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STUDIES ON THE DEVELOPMENT AND NUTRITION OF THE CARIDEAN PRAWN *MACROBRACHIUM ROSENBERGII* (DE MAN) (CRUSTACEA: DECAPODA)

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BY

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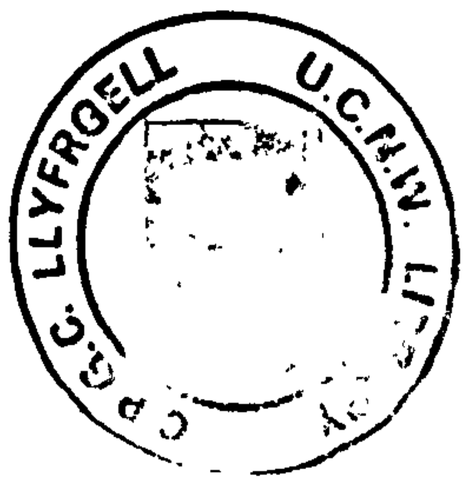

Dedicated to:

my parents, **Colette and Désiré**, for their love and constant trust

and

my wife, **Liliana**, for her understanding and support during all difficulties

" Notre surprise est venue des chevrettes, seul produit que nous ne connaissions pas. Nous avons été subjugués, et nous étions nombreux dans ce cas, par le goût de ces Macrobrachium, présentés tout simplement grillés "

Quincy & Barbier
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SUMMARY

It is confirmed that Macrobrachium rosenbergii contains much lower w3 HUFA levels than marine prawns. For larvae which have a low HUFA profile at stage I, survival and growth vary in relation to dietary linolenic acid which is the precursor of these long chain fatty acids. Diets containing different levels of w6 fatty acids produced large differences in survival and slight differences in growth. Recently hatched larvae utilize saturated and monounsaturated fatty acids for their energy requirements, as PUFA's increase from stage I to stage II, suggesting that larvae are able to chain elongate.

Heavier M. rosenbergii females have heavier clutches and higher numbers of eggs. However, female weight does not influence weight per egg, nor the volume of each egg. The length of incubation does not significantly influence the larval survival though a negative trend is observed. The ratio between the weight of the parent female and the weight per egg (Wegg/W) is found to be 0.036 which reflects a poor fecundity.

Recently hatched larvae consume microencapsulated feed, but do not survive beyond day 13. However, artificial diets can be fed successfully from stages VI-VII, the best growth occurring at a feeding rate of 8-16mg of microcapsules per litre.

For the caridean M. rosenbergii and penaeid Penaeus monodon larvae, edge index increases from 641 and 223, respectively, at the beginning of their development to 1847 and 750 at the end, reaching 2817 and 5000 at postlarval metamorphosis. These results confirm the herbivorous, omnivorous and raptorial feeder classification given by Itoh (1970).

Recently hatched M. rosenbergii larvae show poor ability to crush their food and rely heavily on their embryonic lipid droplet reserves. Later, they feed on live diets, such as Artemia and rotifers, containing their own enzymes which aid larval digestion. Between stages V and VII, the cardiac foregut becomes muscular, the pyloric filter apparatus is functional, and the hepatopancreas increases rapidly in size. Residence time of food becomes longer in the foregut, but remains the same, and later decreases, in the midgut. Survival and growth on artificial feed coincide with these changes, suggesting that larval enzyme production is sufficient to digest and assimilate such diets.

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GENERAL INTRODUCTION

By definition, prawns have a well developed forwardly projecting rostrum between the eyes, which is greatly reduced or absent in shrimps (Jones, 1988). Cultured prawns contain the families Penaeidae and Palaemonidae, separated taxonomically by the pleuron of the second abdominal somite which is overlapped by the first in the former, but overlaps the first in the latter. Table 1 presents the taxonomy of these two families with some of their characteristics. Macrobrachium rosenbergii (de Man) belongs to the Palaemonidae and is commonly known as the " giant freshwater prawn ". Hedgepeth (1947) states that over a hundred species are known to exist and over a quarter of these being found in the Americas. Holthuis (1980) has provided useful information on the distribution, local names, habitats and maximum sizes of commercial species of Macrobrachium. However, Macrobrachium culture, at present, is synonimous with the culture of Macrobrachium rosenbergii.

TABLE 1. Shrimp and prawn taxonomy and some of their characteristics (1)

Phylum - Arthropoda
 Class - Crustacea (>26,000 spp.)
 Sub-class - Malacostraca (>18,000 spp.)
 Serie - Eumalacostraca
 Superorder - Eucarida (>8,600 spp.)
 Order - Decapoda, Latreille, 1803 (>8,321 spp.):
. 5 pairs of legs
. cephalothorax
. pereiopods bearing chela
 Suborder - Natantia (1930 spp.):
. body, dorso-ventrally compressed
. first abdominal segment, same size than others
. large antennal scale
. 3 pairs of pereiopods, large & chelated (others, small & slender)
. pleopods, well developed for SWIMMING

 Section - Penaeidea (318 spp.)

 Family - Penaeidae, Rafinesque, 1815: gambas

Metapenaeus spp.
Penaeus spp. (109 spp.)

 Section - Caridea (1,590 spp.)

 Family - Palaemonidae, Rafinesque, 1815 (399 spp.): scampi

Palaemon spp.
Macrobrachium spp. (>100 spp.)

 Suborder - Reptantia (>6,391 spp.):
. body, dorso-ventrally flat
. first abdominal segment, smaller than others
. relatively small antennal scale
. 2nd and 3rd pair of pereiopods, never heavier than 1st (others, adapted for CRAWLING)
. pleopods, if present, not developed for swimming
 Family - Palinuridae
Spiny lobster
 Family - Homaridae
Lobster
 Family - Astacidae
Crab
Crayfish

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(1) adapted from Lawrence (1983) and Rouse (1984)

M. rosenbergii is indigenous in the whole of the South and Southeast Asia areas as well as in Northern Oceania and in the Western Pacific islands (New & Singholka, 1985). This species has been introduced into almost every continent initially for research purposes (Malecha, 1980). At the present time, M. rosenbergii is farmed in considerable quantity in many other countries, including Hawaii, Honduras, Costa Rica, Mexico, French Guyana, Mauritius, Zimbabwe and Israel (New & Singholka, 1985).

M. rosenbergii occurs in both fresh and brackish waters throughout the year. It inhabits most tropical rivers, especially the lower reaches which are influenced by tides, but also occurs up to at least 200 Km from the coast, and is present in lakes, water reservoirs, mining pools, irrigation canals and even some paddy fields which have direct or indirect access to the rivers (Ling, 1969a).

M. rosenbergii is found in the habitats of about 25°C in Malaya (Johnson, 1967) and from 27 to 34°C in India (John, 1957; Rao, 1967). Fujimura (1966) suggests that the survival of M. rosenbergii ranges between 23.8 to 30.5°C. Ling (1962) reports a slightly wider temperature tolerance of 22 to 32°C for 2-3 month old juveniles.

Generally, prawns have a life span of one to two years.

However, the giant freshwater prawn has a life span of seven to eight years or longer (Tseng, 1988). After living 3-6 months in a river, the female becomes mature and the cephalothorax becomes yellowish purple at the time when its ovary is mature. Mating, generally, occurs during the night. The male deposits sperm at the basis of the ventral thoracic legs of the female, but this sperm is not retained for long periods of time as in marine penaeids (Bardach et al., 1972). Egg-laying takes place six to twenty hours later. The eggs are held in grape-like bundles on the ovigerous setae of the first four pairs of pleopods (Ling, 1969a). The gravid female prawn takes care of her brood, removing any dead eggs until they hatch. The pleopods beat back, and forth intermittently to provide aeration. Unfertilized eggs are normally lost within 2-3 days of spawning. The whole incubation period is about 19-22 days at 28°C. From about the twelfth day, the colour of the eggs, originally bright orange, gradually becomes light grey, darkening to slate grey when hatching is imminent (Ling, 1969a). As eggs hatch, a process which is normally completed for the whole brood within one or two nights, the larvae are dispersed by rapid movements of the abdominal appendages of the parent. From the first minutes of life, M. rosenbergii larvae are active swimmers, tail first, ventral side uppermost. They are planktonic (New & Singholka, 1985), feeding primarily on zooplankton, but in absence of an adequate supply of live

animal food, they will take minute bits of dead material or plants (Bardach et al., 1972). Some authors (Cohen et al., 1976; Wickins, 1976) claim that larvae may passively ingest phytoplankton cells, but they do not assimilate them.

Macrobrachium species apparently evolved " out of the sea " (Johnson, 1960), as their larvae generally require brackish water for their development. The Tahitian prawn Macrobrachium lar has spread to all major islands of Hawaii after having been introduced on one island, this was only possible as the animal is able to live in full seawater. Its larvae must remain in 35%. seawater from hatching until the fifth to seventh stages (Muranaka, 1977) and its postlarvae must be kept in salinities at least 25%. . The " break through " in closing the life cycle of Macrobrachium rosenbergii came when Ling (1969b) raised (accidently, with soya sauce...) the salinity in his culture aquaria to 16%. Previous attempts to rear larvae to metamorphosis failed because only freshwater was used on the assumption that the species were freshwater (New & Singholka, 1985). Adults were captured in freshwater fisheries, but studies of larvae distribution in plankton tows from estuaries were not available (Malecha, 1978). Thus, prawns are catadromous creatures that spend their lives in freshwater, returning to salty water to hatch their eggs (Hanson & Goodwin, 1977). However, postlarvae exhibit good tolerance to a wide range

of salinities which is a characteristic of freshwater prawns: newly metamorphosed postlarvae can be rapidly transferred from brackish water to completely freshwater (New & Singholka, 1985). Indeed, within three or four weeks they begin to migrate upstream into freshwater areas as soon as they are able to swim against rapidly flowing currents (Ling, 1969a). However, there is also observational evidence that not all prawn individuals migrate to freshwater. Some apparently stay in the estuaries (Hanson & Goodwin, 1977).

Freshwater is normally used for rearing freshwater prawns from postlarvae to market size, although the successful experimental use of partially saline water has been reported. Tidal water fluctuating between 12‰ and 25‰ has been utilized in Western Samoa (Popper & Davidson, 1982), while salinities of at least up to 10‰ gave as good results as freshwater in South Carolina (Smith et al., 1982). Sandifer et al. (1975) also grew prawns in brackish water and Barnes (1982) has reported the use of brackish water for freshwater prawn culture in Israel. Clearly, therefore, freshwater prawn farming need not necessarily be restricted to sites with freshwater supplies. Since the results mentioned above are from research rather than commercial units, some caution should be applied to the use of brackish water sites for farms, for the moment (New & Singholka, 1985). Wickins (1972) reported that postlarvae grew better in freshwater than that in brackish water.

M. rosenbergii has a fast growth (Ling, 1969a; Smith et al., 1976; Malecha, 1978), is easy to reproduce (Liao et al., 1973; Chao & Liao, 1977; New & Singholka, 1985), but has a long larval life (Ling, 1969a,b; Wickins & Beard, 1974) and, when sufficiently hungry, larvae may even become cannibalistic (Ling, 1969a; AQUACOP, 1977; New & Singholka, 1985; Takeuchi & Ohono, 1986).

There are two critical periods during its larval cycle: the first is around the seventh and eighth stages, and the second is when passing to postlarvae. Chao and Liao (1977) report that heavy mortality can be prevented at these periods when food supply is reduced by feeding only a small amount of Artemia nauplii instead of the rich diet given in the days preceeding and during the critical periods.

Larval culture often includes use of algae (Wickins, 1972; Cohen et al., 1976; Maddox & Manzi, 1976; Joseph, 1977; Hanson & Goodwin, 1977; Adisukresno et al., 1977; Malecha, 1978; Corbin et al., 1983; Ang & Cheah, 1986). It appears that the addition of algae may decrease the length of time to postlarval metamorphosis (Corbin et al., 1983). However, New (1982) observed that higher survival is reached without use of algae. Several larval rearing systems are currently in practice, the main differences being in the water

management. Commercial-scale larval rearing techniques for M. rosenbergii are mainly based on two types: the Anuenue " green water " method developed in Hawaii, and the Aquacop " clear water " method developed by the French (Sandifer et al., 1977). In the " green water " method, larvae are reared in water containing about $5x10^5$ to $1x10^6$ cells (usually, Chlorella sp.) per ml. The phytoplankton increases survival by maintaining good water quality through waste removal (ammonia and nitrite) and acts as a biological filter (Malecha, 1978). In the " clear water " method, feed must supply the whole dietary larval requirement (AQUACOP, 1977). The " green water " system seems to give the best larval production (Maddox & Manzi, 1976; Manzi et al., 1977).

In the natural environment, the giant prawn M. rosenbergii is able to satisfy its particular nutrient requirements from a variety of sources which include insects, such as chironomids (Jhingran, 1977) and, mainly, zooplankton, such as rotifers, copepods, minute crustaceans, very small worms and larval stages of other aquatic invertebrates (Ling, 1969a). The same author mentions that postlarvae utilize larger pieces of organic material, both of animal and vegetable origin. Their diet eventually includes algae, nuts, grains, seeds, fruits and fishes.

Food is located mainly by smell and touch. When searching for food the filaments of the antennae and antennules sweep

about actively. When a large piece of food is found, it is picked up and brought to the mouth by the first and second pairs of thoracic legs together. If small pieces of food, such as grains of rice, are found, they are picked up individually and are brought to the mouth one by one by the pincers of the first pair of thoracic legs (Ling, 1969a). Immediately after the grain is taken by the mouth, the empty pincer is again employed for picking up another piece, and an actively feeding specimen may frequently be seen holding two pieces of food, one in each chela, while at the same time there is already one piece in its mouth.

In recent years, several empirical diets have been evaluated under both laboratory and culture conditions to provide appropriate levels of nutritionally balanced feeds. The majority of these formulations have proved rather unsatisfactory with the exception of a very limited number of particular feeds which are commercially available to prawn culturists (Hanson & Goodwin, 1977). Unfortunately, even these are not totally satisfactory in promoting growth to the same degree as achieved when prawns are fed live or fresh food. Therefore, aquiculturists continue to rely on the use of live/natural feeds, particularly for the production of postlarvae and juveniles.

Prepared foods are also used for the first days of life in hatcheries:a mixture of fish flesh and egg custard steamed together, drained and passed through several screens

provides particles of appropriate sizes (Ling, 1969a). New & Singholka (1985) used successfully a mixture of egg and mussels which is blended, chopped, steamed and screened to size. This may used for feeding directly or refrigerated for a few days for later use. Fish flesh (skipjack tuna, bonito or mackerel) may also be used as an ingredient partially or totally replacing mussel.

Generally, technicians rely on live feeds such as diatoms, rotifers (Brachionus plicatilis) (Anderson & Smith, 1983; Lovett & Felder, 1988), or Artemia at 5-15 nauplii per ml (Minamizawa & Morizane, 1970; Bardach et al., 1972; Hanson & Goodwin, 1977). However, mass rotifer culture is limited by difficulties (Schluter et al., 1987; Snell et al., 1987) and the nutritional requirement for quality feeds high in polyunsaturated fatty acids (PUFA's) is often impossible to achieve due to great variation in nutritional quality of rotifers (Watanabe et al., 1983). At the present time, Artemia cysts are used in most of the hatcheries (Sorgeloos, 1977, 1980). However, problems and constraints related to their use have been reported by several workers, these include the presence of cyst contaminants affecting tank hygiene (Gilmour et al., 1975; Austin & Allen, 1982), rapid post-hatch depletion of Artemia nauplii lipids (Wickins, 1976); Benijts et al., 1976), and nutritional variability between Artemia strains (Watanabe et al., 1980, 1983; Leger et al., 1985, 1986).

Many foods are used to supplement the Artemia diet during the latter half or two-thirds of larval development (Perez, 1976; Hanson & Goodwin, 1977). According to these authors, the most widely used supplement is minced fish flesh (primarily tuna), followed by frozen adult Artemia, fish eggs and bits of chicken egg.

The use of live feeds is found in most crustacean larval cultures (Bardach et al., 1972; Hanson & Goodwin, 1977; McVey & Moore, 1983; Harms & Seeger, 1989). However, for some groups, e.g. penaeids, it has been possible to eliminate part or all live feeds by using microencapsulated artificial feeds (Jones et al., 1979a, 1987; Kanazawa et al., 1982). The use of the process of microencapsulation to feed artificially compounded diets to filter feeding invertebrate larvae was first suggested by Meyers et al. (1972), but microencapsulated diets were first shown to be acceptable to crustacean larvae by Jones et al. (1974). Microcapsules are suitable for laboratory trials and have become an accepted tool for the study of larval nutritional requirements (Jones et al., 1979; Langdon and Waldock, 1981; Chu et al., 1982; Sakamoto et al., 1982; Teshima et al., 1986). The results can produce a balanced diet for the studied larvae but require examination of larval physiology and feeding behaviour (Pruder et al., 1982; Gibson, 1983; Yule & Crisp, 1983; Levine & Sulkin, 1984; Langdon et al., 1985; Jones & Kurmaly, 1987; Kurmaly et al., 1989). As a

result of these studies it has recently been possible to replace all live food items in the culture of penaeid larvae and complete larval metamorphosis on artificial feeds (Kurmaly et al., 1989).

The present thesis aims to investigate the possibility of replacing live feeds used in the larval culture of Macrobrachium rosenbergii with artificial diets. As Macrobrachium is a caridean rather than a penaeid prawn, and in addition spends much of its life away from the sea in fresh water, it might be expected that at least some modification of successful penaeid artificial diets will be necessary.

In section I, the total lipid and fatty acid requirements of M. rosenbergii larvae are investigated. Dietary lipid requirements were chosen as a priority because the literature suggests that, unlike marine organisms, most freshwater species are able to desaturate and chain elongate saturated fatty acids and hence do not have an essential requirement for polyunsaturated fatty acids (PUFA's). As it appeared from preliminary experiments that early stage Macrobrachium larvae would not accept the standard penaeid microencapsulated diets, feeding experiments concentrated on supplying larvae with Artemia nauplii fed and unfed on high lipid boost diets. This produced a range of lipid levels in

acceptable diets which were investigated in the Macrobrachium larvae.
Relationships between eggs, larvae and their parent female are examined in section II.

Once the acceptable dietary lipid levels were ascertained, feed trials using microencapsulated artificial feeds were attempted (section III). Although these experiments allowed some of the larval culture requirements of Macrobrachium larvae to be defined, it proved impossible to maintain the early larval stages of M. rosenbergii on artificial diets alone.

As the use of artificial diets was not successful in sustaining early stages of M. rosenbergii, sections IV and V of this thesis are devoted to studies on the development of the edge index derived from the measurement of the cutting edges on the larval mandible, and on the functional morphology of the freshwater prawn gut throughout larval development, respectively. Results are compared to similar studies on penaeid larvae which feed successfully on encapsulated diets in an attempt to identify differences in feed ingestion and processing within the gut which could account for differences in growth and survival of penaeidea and caridea on artificial diets.

Section I

Total lipid and fatty acid requirements of *Macrobrachium rosenbergii* larvae

INTRODUCTION

It is well known that lipids are the most energy rich of nutrient classes, providing approximately 39.558 $kJ.g^{-1}$ compared to 17.372 and 23.651 $kJ.g^{-1}$ for carbohydrates and proteins, respectively. Figure 1 gives the general pattern of utilization of lipids in animals (Elovson, 1980). The principal components of most lipids are fatty acids. Burr and Burr (1929 & 1930) have shown that animals have an essential dietary requirement for specific types of fatty acids.

With due care and attention to detail, a complete analysis by thin layer and gas liquid chromatographic techniques of the fatty acids of an aquatic animal lipid can reveal up to 50 or 60 components (Ackman, 1973). Fewer are necessary (Ackman & Eaton, 1970), and only about fourteen need to be discussed as important in terms of weight per cent composition. These belong to the four basic series typified

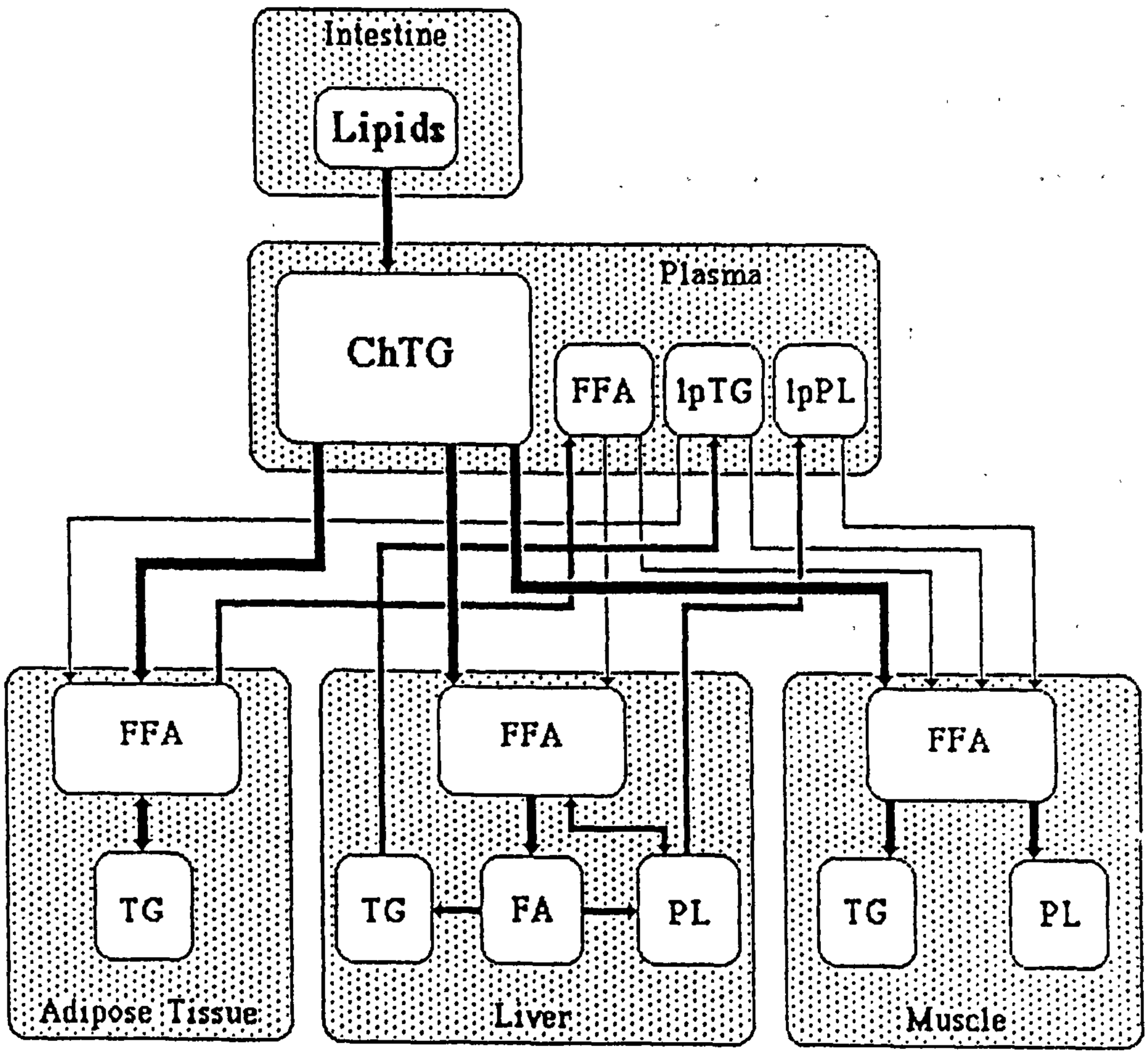
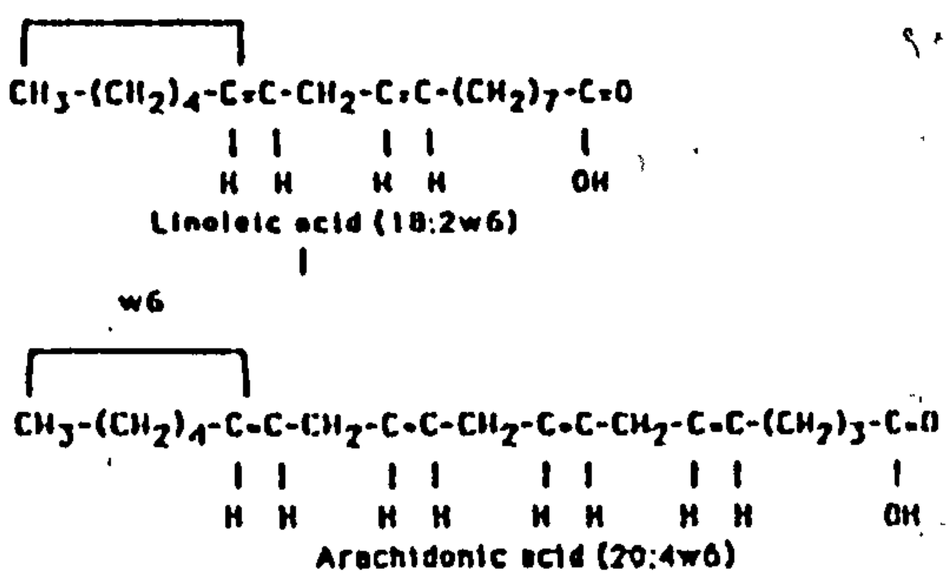


Figure 1. Simple scheme of fatty acid transport, esterification and metabolism. FA, fatty acids; FFA, albumin-bound free fatty acids; TG, triglycerides; PL, phospholipids; lpPL, lipoprotein phospholipids; lpTG, lipoprotein triglycerides; chTG, chylomicron triglycerides (transformed from Elovson, 1980)

by C18 acids. These have no double bond (18:0 or stearic acid); one double bond (18:1w9 or oleic acid); two double bonds (18:2w6 or linoleic acid); and three double bonds (18:3w3 or linolenic acid). Fatty acids are " saturated " when they do not include any double bond. They are called " mono- " and " polyunsaturated " (or PUFA) when they contain one or several double bonds, respectively. " Highly " polyunsaturated fatty acids (or HUFA) refered in this work to the eicosapentaenoic (20:5w3) and docosahexaenoic (22:6w3) acids having five and six double bonds, respectively. Figure 2 shows the path followed by these fatty acids when they are desaturated and elongated through animal metabolism (Ackman, 1973). The key difference between w3 and w6 fatty acid families is that animals cannot convert 20 carbon w6 fatty acids to 22 carbon w3 fatty acids, but can readily do so with w3 fatty acids (Huner, 1989).

It has been established since the late 1920's that fatty acids of the w6 family were essential in animal diets, although the w3 fatty acids reduce some of the symptoms of essential fatty acids (EFA) deficiency (Galli, 1988). Rats will survive and grow normally on a diet containing only w6 type fatty acids (Lamptey & Walker, 1976). They may be reared successfully for even three generations on diets that contain w6, but lack w3 fatty acids. Capucin and patas monkeys require both w6 and w3 fatty acids in their diets to

a.



b.

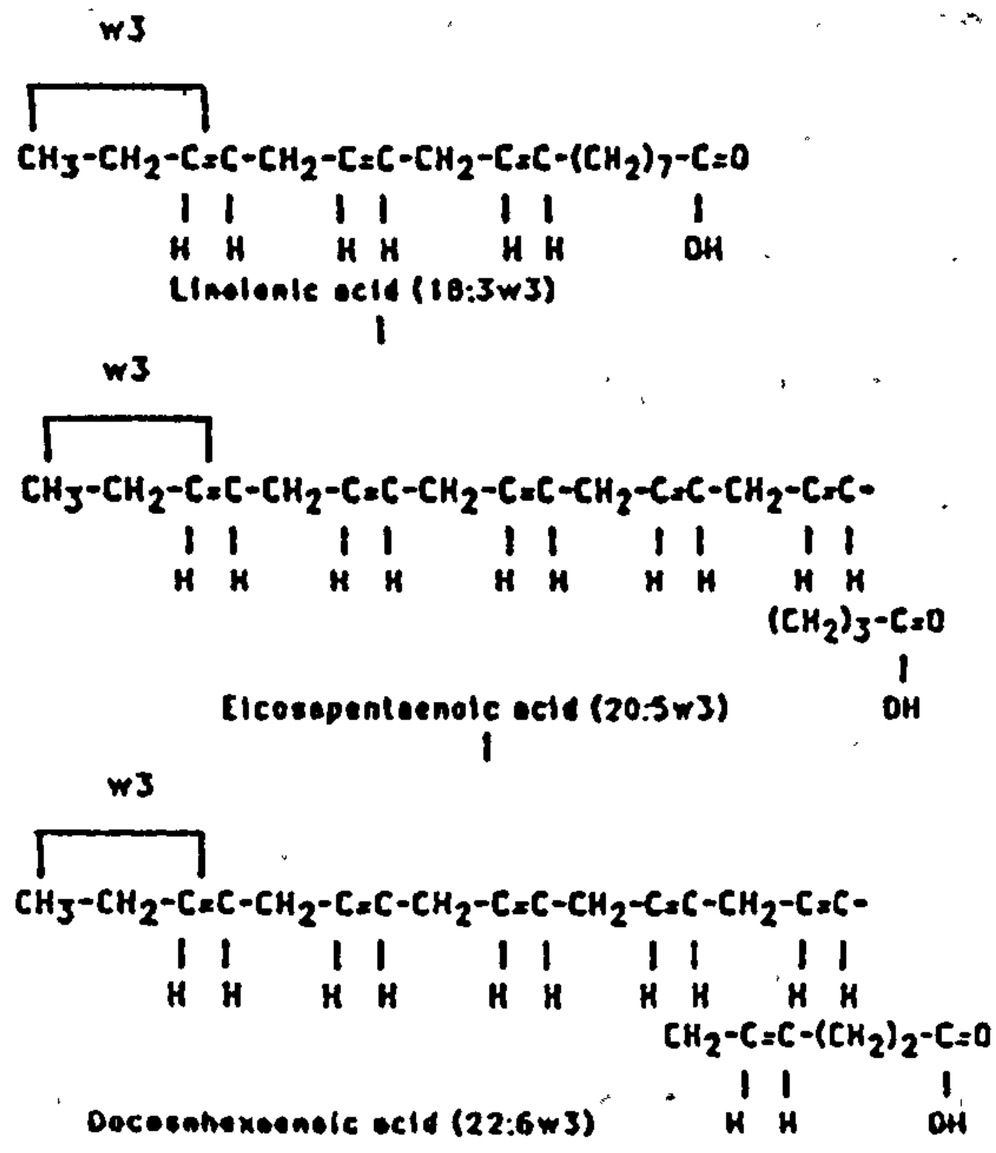


Figure 2. Conversion of linoleic acid to arachidonic acid (a) and linolenic acid to eicosapentaenoic and docosahexaenoic acids (b). Note retention of w6 (a) and w3 (b) structures as all modification in animals takes place at the carboxyl end of the chain (Ackman, 1973)

prevent EFA deficiency (Sinclair et al., 1974). Many of these are polyenoic fatty acids containing two or more double bonds in the cis- configuration separated by a methylene group (Bell et al., 1986). The commonest ones found in fish tissues are listed in table 1.

It is generally admitted that EFA's have two functions. First, they have a dynamic function as precursors of prostanoids and linear oxygenated derivates with local cellular actions. They act specially in various phenomena such as inflammations, the hemostasis and the muscular contraction of unstriped fibers. Second, they have an important structural membrane role (Leger et al., 1987).

The EFA requirement of homeothermic animals is generally satisfied by fatty acids of the linoleic (18:2w6) acid series (Holman, 1968). Fish, poikilothermic animals, have been shown to have varying requirements for either linoleic (18:2w6), linolenic (18:3w3) or mixtures of fatty acids from both series (Castell, 1979; Castell et al., 1981). The nutritional studies of fish and crustaceans for lipids using feeding trials have shown that EFA requirements of aquatic animals vary with the species (Kanazawa et al., 1979b). Differences in the effect of dietary 18:3w3 on the growth of aquatic animals are ascribed to the discrepancy in the capacity for bioconversion of 18:3w3 to w3 series of HUFA

TABLE 1. Polyunsaturated fatty acids commonly found in fish

Common Name	Systematic Name	Abreviation
Linoleic	9,12-octadecadienoic	18:2w6
Arachidonic	5,8,11,14-eicosatetraenoic	20:4w6
Timnodonic	5,8,11,14,17-eicosapentaenoic	20:5w3
Clupanodonic	7,10,13,16,19-docosapentaenoic	22:5w3
Cervonic	4,7,10,13,16,19-docosahexaenoic	22:6w3

Note: the " w " nomenclature (used in this review) numbers the double bonds from the methyl end of the fatty acyl chain

(Yu & Sinnhuber, 1972; Fujii et al., 1976; Takeuchi & Watanabe, 1977; Kanazawa et al., 1978; Teshima, 1978). An essential dietary requirement for w3 HUFA has been reported for different species of fish and shrimps (Wickins, 1972; Fuji & Yone, 1976; Cowey & Sargent, 1972, 1977, 1979; Kanazawa et al., 1979b; Jones et al., 1979a, b; Bottino et al., 1980; Waldock & Holland, 1984).

The fatty acid composition of crustacean lipids has been shown to be affected by many factors. Variation between different species is probably due to a combination of genetics, diet, environmental temperature and other biotic and abiotic factors (Castell, 1981). Variable effects of lipid supplementation in shrimp diets has shown specific fatty acid composition to be more important than total lipid content (New, 1976). As temperature decreases, monounsaturated (16:1 and 18:1) and polyunsaturated (20:4, 20:5, and 22:6) fatty acids increase at the expense of the saturated fatty acids (16:0 and 18:0) (New, 1976). Even within single species, fatty acid patterns may be significantly altered by one or more of these factors. The fatty acids vary amongst different tissues and organs and even amongst different lipid classes of any tissue or organ. Variations occur between wild and pond reared crustaceans (Pigott, 1989). In spite of this complex fatty acid composition picture, there are a number of consistant

patterns that might be valuable in predicting EFA requirements of crustacean species of interest to aquiculture. The ability to elongate and desaturate fatty acids of the linoleic (18:2w6) and linolenic (18:3w3) series has been demonstrated in a number of crustaceans (Kayama et al., 1963; Kanazawa et al., 1979b). Fatty acids of either linoleic (18:2w6) and linolenic (18:3w3) series obtained from dietary sources could, however, be chain elongated and further desaturated. Thus, the fatty acid pattern of crustacean lipids could be expected to represent a combination of those fatty acids synthesized de novo and those obtained from diets (Davis & Robinson, 1986). Similarly, coldwater crustaceans might be expected, based on differences in fatty acid patterns, to require more linolenic (18:3w3) series fatty acid than warmwater species (Castell, 1981).

Freshwater and terrestrial animals contain larger quantities of C16 and C18 saturated fatty acids (Wolfe et al., 1965; Zandee, 1966; O'Connor & Gilbert, 1968; Chapelle, 1977). Freshwater fish, specially commercial cultivated fish, tend to have high levels of 18:2w6 (Ackman, 1967). This reflects inexpensive terrestrial feed sources rich in 18:2w6, usually accompanied by 18:3w3. Table 2 shows the trends of fatty acids in lipid for some freshwater fishes, and it can be seen that type of fatty acid and the w6/w3 ratio vary in a

TABLE 2. Trends of fatty acids in lipid for some fishes and crustaceans living in " freshwater " environment

Species	Common name	Sat.	Mono.	PUFA	HUFA	ω6	ω3	ω6/ω3	Source
FISH									
Cuprinus carpio	Common carp	20.7	56.7	22.6	8.4	13.7	8.9	1.54	Ackman, 1973
Ictalurus punctatus	Channel catfish	26.7	56.9	16.4	3.3	12.9	3.5	3.69	Pigott, 1989
Salmo gairdneri	Rainbow trout	39.5	27.6	32.9	15.8	11.1	21.8	0.51	Ackman, 1973
Sarotheredon niloticus	Tilapia	32.1	47.6	20.3	5.4	3.6	16.7	0.22	Pigott, 1989
X		29.7	47.2	23.1	8.2	10.3	12.7	1.49	
Range		20.7-39.5	27.6-56.9	16.4-32.9	3.3-15.8	3.6-13.7	3.5-21.8	0.22-3.69	
CRUSTACEAN									
Astacus astacus	Noble crayfish	40.6	39.4	20.0	5.9	19.0	1.0	19.00	Collatz, 1969
Macrobrachium rosenbergii	Giant fw prawn	26.4	22.4	51.2	11.2	37.5	13.7	2.74	Sandifer & Joseph, 1976
Palaemon paucidens (1)	Sujiebi shrimp	17.8	41.5	40.7	24.7	8.5	32.2	0.26	Teshima et al., 1976
Sesarma dehaani (2)	Crab	34.1	37.5	28.4	8.3	13.1	14.3	0.86	Teshima et al., 1976
X		29.7	35.2	35.1	12.5	19.5	15.6	5.72	
Range		17.8-40.6	22.4-41.5	20.0-51.2	5.9-24.7	8.5-37.5	1.0-32.2	0.26-19.00	
TOTAL X		29.7	41.2	29.1	10.4	14.9	14.2	3.61	
TOTAL RANGE		17.8-40.6	22.4-56.9	16.4-51.2	3.3-24.7	3.6-37.5	1.0-32.2	0.22-19.00	

(1) harvested in the freshwater lake of Ikeda, Kagoshima
(2) terrestrial crab called Kurobenkeigani (in Japanese)

wide range. In some studies with rainbow trout Salmo gairdneri, Yu and Sinnhuber (1975) showed that relatively high levels (5%) of linoleic acid (18:2w6) inhibited growth and increased mortality, while diets with 1% linolenic acid (18:3w3) alone supported rapid fish growth with high survival. The same authors (1976) showed that diets high in w6 and deficient, or low, in w3 fatty acids did not support rapid growth in trout when compared to diets containing high level of w3, but low level of w6 fatty acids. In carp Cyprinus carpio, both 18:2w6 and 18:3w3 were effective (Watanabe et al., 1975). Leray & Pelletter (1985) also showed that rainbow trout fed for one year grows better on a diet high in w3 series than high in w6 series fatty acids. According to Kanazawa et al. (1979b), linolenic acid (18:3w3) is converted to 20:5w3 and 22:6w3 intensively in the farmed fish.

Table 2 also shows the trends of fatty acids in lipid for some freshwater crustaceans. Type of fatty acid and w6/w3 ratio vary in a much wider range than for freshwater fishes. The freshwater species tend to have higher levels of linoleic (18:2w6) type fatty acids (Castell, 1981; Ackman, 1980). The de novo synthesis of fatty acids from C14 labeled acetate has been demonstrated in crayfish Astacus astacus (Zandee, 1966). Macrobrachium rosenbergii adult has more PUFA's but these reflect the high content of w6 fatty acids

(Sandifer & Joseph, 1976). The terrestrial crab Sesarma dehaani contains more monoenoic acids (Teshima et al., 1976). The freshwater shrimp Palaemon paucidens was surprisingly rich in PUFA and its w6/w3 ratio relatively low (Teshima et al., 1976). They could reflect some possible marine ancestral origin as most of the species of this family inhabit the sea.

Freshwater fishes and crustaceans both have low HUFA's and low w3 fatty acids (table 2).

Marine animals contain relatively large amounts of long-chain polyunsaturated fatty acids (principally 20:5 and 22:6) which are mainly incorporated into phospholipids and especially into phosphatidylethanolamine (Chapelle, 1977). The conservation of high levels of PUFA's in lipids during embryogenesis and early larval development of atlantic herring Clupea harengus L. reflects the importance of these fatty acids during development (Tocher et al., 1985). The effect of linolenic acid (18:3w3) on the growth of fingerling milkfish Chanos chanos (Forsskal) is better than that of linoleic acid (18:2w6), but addition of both of them, alone or in combination, increases the levels of PUFA's (Bautista & De la Cruz, 1988). Marine fishes show higher levels of HUFA's expressed mainly by a higher content in w3 fatty acids than w6 fatty acids and, consequently, the w6/w3 ratios are very low (2.4% and 0.11, respectively) (table 3).

TABLE 3. Trends of fatty acids in lipid for some fishes and crustaceans living in " marine " environment

Species	Common name	Sat.	Mono.	PUFA	HUFA	ω6	ω3	ω6/ω3	Source
FISH									
Clupea harengus	Atlantic herring	21.2	59.9	18.9	14.1	1.5	17.4	0.09	Ackman, 1973
Engraulis capensis	Anchovy	33.3	35.6	31.1	25.6	1.5	29.6	0.05	Young, 1982
Gadus morhua	Cod	20.6	47.7	31.7	24.0	3.4	28.3	0.12	Young, 1982
Mallotus vilosus	Capelin	21.3	65.7	13.0	8.1	1.9	11.1	0.17	Ackman, 1973
Salmo salar	Atlantic salmon	23.8	45.0	31.2	13.4	3.5	27.7	0.13	Pigott, 1989
X		24.0	50.8	25.2	17.0	2.4	22.8	0.11	
Range		20.6-33.3	35.6-65.7	13.0-31.7	8.1-25.6	1.5-3.5	11.1-29.6	0.05-0.17	
CRUSTACEAN									
Palaemon elegans	Common prawn	29.7	32.5	37.8	24.8	9.0	28.8	0.31	Myers, 1979
Pandalus borealis	Deepwater shrimp	23.0	47.0	30.0	17.3	6.6	23.4	0.28	Hayashi, 1976
Penaeus japonicus	King shrimp	26.1	30.7	43.2	27.0	11.0	32.2	0.34	Guary et al., 1975
Penaeus monodon	Tiger shrimp	33.5	29.2	37.3	16.8	14.3	23.0	0.62	Millamena et al, 1988
Temora longicornis	Copepod	37.7	30.4	31.9	26.4	3.3	28.6	0.12	Young, 1982
X		30.0	34.0	36.0	22.5	8.8	27.2	0.33	
Range		23.0-37.7	29.2-47.0	30.0-43.2	16.8-27.0	3.3-14.3	23.0-32.2	0.12-0.62	
TOTAL X		27.0	42.4	30.6	19.8	5.6	25.0	0.22	
TOTAL RANGE		20.6-37.7	29.2-65.7	13.0-43.2	8.1-27.0	1.5-14.3	11.1-32.2	0.05-0.62	

However, linolenic acid (18:3w3) is moderately converted to 20:5w3 and 22:6w3 in the sea ayu Plecoglossus altivelis and the river eel Anguilla japonica, but only slightly in the red sea bream Chrysophrys major, the marine rockfish Sebastiscus marmoratus, and the sea globefish Fugu rubripes rubripes (Kanazawa et al., 1979b). Yone & Fujii (1975) observed that neither 18:2w6 nor 18:3w3 exerted a growth-promoting effect for the red sea bream Chrysophrys major.

The ability to synthesize saturated fatty acids from acetate was shown to be present in several species of marine crustaceans studied to date (Zandee, 1967; Kanazawa & Teshima, 1977; Kanazawa et al., 1979a; Castell, 1981). The de novo synthesis of fatty acids from C14 labeled acetate has been demonstrated in the lobster Homarus gammarus (Zandee, 1967), and the prawns Penaeus japonicus (Kanazawa & Teshima, 1977), Penaeus monodon and P. merguiensis (Kanazawa et al., 1979c). Marine mysid Neomysis integer Leach has even been shown to convert starch or short-chain saturated fatty acids to long chain polyunsaturated fatty acids, which were incorporated into the triglyceride and phospholipid fractions (Morris et al., 1973).

Marine crustaceans tend to have higher levels of linolenic (18:3w3) series fatty acids and higher amounts of 20 and 22

carbon PUFAs than freshwater crustaceans (Castell, 1981; Ackman, 1980; Watanabe, 1982). Table 3 also shows a tendency for higher levels of HUFA's and w3 fatty acids in marine crustaceans. Watanabe (1982) observed that penaeids may require eicosapentaenoic (20:5w3) or docosahexaenoic (22:6w3) acid to satisfy their EFA requirement.

Furthermore, linolenic acid (18:3w3) was found to be moderately converted to 20:5w3 and 22:6w3 in the farmed prawn Penaeus japonicus (Kanazawa et al., 1979b). The lipids obtained from this marine prawn and brackish water crab Helice tridens tridens are rich in PUFA's and deficient in the long chain monoenoic acids, particularly in palmitoleic (16:1w7) and oleic (18:1w9) acids (Teshima et al., 1976). They also totaled less monoenoic acids than the freshwater shrimp P. paucidens and the terrestrial crab S. dehaani. The inverse relationship shown between the 18:3w3 content and the levels of 20:5w3 and 22:6w3 acids in the triglyceride and the phospholipid fractions of Penaeus aztecus juveniles suggests that the former resembles dietary lipid while the latter mirrors the biochemical pathways of the animal (Shewbart et al., 1973). Palaemon serratus appears to be capable of synthesizing C20:5w3 and C22:6w3 from C18:2w6 and C18:3w3, but growth rates are improved when C20:5w3 and C22:6w3 are supplied directly in the diet (Martin, 1980).

The marine fish and crustaceans seem to be inferior to freshwater analogues in their capacity for elongation and

desaturation of dietary 18:3w3 to w3 HUFA (Kanazawa et al., 1979b). Tables 2 and 3 also clearly show that lipids in marine animals have more HUFA's, due to a higher w3 fatty acid content than freshwater animals (19.8% vs 10.4% and 25.0% vs 14.2%, respectively). Their w6 fatty acid content and, therefore, their w6/w3 ratios are also much lower than their homologues in freshwater species (5.6% vs 14.9% and 0.22% vs 3.61%, respectively).

Lipid content in larvae of Penaeus monodon decreases with developmental stage indicating utilization of lipids as an energy source during larval development and metamorphosis (Ward et al., 1979; Millamena, 1985). As the larva develops, levels of 16:1 and 18:1 fatty acids decrease with a corresponding increase in PUFA's, particularly 20:5w3 and 22:6w3. This indicates the importance of PUFA's as dietary components in this marine prawn. When fed with brine shrimp boosted on algae, P. monodon display better postlarval survival and significantly higher growth which is related to the content of PUFAs in Artemia sp. (Millamena et al., 1988).

Since the initial successful rearing of Macrobrachium rosenbergii by Ling (1962), several studies have been conducted to improve larval culture (Ling, 1969a, 1969b; Morizane et al., 1974; Sick & Beaty, 1974; Durgan et al.,

1975; Hagwood & Willis, 1976; Manzi et al., 1977; AQUACOP, 1977; Murai & Andrews, 1978; Menasveta et al., 1984). As a result, larviculture technology is relatively advanced, but remains to be optimized both in terms of production and efficiency. Feed quality and feeding technology are amongst the major aspects requiring improvement and standardisation. At present many types of the diets are used for Macrobrachium larvae, but all are used as a supplement to Artemia nauplii.

