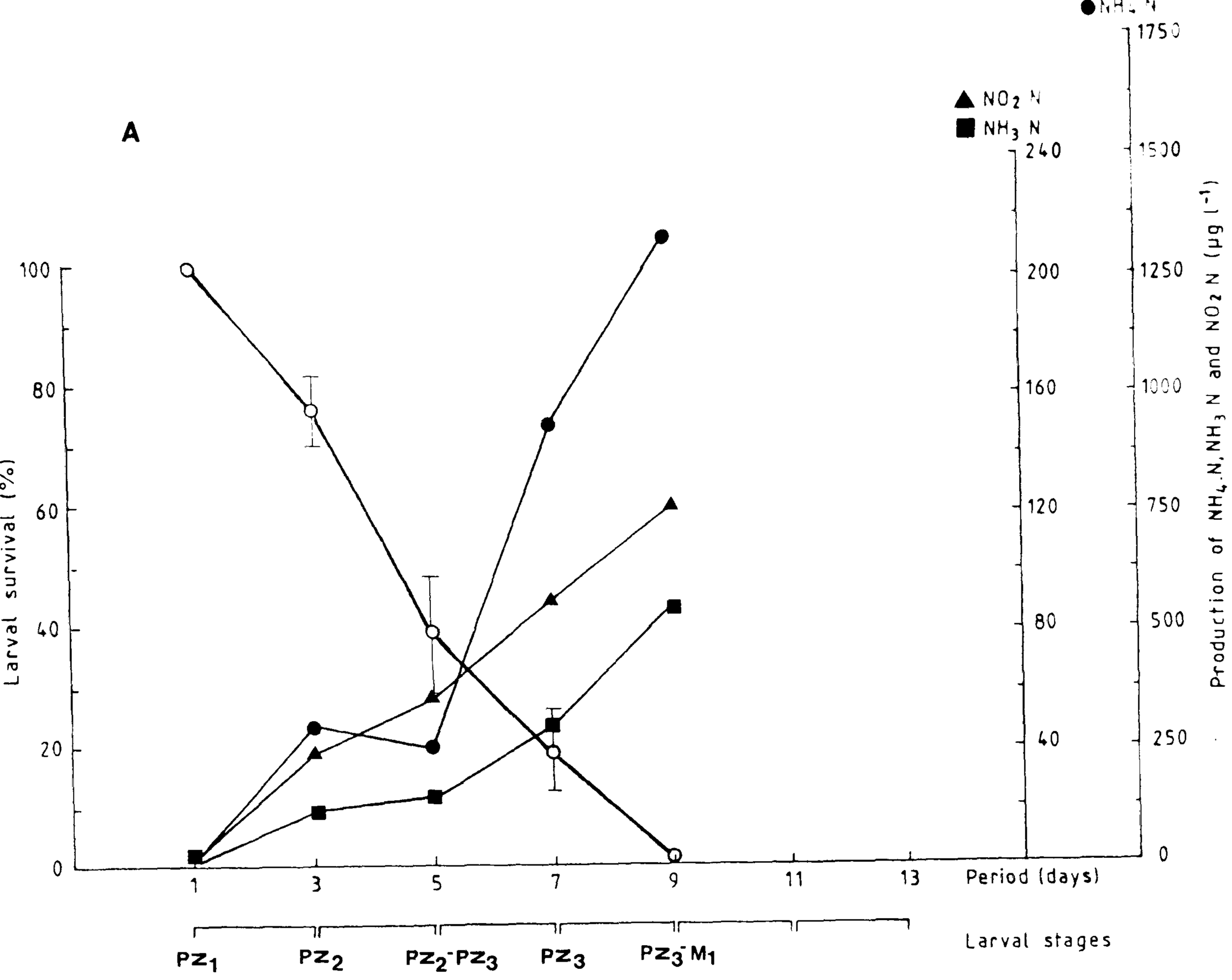

● NH4.N
▲ NO2.N
■ NH3.N
Larval growth (mm).
Production of NH4.N, NH3.N and NO2.N (µg.l-1)
Period (days)
0
1·0
2·0
3·0
4·0
5·0
6·0
1
3
5
7
9
11
13
0
40
80
120
160
200
240
250
500
750
1000
1250
1500
PZ1
PZ2
PZ3
PZ3-M1
M2
M2 - M3
PL1
Larval stages

Figure 18a. P. monodon larval survival (○) on microparticulate diet 'G' supplemented by 10 cells μl^{-1} microalgae, plotted together with NO_2.N (▲), NH_3.N (■) and NH_4.N (●) levels in replicated feed trials. Vertical bars are standard deviation.

Figure 18b. Larval growth (Δ) and development of P. monodon larvae on diet 'G', plotted together with NO_2.N, NH_3.N and NH_4.N levels in replicated feed trials.

A


Larval survival (%)
100
80
60
40
20
0
1
3
5
7
9
11
13
Period (days)
PZ1
PZ2
PZ2-PZ3
PZ3
PZ3-M1
Larval stages
NH4 N
NO2 N
NH3 N
1750
1500
1250
1000
750
500
250
0
240
200
160
120
80
40
Production of NH4.N, NH3.N and NO2.N (µg l-1)

B

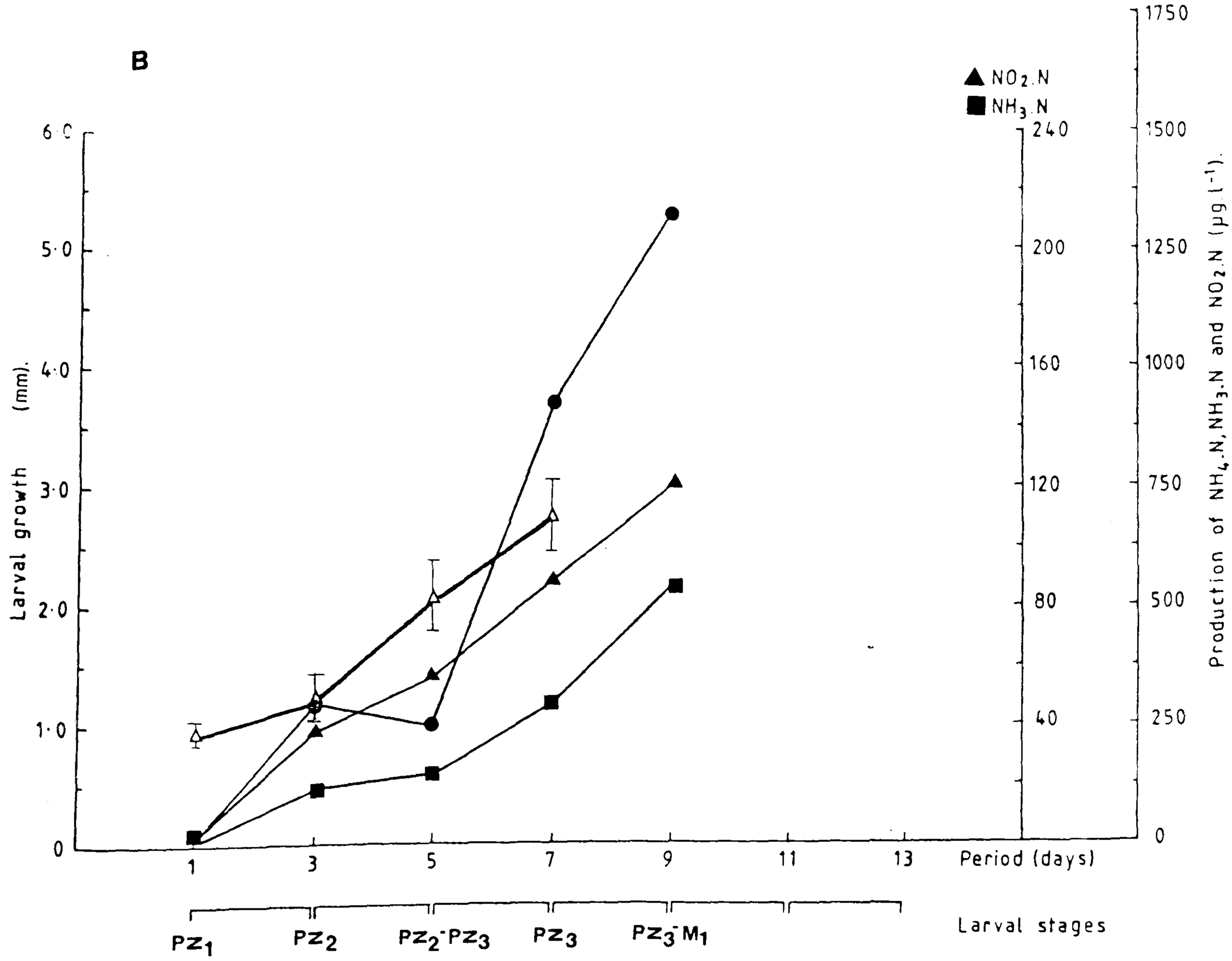
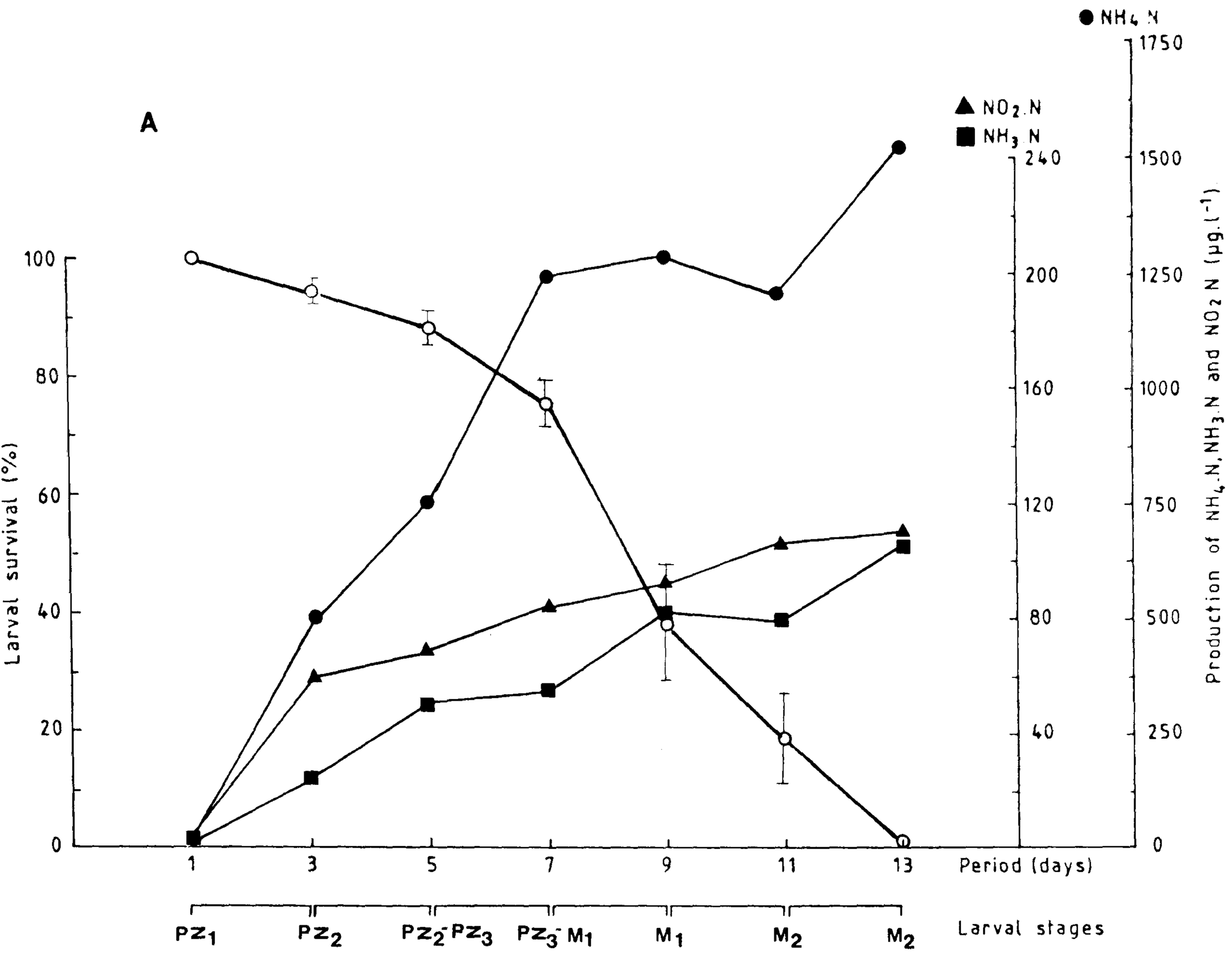

Larval growth (mm).
6·0
5·0
4·0
3·0
2·0
1·0
0
1
3
5
7
9
11
13
Period (days)
PZ1
PZ2
PZ2-PZ3
PZ3
PZ3-M1
Larval stages
NH4.N
NO2.N
NH3.N
1750
1500
1250
1000
750
500
250
0
240
200
160
120
80
40
Production of NH4.N, NH3.N and NO2.N (µg l-1).

Figure 19a. P. monodon larval survival (○) on spray dried algae diet 'E' supplemented by 10 cells μl^{-1} microalgae, plotted together with NO_2.N (▲), NH_3.N (■) and NH_4.N (●) levels in replicated feed trials. Vertical bars are standard deviation.

Figure 19b. Larval growth (Δ) and development of P. monodon larvae on spray dried algae diet 'E', plotted together with NO_2.N, NH_3.N and NH_4.N levels in replicated feed trials.


A
● NH4 N
▲ NO2 N
■ NH3 N
Larval survival (%)
Production of NH4.N, NH3.N and NO2.N (µg.(-1))
Period (days)
Larval stages
PZ1
PZ2
PZ2-PZ3
PZ3-M1
M1
M2
M2

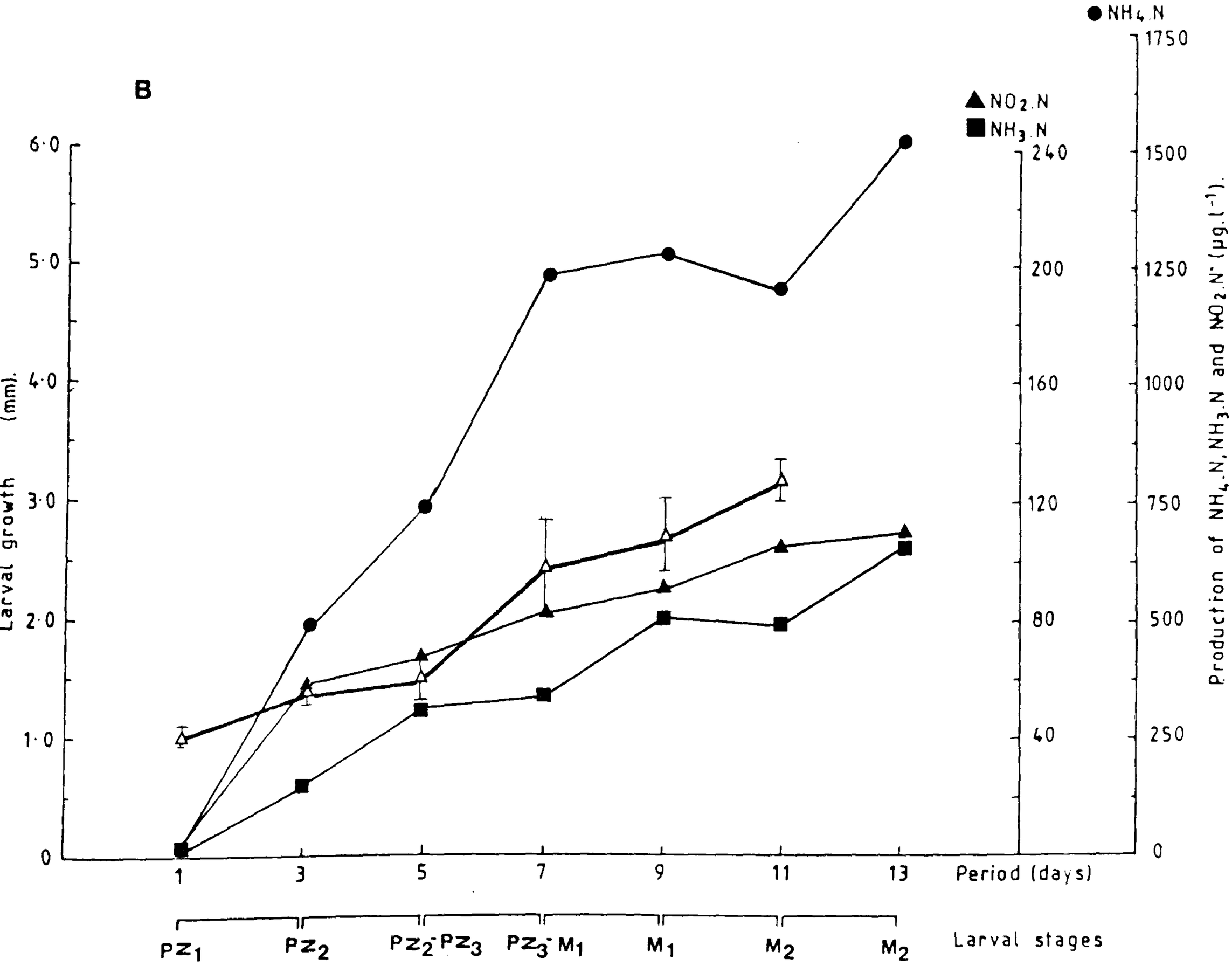

B
● NH4 N
▲ NO2 N
■ NH3 N
Larval growth (mm).
Production of NH4.N, NH3.N and NO2.N (µg.(-1))
Period (days)
Larval stages
PZ1
PZ2
PZ2-PZ3
PZ3-M1
M1
M2
M2

Figure 20. Mean and range of particles selected by P. monodon larvae a) supplied with carbon powder 1-45µm (●), b) supplied with latex beads 1-30µm (○).

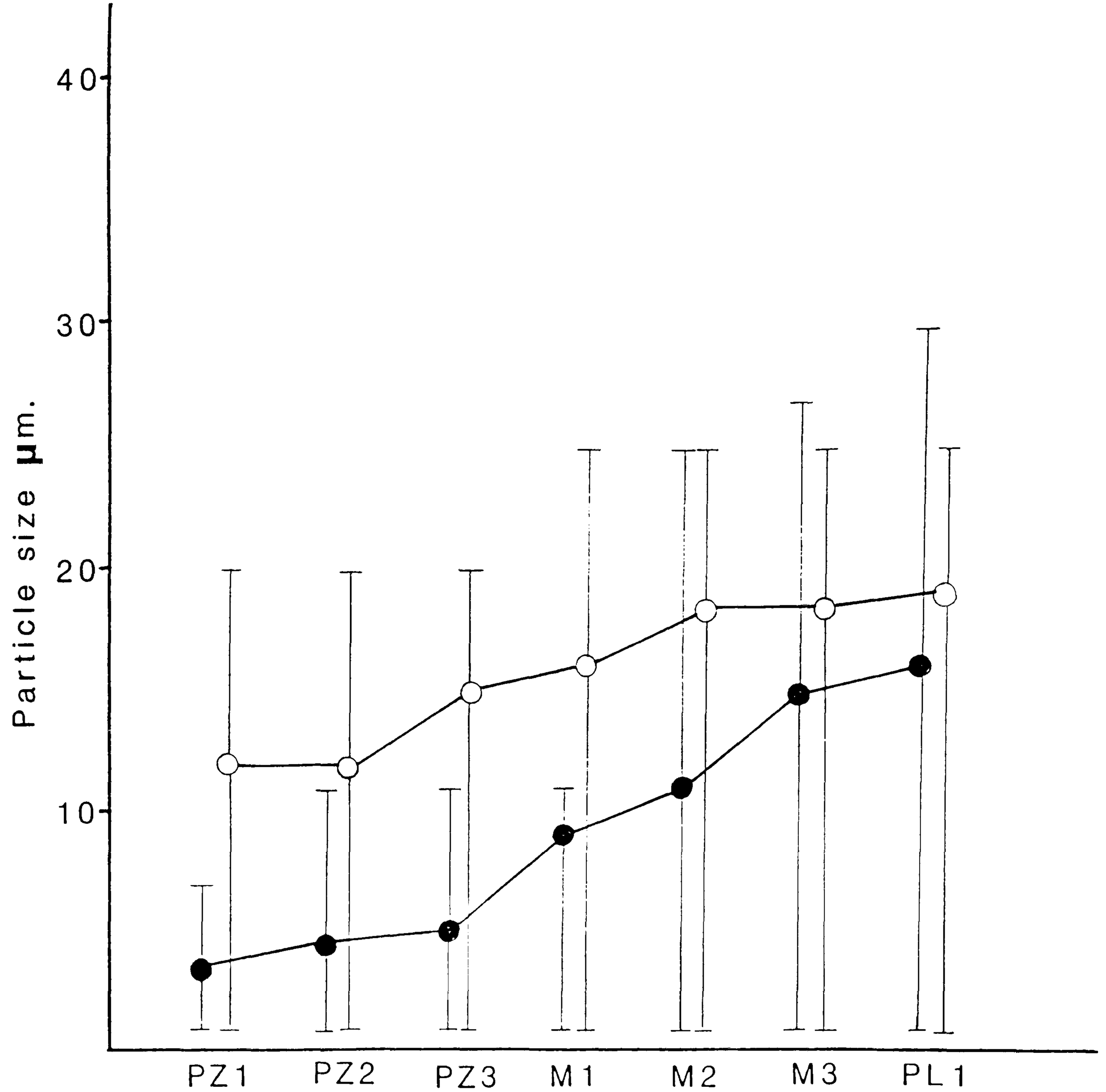

Particle size µm.
40
30
20
10
PZ1
PZ2
PZ3
M1
M2
M3
PL1

Figure 21. Median particle size of leading brands of artificial larval diets measured as dry, 1h and 24h rehydration in seawater at 28°C. Feed particle sizes are mean of two replicates. Diet codes are listed in table 1.

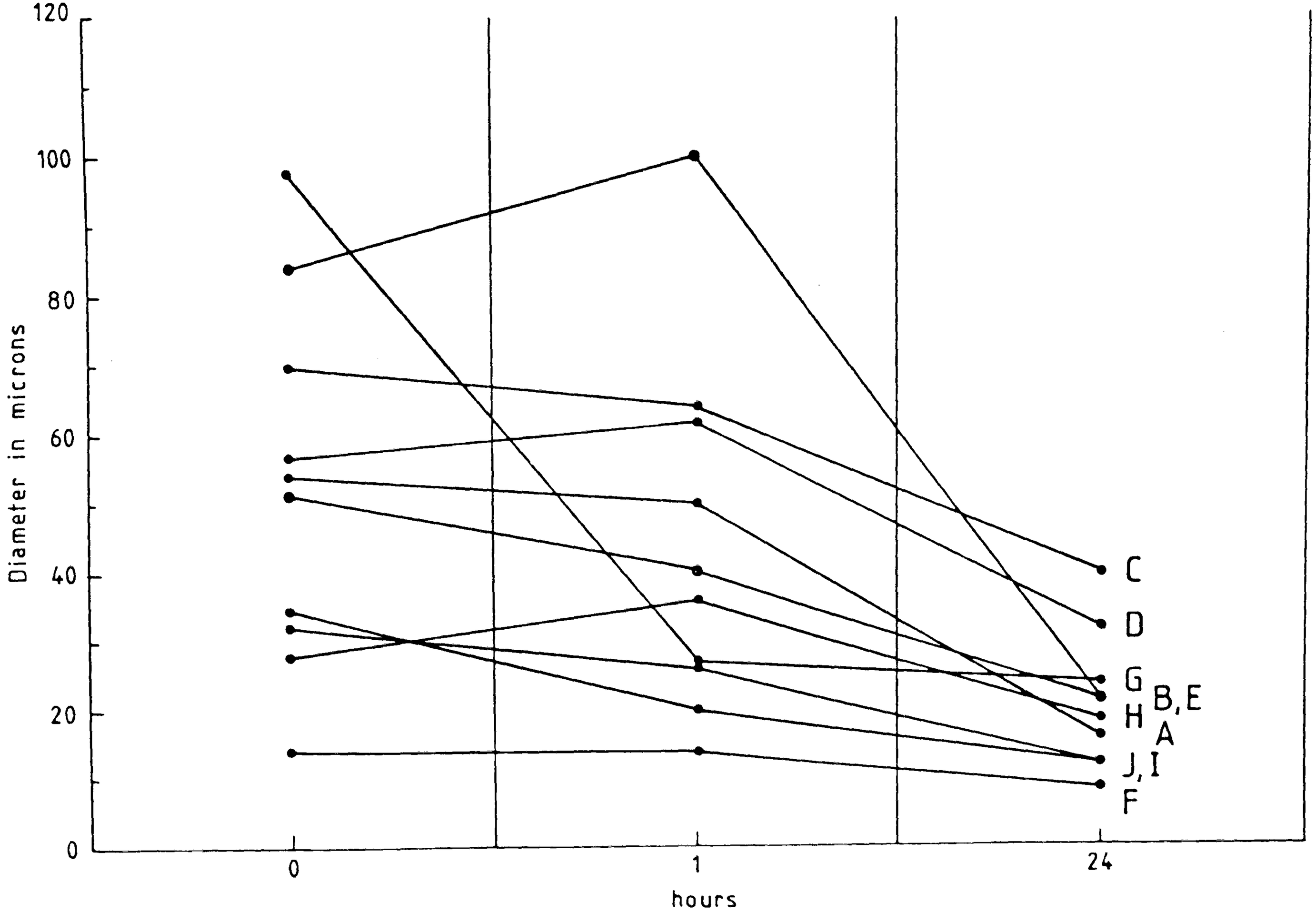
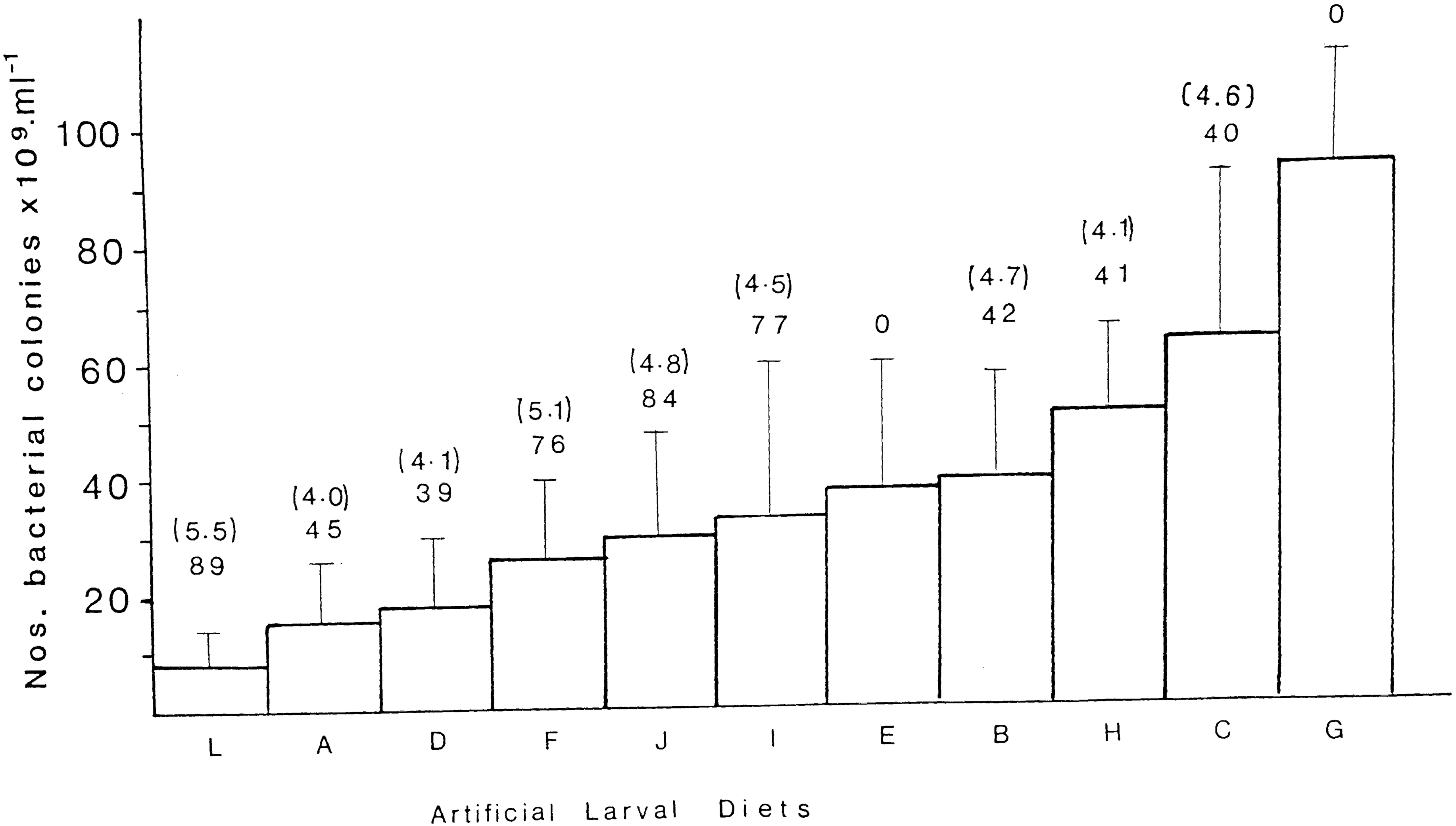

120
100
80
60
40
20
0
Diameter in microns
0
1
24
hours
C
D
G
H
B, E
A
J, I
F

Figure 22. Number of bacterial colonies from water samples taken in replicate from each feed regime after 24h at 28°C. Figures in bracket are growth (mm) of P. monodon postlarvae and percentage survival achieved in feed trials on each diet. Vertical bars are standard deviation. Diet codes are listed in table 1.


Nos. bacterial colonies x10⁹.ml⁻¹
100
80
60
40
20
(5.5)
89
(4·0)
45
(4·1)
39
(5.1)
76
(4·8)
84
(4·5)
77
0
(4.7)
42
(4.1)
41
(4.6)
40
0
L
A
D
F
J
I
E
B
H
C
G
Artificial Larval Diets

SECTION 3

Role of microalgae in feed trials

This section was presented at the World Aquaculture Conference in Los Angeles, California, February 1989.

Title : **Role of Algae in Conditioning Water Quality for Penaeid Larval Culture.**

Authors : S. Amjad and D.A. Jones.

Journal of the World Aquaculture Society, Vol. 20 (1) : 12A.

INTRODUCTION

Apart from supplying all the nutrients essential for larval development microalgae may play an important role in conditioning water quality for penaeid larval culture. As the natural food for penaeid larvae it might be expected that algal metabolic products may act as chemoattractants and feeding stimulants which also facilitate larval growth and development.

Recently it has been demonstrated that modification of the 'green water' larval culture system (Ling, 1969) for *Macrobrachium* spp. allows the production of postlarvae without any water change, despite extensive use of artificial diets (Ang and Cheah, 1986). Ammonia and its intermediate product nitrite, are common toxicants in prawn hatcheries. The accumulation of ammonia in culture water occurs due to protein catabolism, nitrogenous excretion by crustaceans (Hartenstein, 1970; Hochachka and Somero, 1973) and by the breakdown and nitrification of uneaten organic matter (artificial diets) added as food. Between 40 to 90% of the nitrogen eliminated by crustaceans is ammonia (Parry, 1960), and is generally considered a major cause of mortality in unconditioned recirculatory culture systems (Spotte, 1970). In a conditioned system, ammonia stimulates nitrofying bacteria which first oxidize liberated ammonia and convert it into nitrite (NO_2) by *Nitrosomanas*. Nitrite is oxidized again to relatively non-toxic nitrate (NO_3) by *Nitrobactor*.

Total ammonia nitrogen includes nitrogen in both ionic states (ionised [NH_4^+] and unionised [NH_3]). Unionised ammonia

is considered the principle toxic form to aquatic species. The free base [NH_3] has a relatively high lipid solubility because it carries no charge, and is therefore able to diffuse quite readily across cell membranes in the gills (Fromm and Gillette, 1968). The ionised ammonia [NH_4^+] on the other hand penetrates these membranes less readily since it is charged and hydrated and consequently has a low lipid solubility (Whitfield, 1974). Ionised and un-ionised ammonia exist in an equilibrium state ($NH_4^+ \rightleftharpoons NH_3$) in water. Factors which influence shifts between these two ionic states include temperature, salinity, and pH (Wickins, 1976). A shift of one pH unit (7 to 8) will increase NH_3 levels ten fold (Armstrong et al., 1978). Toxicity of ammonia is therefore greater at higher pH. The toxicity of nitrite increases with a decrease in pH because the relative portion of toxic un-ionised nitrous acid (HNO_2) increases (Russo et al., 1974; Colt and Tchobanaoglous, 1976). The toxic action of ammonia in aquatic fish, is due to the retention of ammonia in the blood, which interferes with the ability of hemoglobin to transport oxygen (Spotte, 1979). The toxic action of nitrite is to oxidize the iron in hemoglobin to methemoglobin in fish which is unable to transport oxygen (Smith and Williams, 1974; Smith and Russo, 1975). This results into hypoxia and cyanosis (Kiese, 1974). It is likely that the same reaction may occur within the copper of crustacean hemocyanin and produce a detrimental effect on the growth and development of prawns and their larvae (Chen et al., 1986). *P. monodon* larvae are commercially reared in static water hatcheries in Taiwan (Liao, 1977). Accumulation of

ammonia and nitrite in these static systems adversely affects the survival of larvae. Wickins, (1976) and more recently Chen and Chin (1987; 1988) have demonstrated the toxicity of both ammonia and nitrite to penaeid larvae such as *P. monodon,* and measured accumulation of these toxicants in hatcheries. Toxicity levels of NH_4^+.N, NH_3.N and NO_2.N affecting prawn culture have been cited from literature and are listed in table 1.

Present work examines the use of six algal species, *Skeletonema costatum* (Greville), *Chaetoceros calcitrans* (Paulsen), *Rhodomonas baltica* (Karsten), *Pavlova lutheri* (Droop), *Tetraselmis chuii* (Butcher) and *Chlorella japonica* (Shihara and Krauss) at four levels of cell concentration, 500, 1000, 1500 and 2000 μl^{-1} to act as 'biological filter' system, to control and detoxify levels of ammonia and nitrite in *P. monodon* larval cultures using artificial diets. The chemoattractability of live microalgae which may result from metabolic products secreted into the culture medium, and their effect on *P. monodon* larval growth and survival is also evaluated in a feed trial.

MATERIAL AND METHODS

All experiments were conducted in 2 litre round bottom flasks containing aerated U/V sterilised sea water at 28±1°C at a salinity of 32‰ . Continuous illumination of 6.6 x 10^{15} quanta $sec.^{-1}$ cm^2. was maintained inside the flask by suspending a light source directly above the culture flask. A portable laboratory Quantum Scalar Irradiance meter model QSL - 100 was used to measure light.

PRODUCTION OF AMMONIA

Ammonia production in round bottom culture flasks was achieved by artificial diet, added at 4 mg. $litre^{-1}.day^{-1}$ (PZ1 to PZ3) for six days and 8 mg. $litre^{-1}$ day^{-1} (M1 to PL1) for the next four days, with no water change and in the absence of larvae and microalgae. This feed level mimics actual amounts normally fed to *P. monodon* larvae. Ammonia and nitrite accumulate as a result of breakdown of feed components, levels of each was measured on alternate days. Ammonia (NH_4^+.N) was measured by the Phenolhypochlorite method (Solarzano, 1969) and the unionised ammonia (NH_3.N) calculated from Bower-Bidwell (1978). Nitrite (NO_2.N) was measured from procedures described by Bendschneider and Robinson (1952). pH was measured by WPA instrument CD 300. On day 11 the total volume of water in the culture flask was filtered through Watmans filter paper (No.4) to remove remaining fragments of feed. The filtered water was returned to clean flask and algae species were introduced separately at four cell concentrations.

ALGAL CULTURES

Non-axenic algal cultures of *S. costatum*, *C. calcitrans*, *P. lutheri*, *T. chuii*, *R. baltica* and *C. japonica* maintained in exponential phase in Conway medium (Walne, 1966) were harvested and centrifuged at 2000 r.p.m. for 3 minutes to remove algal metabolites. The supernatant was decanted and algae resuspended in fresh sea water. Cell counts were made using Coulter Counter model ZB and haemocytometer. Four cell concentrations (500, 1000, 1500, 2000 μl^{-1}.) from each of the six algal species were introduced separately, into the culture flask containing known concentrations of ammonia and nitrite liberated from artificial diet.

In another set of experiments cells of *chlorella* (500, 1000, 1500 and 2000 μl^{-1}) were contained in a hollow transparent cylinder measuring 100mm in length and 20mm in diameter, the top and bottom ends were covered by a 5 μm mesh cloth to prevent cells escaping into the culture medium. The cylinder containing algae was suspended vertically in the culture flask, so that water could flow through the cylinder with the aid of air bubbles to encourage water circulation. All other experimental conditions were similar.

Reduction in the levels of ammonia and nitrite concentrations were measured for each of the six algal species at four concentrations after 24 and 48h. respectively. All experiments were replicated.

ALGAE AS CHEMOATTRACTANTS

Microalgae *R. baltica* and *T. chuii* at 40 cells μl^{-1} in a ratio of 1:1 were contained in a transparent cylinder, (same procedure, described in the above section) together with *P. monodon* larvae. PZ1 stage larvae were provided with artificial diet in the presence of 'contained algae' suspended in the culture medium. No algal supplements were given. The purpose of this experiment was to examine, whether larval appetite or the digestion process would be stimulated in the presence of algae which could not be ingested. Larval growth and percentage survival were evaluated and compared with controls. Statistical analyses were employed using one-way ANOVA, together with Tukey's pairwise comparison.

RESULTS

AMMONIA PRODUCTION FROM ARTIFICIAL DIET

Total ammonia liberated from rehydrated artificial larval diet, together with nitrite levels, measured under culture conditions at 2 days interval for 11 days are shown in fig. 1. pH values and unionised ammonia (NH_3.N) are tabulated in table 2. Ammonia (NH_4^+.N) levels steadily increased from 222 $\mu g.\ l^{-1}$ on day 3 to 2116 $\mu g.\ l^{-1}$ on day 11. Unionised ammonia (NH_3.N) also increased from 11.81 $\mu g.\ l^{-1}$ to 139.86 $\mu g.\ l^{-1}$. Nitrite (NO_2.N) levels ranged from 21.62 to 314.7 $\mu g.\ l^{-1}$, and pH values remained between 7.96 to 8.1. In blank controls, levels of NH_4^+.N were 15 - 18.4 $\mu g.\ l^{-1}$, NH_3.N ranged from 0.65 to 1.20 $\mu g.\ l^{-1}$, NO_2.N measured between 7.3 to 8.55 $\mu g.\ l^{-1}$. and pH values were 7.9 to 8.1.

Levels of NH_4^+.N, NH_3.N and NO_2.N in control flask remained well below the recommended safe limits (table 1).

ASSIMILATION OF AMMONIA BY ALGAE

Six algal species (*S. costatum, C. calcitrans, P. lutheri, T. chuii, R. baltica, C. japonica* (free floating) and *C. japonica* (trapped) were added to the water containing ammonia and nitrite from the above experiment at 500, 1000, 1500 and 2000 cells μl^{-1} in separate experiments to ascertain their effect upon ammonia and nitrite. Algae reduced toxicant levels to safer limits in culture medium after 48h. Algae at all four cell concentrations assimilated ammonia and nitrite. Reduction in levels of ammonia in culture water by different

algae and cell concentrations are given below.

Reduction of ammonia by algae at 500 cells μl^{-1}.

Fig. 2 and table 3, shows that algal species tested at 500 cells μl^{-1}. were effective in assimilating ammonia from the culture medium after 24 and 48h. Trapped *C. japonica* reduced initial ammonia levels from 2116 $\mu g.\ l^{-1}$ to 1190 $\mu g.\ l^{-1}$ in 24h and to 999.6 $\mu g.\ l^{-1}$ after 48h. Total ammonia assimilated by trapped *C. japonica* cells was 52.7%. Free floating cells of *C. japonica* at 500 cells μl^{-1} assimilated 43.7% ammonia after 24h. A slight increase (9%) in ammonia levels was observed after 48h. The increase in ammonia level was probably due to cell mortality as dead algal cells liberate assimilated ammonia. Total ammonia uptake by *C. calcitrans* at 500 cells μl^{-1} was 46.9% after 48h. *T. chuii* effectively assimilated 44.5% of ammonia after 48h, followed by *P. lutheri* which absorbed 43.2% of ammonia after 48h. *R. baltica* at 500 cells μl^{-1} was less effective and only 15.6% ammonia was taken up from the culture water after 24h. An increase in ammonia levels by 2.7% was observed after 48h. Total ammonia absorbed by *R. baltica* was only 13.2% after 48h. *S. costatum* also assimilated 13.2% ammonia after 24h, levels of ammonia increased by 10% after 48h. *S. costatum* at 500 cells μl^{-1} was least effective, reducing ammonia levels by only 3% after 48h.

Reduction of ammonia by algae at 1000 cells μl^{-1}.

Amongst the six algal species tested at 1000 cells μl^{-1}, free floating *C. japonica* was most effective in utilizing

ammonia (Fig. 3, table 4). Ammonia levels declined in the culture flask from 2116 to 295.8 μg. l^{-1} in 24h and to 36 μg. l^{-1} in 48h, 98% ammonia was absorbed. Trapped *C. japonica* reduced ammonia levels by 73.4% after 48h. *T. chuii* was also effective in lowering ammonia levels by 33.3% in 24h and by 63.8% after 48h. *P. lutheri* removed 51.7% ammonia from the medium after 48h. *C. calcitrans* reduced ammonia levels by 36.3% in 24h and 49.3% after 48h. *S. costatum* was less effective in absorbing ammonia, only 13% was taken up by the diatom after 48h. *R. baltica* was the least effective, ammonia levels were reduced by 13% after 24h, ammonia levels increased by 11% after 48h. Increase in ammonia levels was due to cell mortality, cell lysis giving off assimilated ammonia.

Reduction of ammonia by algae at 1500 cells μl^{-1}.

Fig. 4 and table 5 show that algal species tested at 1500 cells μl^{-1} were also effective in reducing ammonia levels in culture medium. Free floating *C. japonica* were most effective. Levels were reduced from 2116 to 255 μg. l^{-1} (87.9%) in 24h and to 18.4 μg. l^{-1} (99%) after 48h. Trapped *C. japonica* removed 51.7% ammonia in 24h and 78.8% after 48h. *C. calcitrans* assimilated 52% ammonia from the culture medium in 24h and 54.2% after 48h. *T. chuii* was also effective in absorbing 51.7% ammonia after 48h. *P. lutheri* assimilates 32.5% ammonia after 48h. *S. costatum* was effective in removing 27.6% ammonia after 48h. *R. baltica* was the least effective in reducing ammonia levels and only 13.2% was assimilated by the

algae after 48h.