In general, Artemia allows complete larval development of many species up to juvenile stage or beyond with reasonably consistent duration and morphological sequence (Leger et al., 1986). However, there are some constraints related to the use of Artemia. One of the major problems is the variation in nutritional value amongst different geographical strains, even amongst the batches harvested from the same lake, and sometimes from year to year (Reeve, 1969; Wickins, 1972; Matsuoka, 1975; Watanabe et al., 1978; Fujita et al., 1980; Holland & Jones, 1981). Some strains contain high amounts of essential w3 HUFA, whilst these may be low or absent in others. They can be divided into three types: one group being low in both 18:3w3 and 20:5w3; a second group, low in 18:3w3, but high in 20:5w3; and a third group having high amount of 18:3w3, but very low in 20:5w3 (Fujita et al., 1980; Schauer et al., 1980). When Artemia

were fed with yeast and algae, Fujita et al. (1980) obtained an improvement in the lipid pattern of the brine shrimp which previously contained low amounts of 20:5w3 (Fujita et al., 1980).

Detailed biological and biochemical analysis revealed that the nutritional composition of particular Artemia strains does not always meet the requirements of the predating larvae. Sometimes, considerable variation takes place in highly unsaturated long chain fatty acids, such as eicosapentaenoic acid (20:5w3), which are essential for marine fish and crustacean larvae (Leger & Sorgeloos, 1985). A positive correlation was found by Sorgeloos et al. (1983) between poor larval survival of mysid Mysidopsis bahia and low levels of the polyunsaturated fatty acids in Artemia.

Diet can improve the fatty acid composition of cultured brine shrimp (Dobbeleir et al., 1980). Fatty acids such as 20:5w3 and 22:6w3 supplied in encapsulated diets appear in the tissues of Artemia nauplii fed on these diets. This improves the nutritional value of Artemia as a live food for marine fish and invertebrate larvae, which have a dietary requirement for long chain PUFA (Sakamoto et al., 1982). Walford and Lam (1987) improved the nutritional value of rotifers and Artemia naulpii by feeding them with microcapsules containing a high percentage of total lipids

and HUFA's. Rainuzzo et al. (1989) also positively influenced the fatty acid composition of the rotifer Brachionus plicatilis by giving enrichment diets high in w3 HUFA. But both authors did not record survival and growth of marine fishes fed on these boosted live diets.

Artemia salina is used intensively as a principal feed in Macrobrachium larviculture, but little information is available on the effect of high and low w3 HUFA containing Artemia on larval survival and growth, although Matsuoka (1975) reported that Chinese Artemia nauplii are toxic to Macrobrachium rosenbergii larvae due to high levels of BHCs and DDT.

The total lipid content of the freshwater prawn, M. rosenbergii, was found by Chanmugan et al. (1983) to be greater than that of marine shrimp (3.18% vs 1.33%) due to much higher levels of triglycerides in freshwater prawns as compared to marine shrimp. The w3 PUFA predominated in marine shrimp, primarily due to the greater concentration of linoleic (18:2w6) in freshwater organisms(16.3% vs 2.9%). However, lipid supplementation in shrimp diets has shown that specific fatty acid composition is more important than total lipid content (New, 1976).

Sandifer and Joseph (1976) observed that increasing

percentages of w3 fatty acids and saturated acids in the diet of the juvenile M. rosenbergii resulted in better survival after only a 12 week trial in laboratory. Ahmed (1988) observed that better growth and survival of M. rosenbergii were obtained when larvae were fed on Artemia with a high w3 HUFA profile. In contrast, Ismail (1989) reported no significant increase in growth and survival for the same prawn larvae when fed on newly hatched Artemia nauplii with increased content of total lipid and w3 PUFA's. However, plots of total lipid content and w3 PUFA's in diet against survival and growth indicate that there is an increasing tendency for better growth and survival with increasing total lipid and w3 PUFA. 45 day old juvenile M. rosenbergii only showed significantly greater mean weight gains when fed HUFA's for 4.5 months (D'Abramo & Sheen, 1989). However, they did not vary in mean weight gain when prawns were fed diets containing different w6 and w3 PUFA levels. According to these authors, dietary lipid level did not appear to influence survival and molting frequency.

M. rosenbergii spends its larval cycle in brackish water (estuary) and its adult life in freshwater (Ling, 1969a). Therefore, the PUFA requirement for the larvae of this commercial species needs to be known to improve dietary strategy leading to better survival and growth in a controlled environment. The present work describes the

results when several different levels of w3 PUFA containing Artemia were fed to M. rosenbergii larvae. With the aim of better defining the importance of PUFA/HUFA's and total lipid levels on larval development, survival and growth are measured and comparisons are made with the lipid composition of M. rosenbergii whole-body larvae and any indirect influence of diets received by the parent broodstock before spawning.

MATERIALS AND METHODS

Male and female Macrobrachium rosenbergii were maintained at a 1:3 ratio in a Fastank raceway within the tropical unit, Marine Science Laboratories, Menai Bridge. They were fed daily with 3% body weight frozen shrimp (Pandalus borealis) on days 1, 3 and 5 (on a weekly basis), fresh mussel (Mytilus edulis) on days 2 and 6, frozen squid (Loligo vulgaris) on day 4, and pellets on day 7 (see table 1.0, in appendix, for composition). Berried females were isolated and special care was given as soon as eggs were deposited on the pleopods. When eggs hatched, larvae were removed to 2 litre round flasks at a density of 50 larvae per litre. All dietary treatments were triplicated.

Artemia nauplii (San Francisco Bay Brand Inc., CA, U.S.A.) were supplied at 12 per ml in all controls.

Cysts of Artemia hatched after a 18-24h period of strong

aeration in salt water, the nauplii were then separated from unhatched cysts by tilting the conical vessel, concentrated with a light (positive phototropism), and siphoned off. They were then washed in brackish water and added to the flasks containing Macrobrachium rosenbergii larvae. Artemia density were estimated by repeated ml counting with an automatic calibrated micropipette. Uneaten feed, faecal matter, dead larvae and exuviae were removed daily. At 48h intervals, water was renewed and numbers of larvae were recorded.

A thermostat and electrical heater were used to maintain water temperature in rearing flasks at 29°C. Salinity was brought to 12‰ and checked with a refractometer. Water was previously cartridge filtered to 0.2 µm and irradiated with U.V. light to reduce bacterial contamination. Each flask was gently aerated.

As several authors have shown strong interactions of algae on larval development of Macrobrachium rosenbergii (Ling, 1969; Wickins, 1972; Cohen et al., 1976; Madox & Manzi, 1976) and light (Lu et al., 1976; Chao & Liao, 1977; AQUACOP, 1977; Iwai, 1979; Deru, 1980), preliminary experiments were run to determine the best conditions for rapid larval development under controlled conditions.

Preliminary experiment 1: algal requirements

Chlorella vulgaris (Beijer), cultured in the algal unit, Marine Science Laboratories, was added to the medium, in which newly hatched Macrobrachium larvae were reared, at the following average concentrations:

Level	Algal cell count per µl
1	300
2	400
3	450
4	500
5	550
6	600
7	650
8	750
9	1500

Algae were evaluated with a Zb Coulter Counter supplied by Coulter Electronics Ltd. (Northwell Drive, Luton, England). Macrobrachium larvae also received twelve Artemia nauplii per ml from stage II. After eight days, the experiment was terminated and survival and final larval stage were recorded.

Growth was expressed in terms of the mean larval stage (M.L.S.) and recorded as the sum of the stage values divided

by the number of individuals. Identification of stages was carried out using the keys provided by Ling (1967), Uno and Kwon (1969) and New and Singholka (1985). Table 2.0 (appendix) summarizes the identification of Macrobrachium rosebergii larval stages for all experiments.

Preliminary experiment 2: light requirements

Larvae were reared under 2 different light intensities. Half of the flasks were covered with a black plastic sheet to reduce the illumination levels, the other flasks were uncovered. Light intensities were measured with an Irradiance Collector with Solid Teflon Sphere, model QSL-100 from Biospherical Instruments Inc. (San Diego, CA, U.S.A.). Macrobrachium larvae received daily 12 Artemia nauplii per ml and Chlorella was given at 550 cells per µl from the beginning of the experiment. Cell counting was evaluated using a Zb Coulter Counter, supplied by Coulter Electronics Ltd. (Northwell Drive, Luton, England). Meristic characteristics of Macrobrachium post-larvae were recorded, and the experiment terminated at post larvae I stage.

Experiment 1: effect of increasing dietary w3 fatty acids

Macrobrachium rosenbergii larvae were reared on four strains of Artemia containing increasing concentrations of w3 PUFA.

Frippak Boost microcapsules were used to enhance the w3 PUFA and total lipid content of all strains of Artemia. The 13% lipid microcapsules were 0-30μm in size. The artificial diets were prepared fresh every day by adding dried microcapsules to distilled water at the concentration of 8mg per litre.

The fatty acid composition of 5000 pooled Artemia nauplii lipids was determined by total lipid extraction (Folch et al., 1957), methyl esterfication (Morrison & Smith, 1964) and separation by HRG-Chromatography (Carbo Erba Strumentazione equipped with a carbo wax 20m capillary column). GC output was fed to a Hewlett Packard 3390 A Integrator and peaks identified by comparison with known standards.

Experiment 2: fatty acid evaluation of other diets

Six different regimes commonly used in M. rosenbergii hatcheries were compared for nutritive value to the results of experiment 1.

Total lipid and fatty acid composition of unfed Artemia, Chlorella vulgaris (Beijer) and Dunaliella viridis (Teodor) prefed Artemia were analysed following the method described previously. Data from microcapsules, chlorella and Dunaliella were taken from Frippak Feeds Ltd. and Steward

(1974), respectively.

Experiment 3: fatty acids in M. rosenbergii

Newly hatched larvae of twenty Macrobrachium females were analysed for fatty acid patterns. Pooled larval samples of a thousand were treated and analysed in triplicate with the HRG-Chromatography as described in trial 1.

Experiment 4: " weak " vs " strong " M. rosenbergii larvae

Fatty acids were compared in " strong " versus " weak " newly hatched larvae. " Strong " was evaluated according to the stage reached after eight days starvation. For this evaluation, fifty larvae of each batch, in triplicate, were starved and daily survival and final stage were recorded.

Experiment 5: fatty acid profile of stages I and II for M. rosenbergii larvae

To measure the fatty acid depletion between the two first larval stages, 1000 pooled Macrobrachium larvae of stages I (non feeding or not actively feeding) and II (starting to feed actively) were treated and analysed using the HRG-Chromatography as previously described. The stage II sample was not allowed to feed.

Experiment 6: lipid composition of M. rosenbergii parent diets

As yolk reserve is greatly influenced by feed quality given to the parents (Cohen et al., 1976; Maddox & Manzi, 1976), total lipid and fatty acid analysis were undertaken on the adult diet e.i. shrimp Pandalus borealis, squid Loligo vulgaris, both bought frozen from Kimfish Co. (Bangor), fresh mussel Mytilus edulis collected from Menai Bridge and Beaumaris, and pellets made in the nutrition unit, Marine Science Laboratories (table 1.0, appendix). Pellet composition includes frozen squid, shrimp meal, wheat flour, rice starch, tapioca, cod liver oil and microcapsules as vitamins and mineral premix.

RESULTS

Preliminary experiment 1: algal requirements

Survival and final stage of 8-day M. rosenbergii larvae reared at nine different concentrations of Chlorella vulgaris (Beijer) are presented in tables 4 and 3.0 (appendix), and represented in figures 3a and b. The best rearing medium was found to be between 537 and 739 cells per µl. The one-way ANOVA carried out on final stages was highly significant ($F = 79.661^{**}$; D.F.num. = 8; D.F. denom.= 216; $\alpha = 0.01$). A Duncan's multiple range test (Zar, 1984) shows clear differences (at $\alpha = 0.05$) between the algal concentrations.

Preliminary experiment 2: light requirements

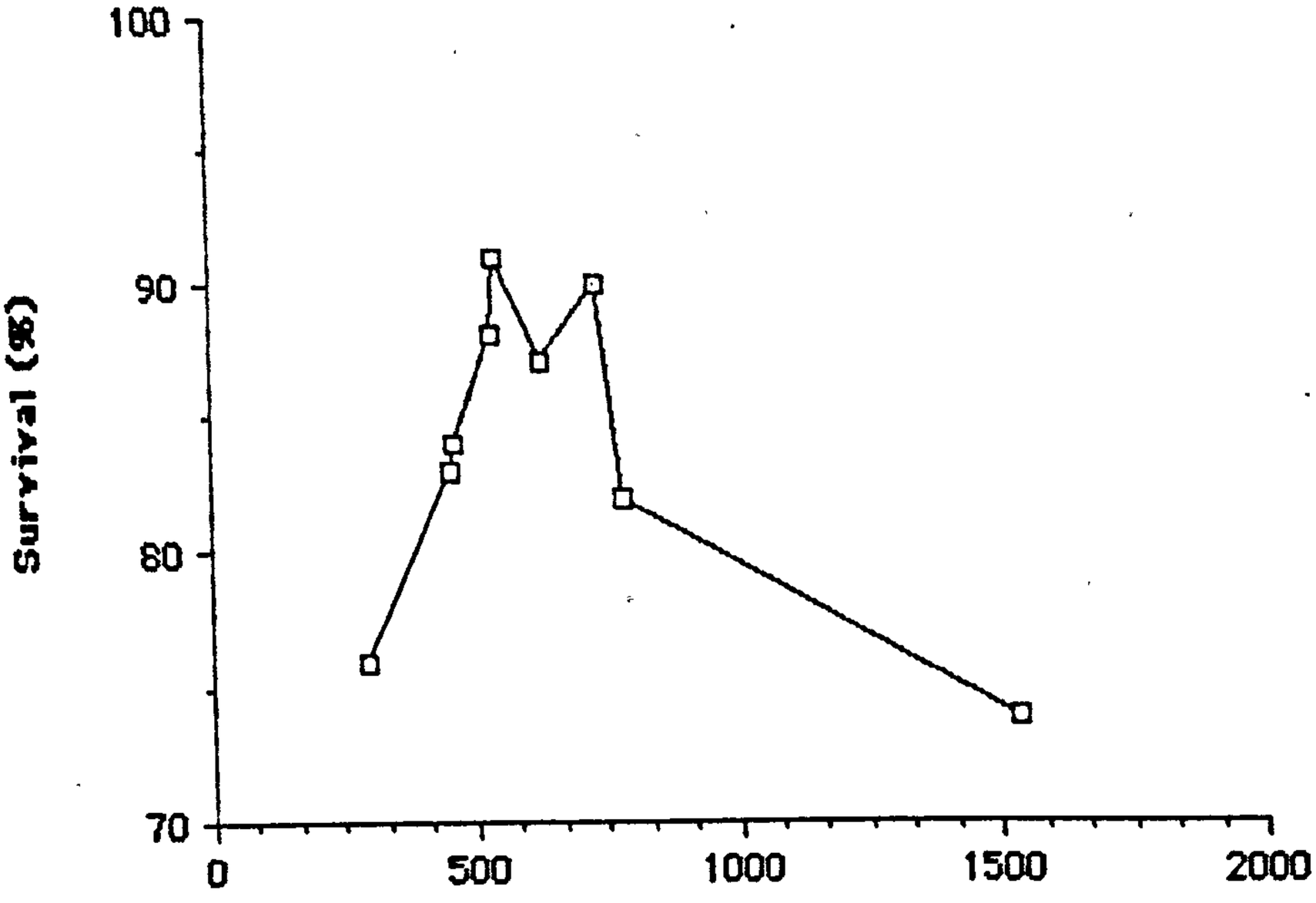
Total length of M. rosenbergii post-larvae (at PLI) are

TABLE 4. Evaluation of algal (Chlorella vulgaris Beijer) addition to Macrobrachium rosenbergii larval rearing water

Level	Algal Concentration (Cells per µl) (1)								% Survival (2)	Final stage (3)	Visual evaluation
	D1	D2	D3	D4	D5	D6	D7	X			
1	285	313	305	280	309	310	261	295	76	3.00a	very light
2	433	501	475	460	415	452	455	456	83	3.08a	light
3	429	439	483	470	459	452	493	461	84	4.00b	slightly light
4	621	517	523	530	489	544	535	537	88	4.92c	good
5	579	521	547	535	497	555	583	545	91	4.88c	good
6	635	653	657	627	613	633	593	630	87	4.80c	good
7	699	757	733	744	693	734	811	739	90	4.72c	good
8	737	829	763	783	777	779	801	781	82	3.92b	slightly dark
9	1759	1511	1439	1606	1537	1460	1419	1533	74	2.88a	dark

(1) using a Zb Coulter Counter, supplied by Coulter Electronics Ltd., Northwell Drive, Luton, England, U.K.
(2) after a 8-day larval rearing; 100 larvae per liter, in triplicate; larvae fed with 12 Artemia per ml; at 29oC
(3) means followed by the same letter are not different at 95% confidence

a.



b.

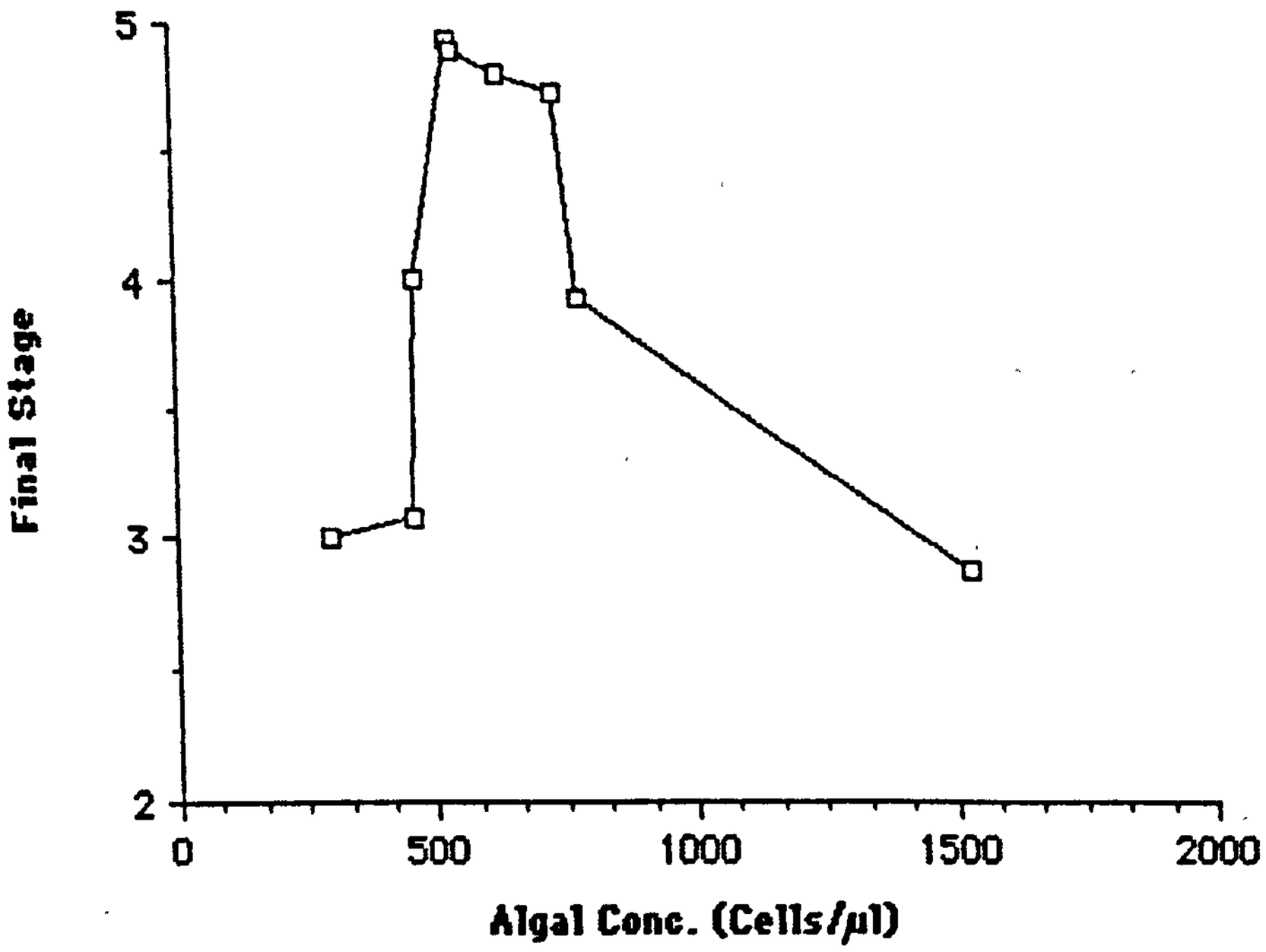


Figure 3. Percentage survival (a) and final stage (b) of 8-day M. rosenbergii fed Artemia in presence of different concentrations of Chlorella vulgaris (Beijer)

summarized in table 5. Tables 4.0 and 4.1 (appendix) give the replicates when larvae were reared in light and dark, respectively. Figure 4a shows that length of post-larvae (at PLI) was greater and survival higher when the larval cycle occurred in a three-fold reduction of light (4.82 vs 12.4 X 10^4 microeinstein.s^{-1}.cm^{-2}). Figure 4b also shows that this larval cycle is shorterned when light intensity was reduced during the rearing. A t-test confirms a significant difference ($t = 3.8648^*$; D.F.= 38; $\alpha = 0.05$) between total length of post-larvae (at PLI) reared at both intensities.

Experiment 1: effect of increasing dietary w3 fatty acids

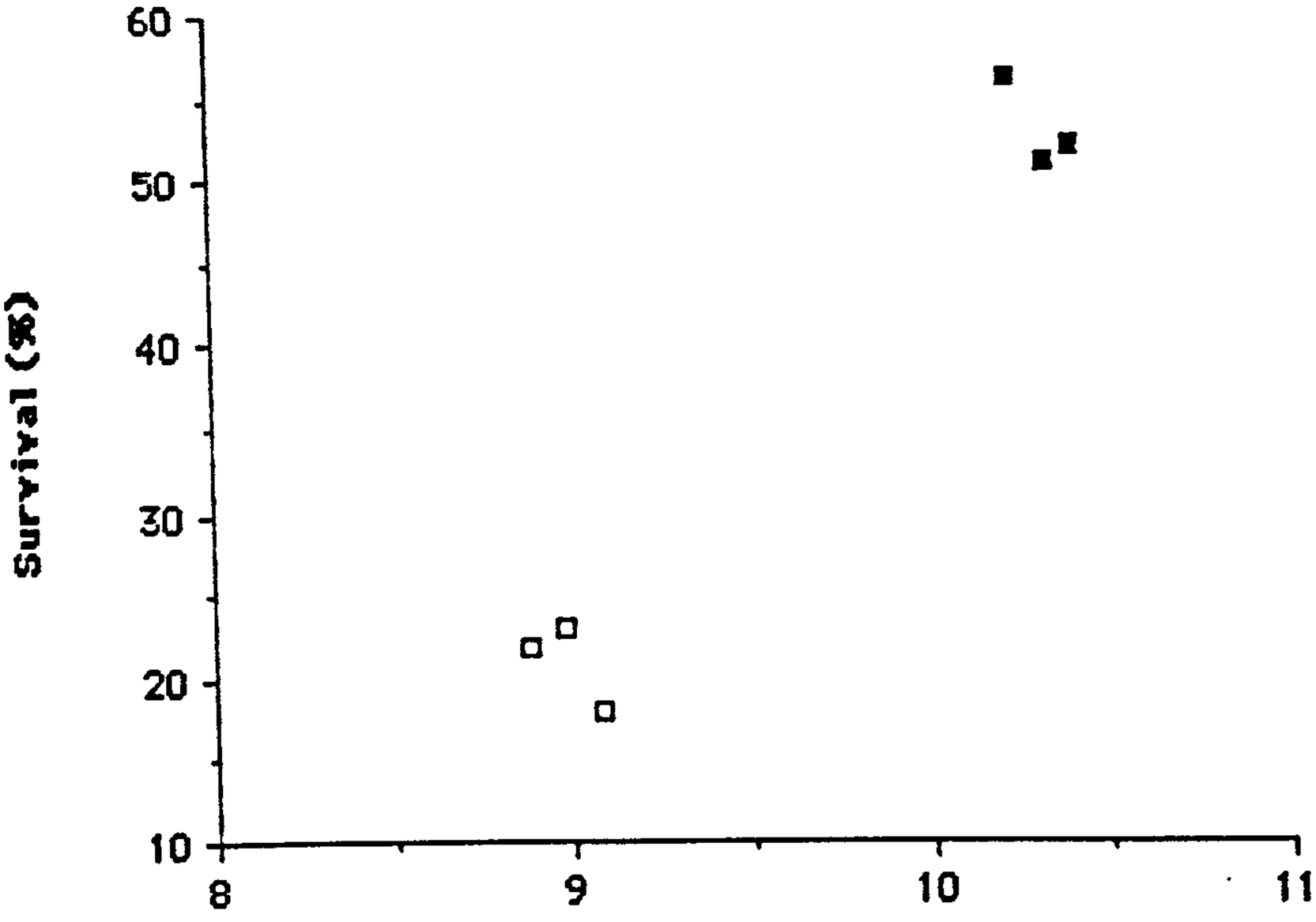
Fatty acid analysis of four strains of Artemia containing increasing concentrations of w3 PUFA's is summarized in table 6. Replicates of these results can be seen in tables 5.0, 5.1, 5.2 and 5.3 (appendix). Table 6 also shows final survival of M. rosenbergii larvae and number of days to reach PL's, or growth, when fed on the related strain of Artemia. Replicates of survival and growth can be seen in tables 6.0, 6.1, 6.2 and 6.3 (appendix). Figure 5 shows the survival of larvae fed on increasing concentrations of w3 PUFA containing Artemia. Tables 7.0, 7.1 and 7.2 (appendix) show the concentrations of ammonia (in mg NH_4-N.l^{-1}), un-ionized ammonia (in µg NH_3-N.l^{-1}) and nitrite (in

TABLE 5. Total length of *Macrobrachium rosenbergii* post-larvae I fed on *Artemia* (1) at 2 different light intensities (2)

	Length (mm)	
PLI #	Light (12.044 x 1014 me.s-1.cm-2)	Dark (4.8176 x 1014 me.s-1.cm-2)
1	7.50	10.00
2	8.25	9.75
3	10.25	11.00
4	9.00	11.50
5	7.50	10.50
6	8.25	9.50
7	8.00	8.75
8	8.50	10.00
9	8.50	12.00
10	10.25	12.25
11	9.00	9.50
12	9.75	10.50
13	9.50	12.00
14	8.25	8.75
15	10.50	9.50
16	7.75	9.25
17	7.50	10.50
18	11.00	9.00
19	10.50	11.50
20	9.75	10.75
x	8.96	10.33
S.E.	1.12	1.11
range	7.50-11.00	8.75-12.25
Survival %	21	53
S.E.	(2.65)	(2.65)
Days to reach PL's	36	24
S.E.	(5.20)	(1.00)

(1) density: 12 *Artemia* per ml
(2) with *Chlorella vulgaris* at 553 ± 71 µl-1; temperature: 29 + 0.5 oC; pH: 7.76 + 0.39; larval density: 75 ml-1

a.



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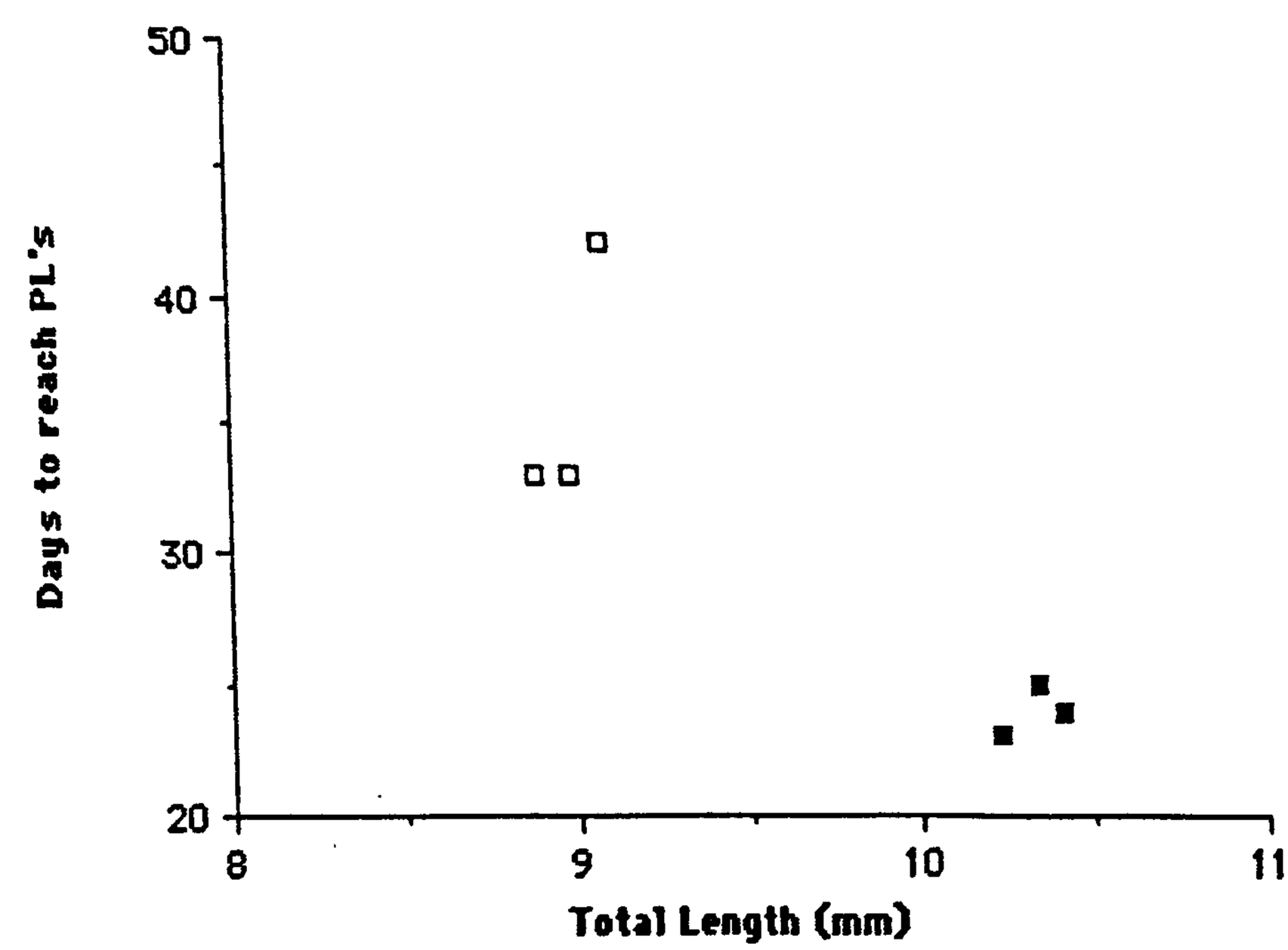


Figure 4. Percentage survival (a) and growth (b) relations to total length of M. rosenbergii larvae when reared in lightness (□) and darkness (■)

TABLE 6. Fatty acid composition and total lipid content of 8 dietary regimes resulting from 4 strains of *Artemia* unfed and fed on Frippak Boost microcapsules

Fatty acid	Low ω3 PUFA *Artemia*		Medio-low ω3 PUFA *Artemia*		Medio-high ω3 PUFA *Artemia*		High ω3 PUFA *Artemia*	
	Unfed	Fed	Unfed	Fed	Unfed	Fed	Unfed	Fed
14:0	0.5	2.2	0.7	0.6	1.2	0.5	1.0	4.8
16:0	16.0	15.1	23.5	18.8	13.9	11.6	12.1	16.8
16:1ω7	16.4	10.2	3.3	2.3	3.5	3.4	1.9	2.1
18:0	5.0	5.8	13.7	13.3	3.3	6.6	6.0	8.4
18:1ω9	27.4	30.8	20.8	23.8	28.6	27.2	21.9	29.4
18:1ω7	17.9	19.5	10.2	12.2	9.1	13.9	7.6	9.8
18:2ω6	5.3	4.9	8.2	4.4	7.9	8.0	7.3	4.3
18:3ω3	3.8	3.0	11.3	12.9	27.8	19.9	34.1	18.5
18:4ω3	0.2	0.2	2.8	3.0	1.7	1.7	7.0	2.3
20:1ω9	0.4	0.8	0.5	1.1	0.3	0.9	0.6	1.2
20:4ω6	1.3	0.8	0.4	1.3	1.1	2.6	0.3	0.3
20:3ω3	nd (1)	nd	0.4	4.2	nd	nd	nd	nd
20:4ω3	nd	nd	0.4	1.0	nd	nd	nd	nd
20:5ω3	4.2	6.1	3.1	0.6	1.6	3.8	0.4	1.2
20:1ω11	nd	nd	0.1	nd	nd	nd	nd	nd
22:5ω3	t (2)	t	0.1	nd	t	t	t	t
22:6ω3	1.6	0.8	0.6	0.7	0.2	t	t	1.0
SAT	21.5	23.1	37.9	32.7	18.4	18.7	19.1	30.0
MUFA	62.1	61.3	34.9	39.4	41.5	45.4	32.0	42.5
SAT + MUFA	83.6	84.4	72.8	72.1	59.9	64.1	51.1	72.5
PUFA	16.4	15.6	27.2	27.9	40.1	35.9	48.9	27.5
20:5ω3 + 22:6ω3	5.8	6.9	3.7	1.3	1.8	3.8	0.4	2.2
ω6	6.6	5.7	8.6	5.7	9.0	10.6	7.5	4.6
ω3	9.8	10.1	18.7	22.4	31.3	25.4	41.5	23.0
ω6/ω3	0.67	0.56	0.46	0.25	0.29	0.42	0.18	0.20
% total lipid in dry wt *Artemia*	9.3	19.5	11.5	15.8	18.3	25.3	19.5	27.8
Survival %	38	41	21	37	32	39	50	55
(S.E.)	(3.51)	(3.21)	(9.45)	(3.51)	(3.21)	(3.79)	(2.08)	(4.36)
Days to PL	38	32	50	32	32	37	34	36
(S.E.)	(4.04)	(3.06)	(3.61)	(4.51)	(1.73)	(4.04)	(3.06)	(4.93)

(1) not detected
(2) < 0.02 %

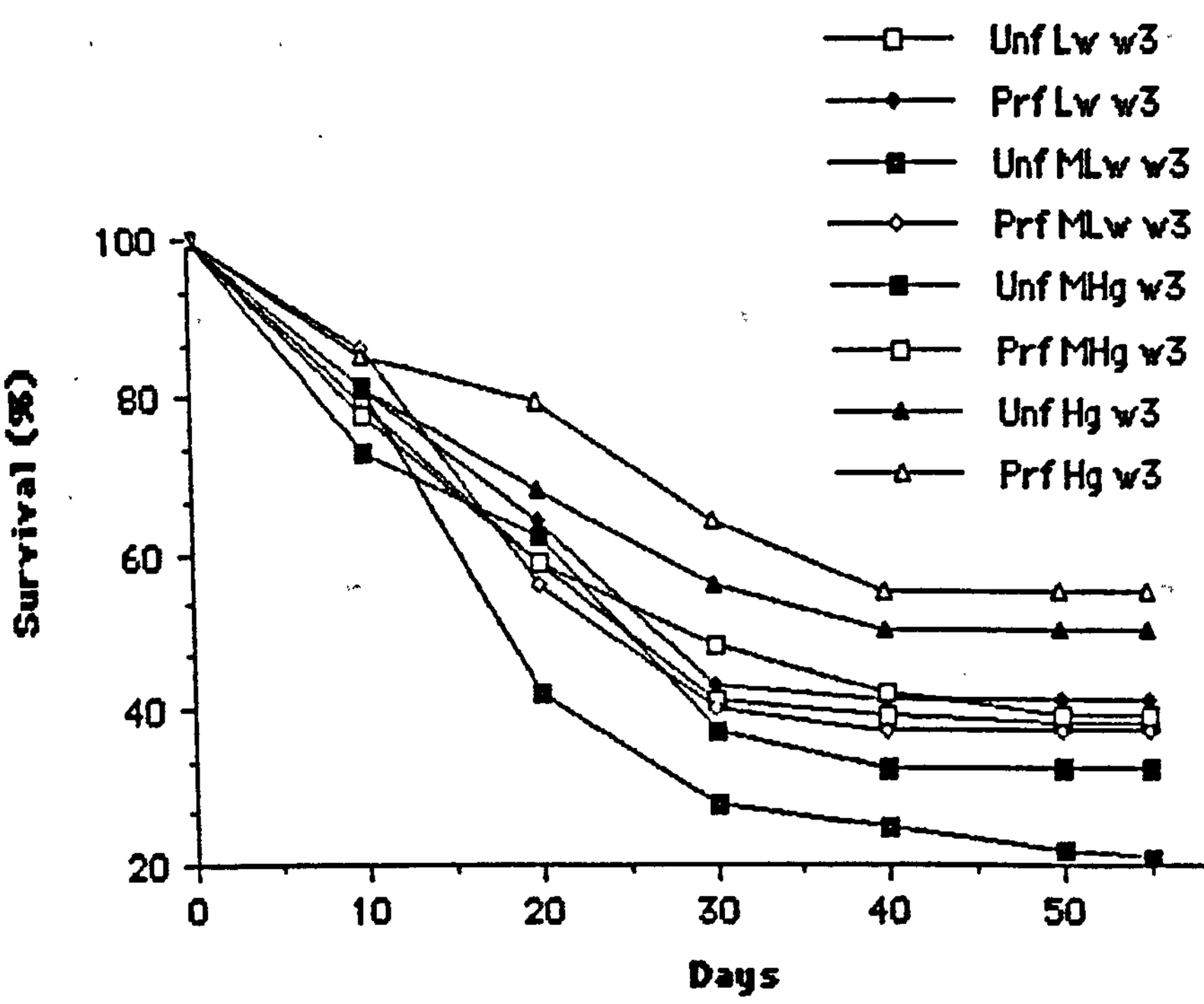


Figure 5. Percentage survival of M. rosenbergii larvae fed on increasing concentrations of w3 PUFA containing Artemia

μg NO_2-N.l^{-1}) during M. rosenbergii larval rearing when fed on a medio-low w3 PUFA containing Artemia, unfed and prefed on artificial microcapsules. Table 7 shows the correlation coefficients (r) of the regression analysis of total lipid and fatty acids of increasing w3 PUFA containing Artemia on survival and growth for M. rosenbergii larvae. Figures 6, 7 and 8 respectively represent the relations between total lipid, w6 PUFA's and linoleic acid (18:2w6) containing Artemia (unfed and prefed on artificial microcapsules) and survival and growth of M. rosenbergii larvae.

Results of these analysis show that:

1° In spite of a non-significant statistic (at $\alpha = 0.05$), survival and growth of M. rosenbergii larvae reveal a better trend (higher survival and shorter cycle, respectively) with Artemia of increasing total lipid (ref.Fig.6);

2° Survival and growth of M. rosenbergii larvae can not be related to PUFA's of Artemia as different results are obtained for unfed, prefed and unfed + prefed Artemia;

3° Survival of M. rosenbergii larvae seems to be slightly negatively related with the HUFA's in Artemia, mainly due to eicosapentaenoic acid (20:5w3), but this is not

TABLE 7. Correlation coefficients (r) of the regression analysis of total lipid and fatty acids containing *Artemia* on survival and growth of *M. rosenbergii* larvae

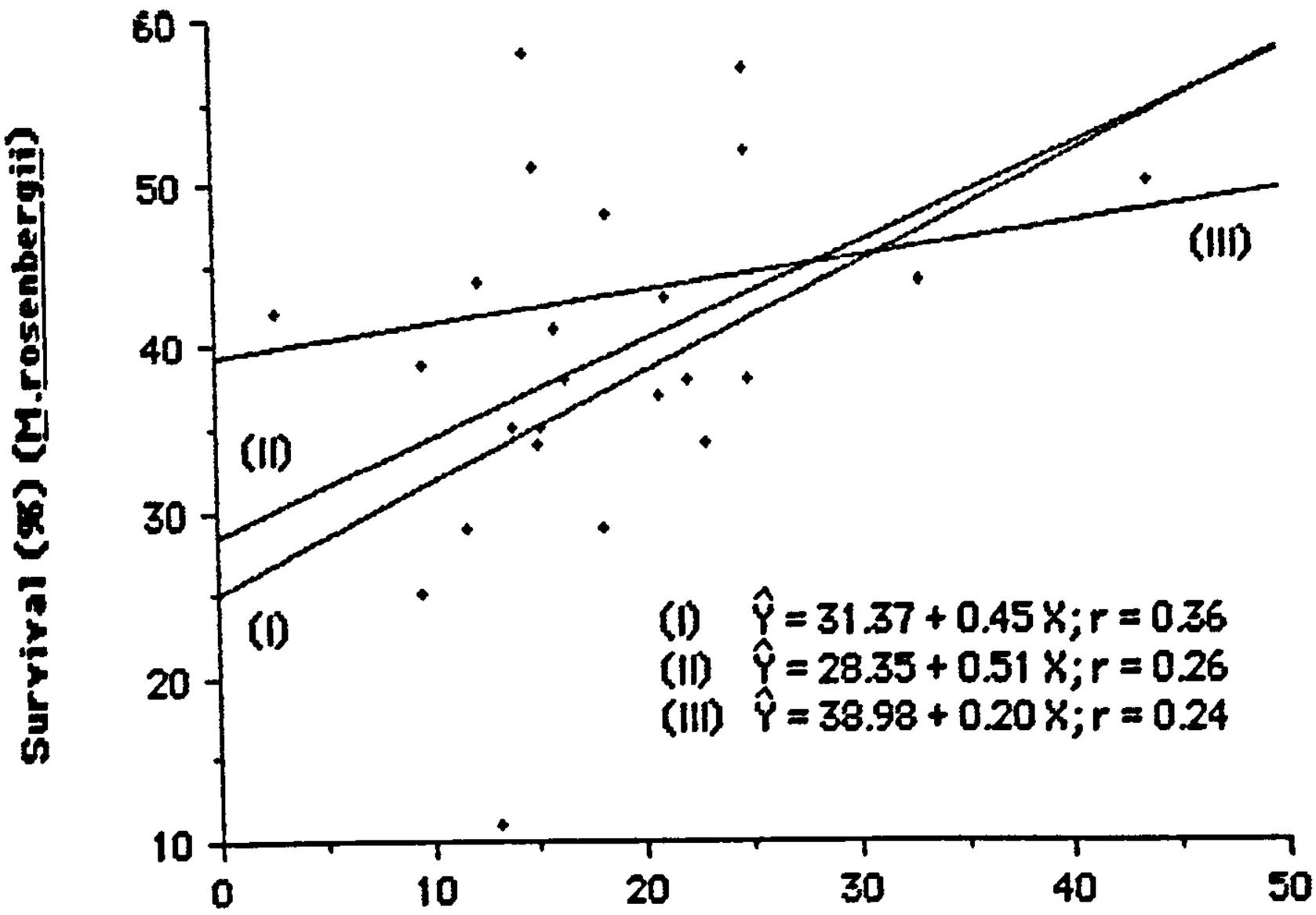
		Unfed *Artemia*	Prefed *Artemia*	All data	Trend
16:0	Survival	-0.73*	0.17	-0.44*	
	Growth	0.72*	-0.42	0.40*	
16:1w7	Survival	0.06	-0.21	-0.08	
	Growth	-0.02	-0.30	-0.03	-
18:0	Survival	-0.56*	-0.20	-0.32	-
	Growth	0.81*	-0.35	0.36	
18:1w9	Survival	0.03	0.41	0.31	+
	Growth	-0.60*	0.53	-0.22	
18:1w7	Survival	-0.09	-0.41	-0.07	-
	Growth	0.09	-0.08	-0.07	
18:2w6	Survival	-0.32	-0.38	-0.45*	-
	Growth	0.28	0.33	0.38	+
18:3w3	Survival	0.45	0.25	0.25	+
	Growth	-0.53	0.46	-0.17	
20:5w3	Survival	-0.42	-0.30	-0.26	-
	Growth	0.53	-0.25	0.11	
Saturated	Survival	-0.67*	0.21	-0.29	
	Growth	0.79*	-0.35	0.37	
MUFA	Survival	0.01	-0.05	0.07	
	Growth	-0.15	0.09	-0.13	
Sat.+ MUFA	Survival	-0.43	0.12	-0.13	
	Growth	0.38	-0.18	0.12	
PUFA	Survival	0.43	-0.12	0.13	
	Growth	-0.38	0.18	-0.12	
HUFA	Survival	-0.31	-0.19	-0.18	-
	Growth	0.47	-0.27	0.13	
w6	Survival	-0.34	-0.53	-0.46*	-
	Growth	0.20	0.14	0.22	+
w3	Survival	0.47	0.06	0.23	+
	Growth	-0.42	0.15	-0.18	
w6/w3	Survival	-0.30	-0.47	-0.37	-
	Growth	0.31	-0.15	0.21	
Total Lipid	Survival	0.26	0.24	0.36	+
	Growth	-0.35	-0.10	-0.32	-

(1) % survival

(2) Growth = number of days to reach the post-larval metamorphosis

* Significative at 95% confidence level

a.



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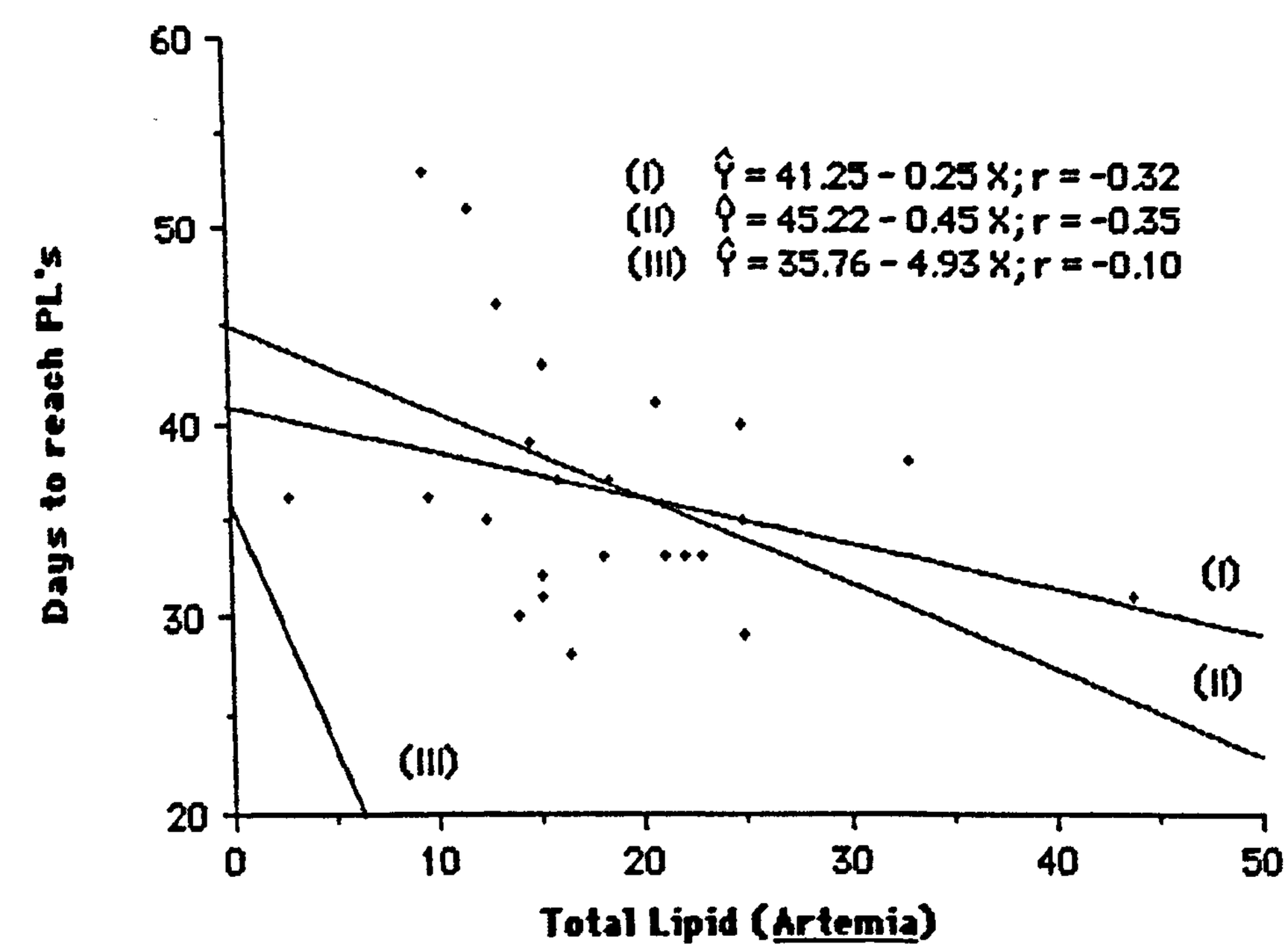
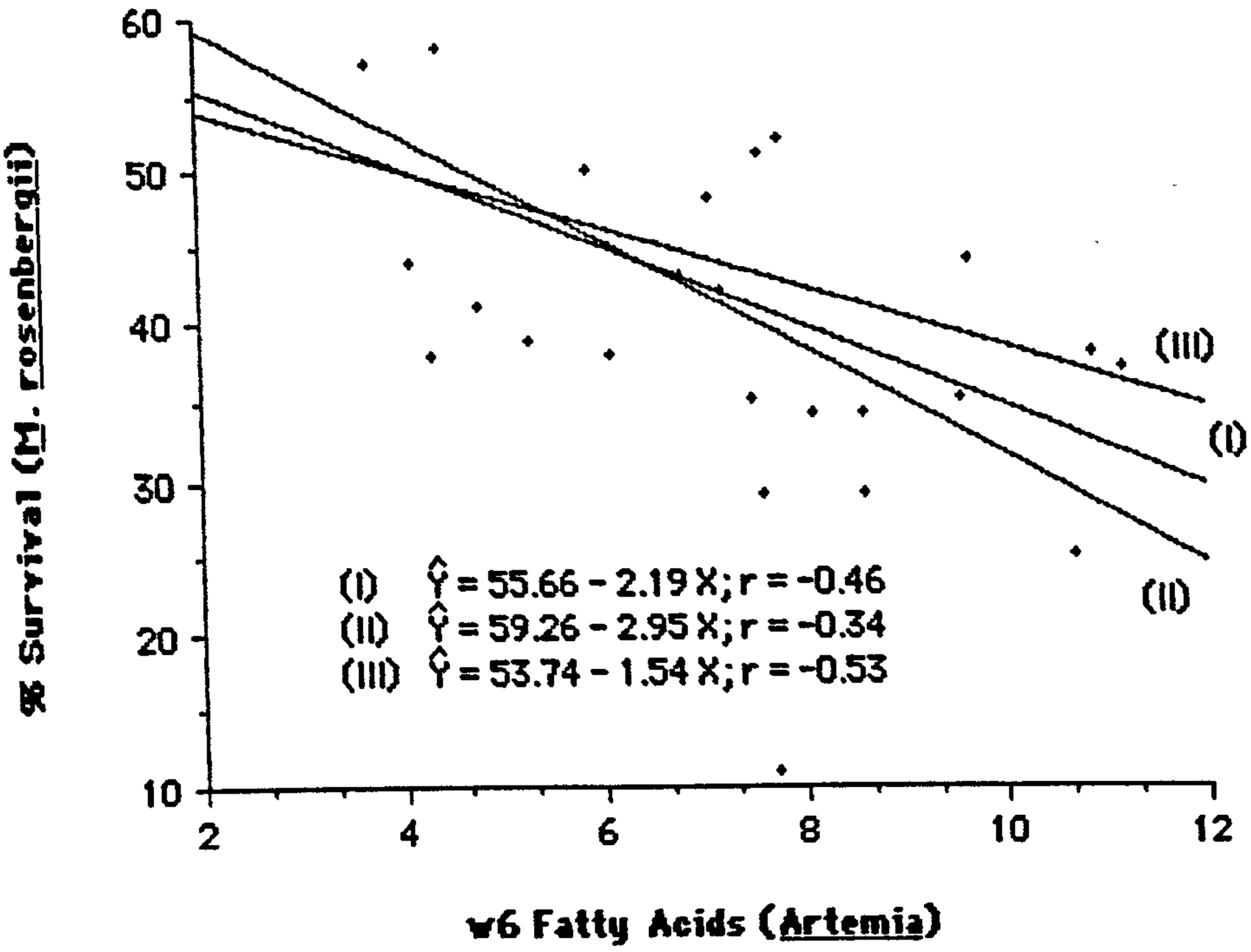


Figure 6. Percentage survival (a) and growth (b) of M. rosenbergii larvae fed Artemia containing different levels of total lipid; (I) all data, (II) unfed Artemia, (III) prefed Artemia

a.



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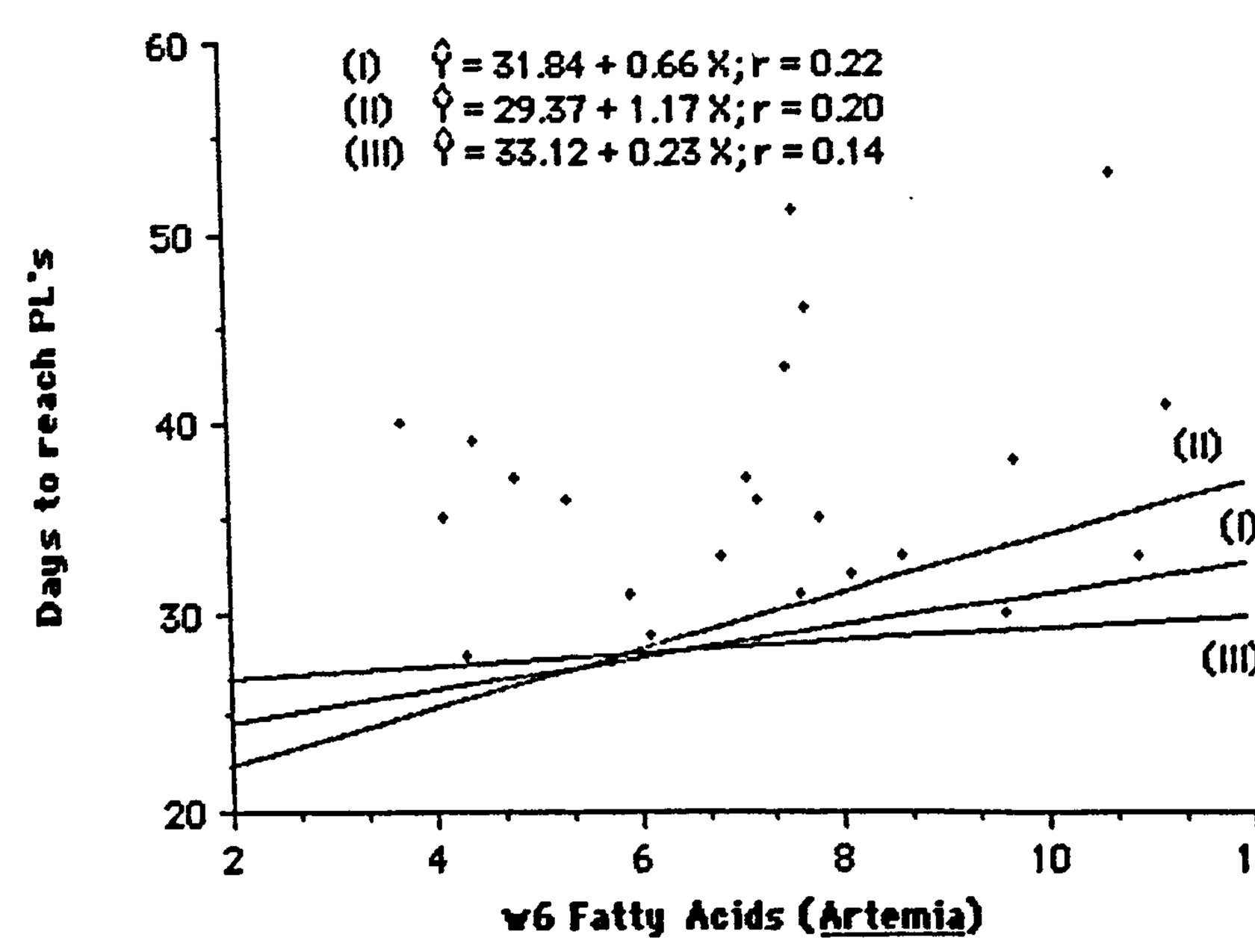
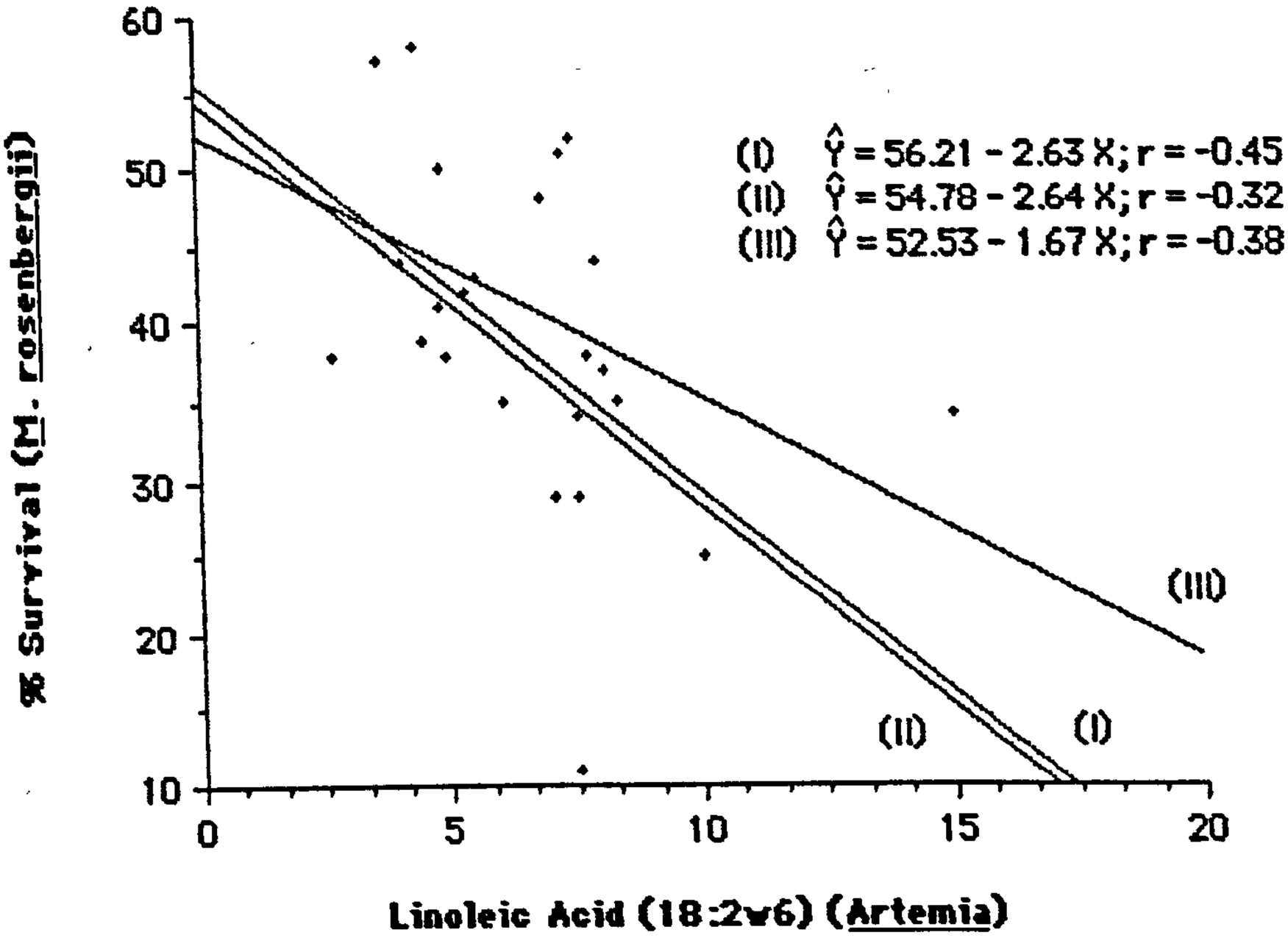


Figure 7. Percentage survival (a) and growth (b) of M. rosenbergii larvae fed on Artemia containing different levels of ω6 fatty acids; (I) all data, (II) unfed Artemia, (III) prefed Artemia

a.



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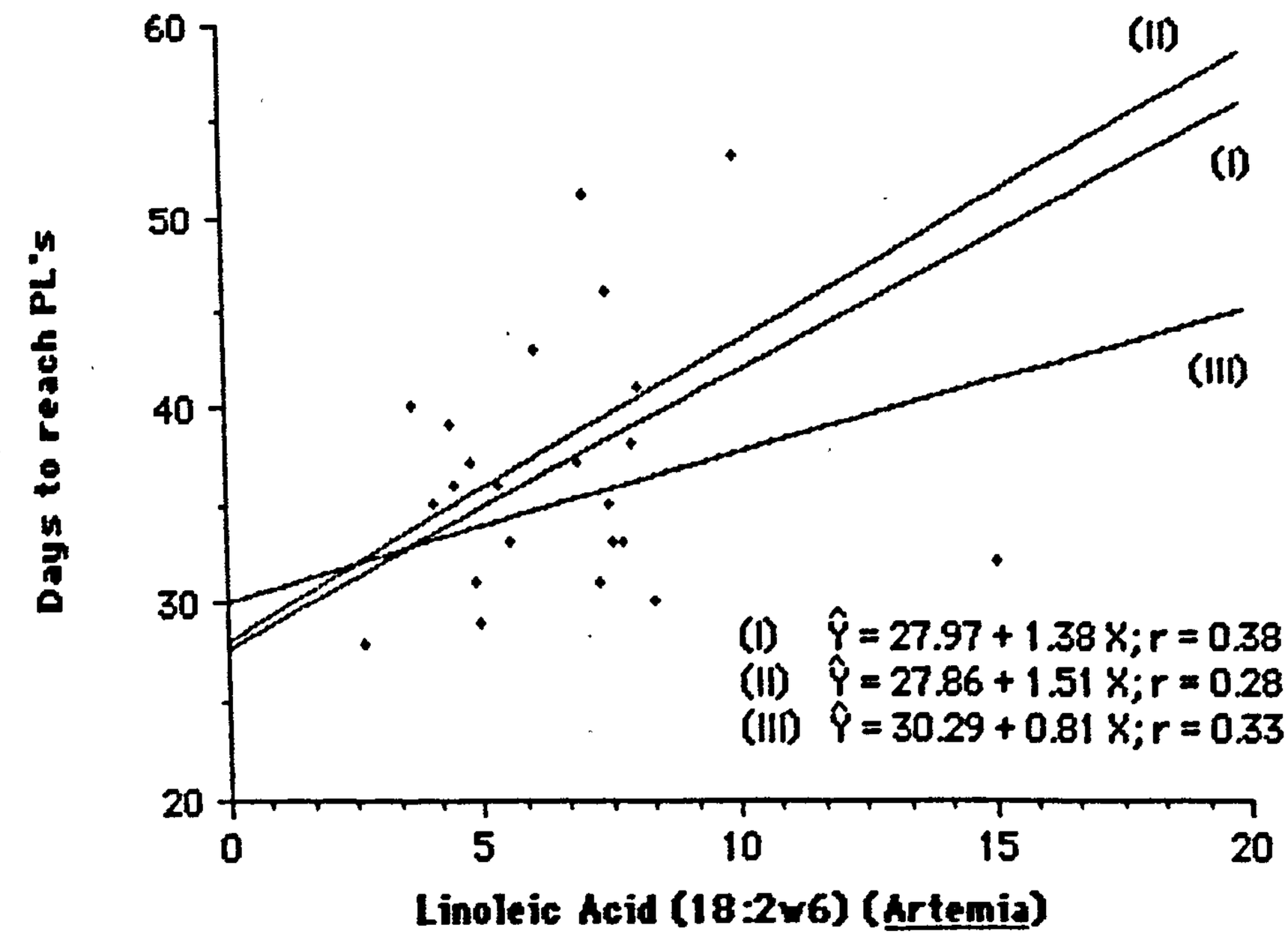


Figure 8. Percentage survival (a) and growth (b) of M. rosenbergii larvae fed Artemia containing different levels of linoleic acid (18:2w6); (I) all data, (II) unfed Artemia, (III) prefed Artemia

significant at 95% confidence level;

4° Survival and growth of M. rosenbergii larvae are not significantly related to w3 fatty acids of Artemia, however here also results respectively show both positive and negative trends;

5° Survival of M. rosenbergii larvae is significantly related (at $\alpha = 0.05$) to w6 but shows a negative relationship (ref.fig.7). Growth, however, is not significantly related (at $\alpha = 0.05$), but suggests a positive relationship. This is mainly due to linoleic acid (18:2w6) which shows the same tendencies (lower survival and longer larval development) (ref.fig.8);

6° Relationships between survival and growth of M. rosenbergii larvae and saturated and monounsaturated fatty acids in Artemia are not significant (at $\alpha = 0.05$), but are found to be opposite to PUFA relations. Survival and growth of M. rosenbergii larvae are significantly related to the saturated fatty acids contained in the four strains of Artemia when these are unfed (lower survival and longer development). This is found to be mainly due to palmitic (16:0) and stearic (18:0) acids which represent 17.2% and 37.2% of all the fatty acids. Palmitic acid alone represents 11.6 to 23.5% of all fatty

acids and increased content in Artemia clearly leads to a lower survival and longer cycle in M. rosenbergii larvae.

Experiment 2: fatty acid evaluation of other diets

The total lipid and fatty acid composition of CAR003 Frippak microcapsules, unfed Artemia, Chlorella vulgaris (Beijer), Dunaliella viridis (Teodor), and Chlorella and Dunaliella prefed Artemia are summarized in table 8 and detailed in tables 8.0, 8.1, 8.2, 8.3 and 8.4 (appendix). Microcapsules have the highest content in saturated fatty acids (30.0%), HUFA's (20.3%) and total lipid (19.5% dry weight). Chlorella contains the highest level of w6 fatty acids (33.7%) and the highest ratio w6/w3 (3.83), but the lowest PUFA's (non detected), w3 fatty acids (8.8%) and total lipid (1.5% dry weight). Dunaliella has the lowest ratio w6/w3 (0.16) and is also very low in total lipid (1.7%). When Chlorella or Dunaliella is given to Artemia, the saturated and monounsaturated fatty acid content of Artemia increases from 49.6% to 65.8% and 66.4%, respectively.

Experiment 3: fatty acids in M. rosenbergii larvae

TABLE 8. Fatty acid composition and total lipid content of 6 different feed regimes

Fatty acid	CAROO3 micro-caps. (5-30µ)	unfed Artemia	Chlo-rella	Duna-liella	Chlo-rella prefed Artemia	Duna-liella prefed Artemia
14:0	6.3	0.4	1.4	7.1	0.1	0.3
16:0	19.7	13.5	23.9	19.3	13.6	10.9
16:1ω7	8.5	2.3	11.6	10.3	2.0	1.9
18:0	4.0	6.0	3.2	1.7	10.7	10.5
18:1ω9 + ω7	14.6	27.0	51.2	12.9	38.5	41.8
18:2ω6	15.7	7.5	8.8	6.0	6.1	5.5
18:3ω3	2.2	33.2	nd (1)	10.5	21.0	19.9
18:4ω3	2.2	7.0	nd	8.5	4.8	5.2
20:1ω9	2.3	0.5	nd	3.1	0.8	0.8
20:4ω6	2.7	0.1	nd	0.5	0.2	0.3
20:3ω3	nd	1.2	nd	1.3	0.9	1.1
20:4ω3	nd	1.2	nd	2.6	0.9	0.9
20:5ω3	13.1	0.1	nd	14.6	0.4	0.9
20:1ω11	nd	t (2)	nd	nd	nd	nd
22:5ω3	1.7	nd	nd	2.8	nd	nd
22:6ω3	7.2	0.1	nd	nd	t	t
SAT	30.0	19.9	28.4	27.8	24.5	21.8
MUFA	25.4	29.7	29.1	25.9	41.4	44.6
SAT + MUFA	55.4	49.6	57.5	53.7	65.8	66.4
PUFA	44.8	50.4	42.5	46.3	34.2	33.6
20:5ω3 + 22:6ω3	20.3	0.2	nd	14.6	0.5	0.9
ω6	18.4	7.6	33.7	6.5	6.2	5.8
ω3	26.4	42.8	8.8	40.2	27.9	27.9
ω6/ω3	0.70	0.18	3.83	0.16	0.23	0.20
Total lipid dry wt	19.5	14.7	1.5	1.7	18.8	19.2

(1) not detected
(2) < 0.02 %

Fatty acid composition of 20 batches of M. rosenbergii at the first larval stage is presented in table 9. Saturated and monounsaturated fatty acids represent more than 80% (average: 85.5%) of the total fatty acids, due principally to palmitic (16:0) and oleic (18:1w9) acids, with an average of 29.8% and 25.1%, respectively. PUFA's represent less than 20% with an average of 14.5% . Eicosapentaenoic (20:5w3) and docosahexaenoic (22:6w3) acids are the major PUFA components and together score an average of 10.2% . W6 fatty acids are generally low (3.4%) and w3 fatty acids are much higher (11.1%). W6/w3 ratio is relatively low having an average value of 0.31 (range: 0.20-0.53).

Experiment 4: " weak " vs " strong " M. rosenbergii larvae

Table 10 gives the fatty acid composition of " weak " versus " strong " larval groups of unfed M. rosenbergii larvae at stage I. Tables 9.0 and 9.1 (appendix) show the replicates for the " weak " and the " strong " larval groups, respectively. Figures 9a and b show the difference in the fatty acid pattern of both groups. " weak " and " strong " larvae reach an average stage of 2.67 and 4.50, respectively, when fed the same diet for a 8-day period. " Weak " larvae have a higher content of PUFA's (12.4% more) mainly due to the HUFA's (26.9% more) than the " strong "

TABLE 9. Summary of fatty acid composition of 20 groups of *Macrobrachium rosenbergii* larvae at stage I (1)

Fatty acid	group # I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI	XVII	XVIII	XIX	XX	x	S.E.	Range
14:0	1.5	1.7	0.9	1.5	1.6	1.4	1.9	1.5	1.4	1.4	1.4	1.8	1.9	2.1	2.6	1.4	1.6	1.8	1.4	1.5	1.6	0.27	0.9-2.1
16:0	29.5	27.0	31.6	29.3	28.7	30.0	28.9	30.2	30.2	29.2	29.6	29.4	30.2	28.7	24.8	30.0	30.8	31.3	31.6	30.4	29.8	1.13	27.0-31.3
16:1ω7	9.3	9.5	9.3	10.7	8.4	10.9	7.5	10.2	8.9	8.9	8.8	8.6	8.2	8.7	11.3	11.3	7.9	8.0	9.1	9.3	9.1	1.02	7.5-11.3
18:0	8.4	7.8	8.2	8.5	9.1	8.3	9.1	7.3	8.1	8.9	9.0	8.9	9.7	9.7	6.5	8.3	11.1	9.9	9.5	8.7	8.9	0.86	7.3-11.1
18:1ω9	24.2	24.8	25.5	22.5	26.0	23.4	22.9	26.4	27.1	26.6	26.0	23.0	24.2	26.4	24.4	24.5	26.7	25.0	25.8	26.3	25.1	1.46	22.5-26.7
18:1ω7	8.6	10.0	10.1	8.4	10.9	10.5	9.3	10.1	10.8	8.7	8.6	10.5	8.5	8.8	7.6	7.4	8.2	9.5	9.1	8.4	9.3	1.01	7.4-10.9
18:2ω6	2.2	1.9	0.8	1.2	1.0	1.2	2.8	1.7	1.7	2.2	0.9	1.3	1.6	2.5	3.0	1.2	1.6	2.4	1.9	1.8	1.7	0.57	0.9-2.8
18:3ω3	0.4	0.6	0.2	0.0	0.3	0.3	0.5	0.2	0.3	0.5	0.3	0.3	0.3	0.5	0.7	0.5	0.5	0.5	0.4	0.5	0.4	0.15	0.0-0.6
18:4ω3	0.2	0.4	0.2	0.0	0.2	0.1	0.3	0.1	0.1	0.3	0.1	0.1	0.3	0.3	0.4	0.3	0.2	0.2	0.2	0.1	0.2	0.09	0.0-0.4
20:1ω9	1.1	1.5	1.5	1.8	1.3	1.4	1.5	1.2	1.1	1.5	1.4	1.6	1.4	2.1	1.6	2.2	1.5	1.8	1.6	1.4	1.5	0.30	1.1-2.2
20:4ω6	2.0	1.9	1.4	2.0	1.7	1.7	2.3	1.3	1.4	1.7	1.9	2.4	1.8	1.7	2.8	1.6	1.6	1.9	1.4	1.5	1.8	0.29	1.3-2.4
20:3ω3	0.0	0.0	nd(2)	0.0	0.0	0.0	0.0	0.1	0.1	0.1	0.0	0.1	0.1	0.0	0.1	0.1	0.2	nd	nd	nd	0.1	0.05	nd-0.2
20:4ω3	0.2	0.1	nd	0.1	0.1	0.3	0.2	0.1	0.1	0.1	0.2	0.1	0.3	0.1	0.2	0.1	0.1	nd	0.1	0.1	0.1	0.06	nd-0.3
20:5ω3	7.9	7.7	7.9	8.3	6.9	8.1	8.9	5.9	5.4	6.0	7.6	7.9	6.7	5.0	8.0	6.1	4.8	5.1	4.8	8.0	6.7	1.33	4.8-8.9
22:1ω11	0.1	0.1	nd	0.4	0.2	0.0	0.3	0.1	0.1	0.3	0.0	0.1	0.0	0.3	0.1	0.1	0.2	0.3	0.2	0.2	0.2	0.12	nd-0.4
22:5ω3	0.3	0.1	0.3	0.3	0.2	0.2	0.1	0.2	0.2	0.3	0.1	0.1	0.3	0.2	0.5	0.3	0.3	0.4	0.2	0.2	0.2	0.08	0.1-0.4
22:6ω3	4.2	4.8	2.2	4.9	3.3	2.2	3.4	3.3	3.2	3.4	4.0	3.9	4.6	2.8	5.7	4.8	2.8	1.9	2.8	3.7	3.5	0.91	1.9-4.9
SAT	39.4	36.5	40.7	39.3	39.5	39.7	39.9	39.1	39.7	39.5	40.0	40.1	41.7	40.5	33.8	39.7	43.6	42.9	42.5	40.6	40.3	1.59	36.5-43.9
MUFA	43.2	46.0	46.3	43.8	46.7	46.2	41.6	48.0	48.0	45.9	44.9	43.8	42.3	46.4	44.9	45.5	44.5	44.7	45.8	45.6	45.2	1.71	41.6-48.0
SAT + MUFA	82.6	82.5	87.0	83.1	86.2	85.9	81.6	87.1	87.7	85.4	84.9	83.9	84.0	86.9	78.7	85.1	88.1	87.6	88.3	86.2	85.5	2.05	78.7-88.3
PUFA	17.4	17.5	13.0	16.9	13.8	14.1	18.4	12.9	12.4	14.6	15.1	16.2	16.0	13.1	21.3	14.9	12.1	12.4	11.7	13.9	14.5	2.04	11.7-21.3
20:5ω3 + 22:6ω3	12.0	12.5	10.2	13.2	10.2	10.3	12.3	9.2	8.5	9.4	11.7	11.7	11.3	7.8	13.7	10.9	7.6	7.0	7.5	9.6	10.2	1.87	7.0-13.2
ω6	4.2	3.8	2.2	3.2	2.7	2.9	5.0	3.1	3.1	4.0	2.8	3.7	3.4	4.2	5.8	2.8	3.2	4.3	3.3	3.3	3.5	0.69	2.2-5.0
ω3	13.2	13.3	10.8	13.8	11.1	11.2	13.4	9.8	9.3	10.6	12.3	12.4	12.6	9.0	15.6	12.2	8.8	8.1	8.4	10.6	11.1	1.82	8.1-13.8
ω6/ω3	0.32	0.29	0.20	0.23	0.25	0.26	0.38	0.31	0.33	0.37	0.23	0.30	0.27	0.46	0.37	0.23	0.37	0.53	0.39	0.31	0.31	0.09	0.20-0.53

(1) in triplicate
(2) not detected

TABLE 10. Fatty acid composition of " weak " vs " strong " groups of unfed *Macrobrachium rosenbergii* larvae at stage I

Fatty acid	weak	strong	% Difference (1) Decrease	% Difference (1) Increase
14:0	1.5	1.8		21.8
16:0	29.4	30.1		2.2
16:1ω7	9.5	9.0	5.0	
18:0	8.8	9.0		1.7
18:1ω9	25.5	25.9		1.7
18:1ω7	9.0	9.5		5.8
18:2ω6	1.0	2.2		114.6
18:3ω3	0.4	0.4		20.0
18:4ω3	0.2	0.2		5.0
20:1ω9	1.6	1.7		5.5
20:4ω6	1.7	1.6	5.2	
20:3ω3	t (2)	0.1		66.7
20:4ω3	0.1	0.1	57.1	
20:5ω3	6.9	5.3	22.7	
20:1ω11	0.1	0.2		118.2
22:5ω3	0.2	0.3		18.2
22:6ω3	4.0	2.7	34.0	
SAT	39.7	40.9		2.8
MUFA	45.7	46.3		1.5
SAT + MUFA	85.4	87.2		2.1
PUFA	14.6	12.8	12.4	
20:5ω3 + 22:6ω3	10.9	8.0	26.9	
ω6	2.7	3.8		39.6
ω3	11.8	9.1	24.4	
ω6/ω3	0.23	0.44		91.0
Stage (3)	2.67	4.50		73.2

(1) " strong " compared to " weak "
(2) < 0.02 %
(3) after a 8-day larval rearing

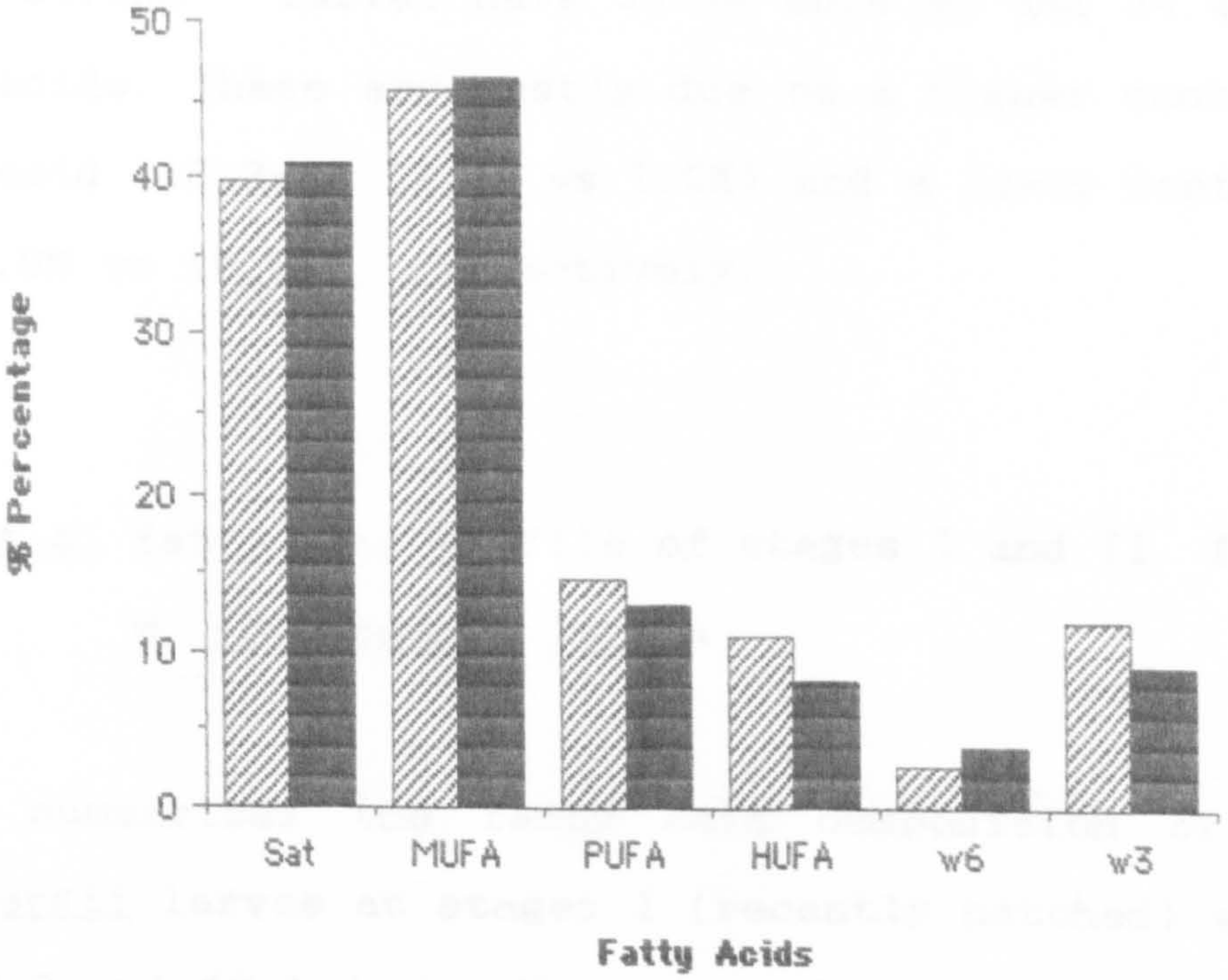


Figure 9. Fatty acid composition of "weak" vs "strong" batches of unfed M. rosenbergii larvae at stage I (a & b); ▨ Weak, ■ Strong

larvae. " strong " larvae have 39.6% more w6 and 24.4% less w3 fatty acids. These are mostly due to a higher content of linoleic acid (18:2w6) (2.2% vs 1.0%) and a lower content of HUFA's (8.0% vs 10.9%), respectively.

Experiment 5: fatty acid profile of stages I and II for M. rosenbergii larvae

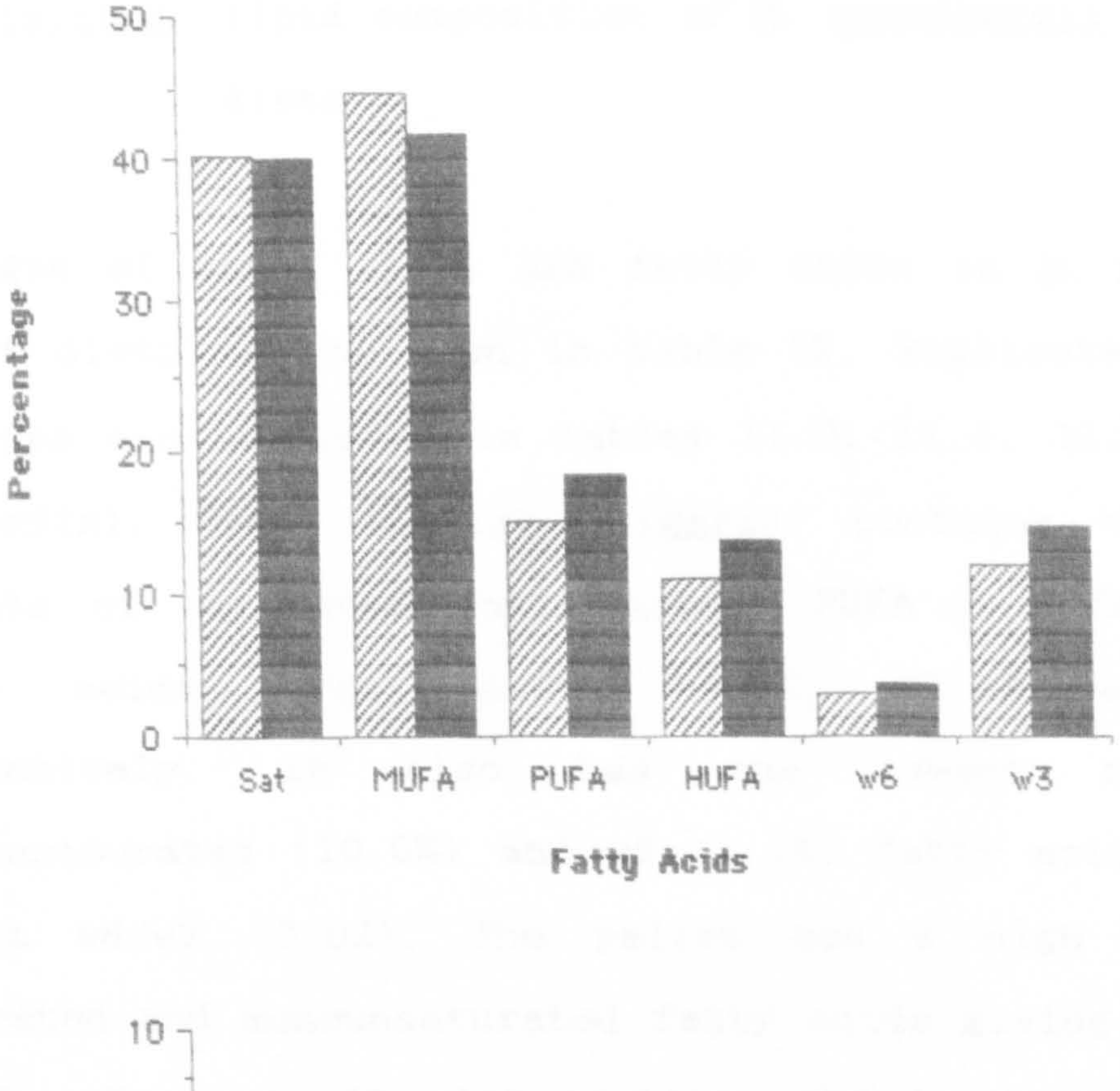
Table 11 summarizes the fatty acid composition of unfed M. rosenbergii larvae at stages I (recently hatched) and II. Tables 10.0 and 10.1 (appendix) show the replicates for the stages I and II, respectively. Figures 10a and b visualize the contrasts amongst certain fatty acid levels between the two stages. Depletion mainly occurs for monounsaturated fatty acid (44.6% to 41.8%), as palmitoleic (16:1w7) and oleic (18:1w9) acids are decreasing from 8.5% and 25.4% to 6.7% and 23.2%, respectively. However, PUFA's increase by 21.2% due to both short and long chains, w6 and w3 increasing by 25.0% and 20.3%, respectively. These increases come mostly from arachidonic acid (20:4w6) (43.6% higher), on one side, and from eicosapentaenoic (20:5w3) and docosahexaenoic (22:6w3) acids (26.2% and 19.1% higher, respectively), on the other. No change is observed in the w6/w3 ratio.

TABLE 11. Depletion of fatty acids in unfed *Macrobrachium rosenbergii* larvae between stages I and II

Fatty acid	Stage I	Stage II	% Difference (1) Decrease	% Difference (1) Increase
14:0	1.6	1.3	20.2	
16:0	29.5	28.8	2.4	
16:1ω7	8.5	6.7	20.6	
18:0	9.3	10.0		7.7
18:1ω9	25.4	23.2	8.5	
18:1ω7	9.3	10.4		12.1
18:2ω6	1.2	1.1	3.4	
18:3ω3	0.3	0.3		
18:4ω3	0.2	0.2		
20:1ω9	1.4	1.3	8.1	
20:4ω6	1.8	2.6		43.6
20:3ω3	0.0	0.0		
20:4ω3	0.2	0.1	58.1	
20:5ω3	7.1	8.9		26.2
22:1ω11	0.1	0.2		77.8
22:5ω3	0.2	0.2	18.2	
22:6ω3	4.0	4.7		19.1
SAT	40.4	40.1	0.8	
MUFA	44.6	41.8	6.3	
SAT + MUFA	85.0	81.8	3.7	
PUFA	15.0	18.1		21.2
20:5ω3 + 22:6ω3	11.0	13.7		23.6
ω6	3.0	3.7		25.0
ω3	12.0	14.5		20.3
ω6/ω3	0.25	0.26		4.1

(1) Stage II compared to stage I

a.



b.

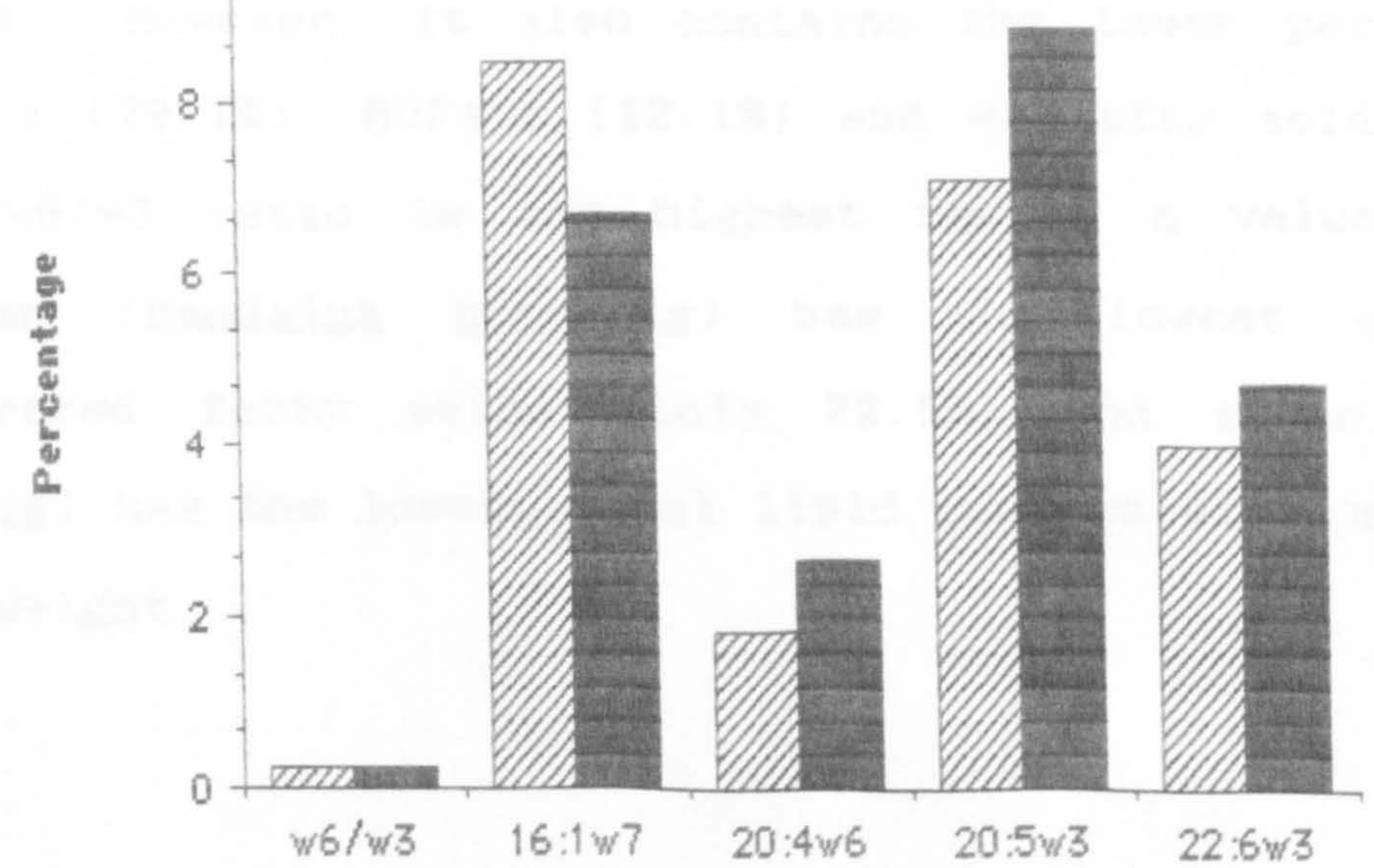


Figure 10. Fatty acid composition of unfed M. rosenbergii larvae at stages I and II (a & b); ▨ stage I, ■ stage II

Experiment 6: lipid composition of M. rosenbergii parent diets

Averages of total lipid and fatty acids in M. rosenbergii parent diets can be seen in table 12. Replicates of these analyses are presented in tables 11.0, 11.1, 11.2 and 11.3 (appendix). Squid (Loligo vulgaris) contains the highest amounts of saturated fatty acids, PUFA's, HUFA's and w3 fatty acids with 34.4%, 54.7%, 53.9% and 54.7%, respectively. It also has the lowest content of monounsaturated (10.9%) and w6 (1.1%) fatty acids, and the lowest w6/w3 (0.02). The pellet has a high content of saturated and monounsaturated fatty acids giving a total of 70.9% . However, it also contains the lower percentage of PUFA's (29.1%), HUFA's (12.1%) and w3 fatty acids (17.2%). The w6/w3 ratio is the highest having a value of 0.70. Shrimp (Pandalus borealis) has the lowest content of saturated fatty acids (only 22.5%) and mussel (Mytilus edulis) has the lowest total lipid content with only 1.5% in dry weight.