Reduction of ammonia by algae at 2000 cells μl^{-1}.

Algal species at 2000 cells μl^{-1} were also effective in assimilating ammonia, from the culture medium. Fig. 5 and table 6 shows that free floating *C. japonica* reduced ammonia levels by 99% in 48h; trapped cells effectively removed 71% of ammonia from the culture medium after 48h. Ammonia levels were reduced by 61.4% after 48h by *T. chuii*. *C. calcitrans* was effective in removing 52% ammonia from the medium. *P. lutheri* assimilated and removed 46.9% ammonia after 48h. *S. costatum* reduced ammonia levels by 37.3% after 48h. *R. baltica* was the least effective in reducing ammonia levels from the culture medium and only 3.5% was assimilated by the flagellate.

ASSIMILATION OF NITRITE BY ALGAE

The six microalgal species tested were effective in reducing nitrite levels accumulated in the culture medium from rehydrated artificial diet (56 mg. l^{-1} dry weight). Nitrite concentration measured ranged between 21.62 μg. l^{-1} on day 3 to 314.7 μg. l^{-1} on day 11 (table 2). The effect of four algal cell densities 500 μl^{-1}, 1000 μl^{-1}, 1500 μl^{-1} and 2000 μl^{-1} upon nitrite concentration in culture medium was ascertained.

Reduction of nitrite by algae at 500 cells μl^{-1}.

Fig. 6 and table 3 shows that free floating *C. japonica* at 500 cells μl^{-1} was most effective in lowering nitrites in the medium. Levels were reduced from 314.7 μg. l^{-1} to

156.4 $\mu g.\ l^{-1}$ after 48h, a decrease of 50.3%. *C. calcitrans* assimilated 35.6% nitrites in 24h and 47.3% after 48h. *T. chuii* and *C. japonica* (trapped) were effective in reducing nitrite levels by 38.9% after 48h. *P. lutheri* lowered nitrite levels by 35.8%. Uptake of nitrite by *R. baltica* was 26.9% after 48h. *S. costatum* was less effective in reducing nitrite levels, only 18.9% was assimilated by the diatom.

Reduction of nitrite by algae at 1000 cells μl^{-1}.

Fig. 7 and table 4 shows that free floating *C. japonica* was effective in reducing nitrite levels, 85.3% of nitrite being assimilated from the medium by the algae after 48h. Trapped *C. japonica* removed 70.7% nitrite after 48h. *C. calcitrans* was effective in reducing levels of nitrite by 53.2% after 48h, followed by *S. costatum* which took up 41.5% nitrite after 48h. Levels of nitrite reduced by *R. baltica* was only 3.5% after 48h. *P. lutheri* was the least effective in lowering nitrite levels from the culture medium, 3.5% nitrite was reduced in 24h but nitrite level in culture medium increased by 6% after 48h.

Reduction of nitrite by algae at 1500 cells μl^{-1}.

Fig. 8 and table 5 show that nitrite levels reduced by free floating and trapped *C. japonica* at cell densities of 1500 μl^{-1} were similar (85.3% and 70%) respectively to nitrite levels reduced by 1000 cells μl^{-1} of *C. japonica*. *P. lutheri* was effective in lowering nitrite levels by 56.1% after 48h. Nitrite levels reduced by both *T. chuii* and *S. costatum* were

26.9% after 48h. *R. baltica* lowered nitrite levels by 12.2% after 48h. Nitrites increased with *C. calcitrans* by 8% after 48h.

Reduction of nitrite by algae at 2000 cells μl^{-1}.

Free floating and trapped *C. japonica* were most effective in lowering nitrite levels, (82 and 73.6% respectively), Fig. 9 and table 6. *S. costatum* was effective in removing 41.2% nitrite in 48h. *P. lutheri* reduced nitrite levels by 35.8% and *T. chuii* assimilated 26.9% nitrites from culture medium in 48h. *C. calcitrans* lowered nitrite levels by 12.2% in 48h. *R. baltica* was less effective comparatively in lowering nitrite levels, only 11% was removed by the flagellate after 48h.

From the above experiments it can be concluded that *C. japonica* is most effective in reducing levels of ammonia and nitrite from culture water environment. Fig. 10a and b summarise reduction in levels of toxicant ammonia and nitrite by *C. japonica* at four cell densities.

P. monodon LARVAL FEED TRIAL ON ARTIFICIAL DIET WITH *C. japonica*

Results showed that *C. japonica* at 1000 cells μl^{-1} was most effective in reducing toxic levels of ammonia and nitrite in the culture medium. An experimental feed trial was set up using *P. monodon* larvae (PZ1 - PL1) fed on an artificial diet in the presence of free floating and trapped *C. japonica* at 1000 cells μl^{-1}. Culture water was not changed during the experiment. *P. monodon* larvae fed on an artificial diet with free floating *C. japonica* collapsed at PZ2/PZ3 on day 7

(fig. 11). Ammonia and nitrite levels in culture water were 81.6 and 18.4 μg. l^{-1}. Larval mortality resulted from ingestion of free floating *C. japonica* cells by the larval stages. *C. japonica* is considered to be a poor food for crustacean larvae due to its thick cell wall. *P. monodon* larvae on an artificial diet in the presence of trapped *C. japonica* reached the intermediate postlarval stage (3.59mm) with a survival of 20% on day 11 (Fig. 12). Low survival and larval growth was due to inadequate nutrition for larval development in the artificial diet and the absence of an algal (10 cells μl^{-1}) supplement. Ammonia and nitrite levels in the culture water were 420 μg. l^{-1} and 230 μg. l^{-1} respectively. *P. monodon* larvae on 100% artificial diet, in the absence of algae did not survive beyond day 9 (Fig. 13). Larval mortality occurred due to a high (320 μg. l^{-1}) level of nitrite in the culture water. Percentage survival on control feed (Fig. 14) *T. chuii* and *R. baltica* were low (9% to PL stages) due to high nitrite (230 μg. l^{-1}) levels as the culture water was not changed during the experiment.

ALGAE AS A CHEMOATTRACTANT

The effectiveness of microalgae to act as chemoattractants was evaluated in a feed trial using artificial diet CAR 335 with *P. monodon* larvae from PZ1 to PZ3/M1 stage. Culture water was changed daily.

Mean larval growth and percent survival of larvae on an artificial diet in the presence of *T. chuii* at 20 cell μl^{-1} and *R. baltica* at 20 cell μl^{-1} contained in a flow through cylinder

(described earlier in materials and methods section) were 2.71mm and 45.5% respectively at PZ3 stage. Larval growth and survival on artificial diet CAR 335 in the absence of algae was 2.58mm and 43% respectively. Larval development on a control diet containing microalgae at 20 cells μl^{-1} of *T. chuii* and 20 cells μl^{-1} of *R. baltica* produced a mean larval growth of 3.16mm and percentage survival of 88% to M1 stage (table 7).

Results of one-way ANOVA reveals that larval growth were significantly different ($P < 0.05$) (table 8a). Tukeys pairwise comparison indicates that larval growth was significantly better on control diets than growth on artificial diet obtained in the presence of trapped algae. Larval growth on artificial diet in the presence of trapped algae was significantly better ($P < 0.05$) than growth obtained on 100% artificial diet (table 8b).

Percentage larval survival was also significantly different ($P < 0.05$) (table 9a). Tukeys pairwise comparison shows that percentage survival on the control diet was significantly better than survival obtained with larvae on artificial diet in the presence of algae, and on 100% artificial diet alone. Percentage survival on artificial diet in the presence of trapped algae was not significantly different from that obtained on 100% artificial diet alone (table 9b).

DISCUSSION

Results show that the toxic range of ammonia and nitrite levels in the culture water can be reduced effectively to safer limits by adding microalgae cells at densities of 1000 to 1500 μl^{-1}, at an illumination of 6.6 x 10^{15} quanta. sec^{-1}. cm^2. Amongst the six algal species tested both free floating and trapped *C. japonica* were effective in absorbing 99.1% ammonia from the culture water after 48h (fig. 10a). *R. baltica* tested at four cell densities (500, 1000, 1500 and 2000 cells μl^{-1}.) was least effective in assimilating and reducing initial ammonia levels (13.2%) after 48h from the culture water. The diatom *S. costatum* was also less effective in absorbing ammonia (37.3%) from the culture water.

Nitrite levels in the culture water were also effectively reduced by both free floating and trapped *C. japonica* (70.7 to 85.3%) in 48h (fig. 10b). *S. costatum* and *R. baltica* were less effective in reducing nitrite levels (fig. 6-9) from culture waters. Algal cells of *S. costatum* and *R. baltica* periodically collapse and decomposition of dead algal cells increases toxicant ammonia and nitrite levels in the water. The effectiveness of algal species in assimilating and reducing ammonia levels in the culture water, demonstrates that ammonia is the preferred nitrogen source of nutrient for live algae in the present culture water environment. Molly and Syrett (1988) also showed that algae in the presence of ammonia and urea assimilated ammonia first, and only when this disappeared from the culture medium were other nitrogen sources utilized.

Since ammonia and nitrite levels reduced by *C. japonica* at cell densities of 1000, 1500 and 2000 μl^{-1} were not significantly different from each other (tables 4, 5 and 6), a minimum cell concentration of 1000 μl^{-1} of free floating *C. japonica* was tested in a feed trial with *P. monodon* larvae fed on artificial diet. Although ammonia (81.6 $\mu g.\ l^{-1}$) and nitrite (18.4 $\mu g.\ l^{-1}$) levels were well below safe limits (1500 and 110 $\mu g.\ l^{-1}$ respectively) larval growth did not proceed beyond the PZ2/PZ3 stages, and larval mortality occurred on day 7. The reason for this is that larvae consumed *C. japonica* as food. Cells of *C. japonica* are nutritionally inferior for penaeid and other molluscan larvae due to their thick cell walls (Web and Chu, 1982). Growth and survival of *P. monodon* larvae on an artificial diet in unchanged culture water in the presence of *C. japonica* at 1000 cells μl^{-1} trapped in a flowthrough transparent cylinder were significantly better. Larval growth on day 11 was 3.59mm and 20% survived to intermediate PL stage (fig. 12). Levels of NH_4^+.N (406 $\mu g.\ l^{-1}$), NH_3.N (13.56 $\mu g.\ l^{-1}$) were within safe limits, NO_2.N (230 $\mu g.\ l^{-1}$) exceeded the recommended safe limit (110 $\mu g.\ l^{-1}$). Larvae on 100% artificial diet in the absence of algae, died on day 9 (fig. 13). Toxicant levels of NH_4^+.N (2161 $\mu g.\ l^{-1}$) and NH_3.N (38.37 $\mu g.\ l^{-1}$) were either close to or within safe limits, NO_2.N (320 $\mu g.\ l^{-1}$) in the culture water far exceeded the safe limit. The control live diet (40 cells μl^{-1} algae + 5. ml^{-1} *Artemia*) produced poor survivals (9%) in unchanged culture water. NH_4^+.N was 800 $\mu g.\ l^{-1}$ and NO_2.N was 230 $\mu g.\ l^{-1}$ (fig. 14). Higher levels of these toxicants may be

due to the presence of the live *Artemia* provided as food for later prawn larvae.

It is well documented (table 1) that accumulated ammonia and nitrite adversely effect growth and survival of penaeid prawns (Wickins, 1976; Mevel and Chamroux, 1981; Jayasankar and Muthu, 1983a, b; Chen et al., 1986; and Chen and Chin, 1988). The result from this study indicates that the algal species *C. japonica* was most effective as a 'biological filter' and efficiently reduced accumulated toxic metabolites from the unchanged culture water environment. Levels of NH_4^+.N and NO_2.N were within or close to recommended safe limits. Early protozoeal stages of *P. monodon* are conceivably most sensitive to nitrite levels above 100 $\mu g.\ l^{-1}$ rather than ammonia. Static water hatcheries must include algae as a biofilter to control and detoxify accumulated levels of both ammonia and nitrite in culture water, alternatively some water changes must be carried out to combat the release of these toxic metabolites.

When the algae *T. chuii* and *R. baltica* (40 cells μl^{-1}) were contained in a flow through cylinder and suspended in the culture water with *P. monodon* fed on a 100% artificial diet, larval growth was significantly different ($P < 0.05$) from larval growth obtained in the absence of trapped algae, (table 8a and 8b). Larval survival however was not significantly different ($P < 0.05$) (table 9b). In this experiment culture water was changed daily preventing accumulation of ammonia and nitrite so that the observed difference in growth cannot be attributed to the effect of higher levels of toxicant in the

absence of algae. It is suggested that algal metabolites secreted by *T. chuii* and *R. baltica* into the culture water may stimulate larval growth.

Although the feeding behaviour of adult crustaceans has been studied intensively (Linstedt, 1971; Heinen, 1980; Ache, 1983; Carr et al., 1987). Far less research has been conducted into the chemosensory feeding behaviour of crustacean larvae. However it has been shown that planktonic larval stages and marine copepods respond positively to the presence of algae in the water. Yule (1982) demonstrated a positive feeding response by two species of barnacle nauplii to the presence of algae in seawater. Similarly, Yule and Crisp (1983) demonstrated increased limb movement for the copepod *Temora* in the presence of algal food (*Skeletonema* sp.). A decrease in limb activity was observed as the algae moved away from the animal. Mechanical and chemical cues were suggested as obvious candidates for the initiation of this response. Evidence is accumulating which suggests that many planktonic crustacean larvae and planktonic copepods assess the concentration of suspended food particles in the surrounding medium by chemical and other methods and adjust feeding rates accordingly (Yule and Crisp, 1983). Strickler (1982) suggested that calanoid copepods are able to predict the trajectory of algal cells on the basis of chemical diffusion of metabolites from algal cells. Most predatory crustacean larvae do not appear to show orientation behaviour towards a food stimulus source, but demonstrate chance encounter feeding behaviour (Langdon et al., 1985). On contact with the mouth parts, both chemical and

textural stimulii become important, and unless the correct stimuli are provided the particle will be rejected (Möller et al., 1979). Most authors are in agreement that amino acids are strong chemoattractants for herbivorous stages of crustacean larvae. Yule (1982) demonstrated a response by barnacle larvae to glycine and glutamic acid, these non-essential amino acids are natural exudates from phytoplankton . In the present experiment, it would appear that metabolites secreted by the trapped algae into the culture water enhanced growth in *P. monodon* larvae feeding on artificial diets.

From the present results it would appear unlikely that the 10 cells μl^{-1} algae added to culture water in the previous section (2), could be effective in reducing levels of toxic metabolites when artificial feeds are used. Especially as 50% of the culture water was changed daily to renew water quality. Present results indicate that in order to reduce toxic levels of both ammonia and nitrite in culture water, frequent water exchange must be carried out. Alternatively high levels of microalgae (*Chlorella* 1000 cells μl^{-1}) must be present at all times during the culture period if the culture water remains unchanged. With frequent water exchanges, it is possible to culture *P. monodon* successfully on Frippak feeds, with 60 - 70% survival to PL stage. However most commercial hatcheries rarely exchange culture water; *P. monodon* larvae are commercially reared in static water hatcheries in Taiwan (Liao, 1977). Accumulation of toxicants in these static systems adversely affects the survival and development of larvae (Chin and Chen, 1987; 1988).

This section further shows that metabolites secreted by live microalgae contained in the culture water may act as feeding/growth stimuli. Enhanced larval growth of 5.1% was recorded on artificial diets fed as total replacement in the presence of contained microalgae (40 cells μl^{-1}) in the culture water. However algae supplied as a cofeed (10 cμl^{-1}) with artificial diet gives better performance, both larval growth and survival are significant compared to larval growth obtained on artificial diets without algal supplements. It is possible that 10 cells μl^{-1} microalgae may provide essential micronutrients such as vitamins and minerals to the diet.

The next section evaluates the dietary composition of microcapsules. Larval performance on different protein sources (plant and animal) incorporated into the diet are investigated together with optimum lipid levels required and the requirement for vitamins and minerals in diet for better larval growth and survival.

Table 1 : Toxicity levels of NH_4^+.N, NH_3.N and NO_2.N for penaeid prawns

Species	Larval stage	Toxin	Exposure Concentration mg. l^{-1}	Duration	Result	Author
P. japonicus	Juveniles	NH_3.N	0.37	3 weeks	Growth reduced by 50% of that of the control	Wickins, 1976
P. occidentalis	Juveniles	NH_3.N	0.40	3 weeks	Growth reduced by 50% of that of the control	Wickins, 1976
P. schmitti	Juveniles	NH_3.N	0.69	3 weeks	Growth reduced by 50% of that of the control	Wickins, 1976
P. semisulcatus	Juveniles	NH_3.N	0.22	3 weeks	Growth reduced by 50% of that of the control	Wickins, 1976
P. setiferus	Juveniles	NH_3.N	0.59	3 weeks	Growth reduced by 50% of that of the control	Wickins, 1976
P. indicus	Juveniles	NO_2.N	6.4	34 day	Growth reduced by approx. 50%	Wickins, 1976
P. monodon	Larvae	NO_2.N	8.5	96h	LCso	Colt and Armstrong, 1981
P. japonicus	Juveniles	NO_2.N	0.1 - 0.6	9 months	Mortality between 5-32%	Mevel and Chamroux, 1981
P. indicus	Larvae	NH_4^+.N	3.5	24h	LCso	Jayasankar and Muthu, 1983a
P. indicus	Larvae	NO_2.N	1.8	9 days	50% larvae did not metamorphise into post larvae	Jayasankar and Muthu, 1983b
P. monodon	Post larvae	NO_2.N	0.11	21 days	4% survival	Chen et al., 1986
P. monodon	Larvae	NH_3.N	0.10	96h	LCso	Chin and Chen, 1987
P. monodon	Larvae	NO_2.N	0.11	96h	LCso	Chen and Chin, 1988
P. monodon	Larvae	NO_2.N	0.23	11 days	20% survival to PL stage	Present study

Table 2 : Accumulation of toxicant levels, NH_4^+.N, NH_3.N and NO_2.N from decomposition of artificial diet over a period of 11 days in unchanged culture water. Levels in control over the same period and culture conditions also given. Values are means of replicates with ± S.D.

TIME/ TOXINS	DAY 3	DAY 5	DAY 7	DAY 9	DAY 11
NH_4^+.N $\mu g.l^{-1}$	222 ± 18	702 ± 12	976.5 ± 7.5	1524 ± 6.0	2116.0±26
NH_3.N $\mu g.l^{-1}$	11.81±0.96	33.723±4.26	51.949 ± 0.399	102.81±1.685	139.86±1.725
NO_2.N $\mu g.l^{-1}$	21.62±1.38	39.9 ± 1.5	75.8 ± 2.2	115 ± 0	314.7±20.3
pH	8.0 ± 0	7.95 ± 0.70	8.0 ± 0	8.1 ± 0	8.1 ± 0

CONTROLS

TOXINS	DAY 1	DAY 3	DAY 5	DAY 7	DAY 9	DAY 11
NH_4^+.N $\mu g.l^{-1}$	15.0 ± 1.27	15.3 ± 0.70	16.7 ± 2.12	15.3 ± 2.82	16.0 ± 2.54	18.4 ± 7.07
NH_3.N $\mu g.l^{-1}$	0.991± 0.70	0.813± .85	0.713± .70	0.653± .42	0.851±.84	1.216±1.13
NO_2.N $\mu g.l^{-1}$	7.3 ± 2.96	7.36 ± 3.53	7.36 ± 5.09	7.36	8.50 ± 6.36	8.55 ± 4.24
pH	8.1 ± 0.1	8.0 ± 0	7.92 ± .28	7.96 ± 0.14	8.0 ± 00	8.1 ± 0

Table 3 : Reduction of NH_4^+.N, NH_3.N and NO_2.N levels by algal species (500 cells μl^{-1}) in the culture medium, after 24 and 48h. Values are means of replicates with ± S.D.

			24h				48h			
Microalgal species	Cell Size µm	Cell Density μl^{-1}	NH_4^+.N $\mu g.l^{-1}$	NH_3.N $\mu g.l^{-1}$	NO_2.N $\mu g.l^{-1}$	pH	NH_4^+.N $\mu g.l^{-1}$	NH_3.N $\mu g.l^{-1}$	NO_2.N $\mu g.l^{-1}$	pH
C. japonica	5	500	1020.0 ±219.20	54.26 ±4.24	138.0 ±49.49	8.0 ±0.0	1122.0 ±141.42	59.69 ±14.14	156.4 ±35.35	8.0 ±0.0
C. calcitrans	5	500	1275.0 ±156.97	67.83 ±15.55	202.4 ±63.63	8.0 ±0.0	1122.0 ±173.94	59.69 ±8.48	165.6 ±46.66	8.0 ±0.0
C. japonica (Trapped)	5	500	1190.0 ±155.56	63.30 ±18.38	211.6 ±127.27	8.0 ±0.0	999.6 ±77.78	53.17 ±11.31	192 ±84.85	8.0 ±0.0
T. chuii	10	500	1275.0 ±226.27	67.83 ±14.14	184.0 ±63.63	8.0 ±0.0	1173.0 ±79.19	62.40 ±5.65	192 ±42.42	8.0 ±0.0
P. lutheri	5	500	1530.0 ±197.98	65.331 ±12.72	230.0 ±96.16	7.9 ±0.14	1200.0 ±155.56	63.84 ±12.72	202.4 ±79.19	8.0 ±0.0
R. baltica	10	500	1785.0 ±254.55	76.219 ±7.07	276.0 ±73.53	7.9 0.0	1836.0 ±212.13	97.67 ±31.11	230 ±73.53	8.0 ±0.0
S. costatum	7	500	1836.0 ±169.70	78.397 ±21.21	184.0 ±35.35	7.9 0.14	2040.0 ±169.70	108.52 ±46.66	255 ±89.09	8.0 ±0.0

Table 4 : Reduction of NH_4^+.N, NH_3.N and NO_2.N levels by algal species (1000 $\mu g.l^{-1}$) in culture medium, after 24 and 48h. Values are means of replicates with ± S.D.

			24h				48h			
Microalgal Species	Cell Size μm	Cell Density μl^{-1}	NH_4^+.N $\mu g.l^{-1}$	NH_3.N $\mu g.l^{-1}$	NO_2.N $\mu g.l^{-1}$	pH	NH_4^+.N $\mu g.l^{-1}$	NH_3.N $\mu g.l^{-1}$	NO_2.N $\mu g.l^{-1}$	pH
C. japonica	5	1000	295.8 ±56.56	19.49 ±4.24	73.6 ±9.89	8.1 ±0.14	36.0 ±11.31	2.37 ±0.84	46.0 ±8.48	8.1 ±0.14
C. japonica (Trapped)	5	1000	714.0 ±70.71	30.48 ±4.24	138 ±28.28	7.9 ±0.14	561 ±89.09	23.95 ±2.82	92 ±8.48	7.9 ±0.14
T. chuii	10	1000	1326.0 ±124.45	56.62 ±2.82	276 ±73.53	7.9 ±0.0	765 ±84.85	32.66 ±7.02	255 ±46.66	7.9 ±0.28
P. lutheri	5	1000	918.0 ±79.19	48.83 ±11.31	303.6 ±93.33	8.0 ±0.14	1020 ±70.71	54.26 ±12.72	322 ±79.19	8.0 ±0.14
C. calcitrans	5	1000	1346.4 ±120.20	57.49 ±5.65	184 ±46.66	7.9 ±0.0	1071 ±120.20	45.73 ±5.65	147.2 ±30.97	7.9 ±0.00
S. costatum	7	1000	1632.0 ±108.89	69.68 ±9.89	230 ±62.22	7.9 ±0.0	1836 ±127.27	97.67 ±28.28	184 ±29.69	8.0 ±0.00
R. baltica	10	1000	1836.0 ±169.70	78.39 ±7.07	322 ±35.35	7.9 ±0.14	2040 ±28.28	108.52 ±22.62	303.6 ±84.85	8.0 ±0.00

Table 5 : Reduction of NH_4^+.N, NH_3.N and NO_2.N by algal species (1500 cells μl^{-1}) in culture medium, after 24 and 48h. Values are means of replicates with ± S.D.

			24h				48h			
Microalgal Species	Cell Size µm	Cell Density μl^{-1}	NH_4^+.N $\mu g.l^{-1}$	NH_3.N $\mu g.l^{-1}$	NO_2.N $\mu g.l^{-1}$	pH	NH_4^+.N $\mu g.l^{-1}$	NH_3.N $\mu g.l^{-1}$	NO_2.N $\mu g.l^{-1}$	pH
C. japonica	5	1500	255 ±39.59	13.56 ±7.07	82.8 ±14.14	8.0 ±0.14	18.4 ±4.24	1.216 ±0.707	46.0 ±2.0	8.1 ±0.14
C. japonica (Trapped)	5	1500	1020 ±138.59	43.55 ±8.48	138 ±16.97	7.9 ±0.0	510.0 ±91.92	21.78 ±5.65	9.2 ±32.52	7.9 ±0.14
C.calcitrans	5	1500	1009.8 ±325.27	43.12 ±7.07	276 ±15.515	7.9 ±0.0	969.0 ±156.97	51.55 ±4.24	340 ±59.39	8.0 ±0.14
T.chuii	10	1500	1122 ±213.54	59.69 ±19.79	294.4 ±32.52	8.0 ±0.0	1020.0 ±155.56	54.26 ±11.31	230 ±72.12	8.0 ±0.0
P. lutheri	5	1500	1326 ±301.22	56.62 ±9.89	184 ±22.62	7.9 ±0.28	1428.0 ±299.8	75.96 ±15.55	138 ±43.84	8.0 ±0.0
S.costatum	7	1500	1428 ±224.85	75.97 ±21.21	202.4 ±18.38	8.0 ±0.14	1530.0 ±195.16	81.39 ±8.48	230 ±114.55	8.0 ±0.0
R.baltica	10	1500	2040 ±70.71	134.84 ±16.97	340 ±11.31	8.1 ±0.14	1836 ±176.77	97.67 ±12.72	276 ±73.53	8.0 ±0.0

Table 6 : Reduction of NH_4^+.N, NH_3.N and NO_2.N levels by algal species (2000 cells μl^{-1}) in culture medium, after 24 and 48h. Values are means of replicates with ± S.D.

			24h				48h			
Microalgal Species	Cell Size μm	Cell Density μl^{-1}	NH_4^+.N $\mu g.l^{-1}$	NH_3.N $\mu g.l^{-1}$	NO_2.N $\mu g.l^{-1}$	pH	NH_4^+.N $\mu g.l^{-1}$	NH_3.N $\mu g.l^{-1}$	NO_2.N $\mu g.l^{-1}$	pH
C. japonica	5	2000	265.2 ±57.27	11.32 ±2.82	92.0 ±7.07	7.9 ±0.0	20.40 ±2.82	0.87 ±0.70	55.2 ±7.07	7.9 ±0.0
C. japonica (Trapped)	5	2000	714 ±138.59	30.48 ±9.89	174.8 ±50.34	7.9 ±0.0	612.0 ±63.63	26.13 ±8.48	82.8 ±11.31	7.9 ±0.0
T. chuii	10	2000	1020 ±141.42	54.26 ±11.31	276 ±73.53	8.0 ±0.0	816.0 ±169.70	34.84 ±21.21	230.0 ±36.76	7.9 ±0.14
C. calcitrans	5	2000	1122 ±144.24	59.69 ±7.07	257.6 ±53.74	8.0 ±0.0	1020.0 ±173.94	54.26 ±16.97	276.0 ±91.92	8.0 ±0.0
P. lutheri	5	2000	1224 ±173.94	65.11 ±12.72	193.2 ±77.78	8.0 0.14	1122.0 ±205.06	59.69 ±16.97	202.0 ±70.71	8.0 ±0.0
S. costatum	7	2000	1275 ±247.48	67.83 ±15.55	165.6 ±32.52	8.0 ±0.0	1326.0 ±173.94	87.64 ±18.38	185.0 ±35.35	8.1 ±0.0
R. baltica	10	2000	2550 ±226.27	135.66 ±21.21	294.4 ±79.19	8.0 ±0.0	2040.0 ±212.13	134.84 ±34.76	280.0 ±63.63	8.1 ±0.14

Table 7 : *P. monodon* larval growth and survival (PZ1 - PZ3) on artificial diet, artificial diet + trapped algae (*T. chuii* + *R. baltica* 40 cμl $^{-1}$) and controls

	Larval growth (mm)		Larval survival (%)	
TREATMENT	MEAN	STANDARD DEVIATION	MEAN	STANDARD DEVIATION
Artificial diet	2.5871	.1756	43.0000	14.1421
Artificial diet + Trapped algae	2.7163	.1126	45.5000	4.9497
Controls	3.1686	.0926	88.0000	7.0711

Table 8a: ANOVA table, Larval growth against treatments with artificial diet, artificial diet + trapped algae (*T.chuii* + *R.baltica* 40 cμl^{-1}) and controls

ANALYSIS OF VARIANCE

SOURCE	D.F.	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB
BETWEEN GROUPS	2	3.7289	1.8645	107.3923	.001
WITHIN GROUPS	57	.9896	.0174		
TOTAL	59	4.7185			

Tests for Homogeneity of Variances

Cochrans C = Max. Variance/Sum (Variances) = .5922, P = .008

Bartlett-Box F = 4.148, P = .016

Table 8b: Tukeys pairwise comparison between different treatment

		TREATMENTS		
MEAN	TREATMENT	1	2	3
2.5871	1			
2.7163	2	*		
3.1686	3	*	*	

(*) Denotes pairs of groups significantly different at the 0.050 level

1 = Artificial diet

2 = Artificial diet + trapped algae

3 = Controls

Table 9a : ANOVA table, larval survival against treatments with artificial diet, artificial diet + trapped algae (*T.chuii* + *R.baltica* 40 cμl^{-1}) and controls

ANALYSIS OF VARIANCE

SOURCE	D.F.	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB
BETWEEN GROUPS	2	2558.3333	1279.1667	13.9800	.0302
WITHIN GROUPS	3	274.5000	91.5000		
TOTAL	5	2832.8333			

Tests for Homogeneity of Variances

Cochrans C = Max. Variance/Sum (Variances) = .7286, P = .439

Bartlett-Box F = .387, P = .684

Table 9b : Tukeys pairwise comparisons between treatments

MEAN	TREATMENTS	TREATMENTS 1	2	3
43.0000	1			
45.5000	2	ns		
88.0000	3	*	*	

(*) Denotes pairs of groups significantly different at the 0.050 level

ns indicates a non-significant result

1 = Artificial diet
2 = Artificial diet + trapped algae
3 = Controls

Figure 1. Total ammonia and nitrite production for 56mg l^{-1} artificial larval diet incubated at 28°C in seawater over 11 days together with blank controls.

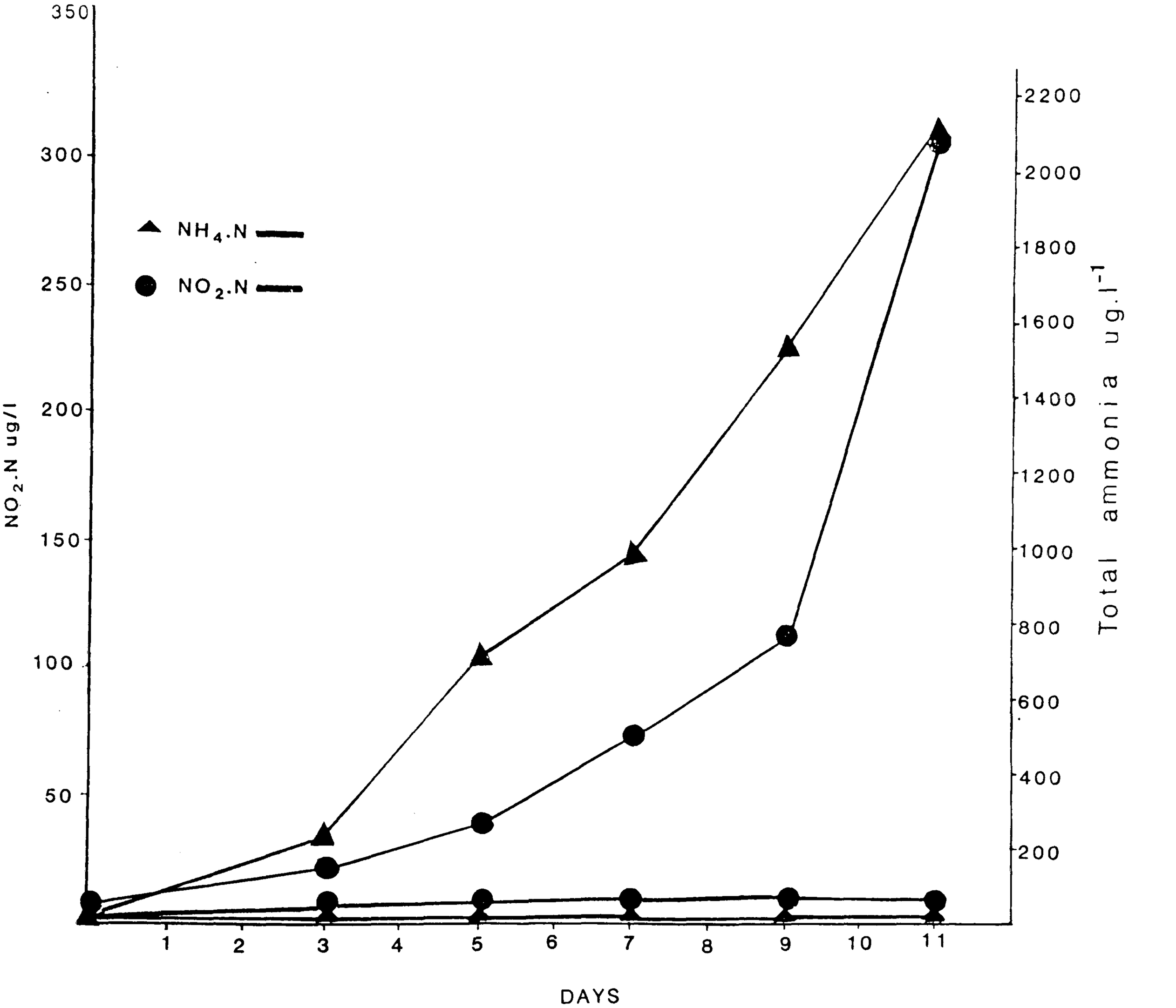

350
300
250
200
150
100
50
$NH_4.N$
$NO_2.N$
$NO_2.N$ ug/l
2200
2000
1800
1600
1400
1200
1000
800
600
400
200
Total ammonia ug.l^{-1}
1
2
3
4
5
6
7
8
9
10
11
DAYS

Figure 2. Reduction in ammonia levels produced in seawater by artificial larval diet over 11 days using different live microalgae at 500 cells μl^{-1}.

Legend

Skeletonema costatum △

Rhodomonas baltica ■

Pavlova lutheri ▲

Tetraselmis chuij □

Chaetoceros calcitrans ✱

Chlorella japonica ○

C. japonica (trapped) ●

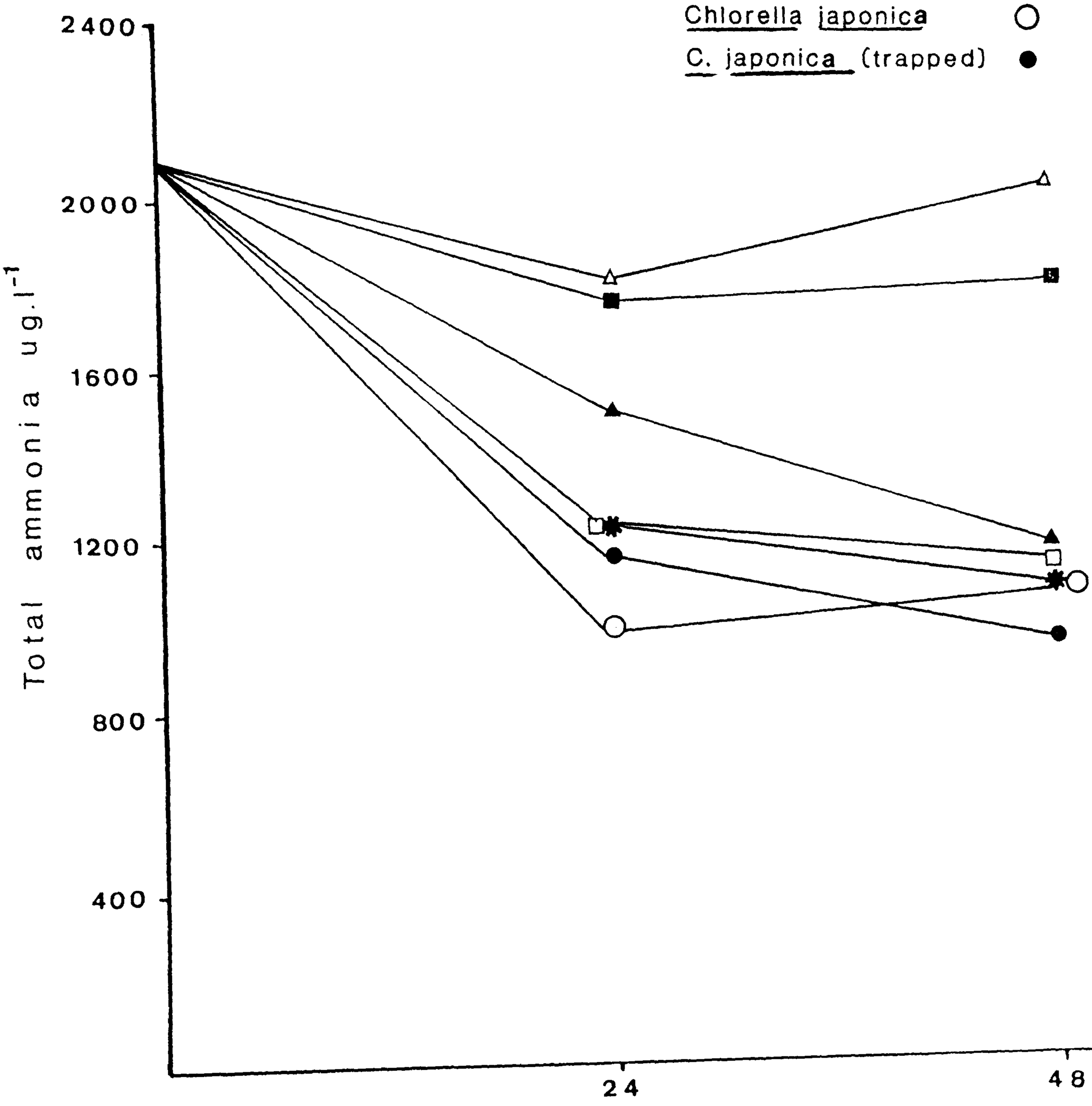


Figure 3. Reduction in ammonia levels produced in seawater by artificial larval diet over 11 days using different live microalgae at 1000 cells μl^{-1}.

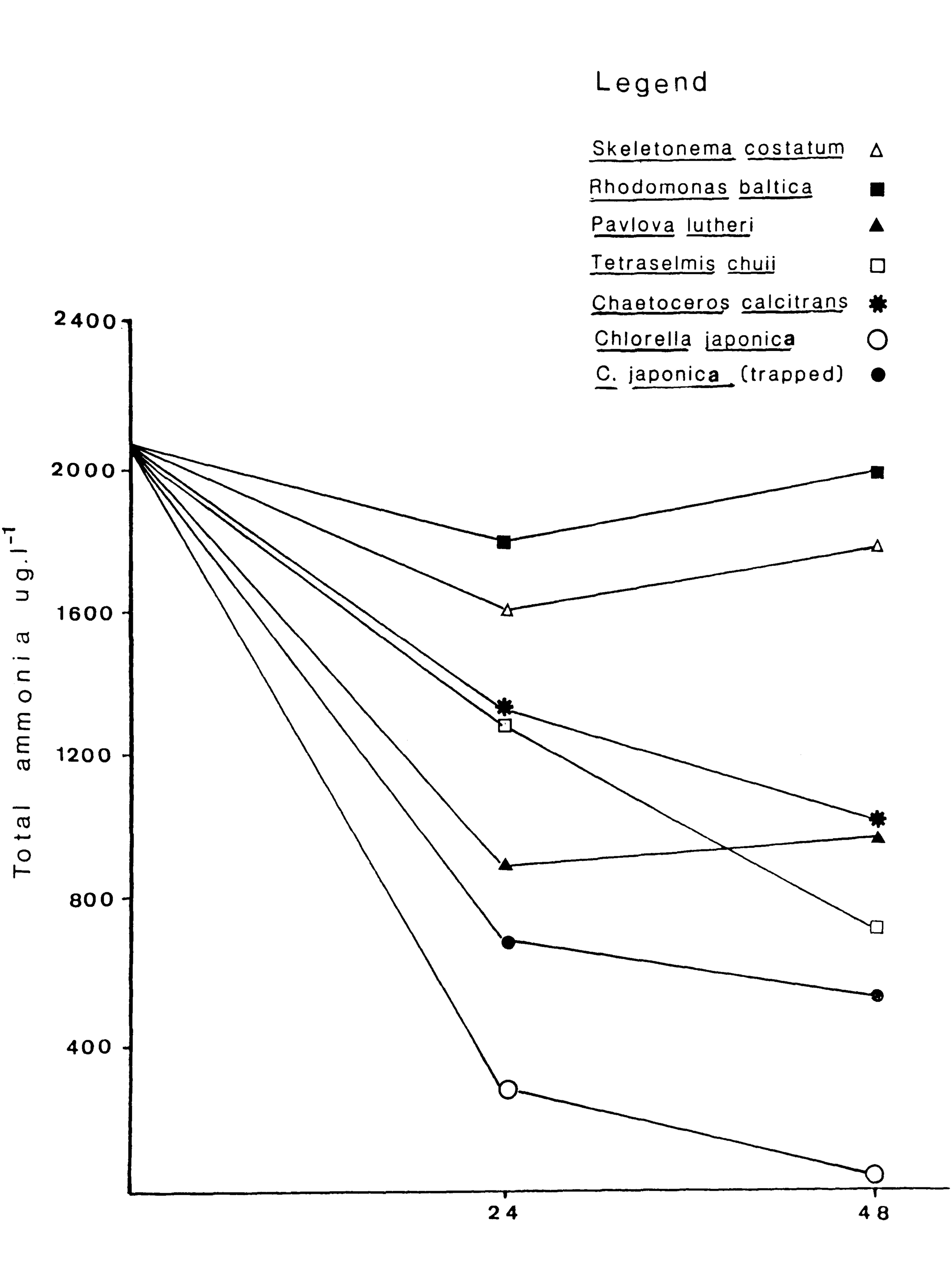

Legend
Skeletonema costatum
Rhodomonas baltica
Pavlova lutheri
Tetraselmis chuii
Chaetoceros calcitrans
Chlorella japonica
C. japonica (trapped)
2400
2000
1600
1200
800
400
Total ammonia ug.l-1
24
48
Time (hours)

Figure 4. Reduction in ammonia levels produced in seawater by artificial larval diet over 11 days using different live microalgae at 1500 cells μl^{-1}.

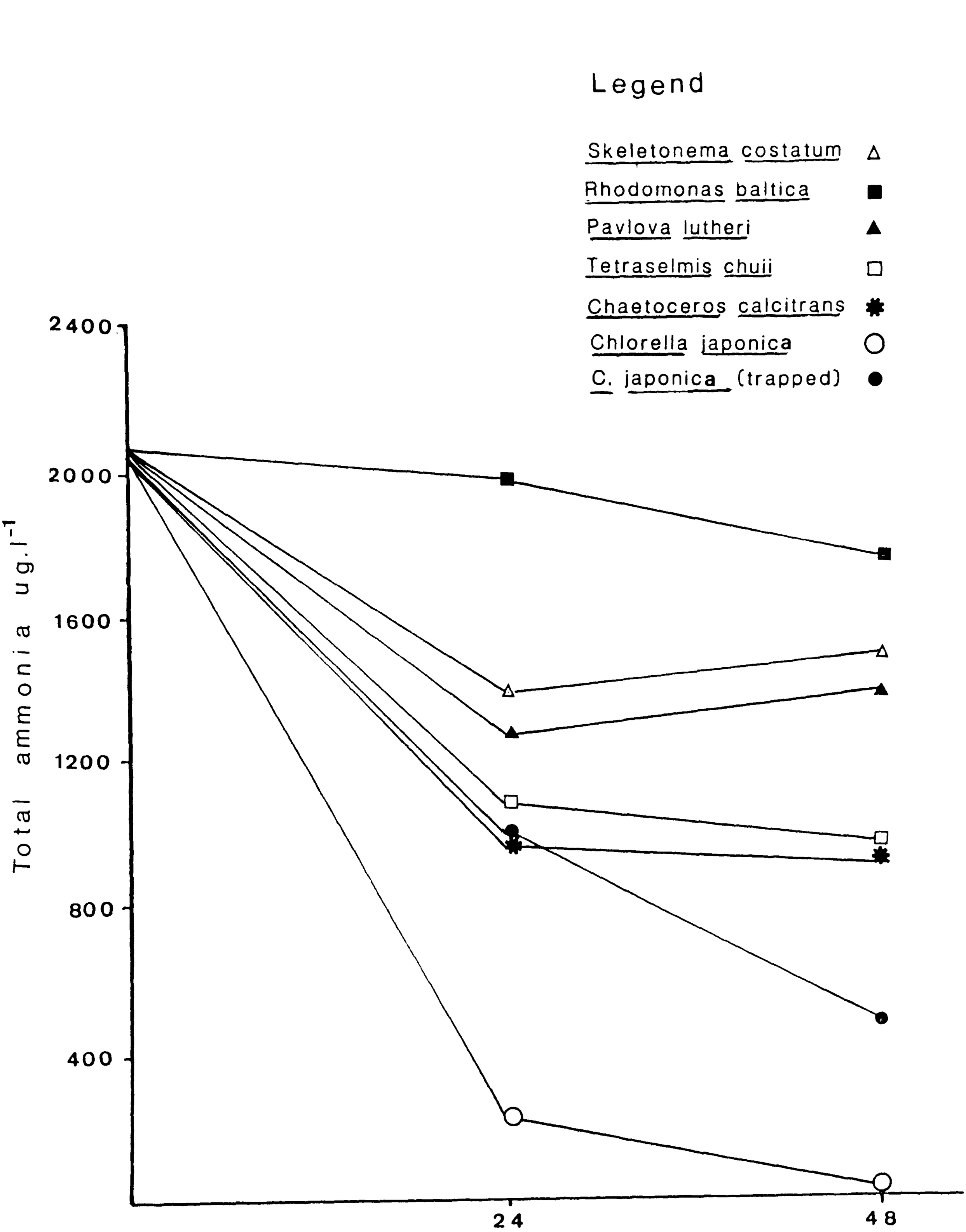

Legend
Skeletonema costatum
Rhodomonas baltica
Pavlova lutheri
Tetraselmis chuii
Chaetoceros calcitrans
Chlorella japonica
C. japonica (trapped)
2400
2000
1600
1200
800
400
Total ammonia ug.l-1
24
48
Time (hours)

Figure 5. Reduction in ammonia levels produced in seawater by artificial larval diet over 11 days using different live microalgae at 2000 cells μl^{-1} .

Legend

Skeletonema costatum △

Rhodomonas baltica ■

Pavlova lutheri ▲

Tetraselmis chuii □

Chaetoceros calcitrans ✸

Chlorella japonica ○

C. japonica (trapped) ●

Total ammonia ug.l^{-1}

2400
2000
1600
1200
800
400

24
48

Time (hours)

Figure 6. Reduction in nitrite levels produced in seawater by artificial larval diet over 11 days using different live microalgae at 500 cells μl^{-1}.

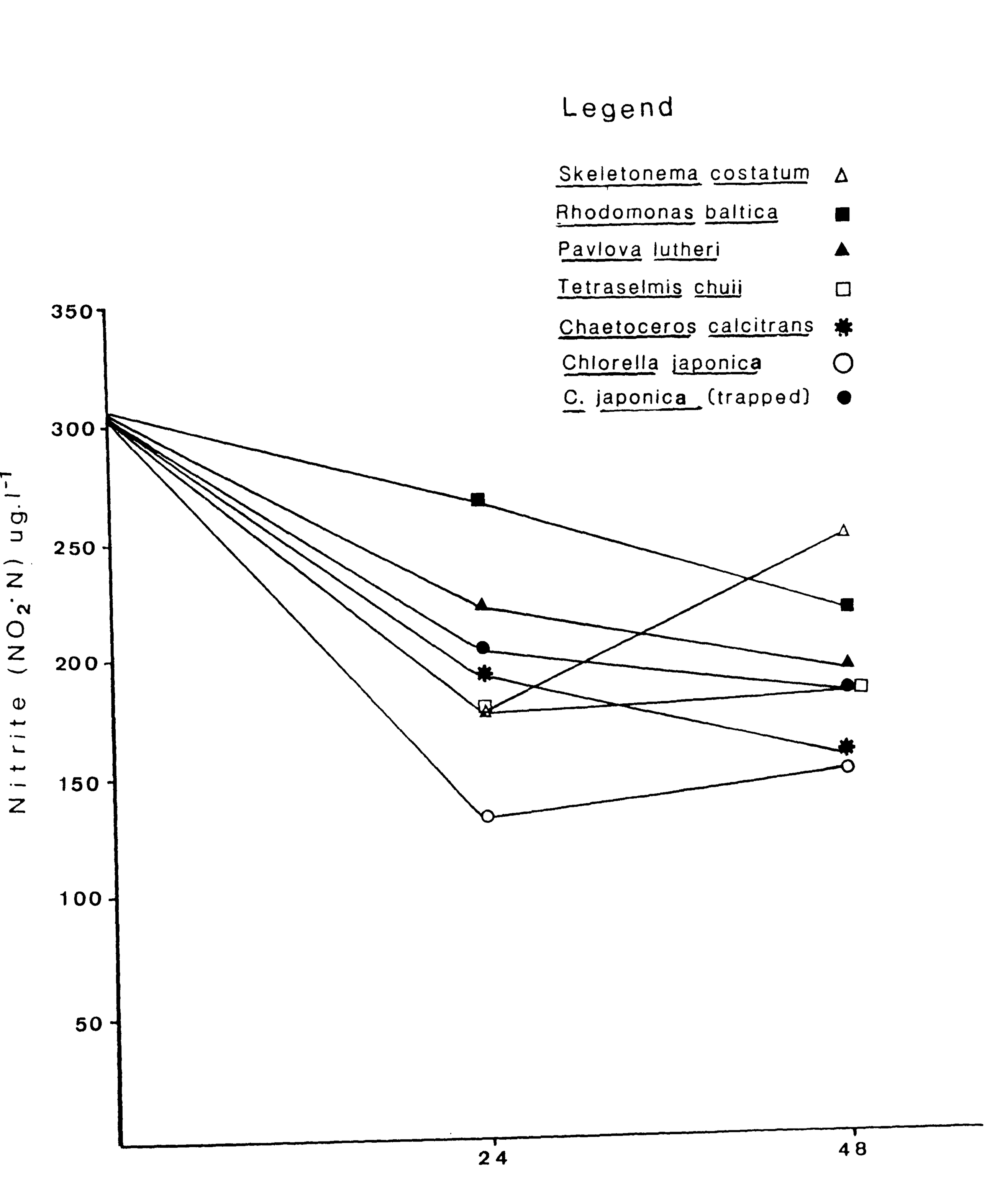

Legend
Skeletonema costatum
Rhodomonas baltica
Pavlova lutheri
Tetraselmis chuii
Chaetoceros calcitrans
Chlorella japonica
C. japonica (trapped)
350
300
250
200
150
100
50
Nitrite (NO_2-N) ug.l^{-1}
24
48
Time (hours)

Figure 7. Reduction in nitrite levels produced in seawater by artificial larval diet over 11 days using different live microalgae at 1000 cells μl^{-1}.

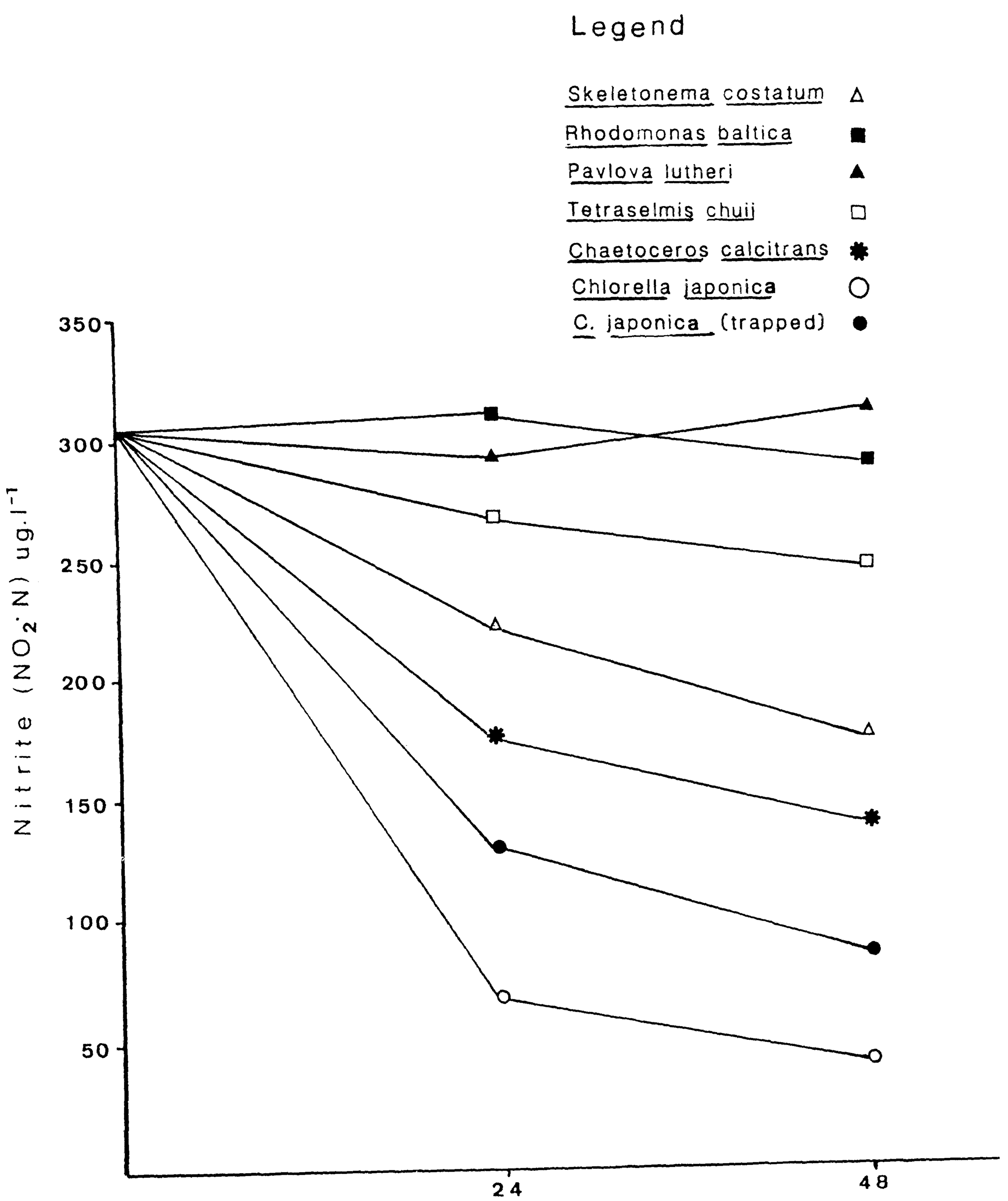

Legend
Skeletonema costatum
Rhodomonas baltica
Pavlova lutheri
Tetraselmis chuii
Chaetoceros calcitrans
Chlorella japonica
C. japonica (trapped)
350
300
250
200
150
100
50
Nitrite (NO_2-N) ug.l^{-1}
24
48
Time (hours)

Figure 8. Reduction in nitrite levels produced in seawater by artificial larval diet over 11 days using different live microalgae at 1500 cells μl^{-1}.

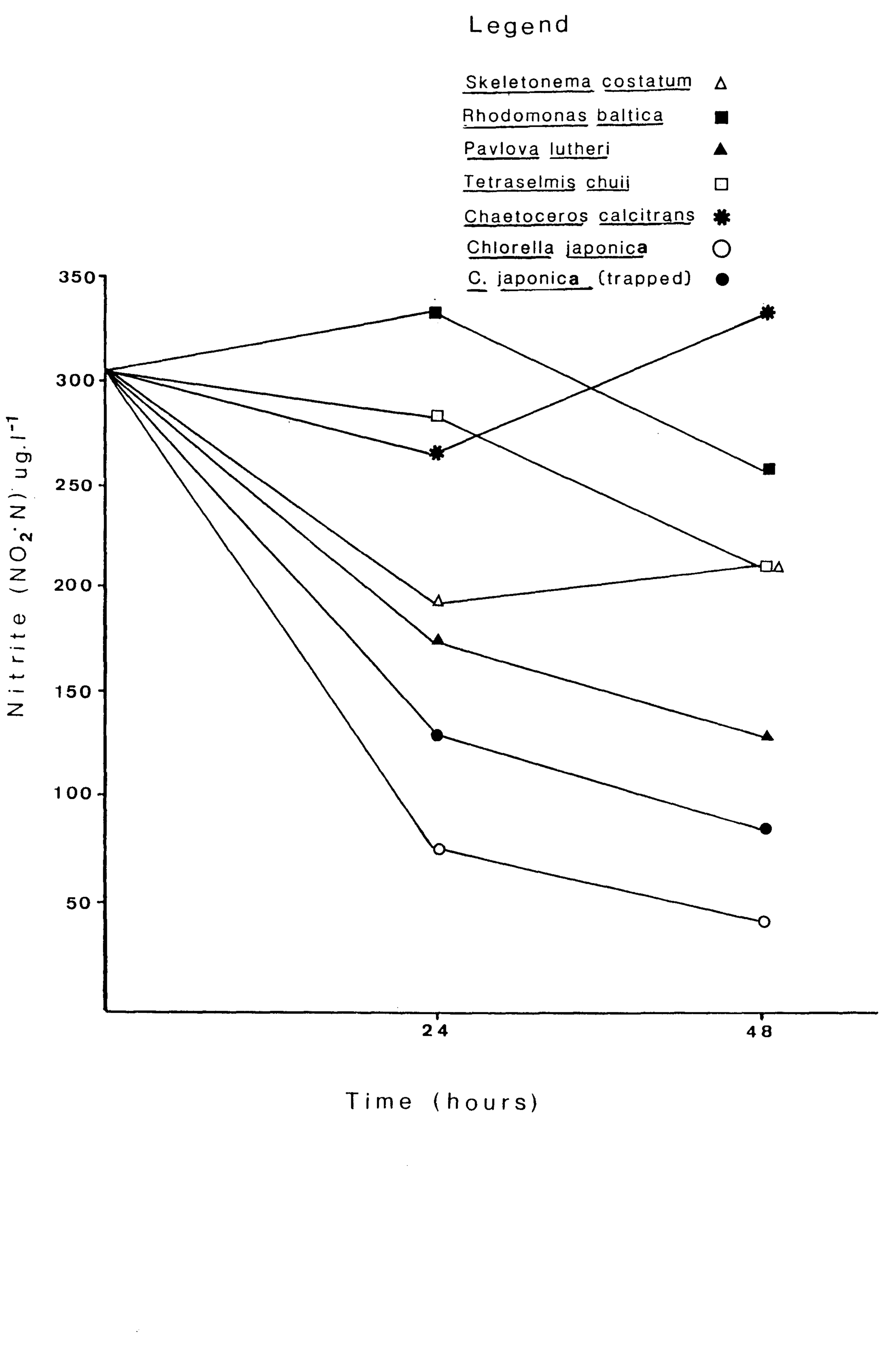

Legend
Skeletonema costatum
Rhodomonas baltica
Pavlova lutheri
Tetraselmis chuii
Chaetoceros calcitrans
Chlorella japonica
C. japonica (trapped)
350
300
250
200
150
100
50
Nitrite (NO_2-N) ug.l^{-1}
24
48
Time (hours)

Figure 9. Reduction in nitrite levels produced in seawater by artificial larval diet over 11 days using different live microalgae at 2000 cells μl^{-1}.

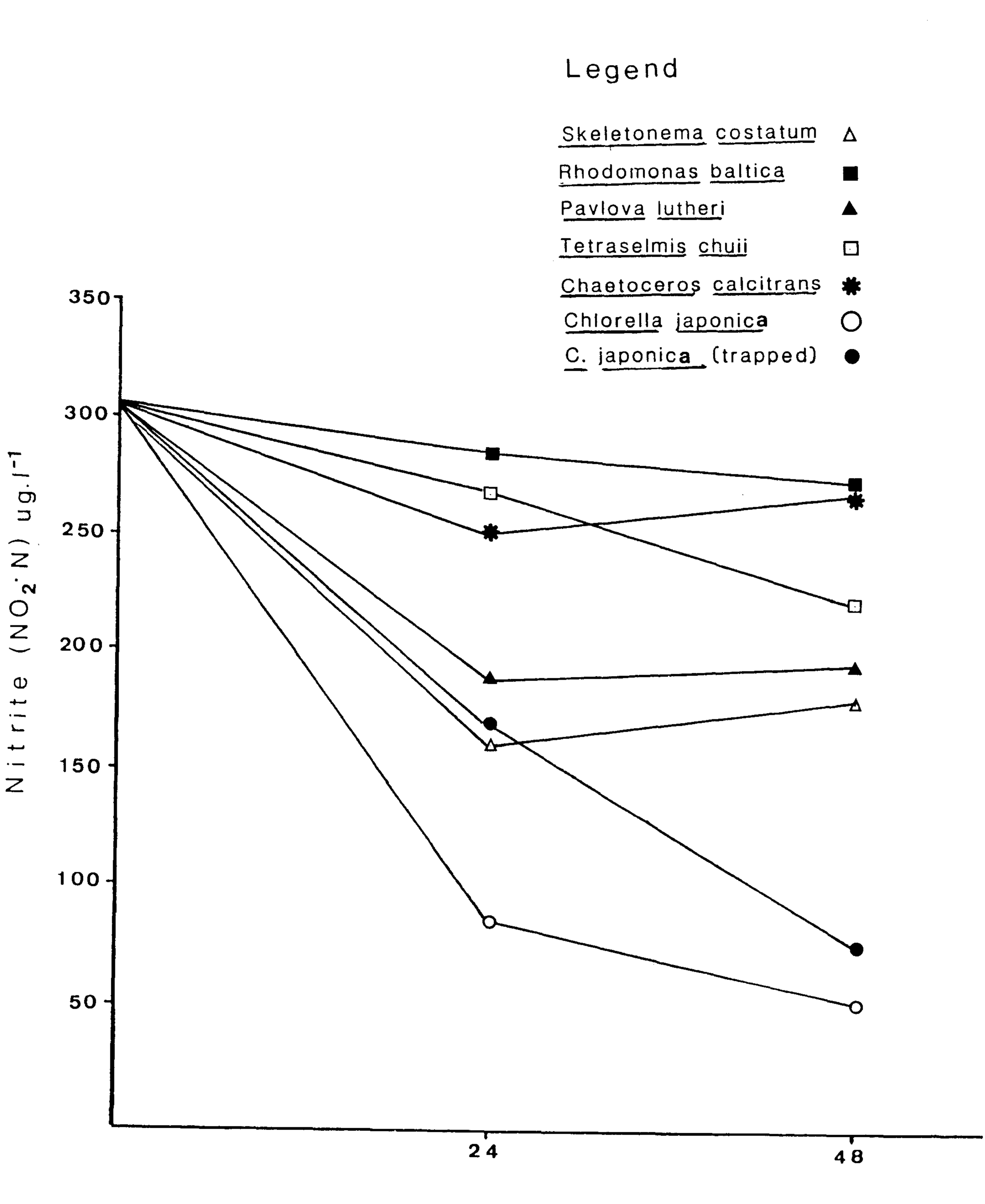
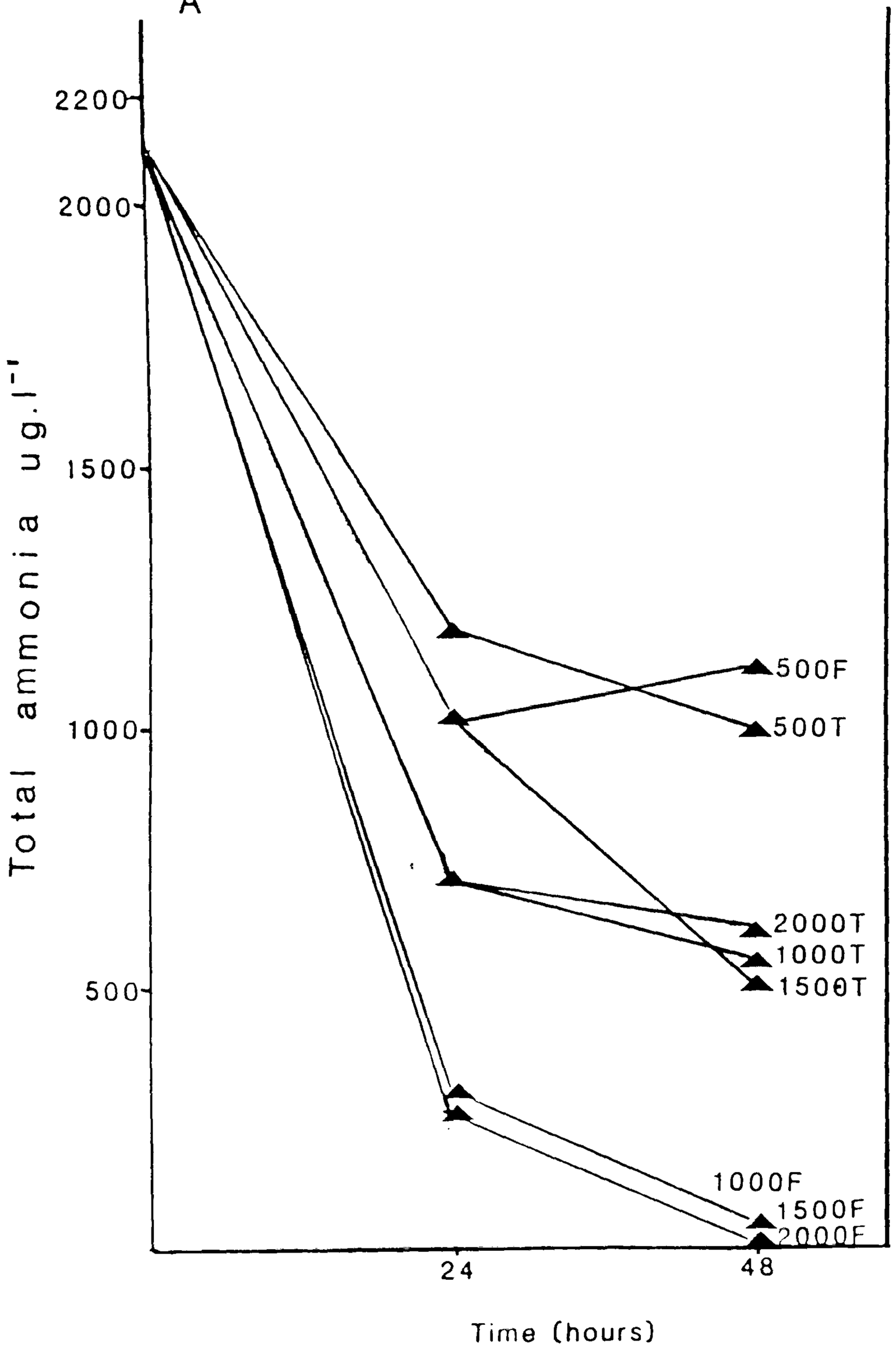

Legend
Skeletonema costatum
Rhodomonas baltica
Pavlova lutheri
Tetraselmis chuii
Chaetoceros calcitrans
Chlorella japonica
C. japonica (trapped)
350
300
250
200
150
100
50
Nitrite (NO_2-N) ug.l^{-1}
24
48
Time (hours)

Figure 10. Reduction in A) total ammonia (▲) and B) nitrite (●) levels in seawater after 24h and 48h using live *Chlorella japonica* at four different cell levels. T = Trapped *Chlorella*. F = Free floating *Chlorella*.

A


2200
2000
1500
1000
500
Total ammonia ug.l⁻¹
500F
500T
2000T
1000T
1500T
1000F
1500F
2000F
24
48
Time (hours)

B

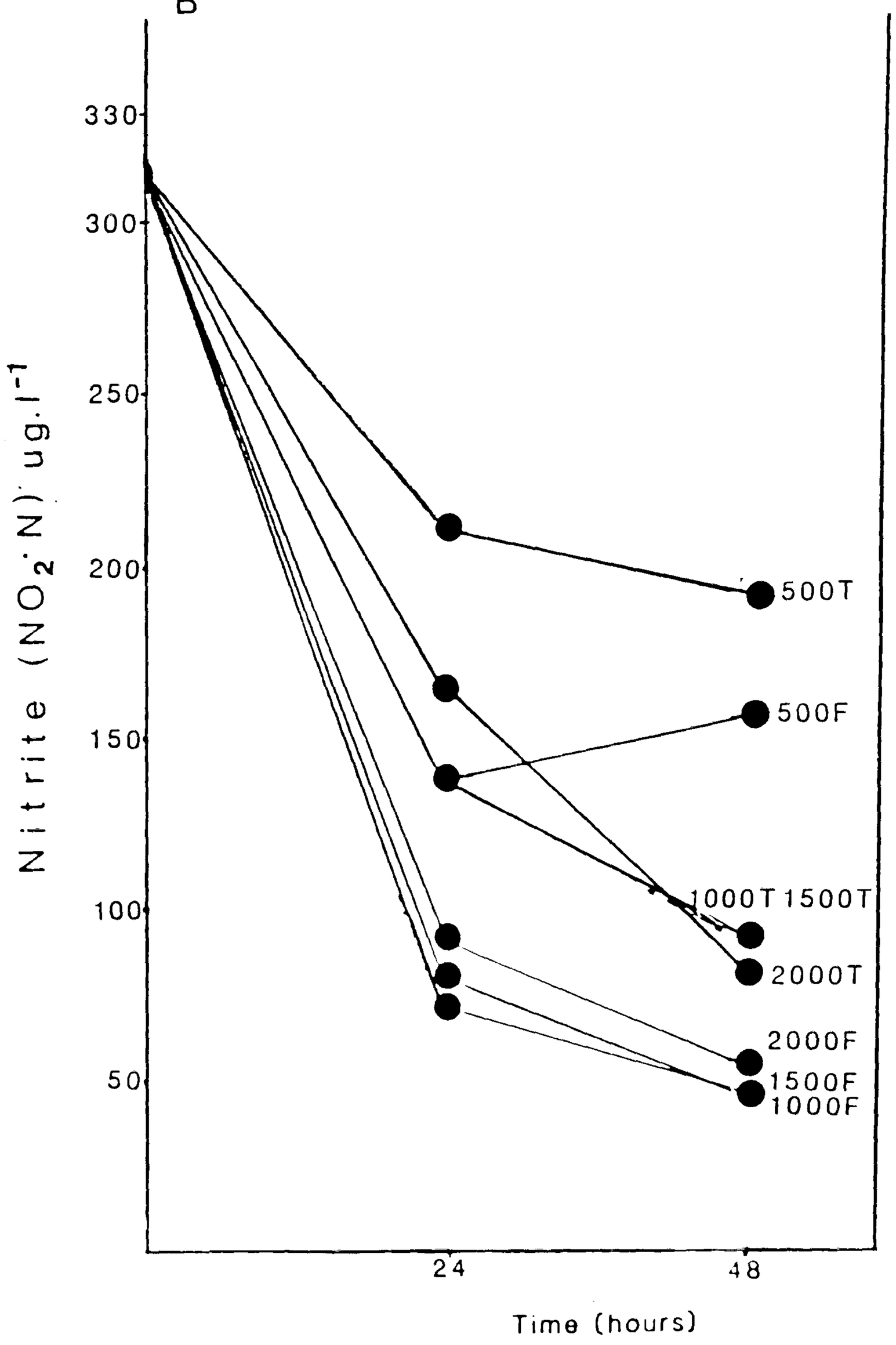

330
300
250
200
150
100
50
Nitrite (NO2·N) ug.l⁻¹
500T
500F
1000T
1500T
2000T
2000F
1500F
1000F
24
48
Time (hours)

Figure 11. The survival of P. monodon larvae fed on an artificial diet at 8mg.l^{-1} in the presence of free floating Chlorella at 1000 cells μl^{-1}, ammonia (▲) and nitrite (●) levels are also plotted, no water exchange occurred and illumination was constant at $6.6x10^{15}$quanta.$sec^{-1}cm^{2}$.

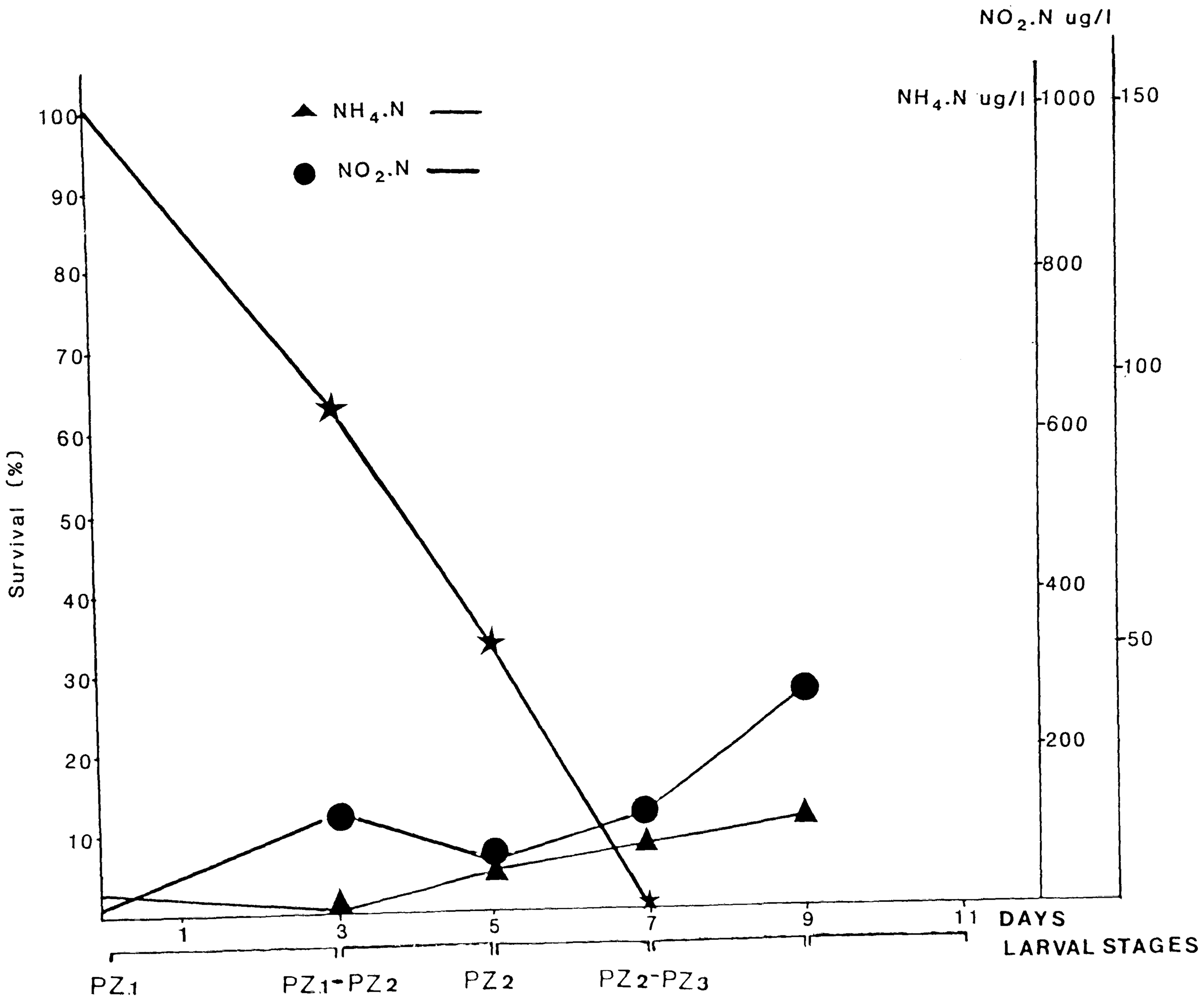

NO_2.N ug/l
NH_4.N ug/l
1000
800
600
400
200
150
100
50
NH_4.N
NO_2.N
Survival (%)
100
90
80
70
60
50
40
30
20
10
1
3
5
7
9
11
DAYS
LARVAL STAGES
PZ1
PZ1-PZ2
PZ2
PZ2-PZ3

Figure 12. Percentage survival of P. monodon larvae fed on an artificial diet at 8mg.$l^{-1}d^{-1}$in the presence of trapped live Chlorella at 1000 cells μl^{-1}, other conditions as before.

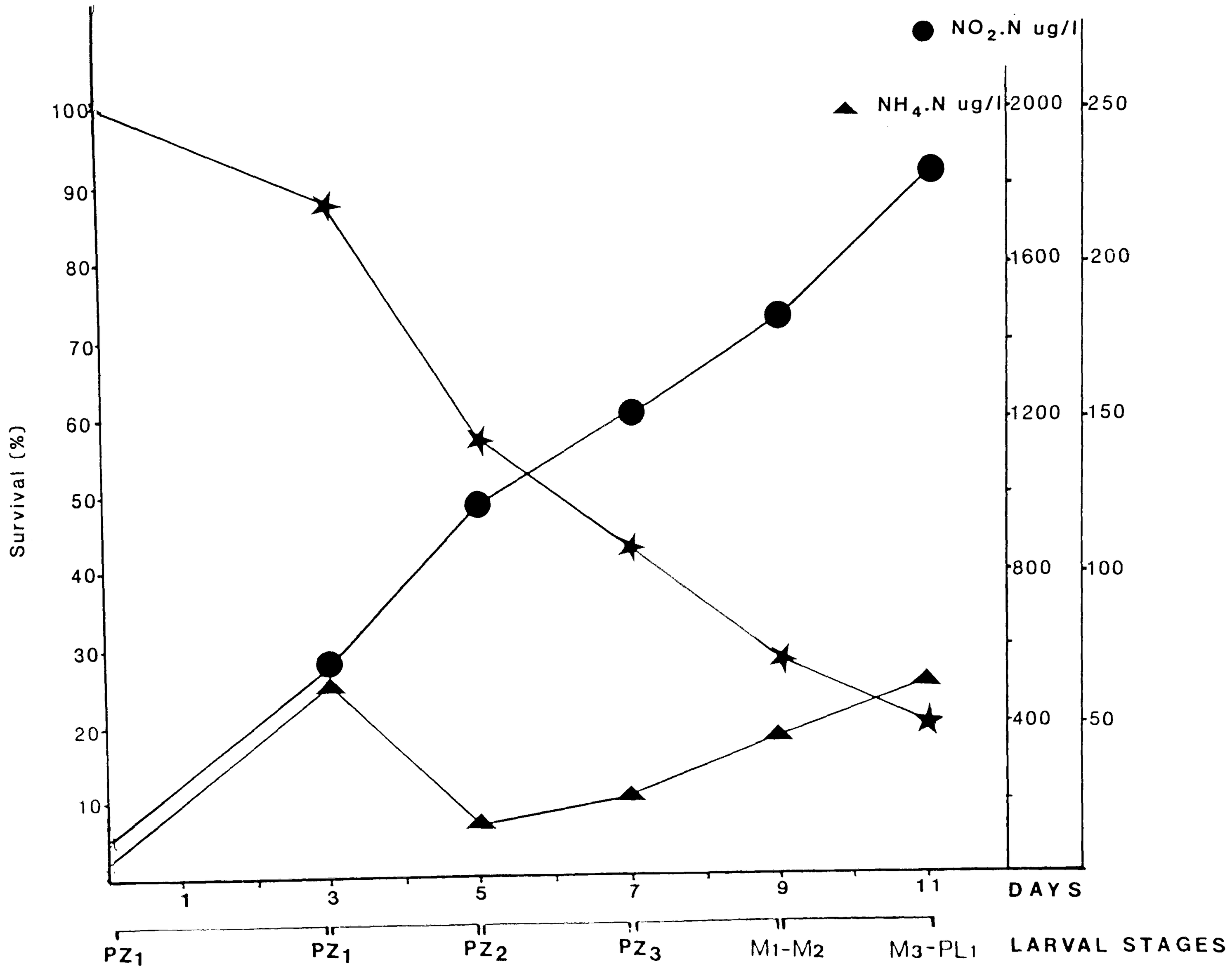

NO_2.N ug/l
NH_4.N ug/l
Survival (%)
100
90
80
70
60
50
40
30
20
10
2000
1600
1200
800
400
250
200
150
100
50
1
3
5
7
9
11
DAYS
PZ_1
PZ_1
PZ_2
PZ_3
M1-M2
M3-PL_1
LARVAL STAGES

Figure 13. The survival of P. monodon larvae fed on an artificial larval diet at 8mg l^{-1} d^{-1} in the absence of microalgae. Ammonia (▲) and nitrite (●) levels are plotted throughout the experiment during which no water exchange occurred.

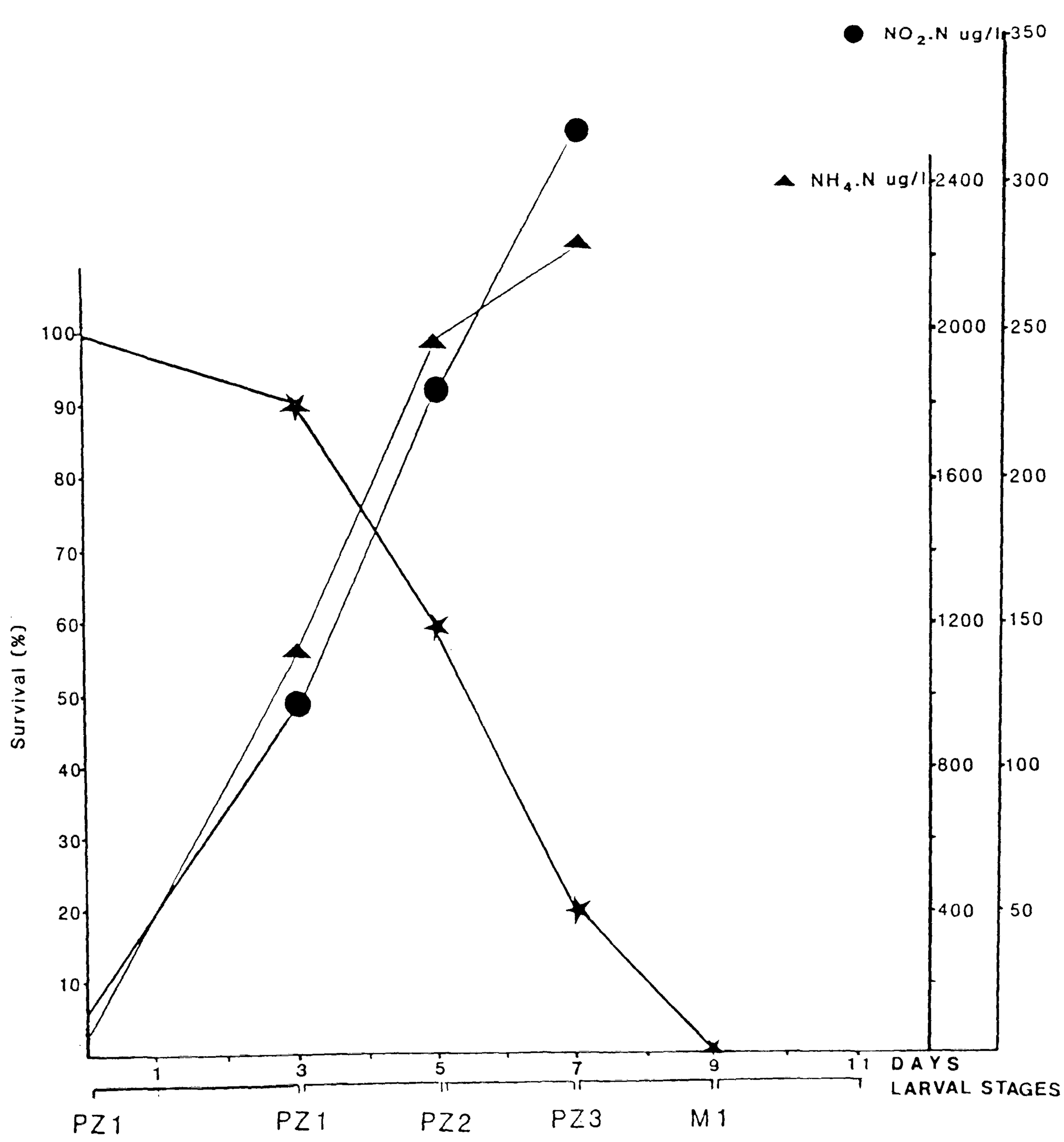
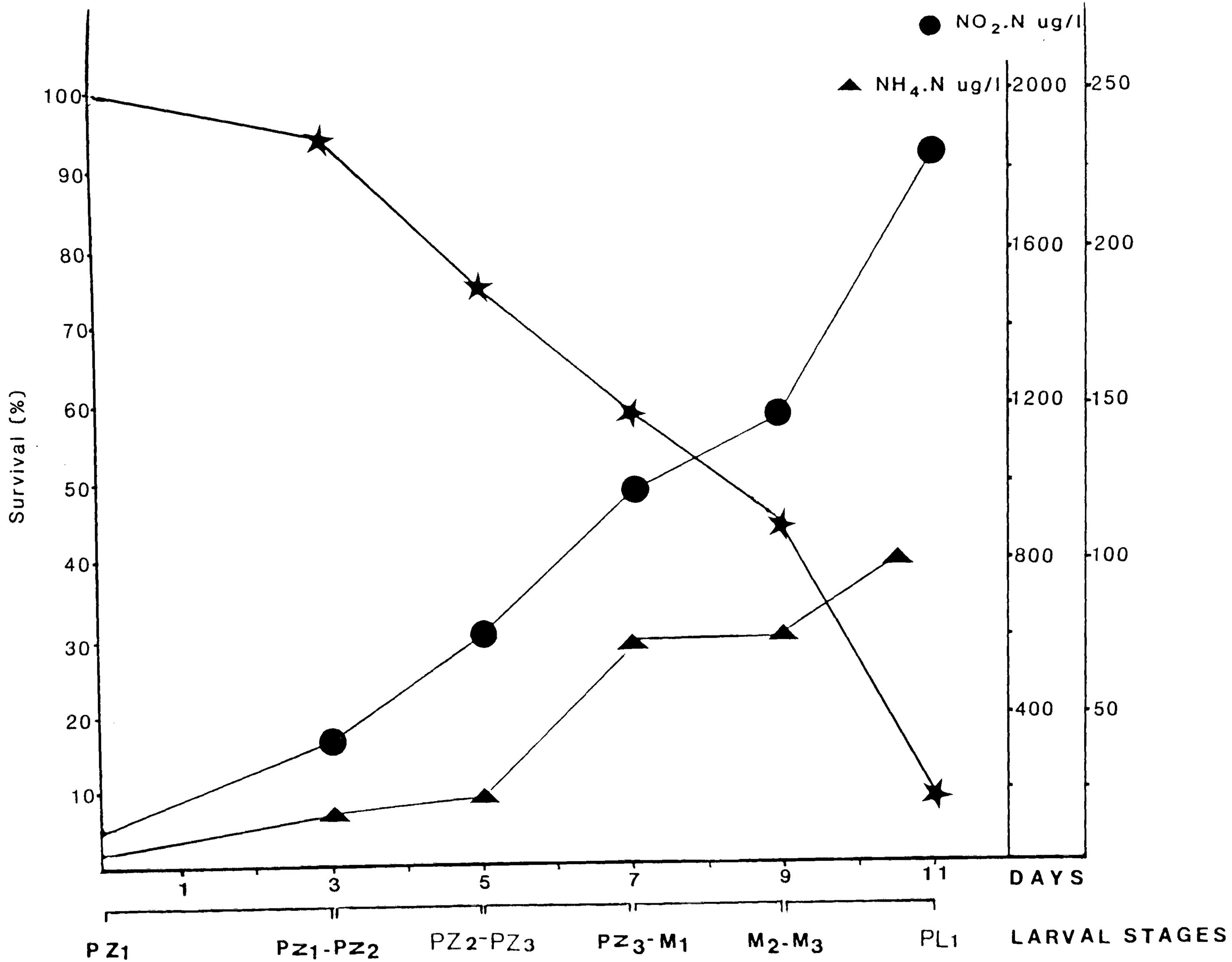

NO_2.N ug/l
NH_4.N ug/l
Survival (%)
100
90
80
70
60
50
40
30
20
10
350
300
250
200
150
100
50
2400
2000
1600
1200
800
400
1
3
5
7
9
11
DAYS
LARVAL STAGES
PZ1
PZ1
PZ2
PZ3
M1

Figure 14. Percentage survival of P. monodon larvae fed on a control diet of 20 cells of Rhodomonas and Tetraselmis μl^{-1} with 5. Artemia nauplii ml^{-1} from mysis (M1). Ammonia (▲) and nitrite (●) levels are measured in unchanged culture water throughout the experiment.