TABLE 12. Fatty acid composition and total lipid content of 4 diets given to *Macrobrachium rosenbergii* females

Fatty acid	Shrimp (1)	Squid (1)	Mussel (2)	Pellet (3)
14:0	0.9	2.4	0.8	2.6
16:0	17.7	29.4	16.2	18.5
16:1ω7	8.4	1.2	10.0	5.0
18:0	3.9	2.6	9.2	12.4
18:1ω9	14.7	3.3	3.1	17.2
18:1ω7	9.1	2.2	2.0	3.8
18:2ω6	0.9	0.3	1.1	9.5
18:3ω3	0.6	0.1	0.5	1.4
18:4ω3	0.9	0.2	0.6	1.1
20:1ω9	1.4	4.1	8.0	5.8
20:4ω6	3.2	0.8	7.4	2.4
20:3ω3	0.3	0.2	0.1	0.1
20:4ω3	0.4	0.1	3.5	0.6
20:5ω3	23.8	16.8	15.5	5.9
20:1ω11	0.3	0.2	0.2	5.6
22:5ω3	1.0	0.2	1.5	2.0
22:6ω3	12.6	37.1	20.5	6.2
SAT	22.5	34.4	26.2	33.5
MUFA	33.9	10.9	23.2	37.4
SAT + MUFA	56.3	45.3	49.4	70.9
PUFA	43.7	54.7	50.6	29.1
20:5ω3 + 22:6ω3	36.4	53.9	36.0	12.1
ω6	4.1	1.1	8.5	11.9
ω3	39.6	54.7	42.2	17.2
ω6/ω3	0.10	0.02	0.19	0.70
Total lipid (dry wt)	4.1	4.6	1.5	3.4

(1) *Pandalus borealis* and *Loligo vulgaris*, respectively; from Kim Fish Co.
(2) *Mytilus edulis*: 1 & 2, from Beaumaris shores, and 3, from Menai Bridge shores
(3) made in the Marine Science Laboratories, U.C.N.W., Menai Bridge

DISCUSSION

Algal requirements

Survival and growth of M. rosenbergii larvae are improved when the larval cycle occurs in presence of 550-750 cells of Chlorella vulgaris (beijer) per µl of rearing water. Outside these concentrations, results show poorer survival and longer larval cycle. This suggests that algae, as well as Artemia and M. rosenbergii larvae, have to be present at an appropriate level in the common rearing medium. By examining the gut contents, it was observed that Chlorella was consumed by Artemia which, in its turn, was ingested by Macrobrachium. The nutritional value of Artemia nauplii apparently decreases rapidly after hatching because the high rates of metabolism and growth which results in rapid utilization of yolk material (Maddox & Manzi, 1976; Sorgeloos, 1977). This practice of feeding Artemia enhances the nutritional value of Artemia and hence improves the

Macrobrachium diet. This was also observed by Ling (1969b), Wickins (1972), Maddox & Manzi (1976), AQUACOP (1979), Malecha (1983), Lovett & Felder (1988) and Millamena et al. (1988). Wickins (1972) considers that the beneficial effect of the algae is due either to the larvae ingesting quantities of algae with their prey (Artemia), or to the improved nutritional value of Artemia which have consumed the algae. According to Millamena et al. (1988), the Artemia shows a better fatty acid profile for prawn larvae. Work by Manzi et al. (1977) has demonstrated that addition of high densities of monospecific algal cultures, especially of Phaeodactylum tricornutum, has marked beneficial effects on survival and growth of M. rosenbergii larvae. Hanson & Goodwin (1977) indicate that, while algal cells are frequently ingested by larvae, there is no evidence to suggest that larvae derive any direct nutritive value from algae. Cohen et al. (1976) found that the algae were not assimilated by M. rosenbergii larvae, and concluded that algae enhance the growth of prawn larvae only indirectly by removing toxic substances, notably ammonia, from the rearing medium. Of the nitrogen sources in the sea that are well-characterized, ammonium, nitrite, nitrate and urea are the most abundant (Parsons et al., 1984a). The results of several studies on Chlorella emersonii (Shihara et Krauss), formerly Chlorella fusca, and Phaeodactylum tricornutum, particularly, those in which ammonium-nitrate interactions

are concerned, suggest that ammonium is the preferred nitrogen source for uptake and assimilation of the phytoplankton growth (Molloy & Syrett, 1988).

Another possibility is that the algae add a growth factor to the culture water or act as a bactericidal agent (Hanson & Goodwin, 1977). Certain algae are known to produce substances which can cause a drag reduction in water similar to that produced with synthetic long-chain polymers (Hoyt, 1966). At least one of these synthetic polymers, polyethylene oxide, has been shown to beneficially affect survival of other caridean shrimp larvae (Palaemonetes spp.) in an unknown manner (Sandifer et al., 1975).

Other authors (New, 1976; Wickins, 1976; Ang & Cheah, 1986) also claim that the algae can act as a buffer on water quality against toxic ammonia build up (ammonia, un-ionized ammonia, nitrite). " Green " water is a mixed phytoplankton culture in which Chlorella sp. is frequently dominant (New & Singholka, 1985). Its density is about 750-1500 cells per µl. Phytoplankton-rich water is added to the larval tanks regularly and exchanged every 1 to 2 days. Corbin et al. (1983) says that phytoplankton densities of 100-1000 cells per µl are maintained in the green water system. Usually the chlorophytes, Chlorella or Palmellococcus, or the cyanophytes Nannochloris are the dominant species. Algal concentration in larval tanks can not easily be controlled. Therefore, a minimum of 5 cells

per µl is generally sufficient for prawn larvae, but levels exceeding 70, and even 600, cells per µl has been commonly recorded (Liao et al., 1976). The same authors mention that typical densities produced in the mass culture of penaeid tanks range between 275 to 450 cells per µl with a record of 1500 cells per µl.

The present experiment suggests that, below 550 cells of Chlorella per µl, Artemia does not graze properly or toxic nitrogen is not efficiently removed from the rearing water. This was already demonstrated with rotifers (Brachionus plicatilis) when grazing on only 2 cells of Chlorella per µl (Lovett & Felder, 1988). Above 750 cells of Chlorella per µl, algal concentration could be so dense that it can compete for oxygen with Artemia and M. rosenbergii larvae. Artemia fed Chlorella increases in saturated and monosaturated fatty acids, but PUFA's profile was not improved as HUFA's remain the same and w3 fatty acids decrease (table 8).

A survey of Macrobrachium hatchery operators found feeding rates of 5 to 15 Artemia nauplii per ml depending on the stage of M. rosenbergii larvae (Corbin et al., 1983). By adding an alga, such as Chlorella, to the rearing water, it does not only improve the fatty acid profile of Artemia (Sorgeloos, 1977) and control water quality, but it also

allows the use of less Artemia per ml to satisfy Macrobrachium larval energetic requirements.

Light requirements

Environmental factors other than diet also affect the survival, development and duration of decapod larval life (Ling, 1969a,b). Even under optimal dietary conditions each decapod species has absolute ranges of temperature, salinity and light conditions beyond which development will not continue. In the present experiment, M. rosenbergii post-larvae (at PL1) were longer in length (10.33 vs 8.96mm), survived better (53 vs 21%) and grew faster (24 vs 36 days to reach PL's) when the larval cycle occurred under a strong reduction of light (4.82 vs 12.04 X 10^4 me.s^{-1}.cm^{-2}). These results agree with Templeman (1936) who found that larvae reared in darkness survived better than those in natural light. They also agree with Brohmanonda & Sahavacharin (1968) who shaded some part and obtained a shorter cycle (38 vs 47 days). Uno & Kwon (1969) also partially wrapped their M. rosenbergii cultures with black paper and the larval development was reduced from 43 days (in light) to 33 days. Finally, these results also agree with Fujimura & Okamoto (1970) who partially covered M. rosenbergii culture with plywood panel and reached 21%

survival compared to 15% in light. However, these results contrast with Sandoz & Rogers (1944) and Reeve (1969) who found that larvae reared in light had a higher survival. Foster & Wickins (1967) have shown that spectral composition of the light may be important, as development of the palaemonid (Palaemon serratus) is delayed by red light, while survival is slightly improved by blue light in comparison to results obtained in white light. According to the same authors, M. rosenbergii may well experience difficulty in trapping their food in the absence of complete light. Shelbourne (1973) reports similar difficulties for the plaice larvae which also feed on Artemia. Oonchit (1974) reports that M. rosenbergii larvae reared in the natural daylight suffer a 61% mortality, but larvae reared in dark room were all dead by the eighth day after hatching. None of these authors recorded total length of their post-larvae (at PL_I).

In nature, many decapod larvae generally migrate to bodies of water less exposed to direct light and look for some shade, such as the cover of trees, stones, roots or algal blooms (Gurney, 1960; Bauchau, 1966; Ling, 1969a; Arrignon, 1981; Buggren & Mc Mahan, 1988; personal observations).

The present experiment, in which larvae were not reared in complete darkness, but in a three-fold reduced natural

strong light, suggests, like New & Singholka (1985), that exposure to direct sunlight appears to be harmful to M. rosenbergii larvae, especially in a " clearwater " rearing system. New & Singholka recommend that 90% of the rearing tank be covered. However, the algal requirements for light and the covered tank for M. rosenbergii larvae are incompatible and can be difficult to achieve, unless both productions are cultured in separate units before being periodically mixed such as in the Anuenue " green water " method. Ang and Cheah (1986) manage M. rosenbergii larvae by using deep tanks where light is low due to algal shading.

Effect of increasing dietary w3 fatty acids

To date, few studies on essential fatty acid deficiencies or the relationship between deficiencies and growth in freshwater crustaceans can be found for larvae. Consequently, the relative importance of specific fatty acids in the nutrition of M. rosenbergii larvae is poorly known. In the present experiment, survival of M. rosenbergii larvae seems to be related (positive trend) to the total lipid of Artemia. However, this result is only significant at $\alpha = 0.1$ (90% confidence level) ($r = 0.36$; D.F.= 23). This finding is in agreement with Ismail (1989) who also found that survival of Macrobrachium larvae was positively

correlated with increasing levels of total lipid in the diet but could not identify a statistical correlation. Furthermore, if data of Ahmed (1988), Ismail (1989) and present results are considered together, statistical analysis reveals no differences between the three sets of data (for one-way ANOVA: $F = 1.76$; D.F.num = 2; D.F. denom = 27; $\alpha = 0.05$) and regression equation is found to be $Y = 0.48X + 32.4$ with an equally significant correlation coefficient at 90% confidence level ($r = 0.35$; $\alpha = 0.1$). In contrast, Ismail (1989) found that survival of marine caridean, Palaemon elegans, was clearly significatively increased with an increase in dietary Artemia lipid.

Increased growth of M. rosenbergii larvae in relation to increasing total Artemia lipid was not significant, but a trend towards a shorter larval cycle was indicated, and this result agrees with Ismail (1988). D'Abramo and Sheen (1989) fed M. rosenbergii juveniles with several isoenergetic and isonitrogenous purified diets with total lipid levels ranging from 0 to 12% . They found a significant reduction in growth when total lipid overpassed 10%, but total lipid did not appear to influence survival. In the present experiment, total lipid varies from 9.3 to 27.8% and results suggest that growth was slightly improved shortening the larval development at the higher levels. Here also, total lipid did not appear to significantly influence survival of

M. rosenbergii larvae, although a slight positive trend could be detected.

Macrobrachium rosenbergii (de Man) spends the adult part of its life cycle in freshwater and the larval part in brackish water and it is of interest to determine whether it retains a dependency upon dietary w3 fatty acids. In the four strains of Artemia used in the present work, w3 PUFA content is mainly represented by linolenic acid. Linolenic acid (18:3w3) is the precursor of eicosapentaenoic (20:5w3) and docosahexaenoic (22:6w3) acids (Kanazawa et al., 1979b; Ackman, 1980; Castell, 1982) which might supply the HUFA's needed for rapid growth in M. rosenbergii larvae.

Statistically, no better survival and growth of larvae was achieved when M. rosenbergii larvae were fed on Artemia containing increasing amounts of w3 fatty acid, although from tables 6 and 7, it can be seen that there is an increasing tendency for better survival and growth with increasing w3 fatty acid content. This is in agreement with Sandifer & Joseph (1988) and Ismail (1989). However, working on M. rosenbergii juveniles, Sandifer & Joseph (1988) detected significantly better growth (larger animals) only after a treatment for a 6-week period of time. In the present work, the increase in w3 fatty acids hides a decrease of HUFA's in the different strains of Artemia, and

this decrease of HUFA's may be corrected for the increase of linolenic acid (18:3w3), the precursor of HUFA's. If HUFA content of Artemia had been higher initially, together with the metabolised end products of 18:3w3 (i.e. 20:5w3 & 22:6w3), survival and growth might have been very different from the present results. Romdhane et al. (1990) and Devresse et al. (1990) in a similar study found better growth, higher survival, faster metamorphosis and greater resistance of M. rosenbergii larvae when they were fed over increasing periods with w3 HUFA enriched Artemia. However, their experiments were conducted using a unique strain of Artemia which was apparently high in w3 fatty acids but with a low w6/w3 ratio. Moreover, the fatty acid profile of M. rosenbergii larvae at stage I was also higher in HUFA's than larvae in the present experiments. These authors acknowledge that the highest w3 HUFA incorporation levels in the tissue of postlarvae at stage I did not necessarily give better culture performance, as the highest amount of w3 HUFA did not yield the best result in terms of growth and survival. Webster and Lovell (1990) also reveal that Artemia containing the highest levels of fatty acids do not always give the best results, but indicate that larvae can more effectively digest the long-chain w3 fatty acids than shorter ones.

It has to be noted that, in the present experiments, all Artemia prefed with HUFA-rich encapsulated feed gave better

survival amongst M. rosenbergii larvae compared with all respective unfed Artemia. It is also interesting to observe that Artemia prefed on encapsulated diets gained in w3 PUFA content when w3 PUFA's were initially low (< around 20%), but lost in w3 PUFA content when w3 PUFA's were initially high (> around 20%).

Freshwater fishes possess the ability to biosynthesize HUFA's (Kanazawa et al., 1979b), whereas marine fishes and prawns have an essential dietary requirement for these fatty acids (Yone & Fujii, 1975; Jones et al., 1979a; Castell, 1982). No relation could be found in the present work between survival and growth of M. rosenbergii larvae and PUFA content in Artemia. However, survival was slightly negatively affected with HUFA's, mainly due to the presence of eicosapentaenoic acid (20:5w3). In contrast, Devresse et al. (1990) could not distinguish a possible negative effect due to w3 HUFA excess, and Ahmed (1988) found better survival for M. rosenbergii larvae with an HUFA increase in Artemia, and significantly lower survival and growth were obtained for marine Palaemon elegans larvae fed on low HUFA Artemia (McColgan, 1988). When this Artemia was boosted (prefed) with HUFA's using encapsulated feed, growth and survival of marine Palaemon larvae were significantly improved. Present results tend to agree with D'Abramo & Sheen (1989) in that significantly greater growth can only

be detected after long periods of feeding with diets containing higher levels of PUFA's and HUFA's.

The genus Macrobrachium contains many species which already have successfully colonised freshwaters, and M. rosenbergii is regarded as a transitional species (Wong, 1988). The loss of an obvious dependency upon HUFA's may be a biochemical stage in this transition from marine to freshwater together with the acquisition of the capacity for biosynthesis of these fatty acids.

In this experiment, higher levels of w6 fatty acids in dietary Artemia seem to have an inhibitory effect on survival and growth of M. rosenbergii larvae, possibly due to the presence of linoleic acid (18:2w6). This was also found by New (1976) and Sandifer & Joseph (1976) who reached the same conclusion for M. rosenbergii juveniles. However, D'Abramo & Sheen (1989) found no differences in growth when juveniles were fed diets containing w3 and w6 fatty acids. The experiments of Devresse et al. (1990) did not allow confirmation of any requirement for w6 fatty acids in larvae. The present findings are in contrast to Castell (1982) who states that freshwater species may require more linoleic series fatty acids or a mixture of linoleic (18:2w6) and linolenic (18:3w3) acids. According to Castell, species grow better on a mixture rather than on one alone, depending on the rate at which they are able to desaturate

and elongate fatty acids. The differing requirements for EFA's with changing salinities is probably because fats have been shown to play a role in activation of enzymes, for example, those involved in the transport of salts accross the gills and the skin (Matty, 1989). Sandifer & Joseph (1976) found that when M. rosenbergii juveniles were fed on a higher w3 fatty acid diet, their tissues contained about the same percentage of w3 fatty acids as the diet, indicating conservation of these dietary acids and/or biosynthesis. In contrast, the animals contained substantially less linoleic (18:2w6) than their diets, suggesting that some of this fatty acid may have been utilized as an energy source. Present data suggest that long chain arachidonic acid (20:4w6) in dietary Artemia does not influence survival and growth of M. rosenbergii larvae, but short chain linoleic acid (18:2w6) lowers survival significantly and seems to reduce growth (longer larval cycle). The dietary requirements in M. rosenbergii larvae are probably very different from those in post-larvae where w6 fatty acids, according to the literature, play a more important role (Reigh & Stickney, 1989). Generally, fish and crustaceans are not capable of synthesizing either 18:2w6 and 18:3w3 from acetate and palmitate as well as mammals (Kanazawa et al., 1979b). However, freshwater fishes seem to be superior to marine fishes and crustaceans in their capacity for elongation and desaturation of dietary 18:3w3

to 20:5w3 and 22:6w3. Thus, freshwater organisms appear to require less longer chain PUFA's (Watanabe, 1982). The w6/w3 ratio may be a more critical factor than the actual level of either type of fatty acid. Low ratio diets appear to be beneficial to penaeids and carideans (New, 1976). W6/w3 ratio in the present experiment varies between 0.18, for a high w3 PUFA Artemia, and 0.67, for a low w3 PUFA Artemia, but it does not appear to confirm the statement of New: neither survival nor growth of M. rosenbergii larvae seems to be significatively influenced by this ratio, though the former shows a slight negative response to the higher ratio.

The saturated and monosaturated fatty acids of dietary Artemia did not influence survival and growth of M. rosenbergii larvae significantly, but the effects were exactly opposite to PUFA relationships. However, saturated fatty acids in all unfed Artemia treatments strongly influenced survival and growth negatively (longer larval cycle), principally due to palmitic (16:0) and stearic (18:0) acids. Sandifer & Joseph (1976) obtained significantly better growth when M. rosenbergii larvae were fed high amounts of saturated fatty acid contained in Artemia, but their study diets also had large amounts of w3 fatty acids.

In summary, the present experiment shows that diets varying

widely in total lipid only show a slight influence on survival and growth for M. rosenbergii larvae.

Diets varied in w3 fatty acids showed no difference in survival and growth. No difference was observed in relation to the dietary long chain fatty acids (20:5w3 & 22:6w3), but there are differences in relation to linolenic acid (18:3w3) which is the precursor of long chains. However, the elongation of the chain was limited by an unkown factor. W3 fatty acids are not so important in freshwater organisms as in marine organisms. From the present data, they also seem to be unimportant for the brackish water prawn larvae. However, this conclusion is based on experiments with specific dietary PUFA's, HUFA's and w6/w3 ratios.

On the other hand, diets containing differences in w6 fatty acids are reflected in strong differences in survival and slight differences in growth of M. rosenbergii larvae. These differences in survival may be due to the presence of short chain linoleic acid (18:2w6).

It would be interesting to see the effect on survival and growth for M. rosenbergii larvae with diets showing large differences between long chain w3 fatty acids (mainly 20:5w3 & 22:6w3) whilst maintaining the other fatty acid contents at the same level.

Fatty acid evaluation of other diets

Unfed Artemia used in experiment 2 were low in total lipid (14.7% vs 9.3-27.8% in experiment 1), very low in monounsaturated fatty acids (MUFA's) (29.7% vs 32.0-62.1%), in HUFA's (0.2% vs 0.4-6.9%) and for w6/w3 ratio (0.18 vs 0.18-0.67), but high in w3 fatty acids (42.8% vs 9.8-41.5%) due mainly to the high PUFA content (50.4% vs 15.6-48.9%).

Artemia prefed Chlorella and Dunaliella gained in total lipid and w3 Fatty acids due principally to an increase in 18:3w3. This was also reported by Fujita et al. (1980) and Millamena et al. (1988). In contrast, PUFA's in Artemia decreased when boosted with both micro-algae. Fujita and Millamena found no major changes in PUFA's. This is may be due to the poor PUFA content of their strains initially and the very high PUFA's in the Artemia during the present work (40.9% and 37.5% vs 50.4%).

Experiment 1 suggested that survival and growth of M. rosenbergii larvae could be improved on a diet possibly higher in total lipid and, lower in w6 fatty acids such as linoleic acid (18:2w6). Encapsulated diet and boosted Artemia could be utilized to provide the extra total lipid required. However, from the present results, only unfed and

boosted Artemia contain low w6 fatty acids (ref.table 8). In experiment 1, dietary w6/w3 ratio also showed an inverse relationship with survival and growth of M. rosenbergii larvae. In experiment 2, unfed and boosted Artemia gave a lower w6/w3 ratio than the microencapsulated diets. Thus, Artemia prefed algae present better fatty acid profiles for M. rosenbergii. Manzi & Maddox (1980) also found better survival and growth on algae prefed Artemia, and Jones et al. (1975) could not rear M. rosenbergii larvae beyond stage IV when fed on a protected artificial diet.

Fatty acids in M. rosenbergii larvae

The total lipid content of M. rosenbergii adults is found to be greater than that of marine shrimp P. japonicus (3.18 vs 1.33%) (Chanmugan et al., 1983). According to the same author, this is due to much higher levels of triglycerides in the freshwater prawn. Freshwater fishes and prawns also appear to have less longer chain PUFA's (Watanabe, 1982). This is due mainly to lower levels of linolenic series fatty acids (18:3w3) and lower levels of HUFA's (20:5w3 & 22:6w3). Several authors (O'Connor & Gilbert, 1968; Sick & Beaty, 1974; Wickins, 1976; Jones et al., 1979a; Kanazawa et al., 1979b; Watanabe et al., 1980; Bautista & De La Cruz, 1988) provide evidence to suggest that lipids from marine

organisms contain more long-chain PUFA's than do the lipids of freshwater organisms, which in turn have a high proportion of C_{16} and C_{18} fatty acids. Tables 2 & 3 also confirm these findings: freshwater crustaceans have much lower w3 fatty acids (15.6 vs 27.2%) and lower HUFA's (12.5 vs 22.5%) than marine crustaceans. In the present experiment, w3 PUFA's in M. rosenbergii are found to have an average of 11.1% . This is much higher than was found by Chanmugan et al. (1983) for adults M. rosenbergii (2.9 vs 16.3% for marine shrimps). Present findings may be explained as Stewbart et al. (1973) pointed out that linolenic acid is found in greater amounts in rapidly growing juvenile stages than in adults. In experiment 3, linolenic acid represents an average of 0.4% .

Freshwater species tend to have higher levels of linoleic type fatty acids (18:2w6) than do the marine species. Tables 2 & 3 show that freshwater crustaceans have an average of 19.5% w6 fatty acid content compared to only 8.8% for marine crustaceans. The present results reveal an average of only 3.5% w6 fatty acids (range: 2.2-5.0%), with an average of 1.7% linoleic acid (18:2w6) (range: 0.9-2.8%) and 1.8% arachidonic acid (20:4w6) (range: 1.3-2.4%). This contrasts with table 2 based on adult prawn analysis (only data available). Experiment 1 clearly demonstrated that a high w6 content may have a negative impact on survival for these

larvae. It is possible that the small amount of w6 PUFA series found here may be related to the finding of experiment 1.

Finally, two major fatty acids dominate in M. rosenbergii larvae: palmitic acid (16:0) (average: 29.8%) and oleic acid (18:1w9) (average:25.1%). Palmitic (16:0) and oleic (18:1w9) acids are also the major fatty acids in marine penaeid protozoea besides arachidonic acid (20:4w6) (Lawrence, 1976; Ward et al., 1979). However, these marine larvae have a higher palmitic acid content (average: 42.3%) and a lower oleic acid content (average: 11.9%). The fatty acid analysis of M. rosenbergii reveals that larvae conserve some of the marine pattern for that they contain some 20:5w3 (6.7%) and 22:6w3 (3.5%) which remains at these levels later in their development. For M. rosenbergii, palmitic and oleic acids decrease in juveniles to averages of 14.9 and 19.7%, respectively, and linoleic acid (18:2w6) becomes more important (average: 29.8%) (Sandifer & Joseph, 1976) and characterizes crustaceans living in more freshwater environment. For penaeid shrimps, the main fatty acids in juveniles are 16:0 (23.9%), 18:1w9 (16.3%), 20:5w3 (12.6%) and 22:6w3 (13.3%) (Guary et al., 1975; Kanazawa et al., 1979a) which characterize crustaceans living in marine environment.

" Weak " vs " strong " M. rosenbergii larvae

Saturated and monounsaturated fatty acids in M. rosenbergii larvae seem to be of the same level in the two larval groups. However, " strong " larvae contained 39.6% higher w6 and 24.4% lower w3 fatty acids compared to " weak " larvae. Short-chain linoleic acid (18:2w6), in the former, and long-chain eicosapentaenoic (20:5w3) and docosahexaenoic (22:6w3) acids, in the latter, are mainly responsible for these differences. Possibly, like freshwater fishes (Ackman, 1980), M. rosenbergii larvae are able to convert some short-chain fatty acids more efficiently than long-chain fatty acids.

The results of this analysis seem to contrast with experiment 1 in which findings suggested that growth can be better (shorter larval cycle) if M. rosenbergii larvae are reared on lower w6 or higher w3 fatty acid levels. However, in that case " dietary " differences were examined with respect to impact on the growth of the freshwater prawn larvae. Present results come from analysis of " whole body " composition of M. rosenbergii larvae. The amounts of fatty acids found could represent the optimum metabolic equilibrium in recently hatched larvae which stimulates growth in the absence of any dietary influence. The high w6 and low w3 fatty acid levels found in " strong " larvae

suggest the unimportant or non-essential role of PUFA's for M. rosenbergii larvae at the begining of its life cycle. These findings also seem to agree with the general tendency shown by adults of freshwater crustaceans which are characterized by higher levels of w6 compared to w3 (ref.table 2).

W6/w3 ratio is also twice higher for " strong " larvae than for " weak " larvae (0.44 vs 0.23). This higher ratio foreshadows the higher w6/w3 ratio found in adults of freshwater crustaceans (ref.table 2).

Present results suggest that recently hatched M. rosenbergii larvae at the first stage reflect, before receiving any feed, the fatty acid profile of their parents as far as w6 and w3 are concerned. Later, diet modifies this profile as larvae start to consume feed, because diet influences body composition (Halver & Tiews, 1979; National Research Council, 1983).

Fatty acid profile of stages I and II for M. rosenbergii larvae

Results indicate that larvae apparently utilize saturated and monounsaturated fatty acids for their energy requirements: myristic (14:0), palmitic (16:0), palmitoleic

(16:1w7), oleic (18:1w9) and gondoic, or eicosanoic, (20:1w9) acids decrease by 20.2; 2.4; 20.6, 8.5 and 8.1%, respectively. Tandler et al. (1989) also found that food deprivation in red seabream (Pagrus major) larvae revealed a decline in saturates and monoenes. In particular, oleic acid (18:1w9), the most abundant crustacean monoene (Castell, 1982), is partially shortened into stearic acid (18:0) which increases by 7.7%, whilst cis-vaccenic acid (18:1w7) increases by 12.1% .

PUFA's also increase by 21.2% from stage I to stage II which indicates that M. rosenbergii larvae are able to chain elongate for building structures in membranes. This is expected as when an animal grows, the number of its cells increases and its membranes therefore increase requiring structural synthesis (PUFA's). In the case of short chains, this is obvious as linoleic acid (18:2w6), the precursor of arachidonic acid (20:4w6), decreases by 3.4% and 20:4w6 increases by 43.6% . As far as the long-chain linolenic series is concerned, it is less obvious for linolenic acid (18:3w3), the precursor of eicosapentaenoic (20:5w3) and docosahexaenoic (22:6w3) acids, which remains at the same level. but 20:5w3 and 22:6w3 increase by 26.2 and 19.1%, respectively. Working on the red seabream larvae, Tandler et al. (1989) also found that 18:3w3 was lost at a slower rate. In contrast with previous findings, this experiment

demonstrates that PUFA's, and more specifically HUFA's, are important in M. rosenbergii larval development.

Lipid composition of M. rosenbergii parent diets

Experiment 5 revealed the importance of PUFA's in recently hatched larvae of M. rosenbergii. Endowment of adequate lipid content in the eggs and in recent hatchedly larvae is dependent upon the ovary fatty acid profile (Ward et al., 1979). Results of experiment 4 reveal a wide range in the fatty acid profile of M. rosenbergii. Lipids are concentrated in the ovary of mature crustaceans (Allen, 1971; Morris, 1973; Guary et al., 1974). A linear relation is observed between the accumulation of fatty acids from dietary sources and that in marine shrimp tissues, particularly, in the ovary (Guary et al., 1975). But according to the same authors, mature females of marine decapods tend to have more MUFA's and less PUFA's.

Present analysis shows that squid and mussel have a high PUFA content, having 54.7 and 50.6%, respectively (the former presenting a better HUFA profile, with 53.9%, than the latter, with 36.0%). However, in agreement with results from experiment 1 which showed that total lipid in the diet enhances survival and growth of M. rosenbergii larvae, squid

appears to offer a better profile than mussel on an equal weight basis (4.6 vs 1.5% total lipid). Experiment 1 also showed that a high dietary w6 profile did not promote larval survival. Present findings showed that shrimp and squid contain lower w6 ratios (ref.tab.12) than do mussel and pellet, having 4.1, 1.1, 8.5 and 11.9%, respectively. Squid appear to be a valuable food to include in M. rosenbergii diet particularly for maturation of females.

In summary, two major fatty acids, palmitic and oleic acids, dominate in M. rosenbergii larva. Diets varying widely in total lipid only influence survival and growth in larvae slightly. Diets varying in w3 fatty acid levels produce no difference in survival and growth, but there are differences in relation to linolenic acid which is the precursor of these long chain fatty acids, for larvae which have a very low HUFA profile at stage I. Diets containing different levels of w6 fatty acids produced large differences in survival and slight differences in the larval growth. These differences in survival are probably due to the presence of short chain linoleic acid. Finally, recently hatched larvae utilize saturated and monounsaturated fatty acids for their energy requirements, as PUFA's increase from stage I to stage II suggesting that M. rosenbergii larvae are able to chain elongate.

Section II

RELATIONSHIPS BETWEEN EGGS, LARVAE AND PARENT FEMALE IN *Macrobrachium rosenbergii* (de Man)

INTRODUCTION

Crustaceans play an important part in the life of both marine and continental waters and have long attracted the attention of scientists. Many aspects of crustacean biology have already been thoroughly studied. Fecundity of the females is one which has frequently been considered. Table 1 gives some characteristics of eggs laid by important decapod crustaceans. Several authors (Crisp, 1954; McLaren, 1965; Patel & Crisp, 1960) reported that, for each species, the weight of the eggs is not a constant value, but varies in relation to many factors. Amongst them, they mention environment, season, temperature, size of the female and possible unknown factors. The differences noted in weight of eggs can vary from 14 to 100% (Khmeleva, 1972). Kuznetsov (1964) showed clearly the relationship between egg number and parent female size in the caridean decapods. Ivanova and Vassilenko (1987) studied 16 orders and suborders of crustacea looking for relationships between number of eggs

TABLE 1. Meristic characteristics of several decapod crustacean eggs

Species	Wet weight (µg)	Dry weight (µg)	Dw/Ww ratio (%)	Diameter (mm)	Number	Colour	Incubation	
							days	oC
Macrobrachium rosenbergii (1), (2) & (3)	84.2	35.3	41.9	0.5	5000-120000	yellow-orange-grey	18-23	28
Crangon crangon (4) & (5)	54.0	17.0	31.5	1.4-2.1	400-5750	greenish-grey	18	15
Palaemon elegans (2), (6) & (8)	113.0	50.0	44.2	0.5-2.7	350-4282	bright green-greenish brown	20-39	17
Pandalus latirostris (9) & (10)	3940.0	1390.0	35.2	0.6-0.8	250-859	orange	21	?
Pandalus borealis (9) & (11)	556.0	234.0	42.1	0.9-1.2	300-3000	?	150-210	14
Penaeus monodon (3) & (12)	116.2	?	?	0.2-0.3	110000-1300000	yellowish green-grey	0.5-1.0	28
Homarus gammarus (5) & (9)	3690.0	1700.0	46.1	1.6	5000-30000	green-brown	241-330	13
Homarus americanus (9), (10), (13) & (14)	2203.0	965.0	43.8	<1.6 ?	3000-75000	green-brown	235-330	14
Nephrops norvegicus (5) & (15)	?	?	?	?	900-6000	green-brown	180-300	12
Astacus astacus (16), (17) & (18)	13910.0	5610.7	40.0	2.0-3.5	75-204	black-brown-red-brownish purple	150-240	16
Procambarus clarkii (19) & (20)	21000.0	?	?	1.5-2.0	130-480	white-yellow-dark brown	14-21	24
Carcinus maenas (4) & (13)	?	?	?	0.2	200000	orange-black	37	15

(1) Balasundaran & Pogyamoli, 1984
(2) Magalhaes & Walker, 1988
(3) Hanson & Goodwin, 1977
(4) Gurney, 1960
(5) Khmeleva & Goloubev, 1968
(6) Holthuis, 1975
(7) Yagi & Ceccaldi, 1984
(8) Sorbe, 1983
(9) Cobb & Phillips, 1980
(10) Allen, 1895
(11) Allen, 1959
(12) Hiramatsu, 1984
(13) Iversen, 1971
(14) Talbot et al., 1984
(15) Wear, 1974
(16) Kukerzis, 1970
(17) Le Louarn, 1985
(18) Arrignon, 1981
(19) Huner & Barr, 1984
(20) Corey, 1987

(Ng), clutch weight (Wg), and body length (L) and weight (W) of parent females. They found that comparison of data for some orders has shown that a single equation describes the relationship between clutch weight and female body weight for the whole superclass. On the average for the superclass Crustacea, the Wg/W ratio appeared to be 0.16 (16%). In Brachyura, this ratio varies within a narrow range from 0.04 to 0.15. However, the material available showed that for the Caridea there appears to be no correlation between egg and female size, although a tendency for larger species to produce larger eggs is discernible. The relative clutch weight in Caridea varied from 0.01 to 0.40 (1 to 40%). Tseytlin (1988) found in a long study that the relationship of clutch weight (Wg) to the body weight of females (W) had the form $Wg = 0.25W^{k}$ in decapods where $k = 0.93 \pm 0.07$. He called this equation the " generative production " of the aquatic animals and observed that it was an important index which could contribute to an estimate of the vital activity of these animals. The measure of generative production that is most widely used in practice is fecundity, absolute or relative. The " absolute " fecundity, for crustacean, is the number of eggs per clutch. The " relative " fecundity is the ratio of the weight of eggs per clutch to the body weight of the parent female. All these rules try to characterize relationships between females and their eggs and can be of valuable interest in

theorical studies for the acclimatation or the development of some potential important species.

The data used in this work were taken during a two year study on Macrobrachium rosenbergii reproducing in a closed system, and aim to investigate the particular relationships between females and their eggs and larvae hatching from these eggs.

MATERIALS AND METHODS

The M. rosenbergii prawns used in this study were hatched in the tropical unit of the Marine Science Laboratories, Menai Bridge. The brackish water was produced by mixing the local tap water and the seawater from the laboratory supply. It was recycled continously through a percolating biological filter of plastic media, specific surface area 164 $m^2.m^{-3}$, volume 0.76 m^3, hydraulic load 114 $m^3.m^{-3}.d^{-1}$ (Forster & Wickins, 1972; Richards & Wickins, 1979).

Three days after spawning, 10 eggs per group were measured with a lower-power binocular fitted with an eye graticule. Number of days to reach hatching was recorded. Females were weighed 8 days before hatching and again after the release of larvae. " Clutch " is defined as the total set of eggs per gravid female. For each group, 100 recently hatched larvae were starved and larval resistance was evaluated by the number of days for survival.

RESULTS

Tables 2 and 3 present the meristic characteristics of M. rosenbergii eggs in relation to parent female, and the relations between M. rosenbergii females, the incubation of their eggs and the resistance of the larvae hatched from these eggs, respectively. Table 12.0 (appendix) shows the detail of the starving resistance for the different larval groups. Figures 1, 2, 3 and 4 respectively represent the following relationships:

- weight and total length of the female (from tables 2 & 3)
- clutch weight and egg number
- wet weight and volume of egg
- weight (a) / total length (b) of the female and egg number

An analysis of regression gives the following equations, with their respective slopes, for the previous fitted lines:

TABLE 2. Meristic characteristics of Macrobrachium rosenbergii eggs in relation to parent female

	Female					Eggs (1)					
#	Height without clutch (g)	Height with clutch (g)	Clutch weight (g)	Clutch %	Total length (cm)	Wet weight (µg)	Number	Large axis length (mm)	Small axis width (mm)	Volume (mm3)	Colour
1	49.16	50.05	0.89	1.8	15.0	44.3	20090	0.520	0.474	0.064	yellowish orange
2	49.43	51.17	1.74	3.5	15.5	80.2	21695	0.529	0.474	0.066	dark yellow
3	47.11	48.34	1.23	2.6	14.5	72.8	16895	0.515	0.444	0.058	dark yellow
4	11.77	12.11	0.34	2.9	8.5	70.5	4822	0.515	0.476	0.064	pale yellow
5	34.37	35.50	1.13	3.3	12.5	84.7	13341	0.524	0.478	0.066	yellowish orange
6 (2)	38.50	41.03	2.53	6.6	14.5	147.0	17210	0.605	0.564	0.105	dark yellow
7	27.33	28.15	0.82	3.0	12.0	81.7	10036	0.550	0.488	0.073	yellow
8	24.94	26.08	1.14	4.6	13.3	94.3	12089	0.527	0.481	0.067	yellowish orange
9	62.45	65.65	3.20	5.1	16.0	85.3	37514	0.536	0.478	0.068	pale yellow
10	62.08	63.62	1.54	2.5	15.3	80.9	19035	0.513	0.478	0.064	yellowish orange
x	40.71	42.17	1.46	3.6	13.7	84.2	17272	0.533	0.484	0.070	-

(1) all eggs were measured 3 days after spawning;
(2) partially aborted

TABLE 3. Relations of *Macrobrachium rosenbergii* females, incubation of their eggs and resistance of the larvae hatched from these eggs

	Female			
#	Weight (g)	Total length (cm)	Egg incubation (days)	Larval resistance (days)
1	15	11.50	20	8
2	29	12.25	14	9
3	23	12.75	17	11
4	25	12.75	22	10
5	25	13.00	21	7
6	40	13.20	19	7
7	30	13.25	18	10
8	23	13.50	19	8
9	30	13.50	20	9
10	35	13.50	23	8
11	37	13.50	20	8
12	35	14.00	21	11
13	38	14.00	20	10
14	40	14.00	20	8
15	35	14.50	18	9
16	49	14.50	20	8
17	38	14.75	20	10
18	30	15.00	18	10
19	40	15.00	19	7
20	41	15.00	19	8
21	45	15.50	20	7
Range	11.50-15.50	15-49	14-23	7-11
X	13.76	33.48	19.43	8.71
Std.Dev.	1.02	8.30	1.86	1.31
Coeff. of Var.	7.40	24.81	9.57	15.02

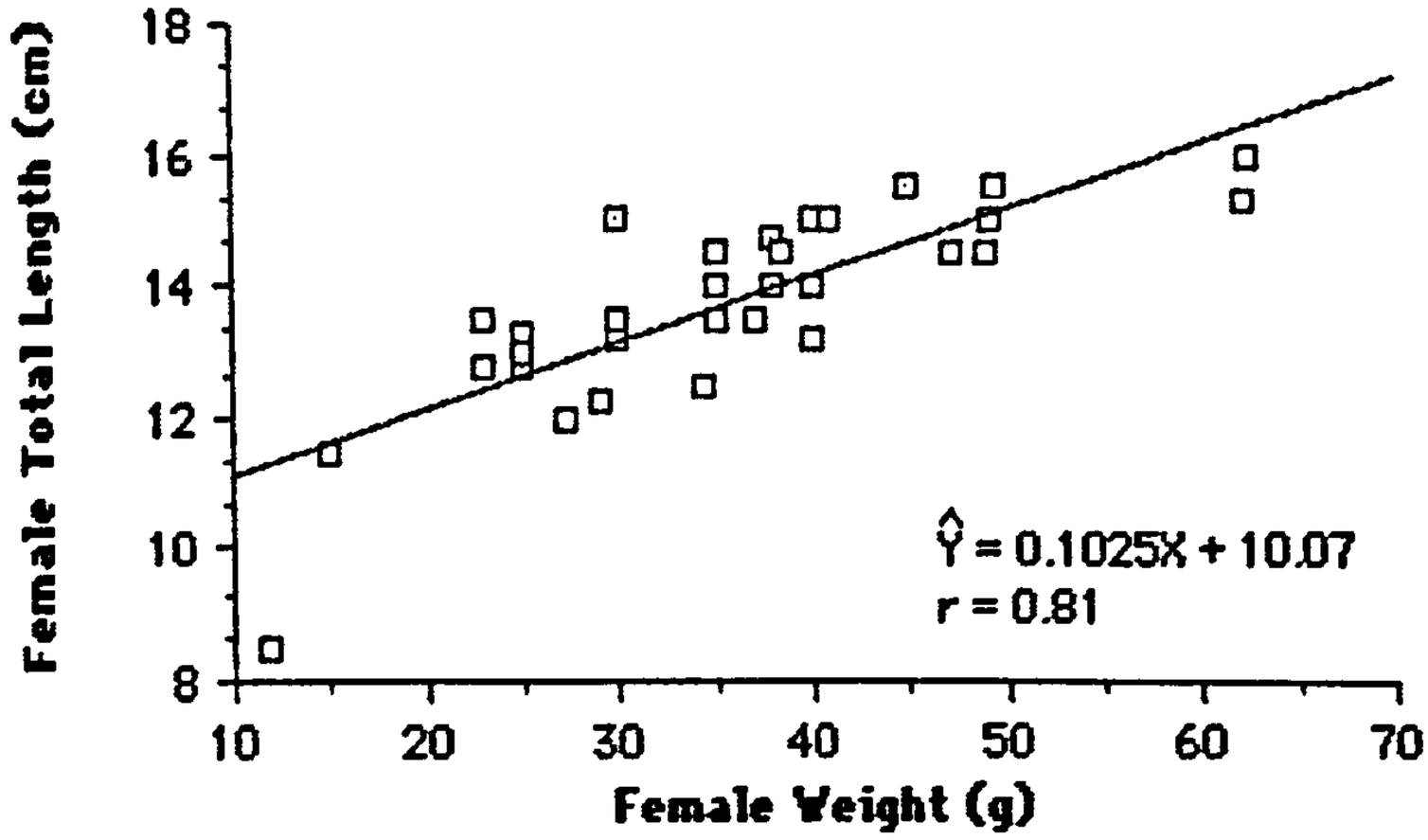


Figure 1. Relationship between weight and total length of M. rosenbergii female (from tables 2 & 3)

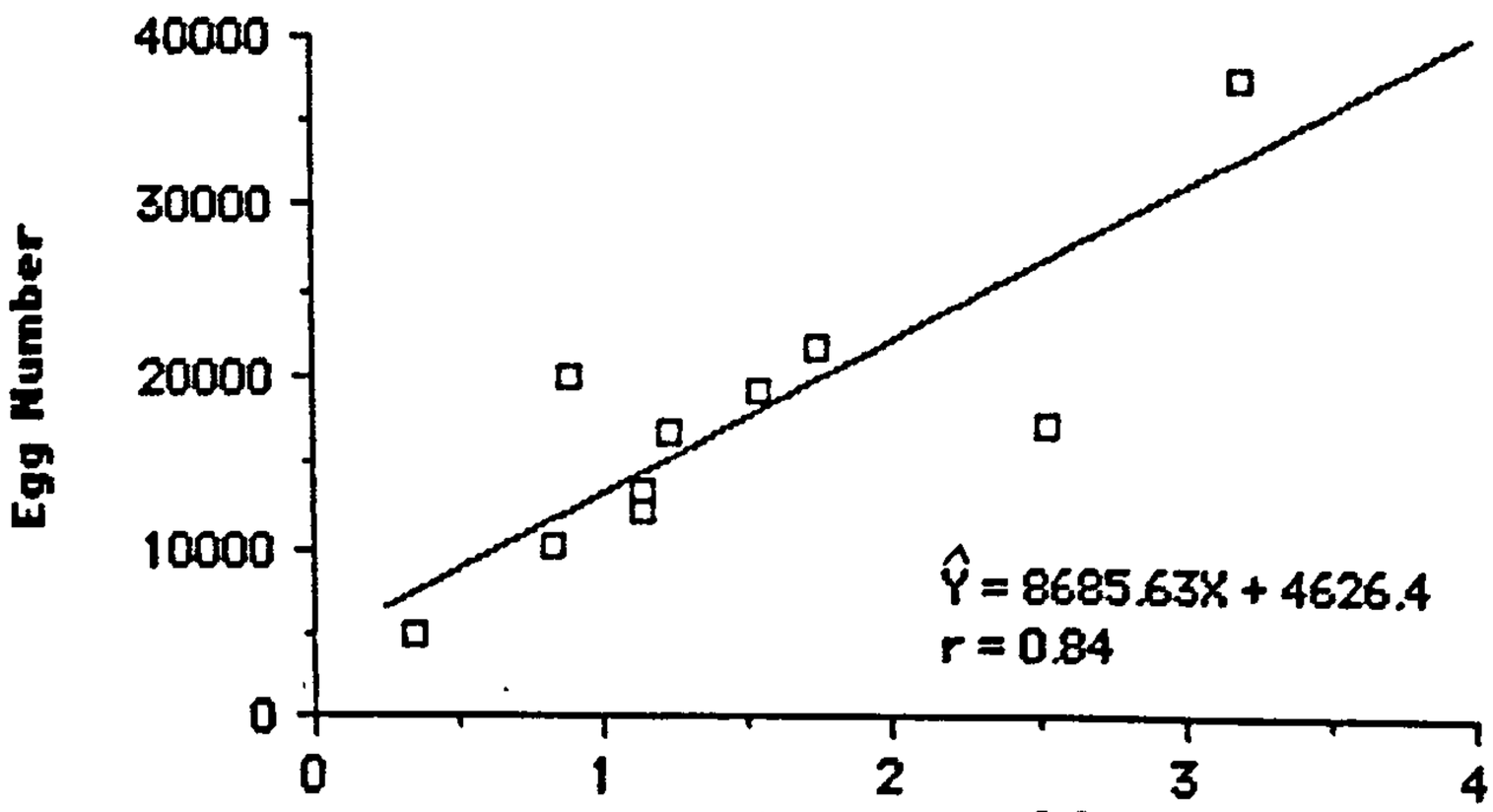


Figure 2. Relationship between weight of the clutch and number of the eggs for M. rosenbergii

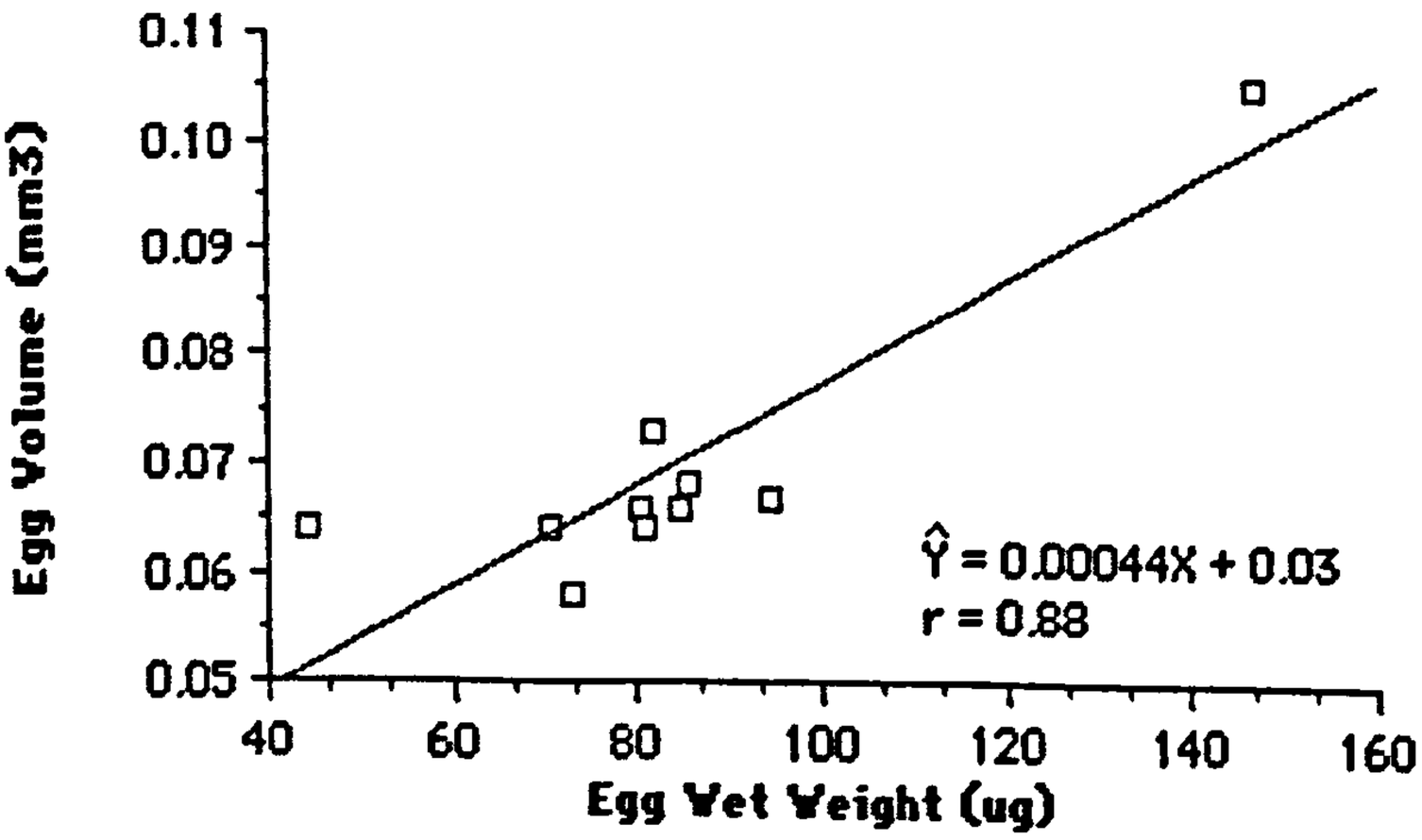
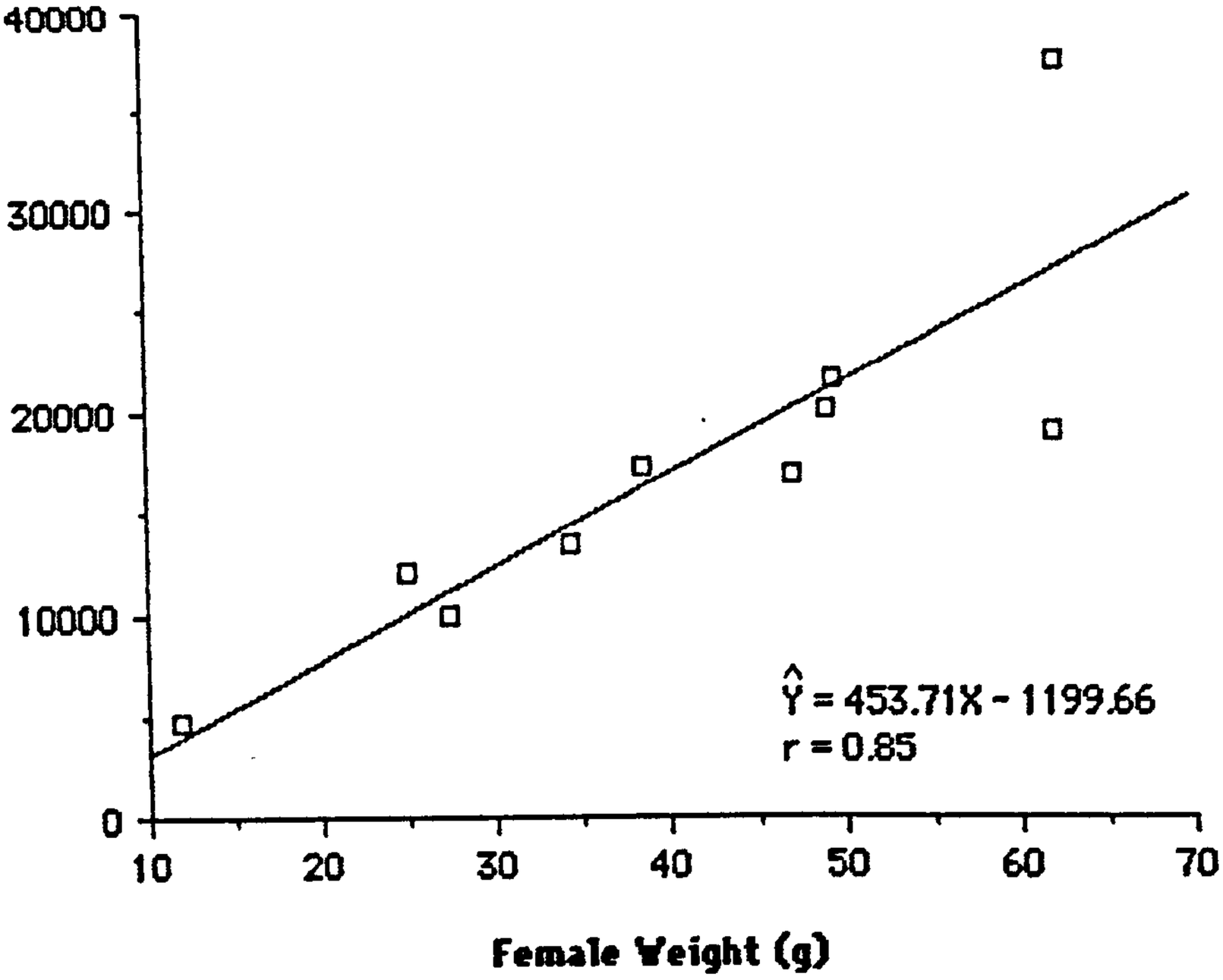


Figure 3. Relationship between weight and volume of M. rosenbergii egg

a.



b.