NO_2.N ug/l
NH_4.N ug/l
Survival (%)
100
90
80
70
60
50
40
30
20
10
2000
1600
1200
800
400
250
200
150
100
50
1
3
5
7
9
11
DAYS
PZ1
PZ1-PZ2
PZ2-PZ3
PZ3-M1
M2-M3
PL1
LARVAL STAGES

SECTION 4

Effect of diet composition on *P. monodon* larvae

Part of this section was presented at the Forum on New Technologies in Aquaculture, Kuala Lumpur, Malaysia. August 1989.

Title : **Advances in Penaeid Larval Feed Technology.**

Authors : S. Amjad, D.A. Jones and K. Chitravedivelu.

Proceedings of the Forum on New Technologies in Aquaculture. Jointly organised by the Malaysian Fisheries Society and the Ministry of Science, Technology and Environment in conjunction with National Science and Technology Congress (in Press).

INTRODUCTION

One of the most important factors in intensive aquaculture is good nutrition. Increased production of cultured prawn larvae in an intensified culture system is dependent upon nutritionally effective and acceptable diet for each of the different developmental stages. New (1976; 1980) presented an extensive bibliography, directed toward understanding the basic requirements of penaeid nutrition but found that specific information concerning essential nutritional requirements for penaeid larvae is limited. With intensification of penaeid larval culture the requirement for a formulated, supplementary or complete, feed is becoming paramount. The major components of formulated diets are, protein, lipid, carbohydrate, fibre, minerals and vitamins.

Dietary proteins are essential nutrients for growth and maintenance of larval and adult prawns. Proteins act as an energy source as well as contributing to tissue construction. Prawns cannot synthesize proteins from simple inorganic material, but rely on ingesting them through diet. The optimum dietary level of protein in diet which produces maximum growth varies in different species of juvenile and adult prawns, but ranges between 30 to 60% (New, 1976). Several groups of workers have reported on optimum protein levels for adult and juvenile *P. monodon*, 46% (Lee, 1971), 40% (Aquacop, 1977; Khannapa, 1977), 35% (Bages and Solonane, 1981). Growth response to different protein levels in diet will also vary according to the protein sources used. Growth is related to

the contents of essential amino acids and the nutritive value of proteins used (Deshimaru and Shigueno, 1972; Deshimaru, 1982; Hew and Cuzon, 1982). Proteins are composed mostly of amino acids linked with peptide bonds and cross linked between chains with sulphydral and hydrogen bonds. Prawns have been shown to require ten amino acids i.e. arginine, methionine, valine, threonine, isoleucine, leucine, lysine, histidine, phenylalanine and tryptophan (Kanazawa and Teshima, 1981). Deshimaru (1982) also showed that diets containing only amino acids instead of protein gave a very poor growth and high mortality in feeding trials with *P. japonicus* juveniles.

Dietary lipids have also been shown to play an important role in prawn nutrition, not only as an energy source, but also as an essential dietary component. Some fatty acids are essential and cannot be synthesized in sufficient amounts by the animal itself. Kanazawa et al., (1977a) showed that the addition of 20:5ω3 and 22:6ω3 fatty acids to the diet of *P. japonicus*, increased survival and weight gain, compared to diets high in 18:2ω6 and 18:3ω3 fatty acids. Jones et al., (1979a), using microcapsules containing ^{14}C-palmitic acid, showed that *P. japonicus* larvae can synthesize 20:5ω3 and 22:6ω3 from 18:3ω3 but only in small amounts. Additionally ω3 fatty acids assist in transportation of other lipids such as cholesterol, activate several specific enzymes and regulate vital processes by prostaglandins (Castell, 1970). Jones et al., (1979b) have pointed out the necessity of ω3 fatty acid for growth of zoeal and juvenile stages of prawns. New, (1987) also suggests that lipids are important factors in palatability

of feeds.

Carbohydrate utilization by penaeids in the form of polysaccharides has been demonstrated by Sick and Andrew, (1973); Alva and Pascul (1987). Prawns vary in their ability to digest carbohydrates effectively. Complex carbohydrates are more readily utilized by prawns than simple sugars (New, 1987).

Mineral elements are important aspects of prawn metabolism. They provide strength and rigidity to the exoskeleton, which is rich in minerals and is lost during moulting. In body fluids minerals are involved mainly with the maintenance of osmotic equilibrium with the aquatic environment, and in the nervous and endocrine system. They are also involved with metabolic processes concerned with energy transport (New, 1987).

Vitamins are complex organic compounds required in trace amounts for normal growth, reproduction and general metabolism. The effect of vitamins on growth and survival of *P. japonicus* larvae has been demonstrated by Kanazawa (1982). Vitamin C deficiency causes blackening of cuticle, hind gut wall and gills in penaeids (Lightner et al., 1977).

Despite recent advances toward understanding the basic nutritional requirement of penaeid prawns, much less emphasis has been given to determining specific requirements essential for penaeid larval growth and development. Specific information concerning essential nutrient requirements of individual species still remains to be elucidated (Jones et al., 1984).

This section evaluates *P. monodon* larval development on a

series of nutritionally improved microencapsulated feeds as total replacements for live feeds. Larval growth and survival were evaluated using six different protein sources. Protein components of the diet were, mussel, cod-roe, beef liver, cooked egg albumin, casein, soya protein and a combination of cooked mussel and soya protein. To determine optimum levels of lipids required by larval stage, lipids high in ω3 and ω6 were included in microcapsules at 0, 5, 10 and 15% level. Vitamins, trace minerals and minerals plus vitamin supplements were added to microcapsules, larval performance was compared in feed trials to diets which did not contain vitamin and minerals. This study also compares growth and survival of *P. monodon* larvae on another series of microencapsulated feeds as total replacements supplemented by extracts taken from live feed. When these are fed together with nutritionally improved Frippak microcapsules, both larval growth and survival are not significantly different from live algal and *Artemia* fed controls.

MATERIALS AND METHODS

A series of larval feed trial experiments was conducted to evaluate the effectiveness of nutritionally improved microencapsulated feeds, developed for penaeid larvae as a total replacement to all live conventional feeds. Table 1 lists microencapsulated feeds formulated from six different protein sources at 60% level (soya, mussel, beef liver, casein, cod-roe, cooked egg albumin and a combination of soya protein and cooked mussel). Lipid (marine and vegetable oils) levels included in diet were 0, 5, 10 and 15%. Trace minerals (copper, zinc and manganese as sulphates) and vitamin mixes of biotin, folic acid, riboflavin, pantothenic acid, thiamine, pyridoxine and niacin were added to encapsulated feeds. The exact formulation of microcapsule manufacture is proprietory information (British Patent No's 79437454 and 2103568). In another feed trial, nutritionally improved microcapsules (table 2) were evaluated for their performance on larval development, fed as total replacement, cofeed with 10 cells μl^{-1} algae and supplemented by homogenised algal additives taken from live feeds.

Homogenised Algal Additives

Microalgae *T. chuii* and *R. baltica* maintained in the laboratory were harvested during the late exponential growth phase. Harvested algae were concentrated by centrifugation in Model MSE cool spin rotor. *T. chuii* was centrifuged at 3000 rpm for 30 min, and *R. baltica* at 1000 rpm for 10 min. The

supernatant was decanted and algal concentrate resuspended in 50 ml autoclaved seawater. 25 µl of resuspended algae was used to estimate cell concentration using Coulter Counter Model Z_B.

Algal concentrates were homogenised separately in homoginser Model K43-TRI-R/STIR-R. *R. baltica* and *T. chuii* were homogenised at 10,000 rpm for 20 and 30 min respectively. The homogenised algae was further centrifuged in centrifuge Model MSE/Superspeed 65 at 40,000 rpm for 40 min to separate the soluble from the insoluble component. The insoluble component was resuspended in 50 ml autoclaved seawater for subsequent treatment and use, unused portions were kept frozen at -18°C. Prior to feeding homogenised algae was thawed, an amount equivalent to 10 cells μl^{-1} was provided as a supplement to encapsulated feeds.

FEED TRIAL PROCEDURE

P. monodon larvae obtained from a single spawning were fed on microencapsulated feed (table 1) as a total replacement to all live feeds in replicated trials, growth and percentage survival of larvae were recorded to evaluate diet performance.

The larvae at PZ1 stage were stocked at 100 litre $^{-1}$ in 2-litre round bottom flasks using U/V sterilised seawater filtered through 0.2 µm filter, salinity of culture water was 32‰ and was maintained at 28 ± 1°C. Gentle aeration was supplied through a glass tubing at the bottom of the flask at the rate of 2-3 air bubbles sec^{-1} which provided not only oxygen but also kept the microcapsules in suspension.

Rehydrated microencapsulated feeds were given to larvae as equal rations 4 times a day. 50% of the water in the culture flask was exchanged daily to renew water quality. Controls were fed on live feeds of microalgae (20 $c\mu l^{-1}$ *R. baltica* and 20 $c\mu l^{-1}$ *T. chuii*) from PZ1 - PZ3, and newly hatched *Artemia* nauplii at 5 ml^{-1} from M1 to PL1. Percentage survival and growth of larvae was monitored from each treatment every other day.

In order to determine whether differences amongst diet treatments and final mean survival and growth of larvae were statistically significant, a test of homogeneity of variance (Bartletts test) and an analysis of variance (One-way ANOVA) followed by Tukeys multiple pairwise comparison at 5% level of significance were performed.

RESULTS

LARVAL GROWTH AND SURVIVAL ON DIFFERENT PROTEIN SOURCES

In a feed trial with *P. monodon* larvae (PZ1 - PL1), microencapsulated feed containing different protein sources fed as total replacement to all live feeds, gave variable responses depending on the protein source incorporated into the diet. All microcapsules contained 60% protein as a dietary component. Larval growth and survival responses to each of the different protein sources (table 1) has been evaluated in replicated feed trials.

Larval Growth (PZ1 - PL1).

Larval Growth response to different protein sources added to microcapsule diets ranged from 4.73mm to 5.10mm (table 3 and fig. 1). One-way analysis of variance (table 3a) shows that larval growths were highly significant ($P < 0.05$) with different protein sources. Multiple pairwise comparison (table 3b) shows that comparatively good larval growth was obtained on live control diets (5.1mm) which differed significantly from all other microcapsules used in the feed trial. Larval growth on microcapsule feed CAR 409 + CD 396 (cooked egg albumin), was significantly better than larval growth obtained on microcapsules CAR 399 + CD 391, containing proteins from mussels and microcapsule CAR 407 + CD 392 which contained cod-roe as a protein source. Larval growth on microcapsules containing cooked egg albumin (CAR 409 + CD 396) was not

significantly different ($P < 0.05$) from growth obtained on microcapsules with casein (CAR 406 + CD 389), soya protein (CAR 335 + CD 315), beef liver (CAR 405 + CD 393) and soya protein + cooked mussel (CAR 408 + CD 397). Significantly lower larval growth was obtained with mussel and cod-roe proteins. Growth obtained on other protein sources (cooked egg albumin, soya protein, beef liver, soya protein + cooked mussel and casein) were not significantly different (table 3b).

Larval Survival (PZ1 - PL1)

Table 4 shows percentage survival of *P. monodon* larvae on different protein sources, which ranged from 21 to 84%. Analysis of variance (table 4a) shows that larval survival on microcapsules containing proteins from different sources was significant ($P < 0.05$). Multiple pairwise comparisons (table 4b) shows that larval survival on live feed controls was significantly better than survivals obtained on microencapsulated feeds CAR 407 + CD 392 (cod-roe protein), CAR 399 + CD 391 (mussel protein), CAR 409 + CD 396 (cooked egg albumin) and CAR 406 + CD 389 (casein), but larval survival on live feed controls do not differ significantly from microencapsulated feeds CAR 405 + CD 393 (beef liver), CAR 335 + CD 315 (soya protein) and with CAR 408 + CD 397 (a combination of soya protein and cooked mussel). Percentage survival on microcapsules feed CAR 408 + CD 397 was significantly better from feeds CAR 407 + CD 392 (cod-roe) and CAR 399 + CD 391 (mussel protein), but percent survival did not differ significantly from other microencapsulated feeds.

Survival obtained on feed CAR 335 + CD 315 (soya protein) differed significantly from survival obtained on CAR 407 + CD 392 (cod-roe proteins). Larval survival on other protein sources in microcapsules do not differ significantly at P = 0.05 level of significance.

EFFECT OF LIPID LEVEL ON LARVAL GROWTH AND SURVIVAL

Lipids from vegetable and marine animal sources incorporated into microcapsules at four levels (0, 5, 10 and 15%) gave variable larval growth and survival (Fig. 2a, b) responses in a feed trial. Highest larval growth (5.1mm) and survival (86%) from PZ1 to PL1 was obtained on live feed controls followed by microcapsules containing 5% lipid. Inclusion of lipid at levels higher than 5% gave comparatively inferior larval growth and survival.

Larval Growth (PZ1 - PL1)

Table 5 shows that highest larval growth from PZ1 to PL1 was obtained on live feeds (40 cells μl^{-1} *T. chuii* + *R. baltica* and 5 *Artemia*. ml^{-1}.) Larval growth on microcapsule with 5% lipid gave the next best growth (4.91mm). Larval growth on microcapsules without lipid was 4.73mm. Larval growth on microcapsules with lipid levels of 10% gave inferior growth (4.67mm) and when fed microcapsules with a higher lipid content (15%) larvae did not metamorphose into subsequent developmental stages. Larval mortality was high (78%) on day 5 at PZ2 and PZ3 stages.

One-way analysis of variance (table 5a) between larval growth and lipid levels in feeds was significant ($P < 0.05$). Multiple pairwise comparison (table 5b) shows that larval growth obtained on the live control diet was significantly better than growth obtained on feeds with lipid levels of 15% (CAR 378 + CD 379), 10% (CAR 376 + CD 377), and 0% (CAR 372 + CD 368) but larval growth was not significantly different from growth obtained on 5% lipid levels (CAR 374 + CD 375). Comparatively larval growth on 5% lipid was significantly better than growth obtained on feeds with 15% and 10%. But larval growth on 5% lipid was not significantly different from growth measured on feeds without any lipid content. Larval growth on feeds with 10% lipid was significantly better than growth obtained on feeds with 15% lipid.

Larval Survival (PZ1 - PL1)

Table 6 shows that highest mean percentage larval survival was obtained on live control feeds (86%), followed by larvae fed on microcapsule feed (CAR 374 + CD 375) with 5% lipid (60%). Survival to PL1 on microcapsule feed CAR 372 + CD 368 without lipids was 25%, survival on feed containing 10% lipid (CAR 376 + CD 377) was 20% to PL1 stage. Larvae on microcapsule feed CAR 378 + CD 379, containing 15% lipid collapsed on day 7.

One-way analysis of variance (table 6a) shows that larval survival when fed microcapsules with different lipid levels gave a significant ($P < 0.05$) response. Multiple pairwise comparison (table 6b) shows that larval survival on live feed

control diets was significantly better than survival obtained on microcapsules with 15%, 10% and 0% lipid levels, but do not differ significantly to survival obtained on microcapsules with 5% lipid. Larval survival on microcapsules with 5% lipids were significantly better than survival obtained on microcapsules with 15% and 10% lipid levels, but survival do not differ significantly with microcapsule feed without any lipid contents. Larval survival was not significantly different on microcapsules CAR 372 + CD 368, CAR 378 + CD 379 and CAR 376 + CD377.

EFFECT OF VITAMINS AND TRACE MINERALS ON LARVAL GROWTH AND SURVIVAL

Additive vitamin mixes (13.9%) and trace mineral, copper sulphate (100ppm), zinc sulphate (100ppm) and manganese sulphate (50ppm) included in microcapsule feeds (table 1) tested in replicated feed trials, gave variable larval growth and survival from PZ1 to PL1 (Fig. 3a, b).

Larval Growth (PZ1 - PL1).

Table 7 shows that highest larval growth (4.99mm) from PZ1 to PL1 was obtained on live feed controls, followed by growth (4.97mm) on microcapsules feed CAR 320 + CD 315 (vitamin + mineral). Larval growth on CAR 319 + CD 314 (vitamin only) was 4.80mm. Larval growth obtained on CAR 321 + CD 316 (minerals only) were 4.1mm and microcapsule feeds CAR 271 + CD 272 (without vitamin and minerals) supported larval growth (3.67mm)

to M3/intermediate PL stages.

One-way analysis of variance (table 7a) shows that larval growth responses were highly significant ($P < 0.001$) on different treatments of vitamins and minerals in microencapsulated feeds. Multiple pairwise comparison (table 7b) shows that larval growth obtained in live feed controls was significantly better than growth obtained on feeds devoid of vitamins and minerals (CAR 271 + CD 272), and with microencapsulated feed (CAR 321 + CD 316) with mineral additives only. Larval growth on live feed controls however do not differ significantly from growth obtained on microcapsule (CAR 319 + CD 314) with vitamins and with microcapsule feed (CD 320 + CD 315) containing vitamin mix and minerals. Larval growth obtained on feed with vitamins and minerals differed significantly from growth obtained on feeds with no added vitamins and minerals and with feeds containing only a mineral source. Larval growth was not significantly different from microencapsulated feed (CAR 319 + CD 314) with only vitamins as an added source. Larval growth on microencapsulated feed with vitamins only was significantly better than feeds CAR 271 + CD 272 (with no vitamins and minerals) and CAR 321 + CD 316 (minerals only). Larval growth was significantly better on feeds with minerals compared with feed devoid of vitamins and minerals.

Larval Survival (PZ1 - PL1).

Table 8 shows that larval survival was highest (90%) on live feed controls, followed by survival (65.5%) on

microencapsulated feed CAR 320 + CD 315 (vitamins and minerals). 41% survival was obtained with added minerals (CAR 321 + CD 316). 32% larvae survived to PL stages on feeds containing vitamins only (CAR 319 + CD 314) and 26% survived on feeds with no vitamins and mineral additives (CAR 271 + CD 272).

Analysis of variance (table 8a) shows that larval survival in response to microencapsulated feeds with vitamins and minerals differed significantly ($P < 0.05$). Multiple pairwise comparison (table 8b) shows that larval survival on live feed controls was significantly different from microencapsulated feeds CAR 271 + CD 272 (no vitamins and minerals), CAR 319 + CD 314 (vitamin only), and CAR 321 + CD 316 (minerals only). Larval survival on live feeds do not differ significantly from survival obtained on microencapsulated feed CAR 320 + CD 315 (vitamin + minerals). Larval survival obtained on feeds CAR 320 + CD 315 were significantly better than feeds with no vitamins and minerals. Larval survival was also not significantly different from survival obtained on microencapsulated feed CAR 319 + CD 314 (vitamin supplements). No significant difference in larval survival was obtained with microencapsulated feed CAR 321 + CD 316 (minerals), compared to CAR 271 + CD 272 (devoid of vitamins and minerals). Microencapsulated feed CAR 271 + CD 272 (no additives) was able to support lower survival (26%) to PL stages.

FROZEN AND HOMOGENISED ALGAE AS SUPPLEMENT TO ENCAPSULATED DIET

Larval Development in Presence of Algal Additives.

Improvements in the dietary composition of microencapsulated feeds, tested as total replacement to live feeds, produced better larval growth and survival in replicated feed trials. However, larval development on these improved microcapsule feeds, were still not comparable to live feed controls. Thus extracts taken from live algae were used to supplement microencapsulated feeds. This combination feed gave larval growth and survival similar to that achieved on live feed controls (Fig. 4a, b).

Larval Growth (PZ1 - PL1).

Table 9 shows that highest larval growth (5.10mm) was achieved on encapsulated feed + 10 cells μl^{-1} of algae (*T. chuii* + *R. baltica* 1:1), followed by larval growth of 4.99mm on live feed controls. Larval growth of 4.92mm to PL1 stage was obtained on a combination of microencapsulated feed and frozen homogenised algae. Larval growth on encapsulated feeds used as total replacements was 4.77mm to PL1 stage of development.

One-way analysis of variance (table 9a) shows that larval growth differed significantly ($P < 0.05$) with different feed treatments. Multiple pairwise comparison (table 9b) shows that encapsulated feed 554 + 10 cells μl^{-1} algae gave significantly better larval growth compared to growth obtained on encapsulated feed 554 used as total replacement. Larval growth

however was not significantly different to growth achieved on encapsulated feed 554 + homogenised frozen algae and live feed controls. Larval growth on live feed controls was significantly better than growth obtained on 100% replacement of live feeds by encapsulated feed 554, but larval growth to PL stages obtained on live feeds, did not differ significantly from encapsulated feed 554 + frozen homogenised algae.

Larval Survival (PZ1 - PL1).

Table 10 shows that larval survival to PL stage on different feed treatments was high and ranged from 74.5 to 93.5%. Highest survival (93.5%) was obtained on live feed controls, followed by 93% survival on encapsulated feed 554 + homogenised frozen algae. 80.5% larval survival to PL1 stage was obtained on 100% replacement encapsulated feed 554. Survival on encapsulated feed 554 + 10 cells μl^{-1} algae was 74.5% to the PL1 stage.

One-way analysis of variance (table 10a) shows that larval survival to the PL stage does not differ significantly ($P < 0.05$) with different feed treatments.

DISCUSSION

Amongst the six protein sources evaluated, for larval growth and survival in a feed trial, highest survival (61%) was obtained on a microencapsulated feed containing a protein combination of soya + cooked mussel protein. Larval growth to PL1 stage on the same diet was 4.90mm. Percentage survival on the soya protein component alone in the microcapsule was 59% and larval growth was 4.94mm. Microcapsules containing beef liver as a protein source supported 52% survival to PL1. Mean larval growth was 4.93mm at PL1 stage. Percentage survival on microcapsules with casein as dietary protein was 47.5%, while larval growth was 4.90mm to PL1 stage. *P. monodon* larval growth on cooked egg albumin as a dietary protein source in microcapsule was higher (4.96mm), but larval survival on the same diet was relatively low (35%). Proteins incorporated from mussel tissues gave 25.5% survival and 4.73mm larval growth to the PL1 stage. Larval survival (21%) and growth (4.75mm) obtained on microcapsules with protein sources derived from cod-roe were relatively poor.

Variability in growth response to protein sources is attributed to the nutritive value of dietary proteins. Proteins differ in the relative proportions of the amino acids they contain. Amino acids considered essential for development are methionine, arginine, threonine, tryptophan, histidine, isoleucine, leucine, lysine, valine and phenylalanine (Cowey and Foster, 1971; Shewbart et al., 1972; Kanazawa and Teshima, 1981).

A combination of protein soya + cooked mussel supported highest larval survival. Soya protein on its own also supported a comparatively higher survival and larval growth. Akiyama (1988) showed that processed soybean protein was not only palatable to the marine shrimps *P. monodon, P. japonicus* and *P. vannamei* but protein digestibility was also higher than for other marine animal protein sources. Egg albumin supported good larval growth but was ineffective in sustaining higher survival to the PL1 stage. Deshimaru (1982) found egg protein deficient in lysine and arginine. Casein also supported better larval growth to the PL stage and larval survival (47.5%) to PL1 stage was better than that achieved on egg albumin (35%). Teshima et al., (1986) also obtained better survival with *P. japonicus* larvae on diets containing casein than with egg albumin. Poor larval growth and survival on meat meals (beef liver) may be due to the low levels of isoleucine, methionine and cystine present (New, 1987). Prior to feed processing, cod-roe and mussel protein contain all the essential amino acids in sufficient quantities (Kanazawa et al., 1970). During feed processing the free α-amino group of lysine becomes bound to other molecules, rendering it unavailable to the consumer animal (New, 1987). Amino acids are used by the tissues to synthesize new proteins. Inadequate proteins in the diet results in a reduction of growth, due to withdrawal of proteins from tissues to maintain the vital functions (Akiyama and Dominy, 1989). Sub-optimal larval growth therefore occurs due to imbalance or deficient essential amino acid in the diets ingested by the larvae.

Effects of lipids on growth and survival of *P. monodon* larvae shows that inclusion of 5% lipid levels to microencapsulated feeds gave the highest larval growth (4.91mm) and survival (60%). Lipids incorporated at the 10% level to microcapsules gave reduced larval growth (4.67mm) and survival (20%). Microencapsulated feed containing 15% lipid did not support larval development to PL stage. The larvae suffered mortality before reaching the mysis stage. Aquacop (1978) demonstrated that inclusion of lipids at 7% to the diet of *P. merguiensis* significantly improved growth and survival. In general, Akiyama and Dominy (1989) have shown that decreased growth and increased mortalities are associated with lipid levels exceeding 10% in diets.

It has been demonstrated that prawn larvae have a requirement for ω3 and ω6 essential fatty acids. Jones et al., (1979b) and Teshima and Kanazawa (1984) pointed out that ω3 - HUFA is essential for growth and survival of the larval stages of *P. japonicus*. Generally, plant lipids are high in 18:2ω6 (linoleic fatty acid) and 18:3ω3 (linolenic fatty acid), while lipids from marine animal sources are high in 20:5ω3 (eicosapentaenoic fatty acid) and 22:6ω3 (decosahexaenoic fatty acid).

Dietary lipids serve as a carrier of fat soluble vitamins and provide other compounds, such as sterols and phospholipids, which are essential for the normal metabolic functions of prawn larvae and adults. Phospholipids and sterols are important structural components of cells and organelles. Phospholipids help in maintaining the fluid and flexible nature of membranes.

Sterols are important in the synthesis of steroid hormones and the hormone-like prostaglandins.

Present trial shows that *P. monodon* larvae requires 5% lipid (from vegetable and marine sources) in the diet for optimum growth and survival to postlarval stages. Inclusion of lipid in feeds at levels of 10% and higher resulted in decreased larval growth and survival.

Effects of supplementary vitamin premix and minerals on larval growth and survival shows that *P. monodon* larvae, require both vitamins and minerals for optimum development. Growth and survival on microencapsulated (CAR 271 + CD 272, vitamin and mineral free) were relatively poor (3.67mm and 26% respectively) compared to microencapsulated feed containing either vitamin (growth 4.80mm and survival of 32%), trace minerals (growth 4.1mm and 41% survival) or vitamin plus minerals (larval growth 4.97mm and 65.5% survival). Kanazawa (1982) demonstrated that *P. japonicus* larvae fed on vitamin-free diets did not reach the postlarval stage suffering 100% mortality in the mysis stage. This author further demonstrated, that addition of vitamin C to a squid based diet for juvenile *P. japonicus* accelerated the growth rate. Excess of vitamin C however, inhibited growth rates. Sedgwick (1980) found that addition of minerals to vitamin-enriched diet (freeze-dried *Mytilus edulis*) improved the growth rate of *P. merguiensis*. Lightner et al., (1977) have found that *P. californiensis* and *P. stylirostris* sometimes show an abnormal symptom, named "black death", with characteristic blackening of the esophagus wall, cuticle, gastic wall, hind

gut wall and gills. "Black death" has been recognised as a symptom of vitamin C deficiency (Margarelli et al., 1979). More recently, Catacutan and Cruz (1989), showed that absence of ascorbic acid (vitamin C) from the diet of *P. monodon* juveniles resulted in poor weight gains and the most severe mid-gut pathology with necrosis and detachment of the epithelial cells.

Larvae may absorb minerals from seawater to some extent, but a dietary source of certain minerals is required due to repeated loss of certain minerals during moulting. Minerals are essential components of enzymes, blood and pigments. They are involved in the metabolic processes concerned with energy transport. Copper is an integral component of oxygen transporting protein hemocyanin, it is also a constituent of enzyme such as cytochrome oxydase. Zinc is a constituent of enzyme and is required for the activity of enzymes such as arginase and aminopeptidase. Manganese requirement in trace amounts is for enzyme activity e.g. an RNA polymerase (Jorgensen, 1977). Deficiency of zinc and manganese in common carps causes slow growth, there is a loss of appetite, high mortality and erosion of the skin and fins (Ogino and Yang, 1980).

Inclusion of minerals and vitamin premix (13.9%) to microencapsulated feed, in the present feed trial, was in excess to ensure that acceptable levels remained in the feed. Water soluble vitamins may be lost as a result of nutrient leach loss in culture water (Goldblatt et al., 1979). Vitamins and minerals are destroyed during feed processing and storage

(New, 1987). The oxidation of vitamins is affected by heat, moisture, pH, the presence of certain minerals, and by lipid oxidation (Stickney et al., 1983).

Improvement in the dietary constituents of microencapsulated feed enhanced larval growth and survival of *P. monodon* in feed trials. However, although growth and survival were significantly better than that achieved on previous Frippak feeds used as 100% replacements, growth was usually less than that achieved on live algae and *Artemia* (Jones and Kurmaly, 1987). Whilst survival on a 100% replacement diet is not significantly different from live feed controls, growth is significantly lower. Present work has shown that if live algae at 10 cells μl^{-1} is added, growth is similar to that achieved on live feeds. Similar growth has now been achieved if preserved (frozen) extracts from live algae are supplied at a concentration equivalent to 10 cells μl^{-1} together with Frippak diets (Fig. 4).

Despite improvements in the dietary constituents of microencapsulated feeds by using different protein sources, inclusion of lipid at four different levels, addition of vitamin and mineral, larval growth and percentage survival has been lower (6 and 13% respectively) than that achieved on live feed controls. Inclusion of live algae fed at 10 cells μl^{-1} together with microencapsulated feed gives significantly better larval growth and survival. Larval development on 10 cells μl^{-1} of live algae on its own are unable to sustain larval growth. Protozoeal stages do not metamorphose beyond PZ2 development stage. The caloric energy of 10 algal cells

calculated in Section 1 is 0.124 Joules. This energy is insufficient for metabolic processes and protozoeal development to successive stages. Protozoea (PZ1) require 0.746 J. $larva^{-1}.day^{-1}$ for development and metabolic processes (Kurmaly et al., 1989b).

Algal homogenate equivalent to 10 cells μl^{-1} supplemented by soaking microcapsules (8 and 16 $mg.l^{-1}$) for 12h gives larval growth and survival to PL stage similar to that achieved on live feed controls (table 9 and 10). Larval development similar to that achieved on live feed on artificial diet (microencapsulated feed) supplemented by preserved (frozen) homogenised algae is reported for the first time (Amjad et al., 1989). The freezing procedure (-18°C) for preservation of algal homogenate does not impair or denature the growth/survival promoting substance present in homogenated algae. This substance could be a peptide, which increases the digestive efficiency of larvae, and enhances growth rate.

Further feed trials were set up in an attempt to evaluate the effectiveness of microencapsulated feeds to rear *P.monodon* larvae through metamorphosis from protozoea stage 1 to nursery stage prawns solely on artificial diet. The response of nursery stage prawns to other commercially available diets is also evaluated in section 5.

Table 1 : Nutritionally improved microencapsulated feed used in larval feed trials with *P. monodon* as total replacement for all live feeds

Microencapsulated feed		Diet component included	Ration level PZ1-PZ3 $mg.l^{-1}.d^{-1}$	Ration level M1-PL1 $mg.l^{-1}.d^{-1}$	Cofeed
PZ1-PZ3	M1-PL1				
Protein feed trial					
CAR 335	CD 315	Soya protein	8 mg.	16 mg.	-
CAR 399	CD 391	Mussel protein	8 mg.	16 mg.	-
CAR 405	CD 393	Beef liver	8 mg.	16 mg.	-
CAR 406	CD 389	Casein	8 mg.	16 mg.	-
CAR 407	CD 392	Cod roe	8 mg.	16 mg.	-
CAR 408	CD 397	Soya protein/ cooked mussel	8 mg.	16 mg.	-
CAR 409	CD 396	Cooked egg albumin	8 mg.	16 mg.	-
Lipid feed trial					
CAR 372	CD 368	No lipid	8 mg.	16 mg.	-
CAR 374	CD375	5% lipid	8 mg.	16 mg.	-
CAR 376	CD 377	10% lipid	8 mg.	16 mg.	-
CAR 378	CD 379	15% lipid	8 mg.	16 mg.	-
Vitamin and Minerals feed trial					
CAR 319	CD 314	Vitamins only	8 mg.	16 mg.	-
CAR 321	CD 316	Minerals only	8 mg.	16 mg.	-
CAR 320	CD 315	Minerals+Vitamins	8 mg.	16 mg.	-
CAR 271	CD 272	No Mineral/ Vitamin	8 mg.	16 mg.	-
Controls :					
Algae					
T.chuii	*Artemia*	-	$20c\mu l^{-1}d^{-1}$	$5ml^{-1}day^{-1}$	
R.baltica		-	$20c\mu l^{-1}d^{-1}$		

Table 2 : Additives supplied with microencapsulated feeds in *P. monodon* larval feed trial

Microencapsulated feed	Ration level PZ1-PZ3	Ration level M1-PL1	Additives
Encapsulated feed 554	8mg.l^{-1}.d^{-1}	16mg.l^{-1}.d^{-1}	Homogenised frozen algae equivalent to 10 cells μl^{-1}
Encapsulated feed 554	8mg.l^{-1}.d^{-1}	16mg.l^{-1}.d^{-1}	10 cell μl^{-1} microalgae
Encapsulated feed 554	8mg.l^{-1}.d^{-1}	16mg.l^{-1}.d^{-1}	Total replacement
Control	25cμl^{-1} *T. chuii*	5.ml^{-1}.d^{-1}	Live microalgae
	25cμl^{-1} *R. baltica*	*Artemia* nauplii	Live Instar 1 *Artemia* nauplii

Table 3 : *P. monodon* larval growth (PZ1-PL1) in response to protein sources incorporated in microencapsulated feeds, as 100% replacement to live feeds. Feed trial replicated, ± S.D.

FEED GROUP	MICROENCAPSULATED FEED PZ1-PZ3	M1-PL1	PROTEIN SOURCE	MEAN GROWTH(mm)	STANDARD DEVIATION
1.	CAR399 +	CD391	Mussel protein	4.73	.2052
2.	CAR407 +	CD392	Cod-roe	4.75	.1231
3.	CAR405 +	CD393	Beef liver	4.93	.2155
4.	CAR409 +	CD396	Egg albumin	4.96	.1129
5.	CAR406 +	CD389	Casein	4.90	.1026
6.	CAR335 +	CD315	Soya protein	4.94	.0256
7.	CAR408 +	CD397	Soya protein + cooked mussel protein	4.90	.1077
8.	CONTROL Live feeds		Algae + *Artemia*	5.10	.1231

Table 3a: One-way ANOVA, larval growth against protein sources in microencapsulated feeds

ANALYSIS OF VARIANCE

SOURCE	D.F.	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB
BETWEEN GROUPS	7	1.9580	.2797	14.4959	.001
WITHIN GROUPS	152	2.9330	.0193		
TOTAL	159	4.8910			

Tests for Homogeneity of Variances
Cochrans C = Max. Variance/Sum (Variances) = .3007, P = .001
Bartlett-Box F = 9.964 , P = .001

Table 3b: Tukeys pairwise comparison of mean growth obtained on different protein sources

Mean	Feed Group	FEED GROUPS 1	2	5	7	3	6	4	8
4.73	1								
4.75	2	ns							
4.90	5	*	*						
4.90	7	*	*	ns					
4.93	3	*	*	ns	ns				
4.94	6	*	*	ns	ns	ns			
4.96	4	*	*	ns	ns	ns	ns		
5.10	8	*	*	*	*	*	*	*	

(*) Denotes pairs of groups significantly different at the 0.050 level
ns indicates a non-significant result

Table 4 : Percentage survival of *P. monodon* larvae in response to protein sources in microencapsulated feeds

FEED GROUPS	MICROENCAPSULATED FEED PZ1-PZ3 M1-PL1	PROTEIN SOURCE	MEAN SURVIVAL(%)	STANDARD DEVIATION
1.	CAR399 + CD391	Mussel protein	25.50	5.6569
2.	CAR407 + CD392	Cod-roe	21.00	8.4853
3.	CAR405 + CD393	Beef liver	52.00	11.3137
4.	CAR409 + CD396	Egg albumin	35.00	7.0711
5.	CAR406 + CD389	Casein	47.50	7.0711
6.	CAR335 + CD315	Soya protein	59.00	11.3137
7.	CAR408 + CD397	Soya protein + mussel protein	61.00	9.8995
8.	CONTROL Live feed	Algae + *Artemia*	84.00	4.2426

Table 4a: One-way ANOVA, larval survival against protein sources in microencapsulated feeds

ANALYSIS OF VARIANCE

SOURCE	D.F.	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB
BETWEEN GROUPS	7	6012.7500	858.9643	11.9301	.0011
WITHIN GROUPS	8	576.0000	72.0000		
TOTAL	15	6588.7500			

Tests for Homogeneity of Variances
Cochrans C = Max. Variance/Sum (Variances) = .2222, P = 1.000
Bartlett-Box F = .140 , P = .995

Table 4b: Tukeys pairwise comparison of percentage survival obtained on different protein sources

Mean	Feed Group	FEED GROUPS 2	1	4	5	3	6	7	8
21.0000	2								
25.5000	1	ns							
35.0000	4	ns	ns						
47.5000	5	ns	ns	ns					
52.0000	3	ns	ns	ns	ns				
59.0000	6	*	ns	ns	ns	ns			
61.0000	7	*	*	ns	ns	ns	ns		
84.0000	8	*	*	*	*	ns	ns	ns	

(*) Denotes pairs of groups significantly different at the 0.050 level
ns indicates a non-significant result

Table 5 : *P. monodon* larval growths (PZ1-PL1) in response to lipid levels in microencapsulated feeds

FEED GROUPS	MICROENCAPSULATED FEED PZ1-PZ3 M1-PL1	LIPID LEVEL (%)	MEAN GROWTH(mm)	STANDARD DEVIATION
1.	CAR372 + CD368	0%	4.73	.1539
2.	CAR374 + CD375	5%	4.91	.3078
3.	CAR376 + CD377	10%	4.67	.2052
4.	CAR378 + CD379	15%	.00	
5.	CONTROL Live Feeds Algae + *Artemia*	-	5.10	.1129

Table 5a: One-way ANOVA, larval growth against lipid levels in microencapsulated feeds

ANALYSIS OF VARIANCE

SOURCE	D.F.	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB
BETWEEN GROUPS	4	25.5136	6.3784	147.2532	.001
WITHIN GROUPS	76	3.2920	.0433		
TOTAL	80	28.8056			

Tests for Homogeneity of Variances
Cochrans C = Max. Variance/Sum (Variances) = .5468, P = .001
Bartlett-Box F = 6.695 , P = .001

Table 5b: Tukeys pairwise comparison of mean larval growths obtained on different lipid levels

Mean	Feed Group	FEED GROUPS 4	3	1	2	5
.0000	4					
4.6700	3	*				
4.7300	1	*	ns			
4.9100	2	*	*	ns		
5.1000	5	*	*	*	ns	

(*) Denotes pairs of groups significantly different at the 0.050 level
ns indicates a non-significant result

Table 6 : Percentage survival of *P. monodon* larvae in response to lipid levels in microencapsulated feeds

FEED GROUPS	MICROENCAPSULATED FEED PZ1-PZ3	M1-PL1	LIPID LEVEL (%)	MEAN SURVIVAL(%)	STANDARD DEVIATION
1.	CAR372 +	CD368	0%	25.00	8.4853
2.	CAR374 +	CD375	5%	60.00	5.6569
3.	CAR376 +	CD377	10%	20.00	4.2426
4.	CAR378 +	CD379	15%	.00	
5.	CONTROL Live Feeds Algae + *Artemia*	-		86.00	9.8995

Table 6a: One-way ANOVA, larval survival against lipid levels in microencapsulated feeds

ANALYSIS OF VARIANCE

SOURCE	D.F.	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB
BETWEEN GROUPS	4	7828.2222	1957.0556	35.5828	.0022
WITHIN GROUPS	4	220.0000	55.0000		
TOTAL	8	8048.2222			

Tests for Homogeneity of Variances
Cochrans C = Max. Variance/Sum (Variances) = .4455, P = .874
Bartlett-Box F = .199 , P = .897

Table 6b: Tukeys pairwise comparison of larval survival obtained on different lipid levels

Mean	Feed Group	FEED GROUPS 4	3	1	2	5
.0000	4					
20.0000	3	ns				
25.0000	1	ns	ns			
60.0000	2	*	*	ns		
86.0000	5	*	*	*	ns	

(*) Denotes pairs of groups significantly different at the 0.050 level
ns indicates a non-significant result

Table 7 : *P. monodon* larval growth (PZ1-PL1) in response to vitamin and trace minerals in microencapsulated feeds

FEED GROUPS	MICROENCAPSULATED FEED PZ1-PZ3 M1-PL1	ADDITIVES	MEAN GROWTH(mm)	STANDARD DEVIATION
1.	CAR319 + CD314	Vitamins	4.80	.2360
2.	CAR321 + CD316	Minerals	4.10	.2565
3.	CAR320 + CD315	Vitamins+Minerals	4.97	.1026
4.	CAR271 + CD272	None	3.67	.5130
5.	CONTROL Live feeds Algae + *Artemia*	-	4.99	.1129

Table 7a: One-way ANOVA, larval growth against vitamins and minerals in microencapsulated feeds

ANALYSIS OF VARIANCE

SOURCE	D.F.	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB
BETWEEN GROUPS	4	27.9944	6.9986	85.7893	.001
WITHIN GROUPS	95	7.7500	.0816		
TOTAL	99	35.7444			

Tests for Homogeneity of Variances
Cochrans C = Max. Variance/Sum (Variances) = .6452, P = .001
Bartlett-Box F = 15.561 , P = .001

Table 7b: Tukeys pairwise comparison of mean larval growth obtained on vitamin and minerals

Mean	Feed Group	FEED GROUPS 4	2	1	3	5
3.6700	4					
4.1000	2	*				
4.8000	1	*	*			
4.9700	3	*	*	ns		
4.9900	5	*	*	ns	ns	

(*) Denotes pairs of groups significantly different at the 0.050 level
ns indicates a non-significant result

Table 8 : Percentage survival of *P. monodon* larvae in response to vitamin and trace minerals in microencapsulated feed

FEED GROUPS	MICROENCAPSULATED FEED PZ1-PZ3	M1-PL1	ADDITIVES	MEAN SURVIVAL(%)	STANDARD DEVIATION
1.	CAR319 +	CD314	Vitamins	32.00	11.3137
2.	CAR321 +	CD316	Minerals	41.00	8.4853
3.	CAR320 +	CD315	Vitamins+Minerals	65.50	10.6066
4.	CAR271 +	CD272	None	26.00	7.0711
5.	CONTROL Live feeds		-	90.00	7.0711
	Algae + *Artemia*				

Table 8a: One-way ANOVA, larval survival against vitamins and minerals in microencapsulated feeds

ANALYSIS OF VARIANCE

SOURCE	D.F.	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB
BETWEEN GROUPS	4	5634.400	1408.600	17.0739	.004
WITHIN GROUPS	5	412.500	82.500		
TOTAL	9	6046.900			

Tests for Homogeneity of Variances
Cochrans C = Max. Variance/Sum (Variances) = .3103, P = 1.000
Bartlett-Box F = .064 , P = .992

Table 8b: Tukeys pairwise comparison of larval survival obtained on vitamins and minerals

Mean	Feed Group	FEED GROUPS 4	1	2	3	5
26.0000	4					
32.0000	1	ns				
41.0000	2	ns	ns			
65.5000	3	*	ns	ns		
90.0000	5	*	*	*	ns	

(*) Denotes pairs of groups significantly different at the 0.050 level
ns indicates a non-significant result

Table 9 : *P. monodon* larval growth (PZ1-PL1) in response to microencapsulated feed 554 with frozen homogenated algal supplements

FEED GROUPS	MICROENCAPSULATED FEED	ADDITIVES	MEAN GROWTH(mm)	STANDARD DEVIATION
1.	CONTROL Algae + *Artemia*	50 $c\mu l^{-1}$+5. ml^{-1}	4.99	.1676
2.	554	10$c\mu l^{-1}$ algae	5.10	.2600
3.	554	Homogenised algae	4.92	.0882
4.	554	Total replacement	4.77	.2601

Table 9a: One-way ANOVA, larval growth against microencapsulated feed 554 with homogenated algal supplements

ANALYSIS OF VARIANCE

SOURCE	D.F.	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB
BETWEEN GROUPS	3	1.1192	.3731	8.7210	.001
WITHIN GROUPS	76	3.2510	.0428		
TOTAL	79	4.3702			

Tests for Homogeneity of Variances
Cochrans C = Max. Variance/Sum (Variances) = .3952, P = .106
Bartlett-Box F = 7.514 , P = .001

Table 9b: Tukeys pairwise comparison of mean larval growth obtained on microencapsulated feed 554 with homogenated algal supplements

Mean	Feed Group	FEED GROUPS 4	3	1	2
4.7749	4				
4.9257	3	ns			
4.9932	1	*	ns		
5.1011	2	*	ns	ns	

(*) Denotes pairs of groups significantly different at the 0.050 level
ns indicates a non-significant result

Table 10: Percentage survival of *P. monodon* larvae in response to microencapsulated feed 554 with homogenated algal supplements

FEED GROUPS	MICROENCAPSULATED FEED	ADDITIVES	MEAN SURVIVAL(%)	STANDARD DEVIATION
1.	CONTROL Algae + *Artemia*	50 $c\mu l^{-1}$+5. ml^{-1}	93.50	.7071
2.	554	10$c\mu l^{-1}$ algae	74.50	7.7782
3.	554	Homogenised algae	93.00	1.4142
4.	554	Total replacement	80.50	10.6066

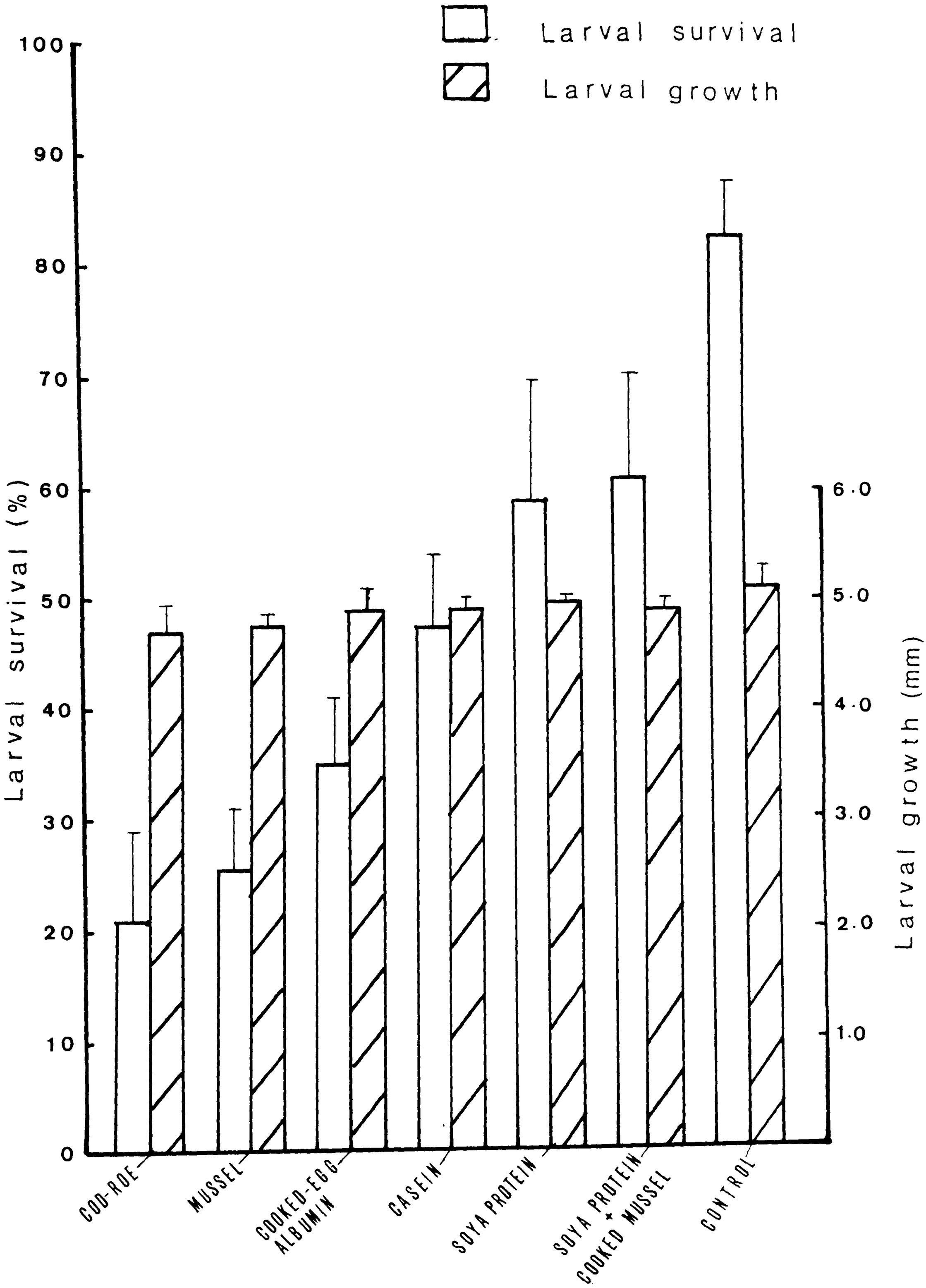
Table 10a : One-way ANOVA, larval survival against microencapsulated feed 554 with honogenated algal supplements

ANALYSIS OF VARIANCE

SOURCE	D.F.	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB
BETWEEN GROUPS	3	532.3750	177.4583	4.0446	.1052
WITHIN GROUPS	4	175.5000	43.8750		
TOTAL	7	707.8750			

Tests for Homogeneity of Variances
Cochrans C = Max. Variance/Sum (Variances) = .6410, P = .414
Bartlett-Box F = 1.599 , P = .211

Figure 1. Percentage survival and growth performance of P. monodon larvae to PL1 stage on different protein sources in microencapsulated feeds. Vertical bars are standard deviation.

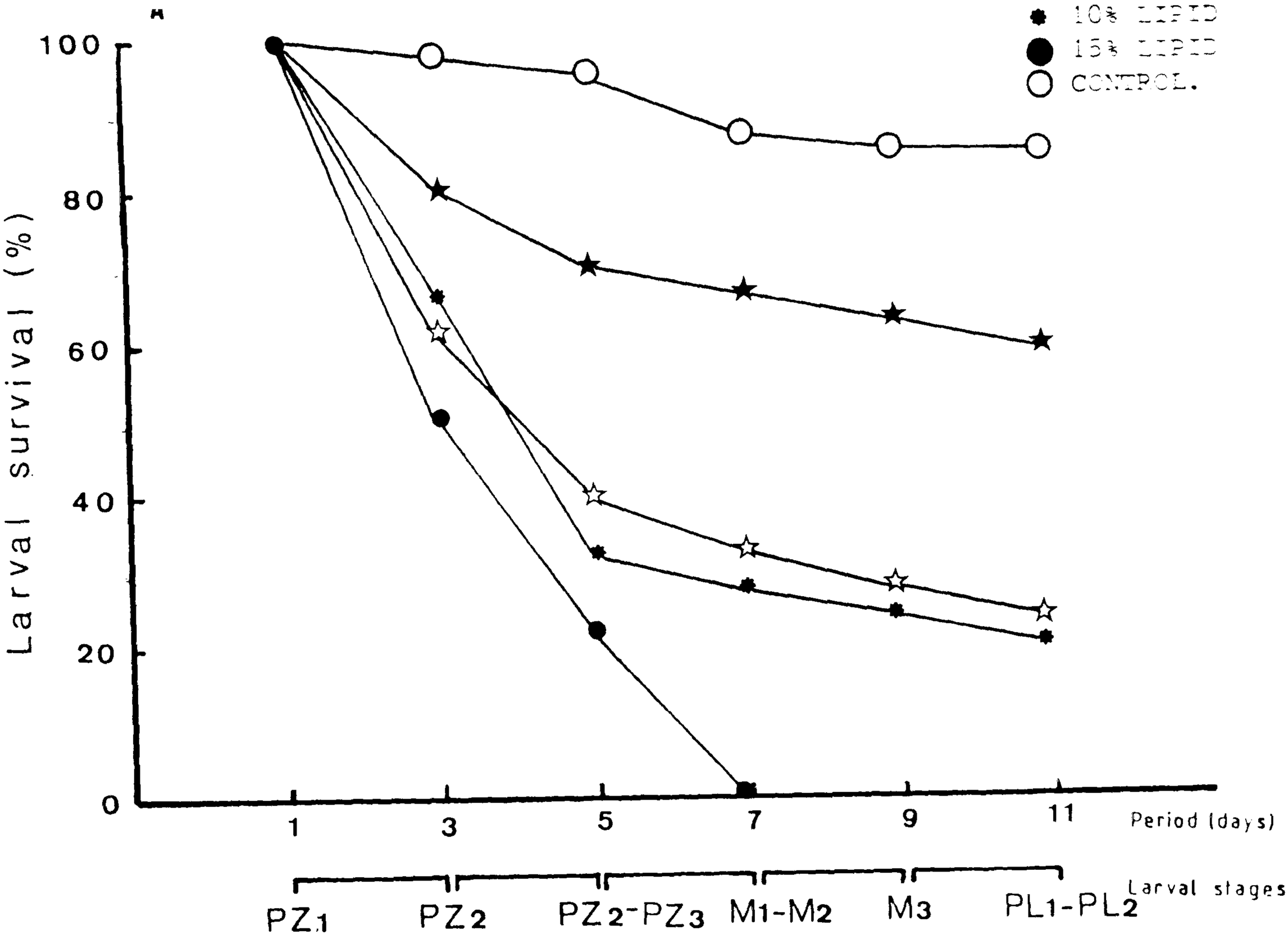


protein sources in microencapsulated feed

Figure 2a. Percentage survival of P. monodon larvae through metamorphosis to PL stages in response to lipid levels in microencapsulated feeds.

Figure 2b. Larval growth of P. monodon larvae through metamorphosis to PL stages in response to lipid levels in microencapsulated feeds.

A


NO LIPID
5% LIPID
10% LIPID
15% LIPID
CONTROL.
Larval survival (%)
100
80
60
40
20
0
1
3
5
7
9
11
Period (days)
PZ1
PZ2
PZ2-PZ3
M1-M2
M3
PL1-PL2
Larval stages

B

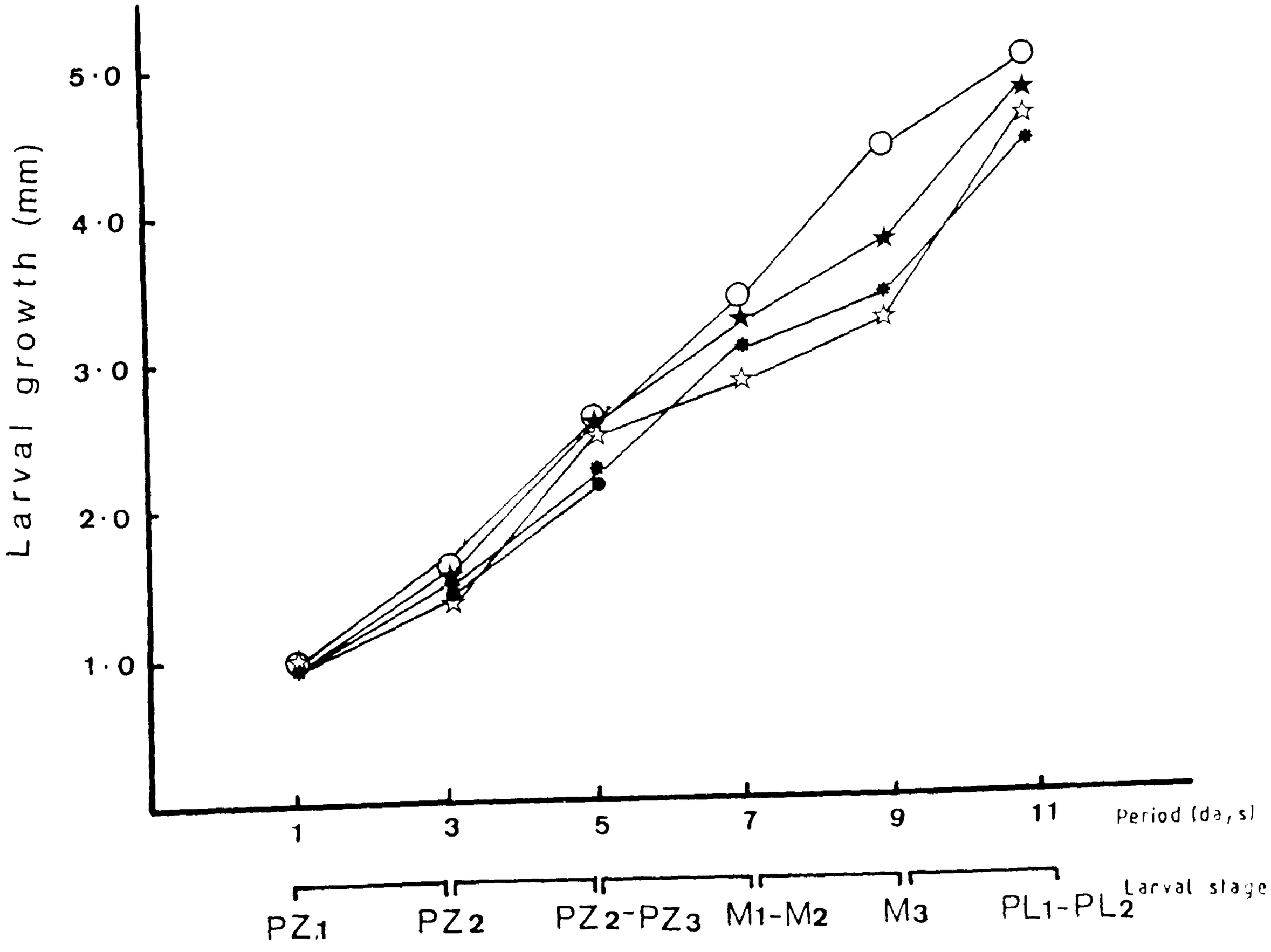
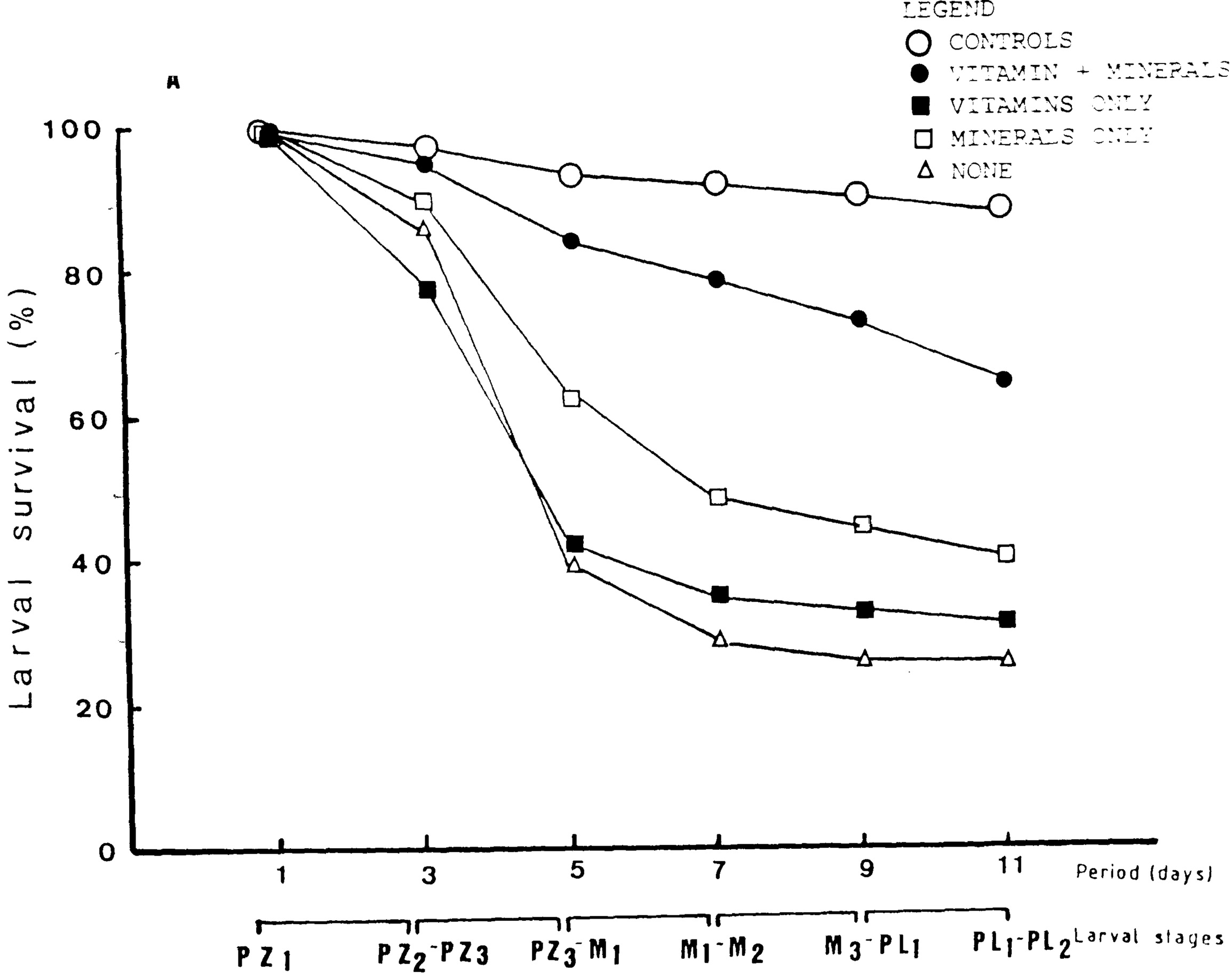

Larval growth (mm)
5·0
4·0
3·0
2·0
1·0
1
3
5
7
9
11
Period (days)
PZ1
PZ2
PZ2-PZ3
M1-M2
M3
PL1-PL2
Larval stages

Figure 3a. Percentage survival of P. monodon larvae through metamorphosis to PL stages in response to vitamin and minerals in microencapsulated feeds.

Figure 3b. Larval growth of P.monodon larvae through metamorphsis to PL stages in response to vitamin and minerals in microencapsulated feeds.

A


LEGEND
CONTROLS
VITAMIN + MINERALS
VITAMINS ONLY
MINERALS ONLY
NONE
Larval survival (%)
100
80
60
40
20
0
1
3
5
7
9
11
Period (days)
PZ1
PZ2-PZ3
PZ3-M1
M1-M2
M3-PL1
PL1-PL2
Larval stages

B

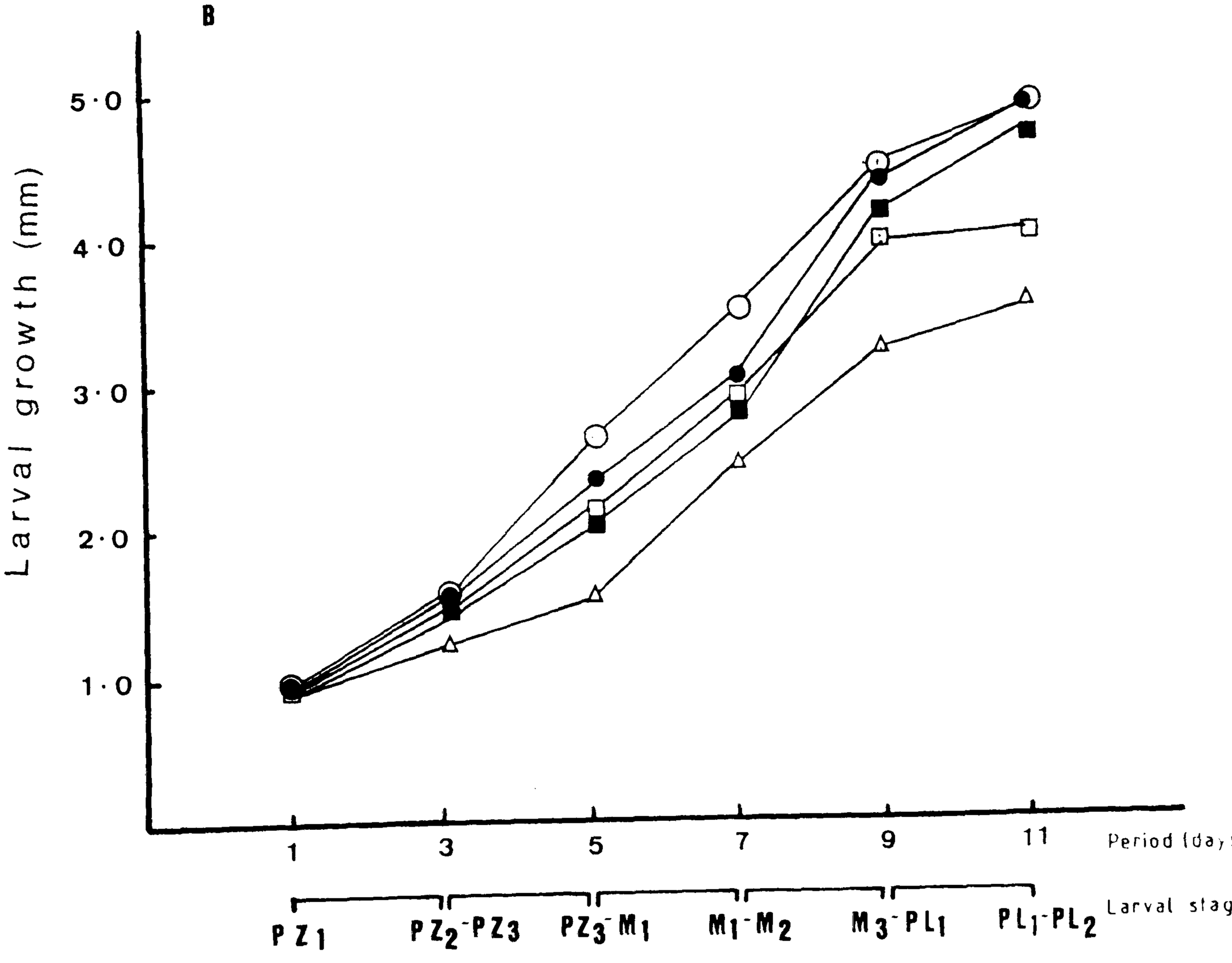
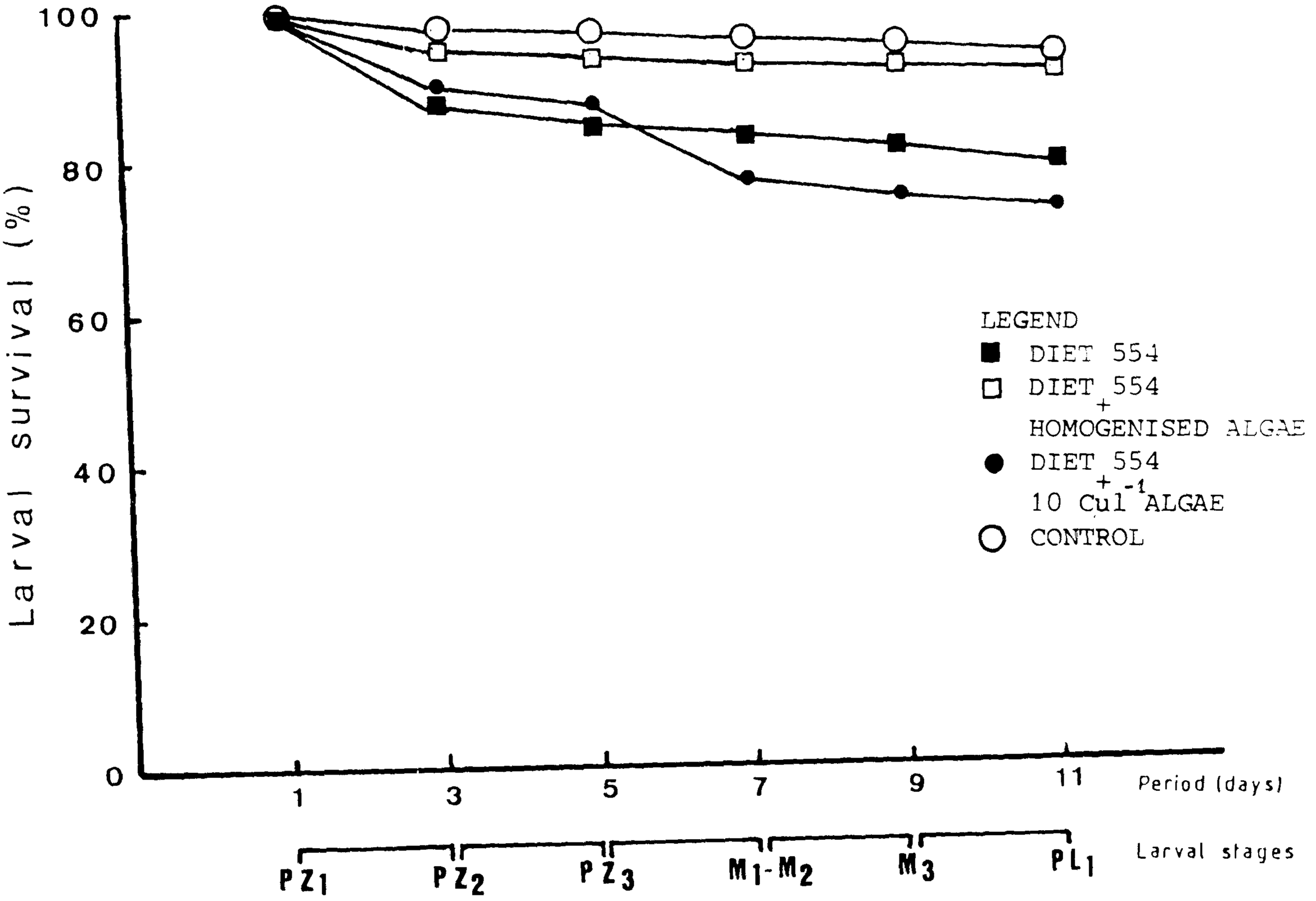

Larval growth (mm)
5·0
4·0
3·0
2·0
1·0
1
3
5
7
9
11
Period (days)
PZ1
PZ2-PZ3
PZ3-M1
M1-M2
M3-PL1
PL1-PL2
Larval stages

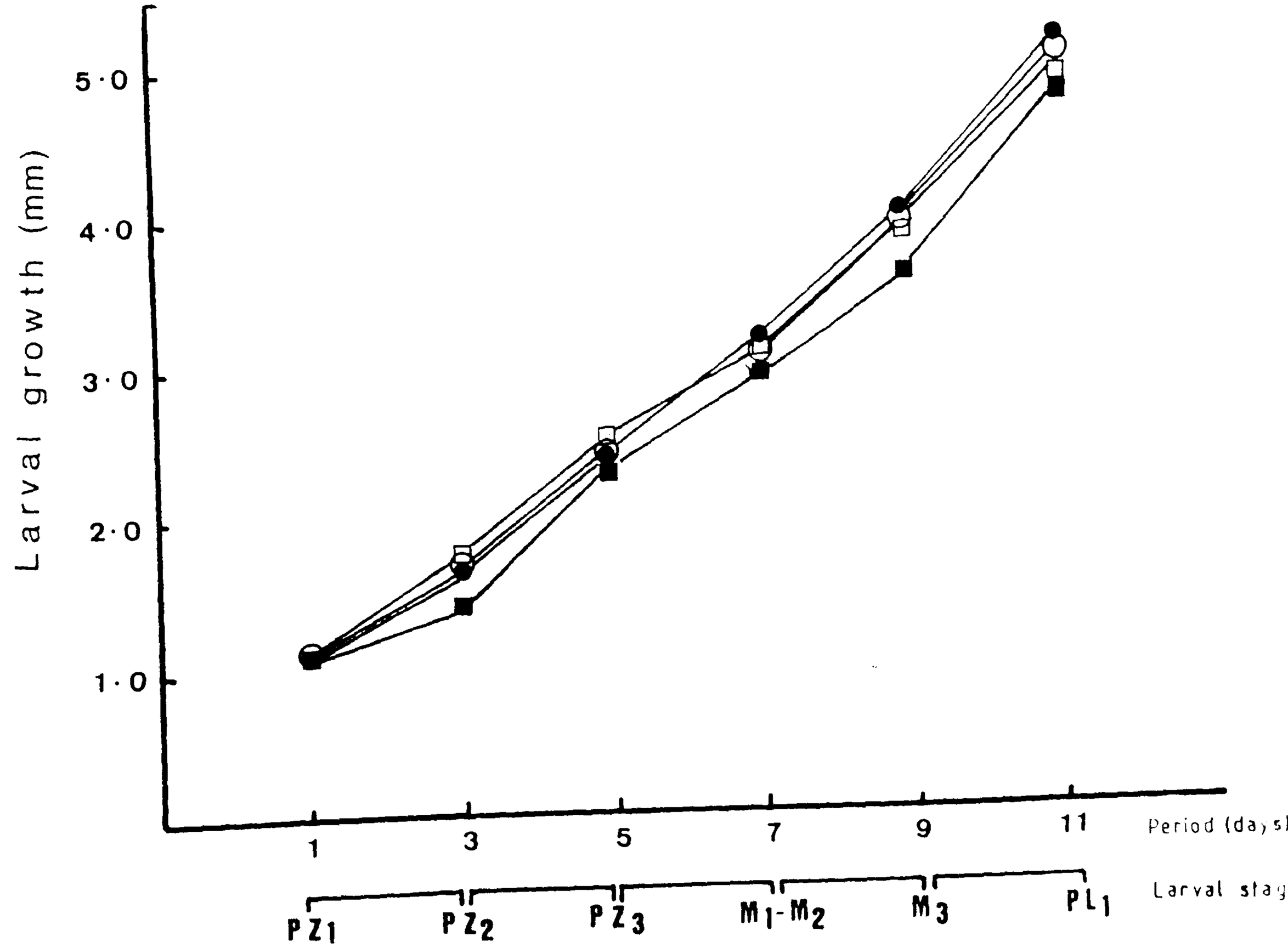
Figure 4a. Percentage survival of P. monodon larvae through metamorphosis to PL stages with microencapsulated diet and homogenated algal supplement.

Figure 4b. Growth of P.monodon larvae to postlarval stages of development on microencapsulated diet and with homogenated algal supplement.

A


100
80
60
40
20
0
Larval survival (%)
1
3
5
7
9
11
Period (days)
PZ1
PZ2
PZ3
M1-M2
M3
PL1
Larval stages
LEGEND
DIET 554
DIET 554 + HOMOGENISED ALGAE
DIET 554 + 10 Cul-1 ALGAE
CONTROL

B


5·0
4·0
3·0
2·0
1·0
Larval growth (mm)
1
3
5
7
9
11
Period (days)
PZ1
PZ2
PZ3
M1-M2
M3
PL1
Larval stage

SECTION 5

Growth and survival of *P. monodon* nursery and juvenile prawns on artificial diets

Part of this section was presented at The World Aquaculture Conference in Hawaii, USA, Jan. 1988.

Title : *Penaeus monodon* : **Nauplius to Juvenile on the same Artificial Diet.**

Authors : K. Kurmaly, S. Amjad and D.A. Jones.

Journal of the World Aquaculture Society. Volume 19 (1) ; Aquaculture Communiqués 43A.

INTRODUCTION

Microencapsulation has become an acceptable laboratory tool for the study of larval nutritional requirement in crustaceans (Jones et al., 1979b; Sakamoto et al., 1982; Levine and Sulkin, 1984; Jones et al., 1987; Kurmaly et al., 1989a), bivalves (Langdon and Waldock, 1981; Chu et al., 1982); and larval and postlarval fish (Gatesoupe et al., 1977; Jones et al., 1984). Refinements in the crustacean encapsulated diet has produced feeds which support the laboratory culture of penaeid larvae *Penaeus japonicus* from nauplius to postlarvae in the absence of all conventional live feeds (Jones et al., 1979a; Kanazawa et al., 1982).

Attempts to culture nursery stage prawns have demonstrated a similar need for artificial pelleted diets that will promote optimal growth. Most dry pellets have poor water stability (Goldblatt et al., 1979; 1980) and disintegrate within a few minutes, it is believed that pellets may act only as a fertilizer for the pond resulting in the production of other organisms which are then consumed by the prawns (Malca, 1983). The lack of an adequate formulated feed is the major remaining problem for commercial grow-out of penaeid prawns. Available diets do not promote the full potential rate of growth possible. Recently Jones et al., (1987) have shown that microencapsulation can successfully be used to deliver a balanced crustacean diet to penaeid larvae and postlarvae of *P. vannamei*, *P. stylirostris* and *P. monodon* in commercial hatcheries of Ecuador, Taiwan and the Philippines.

Postlarval penaeid prawns are usually moved from larval rearing tanks to nursery ponds after five (*P. vannamei*) to fifteen days (*P. monodon*). The nursery ponds constitute from 6 to 15% of the culture area. Postlarvae (PL12 to PL15) at this stage become demersal, and are stocked at 50 - 200 per m^2. These ponds or raceway tanks are usually shallow with a large bottom area, often provided with a natural muddy substrate, both to prevent cannibalism and allow production of some natural food. During the next 20 - 60 days postlarvae are weaned from larval foods onto a mixture of natural and specially prepared flakes, crumble or granulated feeds. For *P. monodon* and other penaeid postlarvae artificial feeds must contain at least 40% proteins and 3 - 4% PUFA rich lipids to sustain the rapid growth that allows juvenile to reach 0.5 - 2 gm by the end of the nursery period (Jones, 1988).

Present work details one of a series of trials in which *P. monodon* larvae were cultured from first feeding protozoea (PZ1) to 18 weeks old juvenile solely on artificial diets. Further, commercially available nursery stage diets (Flakes, crumbles and granulated feeds) were evaluated in a feed trial with nursery stages of *P. monodon* for 4 weeks. Growth, survival and weight gains were monitored to evaluate the effectiveness of each of the diets offered to the nursery stage prawns.

MATERIALS AND METHODS

P. monodon larvae used in feed trials were obtained from a single spawning in the Frippak Research Centre at Findon, U.K., and transported as nauplii (non-feeding stages) by air to the School of Ocean Sciences. At PZ1 stage they were stocked at 100 litre^{-1} in 2-litre round bottom flasks using U/V sterilised seawater filtered to 0.2 μm at 28°C and 33‰ salinity with gentle aeration. 50% of the culture water in the flask was exchanged every other day to renew water quality. At post larvae (PL) stage 1 animals were transferred to shallow plastic boxes (30 x 30 cm) and fed Frippak flakes till they were PL14. Nursery stage prawns were transferred at week 6 to individual 220 μm mesh base compartment (10 x 10 cm) in a recirculating seawater system at 28 ± 1°C with a salinity of 32 - 34‰ . Nursery stage prawns were fed with CD capsule pellets formed by using starch as a binder and extruded through a 1 mm sieve to produce 1 mm thin (diameter) pellets. Experimental diets are given in table 1, together with details of food ration and particle size fed to each stage in the life cycle. All artificial diets in this experiment are of the same basic nutritional composition differing only in manufacture process for presentation.

In a second set of experiments nursery stage prawns (PL15) were reared through metamorphosis to juveniles (PL45) on commercially available artificial diets (table 2). Diet performance was evaluated by monitoring growth, survival and weight gained by the animals. All trials were replicated.

One-way analysis of variance was performed to determine any significant differences (P = 0.05) on growth and survival of larvae, postlarvae, nursery and juvenile stages of *P. monodon* offered various artificial diets. This was followed by Bartletts-Box test (Sokal and Rohlf, 1981) for homogeneity of variance.

RESULTS

PROTOZOEA (PZ1) TO JUVENILE ON ARTIFICIAL DIET

Feed trial data on larval growth and survival obtained during 18 weeks experimental period with artificial diets, has been analysed according to life cycle stages achieved by *P. monodon* larvae. Fig 1 and 2 show growth and survival of *P. monodon* larvae fed on CAR microcapsules up to protozoea 3 (PZ3) stage, CD capsule from mysis (M1) to postlarva 1 (PL1) and flake diets from PL2 to PL14; compared with controls fed on live and natural foods over the same period (fig 3, 4). Growth and survival of nursery stage larvae from week 6 to juveniles (week 18) continuing on artificial diet (table 1) and compared with controls is shown in fig 5a and 5b.

Larval Growth and Survival (PZ1-PZ3)

At PZ3 mean larval growth obtained on CAR diets were 2.80 mm; compared to larval growth achieved on live feed controls (3.3 mm). Analysis of variance (table 3a) shows that larval growth (PZ1 to PZ3) obtained on CAR diet was not significantly different ($P > 0.576$) from growth obtained on live feed controls.

Percentage larval survival (PZ1 - PZ3) fed on CAR diets was 82% as compared to 96% survival achieved on control diets. Analysis of variance (table 3b) shows that percentage survival obtained on live feed controls was not significantly ($P > 0.098$) different from survival obtained on CAR diets at

PZ3 stage.

Larval Growth and Survival (M1 - PL1)

Analysis of variance (table 4a) shows that mean larval growth to PL1 (4.5 mm) achieved on CD microcapsule diet, compared to live feed controls (4.35 mm) was not significantly ($P > 0.830$) different.

Analysis of variance (table 4b) indicates percentage survival to PL1, obtained on live feed controls (85%); compared with 61% survival on CD microcapsules was significantly different ($P < 0.05$) (fig. 2). Although percentage survival on microcapsulated diet to PL1 (61%) was lower in this particular trial than the average attained during similar trials on total replacement diets (> 80% to PL1 : Section 4), growth at PL1 however, was not significantly different from live feed controls (fig. 1).

Postlarval Growth and Survival (PL2 - PL14)

Continuing postlarval feed trial in plastic boxes, fed flake diets, larval growth (9.48 mm) to PL14 (nursery stage) was not significantly different ($P > 0.420$) (table 5a) from growth attained on live feed controls (10.6 mm) (fig. 3). Fig. 4 shows that percentage larval survival to PL14 on flake diets was relatively poor (25%) compared to survival (62.5%) on mussel fed controls.

Analysis of variance (table 5b) shows that larval survival to PL14 was significantly ($P < 0.05$) better on control feeds. Poor PL growth and percent survival were attributed in part to

high stocking density (550 m^2).

Juvenile Growth and Survival on CD Encapsulated Pellets

After a further 2 weeks, replicated groups of 16 on-growing nursery stage prawns, on both flake and mussel diet were transferred to individual compartments (10 x 10 cm). Groups previously fed flake were now fed exclusively on encapsulated CD pellets, whilst controls were fed on frozen mysids (*Neomysis integer*). Mean growth (fig 5a) to juvenile (week 18) on CD pellets was 69.8 mm and 70.0 mm on control feed.