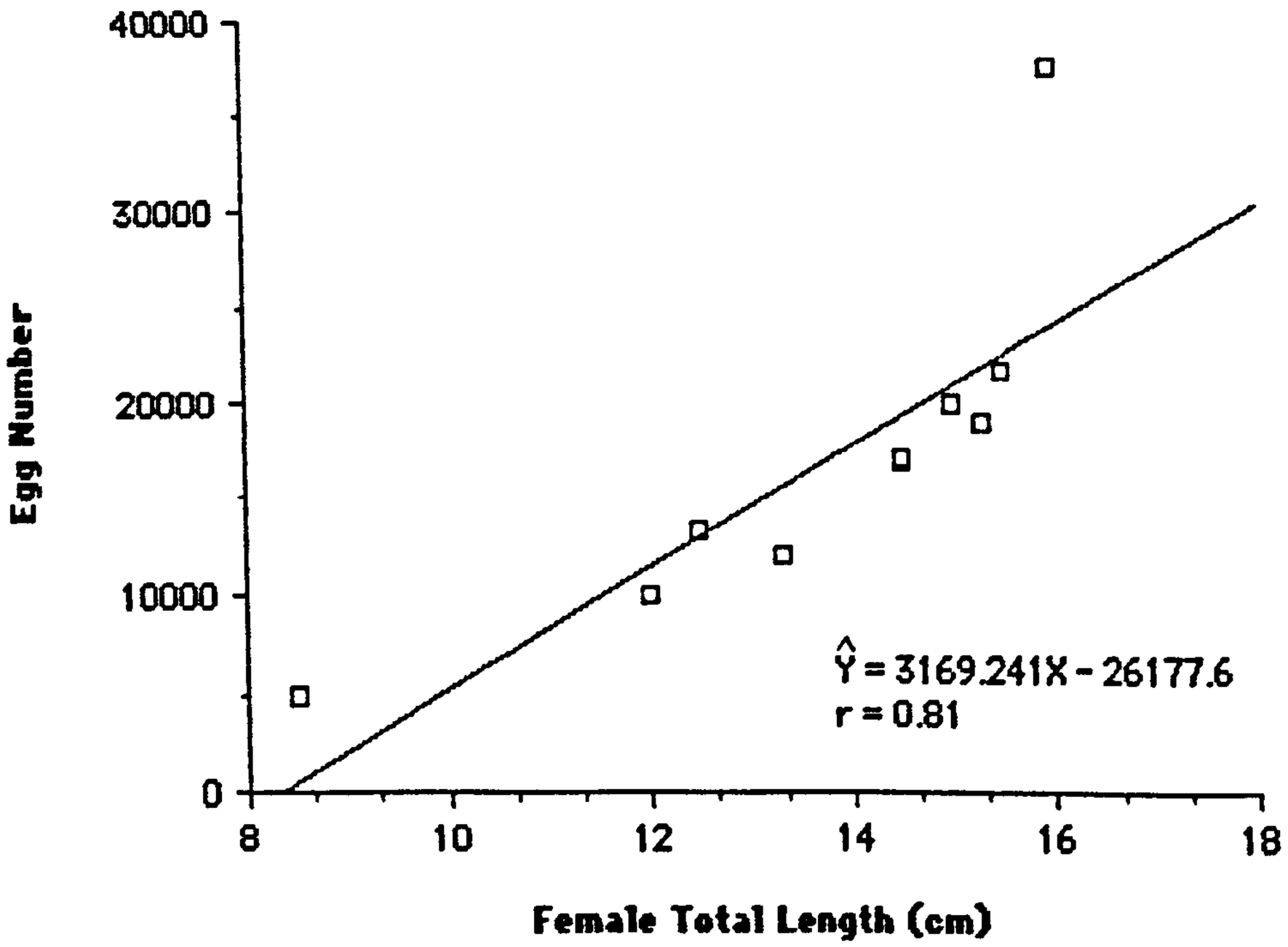


Figure 4. Relationships between weight (a) and total length (b) of M. rosenbergii female and number of their eggs

$$\hat{Y} = 0.1025\ X + 10.07$$

$$r = 0.81^{**} \text{ (D.F.= 30; } \alpha = 0.01)$$

$$\hat{Y} = 8685.63\ X + 4626.4$$

$$r = 0.84^{**} \text{ (D.F.= 9; } \alpha = 0.01)$$

$$\hat{Y} = 0.00044\ X + 0.03$$

$$r = 0.88^{**} \text{ (D.F.= 9; } \alpha = 0.01)$$

(a) $$\hat{Y} = 453.71\ X - 1199.66$$

$$r = 0.85^{**} \text{ (D.F.= 9; } \alpha = 0.01)$$

(b) $$\hat{Y} = 3169.241\ X - 26177.6$$

$$r = 0.81^{**} \text{ (D.F.= 9; } \alpha = 0.01)$$

DISCUSSION

Results show that total length of M. rosenbergii female is significatively related to its weight ($r = 0.81^{**}$; D.F.= 30; $\alpha = 0.01$). This is for animals kept in captivity and maintained in a recirculation system for a long period of time with no apparently major factor affecting their development. Heavier females statistically had heavier clutches ($r = 0.65^{*}$; D.F.= 9; $\alpha = 0.05$) and higher numbers of eggs ($r = 0.85^{**}$; D.F.= 9; $\alpha = 0.01$). Similar relations were found between the total length of a female, clutch weight ($r = 0.7^{*}$; D.F.= 9; $\alpha = 0.05$) and number of eggs ($r = 0.81^{**}$; D.F.= 9; $\alpha = 0.01$). Again, this is for females kept under artificial conditions. Wickins and Beard (1974) also found that larger M. rosenbergii females produced more eggs.

However, the female weight influenced neither the weight per egg ($r = -0.1^{NS}$; D.F.= 9; $\alpha = 0.1$), nor the volume of each egg ($r = -0.11^{NS}$; D.F.= 9; $\alpha = 0.1$). No relation was found

between total length of the female, and the weight per egg ($r = 0.1^{NS}$; D.F.= 9; $\alpha = 0.1$) and the volume of each egg ($r = 0.08^{NS}$; D.F.= 9; $\alpha = 0.1$). Ivanova and Vassilenko (1987) also found that there was no correlation between egg and female size for caridea, though a tendency for larger eggs was detected with larger species.

Weight of the clutch did not influence the weight per egg ($r = 0.54^{NS}$; D.F.= 9; $\alpha = 0.05$) and the volume of each egg ($r = 0.46^{NS}$; D.F.= 9; $\alpha = 0.1$), but influenced the number of eggs ($r = 0.84^{**}$; D.F.= 9; $\alpha = 0.01$). This is in agreement with the present previous findings.

Finally, the number of eggs did not influence the weight ($r = 0.01^{NS}$; D.F.= 9; $\alpha = 0.1$) and the volume ($r = 0.01^{NS}$; D.F.= 9; $\alpha = 0.1$) of each egg, but heavier egg logically had larger volume ($r = 0.88^{**}$; D.F.= 9; $\alpha = 0.01$).

No influence was found between weight and total length of parent female and the length of the period for the incubation of their eggs ($0.11 < r^{NS} < 0.13$; D.F. = 20; $\alpha = 0.1$) and the resistance of their larvae ($-0.12 < r^{NS} < -0.29$; D.F.= 20; $\alpha = 0.1$).

The length of the period for the egg incubation did not significantly influence the resistance of their larvae either, though a negative trend was observed ($r = -0.17^{NS}$;

D.F.= 20: α = 0.1) suggesting that for a longer egg incubation period larvae could be less resistant.

The ratio between the weight of the parent female and the weight per egg (*Wegg*/W) was found to be 0.036 in the present experiment and reflects a poor fecundity when compared to 0.168 obtained by Khmeleva and Goloubev (1986) (table 4). This is probably due to the long artificial maintenance of M. rosenbergii in our facilities, as previous authors collected their data from the wild. However, present findings agree with Ivanova and Vassilenko (1987) who found that this ratio varies between 0.01 and 0.4 for caridea. The relationship of the clutch weight to the body weight of females ($W_g = 0.25W^k$) gave for k a lower value (0.81742) than Tseytlin's value (1988) for decapods (0.81742 < 0.86 < k < 1.00). The index k found for the equation of the " generative production " suggests that relative fecundity is very low in M. rosenbergii under present conditions indicating that these are less than optimal.

TABLE 4. Average weight of parent female, weight per egg, and ratio between the two for populations of some astacidan, anomuran, brachyuran and caridean species

Species	W (mg)	W_{egg} (mg)	W_e /W (1)	Source
Astacidea				
Astacus leptodactylis Escholz	38170	1-5	0.01-0.05	Stypinska, 1979
Astacus astacus Linne	35130	8-12	0.06-0.09	iden
Orconectes limosus	22550	6	0.13	iden
Anomura				
Pagurus anomalus Balls	2200	0.075	0.12	Mikulich & Kosak, 1971
P. platypus Brandt	1014550	0.6	0.06	iden
Brachyura				
Cancer amphioctus Rathbun	5180	0.008	0.125	iden
Carcinus mediterraneus Czerniavsky	31600	0.03	0.06	Khmeleva, 1972
Hyas coarctatus alutaceus Brandt	6450	0.15	0.10	Ivano & Vassilenko,1987
Caridea				
Crangon crangon Linnaeus	1406	0.054	0.07	Bulgurkov, 1970
	2540	0.125	0.14	Khmeleva, 1972
Palaemon elegans Rathke	815	0.094	0.13	Bulgurkov, 1970
	775	0.136	0.18	Khmeleva, 1972
Macrobrachium rosenbergii de Man	-	0.075	0.168	Khmeleva & Goloubev, 1986
	40710	0.084 (2)	0.036 (3)	Present experiment

(1) Clutch weight/parent female weight
(2) Range: 0.044-0.147mg
(3) Range: 0.018-0.066

Section III

Macrobrachium rosenbergii larval rearing on artificial diets

INTRODUCTION

A major difficulty in the development of culture systems for larval crustaceans is their dependence upon supplies of live food organisms. Hence attempts have been made to develop artificial diets to provide an alternative to natural foods in the commercial culture of economically valuable species. In contrast to the numerous satisfactory artificial diets marketed for rearing adult crustaceans (New, 1976), only a few have been reported to support the growth of crustacean larvae to metamorphosis (Sandifer & Williams, 1980).

At present, the only artificial alternatives to encapsulated larval diets are processed natural products such as spray freeze dried algae or yeasts, or micro particulate/micro bound diets (Jones, 1988; Jones et al., 1989). High molecular weight, water-soluble nutrients such as protein and starch can be trapped in gels of calcium carboxymethyl cellulose, calcium alginate, gelatin, carrageenan, agar or

chitosan (Teshima et al., 1982; Langdon, 1983; Levine et al., 1983). Gelatin-acacia capsules are formed by a simple co-acervation reaction between gelatin and acacia at an aqueous-solid or aqueous-liquid interface (Green & Schleider, 1957). Microbound diets are inexpensive to produce, but resultant powders show rapid leaching and particle breakdown due to poor stability (Adron et al., 1974; Jones et al., 1979). Despite the wide range of commercial microbound diets,there doesnot appear to be any evidence to demonstrate that they have been used successfully to totally replace live feeds (Jones, 1988). Lipid-walled capsules are used to prevent the leakage of low molecular weight, water-soluble nutrients from particulate diets immersed in an aqueous medium. They are formed by a process of double emulsion using mainly a mixture of menhaden oil and ethyl cellulose (Langdon, 1983).

Microencapsulation was first applied to biological problems by Chang et al. (1966) who used nylon-protein microcapsules containing erythrocyte hemolysates, as artificial red blood cells, for experimental enzyme therapy. Later, Meyers et al. (1971, 1973) suggested the potential of encapsulated diets for crustacean larvae. However, microencapsulated diets were first shown to be acceptable to crustacean larvae by Jones et al. (1972). Soon after, Jones et al. (1974) grew Artemia salina on nylon-protein encapsulated haemoglobin and starch

under non-axenic conditions. They reported that although mortalities were much higher than with the algal-fed control larvae, a few Artemia reached metamorphosis. Later, the technique was modified by Levine et al. (1983) for the use in studying the nutritional requirements of crustacean larvae. More recently, Sakamoto et al. (1982) reported 35% survival to adult for Artemia reared on encapsulated diets. Microencapsulated diets have been used, with increasing success, to replace live feeds for penaeid prawn larvae. Survival reached 50-78% to postlarval stage 1 (PL1) for Penaeus japonicus Bate (Jones et al., 1979), 80% to PL5-7 for P. merguiensis de Man (Kanazawa et al., 1982), 65% to PL15 for P. vannamei Boone and P. stylirostris Stimpson (Jones et al., 1986), 47% to PL7 for P. monodon Fabricius (Jones et al., 1987), 53-64% to PL1 for P. monodon Fabricius (Kurmaly et al., 1989). Now, microcapsules are used routinely in laboratory as partial or even total replacement for all live feeds for P. monodon larval culture (Jones, 1988; Jones et al., 1989). Table 1 presents the survival of some invertebrate filter feeding larvae when reared on artificial diets.

However, lobster (Homarus gammarus Linnaeus) larvae were unable to survive to metamorphosis on these artificial diets (Brewster, 1987). In the larval foregut, the underdeveloped gastric mill allows food processing to some extent, but

TABLE 1. Use of artificial diets to rear some invertebrate filter feeding larvae

A. Unprotected artificial diets:

Species	Feed Type	Result	Authors
Macrobrachium rosenbergii	Freeze-dried catfish	11% survival to metamorphosis	Sick & Beaty, 1975
M. rosenbergii	Freeze-dried oyster & trout feed	< 2% to metamorphosis	Murai & Andrews, 1978
Palaemonetes pugio	Freeze-dried squid	26.7% surv. to metamorphosis	Sandifer & Williams, 1980
Penaeus monodon	Spray-dried yeast	39% survival	Jones et al., 1989
	Spray-dried algae	No survival	Jones et al., 1989

B. Protected artificial diets:

Species	Feed Type	Result	Authors
Brachionus plicatilis	Nylon-protein microcapsules	Prolonged culture >5 days resulted in decrease of population	Teshima & Kanazawa, 1983
Artemia salina	Idem	35% survival to adult	Sakamoto et al., 1982
M. rosenbergii	Idem	Survival to stage IV only	Jones et al., 1975
P. monodon (1)			
PZI-MI	RDX10 (2)	53.06% survival	Kurmaly et al., 1989
M2-PLI	CD435 (3)		
PZI-MI	RDX24 (2)	64.59% survival	Kurmaly et al., 1989
M2-PLI	CD435		
PZI-PLI	Freeze-dried cross-linked protein mcp (5) + μ-algae (4)	76% survival	Jones et al., 1989

(1) PZI=Protozoea 1; MI=Mysis 1; M2=Mysis 2; PLI=Postlarva 1
(2) 45 microcapsules (mcp).μl^{-1} (3) 20-30mcp.μl^{-1} (4) 10 cells.μl^{-1}
(5) manufactured by Frippak Feeds (British patent Nos. 79437454 and 2103588)

survival of larvae fed on encapsulated diets did not extend beyond day 15. Similarly, Carcinus maenas Linnaeus larvae accept artificial food providing alive diet is also present (Brewster, 1987). Levine and Sulkin (1984b) were also only partially successful in feeding crab zoea on artificial diets. Larvae of the caridean Palaemon elegans Rathke were unable to survive beyond day 9 when fed on microencapsulated diets (Brewster, 1987), and Macrobrachium rosenbergii (de Man) larvae fed on microcapsules only survived to stage IV (Jones et al., 1975; Brewster, 1987). No successful artificial diet has yet been marketed for caridean prawn larvae. Attempts to grow M. rosenbergii larvae on a wide range of encapsulated diets have not been successful (Möller, 1977). These carnivorous larvae do not appear to search for prey using either chemical or visual cues, but rely on contact, whereas under similar conditions postlarvae are capable of both orientation towards and actively approach offered particles (Möller, 1977). Experiments with Macrobrachium postlarvae demonstrate visual, chemosensory, as well as rheotactic response to food stimuli. This sensory capability enhances the efficiency with which postlarvae capture and ingest prey. Möller (1977) suggests that the feeding behaviour seen in M. rosenbergii larvae is probably also utilised by omnivorous and herbivorous crustacean larvae. Once a particle comes in contact and has been grasped, chemo-sensory and mechano-sensory perceptions

determine whether it is rejected or ingested (Kurmaly et al., 1989). However, M. rosenbergii larvae, in common with other decapod larvae, will accept and ingest inert food particles provided these elicit the correct chemo-sensory responses (Jones et al., 1974).

Artificial food substances hitherto presented to various species of crustaceans appear consistently inferior to live diets (Foster & Beard, 1973; Sick & Beaty, 1974; Regnault et al., 1975). The observed limitation of microcapsules as an artificial diet for certain crustacean larvae may be due to one or several factors including: the presence of deterrent substances, the absence of substances stimulating ingestion and growth, congestion of the alimentary passages preventing efficient digestion or poor digestibility (Möller, 1977). The absence of a gastric mill in palaemonidae means that ingested food must contain readily assimilable nutrients and a high energy content if it is to support development. Such a diet, consisting of a new dried cross-linked protein walled microcapsule (frippak Feeds Ltd., England) has recently been shown to successfully support complete larval development in penaeid prawn larvae (Kurmaly et al., 1988, 1989).

Present work details the results of some further studies on

the larval development of Macrobrachium rosenbergii utilising this microcapsule.

MATERIALS AND METHODS

Larval rearing

Macrobrachium rosenbergii larvae were obtained from berried females supplied by the tropical unit, Marine Science Laboratories, Menai Bridge.

Larvae were reared in 2 litre flat-bottomed flasks at a density of 50 larvae per litre and subject to diurnal illumination. A thermostat and electrical heater were used to maintain the temperature of the water at 29°C. Salinity was brought to 12‰ and checked with a refractometer. Water was previously cartridge filtered to 0.2µm and irradiated with U.V. light to reduce bacterial contamination. The cultures were gently aerated with airline attached to a Pasteur pipette.

Ammonia, nitrite and pH were recorded in culture water using respectively the Parsons *et al.* (1984a) method, the

Bendschneider and Robinson (1952)/FWPCA (1969) method, and a photovolt 112pH meter. Un-ionized ammonia concentrations were corrected for the pH values corresponding to the culture water and calculated using Emerson *et al*. (1975) corrected factors.

At the end of stage I, larvae in control (in triplicate) were fed daily with newly hatched *Artemia salina* (San Francisco Bay Brand) at the concentration of 12 nauplii per ml.

Cysts of *Artemia* hatched after a 18-24h period of strong aeration in salt water. *Artemia* nauplii were then separated from unhatched cysts by tilting the conical vessel, concentrating with a light (positive phototropism), siphoned and washed in brackish water before given to *Macrobrachium* larvae. *Artemia* nauplii densities were estimated by repeated 1 ml counting with an automatic calibrated micropipette.

Uneaten feed, faecal matter, dead larvae and exuviae were removed daily. At 48h intervals, water was renewed and numbers of larvae were recorded.

Growth was expressed in terms of the mean larval stage (M.L.S.). M.L.S. was calculated as the sum of the stage values divided by the number of individuals. Identification of stages was carried out using the keys provided by Ling

(1967), Uno and Kwon (1969) and New and Singholka (1985). Table 2.0 (appendix) summarizes the identification of Macrobrachium larval stages.

First trial: recently hatched larvae

Newly hatched Macrobrachium larvae, in triplicate experiments, were fed with Frippak microencapsulated diets scheduled as recommendated (table 13.0, appendix):

Larval stages	Size of microcapsule (µm)
I - III	5-30
IV - V	90

The artificial diets were prepared every day by adding Frippak dried microcapsules to distilled water at 8mg per litre. Survival and M.L.S. were recorded daily.

Second trial: from day 16

Newly hatched Macrobrachium larvae were fed, daily, with 12 Artemia nauplii per ml till day 15. From day 16 (stage VI), they were fed exclusively with Frippak microencapsulated diets scheduled as recommendated (table 13.0, appendix):

Larval stage	Size of microcapsule (μm)
VI - IX	150
X - XII	250

The artificial diets were prepared fresh every day by adding Frippak dried microcapsules to distilled water at 4, 8, 16, 32 and 64mg per litre. Survival and M.L.S. were recorded daily. Number of days to reach post-larvae was also recorded. Ammonia, nitrite and pH were evaluated every 2 days using the methods previously described.

Statistical analysis was conducted using a one-way ANOVA (Zar, 1984) to determine significant differences between survival and larval growth (final stage reached). In addition, Tukey's and Scheffe's multiple pairwise comparison procedures were used to identify differences between individual treatment means.

RESULTS

First trial: recently hatched larvae

Tables 2, 14.0, 14.1 and 14.2 (appendix) present the survival and the final stage reached by recently hatched M. rosenbergii larvae fed on Frippak microencapsulated diets, starved larvae and larvae fed on Artemia. At day 14, control (Artemia fed larvae) produced 71% survival and reached stage VII, whilst total mortality occurred at day 12 and 14 for starved larvae and microcapsule fed larvae, respectively. Figure 1 visualizes these survivals.

A one-way ANOVA analysis reveals significant differences between the three treatments ($F_{survival} = 25.433^{**}$; $D.F.num = 2$; $D.F.denom = 123$; $\alpha = 0.01$ and F final stage $= 124^{**}$; $D.F.num = 2$; $D.F.denom = 6$; $\alpha = 0.01$). Scheffe's multiple pairwise comparisons are presented in table 3 for survival and final stage of M. rosenbergii larvae, and

TABLE 2. Survival (%) at 29oC of recently hatched *Macrobrachium rosenbergii* larvae fed on Frippak microencapsulated diets versus starved larvae and larvae fed on *Artemia*

Trial	Larvae #	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12	D13	D14
Starved larvae	100	100 (1.00) (a)	98.7 (2.00)	96.3 (2.00)	85.7 (2.00)	74.3 (2.00)	62.0 (2.00)	42.0 (2.00)	23.7 (2.00)	7.7 (2.33)	1.3 (3.00)	0.3 (3.00)	0.0	0.0	0.0
Artemia (b)	100	100 (1.00)	99.3 (2.00)	98.3 (2.00)	97.7 (2.67)	97.0 (3.00)	95.7 (3.67)	93.0 (4.33)	92.0 (5.00)	89.0 (5.67)	87.0 (6.00)	84.7 (6.00)	77.7 (6.67)	73.3 (7.00)	71.3 (7.00)
Caps. fed larvae (c)	100	100 (1.00)	95.7 (2.00)	91.7 (2.00)	84.7 (2.00)	77.7 (2.00)	73.3 (2.00)	64.3 (2.33)	47.3 (2.67)	34.7 (2.67)	28.3 (3.00)	8.3 (3.00)	3.3 (4.00)	1.7 (4.00)	0.0

(a) average stage
(b) 12 *Artemia* per ml; 553 + 71 cells of *Chlorella vulgaris* (Beijer) per µl; pH = 7.76 + 0.39
(c) 0-30µm capsules

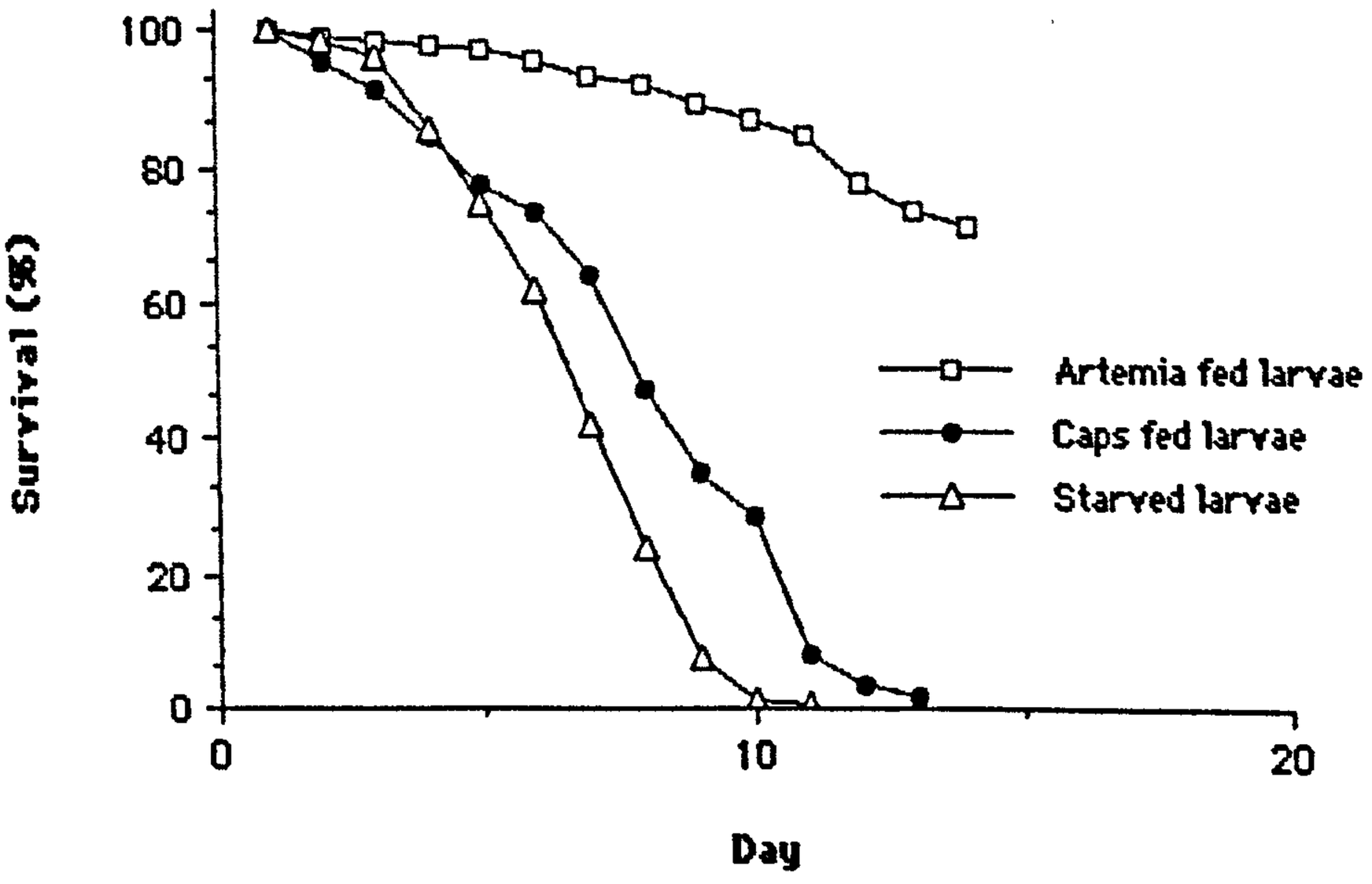


Figure 1. Survival of recent hatched M. rosenbergii larvae when starved or fed Artemia/microcapsules

TABLE 3. Scheffe's multiple pairwise comparisons for survival (a) and growth (final stage reached) (b) of recent hatched M.rosenbergii larvae when starved or fed Artemia/microcapsules.

(a)

	Artemia	Microcapsules	Starved Artemia
Artemia	-		
Microcapsules	**	-	
Starved Artemia	**	NS	-

(b)

	Artemia	Microcapsules	Starved Artemia
Artemia	-		
Microcapsules	**	-	
Starved Artemia	**	NS	-

** Highly significant at $\alpha = 0.01$
NS Not significant at $\alpha = 0.05$

reveal significant differences between the control (Artemia fed larvae) and starved larvae or microcapsule fed larvae. No differences are observed between survival and final stage of starved larvae and microcapsule fed larvae.

Although death occurred at day 14, M. rosenbergii larvae accepted and ingested the artificial diets, as the gut was full of microcapsules and faecal pellets were produced.

Second trial: from day 16

Tables 4 & 5 present the survival and the final stage of M. rosenbergii larvae when they were reared from day 16 on several densities of Frippak microcapsules. In experiment 1, control (Artemia fed larvae) showed 92% survival at day 30 and reached the post-larval stage, whilst 4, 8 & 16mg of microcapsules per litre gave 52, 81 & 80% survival with larvae reaching average stage 9, 10.67 & 10.67, respectively. In experiment 2, control gave 89% survival and reached length stage 11.33, whilst 8, 16, 32 & 64mg of microcapsules per litre give 84, 79, 60 & 46% survival and reached stage 11, 10.67, 10.33 & 10, respectively. Figures 2a & b visualize survival in experiments 1 & 2, respectively.

TABLE 4. Survival of Macrobrachium rosenbergii larvae when reared at 29oC from day 16 on several Frippak microcapsule densities

Experiment #	Day #	Control (1)				4 mg.l-1				8 mg.l-1				16 mg.l-1				32 mg.l-1				64 mg.l-1			
		1	2	3	X	1	2	3	X	1	2	3	X	1	2	3	X	1	2	3	X	1	2	3	X
1	16	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100								
	18	99	99	100	99	96	97	90	94	100	98	96	98	97	100	98	98								
	20	99	99	100	99	95	91	84	90	100	95	96	97	94	96	97	95								
	22	98	98	97	97	95	88	69	84	98	94	92	94	93	83	94	90								
	24	97	97	94	96	87	87	61	78	96	92	87	91	91	78	93	87								
	26	97	96	93	95	70	50	50	56	93	92	80	88	91	77	90	86								
	28	97	92	90	93	65	49	48	54	90	79	79	82	88	76	86	83								
	30	96	91	89	92	62	48	47	52	89	76	78	81	82	76	82	80								
2	16	100	100	100	100					100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
	18	100	98	100	99					99	100	98	99	100	98	100	99	100	99	98	99	98	100	99	99
	20	100	98	96	98					99	93	98	96	99	95	86	96	86	93	76	85	80	88	80	82
	22	99	98	98	98					90	89	98	92	97	94	84	91	84	89	68	80	64	76	58	66
	24	98	98	95	97					87	89	96	90	90	93	80	87	78	83	58	73	52	69	49	56
	26	97	98	95	96					87	87	94	89	89	90	74	84	74	80	50	68	49	65	44	52
	28	93	93	91	92					86	85	88	86	89	89	74	84	69	74	45	62	48	57	43	49
	30	90	91	88	89					84	81	87	84	86	78	73	79	67	70	43	60	47	52	40	46

(1) 12 Artemia.ml-1

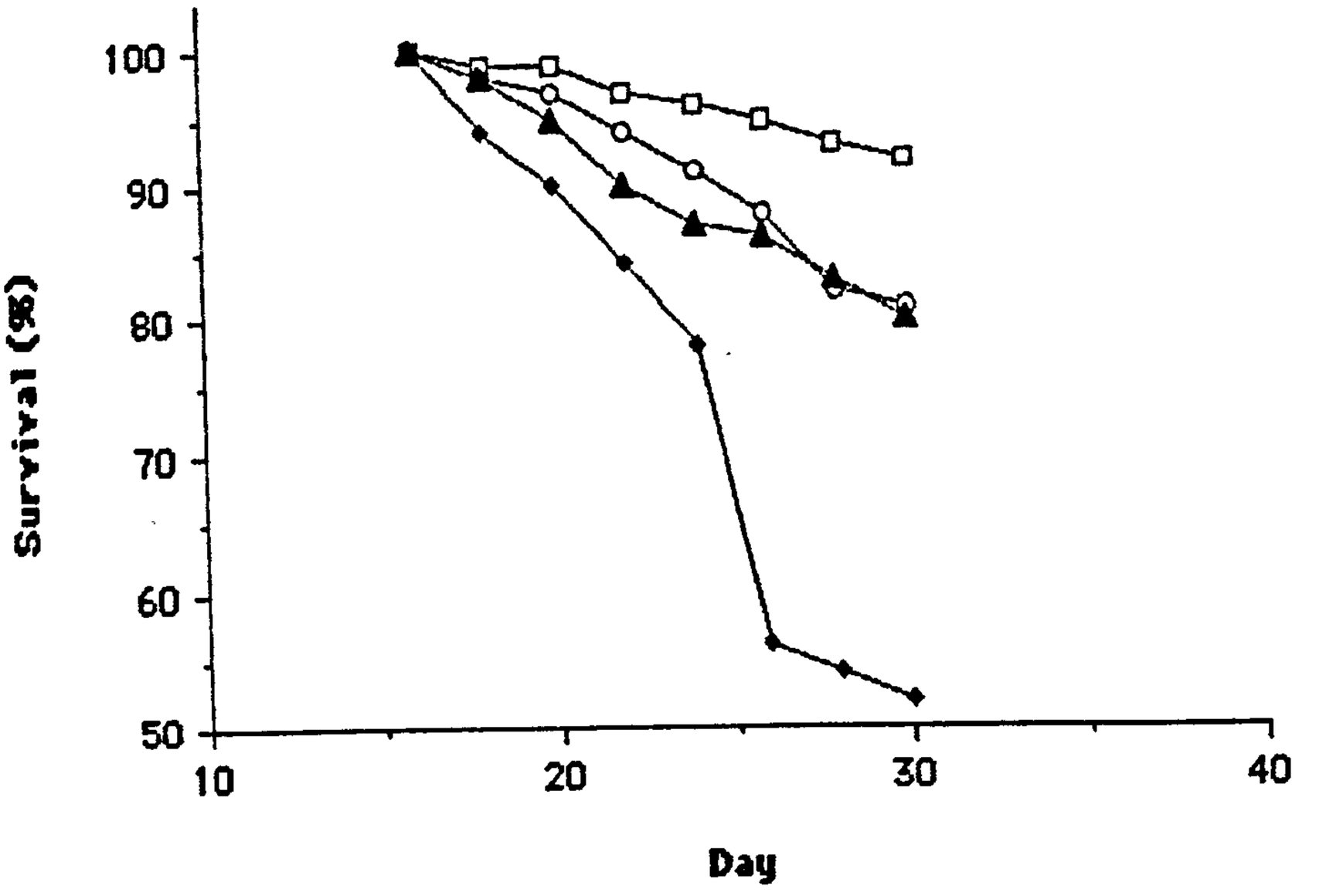
TABLE 5. Stage of Macrobrachium rosenbergii larvae when reared at 29 oC from day 16 on several Frippak microcapsule densities

Experiment #	Day #	Control (1)				4 mg.l-1				8 mg.l-1				16 mg.l-1				32 mg.l-1				64 mg.l-1			
		1	2	3	N	1	2	3	N	1	2	3	N	1	2	3	N	1	2	3	N	1	2	3	N
1	16	6	6	6	6.00	6	6	6	6.00	6	6	6	6.00	6	6	6	6.00								
	18	7	7	7	7.00	6	6	6	6.00	6	6	7	6.33	7	6	7	6.67								
	20	8	8	8	8.00	7	7	6	6.67	7	6	6	6.33	8	7	7	7.33								
	22	9	9	9	9.00	7	7	7	7.00	7	7	7	7.00	8	8	8	8.00								
	24	10	10	10	10.00	8	8	7	7.67	8	7	8	7.67	9	9	9	9.00								
	26	11	11	11	11.00	8	8	8	8.00	9	9	9	9.00	10	9	9	9.33								
	28	12	11	12	11.67	8	9	8	8.33	10	9	10	9.67	11	10	10	10.33								
	30	12	12	12	12.00 (2)	9	9	9	9.00	11	10	11	10.67	11	10	11	10.67								
2	16	6	6	6	6.00	6	6	6	6.00	6	6	6	6.00	6	6	6	6.00	6	6	6	6.00	6	6	6	6.00
	18	7	7	7	7.00	7	7	7	7.00	7	7	7	7.00	7	7	7	7.00	7	7	7	7.00	7	6	7	6.67
	20	8	8	8	8.00	8	7	8	7.67	7	7	7	7.00	7	7	7	7.00	8	7	7	7.33				
	22	9	9	9	9.00	8	8	8	8.00	8	8	8	8.00	7	7	8	7.33	8	8	8	8.00				
	24	9	9	10	9.33	9	9	9	9.00	8	9	9	8.67	8	8	9	8.33	9	9	9	9.00				
	26	10	10	10	10.00	10	10	9	9.67	9	9	9	9.00	8	9	9	8.67	9	9	9	9.00				
	28	10	11	11	10.67	11	11	10	10.67	10	9	10	9.67	9	9	10	9.33	10	10	10	10.00				
	30	11	11	12	11.33	11	11	11	11.00	11	10	11	10.67	10	10	11	10.33	10	10	10	10.00				

(1) 12 Artemia.ml-1

(2) 12 = Postlarval stage 1

a.



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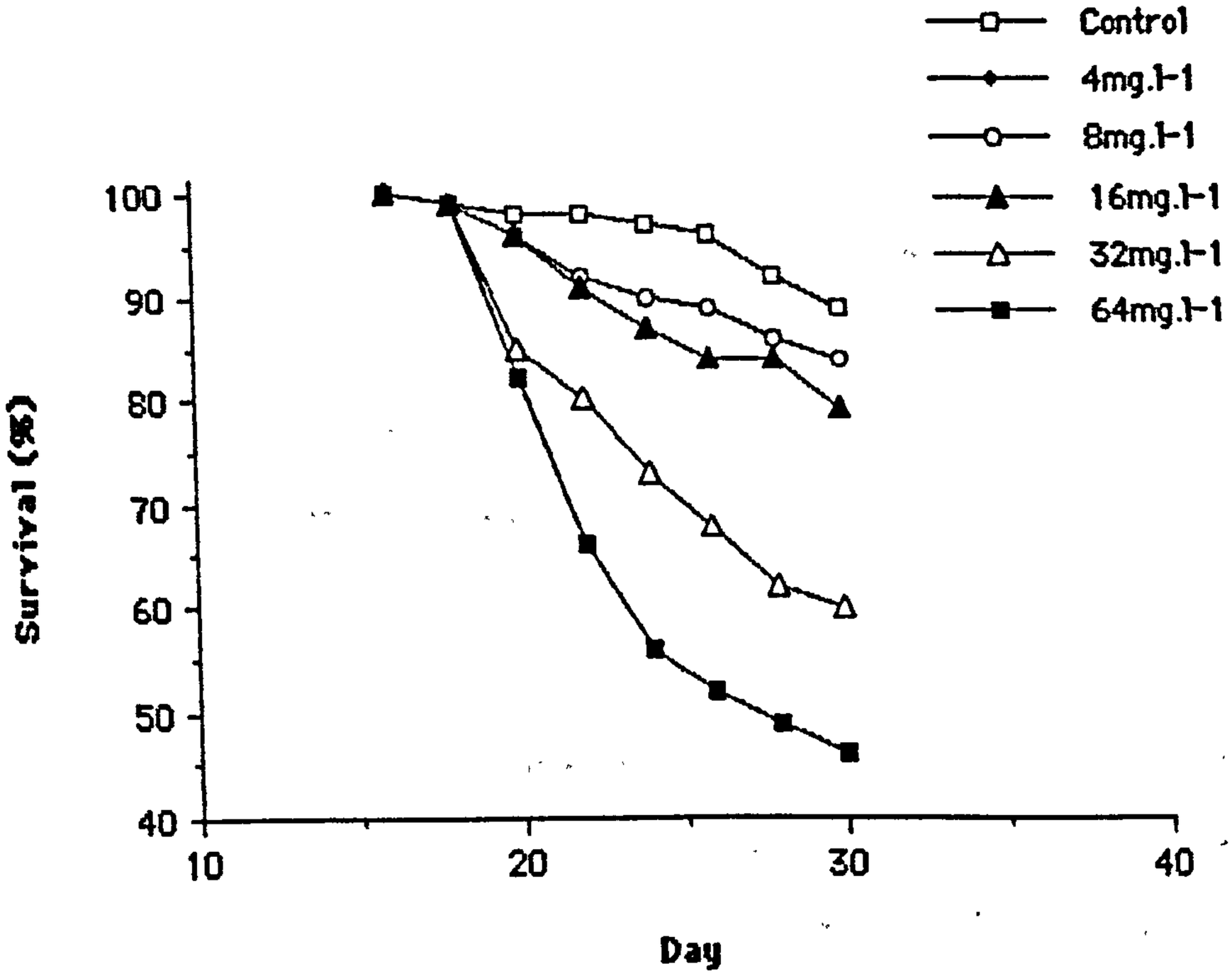


Figure 2. Survival of M. rosenbergii larvae fed on microcapsules from day 16; (a) experiment 1, (b) experiment 2

A one-way ANOVA analysis shows significant differences between all treatments:

experiment 1:

$$F_{survival} = 23.741^{**}$$

(D.F.num = 3; D.F. denom = 8; α = 0.01)

$$F_{final\ stage} = 27.167^{**}$$

(D.F.num = 3; D.F. denom = 8; α = 0.01)

experiment 2:

$$F_{survival} = 15.836^{**}$$

(D.F.num = 4; D.F. denom = 10; α = 0.01)

$$F_{final\ stage} = 4.167^{*}$$

(D.F.num = 4; D.F. denom = 10; α = 0.05)

Tukey's multiple pairwise comparisons are presented in tables 6 & 7 for survival and final stage of M. rosenbergii larvae in experiments 1 & 2, respectively. In both experiments, no difference is found between survivals of larvae fed 8 & 16mg of microcapsules per litre. The control groups do not differ from 8 & 16mg treatments in experiments 1 & 2, respectively. However, a significant difference is found in experiment 1 between the control and 16mg treatment, and between 4 & 8mg treatments. A highly significant difference is detected between control and 4mg

TABLE 6. Tukey's multiple pairwise comparisons for final survival *M. rosenbergii* larvae when fed *Artemia* (control) or microcapsules at different concentrations from D16 (a. Experiment 1; b. Experiment 2)

(a)

	Control	4mg.l^{-1}	8mg.l^{-1}	16mg.l^{-1}
Control	-			
4mg.l^{-1}	**	-		
8mg.l^{-1}	NS	*	-	
16mg.l^{-1}	*	**	NS	-

(b)

	Control	8mg.l^{-1}	16mg.l^{-1}	32mg.l^{-1}	64mg.l^{-1}
Control	-				
8mg.l^{-1}	*	-			
16mg.l^{-1}	NS	NS	-		
32mg.l^{-1}	*	NS	NS	-	
64mg.l^{-1}	**	**	**	NS	-

** Highly significant at $\alpha = 0.01$
* Significant at $\alpha = 0.05$
NS Not significant at $\alpha = 0.05$

TABLE 7. Tukey's multiple pairwise comparisons for final stage of *M.rosenbergii* larvae when fed *Artemia* (control) or microcapsules at different concentrations from D16 (a. Experiment 1; b. Experiment 2)

(a)

	Control	4mg.l^{-1}	8mg.l^{-1}	16mg.l^{-1}
Control	-			
4mg.l^{-1}	**	-		
8mg.l^{-1}	*	**	-	
16mg.l^{-1}	*	**	NS	-

(b)

	Control	8mg.l^{-1}	16mg.l^{-1}	32mg.l^{-1}	64mg.l^{-1}
Control	-				
8mg.l^{-1}	NS	-			
16mg.l^{-1}	NS	NS	-		
32mg.l^{-1}	NS	NS	NS	-	
64mg.l^{-1}	*	*	NS	NS	-

** Highly significant at $\alpha = 0.01$
* Significant at $\alpha = 0.05$
NS Not significant at $\alpha = 0.05$

treatment, and between 4 & 16mg treatments. Experiment 2 reveals differences between control and the highest concentrations of microcapsules (significant with 32mg, and highly significant with 64mg).. No difference is found between these highest concentrations.