Analysis of variance (table 6a) shows that no significant ($P > 0.837$) difference in growth was achieved after 18 weeks. Percentage survival of juveniles on artificial diet remained high at 80% over the first 2 weeks (week 8, fig 5b), but fell to 25% by the end of the experiment at week 18. Analysis of variance (table 6b) indicates survival of juvenile prawns was significantly ($P < 0.05$) better on controls than on CD pellets diets. Growth however remained comparable between groups fed on artificial and natural diet, with no significant difference appearing even at the end of week 18.

RESPONSE OF NURSERY STAGE PRAWNS TO COMMERCIALLY AVAILABLE ARTIFICIAL DIETS

In the second feed trial response of nursery stage (PL15) *P. monodon* to commercially available artificial diets (table 2) was evaluated during development of nursery stage prawns into juveniles. Larval growth, weight gains and percentage survival

of 20 prawns on each diet was monitored in a replicated feed trial; over a period of 4 weeks, to evaluate the effectiveness of each diet type.

Growth - Nursery to Juvenile prawns

Fig. 6 demonstrates growth (total length) of nursery stage prawns PL15 (initial mean stocking size 10 mm) to juvenile prawns reared on commercially available artificial diets for 4 weeks. Lowest growth (15 mm) was obtained on particulate feeds (group 2). On granulated feed growth of juveniles was 17.72 mm. Growth of juvenile prawns on crumble feed was 20.13 mm (group 3). Nursery stage prawns fed flake diets (group 4) attained growth of 27.55 mm; the highest growth amongst commercially available diets tested. Mean growth achieved on control feeds (group 5) was 28.65 mm.

Analysis of variance (table 7a) indicates an overall significant ($P < 0.05$) effect of growth on different diets. Multiple pairwise comparisons (table 7b) shows that growth achieved on control diet (group 5) was significantly better than growth obtained on particulate diet (group 2), granulated diet (group 1) and on crumbles (group 3). Juvenile growth achieved on flake diet (group 4) however was not significantly different from growth achieved on control diets (group 5). Growth on flake diet (group 4) was significantly better than growth obtained on groups 2, 1 and 3. Juvenile growth on crumble diet (group 3) was significantly better than growth obtained on group 2. Growth was not significantly different from granulated feed (group 1). Growth obtained on granulated

feed (group 1) was not significantly different from growth obtained on particulate diet (group 2).

Wet Weight Gains - Nursery to Juvenile Prawns

Wet weight gains achieved by nursery stage prawns (PL15, 0.004 g.) to juvenile fed artificial diet for 4 weeks is shown in fig 7. Prawns on control feed (group 5) attained the highest weight (1.02 g.) followed by weight increase of 0.76 g. on flake diet (group 4). Weight gained by juveniles on crumble diet (group 3) was 0.38 g. Nursery stage prawns fed granulated feed (group 1) and particulate diet (group 2) attained 0.031 g. and 0.022 g. respectively.

Analysis of variance (table 8a) shows that the difference in juvenile weights achieved on different diets was significant ($P < 0.05$). Multiple pairwise comparisons (table 8b) demonstrates that juvenile weight gains on control feed (group 5) was significantly better than those on the particulate diet (group 2), granulated diet (group 1) and crumbles (group 3). Juvenile weight gains on flake diet (group 4) were not significantly different from weight gained by juveniles on control feed. Weight gained by juveniles on flake diet (group 4) was significantly better than weight gained by juvenile prawns on particulate diet (group 2), and granulated diet (group 1). Weight gain on the crumble diet (group 3) was not significantly different from weight gained on the flake diet (group 4). Juvenile weight obtained on crumbles (group 3) was not significantly different from weight gained on particulate diet (group 2) and granulated feed (group 1).

Percentage Survival - Nursery to Juvenile Prawns

Percentage survival obtained after 4 weeks experimental period is shown in fig. 8. 100% survival to juvenile was obtained on control feed. 90% survival was achieved on flake diet. Survival of juveniles on crumble feed was 75%, whilst juvenile survival on both particulate and granulated feeds were 55%.

Analysis of variance (table 9) shows that percentage survival of nursery stage prawns to juvenile on different diets was significant ($P < 0.05$). Multiple pairwise comparison indicates that survival obtained on different diets was not significantly different at ($P = 0.05$) level.

DISCUSSION

Growth and survival of protozoeal stages (PZ1 - PZ3), attained on live feed controls do not differ significantly, from growth and survival obtained on CAR microencapsulated feeds (table 3a), which were used as total replacement to live feeds. Both mean growth and percentage survival on microcapsules were comparable to live feed controls (fig. 1). This indicates that CAR microencapsulated feeds are nutritionally adequate and highly acceptable to protozoeal stages of *P. monodon*. Although survival to PL1 (61%) was lower in this particular trial than the average attained during similar trials on total replacement diets (> 80%), growth at PL1 was not significantly different from live feed controls (table 4a). Larval growth and percentage survival during larval stages compares well with hatchery trial data (Jones et al., 1987), although the addition of supplementary algae may increase average survival rates to over 80%. McVey and Fox, (1983) obtained 50% survival to PL1 stage for penaeid larvae (*P. monodon, P. stylirostris* and *P. vannamei*) on live *Artemia* feed. Percentage survival to PL1 (61%) attained solely on microencapsulated diet in the present trial was comparatively better.

Survival of postlarvae continuing on the artificial flake diet still achieved 30% at PL14, as opposed to 60% for PLs on fresh mussel diet (controls). Liao and Chao, (1983) showed survival rates for penaeid larvae from nauplius to PL20 ranged between 25 - 60% on natural diets. More recently Jones et al.,

(1987), using microencapsulated feed on a commercial scale in a Philippines hatchery for the first time showed that survival of *P. monodon* to postlarvae (PL5) ranged between 3 - 26%. Percentage survival (30%) to PL14 achieved in the present trials on artificial diet are similar and do not differ significantly from their results. Recently MacDonald et al., (1989) examined the enzymic activity in *P. monodon* larvae and postlarvae. A minimum or 'enzyme crisis' period was observed between day 11 and day 15, this growth period coincides with postlarval stages PL1 to PL4/PL5. Postlarval mortality of *P. monodon* during these stages in usually high. Growth rate of PLs to nursery stage prawns fed solely on artificial diet was 90.5% of that achieved on mussel fed PLs after 26 days. Both growth and survival rate were comparatively lower during the early postlarval feed trial, attributable in part to the high stocking density, equivalent to 550 m^2. The stocking density is usually 160 animals m^2 in an intensive and 250 m^2 in super intensive systems (Liao and Chao, 1983).

Whilst survival rates of artificial diet fed animals declined rapidly towards the end of the 18 week juvenile experiment, indicating a possible nutritional deficiency, growth was not significantly different from that achieved on frozen mysids (controls). Juvenile growth rates of both artificial and control groups were some 25% less than can be expected in nursery ponds (Ryther and Bardach, 1968; Bardach et al., 1972). Grabner et al., (1981) demonstrated that freeze dried or frozen zooplankton release soluble materials from the freeze-damaged cells into the water. This may be the reason

for lower growth and survival of *P. monodon* juveniles on frozen mysids (controls).

In the second, 4 week feed trial with nursery stage prawns (PL15) reared on commercially available artificial diets to juveniles (PL45), growth (total length) achieved on flake diet (T.L. = 27.55 mm) was not significantly different from growth (T.L. = 28.65 mm) obtained on control (fresh mussel) feed. Juvenile growth on crumble feed (T.L. = 20.13 mm) was significantly better compared with granulated (T.L. = 17.72 mm) and particulate (T.L. = 15.0 mm) feeds (fig.5). Weight gained by juveniles fed different types of artificial feeds for the same period also showed significant differences (table 8a). Multiple pairwise comparison showed that there was no significant difference between weight gained by juvenile prawns fed flake diet and those on the control diet. Highest mean wet weight gained by juveniles amongst artificial diets tested was on flake diet (0.76 g.). This weight gained over 4 week period is similar to average weights (0.78 g.) achieved by juvenile *P. monodon* cultured in the Philippines with supplemental feeding (Bardach et al., 1972). Crumble feeds were less efficient, with only 0.38 g. weight gain by the juveniles. Granulated and particulate feeds gave considerably poorer weight increases (0.031 and 0.022 g. respectively). Leaching of dietary solubles and high concentrations of ammonia and nitrite measured in earlier feed trials (Section 2) may have contributed to poor growth performance with unprotected crumble, granulated and particulate feeds. Conversely, it is possible that the digestive system of prawn may lack certain

enzymes (Stark et al., 1988) capable of extracting nutrients from complex feeds. Mean percentage survival of juveniles obtained by feeding different artificial diet was also shown to be significant (table 9). Amongst the artificial diets, flake feed gave high mean survival (90%), followed by juveniles fed crumble (75%), both particulate and granulated feed gave 55% survival after 4 week trial period. Percentage survival obtained in the present study compares favourably with results achieved with *P. monodon* on natural feeds (Aquacop, 1983) and production of *P. monodon* on formulated feed in Taiwan (Chen et al., 1989).

These experiments demonstrate for the first time that penaeid shrimp may be reared exclusively on artificial feeds from the protozoeal to juvenile stages. It is concluded that the microencapsulated feeds used in the present work are capable of sustaining *P. monodon* throughout larval, postlarval and a substantial part of juvenile life in the absence of any other obvious source of nutrition. Whilst these feeds are not optimal for growth and survival they provide a basis for investigating the optimal nutritional requirements for penaeid prawns.

Table 1: Diets used in feeding trials for *P. monodon*

DIETS	FEED RATION	SIZE OF FOOD	TYPE OF DIET	LIFE CYCLE STAGE
ARTIFICIAL DIETS				
CAR	8 $mg.l^{-1}.d^{-1}$	< 20 µm	microcapsule	PZ1-PZ3
CD	16 $mg.l^{-1}.d^{-1}$	90-150µm	microcapsule	M1-PL1
FLAKE	20 $mg.l^{-1}.d^{-1}$	5-10 mm	flakes	PL1-PL14
PELLETS (CD)	20 $mg.l^{-1}.d^{-1}$	1 mm dia.	CD	juveniles
CONTROLS				
Microalgae	50 $cell.\mu l^{-1}.d^{-1}$	10 µm	*T.chuii* + *R.baltica*	PZ1-PZ3
Artemia	5 $.ml^{-1}.d^{-1}$	0.4 mm	nauplii	M1-PL
Mytilus sp. (mussel)	2x0.5shell.$l^{-1}.d^{-1}$	10-20 mm	fresh	PL-nursery stage
Neomysis sp. (mysid)	5-7ind.$larva^{-1}.d^{-1}$	7-10 mm	frozen	nursery-juveniles

Table 2: Commercially available artificial diets for nursery stage prawns used in feeding trials for *P. monodon*

FEED GROUP	DIETS	TRADE NAME	FEED RATION	SIZE OF FOOD	TYPE OF DIET	LIFE CYCLE STAGE
FORMULATED FEEDS						
4	FLAKES	FRIPPAK	20mg.l^{-1}.d^{-1}	5-10 mm	flakes	Nursery-Juveniles
1	GRANULATED	ARGENT	20mg.l^{-1}.d^{-1}	1-3 mm	granules	Nursery-Juveniles
3	CRUMBLES	NIPPAI	20mg.l^{-1}.d^{-1}	2-5 mm	crumbles	Nursery-Juveniles
2	PARTICULATES	ALMA	20mg.l^{-1}.d^{-1}	1-3 mm	particles	Nursery-Juveniles
CONTROLS						
5	*Mytilus* sp.		2 x 0.5 shell.l^{-1}.d^{-1}	10-20 mm	fresh	Nursery-Juveniles

Table 3a: Analysis of variance. *P. monodon* protozoeal feed trial (PZ1-PZ3) growth obtained by microencapsulated feed and control diet (live microalgae)

SOURCE	D.F.	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB
BETWEEN GROUPS	1	.2926	.2926	.3488	.5763
WITHIN GROUPS	6	5.0332	.8389		
TOTAL	7	5.3258			

Test for Homogeneity of Variance.
Bartlett-Box F 0.262, P = 0.609

Table 3b: Analysis of Variance. Protozoeal feed trial (PZ1-PZ3), percentage survival obtained with microencapsulated against live feed conrols (algae)

SOURCE	D.F.	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB
BETWEEN GROUPS	1	128.0000	128.0000	3.8114	.0987
WITHIN GROUPS	6	201.5000	33.5833		
TOTAL	7	329.5000			

Test for Homogeneity of Variance.
Bartlett-Box F = 4.790, P = 0.031

Table 4a: Analysis of Variance. Mysis feed trial (M1-PL1), growth (body lengths) obtained on CD encapsulated feed and live feed controls (*Artemia*)

SOURCE	D.F.	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB
BETWEEN GROUPS	1	.0131	.0131	.0523	.8303
WITHIN GROUPS	4	.9997	.2499		
TOTAL	5	1.0127			

Test for Homogeneity of Variance.
Bartlett-Box F = 0.046, P = 0.831

Table 4b: Analysis of Variance. Mysis feed trial (M1-PL1), percentage survival obtained on CD encapsulated feed and live feed controls (*Artemia*)

SOURCE	D.F.	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB
BETWEEN GROUPS	1	793.5000	793.5000	13.6029	.0211
WITHIN GROUPS	4	233.3333	58.3333		
TOTAL	5	1026.8333			

Test for Homogeneity of Variance.
Bartlett-Box F = 0.418, P = 0.521

Table 5a: Analysis of Variance. Postlarval feed trial (PL2-PL14), growth (body lengths) obtained on flake diets and by mussel (*Mytilus* sp.) controls

SOURCE	D.F.	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB
BETWEEN GROUPS	1	2.4864	2.4864	.6963	.4203
WITHIN GROUPS	12	42.8486	3.5707		
TOTAL	13	45.3350			

Test for Homogeneity of Variance.
Bartlett-Box F 0.106, P = 0.745

Table 5b: Analysis of Variance. Postlarval feed trial (PL2-PL14), percentage survival obtained on flake diets and by mussel (*Mytilus* sp.) controls

SOURCE	D.F.	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB
BETWEEN GROUPS	1	4235.1607	4235.1607	54.1276	.001
WITHIN GROUPS	12	938.9286	78.2440		
TOTAL	13	5174.0893			

Test for Homogeneity of Variance.
Bartlett-Box F = 0.267, P = 0.605

Table 6a: Analysis of Variance. *P. monodon* juvenile feed trial (nursery stage to juveniles), growth (body length) obtained on CD pellets and mysids (frozen *Neomysis)* control diet

SOURCE	D.F.	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB
BETWEEN GROUPS	1	10.1490	10.1490	.0441	.8372
WITHIN GROUPS	12	2763.6413	230.3034		
TOTAL	13	2773.7904			

Test for Homogeneity of Variance.
Bartlett-Box F = 0.054, P = 0.817

Table 6b: Analysis of Variance. Juvenile feed trial, percentage survival obtained on CD pellets and mysids (frozen *Neomysis*) control diet

SOURCE	D.F.	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB
BETWEEN GROUPS	1	2200.0179	2200.0179	5.1090	.0432
WITHIN GROUPS	12	5167.3571	430.6131		
TOTAL	13	7367.3750			

Test for Homogeneity of Variance.
Bartlett-Box F = 8.771, P = 0.003

Table 7a: Analysis of Variance. Growth of nursery stage (PL15) *P. monodon* to juvenile prawns, on commercially available artificial diets (for feed groups see table 2) and mussel (fresh *Mytilus* sp.) control diet

SOURCE	D.F.	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB
BETWEEN GROUPS	4	2144.6104	536.1526	75.8334	.001
WITHIN GROUPS	70	494.9096	7.0701		
TOTAL	74	2639.5200			

Test for Homogeneity of Variance.
Bartlett-Box F = 1.882, P = 0.111

Table 7b: Multiple pairwise comparison of growth (juveniles) fed artificial and control diet (for feed groups see table 2)

		FEED GROUP				
MEAN	FEED GROUP	2	1	3	4	5
15.0000	Group 2					
17.7273	Group 1	ns				
20.1333	Group 3	*	ns			
27.5556	Group 4	*	*	*		
28.6500	Group 5	*	*	*	ns	

(*) Denotes pairs of groups significantly different at the 0.050 level

ns indicates a non-significant result

Table 8a: Analysis of Variance. Wet weights of *P. monodon* juvenile prawns obtained on artificial diets (for feed groups see table 2) and mussel (fresh *Mytilus* sp.) control diets

SOURCE	D.F.	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB
BETWEEN GROUPS	4	11.8695	2.9674	17.4020	.001
WITHIN GROUPS	71	12.1069	.1705		
TOTAL	75	23.9764			

Test for Homogeneity of Variance.
Bartlett-Box F = 30.363, P = 0.000

Table 8b: Multiple pairwise comparison of weight gained by juvenile prawns on artificial feeds (for feed groups see table 2) and control diet

		FEED GROUP				
MEAN	FEED GROUP	2	1	3	4	5
.0225	Group 2					
.0318	Group 1	ns				
.3867	Group 3	ns	ns			
.7695	Group 4	*	*	ns		
1.0265	Group 5	*	*	*	ns	

(*) Denotes pairs of groups significantly different at the 0.050 level

ns indicates a non-significant result

Table 9 : Analysis of Variance. Percentage survival of juvenile prawns obtained on artificial and control diet

SOURCE	D.F.	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB
BETWEEN GROUPS	4	3300.0000	825.0000	5.5000	.044S
WITHIN GROUPS	5	750.0000	150.0000		
TOTAL	9	4050.0000			

Test for Homogeneity of Variance.
Bartlett-Box F = 0.386, P = 0.764

Multiple pairwise comparison showed no two feed groups were significantly different at 0.050 level.

Figure 1. Growth of P. monodon larvae from protozoea to postlarvae on artificial (CAR capsules, CD capsules) feeds and on live microalgae at 40 cells μl^{-1} and Artemia at 5 ml^{-1} as controls. Vertical bars are standard deviation.

ARTIFICIAL DIET — CAR CAPSULES — CD CAPSULES

CONTROL DIET — T. CHUII + R. BALTICA — ARTEMIA + ALGAE

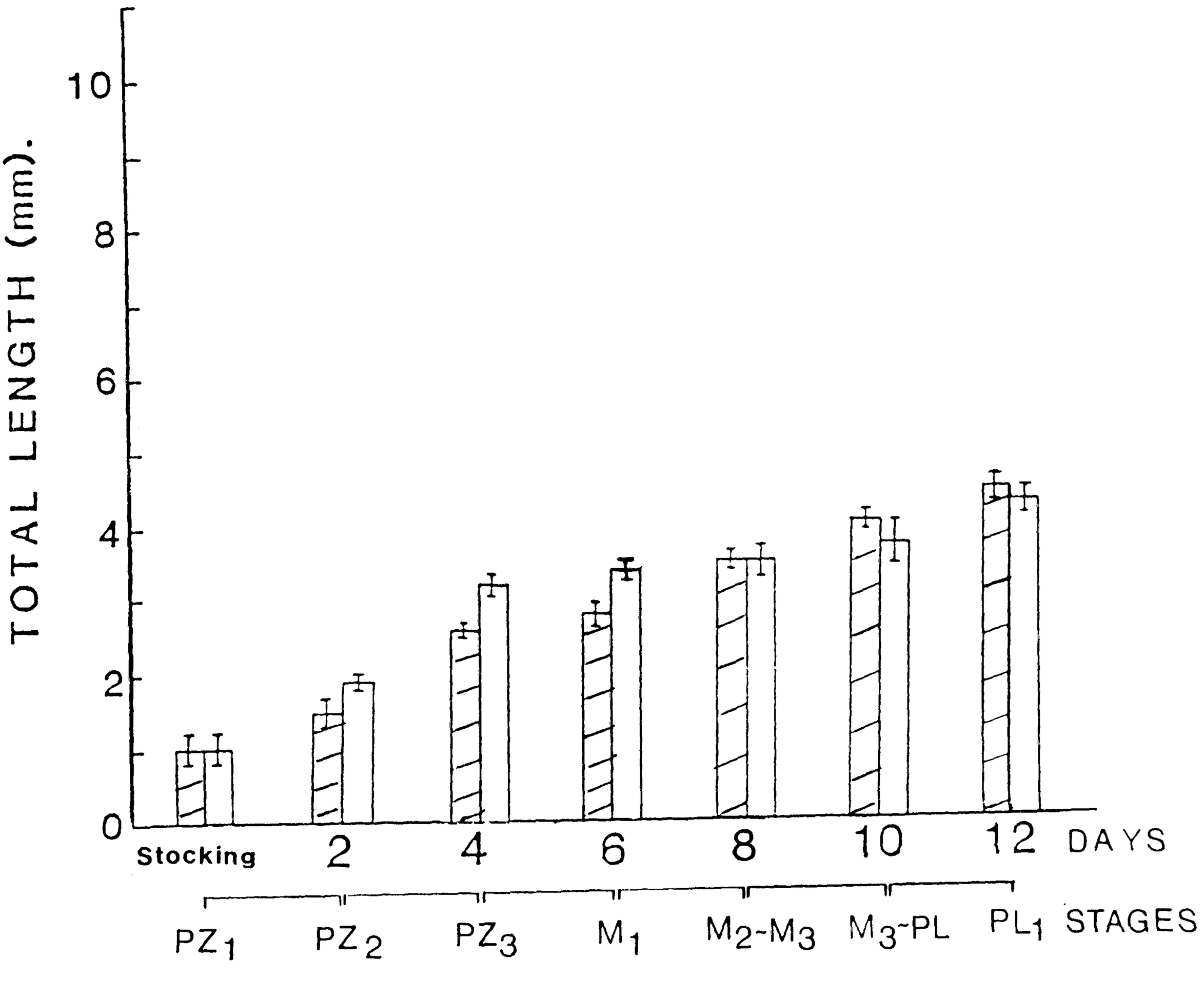


Figure 2. Percentage survival of P. monodon larvae from protozoea to postlarvae on artificial (CAR capsule, CD capsules) feeds and on live microalgae at 40 cells μl^{-1} and Artemia nauplii at 5 ml^{-1} as controls. Vertical bars are standard deviation.

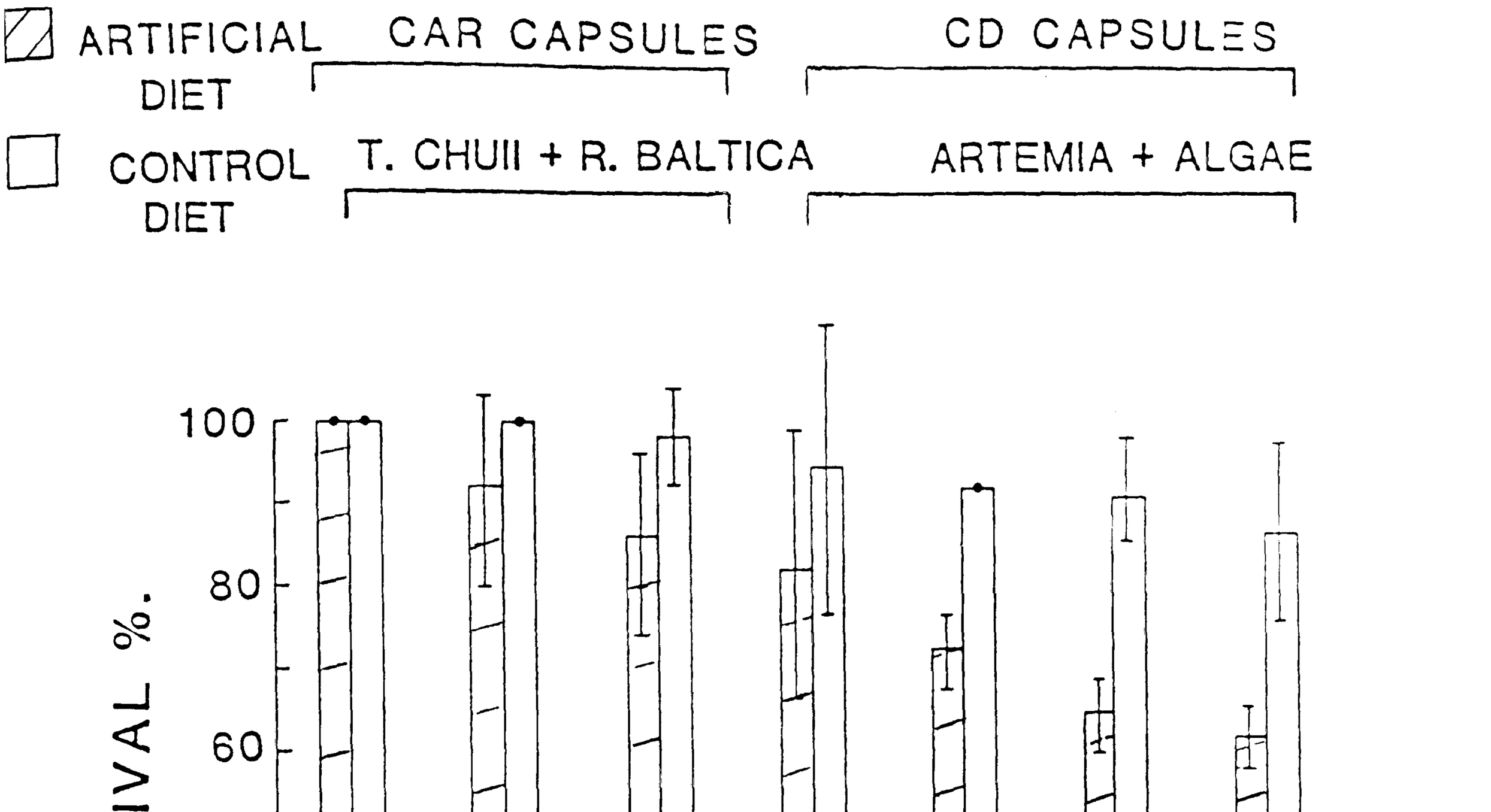

ARTIFICIAL DIET
CAR CAPSULES
CD CAPSULES
CONTROL DIET
T. CHUII + R. BALTICA
ARTEMIA + ALGAE
SURVIVAL %.
100
80
60
40
20
0
Stocking
2
4
6
8
10
12
DAYS
PZ1
PZ2
PZ3
M1
M2-M3
M3-PL
PL1
STAGES

Figure 3. Growth of P. monodon postlarvae on artificial feed and on fresh mussels as control. Vertical bars are standard deviation.

ARTIFICIAL DIET — FRIPPAK FLAKE

CONTROL DIET — MUSSEL

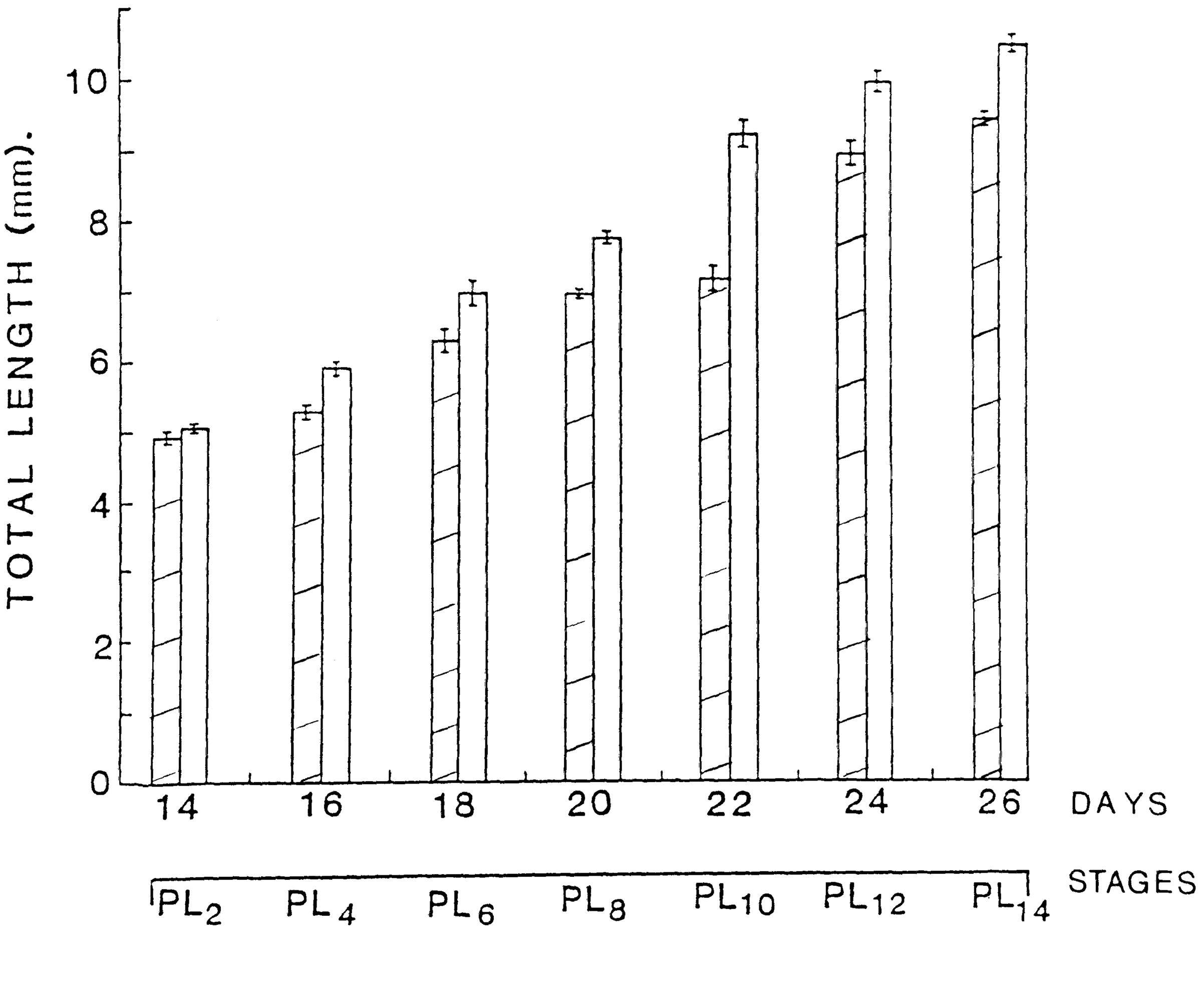


Figure 4. Percentage survival of P. monodon postlarvae on artificial feed and on mussels as control. Vertical bars are standard deviation.

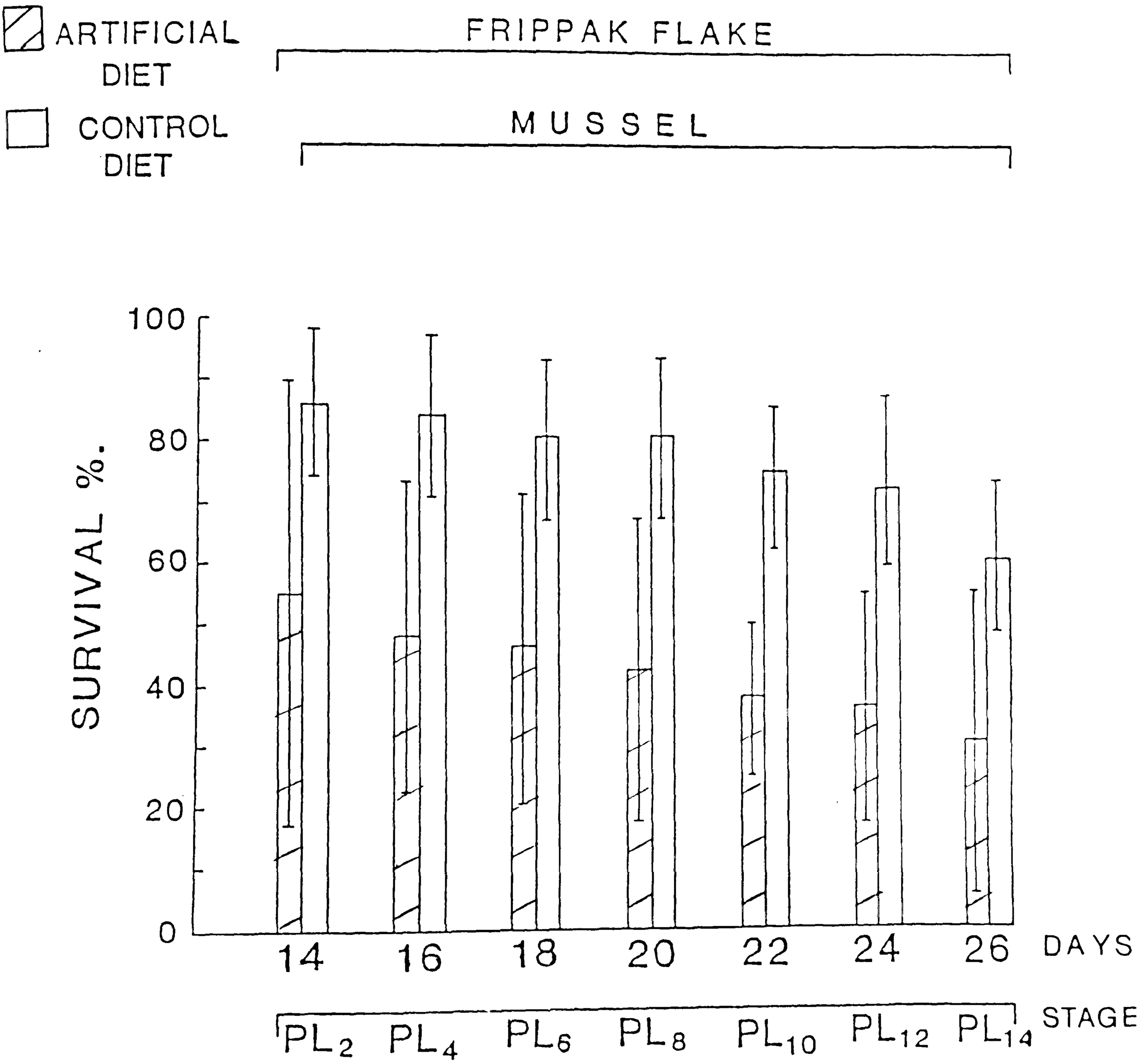
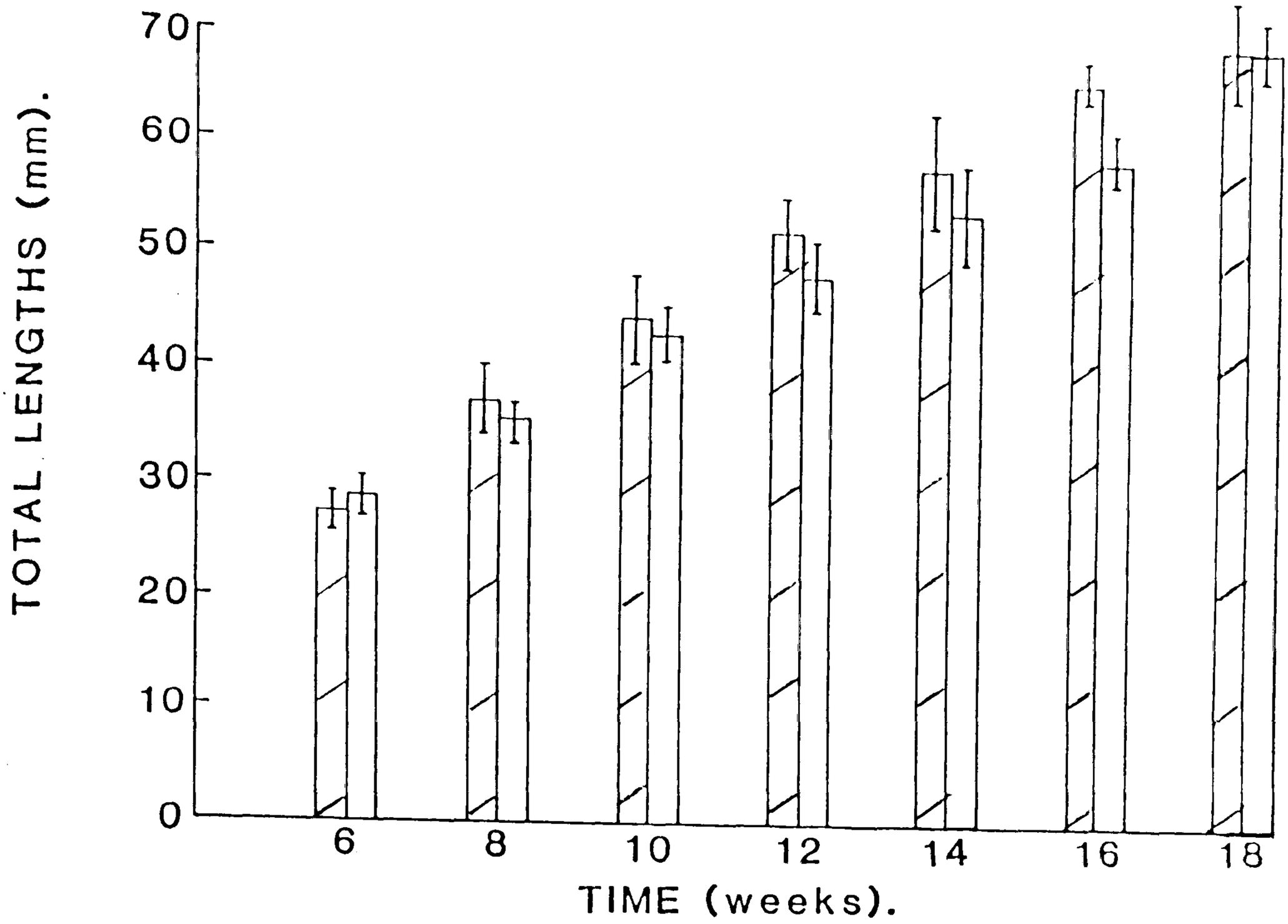

ARTIFICIAL DIET
CONTROL DIET
FRIPPAK FLAKE
MUSSEL
SURVIVAL %.
100
80
60
40
20
0
14
16
18
20
22
24
26
DAYS
PL2
PL4
PL6
PL8
PL10
PL12
PL14
STAGE

Figure 5a. Growth of P. monodon juveniles fed on frozen mysid(□), and CD capsules (▨). Vertical bars are standard deviation.

Figure 5b. Percentage survival of P. monodon juveniles fed frozen mysid (□) as control and CD capsules (▨). Vertical bars are standard deviation.

a


TOTAL LENGTHS (mm).
70
60
50
40
30
20
10
0
6
8
10
12
14
16
18
TIME (weeks).

b

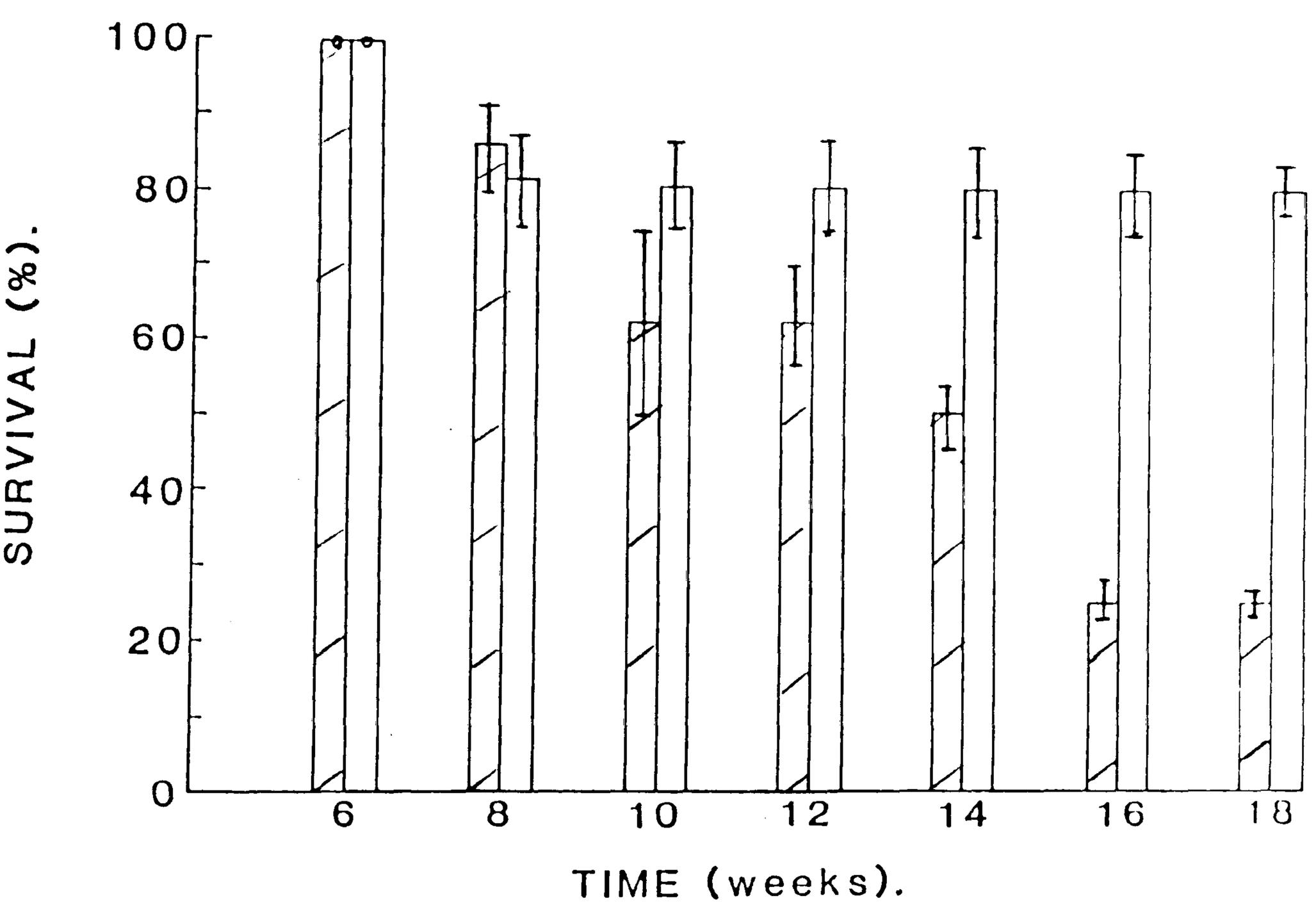

SURVIVAL (%).
100
80
60
40
20
0
6
8
10
12
14
16
18
TIME (weeks).

Figure 6. Growth of P. monodon from nursery stages to juveniles on artificial feeds (for feed groups 1-5 see table 2). Vertical bars are standard deviation.

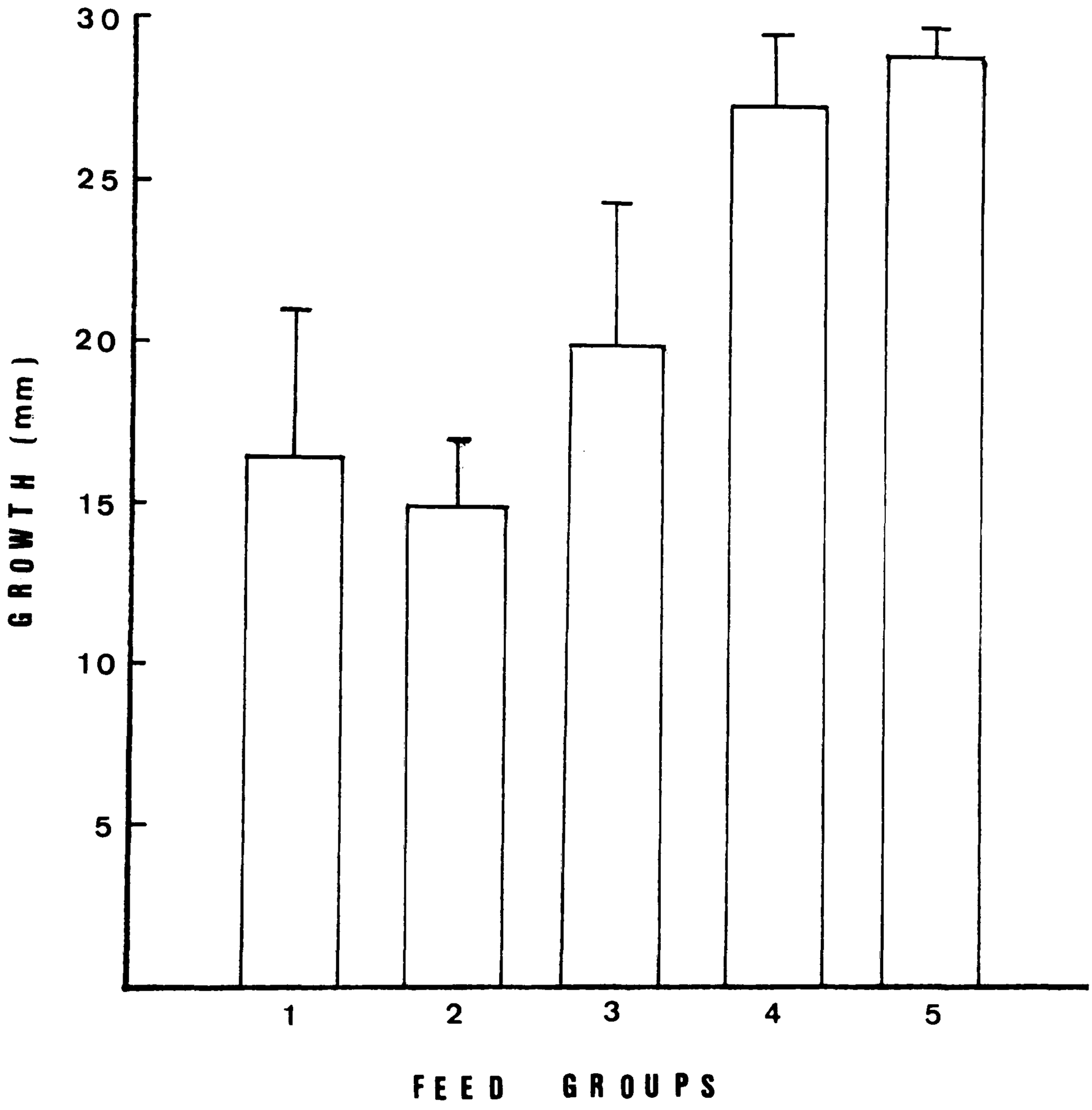

GROWTH (mm)
30
25
20
15
10
5
1
2
3
4
5
FEED GROUPS

Figure 7. Weight gained by P. monodon from nursery to juveniles on artificial feeds (for feed groups 1-5 see table 2). Vertical bars are standard deviation.

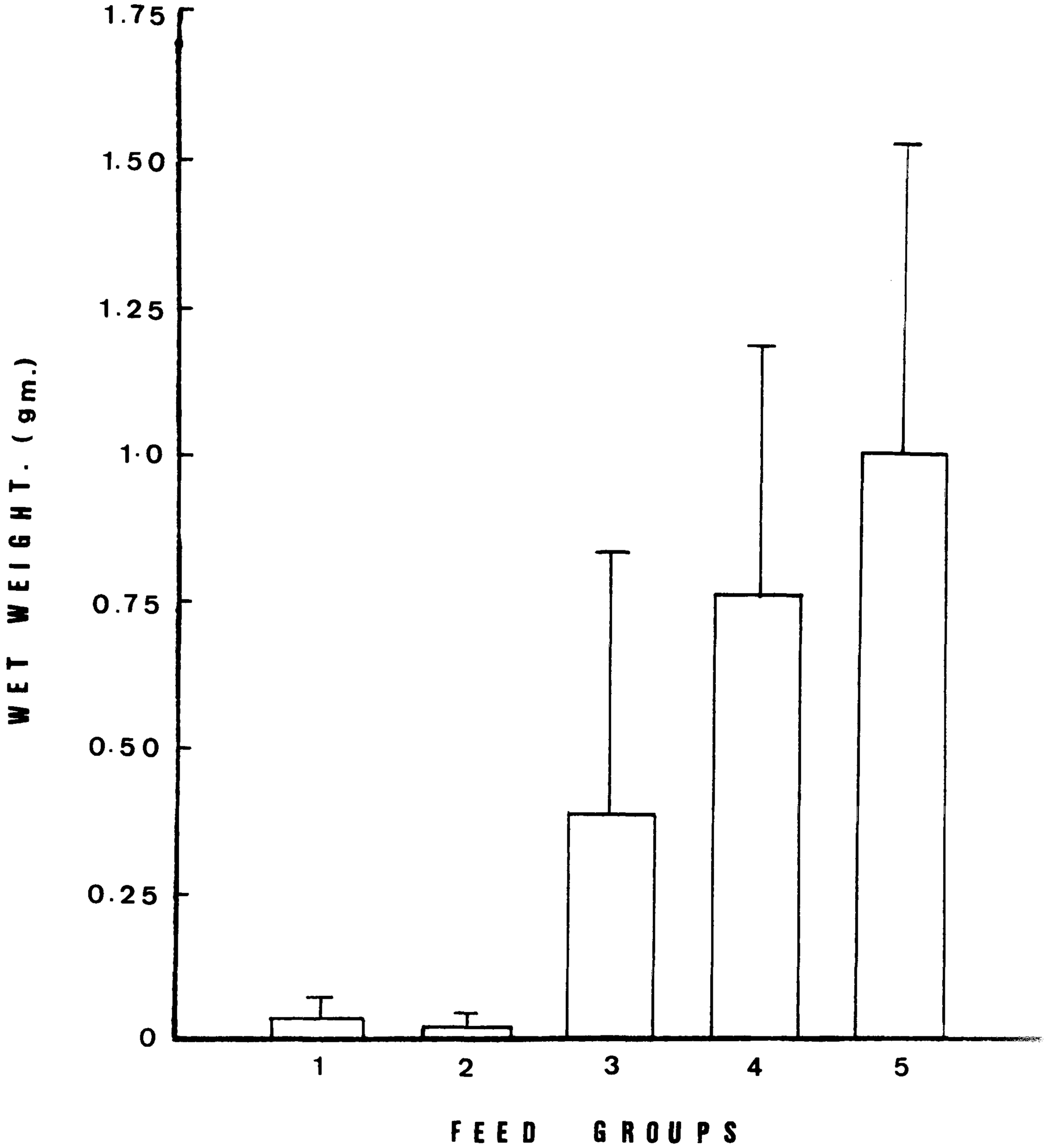
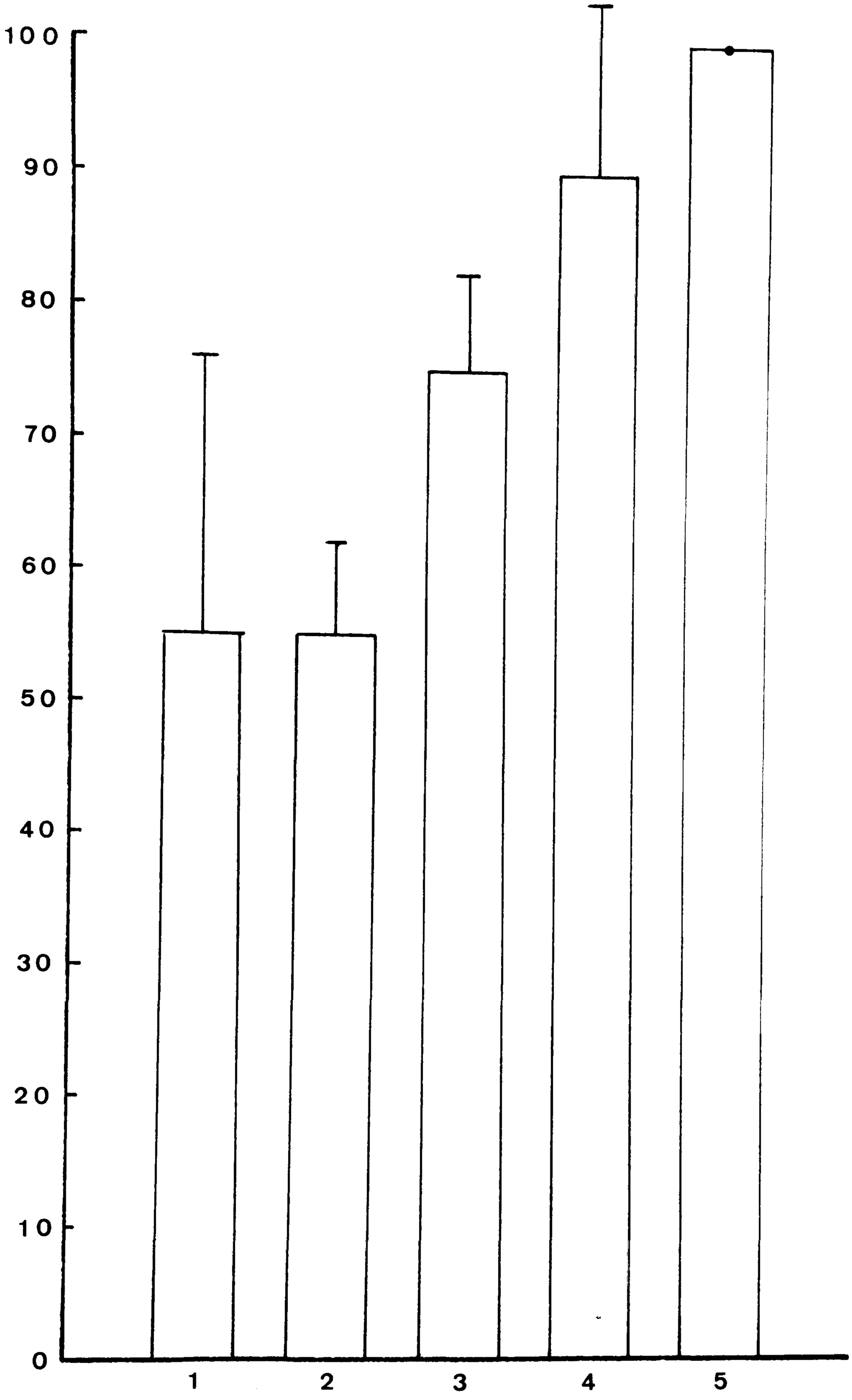

1.75
1.50
1.25
1·0
0.75
0·50
0.25
0
WET WEIGHT. (gm.)
1
2
3
4
5
FEED GROUPS

Figure 8. Percentage survival of P. monodon from nursery stage to juveniles on artificial feeds (for feed groups see table 2.) Vertical lines are standard deviation.


100
90
80
70
60
50
40
30
20
10
0
SURVIVAL (%)
1
2
3
4
5
FEED GROUPS

GENERAL DISCUSSION

GENERAL DISCUSSION

Continued demand for prawns worldwide has intensified prawn farming efforts to meet an ever increasing demand. Fish and shellfish demand is expected to grow to 130 million metric tons by the end of this century. In 1989 out of the total prawns (2.2 million metric tons) placed on the world market, 26% were farm produced - with prawn producing countries consuming 15% of the 1989 crop (Rosenberry, 1989).

Successful prawn farming requires dependable postlarval seed, either from the wild or from prawn hatcheries, nutritionally balanced and readily available diets and proper management. Supply of postlarvae from the wild is unpredictable depending upon environmental factors (Jones, 1988). Natural resources of prawns are diminishing due to over exploitation, destruction and pollution of their environment. Hence there is a genuine demand for cultured prawns (Liao, 1984). To attain the full production potential of hatcheries, a balanced nutritional diet capable of sustaining larval development and optimum survival to postlarval stages is required. The lack of essential nutrients in the diet can result in specific deficiency diseases, inhibit larval growth and cause general mortality. Larval nutrition is therefore one of the most important factors affecting larval development of penaeid prawns (Yufera et al., 1984).

Most prawn hatcheries are dependent on live foods for larval stages. Despite the recognised suitability of microalgae as aquaculture feeds, several problems currently

limit the reliability of algal mass production systems for aquaculture purposes. These constraints include variation in the nutritional value of microalgae due to changing culture conditions (Enright et al., 1986), difficulty in maintenance of monospecific algal cultures (Liao, 1983) and the expense of production and harvest on a mass scale (Fox, 1983; DePauw et al., 1984; Barclay et al., 1987). In present trials growth and survival responses of *P. monodon* protozoeal stages to five species of live microalgae at seven cell concentrations in replicated laboratory feed trial demonstrates that larval development was best on combination algal diet (Section 1) in a ratio of 1:1. Mixed algal diet provides the larvae with better balance of nutrients, such as amino acids, fatty acids and carbohydrates as well as micronutrients such as vitamins and trace minerals. Nutritional deficiency in one algal species may be compensated by its presence in another species. Failure to obtain optimum larval growth on some algal diets offered to protozoeal stages suggests nutritional limitations, or that the larval digestive system lacks enzymes capable of extracting nutrients from the armored frustrule (McConaugha, 1985). Algal food concentrations also play an important role in larval development. Protozoeal stages (PZ1) offered algal cell concentrations of 10 cells μl^{-1} failed to maintain larval growth and survival. The caloric value of 10 cells μl^{-1} was estimated at 0.124 Joules. Kurmaly et al., (1989b) estimated 0.746 Joules.larva^{-1}.day^{-1} of energy requirements for PZ1 stage. In present feed trials a minimum of 40 cells μl^{-1} (0.50 Joules.larva^{-1}.day^{-1}) of microalgae was required to

support good larval growth and survival. The protozoeal filter feeder moves to the next trophic level in the planktonic food chain and the mysis stage larvae become omnivorous with zooplankton as the preferred food. Although *Artemia* nauplii supported good larval growth and survival, they are expensive (Beidenbach et al., 1989) and can be unreliable from nutritional standpoint (Wickins, 1972). Live *Artemia* competes with penaeid larvae for the same source such as algae and dissolved oxygen in prawn hatcheries. *Artemia* cysts obtained from a polluted environment can introduce parasite in the culture system (Spotte, 1971). Hence there is pressure to develop artificial substitute diets for these live feeds.

A wide range of artificial feeds are currently manufactured using different process technologies as substitutes for live feeds used in prawn culture. Leading brands of ten artificial feeds evaluated as total replacements to live feeds, and with microalgae (10 cells μl^{-1}) as cofeed, revealed that Frippak microencapsulated feeds gave an overall significantly better larval growth and survival in replicated feed trials. Inclusion of live microalgae at 10 cells μl^{-1} as a supplemental cofeed with artificial diets produced improved larval growth and survival for most of the feed types tested in replicated feed trials. In an attempt to evaluate factors causing variability in larval growth and survival with artificial feeds, several factors were examined (Section 2). Gross nutritional composition of artificial larval diets compares favourably with that of natural algae and zooplankton. However feed particle stability in artificial larval feeds

appears to be a key factor. A strong correlation was found between leach loss, larval survival and growth. The breakdown and leaching of soluble components from rehydrated artificial feeds not only decrease food availability for larval growth, but also pollute culture water by increasing the production of bacteria and fouling larval cultures (Jones et al., 1974; 1975; 1987; Amjad and Jones, 1989a). Despite water renewals of 50% day^{-1} ammonia and nitrite levels do not decline to recommended safe limits for larval culture. Both ammonia and nitrite levels above 1.5 $mg.l^{-1}$ and 0.1 $mg.l^{-1}$ respectively produce physiological stress on early protozoeal stages, observed as a loss of appetite, revealed by the empty larval gut and the absence of faecal chains when food was abundantly available. Unionised ammonia diffuses quite readily across cell membranes in the gills (Fromm and Gillette, 1968) and interferes with the oxygen transport in fish (Smith and Williams, 1974; Smith and Russo, 1975). The same phenomena is believed to occur in crustaceans (Chen et al., 1986).

Apart from supplying all the nutrients essential for larval development microalgae plays an important role in conditioning water quality for penaeid larval culture (Amjad and Jones, 1989b). It has been demonstrated that modification of the 'green water' larval culture system (Ling, 1969) for *Macrobrachium* sp. allows the production of postlarvae without any water change, despite extensive use of artificial diets (Ang and Cheah, 1986). Amongst the six microalgal species tested at four levels of cell concentration *Chlorella* sp. at a 1000 cells μl^{-1} was most effective in reducing ammonia levels

by 99% and nitrite levels by 85.3% from the culture water (Section 3). Algae in the culture water environment act as a 'biological filter' system utilising ammonia and nitrite.

Metabolic products secreted by algae (40 cells μl^{-1}) trapped in culture water are ineffective as chemoattractants, although larval growth shows slight improvement, percentage survival however is not significantly different.

Improvements made in the dietary constituents of microcapsules (Section 4) by substituting proteins from different sources, lipid levels, vitamin and mineral mixes demonstrated improved larval growth and survival in replicated feed trials. Larval development however was still lower than growth and survival obtained on live feed controls. Algal supplements of 10 cells μl^{-1} to artificial feeds always gave significantly better larval growth and survival to postlarval (PL1) stage compared to larval development on unsupplemented feeds. Live algae supplied at a concentration of 10 cells μl^{-1} are unable to meet the energetic requirement for larval development. This algal cell concentration is insufficient to condition culture water. The metabolic secretions produced by this concentration of live algae are not effective in stimulating larval development. Yet 10 cells μl^{-1} of homogenised microalgae (*R. baltica + T. chuii*) as a supplement to microencapsulated feeds gave larval growth and survival comparable to larval development achieved on live feed controls.

It is believed (Greenberry and Thorndyke, 1989) that peptides which link amino acids to form proteins serve to

regulate the environment of digestive glands by enhancing digestive secretions. Chen and Lin (1989) demonstrated significant improvements in growth of *P. monodon* postlarvae fed compounded diets containing exogenous digestive enzymes. It is possible that peptides in microalgae may enhance digestive efficiency of larvae, resulting in increased growth increments and larval survival. Freezing of algal homogenates do not alter or denature the activity of homogenised algae.

Section 5 demonstrates for the first time that *P. monodon* larvae obtained from a single spawning can be successfully weaned through metamorphosis from protozoea (PZ1) to juvenile prawns solely on artificial feeds. Although larval survival was low at the end of the experiment, larval growth remained comparable between groups fed on artificial feeds and natural diets, with no significant difference appearing even at the end of 18 weeks.

It is concluded that the microencapsulated diets used in present work are capable of sustaining *P. monodon* throughout larval, postlarval and a substantial part of juvenile life in the absence of any other obvious source of nutrition. As such artificial diets form an ideal basis for further research into optimal nutritional requirements of penaeid prawns.

REFERENCES

REFERENCES

ACHE, B.W., 1983 : Chemoreception and thermoreception In. H.L. Atwood and D.C. Sandeman (Eds.), **Biology of Crustacea**, Vol. 3, Neurobiology : Structure and function. Academic Press Inc., pp. 369-398.

ADRON, J.W.; BLAIR, A. and COWEY, C.B., 1974 : Rearing of plaice (*Pluronectes platessa*) larvae to metamorphosis using an artificial diet. **Fishery Bulletin**, 72 : 353-357.

AKIYAMA, D.M., 1988 : Soybean meal utilization by marine shrimp. **Presented at the AOCS World Congress on Vegetable Protein Utilization in human food and animal feed stuff**, Singapore, October 2-7.

AKIYAMA, D.M. and DOMINY, W.G., 1989 : Penaeid shrimp nutrition for the commercial feed industry. **Texas Shrimp Farming Manual**, Volume 1 : Grow-out Technology, pp. 50.

ALAVA, V.R. and LIM, C., 1988 : Artificial diets for milkfish, *Chanos chanos* (Forsskal), fry reared in seawater. **Aquaculture**, 71 : 339-346.

ALVA, V.R. and PASCUL, F.P., 1987 : Carobhydrate requirements of *P.monodon* (Fab.) juveniles. **Aquaculture**, 61 : 211-217.

AMJAD, S.; JONES, D.A. and CHITRAVEDIVELU, K., 1989 : Advances in penaeid larval feed technology. **Presented at the Forum on New Technologies in Aquaculture.** Malaysian Fisheries Society and the Ministry of Science, Technology and Environment, pp. 16. (In press).

AMJAD S. and JONES, D.A., 1989a (Abstract) : A comparison of the stability of some artificial feeds used in penaeid larval culture. **Journal of the World Aquaculture Society** 20 (1) : 12A.

AMJAD, S. and JONES, D.A., 1989b (Abstract) : Role of algae in conditioning water quality for penaeid larval culture. **Journal of the World Aquaculture Society** 20 (1) : 12A.

ANDREW, J.W.; SICK, L.V. and BAPTIST, G.J., 1972 : The influence of dietary protein and energy levels on growth and survival of Penaeid shrimp. **Aquaculture**, 1 : 341-347.

ANG, K.J. and CHEAH, S.H., 1986 : Juvenile production of the Malaysian giant freshwater prawn (*Macrobrachium resenbergii* de Man) using modified static 'green water' system. **Proceedings of International Conference on Development and Management of Tropical Living Aquatic Resources.** Serdang, Malaysia, pp. 141-144.

ARMSTRONG, D.A., CHIPPENDALE, D., KNIGHT, A.W. and COLT, J.E., 1978 : Interaction of ionized and un-ionized ammonia on short-term survival and growth of prawn larvae, *Macrobrachium resenbergii*. **Biological Bulletin**, 154 : 15-31.

AQUACOP. 1977 : Reproduction in captivity and growth of *Penaeus monodon* Fabricius in Polynesia. **Proceedings of the World Mariculture Society**, 8 : 927-945.

AQUACOP, 1978 : Study of nutrition requirements and growth of *Penaeus merguiensis* in tanks by means of purified and artificial diets. **Proceedings of the World Mariculture Society**, 9 : 225-234.

AQUACOP, 1983 : Constitution of broodstock maturation, spawning, and hatching systems for penaeid shrimps in the Centre Oneanologique Du Pacifique. In : **CRC Handbook of Mariculture**, J.P. McVey and J.R. Moore (Editors). Vol. 1. Crustacean Aquaculture. CRC Press Inc. pp. 105-121.

BAGES, M. and SLOANE, L., 1981 : Effects of dietary protein and starch level on growth and survival of *Penaeus monodon* (Fabricus) postlarvae. **Aquaculture**, 25 : 117-128.

BARCLAY, W.R.; TERRY, K.L.; NAGLE, N.J.; WEISSMAN, J.C. and GOEBEL, R.P., 1987 : Potential of new strains of marine and inland saline-adapted microalgae for aquaculture. **Journal of the World Aquaculture Society**, Vol. 18 (4) : 218-228.

BARDACH, J.E.; RYTHER, J.H. and McLARNEY, 1972 : Aquaculture, the Farming and husbandry of freshwater and marine organisms. **A Wiley Interscience publication**, New York.

BAYNE, B.L., 1976 : The Biology of mussel larvae In. **Marine Mussels : Their ecology and physiology.** Eds. B.L. Bayne. Cambridge University Press Cambridge. 81-120.

√BEIDENBACH, J.M.; SMITH, L.L.; THOMSON, T.K. and LAWRENCE, A.L., 1989 : Use of the nematode *Panagrellus redivivus* as an Artemia replacement in a larval penaeid diet. **Journal of the World Aquaculture Society.** 20 (2) : 61-71.

BEN-AMOTZ, A.; FISHLER, R. and SCHNELLER, A., 1987 : Chemical composition of dietary species of marine unicellar and rotifers with emphasis on fatty acids. **Marine Biology,** 95 : 31-36.

BENDSCHNEIDER, K. and ROBINSON, J.P., 1952 : A new spectrophotometric method for the determination of nitrite in seawater. **Journal of Marine Research,** 11 (1) : 87-96.

√BOOKHOUT, C.G. and COSTLOW, J.D., 1970 : Nutritional effects of *Artemia* from different locations on larval development of crabs. **Helolander Wiss Meeres** 20 : 435-442.

BOWER, C.E. and BIDWELL, J.P., 1978 : Ionization of ammonia in seawater : effects of temperature, pH and salinity. **Journal of the Fisheries Research Board of Canada.** 35 (7) : 1012-1016.

BROWN, A., 1972 : Experimental techniques for preserving diatoms used as food for larval *Penaeus aztecus*. **Proc. Natn. Shellfish Association**. 62 : 21-25.

CARR, W.E.S.; ACHE, B.W. and GLEESON, R.A., 1987 : Chemoreceptors of crustaceans : Similarities to receptors for neuroactive substances in internal tissues. **Environ. Health Persp.**, 71 : 31-46.

CASTELL, J.D., 1970 : The essential fatty acid requirements of rainbow trout (*Salmo gairdneri*). Ph.D. thesis, Oregon State University, Corvallis, Oregon, pp. 116.

CASTELL, J.D., 1982 : Fatty acid metabolism in crustaceans. In : Biochemical and physiological approaches to shellfish nutrition. **Proceedings 2nd International Conference on Aquaculture Nutrition**, G.D. Pruder, C.J. Langdon and D.E. Conklin. World Mariculture Society, Special Publication No. 2, Louisiana State University, Louisiana, 124-145.

CATACUTAN, M.R. and DE LA CRUZ, M., 1989 : Growth and mid-gut cells profile of *Penaeus monodon* juveniles fed water-soluble vitamin deficient diets. **Aquaculture**, 81 : 137-144.

CHANG, T.M.S.; MacINTOCH, F.C. and MASON, S.G., 1966 : Semipermeable aqueous microcapsules : Preparation and properties. **Canadian Journal of Physiology and Pharmacology**, 44 : 115-128.

CHEN, H.Y. and LIN, H.F., 1989 (Abstract) : The effects of exogenous digestive enzymes on the growth of early postlarval *Penaeus monodon*. **European Aquaculture Society,** Quarterly Newsletter 53/54.

CHEN, J.C. and CHIN, T.S., 1988 : Acute toxicity of nitrite to tiger prawn, *Penaeus monodon* larvae, **Aquaculture**, 69 : 253-262.

CHEN, J.C.; CHIN, T.C. and LEE, C.K., 1986 : Effects of ammonia and nitrite on larval development of the shrimp (*Penaeus monodon*). In: **The First Asian Fisheries forum.** Editors J.L. MacLean, L.B. Dizon and L.V. Hosillos. The Asian Fisheries Society, Manila, Philippines. pp. 657-662.

CHEN, J.C.; LIU, P.C. and LIN, Y.T., 1989 : Culture of *Penaeus monodon* in an intensified system in Taiwan. **Aquaculture,** 77 : 319-328.

CHIN, T.S. and CHEN, J.C., 1987 : Acute toxicity of ammonia to larvae of the tiger prawn *Penaeus monodon*. **Aquaculture**, 66 : 247-253.

CHU, F.; WEBB, K.L.; HEPWORTH, D. and ROBERTS, M., 1982 : The acceptability and digestibility of microcapsules by larvae of *Crassostrea virginica*. **Journal of Shellfish Research**, 2 (1) : 29-34.

CHU, K.H. and SHING, C.K., 1986 : Feeding behaviour of the shrimp, *Metapenaeus ensis*, on *Artemia nauplii*. **Aquaculture**, 58 : 175-184.

CHU, F.-L.E.; WEBB, K.L.; HEPWORTH, D.A. and CASEY, B.B., 1987 : Metamorphosis of the larvae of *Crassostrea virginica* fed microencapsulated diets. **Aquaculture**, 64 : 185-197.

CHUECAS, L. and RILEY, J.P., 1969 : The component combined amino acids of some marine diatoms. **Journal of the Marine Biological Association**, *U.K.* 49 : 117-120.

CLARK, J.; JONES, D.A. and KURMALY, K., 1986 : Penaeid larval rearing and microencapsulated feeds. **Presented at the 1st Inter-American Congress of Aquaculture**, 14-21 September 1986, Salvador, Brazil.

√CLAUS, C.; BENIJTS, F.; VANDEPUTTE, G. and GARDNER, W., 1979 : The biochemical compostion of the larvae of two strains of *Artemia salina* (L.) reared on two different algal foods. **Journal of Experimental Marine Biology and Ecology**, 36 : 171-183.

COLLINS, M.T.; GRATZEK, J.B.; SHOTTS, E.B.; DAWE, D.L.; CAMPBELL, L.M. and SENN, D.R., 1975 : Nitrification and aquatic recirculation system. **Journal of Fishery Research Board of Canada**, 32 : 2025-2031.

COLT, J.E. and ARMSTRONG, D.A., 1981 : Nitrogen toxicity to crustaceans, fish and molluscs. In: L.J. Allen and E.C. Kinney (Eds.), **Proceedings of the Bio-Engineering Symposium for Fish Culture.** Fish Culture Selection, Northeast Society of Conservation Engineers, Bethesda, MD, pp. 34-47.

COLT, J. and TCHOBANAOGLOUS, G., 1976 : Evaluation of the short-term toxicity of nitrogenous compounds to channel catfish, *Ictalurus punctatus*. **Aquaculture**, 8 : 209-224.

COLVIN, P.M., 1976 : The effect of selected seed oils on the fatty acid composition and growth of *Penaeus indicus*. **Aquaculture**, 8 : 81-89.

COOK, H.L. and MURPHY, M.A., 1966 : Rearing penaeid shrimp from eggs to postlarvae. **Proceedings of the Conference of Southeast Association, Game Commission**, 19 : 283-288.

√COOK, H.L. and MURPHY, M.A., 1969 : The culture of larval penaeid shrimp. **Trans. Am. Fish Soc.**, 98 : 751-754.

COOK, H.L. and MURPHY, M.A., 1971 : Early developmental stages of the brown shrimp, *Penaeus aztecus* Ives, reared in the laboratory. **Fishery Bulletin Fisheries and Wildlife Service,** *U.S.* 69 (1) : 223-239.

COWEY, C.B. and FORSTER, J.R.M., 1971 : The essential amino-acid requirements of the prawn, *Palaemon serratus*. The growth of prawns on diets containing proteins of different amino-acid compositions. **Marine Biology**, 10 : 77-81.

CRISP, D.J., 1956 : A substance promoting the hatching and liberation of young in cirripedes. **Nature**, London. 178 : 263.

CRISP, D.J. and SPENCER, C.P., 1958 : The control of the hatching process in barnacles. **Proceedings of the Royal Society**, 148 : 278-299.

DePAUW, N.; MORALES, J. and PERSOONE, G., 1984 : Mass culture of microalgae in aquaculture : progress and constraints. **Hydrobiologia** 116/117 : 338-342.

DePAUW, N. and PERSOONE, G., 1988 : Micro-algae for aquaculture. In. **Microalgal Biotechnology** eds. M.A. Borowitzka and L.J. Borowitzka. Cambridge University Press, Cambridge. 197-221.

DESHIMARU, O., 1982 : Protein and amino acid nutrition of the prawn *Penaeus japonicus*. In : **Proceedings of the 2nd International Conference on Aquaculture Nutrition : Biochemical and Physiological approaches to shellfish nutrition.** Editors. G.D. Pruder, C. Langdon and D. Conklin. World Aquaculture Society. Louisiana. 106-123.

DESHIMARU, O. and SHIGEUO, K., 1972 : Introduction to the artificial diet for prawn *Penaeus japonicus*. **Aquaculture**, 1 : 115-133.

EMMERSON, W.D., 1980 : Ingestion, growth and development of *Penaeus indicus* larvae as a function of *Thalassiosira weissflogii* cell concentration. **Marine Biology**, 58 : 65-73.

EMMERSON, W.D., 1984 : Predation and energetics of *Penaeus indicus* (Decapoda : Penaeidae) larvae feeding on *Brachionus plicatilis* and *Artemia* nauplii. **Aquaculture**, 38 : 201-209.

ENRIGHT, C.T.; NEWKIRK, G.F.; CRAIGIE, J.S. and CASTELL, J.D., 1986 : Evaluation of phytoplankton as diets for juvenile *Ostrea edulis* L. **Journal of Experimental Marine Biology and Ecology**, 96 : 1-13.

FERNANDEZ-REIRIZ, M.J.; PEREZ-CAMACHO, A.; FERREIRO, M.J.; BLANCO, J.; PLANAS, M.; CAMPOS, M.J. and LABARTA, U., 1989 : Biomass production and variation in biochemical profile (total proteins, carbohydrates, RNA, lipids and fatty acids) of seven species of marine microalgae. **Aquaculture**, 83 : 17-37.

FISHER, L.R., 1960 : Vitamins. In : T.H. Waterman (ed), **The Physiology of Crustacea**, Vol. 1, Acad. Press, New York, N.Y. : 259-289.

FOOD AND AGRICULTURE ORGANISATION OF THE UNITED NATIONS 1982 : **FAO Fisheries Circular No. 722.** FAO, Rome, Italy.

FORSTER, J.R.M., 1976 : Studies on the development of compounded diets for prawns. **Proc. First Int. Conf. Aquacult. Nutr.** NOAA (sea grant) 229-248.

FOX, J.M., 1983 : Intensive Algal Culture Techniques. In : **CRC Handbook of Mariculture Vol. 1. Crustacean Aquaculture** eds. James P. McVey. CRC Press Inc. Florida, 15-41.

FROMM, P.O. and GILLETTE, J.R., 1968 : Effect of ambient ammonia on blood ammonia and nitrogen excretion of Rainbow trout (*Salmo gairdnerii*). **Comparative Biochemistry and Physiology**, 26 : 887-896.

GABAUDAN, J.; PIGOTT, G.M. and HALVER, J.E., 1980 : The effect of processing on protein ingredients for larval diet : Biological evaluation. **Proceedings of the World Mariculture Society**, 11 : 424-432.

GABBOTT, P.A.; JONES, D.A. and NICHOLS, D.H., 1975 : Studies on the design and acceptability of micro-encapsulated diets for marine particle feeders. II. Bivalve molluscs. In : G. Persoone and E. Jaspers (eds.), **Proceedings of the 10th European Symposium of Marine Biology**, Ostend, Belgium, Sept. 17-23, 1 : 127-141.

GATESOUPE, F.J.; GIRIN, M. and LUGUET, P., 1977 : Recherche d'une alimentation artificial adaptee a l'elevage Larvaire du Bar et de la sole. **Actes de Colloques du Centre National Pour l'Exploitation des Oceans**, 4 : 59-66.

GIBSON, R., 1983 : Feeding and digestion in decapod crustaceans. **Proceedings of the Second International Conference on Aquaculture Nutrition**, World Mariculture Society, Special publication No. 2 : 59-86.

GOLDBLATT, M.J.; CONKLIN, D.E. and BROWN, W.D., 1979 : Nutrient leaching from pelleted ratios. In : **Fin Fish nutrition and fishfeed technology**, (J.E. Halver and K. Teinos (Eds), Vol II. H. Heenemann GmbH and Co., Berlin, 117-124.

GOLDBLATT, M.J.; CONKLIN, D.E. and BROWN, W.D., 1980 : Nutrient leaching from coated crustacean rations. **Aquaculture**, 19 : 383-388.

GRABNER, M.; WEISER, W. and LACKNER, R., 1981 : The suitability of frozen and freeze-dried zooplankton as food for fish larvae : a biochemical test program. **Aquaculture**, 26 : 85-94.

GREENBERRY, M.J. and THORNDYKE, M.C., 1989 : Consistency and variability in peptide families : Introduction. **Biological Bulletin** 177 : 167-171.

GUARY, J.C.; KAYAMA, M.; MURAKAMI, Y. and CECCALDI, H., 1976 : The effects of a fat-free diet and compounded diets supplemented with various oils on a moult, growth and fatty acid composition of prawn, *Penaeus japonicus* Bate, **Aquaculture**, 7 : 245-254.

HARTENSTEIN, R., 1970 : Nitrogen metabolism in non-insect anthropods. In : J.W. Campbell (editor), **Comparative Biochemistry of Nitrogen Metabolism** Vol. 1, Academic Press, pp. 299-372.

HEINEN, J.M., 1980 : Chemoreception in decapod crustacea and chemical feeding stimulants as potential feed additives. **Proceedings of the World Mariculture Society**, 11 : 319-334.

HELM, M.M. and LAING, I., 1981 : Cost effective culture of marine unicellular algae. In : F. Vogt (Ed), **Energy Conservation and Use of Renewable Energies in the Bioindustries.** Pergamon Press, Oxford and New York, N.Y. 247-259.

HEW, M. and CUZON, G., 1982 : Effects of dietary lysine and arginine levels, and their ratio, on the growth of *Penaeus japonicus* juveniles. **Journal of the World Mariculture Society.** 13 : 154-156.

HIRATA, H.; ANASTASIOS, M. and YAMASAKI, S., 1985 : Evaluation of the use of *Brachionus plicatilis* and *Artemia* for rearing prawn *Penaeus japonicus* larvae on a laboratory scale. **Memoirs of the Faculty of Fisheries,** Kagoshima University, Japan. 34 (1) : 27-36.

HIRATA, H.; MORI, Y. and WATANABE, W., 1975 : Rearing of prawn larvae, *Penaeus japonicus,* fed soy cake particles and diatoms. **Marine Biology.** Vol. 29 : 9-13.

HOCHACHKA, P.W. and SOMERS, G.N., 1973 : Strategies of Biochemical Adaptation. W.B. Saunders, Philadelphia, Pa., pp. 358.

HOLLAND, D.L., 1978 : Lipid reserves and energy metabolism in the larvae of benthic marine invertebrates. In : **Biochemical and Biophysical Perspectives in Marine Biology.** Editors D.C. Malins and J.R. Sargent. Academic Press, London. 4 : 85-123.

HUDINAGA, M., 1942 : Reproduction, development and rearing of *Penaeus japonicus* Bate. **Japanese Journal of Zoology,** 10 : 305-393.

HUDINAGA, M. and KITTAKA, J., 1967 : The large scale production of young Kuruma prawn, *Penaeus japonicus* Bate. **Bull. Planktol. Japan** (Commemoration Vol., Dr. Matsue's 60th birthday) : 35-46.

JAMES, C.M.; AL-KHARS, A.M. and CHORBANI, P., 1988 : pH dependent growth of *Chlorella* in a continuous culture system. **Journal of the World Aquaculture Society**. Vol. 19 No. 2 : 27-35.

JAYASANKAR, P. and MUTHU, M.S., 1983a : Toxicity of ammonia to larvae of *Penaeus indicus* H. Milne Edwards. **Indian Journal of Fisheries**, 30 (1) : 1-12.

JAYASANKAR, P. and MUTHU, M.S., 1983b : Toxicity of nitrite to the larvae of *Penaeus indicus* H. Milne Edwards. **Indian Journal of Fisheries**, 30 (2) : 231-240.

JONES, D.A., 1988 : Tropical Prawn culture. **Science and Technology Review No.4**. Autumn, University College of North Wales, 51-58.

JONES, D.A.; AMJAD, S. and CHITRAVADIVELU, K., 1989 : Comparison of Artificial feeds Used in penaeid shrimp hatcheries. **Third Egyptian-British Conference on Animal, Fish and Poultry Production**, Alexandria, 15-22.

JONES, D.A. and GABBOTT, P.A., 1976 : Prospects for the use of microcapsules as food particles for marine particulate feeders. In: J.R. Nixon (Ed.), **Proc. 2nd. Int. Symp. Microencapsulation**, Marcel Dekker Inc., New York, NY, pp. 77-91.

JONES, D.A.; HOLLAND, D.L. and JABBORIE, S., 1984 : Current status of microencapsulated diets for aquaculture. **Applied Biochemistry and Biotechnology**. 10 : 275-288.

JONES, D.A.; JAWED, T. and TILY, P., 1972 : The acceptance of artificial particles by planktonic crustacea. **Chemosphere** 3 : 133-136.

JONES, D.A.; KANAZAWA, A. and RAHMAN, S.A., 1979a : Studies on the presentation of artificial diets for rearing the larvae of *Penaeus japonicus* (Bate). **Aquaculture**, 17 : 33-43.

JONES, D.A.; KANAZAWA, A. and ONO, K., 1979b : Studies on the nutritional requirement of larval stages of *Penaeus japonicus* using microencapsulated diets. **Marine Biology**. 54 : 216-267.

JONES, D.A. and KURMALY, K., 1987 : Feeding and assimilation of artificial feeds by crustacean larvae. **Journal of the World Aquaculture Society**, 18 (1) 56-57.

JONES, D.A.; KURMALY, K. and ARSHAD, A., 1987 : Penaeid shrimp hatchery trials using microencapsulated diets. **Aquaculture**, 64 : 133-146.

JONES, D.A.; MOLLER, T.H.; CAMPBELL, R.J.; MUNFORD, J.G. and GABBOTT, P.A., 1975 : Studies on the design and acceptability of micro-encapsulated diets for marine particulate feeders I. Crustacea. In : G. Persoone and E. Jaspers (eds.), **Proceedings of the 10th European Symposium of Marine Biology**, Ostend Belgium. Wetteren, Belgium, Universa Press, 229-239.

JONES, D.A.; MUNFORD, J.G. and GABBOTT, P.A., 1974 : Microcapsules as Artificial food particles for aquatic filter feeders. **Nature**, London., 247 : 233-235.

JORGENSEN, B., 1977 : Nutrition. In : **Animal Physiology**, 3rd edition, Editor Malcolm, G. Macmillan publication, 16-55.

KANAZAWA, A., 1982 : Penaeid nutrition. In : Biochemical and Physiological approaches to shellfish nutrition. **Proceedings 2nd International Conference on Aquaculture Nutrition**. G.D. Pruder, C.J. Langdon and D.E. Conklin. World Mariculture Society. Special publication no. 2, Louisiana State University, Louisiana. 87-105.

KANAZAWA, A., 1989 : Prawn nutrition. Presented at **Forum on New Technologies in Aquaculture**. Putra World Trade Centre, Kuala Lumpur, pp. 1-18.

KANAZAWA, A.; SHIMAYA, M.; KAWASAKI, M. and KASHIWADA, K., 1970 : Nutritional requirements of prawn - I : Feeding on artificial diet. **Bulletin of the Japanese Society of Scientific Fisheries**, 37 : 949-954.

KANAZAWA, A.; TANAKA, N.; TESHIMA, S. and KASHIWADA, K., 1971 : Nutritional requirements for prawn - II : Requirement for Sterols. **Bulletin of the Japanese Society of Scientific Fisheries**, 37 : 211-215.

KANAZAWA, A.; TESHIMA, S.; KOBAYASHI, T.; IWASHITA, T.; KAWASAKI, M., 1983 : Rearing of the Larval crab *Portunus trituberculatus*, with the artificial microparticulate diets. **Mem. Fac. Fish.**, Kagoshima University, Vol. 32 : 121-127.