From both experiments, results are less clear as far as final stage is concerned. No difference again occurs between 8 & 16mg treatments, but 4 & 64mg treatments give slower growth when compared to control.

As with earlier experiments, M. rosenbergii larvae accept and ingest the artificial diet, but apparently more efficiently, as they reached an advanced stage in their development. The best performance was obtained on 8 & 16mg of microcapsules per litre, as by day 30 average stage reached was 10.67 & 11, respectively.

Tables 8, 9, 10, 11 and 12 present the concentrations of brackish/fresh- & seawater pH, total ammonia (NH_4-N), un-ionized ammonia (NH_3-N) and nitrite (NO_2-N), respectively, in the rearing water. Figures 3 and 4 represent these concentrations in relation to larval development M. rosenbergii in experiments 1 & 2, respectively.

TABLE 8. Water pH during *Macrobrachium rosenbergii* larval rearing (1) when fed from day 16 on several Frippak microcapsule densities

Experiment #	Day #	Control (2)				4 mg.l-1				8 mg.l-1				16 mg.l-1				32 mg.l-1				64 mg.l-1			
		1	2	3	X	1	2	3	X	1	2	3	X	1	2	3	X	1	2	3	X	1	2	3	X
1	16	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-								
	18	7.53	7.90	7.80	7.68	7.92	8.03	8.00	7.98	7.92	7.86	7.90	7.89	7.84	7.85	7.87	7.85								
	20	6.49	7.21	7.43	7.04	7.62	7.60	7.54	7.59	7.53	7.54	7.58	7.55	7.43	7.49	7.50	7.47								
	22	7.25	7.42	7.44	7.37	7.60	7.53	7.53	7.55	7.55	7.59	7.57	7.57	7.60	7.61	7.60	7.60								
	24	7.30	7.42	7.32	7.35	7.58	7.56	7.57	7.57	7.53	7.59	7.58	7.57	7.48	7.57	7.52	7.52								
	26	7.48	7.40	7.30	7.39	7.49	7.49	7.48	7.49	7.48	7.52	7.63	7.34	7.33	7.44	7.39	7.39								
	28	7.48	7.38	7.31	7.39	7.46	7.59	7.49	7.52	7.51	7.50	7.58	7.53	7.35	7.45	7.41	7.40								
	30	7.47	7.40	7.33	7.40	7.61	7.45	7.48	7.51	7.47	7.52	7.50	7.50	7.37	7.48	7.42	7.42								
	X				7.37				7.60				7.59				7.52								
2	16	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	18	7.56	7.59	7.53	7.56	7.83	7.71	7.92	7.82	8.04	7.98	8.02	8.01	8.00	7.90	7.91	7.94	7.62	7.56	7.76	7.65				
	20	7.24	7.20	7.24	7.23	7.24	7.27	7.33	7.28	7.27	7.34	7.28	7.30	7.42	7.40	7.41	7.41	7.05	7.07	7.08	7.07				
	22	7.42	7.43	7.33	7.37	7.54	7.51	7.61	7.55	7.59	7.62	7.62	7.61	7.36	7.30	7.27	7.31	7.17	7.12	7.15	7.15				
	24	7.40	7.42	7.28	7.35	7.38	7.50	7.62	7.50	7.48	7.53	7.54	7.52	7.49	7.40	7.43	7.43	7.10	7.22	7.16	7.16				
	26	7.20	7.22	7.21	7.21	7.37	7.52	7.34	7.41	7.28	7.29	7.31	7.28	7.31	7.20	7.21	7.24	7.08	7.13	7.08	7.10				
	28	7.10	7.04	7.07	7.07	7.26	7.32	7.47	7.35	7.26	7.33	7.33	7.31	7.24	7.15	7.14	7.18	7.05	7.12	7.14	7.10				
	30	7.39	7.39	7.38	7.39	7.45	7.43	7.32	7.40	7.38	7.45	7.47	7.42	7.38	7.33	7.37	7.36	7.17	7.18	7.18	7.18				
	X				7.31				7.47				7.49				7.41				7.20				

(1) At 29oC

(2) 12 *Artemia* per ml

TABLE 9. Fresh- and seawater pH during Macrobrachium rosenbergii larval rearing (1) when fed from day 16 on several Frippak microcapsule densities

	Freshwater		Seawater	
Temperature (oC)	Rainfall pH	Tap pH	Salinity (%.)	pH
4.5	7.00	7.54	32.0	7.09
8.0	7.03	8.07	32.5	7.22
12.0	7.29	9.10	33.0	7.63
13.0	7.11	7.37	33.2	7.68
12.5	6.30	8.06	32.0	7.67
12.0	6.84	8.85	32.0	7.82
11.0	7.45	7.82	32.0	7.72
10.0.	7.52	9.34	32.0	7.82
9:0	7.47	9.51	32.1	7.50
8.5	8.38	8.58	32.3	7.68
X	7.24	8.42	32.3	7.58
S.E.	0.54	0.76	0.45	0.25
min.	6.30	7.37	32.0	7.09
max.	8.38	9.51	33.2	7.82

(1) At 29oC

TABLE 10. Water total ammonia concentrations (in mg NH4-N.l-1) during Macrobrachium rosenbergii larval rearing (1) when fed from day 16 on several Frippak microcapsule densities

Experiment #	Day #	Control (2) 1	2	3	X	4 mg.l-1 1	2	3	X	8 mg.l-1 1	2	3	X	16 mg.l-1 1	2	3	X	32 mg.l-1 1	2	3	X	64 mg.l-1 1	2	3	X
1	16	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-								
	18	0.85	0.80	0.25	0.63	0.33	0.41	0.50	0.41	0.31	0.29	0.30	0.30	0.85	0.84	0.76	0.82								
	20	0.91	1.25	0.94	1.03	0.56	0.59	0.54	0.56	0.30	0.38	0.37	0.35	1.10	1.20	1.30	1.20								
	22	0.54	0.51	0.40	0.48	0.55	0.49	0.53	0.52	0.23	0.31	0.31	0.28	1.20	1.05	1.10	1.12								
	24	0.94	0.79	0.75	0.83	0.54	0.51	0.52	0.52	0.27	0.27	0.32	0.29	1.00	0.84	0.94	0.93								
	26	0.69	1.10	0.94	0.91	0.44	0.46	0.49	0.46	0.31	0.33	0.28	0.31	0.85	0.86	0.91	0.87								
	28	0.75	0.89	0.91	0.85	0.40	0.47	0.49	0.45	0.32	0.33	0.25	0.30	0.93	0.97	0.99	0.96								
	30	0.68	0.46	0.39	0.51	0.38	0.51	0.49	0.46	0.34	0.35	0.32	0.34	1.00	1.05	0.76	0.94								
	X				0.75				0.48				0.31				0.98								
2	16	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	18	0.99	0.95	0.60	0.85	0.87	0.65	1.15	0.89	1.25	1.20	1.20	1.22	1.90	2.00	1.85	1.92	3.40	3.60	3.50	3.50				
	20	0.96	0.85	0.48	0.76	0.85	0.82	0.55	0.74	0.80	0.84	0.76	0.80	1.80	1.50	1.70	1.67	2.50	2.30	2.80	2.53				
	22	0.96	1.30	1.45	1.24	0.90	1.09	1.10	1.03	0.75	0.80	0.96	0.84	1.60	1.50	1.80	1.63	2.40	2.30	2.60	2.43				
	24	1.25	1.10	0.35	0.90	0.92	1.12	1.02	1.02	0.70	0.70	0.77	0.72	1.40	1.30	1.30	1.33	2.20	1.90	2.20	2.10				
	26	1.35	0.58	0.25	0.73	0.96	0.98	0.95	0.96	0.81	0.90	0.88	0.86	1.50	1.50	1.60	1.53	2.80	2.80	2.70	2.77				
	28	1.40	0.80	0.49	0.90	0.91	0.96	0.88	0.92	0.93	1.00	0.99	0.94	1.60	1.50	1.60	1.57	2.60	2.60	2.70	2.73				
	30	0.69	0.25	0.36	0.43	1.00	1.05	1.10	1.05	1.10	1.15	1.15	1.13	1.70	1.90	1.80	1.80	2.40	2.60	2.60	2.53				
	X				0.83				0.94				0.93				1.64				2.64				

(1) At 29oC

(2) 12 Artemia per ml

TABLE 11. Water un-ionized ammonia concentrations (in µg NH3-N.l-1) during Macrobrachium rosenbergii larval rearing (1) when fed from day 16 on several Frippak microcapsule densities

Experiment #	Day #	Control (2)				4 mg.l-1				8 mg.l-1				16 mg.l-1				32 mg.l-1				64 mg.l-1			
		1	2	3	X	1	2	3	X	1	2	3	X	1	2	3	X	1	2	3	X	1	2	3	X
1	16	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-								
	18	21	52	14	29	21	31	37	30	20	19	19	19	46	54	49	50								
	20	2	16	20	13	19	21	10	17	7	9	13	10	24	30	32	29								
	22	10	11	9	10	19	12	13	15	8	10	11	10	42	36	38	39								
	24	17	17	14	16	19	18	18	18	7	9	11	9	25	29	23	26								
	26	17	24	17	19	11	11	12	11	8	8	10	9	15	18	20	18								
	28	18	19	16	18	10	16	5	10	8	8	9	8	20	24	21	22								
	30	17	10	7	11	13	18	12	14	8	9	8	8	21	26	16	21								
	X				17				16				10				29								
2	16	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	18	34	33	15	27	48	29	74	50	93	90	90	91	142	129	120	130	118	125	191	145				
	20	14	13	7	11	13	15	10	13	14	15	14	14	39	32	36	36	28	26	32	32				
	22	21	28	26	25	22	27	38	29	26	28	33	29	34	27	33	31	35	26	38	33				
	24	27	24	6	19	20	28	35	28	17	17	19	18	35	28	28	30	25	28	32	28				
	26	20	9	4	11	21	24	17	21	15	16	16	16	27	22	24	24	32	32	31	32				
	28	16	6	6	9	16	17	22	18	17	18	18	18	24	22	18	21	30	30	31	30				
	30	15	5	8	9	25	23	20	23	24	29	29	27	36	34	39	36	35	38	38	37				
	X				16				26				30				44				46				

(1) At 29oC

(2) 12 Artemia per ml

TABLE 12. Water nitrite concentrations (in µg NO2-N.l-1) during *Macrobrachium rosenbergii* larval rearing (1) when fed from day 16 on several Frippak microcapsule densities

Experiment #	Day #	Control (2) 1	2	3	X	4 mg.l-1 1	2	3	X	8 mg.l-1 1	2	3	X	16 mg.l-1 1	2	3	X	32 mg.l-1 1	2	3	X	64 mg.l-1 1	2	3	X
1	16	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-								
	18	30	50	580	220	60	90	100	90	460	120	150	240	110	100	130	110								
	20	20	40	720	260	50	40	60	50	440	60	10	170	60	120	110	100								
	22	50	60	930	350	30	20	10	20	290	40	10	110	30	100	140	90								
	24	20	50	770	280	80	30	60	60	220	50	30	110	80	160	120	120								
	26	30	140	125	470	50	40	50	50	220	60	10	100	60	150	90	100								
	28	20	120	100	80	40	90	110	80	130	50	30	70	70	170	100	110								
	30	100	130	100	110	60	80	50	60	270	40	70	130	80	120	110	100								
	X				253				59				133				104								
2	16	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	18	550	190	450	400	20	200	190	140	140	240	210	200	200	210	260	220	80	50	160	100				
	20	80	390	450	310	250	60	210	170	150	310	220	230	220	180	270	220	100	110	60	90				
	22	100	400	400	300	250	80	220	180	250	230	250	240	200	120	140	150	120	130	170	140				
	24	270	500	460	410	370	90	340	270	110	130	190	140	230	60	70	120	80	100	50	80				
	26	500	530	510	510	430	130	430	330	120	100	40	90	200	100	90	130	40	40	60	50				
	28	420	600	480	500	140	190	260	200	90	70	80	80	60	50	80	60	30	40	110	60				
	30	700	580	620	630	500	220	210	310	120	100	90	100	250	50	60	120	30	30	90	50				
	X				437				229				154				146				81				

(1) At 29oC

(2) 12 *Artemia* per Ml

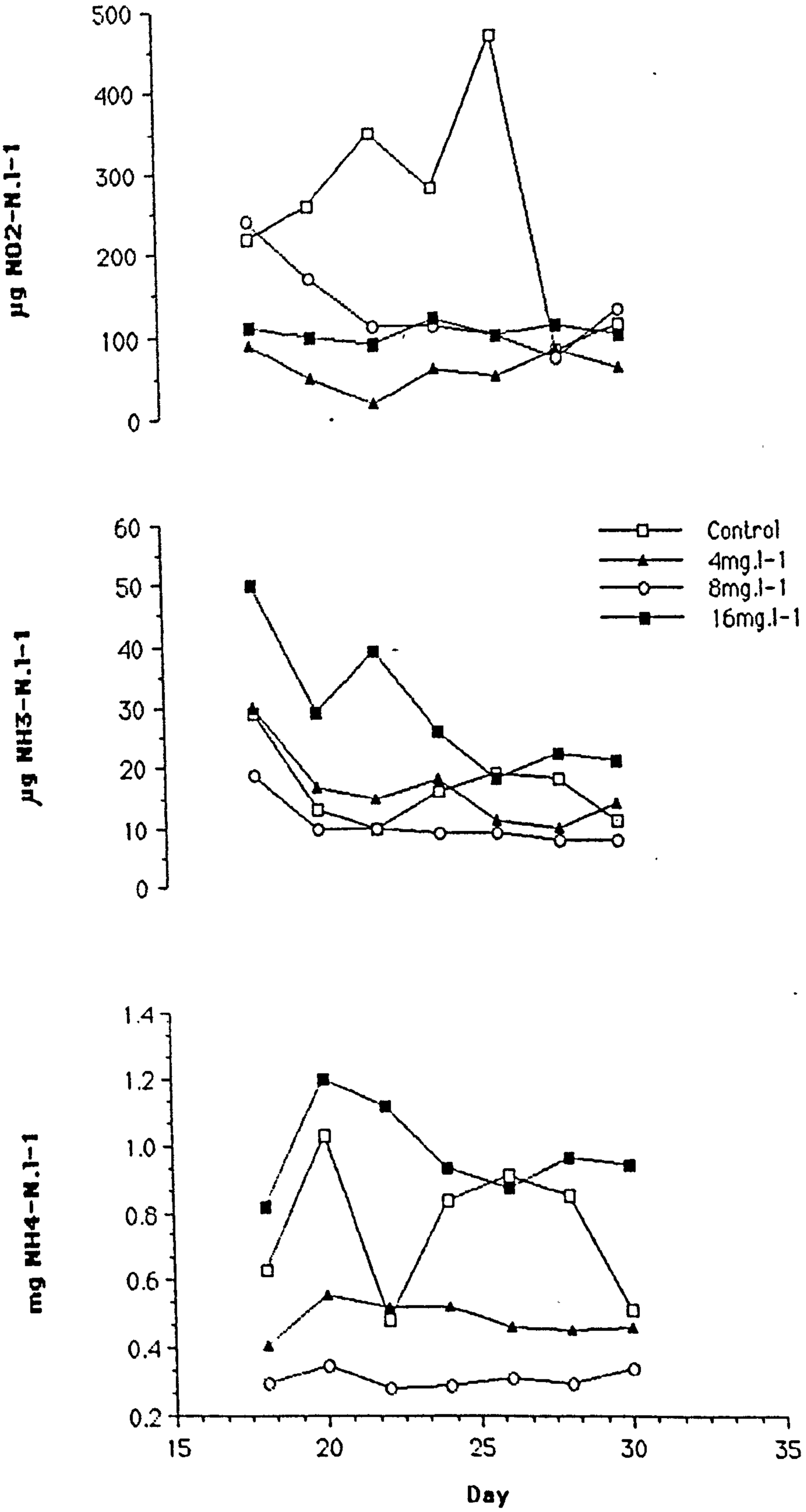


Figure 3. Total ammonia (NH4-N), un-ionized ammonia (NH3-N) and nitrite (NO2-N) of the rearing water in M. rosenbergii experiment 1

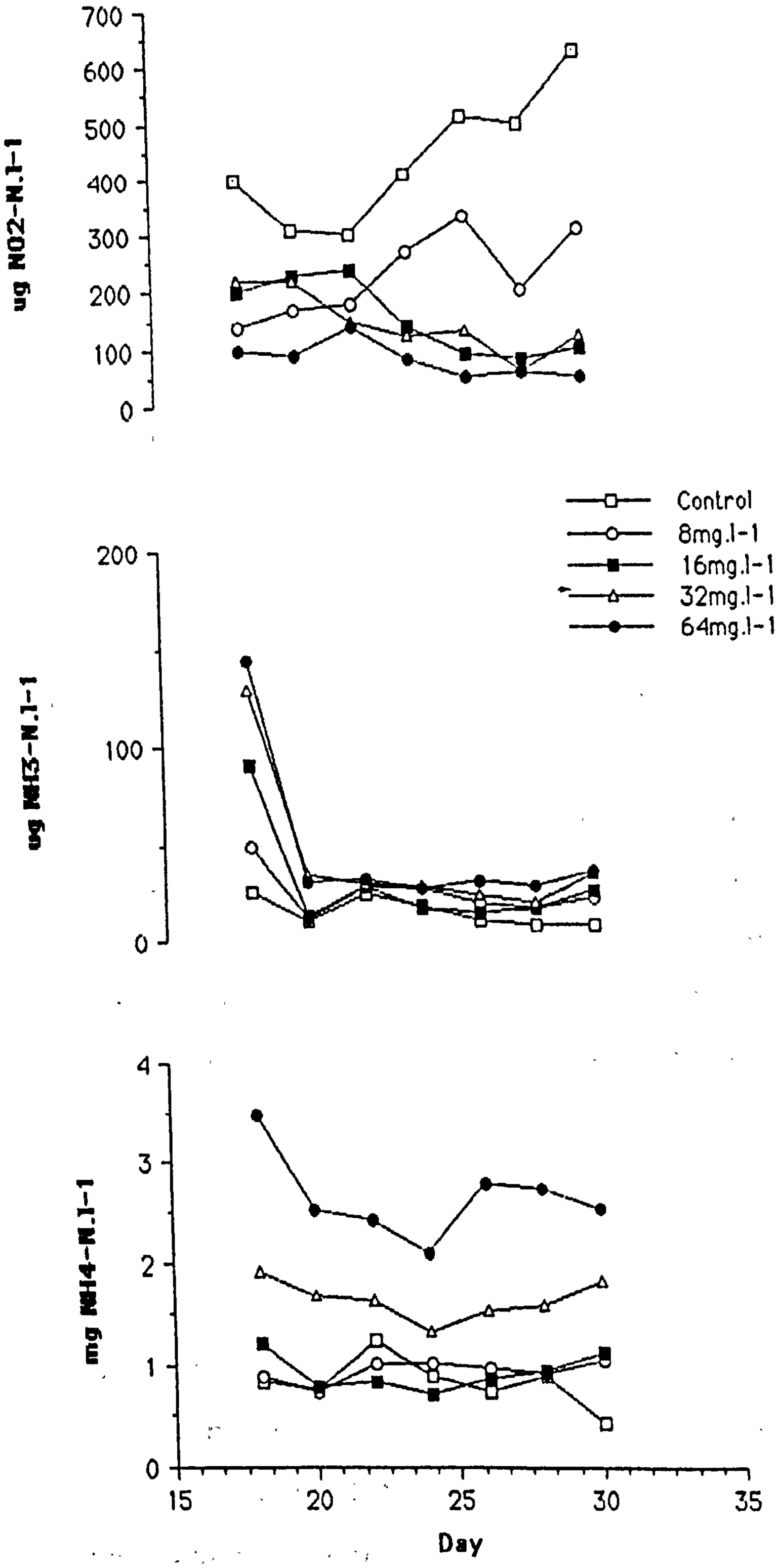


Figure 4. Total ammonia (NH4-N), un-ionized ammonia (NH3-N) and nitrite (NO2-N) of the rearing water in M. rosenbergii experiment 2

DISCUSSION

Prawn larvae at present are routinely reared on live feeds, but there is a consistent inability to duplicate results throughout the larval season (Cook & Murphy, 1969; Mock et al., 1980). M. rosenbergii have shown 47-70% survival to the post-larval metamorphosis when larvae were fed on Artemia in " green water " (Fujimura, 1974; Lu et al., 1976; Perez, 1976; Malecha, 1983), 60-80% survival on Artemia in " clear water " (AQUACOP, 1977, 1983), and even 88% survival on a combination of steamed egg custard, rotifers, Artemia and chopped flesh of fish, shrimp or clam roe in " green " and " clear " waters for early and later larval stages, respectively (Chao & Liao, 1977). Despite these problems, hatcheries are dependent on live feeds due to the lack of suitable alternatives. Unprotected artificial diets have been used rather unsuccessfully to replace live feeds. M. rosenbergii gave only 11% survival to post-larval metamorphosis when larvae were fed on freeze dried catfish

(Sick & Beaty, 1975) and less than 2% when fed on freeze dried oyster and trout feed (Murai & Andrews, 1978).

Present work shows that recently hatched larvae of M. rosenbergii consume protected artificial (Frippak) microcapsules and produce faecal pellets, but they do not survive beyond day 13, and that this survival is not statistically different from the survival of starved larvae. Similar conclusions were also reached by Jones et al. (1975) who found that M. rosenbergii larvae fed on encapsulated diets only survived to stage IV. Later, Brewster (1987) also failed to rear larvae on protected artificial diets. These results suggest that there is a problem related with the diets or the gut when larvae are in their early stages.

However, 16 day-old M. rosenbergii larvae fed sole microcapsules develop to further stages and reach, in some cases, post-larval metamorphosis for the first time.

The experiments demonstrate that artificial diets can be given to M. rosenbergii larvae when they passed through their first critical period or crisis of mortality (at stages VI-VII). Results also indicate that the best growth on Frippak diets occurs at a feeding rate of 8 to 16mg of microcapsules per litre, suggesting that 12mg could be a recommended average concentration.

A feeding level of 4mg of microcapsules per litre gave low total ammonia (NH_4-N), un-ionized ammonia (NH_3-N) and

nitrite (NO_2-N) in the rearing water (figure 3), but was probably too low in concentration to be easily seized by the larvae making them underfed. Feeding levels of 32 and 64mg visibly foiled the water. Total ammonia, un-ionized ammonia and nitrite reached concentrations as high as 1.92 and 3.5mg per litre, 130 and 145µg per litre, and 220 and 140µg per litre, respectively (tables 10, 11 & 12). Slow growth and death may have resulted as pH of the rearing water were the highest amongst all treatments (table 8) promoting diffusion of toxic NH_3 into the larvae (Wickins, 1976; Armstrong et al., 1976, 1978) (figure 4). Nitrite concentrations were the lowest with these feeding levels.

Clearly, the present encapsulated diet can sustain M. rosenbergii during later larval stages providing good growth which is similar to that of larvae fed on live Artemia. Thus this diet, initially designed for penaeid larvae, is consumed efficiently by M. rosenbergii during its later larval cycle, and therefore must be suitable for freshwater caridean larvae, requiring perhaps only slight changes in its composition to meet the specific requirements of M. rosenbergii.

However, despite ingestion (revealed by the production of faecal pellets), microcapsules are not used efficiently early in the larval development as survival and growth are poor. As the artificial diet is nutritionally adequate to

provide good growth in later stages and the microcapsules are eaten by early stage larvae, the problem must be either that not enough capsules are consumed to secure the energy required for maintenance and growth of M. rosenbergii larvae, or the capsules are not sufficiently digestible. During the present work, it was observed that the gut of recent hatched larvae was always fully filled with capsules. So, it is possible that either the diet does not contain sufficient energy or the larvae can not digest them and are thus physiologically starved during early larval stages. This may be because the gut in the early stages is undeveloped. In an attempt to discover why artificial diets fail to support larval development, an examination of the gut development in M. rosenbergii larvae during each stage was undertaken so that comparison could be made between early and later stages, and between M. rosenbergii and penaeid larvae which successfully grow on these diets.

Section IV

Edge index derived from the measurement of the cutting edges of the mandible for *Macrobrachium rosenbergii* (de Man) larvae

INTRODUCTION

Amongst the zooplankton, methods of feeding may be broadly divided into " filter " feeding, which uses different mechanisms to induce a flow of water, and " raptorial " feeding, in which individual prey is seized (Parsons & Takahashi, 1975). These two processes are not mutually exclusive and examples of both feeding types can be found in the same species, especially amongst the planktonic crustaceans.

The position of the planktonic crustaceans, as by far the largest group of suspension feeders, together with their ability to consume a great variety of prey, warrants special consideration of feeding habits of these animals in the food web. The details of feeding appendages vary considerably and their general form and use include means of inducing water currents, filtering out organisms, seizing preys, and cutting or grinding food particles (Parsons & Takahashi,

1975). The second antennae, mandibles and first maxillae and maxillipeds are well developed in " herbivorous " filter feeders, increasing the surface area by setae assuring their efficient use in producing water currents (Anraku & Omori, 1963). In contrast, in " predatory " species, appendages have few setae and, instead, there are modifications in structure which aid the use of these appendages for seizing and holding a prey. Anraku and Omori (1963) also noted differences in the cutting edges of mandibles in different copepods and describe a typical herbivore as having grinding teeth while those of a typical predator have very sharp teeth. Between these two extremes, there are a variety of structures which enable some copepods to be " omnivorous ".

From examination of stomach contents of these animals, it is sometimes possible to identify raptorial feeders, but the inclusion of detritus and diatom fragments often makes it difficult to diagnose whether an animal is exclusively carnivorous. Difference between herbivores, omnivores and carnivores was assessed by Itoh (1970) on the basis of an " Edge Index " derived from measurements of the cutting edges of the mandible for numerous planktonic crustaceans.

The objective of the present investigation was to see whether, at each developmental stage, the edge index of the

larvae of the caridean Macrobrachium rosenbergii (de Man) and penaeidean Penaeus monodon (Fabricius), reflects their feeding habits.

MATERIALS AND METHODS

Macrobrachium rosenbergii (de Man) larvae were obtained from berried females reared in the tropical unit, Marine Science Laboratories, Menai Bridge. Larvae were reared in two litre flat-bottomed flasks at a density of 50 per litre and subject to diurnal illumination. A thermostat and electrical heater were used to maintain the temperature of the water at 29°C. Salinity was brought to 12 ‰ and checked with a refractometer. Water was previously cartridge filtered to 0.2µm and irradiated with U.V. light to reduce bacterial contamination. The cultures were gently aerated with airline attached to a Pasteur pipette. Chlorella vulgaris (Beijer) cultured in the algae unit, Marine Science Laboratories, was added at 500 cells per µl to the medium in which newly hatched larvae were reared to reduce the effect of toxic un-ionized ammonia (NH_3-N) concentration. Larvae were fed with 15 artemia per ml.

Ten larvae were taken at each larval stage and the mandible

extracted under a binocular microscope. Number of edges, individual cutting edges, total width and edge height of mandibles were recorded according to Itoh's method (1970).

Number of edges, individual cutting edges, total width and edge height of mandibles in Penaeus monodon (Fabricius) larvae were taken by direct measurements from published figures (Silas et al., 1978).

Calculations were computed using the following formula:

$$\text{Edge Index (or E.I.)} = \text{Sum}(w_i . W^{-1} . h_i . H^{-1} . 10^4)/N$$

where w_i = width of individual cutting edges

W = total width of the mandible

h_i = individual edge height

H = difference between the basis of the lower edge and the crest of the larger edge

N = edge number

Edge indexes were compared to Itoh's classification (1970) and regression analysis were carried out on number of edges versus edge index to observe eventual relationships.

RESULTS

Tables 1 and 2 gave the number of edges, and the widths and heights of the cutting edges of the mandible for M. rosenbergii and P. monodon at each larval stage. Figures 1a and b represent this edge index in relation to the edge number for M. rosenbergii and P. monodon, respectively. Table 3 presents the edge index corresponding to each larval stage for both decapods. An analysis of regression was conducted on edge index related to edge number for all larval stages, and gave for:

Macrobrachium rosenbergii

$$\hat{Y} = 250.38\ X - 359.1$$

$$r = 0.96^{**} \quad (D.F. = 10;\ \alpha = 0.01)$$

Penaeus monodon

$$\hat{Y} = 142.13\ X - 612.05$$

$$r = 0.80^{*} \quad (D.F. = 5;\ \alpha = 0.05)$$

TABLE 1. Edge index derived from the measurement of the cutting edges of the mandible for Macrobrachium rosenbergii at each larval stage

	Widths and Heights of cutting edges (1)																			
	Individual																Total			
Stage	W1	H1	W2	H2	W3	H3	W4	H4	W5	H5	W6	H6	W7	H7	W8	H8	W	H	Edge Number (N)	Edge Index (E.I.) (2)
I	2.7	1.6	2.9	1.2	2.8	0.5											7.8	4.6	4	641
II	2.3	2.6	4.5	2.3	2.9	1.7	4.1	1.7									10.5	6.5	5	827
III	4.0	2.7	2.5	2.1	1.9	1.8	2.1	1.9									9.6	5.5	5	809
IV	4.2	3.5	3.3	1.9	2.8	2.5	2.9	3.1	1.8	1.1							11.6	5.6	6	993
V	4.2	2.2	3.0	1.8	3.8	1.3	3.2	2.6	2.6	1.7							10.0	5.0	6	1077
VI	4.8	2.4	3.7	1.8	3.9	2.5	3.6	3.0	2.9	1.9							10.8	6.2	6	1101
VII	4.0	5.0	2.5	3.5	2.9	4.0	2.8	4.0	2.0	1.6							11.0	6.0	6	1383
VIII	3.2	4.5	3.5	4.6	3.2	5.3	2.8	6.4	3.7	4.8	2.6	2.9					12.9	7.2	7	1490
IX	4.7	5.9	4.9	3.5	4.2	5.8	5.6	6.1	3.9	5.1	3.8	2.7	3.8	4.1			17.0	6.3	8	1775
X	3.9	6.0	3.4	5.7	2.7	3.9	3.8	5.5	3.3	5.6	3.9	5.8	3.7	5.4	3.3	4.6	15.0	6.2	9	1798
XI	6.6	8.0	4.7	7.9	5.6	7.9	4.3	7.7	3.9	6.8	3.8	7.2	4.0	7.1	3.4	6.8	20.0	8.2	9	1847
PLI	4.6	7.6	14.3	8.8	5.9	4.2											18.3	9.0	4	2817

(1) 0.1mm = 14 units
(2) E.I. = $\mathrm{Sum}(Wi.Hi.10^4/W.H).N^{-1}$

TABLE 2. Edge index derived from the measurement of the cutting edges of the mandible for Penaeus monodon at each larval stage

	Widths and Heigths of cutting edges (1)																			
	Individual																Total		Edge Number (N)	Edge Index (E.I.) (2)
Stage	W1	H1	W2	H2	W3	H3	W4	H4	W5	H5	W6	H6	W7	H7	W8	H8	W	H		
NII	(3)																		0	-
NIV	(3)																		0	-
NVI	(3)																		0	-
PZI	1.4	0.5	2.5	1.1	2.8	1.2	1.8	0.7	1.4	0.5	1.5	0.6					9.7	6.2	7	223
PZII	3.3	0.7	4.5	0.9	2.3	0.6	2.9	0.6	1.7	0.9							13.3	0.9	6	260
PZIII	3.2	1.3	1.8	1.6	1.9	2.5	2.1	0.4	2.0	0.6	3.7	1.1					14.5	4.4	7	401
MI	3.0	2.5	1.8	1.4	2.2	2.3	1.8	2.4	2.8	3.5	2.1	0.5	2.6	0.4	4.0	2.1	20.0	3.8	9	580
MII	2.9	2.5	2.8	1.9	3.8	3.3	2.7	3.2	2.3	2.5	2.5	1.4	3.0	1.3	2.0	0.5	21.5	3.8	9	651
MIII	2.5	0.9	2.7	1.5	2.8	1.7	2.6	2.2	2.7	2.0	4.5	1.3	2.5	1.5			14.3	3.7	8	751
PLI	8.0	1.1															8.0	1.1	2	5000

(1) 0.1mm = 16.5 units
(2) E.I. = Sum (Wi.Hi.10^4/W.H).N^{-1}
(3) No cutting edges of the mandible, only setae

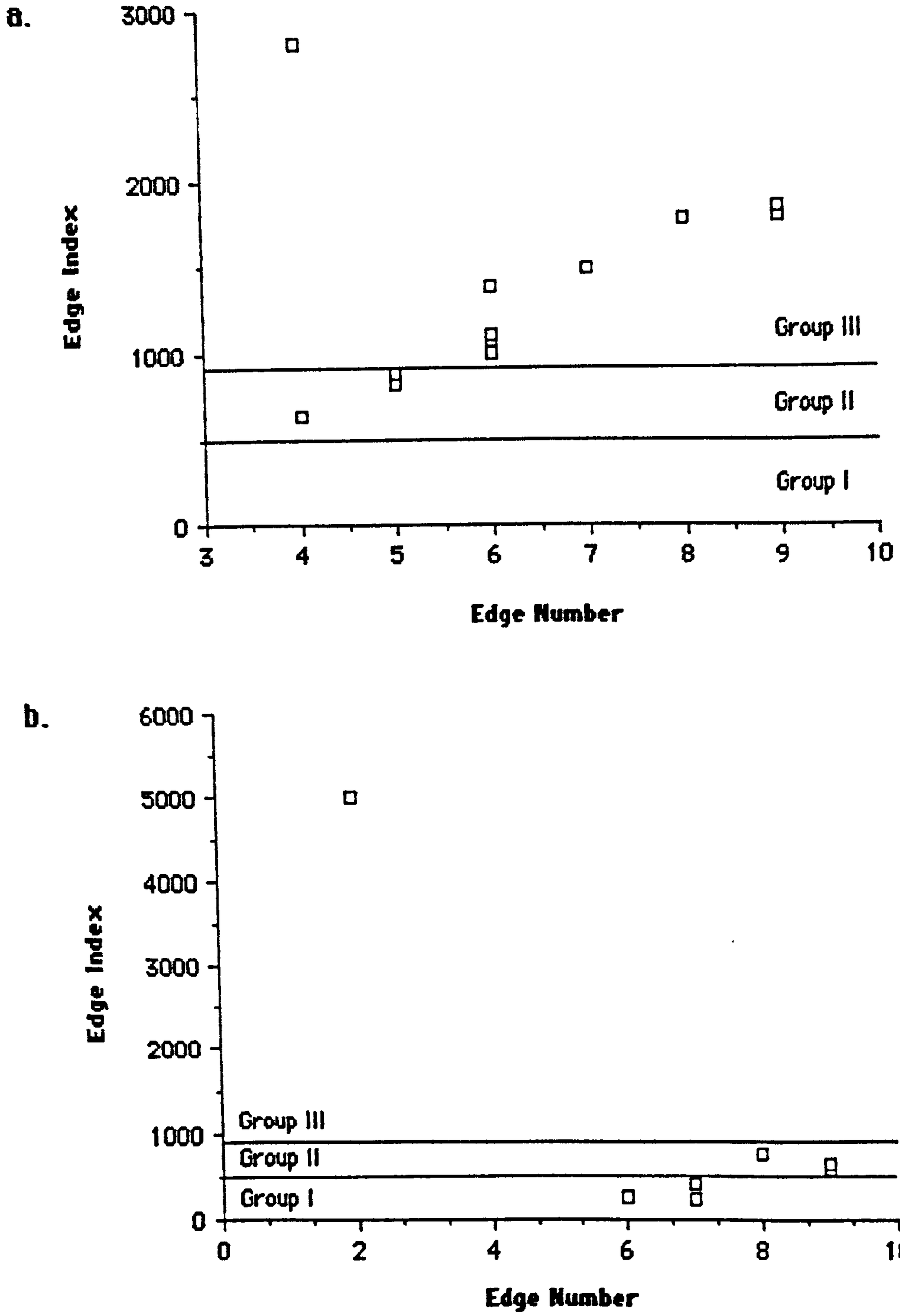


Figure 1. Edge number vs edge index for Macrobrachium rosenbergii (a) and Penaeus monodon (b)

TABLE 3. Edge index for *Macrobrachium rosenbergii* and *Penaeus monodon* at each larval stage

Macrobrachium rosenbergii		*Penaeus monodon*	
Stage	E.I. (1)	Stage	E.I. (1)
I	641	NII	0
II	827	NIV	0
III	889	NVI	0
IV	999	PZI	223
V	1077	PZII	260
VI	1101	PZIII	401
VII	1383	MI	580
VIII	1490	MII	651
IX	1775	MIII	751
X	1798	PLI	5000
XI	1847		
PLI	2817		

(1) E.I. $\leq$ 500 largely herbivorous filter feeder
500 < E.I. $\leq$ 900 generally omnivorous feeder
E.I. > 900 predominantly raptorial (carnivorous) feeder

DISCUSSION

The cutting edge of the mandible of Macrobrachium rosenbergii grows in width and height with larval development (table 1). Edge number also increases during the larval stages, however at postlarval metamorphosis (PLI), it is reduced to a few strong edges. Edge index increases from 641 at stage I to 1847 at stage XI, reaching 2817 at PLI.

Penaeus monodon larvae are none-feeding during nauplius stages. The mandible is functional at protozoeal stage I and grows progressively in width and height during larval development (table 2). As for M. rosenbergii larvae, the edge number of the P. monodon mandible also increases as the larva develops, but is reduced to only two strong edges at the postlarval metamorphosis. Edge index also increases from 223 at protozoeal stage I to 751 at mysis stage III, reaching 5000 at postlarval metamorphosis.

From Itoh's results (1970), pelagic copepods may be divided into three groups: group I, with E.I. ⩽ 500, includes families of zooplanktonic crustaceans which are largely " herbivorous " filter feeders; group II, with 500 < E.I. ⩽ 900, includes generally " omnivorous " feeder families; and group III, with E.I. > 900, includes families of predominantly " raptorial " feeders.

Table 3 shows that the edge index of the three first larval stages of M. rosenbergii fall in the second group of Itoh's classification (500 < E.I.= 641; 827; 889 < 900) which suggests an omnivorous regime. After the fourth larval moult, edge index was clearly in the third group consisting of predominantly raptorial feeders (900 < E.I.= 999-2817). These findings agree with several studies conducted on the feeding habits of the larvae (Ling, 1969a; Wickins, 1976; Malecha, 1978; New & Singholka, 1985) which recognize best development on animal prey such as Artemia and rotifers.

As far as P. monodon larvae are concerned, protozoeal stages show a clear tendency towards group I (E.I.= 223-401 < 500) the herbivorous filter feeders. Edge index of mysis stages falls in Itoh'second group (500 < E.I.= 580-751 < 900) suggesting an omnivorous regime. These feeding habits are clearly confirmed by the strategies used in penaeid hatcheries: algae are given to protozoea, and mysis are generally fed a mixture of algae and animal diets or an artificial diet meeting both requirements (Liao & Huang,

1970; Mock, 1974; Hanson & Goodwin, 1977; Jones, 1988). When postlarval metamorphosis occurs, edge index reaches group III (900 < E.I.= 5000) which indicates a more carnivorous diet. This is also confirmed in penaeid nurseries where mass live or frozen animals, such as Artemia, mysids, mussels, clams, shrimps and squid, are given to the postlarvae.

Thus, Itoh's classification appears to be applicable to the larval stages of caridean and penaeid prawns and may be used to select appropriate feeds for other crustacean larvae under culture.

Section V

The functional morphology during the development of the alimentary canal of *Macrobrachium rosenbergii* larvae

INTRODUCTION

Very little information can be found on the gut histology and its development in decapod larvae as most research has been undertaken on the adults. Jordan (1908, 1912) gave an interpretation of the pyloric foregut function in advanced crustaceans (Malacostraca). Yonge (1924) provided the first complete study of the foregut function of Nephrops norvegicus (Linnaeus 1758) Leach and described its mechanism of feeding, digestion and assimilation. Martin (1964) compared the anatomical and functional characters of Marinogammarus obtusatus foregut (or 'stomodaeum') to other Peracaridae. According to McLaughlin (1983), the simplest form of gastric mill is found in the penaeid shrimp and the most complex in the brachyuran crabs. Rigdon and Mensik (1976) examined the gastrointestinal tract of the brown shrimp Penaeus aztecus Ives histologically and admitted that muscles play an important role in digestion. They also described the hindgut (or 'proctodaeum') and the dorsal

pyloric caecae and suggested that the latter may serve as a " chamber " in the gastrointestinal tract in which particles of food leaving the pyloric stomach are retained for enzymatic action before entering the midgut (or 'mesenteron'). The hindgut caecum may serve a similar function as temporary storage for intestinal contents before their entrance into the hindgut. Jobling (1987), who looked to the influences of food particle size and dietary energy content on patterns of gastric evacuation in fish, noticed that the evacuation of food from the stomach is probably not a continuous smooth process, but may occur in a pulse-like (step-wise) fashion. He also noticed that an exponential function best described the evacuation of small, easily digested prey items, but a linear expression gave the best fit to data on the emptying of large food items. He also mentioned that the major factors likely to affect patterns of emptying are the differences in surface-to-volume ratios between large and small food items and the ease with which different foods can be broken down into fragments.

In adult decapods, both midgut gland and midgut caeca typically are present (Pillai, 1960; Dall, 1967; Bunt, 1968; Stanier et al., 1968; Barker and Gibson, 1977, 1978). The midgut gland, or hepatopancreas, is considered to be an essential organ in the metabolism for decapod crustaceans (Yufera, 1984). It is a bi- or trilobed gland and lies on

either side of the gut, opening ventrally into the gut at the foregut-midgut junction. Secondary ductules arise which further subdivide into a maze of blind-ending tubules. Stanier *et al.* (1968) studied the midgut gland of crab *Carcinus maenas* (Linnaeus 1757) Leach. They found that fat is a very constant feature, but amounts of glycogen and calcium show very wide variation. Glycogen concentration depends possibly on the state of nutrition of the crab and on the time of year, and may be also related to sex and stage of moult cycle (Baumberger & Dill, 1928; Renaud, 1949). The amount of calcium also depends on the stage of moult cycle and calcium and phosphorus content was highest just before the moult, when the Ca:P weight ratio was 1.5 (Robertson, 1937). Ong and Lake (1969) discussed the possible functions of the midgut diverticulum of calanoid copepod *Calanus helgolandicus* Claus, and concluded that it was fundamentally different from the midgut diverticulum of other arthropods due to the fact that its cells did not secrete enzymes, and its epithelial cells did not contain any globule or granules of stored material like fats or any mineral concretions like calcium. The investigations reported by Van Weel (1955) aim to explain the processes of secretion, digestion and resorption in the midgut gland of the tropical freshwater crab *Atya spinipes* Newport. His results showed that cells can perform totally different processes at the same time, such as secretion, resorption of

digested material, and breakdown of fat. In 1970, Van Weel showed the importance of enzymatic activities, such as proteases, lipases and carbohydrases, developed in the crustacean hepatopancreas as far as its digestion is concerned. He compared crustacean to vertebrate and noticed that pepsin was never produced in the former. He also pointed out that certain hormonal factors, such as neuroendocrines, sex and moult hormones, can and do affect the rate of secretion by the midgut gland and the composition of the secretion material. Using light- and electron microscopy, Vogt (1985) differentiated the role of R-, B-, F- and E-cells in the hepatopancreas of Penaeus monodon Fabricius. R-cells (from the German 'Restzellen') are responsible for the uptake of the nutrients. They also accumulate lipids, glycogen and other ions such as Cu, Zn, P and S. B-cells (or 'Blasenzellen') might produce the mucus which can be found on the microvilli border of all cell types. They produce and secrete most, if not all digestive enzymes. B-cells are concerned with the intracellular digestion. F-cells (or 'Fibrillenzellen') may be precursor of B-cells. E-cells (or 'Embryonalzellen') are embryonic cells. F-cells, E-cells are not concerned directly with the processes of digestion. Bunt (1968) examined the hepatopancreas of crayfish Procambarus clarkii Girard, and supported the absorptive role and the function of lipid storage attributed to R-cell. Al-Mohanna (et al., 1985; &

Nott, 1986, 1987) observed the activities of shrimp Penaeus semisulcatus hepatopancreas. He also noticed a smaller and less numerous kind of cell called M-'Midget' cells which, scattered throughout most of the length of tubule, always occur as isolated individuals in the basal part of the epithelium, and whose function could be the storage of some organic reserve. In a study on the hepatopancreas of shore crab Carcinus maenas (Linnaeus 1757) Leach, Hopkin and Nott (1980) recorded that numerous mature B-cells are extruded into the lumen of the hepatopancreas, isolated in a peritrophic membrane and transferred into the midgut to join the faecal column.

The term midgut caecum is used for the smaller dorsal protrusions of the midgut, both anterior and posterior may be present, often in addition to the midgut gland (McLaughlin, 1983). Smith (1978) recognized the diversity of form in the midgut caeca of decapods suggesting a diversity of function. He indicated the possibility that the posterior midgut caecum of brachyuran crabs could have high physiological activity. Young (1959) suggested that this organ may play a part in the osmotic balance in Penaeus setiferus Linnaeus. Midgut caecae are also regarded to play some role in digestion (Mykles, 1977; Smith, 1978; McLaughlin, 1983).

In 1884, Mocquard gave a short description of a lobster

larval gut and noticed the absence of stomacheal armature. He compared his observations with those on other crustaceans and observed a similar simplicity with Crangon, Palaemon and Alphea. Reddy (1935) described the gastric armature of stomatopod Squilla nepa (Latreille) larvae and was the first to explain the grinding function of its cardiac foregut. Later in 1938, he tried to explain digestion and absorption in the crab Paratelphusa (Oziotelphusa) hydrodromus Herbst by looking at its midgut, hepatopancreas and caecae. Regnault (1968) described the foregut of the caridea Hippolyte inermis Leach and followed its larval evolution. This author supplied evidence on the formation of the pyloric filter. He also observed the development of teeth in the cardiac foregut which forecasted the gastric mill. Le Roux (1971) observed the principal gut modifications in the anatomy of Palaemonetes varians Leach during its larval development and its metamorphosis. He pointed out that the disappearance of these teeth at the post-larval metamorphosis is in agreement with the theory generally admitted which considers that the relative simplicity of the adult caridean stomach reflects a regressive evolution. Khan (1976) studied the gut development of Penaeus merguiensis de Man, Macrobrachium rosenbergii de Man and Carcinus maenas during several larval stages. She investigated the possible relationship existing between the nature of the diet, the feeding mechanism and the functional morphology of the

alimentary canal. She mentioned that the protozoeal foregut of P. merguiensis , is simple and totally lacks the development of masticatory and straining mechanisms found in juveniles. This widely differing morphology of the foregut in larvae and juveniles strongly emphasized the differences in diet, initally the pelagic larva living upon phyto-plankton, later becoming an omnivorous scavenger. She also noticed that pre-zoeae and first zoeae in C. maenas lack the food-crushing apparatus and filter apparatus of the adult, but the ventral chamber of the pyloric foregut of the megalopa achieves a high degree of specialization, presenting a most efficient filtering apparatus.

During the early larval stages of Penaeus setiferus, Lovett and Felder (1989, 1990a, b, c) observed that the hepatopancreas consists of two single lateral caeca that extend on each side of the midgut and generally have a diameter greater than that of the midgut. Later the caeca differentiate into various distinct lobes and increase substantially in length. Abubakr (1987) also observed this change in size in Penaeus monodon. During the early postlarval development, the hepatopancreas is fully ramified into small diameter tubules, and by PL35, its development is largely restricted to an increase in size as the individual tubules elongate.

Little information could be gathered on the larval development of the midgut caeca as far as decapod

crustaceans are concerned. Khan (1976) reported the presence of an anterior midgut caecum appearing at the end of the larval stages of Macrobrachium rosenbergii. She also observed the large oblong tubular lobe of the anterior caecum and the uncoiled and pear-shaped posterior caecum of Carcinus maenas zoea. Abubakr (1987) noticed the accumulation of lipid droplets in the lobes of the anterior midgut caeca of Penaeus monodon protozoea. This organic reserve was regressive with hepatopancreas expansion and larval development. As far as the posterior midgut diverticulum (caecum) is concerned, it does not exist during the larval stages and first appears only two weeks after the postlarval metamorphosis in P. setiferus, and consists of a small outpocketing at the end of the midgut, just before the hindgut (Lovett & Felder, 1989). The adult form, a large, cauliflower-shaped structure, is attained three weeks later.

Present work examines the gut morphology and the changes through the development of M. rosenbergii larvae. It compares feeding mechanisms and modes to those of other carideans, penaeids and some reptantia. Size and volume of the hepatopancreas were measured at each stage and compared to its foregut. Embryonic food reserves (yolk) and presence of any midgut diverticulae were also observed. In order to observe the food movements, gastroevacuation times were

recorded and compared to that of other decapods. Finally, a functional interpretation of the M. rosenbergii larval gut is given and possible reasons for the poor acceptability and growth on artificial diets used during its larval cycle are discussed.

MATERIALS AND METHODS

Larvae of Macrobrachium rosenbergii, at each stage, were collected from the tropical unit, Marine Science Laboratories, Menai Bridge. To prevent hardening, they were stored in a Strassburger-Flemming solution (Pantin, 1964) (table 15.0, appendix). They were fixed, first, in brackish Bouin's fluid (Ratcliffe, 1983) (table 15.1, appendix) for 2 weeks and, then, in Davidson solution (Shaw & Battle, 1957) (table 15.2, appendix) for 1 week before further treatment. Excess of fixative was washed out twice with 70% 2-Ethoxyethanol. Decalcification was carried on using Kristensen fluid (Ratcliffe, 1983)(table 15.3, appendix) for another week.

LIGHT MICROSCOPY (LM)

Washing and dehydration were carried out in a graded series of ethanol dilutions, placing the larvae for 1h in 30%, 50%,

70%, 90% and 3 changes in absolute alcohol. Cedarwood oil was used overnight as clearing agent. Larvae were placed in 50:50 xylene:wax (Paramat) for 1h in a oven (57-58°C) with one change, and in molten wax for 1h with 3 changes. The embedded larvae were trimmed into the form of a truncated pyramid, fixed to a small wood holder which was fitted on the microtome, and sectioned with a sharp knife at 6µm. Sections were stained using a modified Heidenhain's azan technique (Pantin, 1964) (table 15.4, appendix) and examined with the light microscope.

Eight sections were drawn at each larval stage according to the following pattern:

Longitudinal section

Transverse sections:

- cardiac foregut
- junction cardiac/pyloric foregut
- pyloric foregut
- junction foregut/midgut
- begining of midgut
- end of midgut
- hindgut

SCANNING ELECTRON MICROSCOPY (SEM)

Newly metamorphosed M. rosenbergii post-larvae were fixed in

2.5% glutaraldehyde at $4^{o}C$ for 2 hours in 0.1M phosphate buffer at a pH of 7.2, washed with filtered brackish water (12%.) for 3h (3 changes), and post-fixed with 1% osmium tetroxide (OsO_4) in buffer for 1h at room temperature. They were washed for 1/2h in buffer (2 changes), rinsed with distilled water for 5 min. (3 changes) and dehydrated at room temperature in a series of increasingly concentrated ethanol solutions. They were transferred from absolute ethanol to acetone. After 30 min., larvae were frozen and fractured under liquid nitrogen (Quenching) and the fragments returned to acetone at room temperature. The tissue was critical-point-dried, mounted on aluminium stubs and coated with gold in a DC Sputter coater before examination in a Cambridge Stereoscan Mark 2a scanning electron microscope.

TRANSMISSION ELECTRON MICROSCOPY (TEM)

Preparation was the same as for SEM until final dehydration when larvae were transferred from absolute ethanol to propylene oxide. Then, they were embedded in Spurr's low-viscosity epoxy resin (Spurr, 1969). Sections of lum were cut on to water with an LKB Ultramicrotome III and picked up on uncoated copper grids. After staining with uranyl acetate and lead citrate (Reynolds, 1963; Venable and

Coggeshall, 1965). They were examined in a GEC-AEI Corinth 275 electron microscope.

For the depletion of embryonic food reserves, larvae were placed alive in watch glasses and observed at the first stage with a binocular microscope.

At each larval stage, length of the different sections of the gut was measured using the eye piece micrometer under high magnification.

The hepatopancreas volume was measured by squaching the animal under a cover slip and using the same technique. Length, width and depth of the hepatopancreas were also estimated on the stained longitudinal and transverse sections to confirm previous measures.

Larval gastroevacuation was recorded as followed:

Ingestion time was determined by feeding the larvae one item at a time to ensure all of it was ingested before another was given. The time required for ingestion was recorded. This process was continued until the larvae refused to take any more food. Ingestion was observed under a binocular

microscope and was found to be completed when the cardiac and pyloric stomachs were full.

Gastroevacuation time was assessed by timing the period taken before various areas of the gut, namely the fore-, mid- and hindgut, became empty.

For each larval stage, 10 larvae were evaluated and their average taken.

ABBREVIATIONS

A.	anus
Ab.	abdomen
A.C.F.	anterior cardiac fold
A.C.M.	anterior cuticular muscle
A.D.F.	antero-dorsal fold
A.G.	abdominal ganglion
A.G.M.	anterior gastric muscle
A.S.D.M.	antero-superior dilator muscle
A.V.	anterior valve
B-	B-(secretory) cell
Bac.	bacteria
B.C.	buccal cavity
B.L.	basal lamina
B.M.	basement membrane
C.	chitin
Ca.	carapace
C.F.	cardiac foregut
C.M.	circular muscle
C.T.	connective tissue
D.C.P.	dorsal chamber of the pyloric foregut
D.C.V.	dorsal cardiac valve
D.G.	digestive gland (hepatopancreas)
E.	epithelium
E-	E-(embryonic) cell
E.M.	extensor muscles
Ep.M.	epithelial mucosa
Ey.	eye
F-	F-(fibrillar) cell
F.A.	filter apparatus
F.M.	flexor muscles
Fs.	folds
G.	gills
G.B.	golgi body
H.	heart
Hae.	haemolymph
H.P.	hastate plate
I.A.A.	inferior abdominal artery
I.P.	incisor process
L.	lumen
Lb.	labium
L.C.R.	longitudinal chitinous ridges
L.F.	lateral fold
L.L.C.F.	lower lateral cardiac fold
L.L.C.R.	lower lateral pyloric fold
L.D.G.	lobes of the digestive gland
Li.	lipid droplets
L.M.	longitudinal muscle
L.P.R.	lateral pyloric ridge
L.R.	longitudinal ridge
Lg.S.	long setae
Ly.	lysosomal-like inclusion
M.	muscle
M.G.	midgut
M.H.J.	midgut hindgut junction
Mi.	mitochondria
M.L.	muscular layer
M.P.	molar process

M.P.R.	median pyloric ridge
Mv.	microvilli
N.	nucleus
N.E.	nuclear envelope
N.G.	nerve ganglia
Nu.	nucleolus
O.	oesophagus
O.A.	oral aperture
O.F.	oesophageal fold
P.	pereiopods
P.C.	posterior caecum
P.D.A.	posterior dorsal aorta
P.D.V.	pyloric dorsal valve
P.F.	pyloric foregut
P.G.M.	posterior gastric muscles
P.L.C.D.M.	postero-lateral cardiac dilator muscle
P.L.V.	pyloric lateral valve
P.V.V.	pyloric ventral valve
R.	chitinous rod borne on the crest of the median pyloric or inter-ampullary ridge
R-	R-(absorptive) cell
R.E.R.	rough endoplasmic reticulum
R.H.P.	rod-like posterior setae of the hastate plate
Ri.	ribosomes
R.M.F.	radiating muscle fibres
R.M.	rectum muscle
S.	setae
S.E.R.	smooth endoplasmic granules
Sm.S.	small setae
Sp.	spines
T.	telson
T.C.M.	transverse cardiac muscle
U.	uropod
U.L.C.F.	upper lateral cardiac fold
U.L.P.F.	upper lateral pyloric fold
V.	vacuole
V.C.	vacuolated cells
V.C.P.	ventral chamber ot the pyloric foregut
V.L.C.G.	ventro-lateral cardiac groove
V.P.D.M.	ventral pyloric dilator muscle
Y.R.	yolk reserve

RESULTS

1. Morphology and ontogenetic changes in the alimentary canal through the larval development

Figures 1, 2 and 3 present the general morphology of M. rosenbergii gut during its larval development. Recently hatched larvae (stage I) have an alimentary canal which resembles a simple pipe without distinct parts in the foregut, however it has a well structured form by the time of postlarval metamorphosis. At the beginning of the larval cycle, the digestive gland is a simple pair of caecae which extend for- and backwards and are full of embryonic lipid droplet reserves (figures 4-A, B, C & D; table 1). An antero-dorsal fold appears during stage VI, but remains underdeveloped. A posterior midgut caecum is present during the whole larval cycle, but consists of a short evagination.

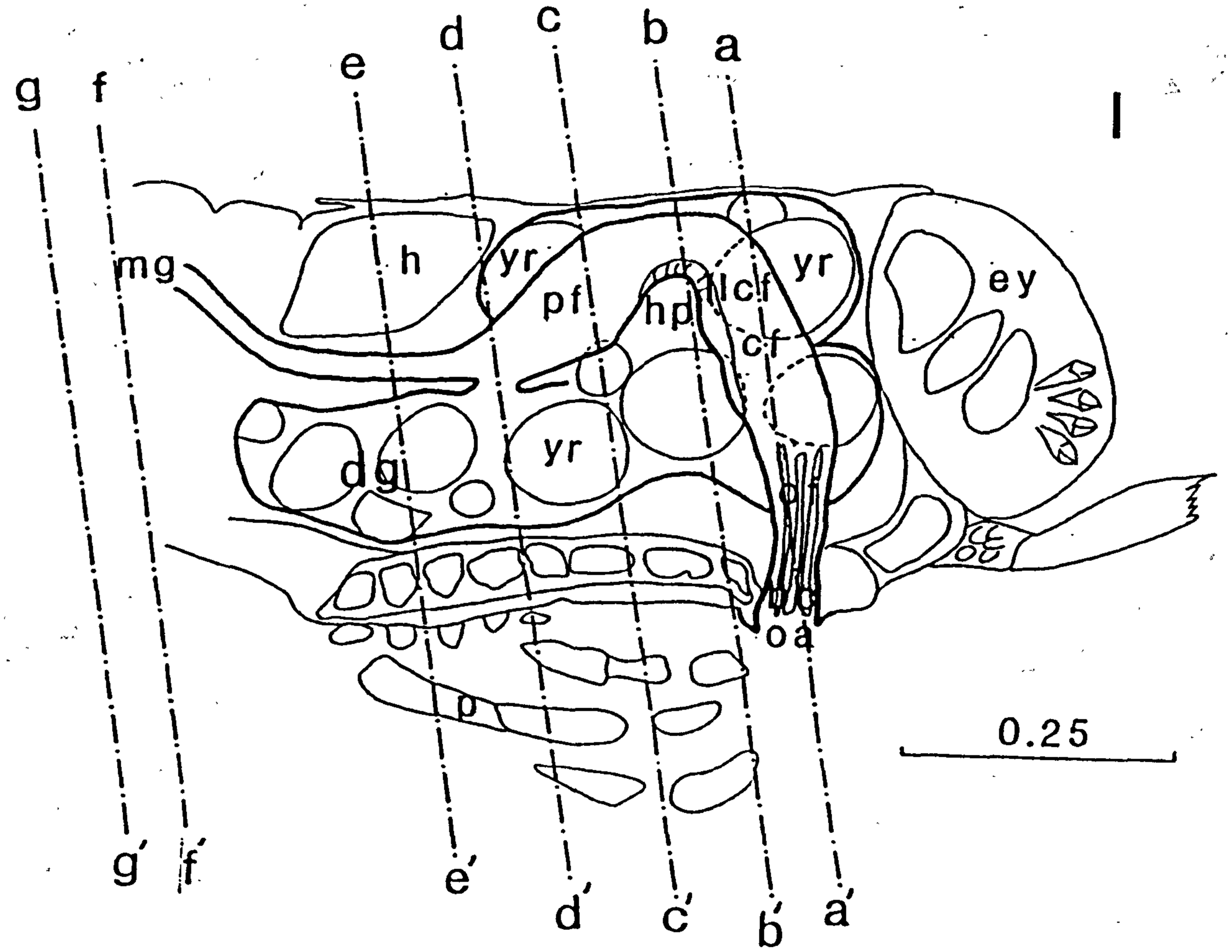


FIGURE 1. Schematic longitudinal section of M. rosenbergii alimentary canal during larval stage I (bar = mm)

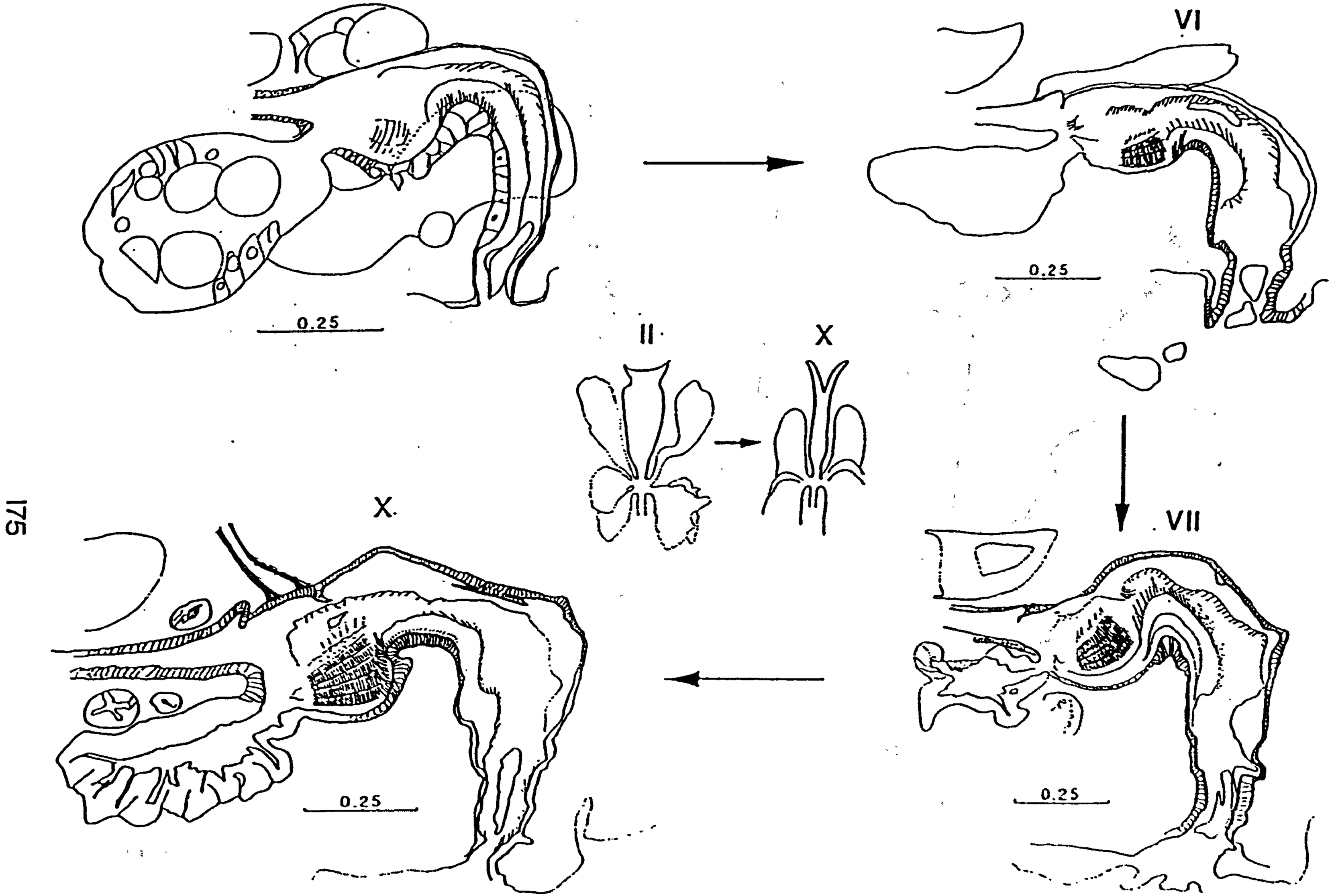


FIGURE 2. Schematic longitudinal section of M. rosenbergii alimentary canal during larval stages II, VI, VII and X (bar = mm)

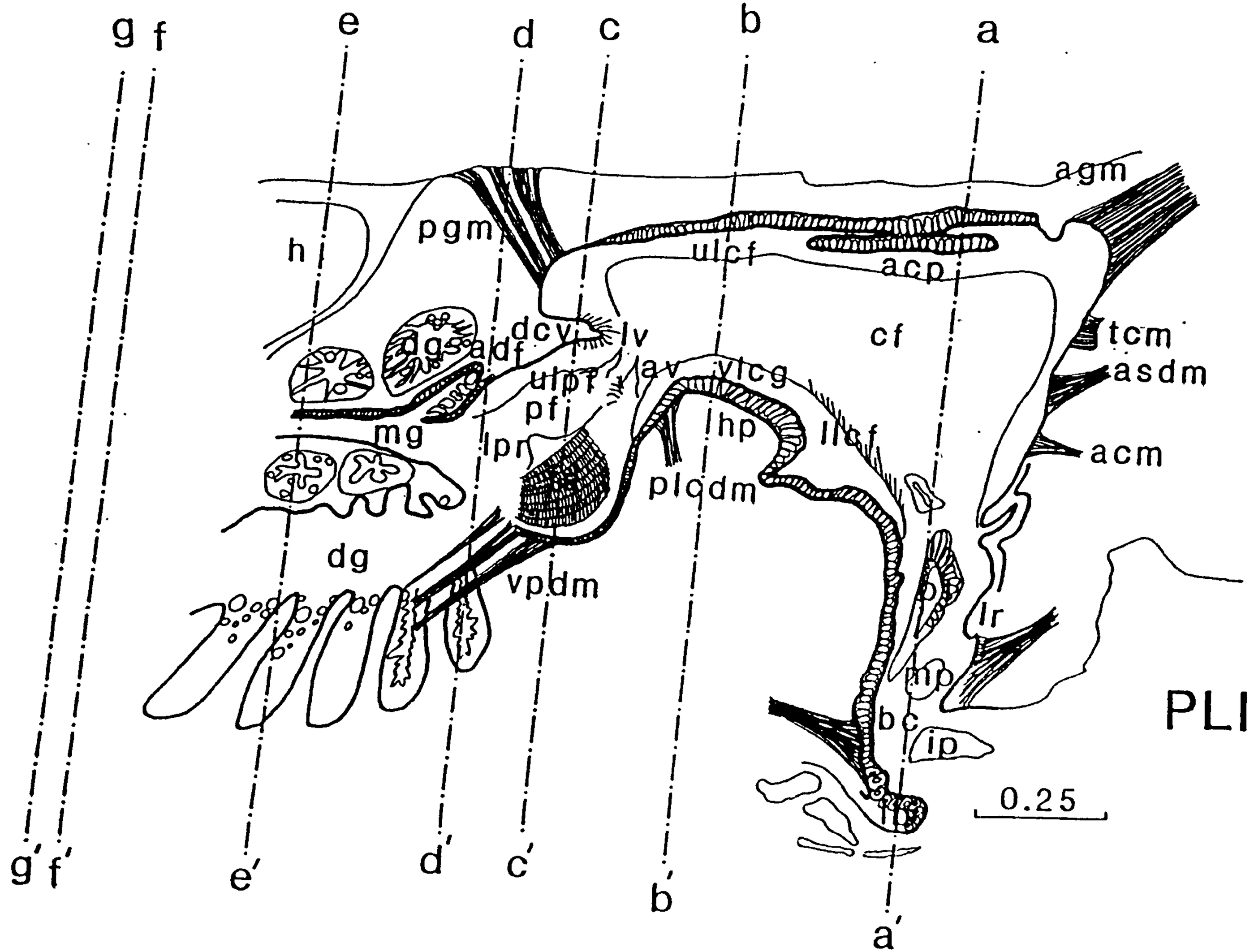
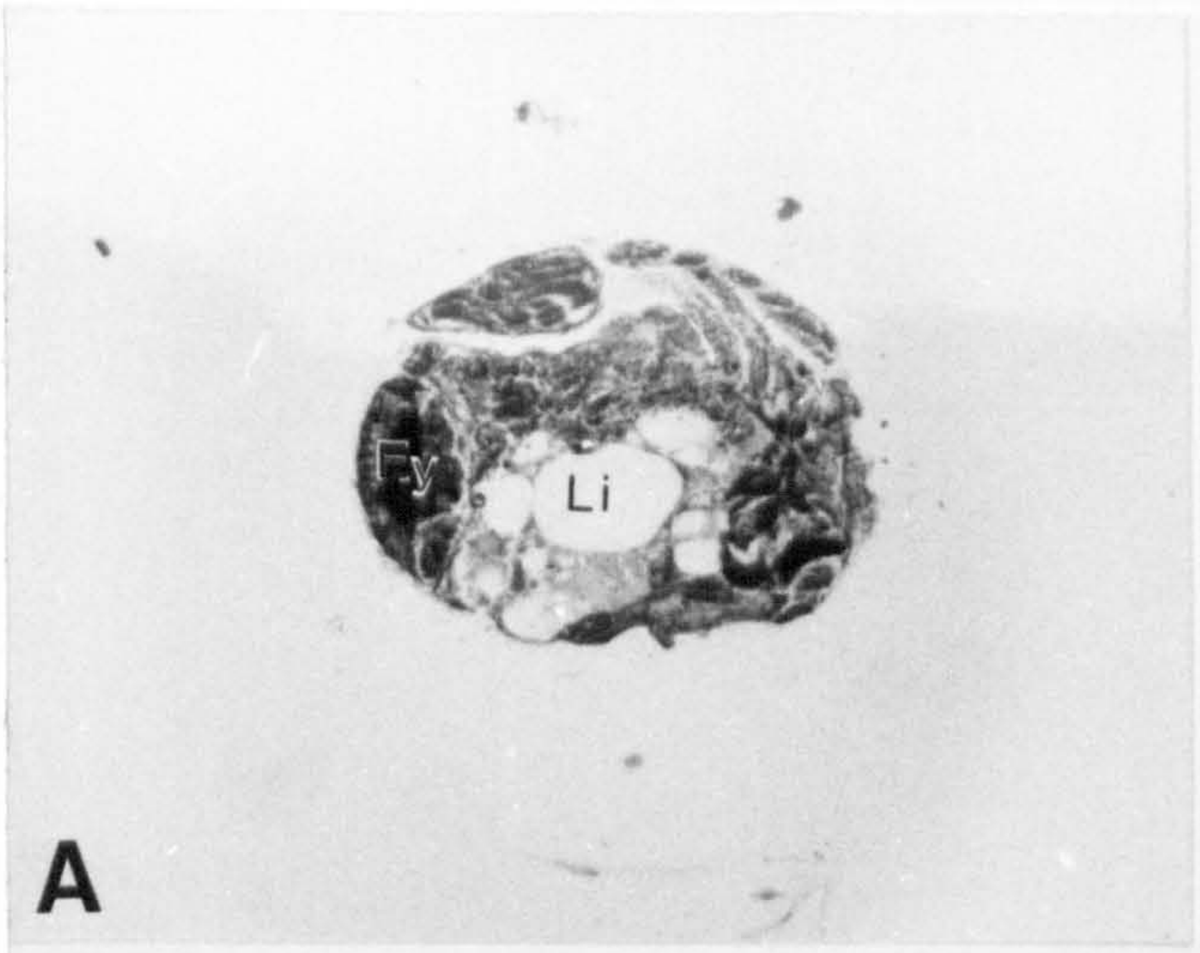


FIGURE 3. Schematic longitudinal section of M. rosenbergii alimentary canal at PLI (bar = mm)

FIGURE 4. Embryonic lipid droplet reserves in M. rosenbergii egg just before hatching (A)(X10) and larvae during stage I (B) longitudinal section (X10), (C) transverse section (fig.1-3, aa')(X40), (D) transverse section in the lipid reserves (TEM X1,500); (E) & (F) transverse section (fig.1-3, aa') showing the oesophagus and cardiac foregut of larvae during stages I (X20) and IV (X20), respectively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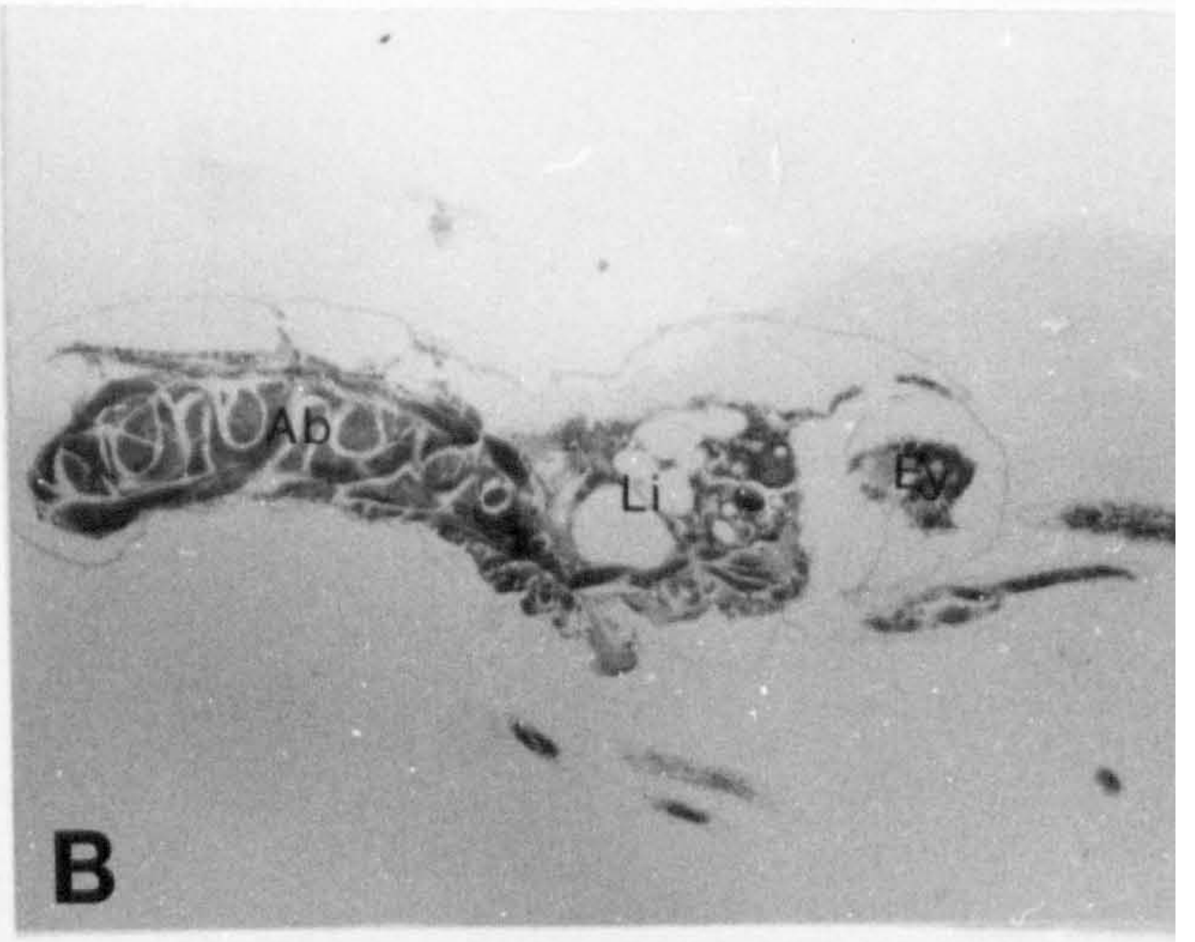
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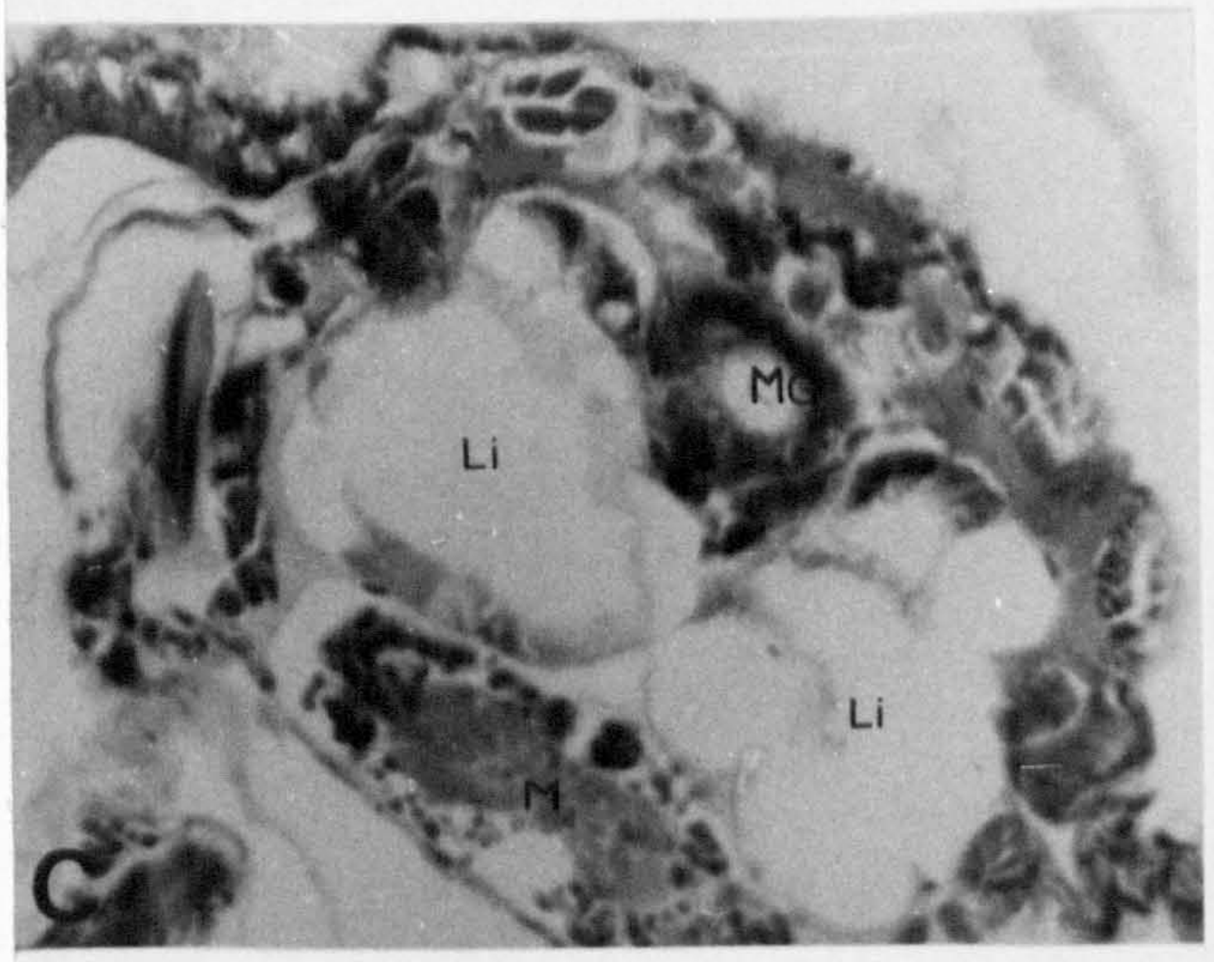
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M
Li
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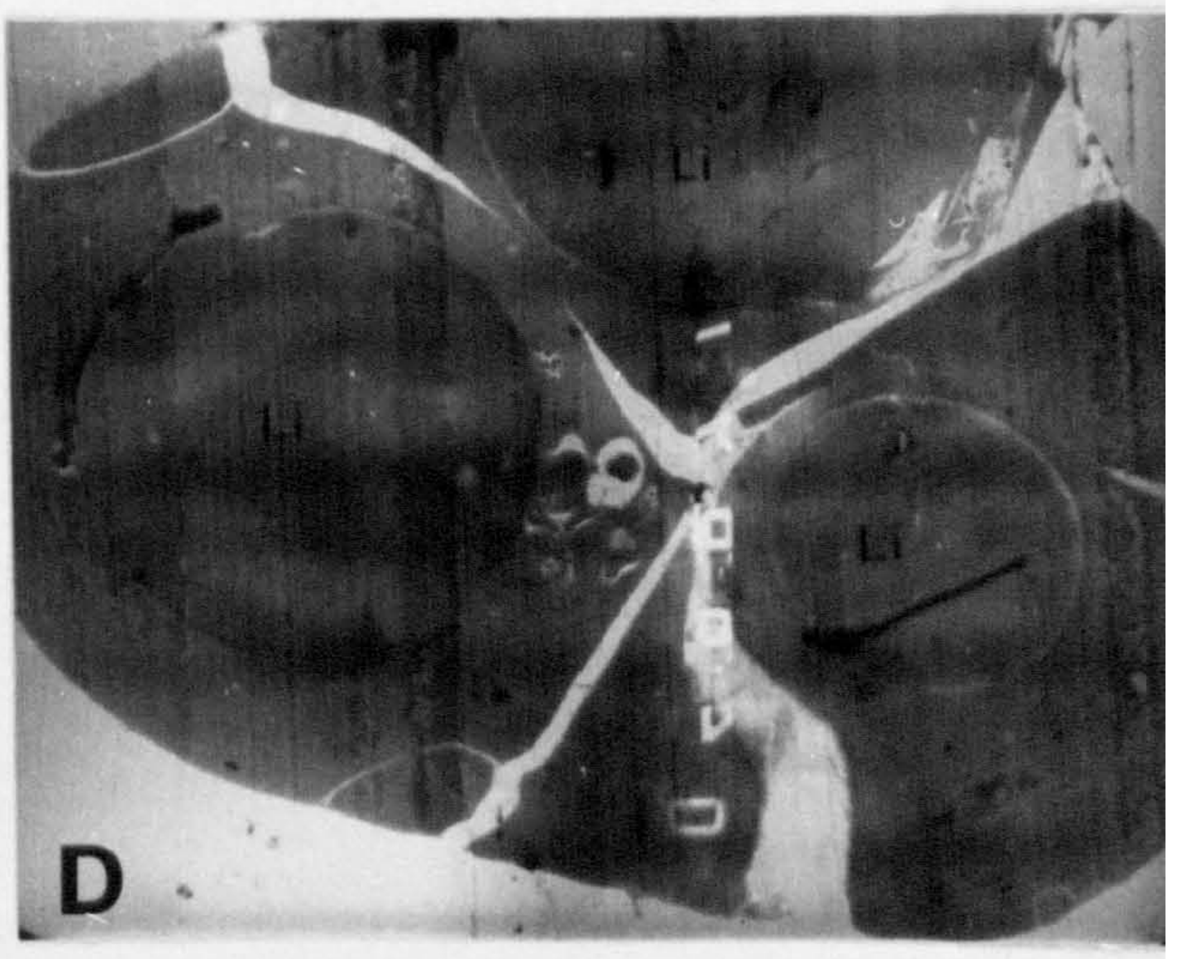
C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CF
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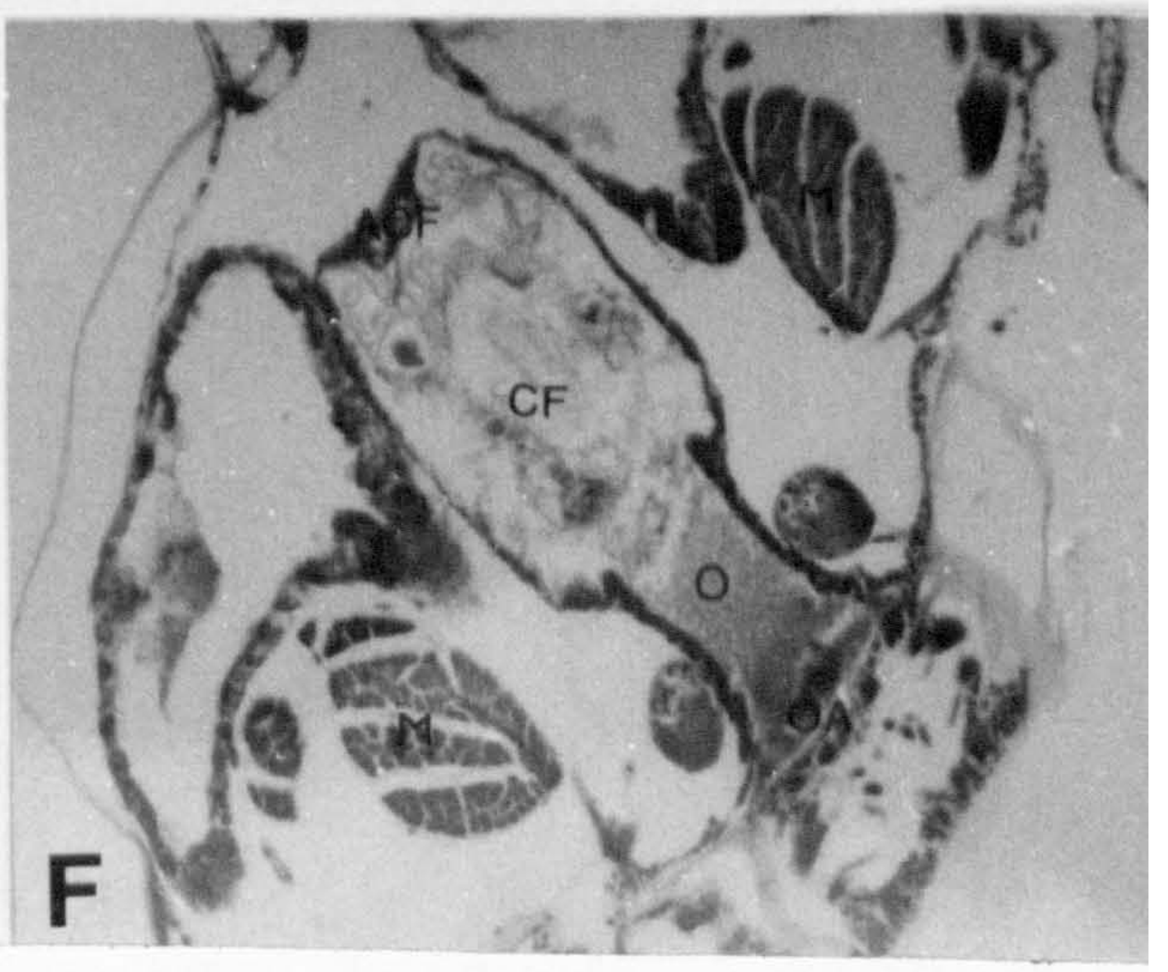
CF
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TABLE 1. Depletion of yolk reserves during the first stages of starved Macrobrachium rosenbergii

Stage	Fatty globules			
	Presence	Number	Size (µm)	Colour
I	+	6-12	36-41	opaque
II	+	9-23	23-28	opaque
III	+	8-17	16-19	opaque
IV	+	3-8	5-11	transparent
V	-	-	-	-

Mouth and its appendages

The mouth width varies from 40 to 225 μm as total body length varies from 1.9 to 10.4 mm (tables 2 and 16.0, appendix). The oesophagus is folded and not very strong during the early stages, but becomes chitinized during the later stages (figures 4-E & F, 5 and 6). Development of the mouth appendages can be seen in figures 7, 8 & 9. These reveal that larvae are poorly able to manipulate and to crush their diet during early stages, but these operations are improved due to the development of a strong mandible and functional first and second pereiopods by the time of postlarval metamorphosis. The mandible in the adult is strong, chitinous and efficiently crushes the feed (figure 10-A).