KANAZAWA, A.; TESHIMA, S. and SAKAMOTO, M., 1985 : Effect of dietary lipids, Fatty acids and phospholipids on growth and survival of Prawn (*Penaeus japonicus*) larvae. **Aquaculture**, 50 : 39-49.

KANAZAWA, A.; TESHIMA, S. and SASADA, H., 1982 : Culture of prawn larvae with microparticulate diets. **Bulletin of the Japanese Society of Scientific Fisheries**, 48 : 195-199.

KANAZAWA, A.; TESHIMA, S. and TOKIWA, S., 1977a : Nutritional requirement of prawn - VII. Effect of dietary lipids on growth. **Bulletin of the Japanese Society of Scientific Fisheries**, 43 : 849-856.

KANAZAWA, A.; TOKIWA, S. and KAYAMA, M., 1977b : Essential fatty acids in the diet of prawn - I. Effects of linoleic and linolenic acids on growth. **Bulletin of the Japanese Society of Scientific Fisheries**, 43 : 1111-1114.

KANAZAWA, A.; TESHIMA, S.; TOKIWA, S.; KAYMA, M. and HIRATA, M., 1979a : Essential fatty acids in the diet of prawn - II. Effect of docosahexaenoic acid on growth . **Bulletin of the Japanese Society of Scientific Fisheries**, 45 : 1151-1153.

KANAZAWA, A.; TESHIMA, S. and ONO, K., 1979b : Relationship between essential fatty acid requirements of aquatic animals and the capacity for bioconversion of Linolenic acid to highly unsaturated fatty acids. **Comparative Biochemistry and Physiology**. 63B : 295-298.

KANAZAWA, A. and TESHIMA, S., 1981 : Essential amino acids of the prawn. **Bulletin of the Japanese Society of Scientific Fisheries.** 47 : 1375-1377.

KHANNAPA, A., 1977 : Effect of various protein levels on growth and survival rates of *Penaeus monodon*. **Quarterly Research Report**, SEAFDEC Aquaculture Department 1 (1) : 24-28.

KIESE, M., 1974 : Methemoglobinemia : A comprehensive Treatise. Editor. M. Kiese. CRC Press, Cleveland, OH, pp. 259.

KITABAYASHI, K.; SHUDO, K,; NAKAMURA, K. and ISHIKAWA, S., 1971 : Studies on formula feed for Kuruma prawn - II : On the Utilization values of glucose. **Bulletin Tokai Reg. Fish. Res. Lab.**, 65 : 109-118.

KITTAKA, J., 1976 : Food and growth of penaeid shrimp. **Proc. First Int. Conf. on Aquacult. Nutr.**, Delaware, NOAA (sea grant), 249-285.

KOSHIO, S.; KANAZAWA, A.; TESHIMA, S. and CASTELL, J.D., 1989 : Nutritional evaluations of crab protein for larval *Penaeus japonicus* fed microparticulate diets. **Aquaculture**, 81 : 145-154.

KUBAN, F.D.; LAWRENCE, A.L. and WILKENFELD, J.S., 1985 : Survival, metamorphosis and growth of larvae from four penaeid species fed six food combinations. **Aquaculture**, 47 : 151-162.

KUBAN, F.D.; WILKENFELD, J.S. and LAWRENCE, A.L., 1983 : Survival and growth of *Penaeus setiferus* L. and *Penaeus aztecus* Ives larvae fed *Artemia* beginning at the protozoea two substage versus the mysis-one substage. **Journal of the World Mariculture Society**, 14 : 38-48.

KURMALY, K.; JONES, D.A.; YULE, A.D. and EAST, J., 1989a : Comparative analysis of the growth and survival of *Penaeus monodon* (Fabricius) larvae from protozoea 1 to postlarva 1, on live feeds, artificial diets and on combination of both. **Aquaculture**, 81 : 27-45.

KURMALY, K.; YULE, A.B. and JONES, D.A., 1989b : An energy budget for the larvae of *Penaeus monodon* (Fabricius). **Aquaculture**, 81 : 13-25.

LANGDON, C.J., 1980 : The nutrition of *Crassostrea gigas* (Thurnberg) larvae and spat fed on artificial diets. Ph.D. dissertation, University College of North Wales, Bangor, U.K.

LANGDON, C.J., 1983 : New techniques and their application to studies of bivalve nutrition. **Proceedings of the Second International Conference on Aquaculture Nutrition**. World Mariculture Society, Special publication No. 2 : 305-320.

LANGDON, C.J., 1989 : Preparation and evaluation of protein microcapsules for a marine suspension-feeder, the Pacific oyster *Crassostrea gigas*. **Marine Biology**, 102 : 217-224.

LANGDON, C.J. and BOLTON, E.T., 1984 : A microparticulate diet for a suspension feeding bivalve mollusc, *Crassostrea virginica* (Gmelin). **Journal Experimental Marine Biology and Ecology**, Vol. 82 : 239-258.

LANGDON, C.J.; LEVINE, D.M.; JONES, D.A., 1985 : Microparticulate feeds for marine suspension-feeders. J. **Microencapsulation**, Vol. 2, No. 1 : 1-11.

LANGDON, C.J. and SIEGFRIED, C.A., 1984 : Progress in the development of artificial diets for bivalve filter feeders. **Aquaculture**, 39 : 135-153.

LANGDON, C.J. and WALDOCK, M.J., 1981 : The effect of algal and artificial diets on the growth and fatty acid composition of *Crassostrea gigas* spat. **Journal of the Marine Biological Association of the United Kingdom**, 61 : 431-448.

LEE, D.L., 1971 : Studies on the protein utilization related to growth of *Penaeus monodon* Fabricius. **Aquaculture**, 1 : 1-13.

LEGER, P.; BENGTSON, D.A.; SIMPSON, K.L. and SORGELOOS, P., 1986 : The use and nutritional value of *Artemia* as a food source. **Oceanography and Marine Biological Annual Review.**, 24 : 521-623.

LEGER, P.; BLEBER, G.F. and SORGELOOS, P., 1985 : International study on *Artemia* XXXIII. Promising results in larval rearing of *Penaeus stylirostris* using a prepared diet as algal substitute and for *Artemia* enrichment. **Journal of the World Mariculture Society**, 16 : 354-367.

LEI, C.H. and SU, H.M., 1985 : Growth and survival of *Penaeus monodon* larvae fed selected food items. **Journal of Fisheries Society**, Taiwan, 12 (2) : 54-69.

LEVINE, D.M. and SULKIN, S.D., 1984 : Ingestion and assimilation of microencapsulated diets by Brachyuran crab larvae. **Marine Biology letters**, 5 : 147-153.

LEVINE, D.M.; SULKIN, S.D. and VAN HEUKELEM, L., 1983 : The design and development of microencapsulated diets for the study of nutritional requirements of brachyuran crab larvae. In. **Culture of Marine Invertebrates:** *Selected Readings,* ed. C.J. Berg Jr. Hutchinson Ross, 193-204.

LIAO, I.C., 1977 : Manual on propagation and cultivation of grass prawn, *Penaeus monodon*. Tungkang Marine Laboratory, Tungkang, Taiwan.

LIAO, I.C., 1984 : A brief review of the larval rearing techniques of penaeid prawns. **Proceedings of the First International Conference on the Culture of Penaeid Prawns/Shrimps**. Iloilo City, Philippines, 1 : 65-78.

LIAO, I.C. and CHAO, N.H., 1983 : Hatchery and grow-out : Penaeid prawns. In : **CRC Handbook of Mariculture**, J.P. McVey and J.R. Moore (Editors). Vol. I. Crustacean Aquaculture, CRC Press Inc. pp. 161-167.

LIAO, I.C. and HUANG, T.L., 1972 : Experiments on the propagation and culture of prawns in Taiwan. In. T.V.R. Pillay (Editors), **Coastal Aquaculture in the Indo-Pacific Region**. Fishing News (Books) Ltd., Farnham, U.K., 328-354.

LIAO, I.C.; KUMENO, F., IIDA, Z. and KOBAYASHI, T., 1988 : Preliminary report on the application of artificial plankton B.P. in *Penaeus monodon* larval production. **19th Annual Meeting of the World Aquaculture Society**, Hawaii, 1-9.

LIAO, I.C.; SU, M.H. and LIN, J.H., 1983 : Larval foods for penaeid prawns In: **CRC Handbook of Mariculture**, Vol. 1 Crustacean Aquaculture. (Ed.) James, P. McVey. CRC Press Inc. Florida, 43-69.

LIGHTNER, O.V.; COLVIN, L.B.; BRAND, C. and DANALD, D.A., 1977 : 'Black Death' - a disease syndrome of penaeid shrimp related to a dietary deficiency of ascorbic acid. **Proceedings Annual Meeting World Mariculture Society**, 8 : 611-624.

LING, S.W., 1967 : The general biology and development of *Macrobrachium resenbergii* (de Man). FAO World Scientific Conference on the Biology and Culture of Shrimps and Prawns, Mexico. **FAO Fishery Report**, 57 (3) : 607-619.

LING, S.W., 1969 : Methods of rearing and culturing *Macrobrachium rosenbergii* (de Man). **FAO Fisheries Report**, 57 (3) : 607-619.

LINSTEDT, K.J., 1971 : Review article. Chemical control of feeding behaviour. **Comparative Biochemistry and Physiology.** 39A : 553-581.

LUMARE, F.; GOZZO, S. and BLUNDO, C.M., 1978 : Studies on the feeding of the protozoea and mysis of *Penaeus Kerathurus* in mass culture. **Atti. Soc. Peloritana Sci. Fis. Mat. Nat.**, Messina, 24 : 315-322.

MacDONALD, N.L.; STARK,J.R. and KEITH, M., 1989 : Digestion and nutrition in *Penaeus monodon*. (Abstract) **Journal of the World Aquaculture Society** Vol. 20 (1) : 53A.

MAGARELLI, P.C.; HUNTER, B.; LIGHTNER, D.V. and COLVIN, L.B., 1979 : Black Death. An ascorbic acid deficiency disease in penaeid shrimp. **Comparative Biochemistry and Physiology**, 63A : 103-108.

MALCA, R.P., 1983 : Penaeus shrimp pond grow-out in Panama. In : **CRC Handbook of Mariculture**, J.P. McVey and J.R. Moore (Editors). Vol I. Crustacean Aquaculture. CRC Press, Inc. pp. 169-178.

McCONAUGHA, J.R., 1985 : Nutrition and larval growth. In: **Larval Growth** Vol. 2. Ed. A.M. Wenner. Rotterdam, Balkema. 127-154.

McVEY, P.J. and FOX, J.M., 1983 : Hatchery techniques for penaeid shrimp utilized by Texas AEM-NMFS Galveston laboratory program. In: **CRC Handbook of Mariculture** Vol. 1., **Crustacean Aquaculture**. Editors J.P. McVey and J.R. Moore. CRC Press, Inc. Florida. pp. 129-154.

MEVEL, G. and CHAMROUX, S., 1981 : A study on nitrification in the presence of prawn (*Penaeus japonicus*) in marine closed system. **Aquaculture** 23 : 29-43.

MEYERS, S.P., 1971 : Crustacean ration formulation research. **Feedstuff**, 43 : 27.

MOCK, C.R.; FONTAINE, C.T.; REVERA, D.B., 1980a : Improvements in rearing larval penaeid shrimp by the Galveston Laboratory method. In: G. Persoone, P. Sorgeloos, O. Roels and E. Jaspers (Eds.), **The Brine Shrimp** *Artemia* Vol. 3 : 331-342. Wetteren, Belgium : Universa Press.

MOCK, C.R., REVERA, D.B. and FONTAINE, C.T., 1980b : Preliminary observations on the larval culture of *Penaeus stylirostris* using modifications of the Galveston technique. **Proceedings of the World Mariculture Society**, 11 : 102-117.

MOCK, C.R. and MURPHY, M.S., 1971 : Techniques for raising penaeid shrimp. In: J.W. Avault, Jr., E. Boudreaux and E. Jasper (Eds.), **Proceedings of the First Annual Workshop World Mariculture Society**. Louisiana State University, Baton Rouge, L.A., 143-156.

MOLLER, T.H.; JONES, D.A. and GABBOTT, P.A., 1979 : Further developments in the microencapsulation of diets for marine animals used in aquaculture. **Proceedings of the Third International Symposium of Microencapsulation** T. Kondo (Editor). Tokyo, : 223-229.

MOLLY, C.J. and SYRETT, P.J., 1988 : Interrelationships between uptake of urea and uptake of ammonium by microalgae. **Journal of Experimental Marine Biology and Ecology**, Vol. 118 : 85-95.

MOTOH, H., 1979 : Larvae of decapod crustacea of the Philippines, III. Larval development of the giant tiger prawn *Penaeus monodon* reared in the laboratory. **Bulletin of the Japanese Society of Scientific Fisheries**, 45 (10) : 1201-1216.

MURAI, T. and ANDREWS, J.W., 1978 : Comparison of feeds for larval stages of the giant prawn (*Macrobrachium rosenbergii*). **Proceedings of the World Mariculture Society**, 9 : 189-193.

NEW, M.B., 1976 : A review of dietary studies with shrimps and prawns. **Aquaculture** 9 : 101-144.

NEW, M.B., 1980 : A bibliography of shrimp and prawn nutrition. **Aquaculture**, 21 : 101-128.

NEW, M.B., 1987 : Feed and feeding of Fish and shrimp - A manual on the preparation and presentation of compound feeds for shrimp and fish in aquaculture. **Aquaculture Development and Coordination Programme ADCP/REP/87/26**. FAO, Rome. pp. 275.

OGINO, C. and YANG, G.Y., 1980 : Requirement of carp and rainbow trout for dietary manganese and copper. **Bulletin of the Japanese Society for Science and Fisheries**. 45 : 967-969.

OPPENHEIMER, C.H. and ZOBELL, C.E., 1952 : The growth and viability of sixty-three species of marine bacteria as influenced by hydrostatic pressure. **Journal of Marine Research**, 11 : 10-18.

PAFFENHÖFFER, G.A. and STRICKLAND, J.D.H., 1970 : A note on the feeding of *Calanus helgolandicus* on detritus. **Marine Biology**, 5 : 97-99.

PARRY, G., 1960 : Excretion. In: T.H. Waterman (Editor), **The Physiology of Crustacea**, Vol. 1. Academic Press, New York, N.Y. pp. 341-366.

PARSONS, T.R.; MAITA, Y. and LALLI, C.M., 1985 : A manual of chemical and biological methods for seawater analysis. Pergamon Press, Oxford, England. pp. 173.

PARSONS, L.M., Stephens, K. and Strickland, J.D.H.,1961: On the chemical composition of eleven species of marine phytoplankters. J. Fish. Res. Bd. Can., 18:1001-1016.

PERSOONE, G. and CLAUS, C., 1980 : Mass culture of algae : a bottle-neck in the nursery culturing of molluscs. In: **Algae Biomass**. Ed. G. Shelef and C.J. Soeder, Elsevier/North Holland Biomedical Press. Amsterdam : 265-285.

QUINITIO, E.; PENA, D. and PASCUAL, F., 1983 : The use of suitable feeds in larval rearing of *Penaeus monodon*. In : **Proceedings of the First International Conference on Warm Water Aquaculture - Crustacea**. (Eds.) G.L Rogers, R. Day and A. Lim. Brigham Young University Hawaii Campus, Honolulu, Hawaii, U.S.A. : 337-342.

RAYMONT, J.E.G., 1980 : Plankton and productivity in the Oceans. Vol 2. **Zooplankton**, Pergamon Press, pp. 824.

ROSENBERRY, B., 1988 : World Shrimp Farming. **Aquaculture Digest**, Editor B. Rosenberry, San Diego, U.S.A. pp. 1-24.

ROSENBERRY, B., 1989 : World Shrimp Farming. **Aquaculture Digest**, Editor B. Rosenberry, San Diego, U.S.A. pp. 1-28.

RUMSEY, G.L. and KETOLA, H.C., 1975 : Amino acid supplementation of casein diet of Atlantic salmon (*Salmo salan*) fry and of soybean meal on rainbow trout (*Salmo gairdneri*) finglerlings. **Journal of Fisheries Research Board of Canada**, 32 : 422-426.

RUSSO, R.C.; SMITH, C.E. and THURSTON, R.V., 1974 : Acute toxicity of nitrite to rainbow trout *Salmo gairdneri*. **Journal of Fisheries Research Board of Canada**. 31 : 1653-1655.

RYTHER, J.H. and BARDACH, J.E., 1968 : The status and potential of aquaculture particularly invertebrate and algal culture. Vol. 2. **Invertebrate and algal culture**. Rep. National Technical Information Service PB 177767, Springfield, U.S.A. pp. 260.

SAKAMOTO, M.; HOLLAND, D.L. and JONES, D.A., 1982 : Modification of the nutritional composition of *Artemia* by incorporation of polyunsaturated fatty acids using microencapsulated diets. **Aquaculture**. 28 : 311-320.

✓SAMOCHA, T.M; UZIEL, N. and BROWDY, C.L., 1989 : The effect of feeding two prey organisms, nauplii of *Artemia* and rotifers, *Brachionus plicatilis* (Müller), upon survival and growth of larval marine shrimp, *Penaeus semisulcatus* (de Haan). **Aquaculture**, 77 : 11-19.

✓SANDIFER, P.A., 1988 : Aquaculture in the West, A Perspective. **Journal of the World Aquaculture Society**, Vol. 19. No. 2 : 73-84.

SANDIFER, P.A. and WILLIAMS, J.E., 1980 : Comparison of *Artemia* nauplii and non-living diets as food for larval grass shrimp *Palaemonetes* spp. : Screening experiments. In: G. Persoone, P. Sorgeloos, O.A. Roels and E. Jaspers (Eds.), **The Brine Shrimp *Artemia***, Vol. 3. Universa Press, Wettern, Belgium, pp. 353-364.

SASAKI, G.C., 1981 : Microencapsulated feeds for larval fish : a preliminary study. **International Council for the Exploration of the Sea, Contribution C.M. 1981/F : 22,** Mariculture Committee.

SCHLUTER, M.; SOEDER, C.J. and GROENEWEG, J., 1987 : Growth and food conversion of *Brachionus rubens* in continuous culture. **Journal of Plankton Research.** 9 : 761-783.

SCOTT, A.P. and MIDDLETON, C., 1979 : Unicellular algae as a food for turbot (*Scopthalmus maximus* L.) larvae - the importance of dietary long-chain poly-unsaturated fatty acids. **Aquaculture**, 18 : 227-240.

SCURA, E.D.; FISHER, J. and YUNKER, M.P., 1984 : (Abstract) The use of micro-encapsulated feeds to replace live food organisms in shrimp hatcheries. **First International Conference on the Culture of Penaeid Prawns/Shrimp,** Philippines.

SEDGWICK, R.W., 1979 : Influence of dietary protein and energy on growth, food consumption and food conversion efficiency in *Penaeus merguiensis* de Man. **Aquaculture,** 16 : 7-30.

SEDGWICK, R.W., 1980 : The requirements of *Penaeus merguiensis* for vitamin and mineral supplement in diets based on freeze-dried *Mytilus edulis* meal. **Aquaculture**, 19 : 127-137.

SHARMA, B. and AHLERT, R.C., 1977 : Nitrification and nitrogen removal. **Water Research.**, 11 : 876-925.

SHEWBART, K.L.; MIES, W.L.; LUDWIG, P.D., 1972 : Identification and quantitative analysis of the amino acids present in protein of the brown shrimp *Penaeus aztecus*. **Marine Biology**, 16 : 64-67.

SHIGUENO, K., 1975 : Shrimp culture in Japan. **Association for International Technical Promotion.** Tokyo, 1-153.

SICK, L.V. and ANDREWS, J.W., 1973 : The effect of selected dietary lipids, carbohydrates and protein on growth, survival and body composition of *Penaeus duorarum*. **Proceedings of the World Mariculture Society**, 4 : 263-276.

SICK, L.V. and BEATTY, H., 1975 : Development of formula foods designed for *Macrobrachium rosenbergii* larvae and juvenile shrimp. **Proceedings of the World Mariculture Society**, 6 : 89-102.

SIMON, C.M., 1978 : The culture of the diatom *Chaetoceros gracillis* and its use as a food for penaeid protozoeal larvae. **Aquaculture**, 14 : 105-113.

SIMON, C.M., 1981 : Design and operation of a large-scale, commercial penaeid shrimp hatchery. **Journal of the World Mariculture Society**, 12 (2) : 322-334.

SIMPSON, K.L.; MacPHEE, G.K. and BECK, A.D., 1983 : Zooplankton as a food source. **Proceedings of the Second International Conference on Aquaculture Nutrition**. World Mariculture Society, special publication No. 2, 180-201.

SMITH, C.E. and RUSSO, R.C., 1975 : Nitrite-induced methemoglobinemia in rainbow trout. **Progressive Fish-Cult.**, 37 (3) : 150-152.

SMITH, C.E. and WILLIAMS, W.G., 1974 : Experimental nitrite toxicity in rainbow trout and chinook salmon. **Trans. American Fisheries Society**. 103 : 389-390.

SNELL, T.W.; CHILDRESS, M.J.; BOYER, E.M. and HOFF, F.H., 1987 : Assessing the status of Rotifermass cultures. **World Aquaculture Society**, 18th Annual Meeting. Guayaquil, Equador. pp. 12.

SOKAL, P.R. and ROHLF, F.J., 1981 : **Biometry**. W.H. Freeman, (Editor) San Francisco, CA, pp. 776.

SOLORANZO, L., 1969 : Determination of ammonia in natural waters by the phenolhypochlorite method. **Limnology and Oceanography**, 14 (5) : 799-801.

SORGELOOS, P., 1978 : The culture and use of brine shrimp, *Artemia salina*, as food for hatchery-raised larval prawns, shrimp, and fish in Southeast Asia. FAO REPORT THA : 75/008/78/WP/3. **Publication of the National Freshwater Prawn Research and Training Centre.** Freshwater Fisheries Division, Department of Fisheries, Ministry of Agriculture and Cooperatives, Bangpagong, Chacheongsao, Thailand. pp. 18.

SORGELOOS, P., 1980 : The use of the brine shrimp *Artemia* in aquaculture. In: G. Persoone, P. Sorgeloos, O. Roels and E. Jaspers (Eds.). The brine shrimp *Artemia*, Vol 3. **Ecology, culturing, use in Aquaculture**, Universa Press, Belgium, pp. 25-46.

SORGELOOS, P.; BASSUYT, E.; LAVINA, E.; BAEZAMEZZA, M. and PERSOONE, G., 1977 : Decapsulation of *Artemia* cysts : A simple technique for the improvement of the use of brine shrimp in aquaculture. **Aquaculture**, 12 : 311-315.

SORGELOOS, P.; BOSSUYT, E.; LAVENS, P.; LEGER, P.; VAN HAECKE, P. and VERSICHELE, D., 1983 : The use of brine shrimp *Artemia* in Crustacean hatcheries and nurseries. In : **CRC Handbook of Mariculture, Vol. 1, Crustacean Aquaculture.** (Ed.) James P. McVey. CRC Press Inc. Florida : 71-96.

SPOTTE, S.H., 1970 : Fish and invertebrate culture, Water Management in Closed Systems. **Wiley-Interscience**, New York, N.Y., pp. 145.

SPOTTE, S.H., 1971 : Four general rules for live foods. S.E.A., Scope., 2 (1).

SPOTTE, S., 1979 : Fish and Invertebrate culture : Water Management in Closed Systems. Editor, S. Spotte, 2nd edition. John Wiley and Sons, New York, N.Y. pp. 179.

STARK, J.R.; MacDONALD, N.L. and GLASS, H.J., 1988 : Digestion of protein in different marine species. **Journal of the World Aquaculture Society**, Aquaculture Communiqueés, Vol 19 (1), 67A.

STICKNEY, R.R.; CASTELL, J.D.; HARDY, R.W.; KETOLA, H.G.; LOVELL, R.T. and WILSON, R.P., 1983 : Nutrient requirements of Warmwater fishes and shellfishes. National Academy Press, Washington, D.C. pp. 102.

STRICKLER, J.R., 1982 : Calanoid copepods, feeding currents, and the role of gravity. **Science**, Vol. 218 : 158-160.

SULKIN, S.D. and EPIFANIO, C.H., 1975 : Comparison of rotifers and other diets for rearing early larvae of the blue crabs, *Callinectes sapidus* Rathbun. **Esturaine and Coastal Marine Science**, 3 : 109-113.

TESHIMA, S.I.; KANAZAWA, A. and SAKOMOTO, M., : 1981 : Attempt to culture the rotifers with microencapsulated diets. **Bulletin of the Japanese Society of Scientific Fisheries**, 47 : 1575-1578.

TESHIMA, S.I. and KANAZAWA, A., 1983 : Effects of several factors on growth and survival of the prawn larvae reared with micro-particulate diets. **Bulletin of the Japanese Society of Scientific Fisheries**, 49 (12) : 1893-1896.

TESHIMA, S. and KANAZAWA, A., 1984 : Effects of protein, lipid and carbohydrate levels in purified diets on growth and survival rates of the prawn larvae. **Bulletin of the Japanese Society for Science and Fisheries**, 50 (10) : 1709-1715.

TESHIMA, S.I.; KANAZAWA, A. and SASADA, H., 1983 : Nutritional value of dietary cholesterol and other sterols to larval prawn, *Penaeus japonicus* Bate. **Aquaculture**, 31 : 159-167.

TESHIMA, S.I.; KANAZAWA, A. and SAKAMOTO, M., 1982 : Microparticulate diets for the larvae of aquatic animals. **Mini review and Data File of Fisheries Research**, Kagoshima University, 2 : 67-86.

TESHIMA, S.I.; KANAZAWA, A. and YAMASHITA, M., 1986 : Dietary value of several proteins and supplemental amino acids for larvae of the prawn *Penaeus japonicus*. **Aquaculture**, 51, No. 3-4 : 225-235.

THOMAS, M.M.; KATHIVEL, M. and PILLAI, N.N., 1976 : Spawning and rearing of the penaeid prawn *Metapenaeus affinis* (H. Milne Edwards) in the laboratory. **Indian Journal of Fisheries**, 21 : 543-556.

TOBIAS-QUINITIO, E. and VILLEGAS, C.T., 1982 : Growth, survival and macronutrient composition of *Penaeus monodon* Fabricius larvae fed with *Chaetoceros calcitrans* and *Tetraselmis chuii*. **Aquaculture**, 29 : 253-260.

VOLKMAN, J.K.; JEFFERY, S.W.; NICHOLS, P.D.; ROGERS, G.I. and GARLAND, C.D., 1989 : Fatty acid and lipid composition of 10 species of microalgae used in mariculture. **Journal of Experimental Marine Biology and Ecology**, 128 : 219-240.

WALNE, P.R., 1966 : Experiments in the large-scale culture of the larvae of *Ostrea edulis* L. **Fishery Investigations**, Ministry of Agriculture, Fisheries and Food. Series II, Vol. 25, No. 4, pp. 53.

WATANABE, T.; OOWA, F.; KITAJIMA, C. and FUJITA, S., 1980 : Relationship between dietary value of brine shrimp, *Artemia salina*, and their content of $\omega 3$ highly unsaturated fatty acids. **Bulletin of the Japanese Society of Science and Fisheries**, 46 : 35-41.

WATANABE, T.; KITAJIMA, C. and FUJITA, S., 1983 : Nutritional value of live food organisms used in Japan for mass propagation of Fish : A review. **Aquaculture**, 34 : 115-143.

WEB, K.L. and CHU, F.E., 1982 : Phytoplankton as a food source for bivalve larvae. **Proceedings of the Second International Conference on Aquaculture Nutrition**. World Mariculture Society, Special Publication No. 2 : 272-291.

WHEELER, R.; YUDIN, A.I. and CLARK, W.H. Jr., 1979 : Hatching events in the cysts of *Artemia salina*. **Aquaculture**, 18 : 59.

WHITFIELD, M., 1974 : The hydrolysis of ammonium ions in sea water :A theoretical study. **Journal of the Marine Biological Association, United Kingdom**. 54 : 565-580.

WICKINS, J.F., 1972 : The food value of brine shrimp *Artemia salina* L. to larvae of the prawn, *Palaemon serratus* Pennant. **Journal of Experimental Marine Biology and Ecology**. 10 : 151-170.

WICKINS, J.F., 1976 : The tolerance of warm-water prawns to recirculated water. **Aquaculture**, 9 : 19-37.

WICKINS, J.F., 1982 : Opportunities for farming crustaceans in Western temperate region. In. **Recent Advances in Aquaculture**. Eds. J.F. Muir and R.J. Roberts. : 89-177. Westview Press, Boulder, Colorado.

WICKINS, J.F., 1986 : Prawn Farming Today : Opportunities, Techniques and developments. **Outlook on Agriculture**, Vol. 15, No. 2, : 52-60.

WILKENFELD, J.S.; FOX, J.M. and LAWRENCE, A.L., 1981 : Consumption of frozen and live *Artemia* by protozoea of *Penaeus setiferus*. **Journal of the World Mariculture Society**, 12 (2) : 250-259.

YUFERA, M.; RODRIGUES, A. and LUBIAM, L.M., 1984 : Zooplankton ingestion and feeding behaviour of *Penaeus kerathurus* larvae reared in the laboratory. **Aquaculture**. 42 : 217-224.

YULE, A.B., 1982 : The application of new techniques to the study of planktonic organism. Ph.D. Thesis, University College of North Wales, Bangor.

YULE, A.B. and CRISP, D.J., 1983 : A study of feeding behaviour in *Temora longicornis* (Möller) (Crustacea : Copepoda). **Journal of Experimental Marine Biology and Ecology**, 71 : 271-282.

APPENDIX

JOURNAL OF THE
WORLD AQUACULTURE SOCIETY

Vol. 20, No. 1
March, 1989

Abstract

A Comparison of the Stability of Some Artificial Feeds Used in Penaeid Larval Culture

S. AMJAD AND D. A. JONES, *School of Ocean Sciences, University College North Wales, Bangor, Menai Bridge, Gwynedd, North Wales, U.K.*

Artificial penaeid larval feeds currently are usually in the form of microcapsules or microparticulates designed to stabilise the dietary components and prevent loss by leaching which leads to fouling of the culture water. There is now information as to the size of particles ingested by the larval stages of at least some penaeid species and feed levels required for successful culture.

A range of commercial artificial larval feeds have been investigated to evaluate their physical characteristics under actual culture conditions. Stability was measured as the change in particle size range in aerated sea water at 28°C over 24 h, together with the loss in weight due to leaching of soluble components of the diet. Estimates of bacterial growth in the culture medium were made together with changes in pH, ammonia and nitrite levels. Each feed was used to culture *Penaeus monodon* larvae following manufacturers instructions. Results indicate that the composition of most artificial feeds alters dramatically within a few hours of immersion, causing significant changes both to food particle size and to the culture water environment.

JOURNAL OF THE
WORLD AQUACULTURE SOCIETY

Vol. 20, No. 1
March, 1989

Abstract

Role of Algae in Conditioning Water Quality for Penaeid Larval Culture.

S. AMJAD AND D. A. JONES, *School of Ocean Sciences, University College North Wales, Bangor, Menai Bridge, Gwynedd, North Wales, U.K.*

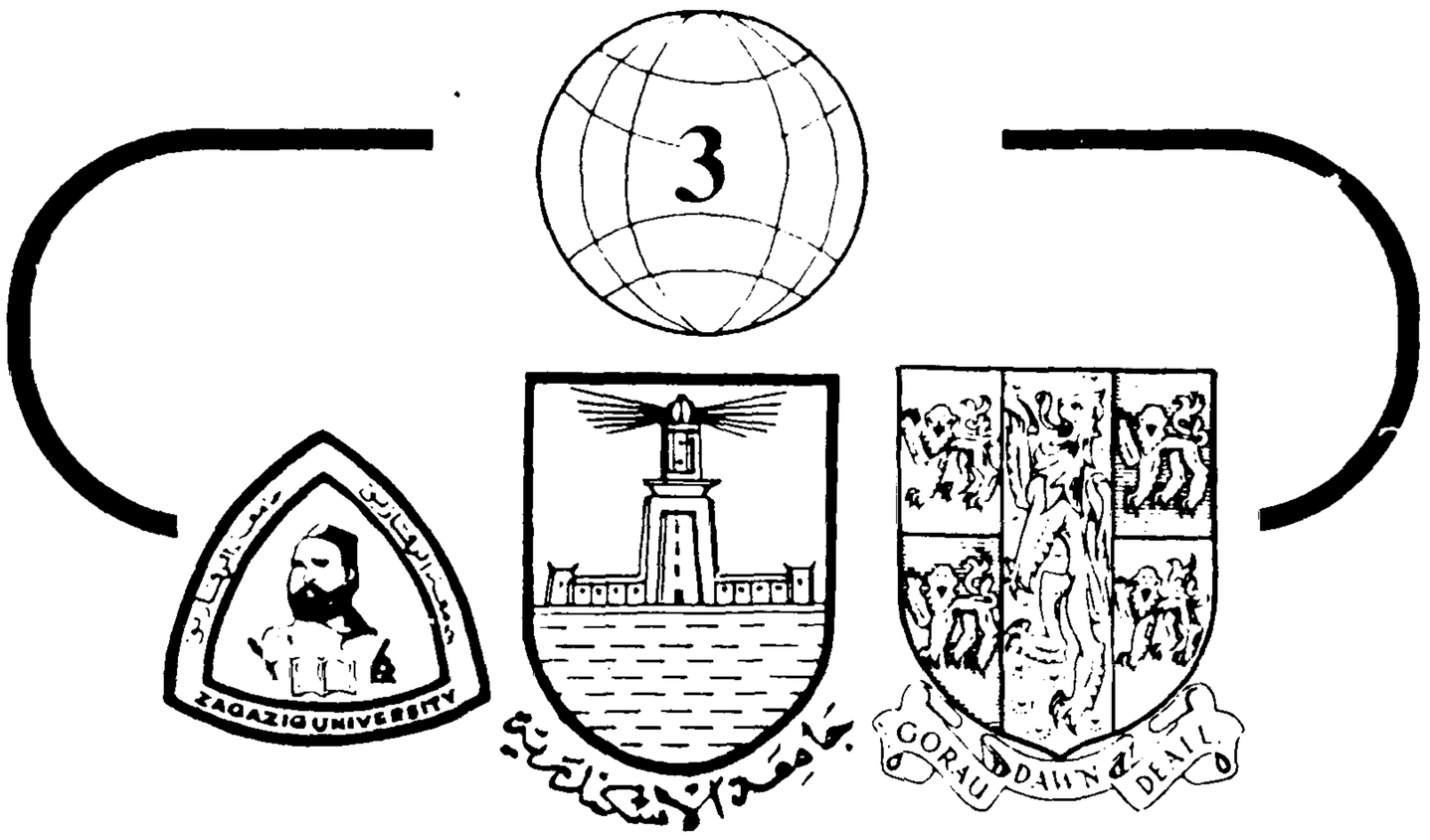
Recently it has been demonstrated that modification of the 'green water' larval culture system (Ling, 1969) for *Macrobrachium* allows the production of post larvae without any water change, despite extensive use of artificial feeds (Ang and Cheah, 1986). Increases in the levels of toxic metabolites such as ammonia and nitrite are also common in penaeid larval culture, especially where excessive amounts of artificial feeds are employed.

Present work examines the use of marine algae as 'biological filter' systems to control levels of ammonia and nitrite in *Penaeus monodon* larval culture using artificial feeds. Preliminary results indicate that toxic metabolite levels may be regulated by manipulation of algal cell and artificial feed concentrations. The use of different levels of illumination provides an additional in- direct mechanism for controlling concentrations of these metabolites in cultures containing algae.

The Third Egyptian British Conference

On

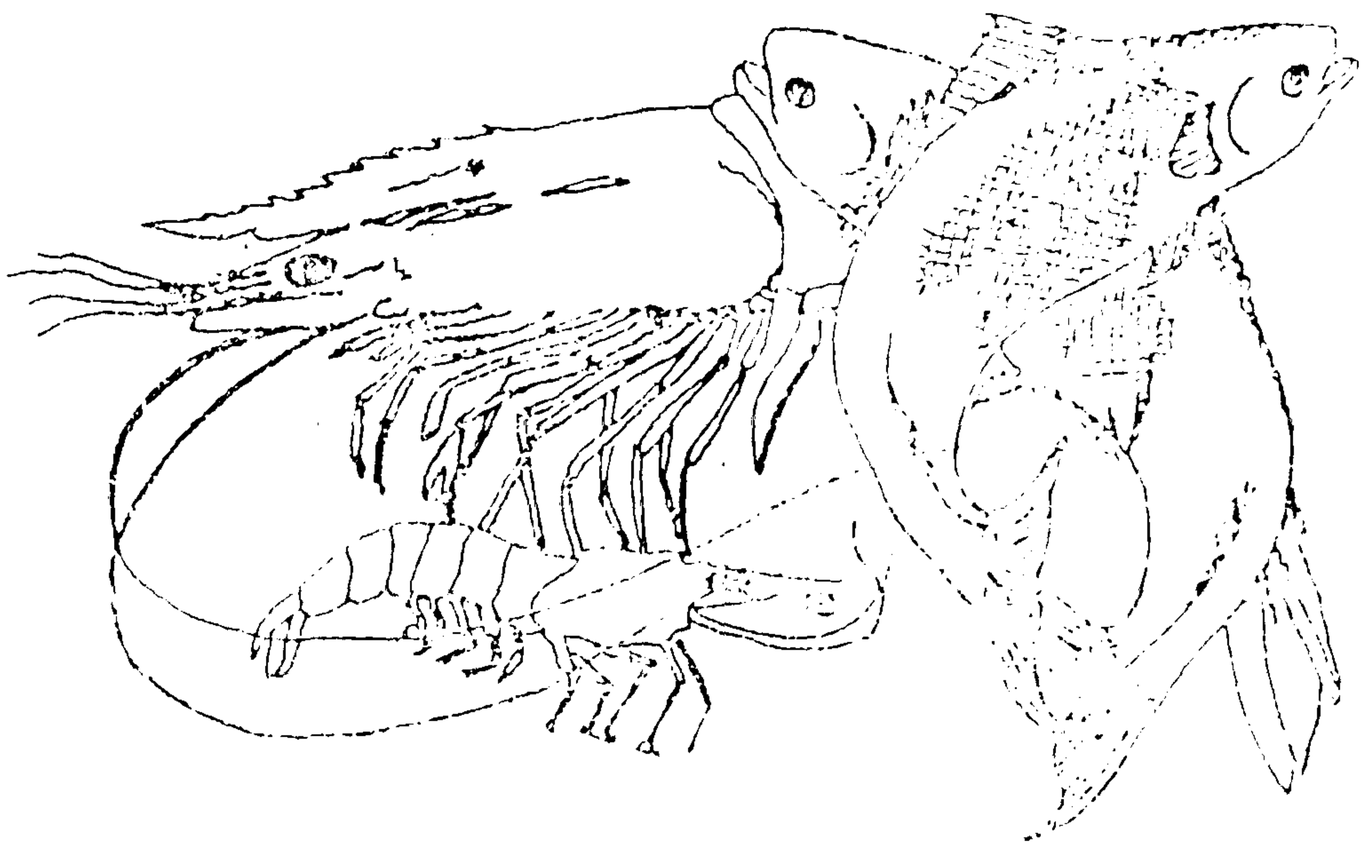
Animals, Fish, and Poultry Production



Faculty of Agriculture
Alexandria University
Alexandria – Egypt

7–10 October, 1989

TEKNOLOGI BARU DALAM AKUAKULTUR



NEW TECHNOLOGIES IN AQUACULTURE

17th August 1989
Putra World Trade Centre
Kuala Lumpur

SEMINAR ANJURAN BERSAMA

PERSATUAN PERIKANAN MALAYSIA
(THE MALAYSIAN FISHERIES SOCIETY)

KEMENTERIAN SAINS TEKNOLOGI
DAN ALAM SEKITAR
(THE MINISTRY OF SCIENCE TECHNOLOGY
AND ENVIRONMENT)

KEMAJUAN DALAM PEMAKANAN LARVA PENAEID

oleh

Amjad, S., K. Chitravadivelu and D.A. Jones
School of Ocean Sciences
University College Bangor
Menai Bridge, Gwynedd, N. Wales, LL59 5EY
United Kingdom

ABSTRAK

Prestasi diet larva buatan yang terkemuka telah dinilai dalam keadaan terkawal dengan menggunakan larva *Penaeus monoduon* (Fab.). Saiz zarah, kadar larut lesap dan kandungan pemakanan kasar telah dikaji bersama-sama dengan faktor mutu air seperti NH_3 dan NO_2. Analisis statistik keatas data kajian menunjukkan kaitan yang kuat diantara pertumbuhan/kemandirian dan kestabilan diet (kehilangan larut lesap/pencemaran air). Kandungan pemakanan dan saiz zarah kelihatan kurang berpengaruh untuk mengakibatkan pertumbuhan dan kemandirian yang rendah, tetapi kedua-dua faktor ini masih dianggap penting. Kekurangan dalam kehilangan larut lesap telah membenarkan pecubaan yang rutin terhadap kultur *P. monodon* sehingga keperingkat pascalarva dengan kadar kemandirian yang tinggi yang hanya begantung kepada diet buatan, tetapi pertumbuhan biasanya kurang daripada yang dicapai dengan makanan hidup. Penyelidikan terbaru menunjukkan kelemahan ini boleh diatasi dengan memasukkan faktor aktif yang diambil daripada makanan hidup ke dalam diet buatan.

ADVANCES IN PENAEID LARVAL FEED

by

Amjad, S., K. Chitravadivelu and D.A. Jones
School of Ocean Sciences
University College Bangor
Menai Bridge, Gwynedd, N. Wales, LL59 5EY
United Kingdom

ABSTRACT

The performance of leading types of artificial larval diets has been evaluated under controlled conditions with *Penaeus monodon* (Fab.) larvae. Particle size, leach rate and gross nutritional content were measured together with water quality factors such as NH_3 and NO_2. Statistical analysis of results reveals a strong correlation between growth/survival and diet stability (leach loss/water pollution). Nutritional content and particle size appear less influential in causing poor growth and suvival, although they remain important factors.

Reduction in leach loss has allowed routine experimental culture of *P. monodon* to postlarval stages with high survival rates solely on artificial diets, but growth has usually been less than that achieved on live feeds. Recent research indicates that this may be corrected by the inclusion of active factors, taken from livefeeds, in the diets.

JOURNAL OF THE
WORLD AQUACULTURE SOCIETY

Vol. 19, No. 1
March, 1988

152. ***Penaeus monodon*: Nauplius to Juvenile on the Same Artificial Diet,**

K. KURMALY, S. AMJAD, AND D. A. JONES,
School of Ocean Sciences, University College of North Wales, Menai Bridge, Gwynedd LL59 5EY, U.K.

Penaeus monodon larvae have been successfully reared using microencapsulated diets (Frippak Feeds) from late nauplius stage VI to early juvenile stages with an average survival of 60%. Development and growth were dependent on capsule size and feed concentration.