Foregut

The cardiac foregut of M. rosenbergii larvae is undifferentiated and smaller than the pyloric foregut during the first stages. It increases in volume by the end of the larval development forming a muscular cavity larger in volume than the pyloric foregut at PLI (figures 5, 6 and 10-B). Table 3 describes the folds, grooves and hastate plate which are formed in the cardiac foregut, and compares

TABLE 2. Meristic characteristics of *Macrobrachium rosenbergii* larvae

Stage	Duration (accumulated days)	Dry Weight (ug) (S.E.)	Carapace Length (mm) (S.E.)	Total length (mm)			
				Present Experiment (S.E.) (1)	Fujimura (1966) (2)	Ling (1962) (3)	Uno & Soo (1969) (4)
I	1-2	71 (0.24)	0.57 (0.02)	1.90 (0.05)	1.70	2.10	1.92
II	2-3	85 (0.89)	0.63 (0.08)	2.20 (0.10)	1.72	2.35	1.99
III	4-5	96 (0.95)	0.64 (0.05)	2.60 (0.61)	2.00	2.75	2.14
IV	6-7	103 (1.21)	0.73 (0.04)	3.00 (0.83)	2.60	2.95	2.50
V	7-9	117 (1.67)	0.86 (0.09)	3.60 (1.22)	3.16	3.25	2.80
VI	9-12	151 (2.03)	1.06 (0.25)	4.30 (1.57)	3.70	3.45	3.75
VII	12-16	278 (4.55)	1.05 (0.21)	5.40 (1.15)	4.40	4.25	4.06
VIII	16-19	392 (4.38)	1.12 (0.30)	6.00 (0.99)	6.15	5.40	4.63
IX	18-24	515 (12.22)	1.20 (0.27)	6.70 (1.68)	7.25	-	6.07
X	20-27	693 (8.77)	1.33 (0.16)	7.50 (1.17)	7.35	-	7.05
XI	23-35	796 (17.36)	1.47 (0.53)	8.30 (1.51)	7.55	-	7.73
PLI	22-47	844 (21.39)	1.85 (0.49)	10.40 (2.08)	7.40	6.25	7.69

(1) Salinity: 11-13‰.; temperature: 29 ± 0.5 oC
(2) Salinity: 13-21‰.; temperature: 26-28.4 oC
(3) Salinity: 20-40‰.; temperature: 26-28 oC
(4) Salinity: 11.9-12.32‰.; temperature: 28 ± 0.5 oC

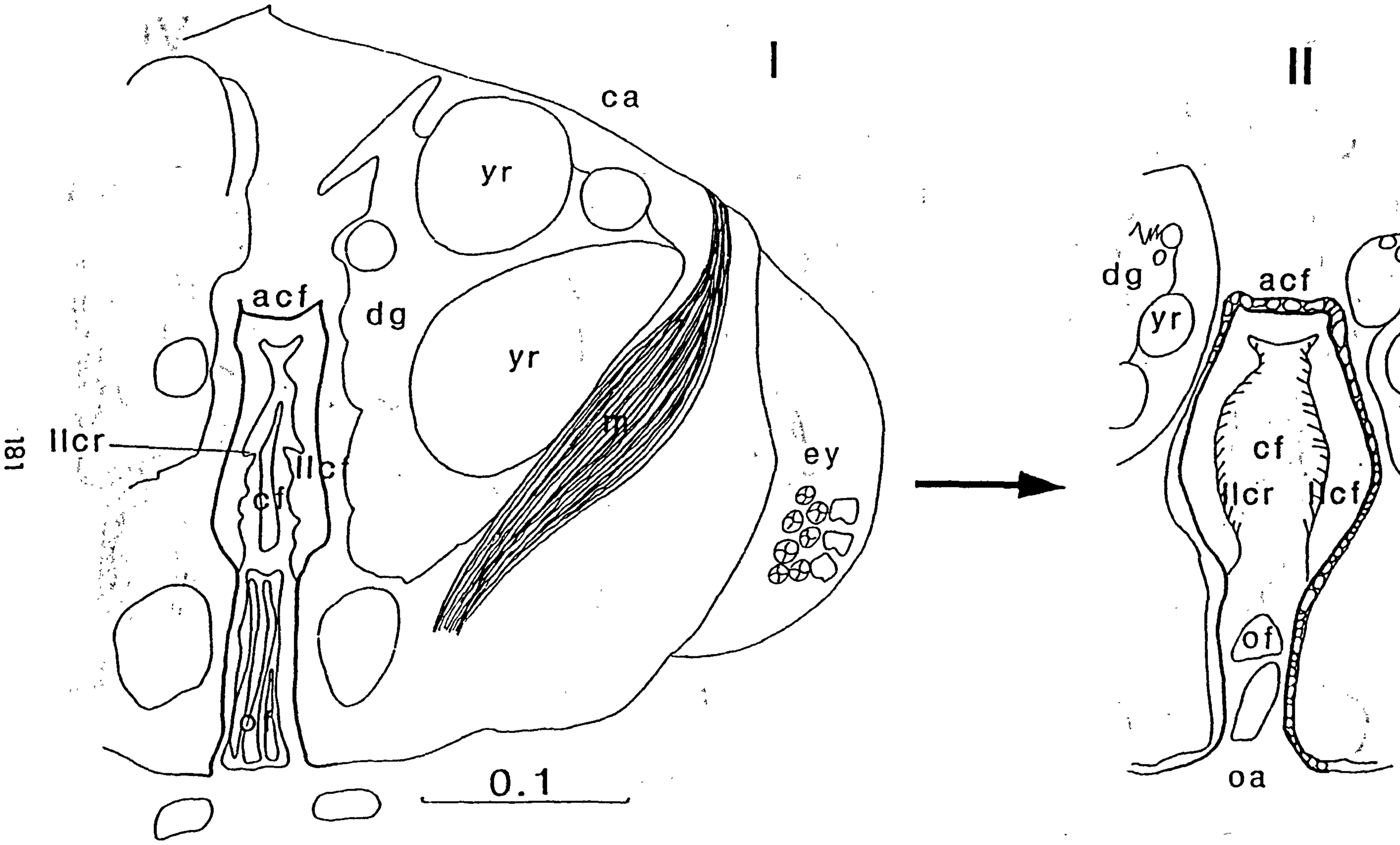


FIGURE 5. Schematic transverse section (fig.1-2, aa') of the oesophagus and cardiac foregut of M. rosenbergii larvae during stages I and II (bar = mm)

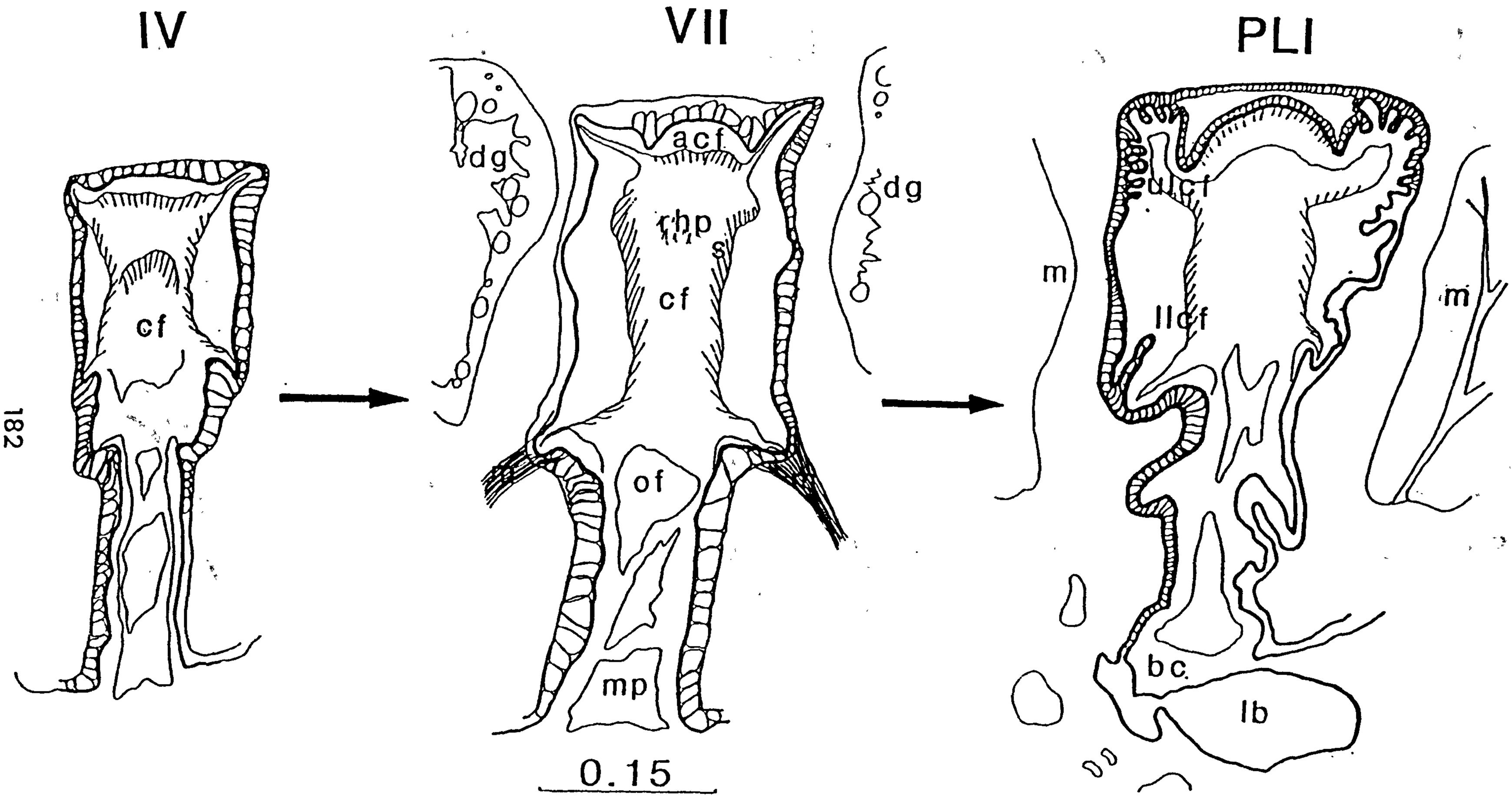


FIGURE 6. Schematic transverse section (fig.2-3, aa') of the oesophagus and cardiac foregut of M. rosenbergii larvae during stages IV, VII and PLI (bar = mm)

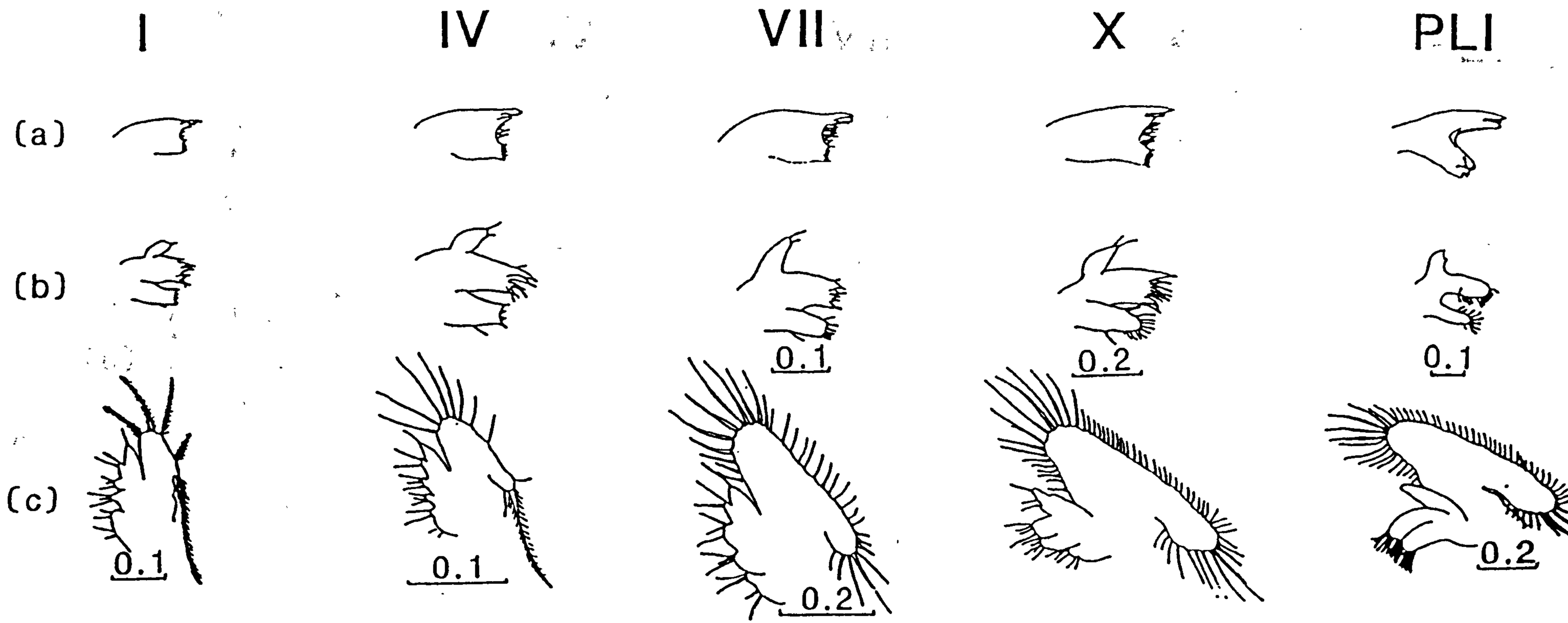


FIGURE 7. Development of the mandible (a), first and second maxillae (b & c, respectively) during the larval development of M. rosenbergii (bar = mm) (adapted from Uno & Kwon, 1969)

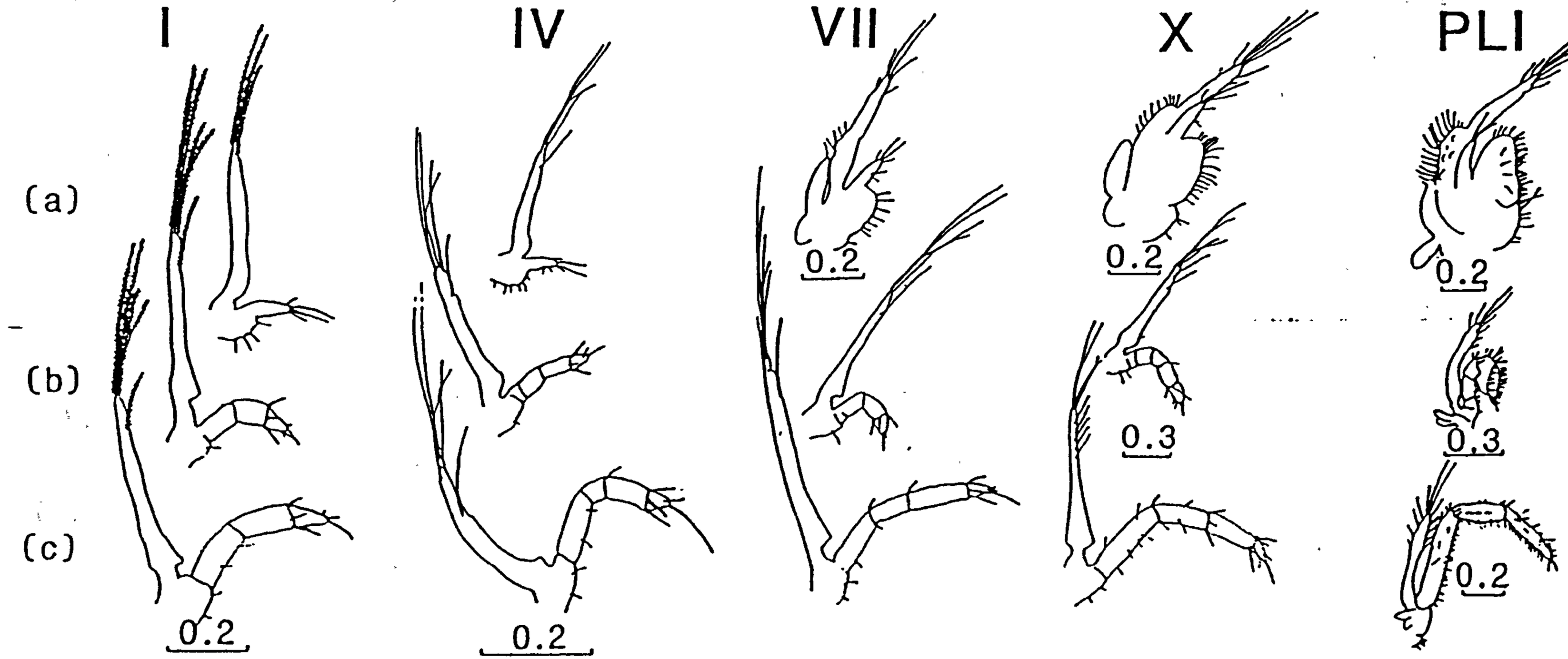


FIGURE 8. Development of the first, second and third maxillipeds (a, b & c, respectively) during the larval development of M. rosenbergii (bar = mm) (adapted from Uno & Kwon, 1969)

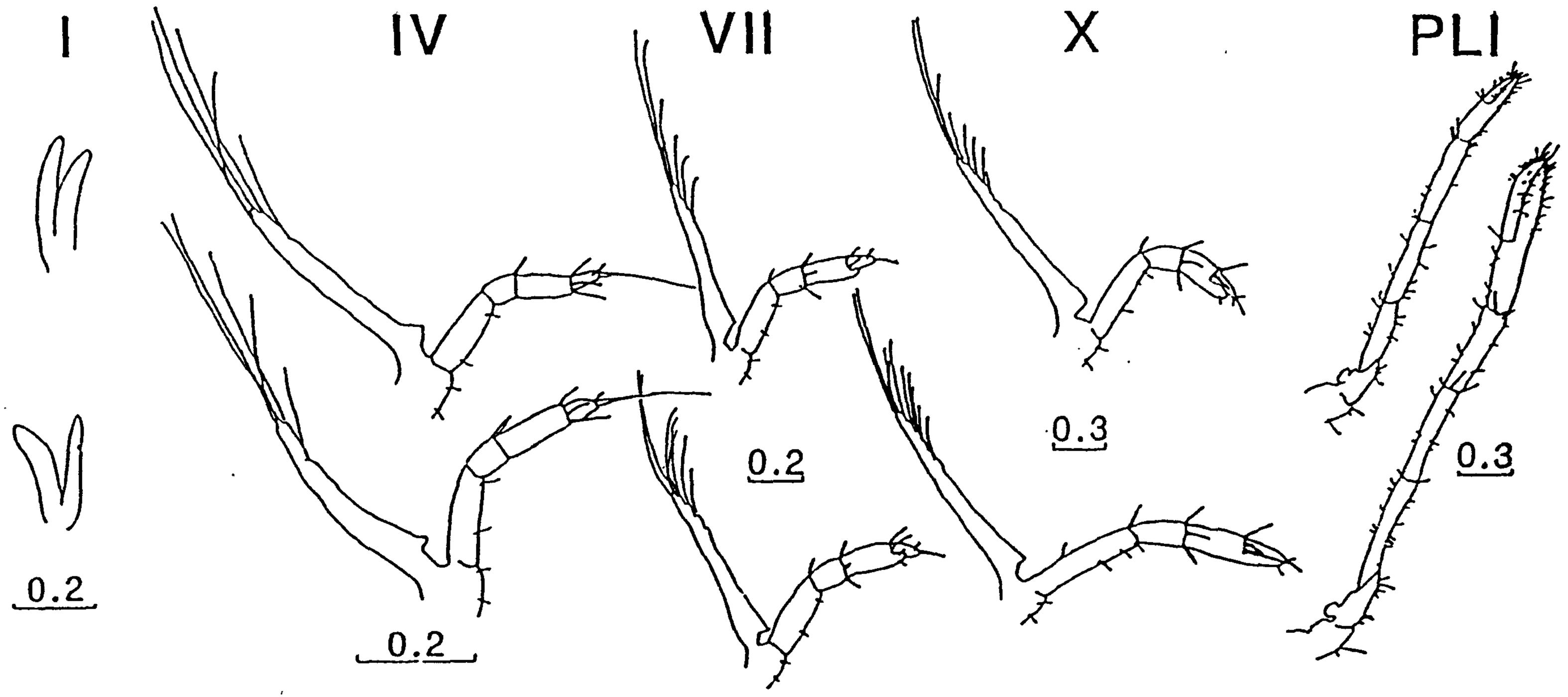
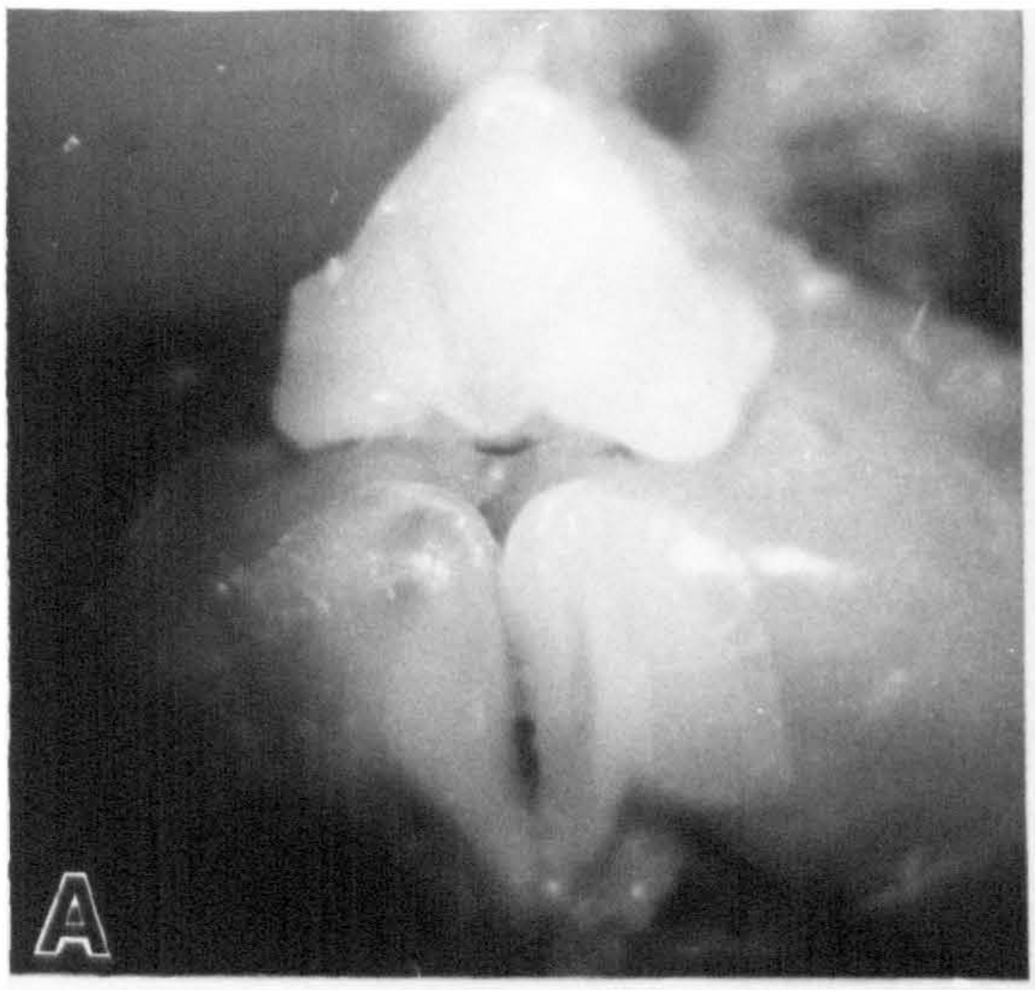
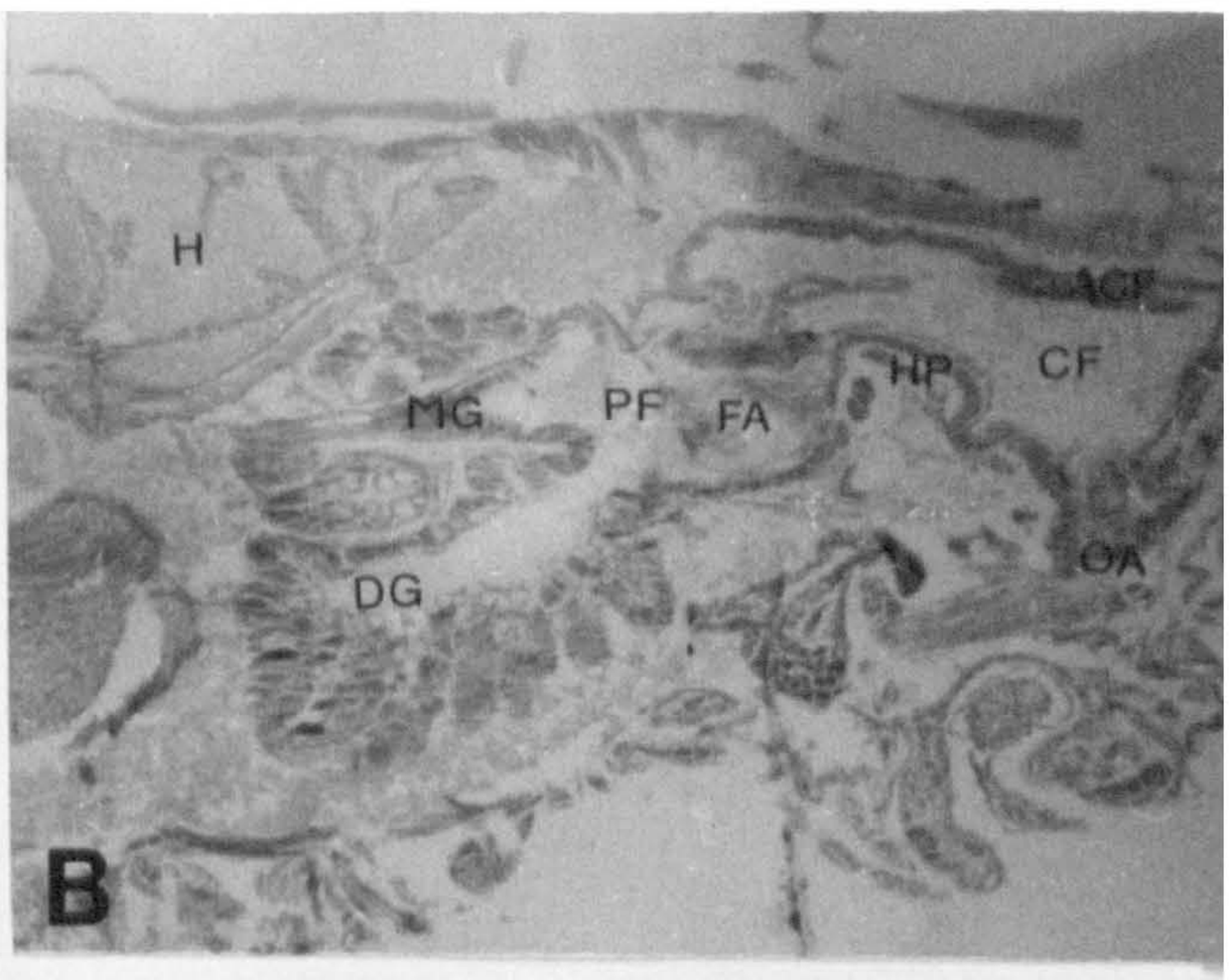


FIGURE 9. Development of the first and second pereiopods (a & b, respectively) during the larval development of M. rosenbergii (bar = mm) (adapted from Uno & Kwon, 1969)

FIGURE 10. (A) Mandible of M. rosenbergii adult (X6); (B) longitudinal section through the foregut at PLI (X10); (C) pyloric foregut adult (X12)(arrow, hepatopancreas entry); (D), (E) & (F) transverse section (fig.1-3, cc') of the pyloric filter during larval stages VII (X40), X (X40) and PLI (X10), respectively

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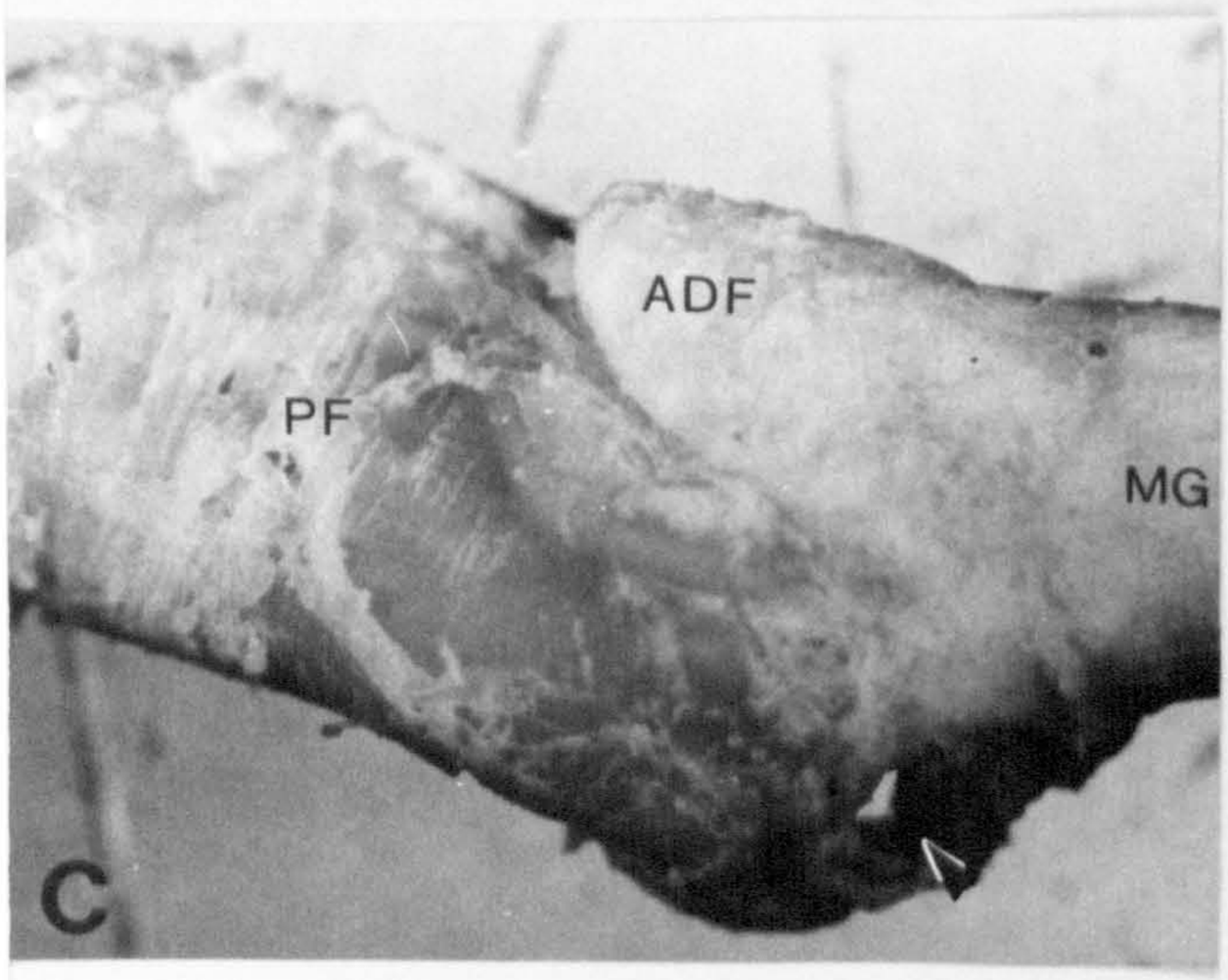
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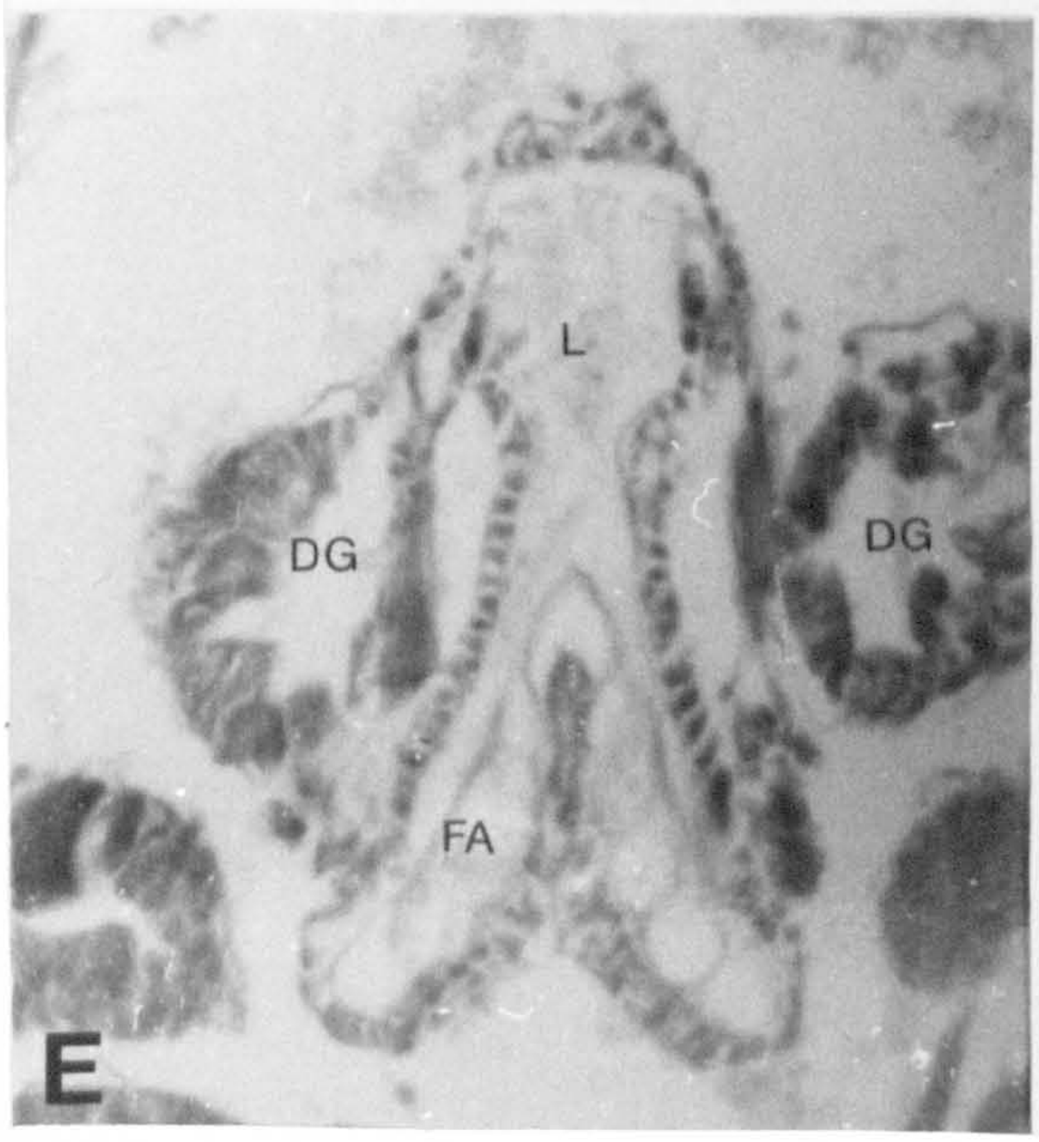
L
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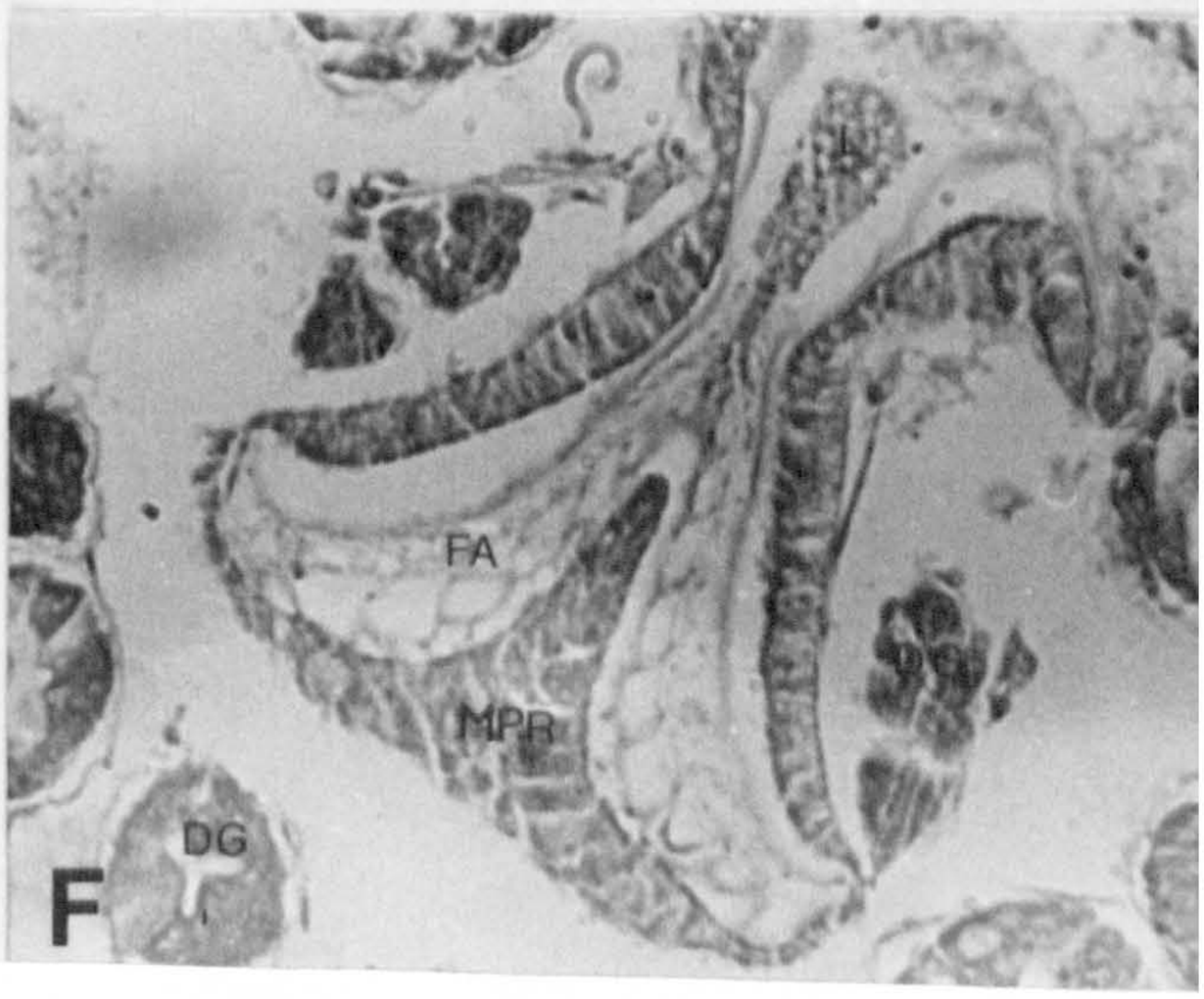
L
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TABLE 3. Comparison of the foregut morphology of two decapods (Palaemonidae) during larval and post-larval stages

Foregut	Stage	*Palaemonetes varians* (Leach)	*Macrobrachium rosenbergii* (de Man)
Cardiac	larval	1 dorsal fold with: anteriorly, 1 groove posteriorly, many barbed spines	1 antero-dorsal fold with few small spines
		2 lateral inferior folds furnished with numerous setae towards the interior of the cardiac foregut + 2 lateral inferior grooves	2 lateral inferior folds 2 lateral superior folds which appear well developed at stage VII 2 ventro-lateral grooves
		1 large ventral fold with many setae orientated backwards and surrounded by strong spines (30 to 35 µm)	1 large ventral fold with long setae orientated backwards and towards the centre of the chamber
		chamber length increases till 130-140 µm	chamber length, first, decreases (225-255 µm at stage I to 205-235 µm at stage V), then increases
	post-larval	chamber length decreases to 110 µm at PLI	chamber length reaches 780-830 µm at PLI
		transverse postero-ventral fold cutting cardiac and pyloric in 2 separate parts	idem
		2 longitudinal lateral ridges	4 lateral folds (2 inferior + 2 superior)
		the posterior barbed spines replaced by setae	predominance of setae over spines
		some folds are more marked such as the anterior and dorsal ones	idem
		new fold on the dorsal roof: formation of the dorsal pocket orientated backwards	no dorsal pocket, but posteriorly dorsal protuberance on the pyloric foregut forming the dorsal valve of Patwardhan
		hastate plate with numerous short setae	hastate plate well developed and chitinous with numerous, delicate and short setae
			chamber widely extended forwards and occupying the greater part of the anterior cephalothoracic cavity
		cardiac region forms a large muscular bag, in which feed is accumulated	idem

TABLE 3. (continued)

Foregut	Stage	*Palaemonetes varians* (Leach) (1)	*Macrobrachium rosenbergii* (de Man)
Pyloric	larval		pyloric foregut broader than the cardiac, with presence of dorsal and ventral chambers
		large ventral fold with 0-4 rows of setae arranged with 1-2µm space, strong spines dorsally	large ventral fold (median pyloric ridge) with 0-6 rows of setae spaced 1-3 µm apart, some spines dorsally
		2 pairs of lateral folds: 2 lateral inferior with 2 latero-inferior grooves 2 lateral superior with 2 latero-superior grooves	iden
		4 valves: 1 dorsal which penetrates deeply in midgut 2 lateral 1 ventral	iden
		posteriorly, 4 digestive gland openings: 2 dorsal orientated forwards 2 lateral tubules, branched, orientated backwards	posteriorly, 2 digestive gland openings: branched tubules, orientated 1 dorsally forwards 2 laterally backwards
	post-larval	transverse postero-dorsal fold which closes the pyloric chamber (= dorsal valve)	iden
		spines on the ventral fold replaced by setae	iden
		filter apparatus has 5 rows of setae spaced at 0.5-1 µm	filter apparatus has 7-8 rows of setae spaced at 0.5-2 µm on longitudinal chitinous ridges
		2 postero-vertical partitions obstruct the lateral inferior canals	(mentioned by Khan, 1976, but the author has not observed this)

(1) Roux, 1971; Khan, 1976

these morphological structures to those seen in other palaemonid decapod, Palaemon varians (Leach).

The junction between the cardiac and the pyloric foregut is narrow at stage I, but rapidly increases in size during later larval stages (figure 11). At the beginning of the larval development, the walls of this junction are smooth. Then, they are furnished with long setae and spines along the building of a filter apparatus in the pyloric foregut. At the postlarval metamorphosis, most of the spines desappear as a strong and muscular cardiac foregut develops (chitinized hastate plate) and the filter apparatus in the pyloric foregut becomes fully operational.

The pyloric foregut is broader than the cardiac foregut during the first larval stages (figures 1 and 12). Reaching stage VII, it is slightly smaller, but is already well differentiated, and a postero-dorsal fold appears (figures 2 and 13). At the postlarval metamorphosis, it appears similar to the adult pyloric foregut (figures 3, 10-C and 14).

Recently hatched larva does not have any pyloric filter (figures 1 and 12). This filter clearly appears at stage III and consists in two pairs of setal rows. The number of rows increases during the larval development reaching 7-8 rows at PLI (figures 2, 3, 10-D, E & F, 13, 14, 15-A, B,C & D;

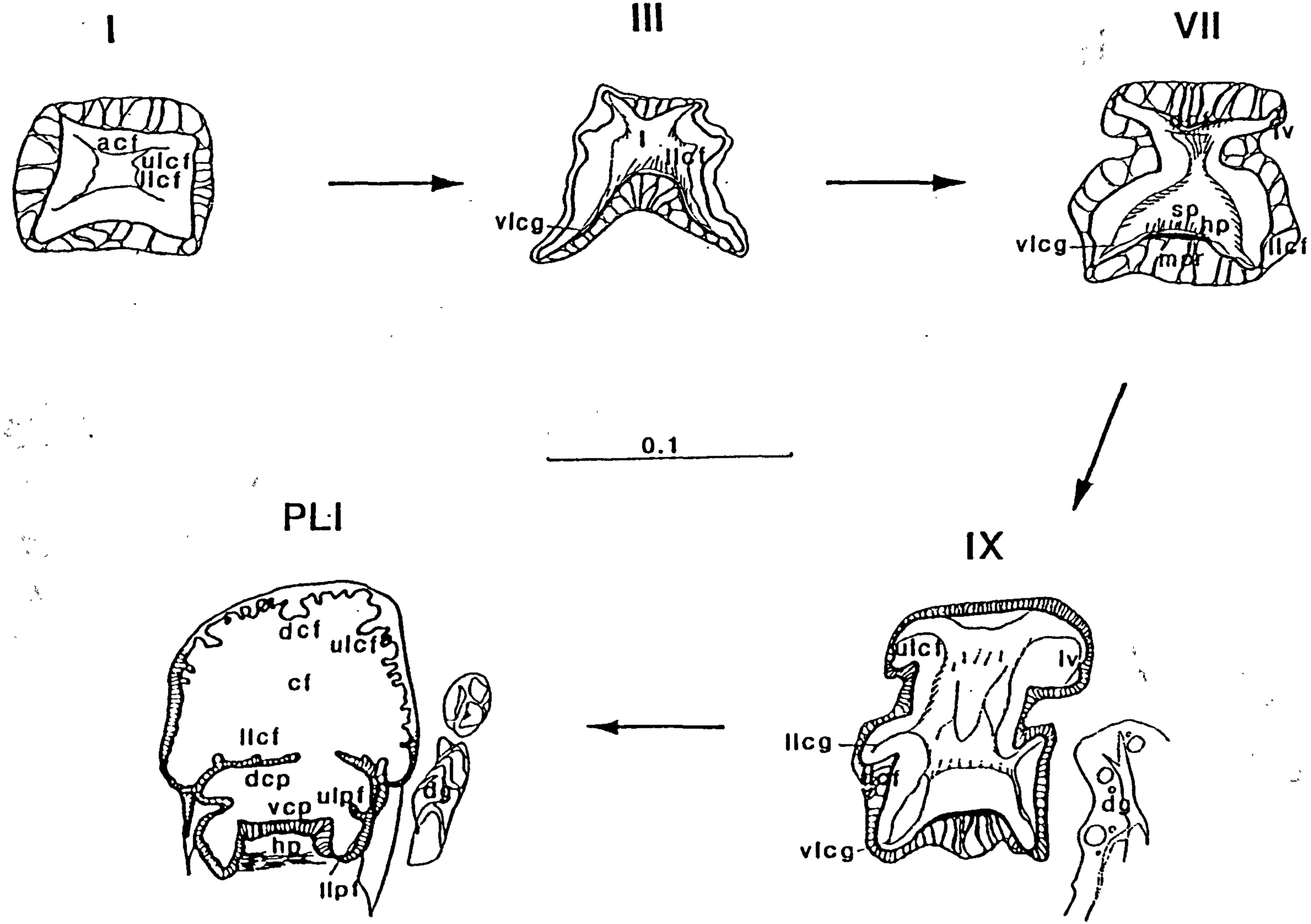


FIGURE 11. Schematic transverse section (fig.1-3, bb') of the junction cardiac/pyloric foregut of M. rosenbergii during its larval development (bar = mm)

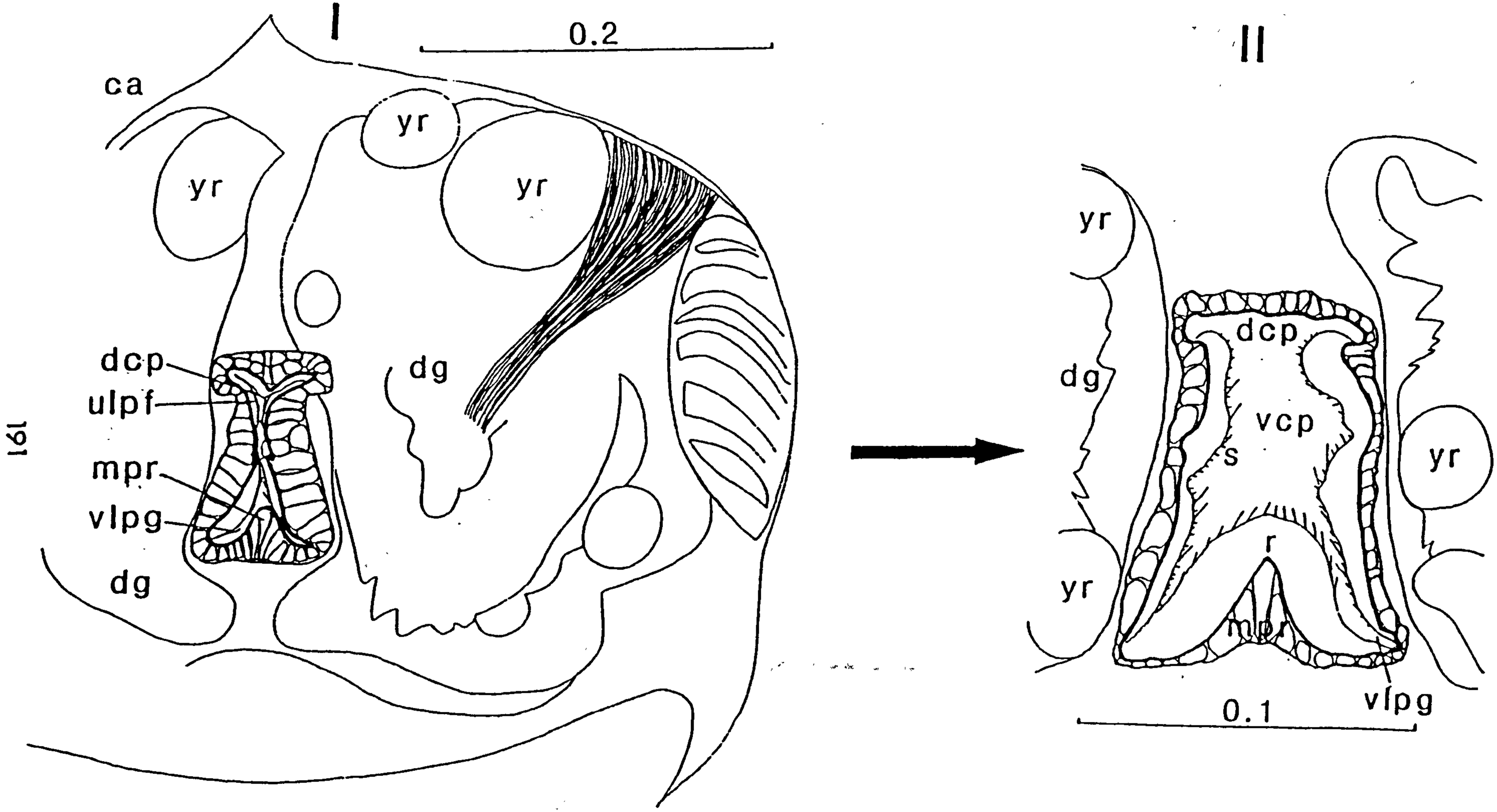


FIGURE 12. Schematic transverse section (fig.1-2, cc') of the pyloric foregut of M. rosenbergii larvae during stages I and II (bar = mm)

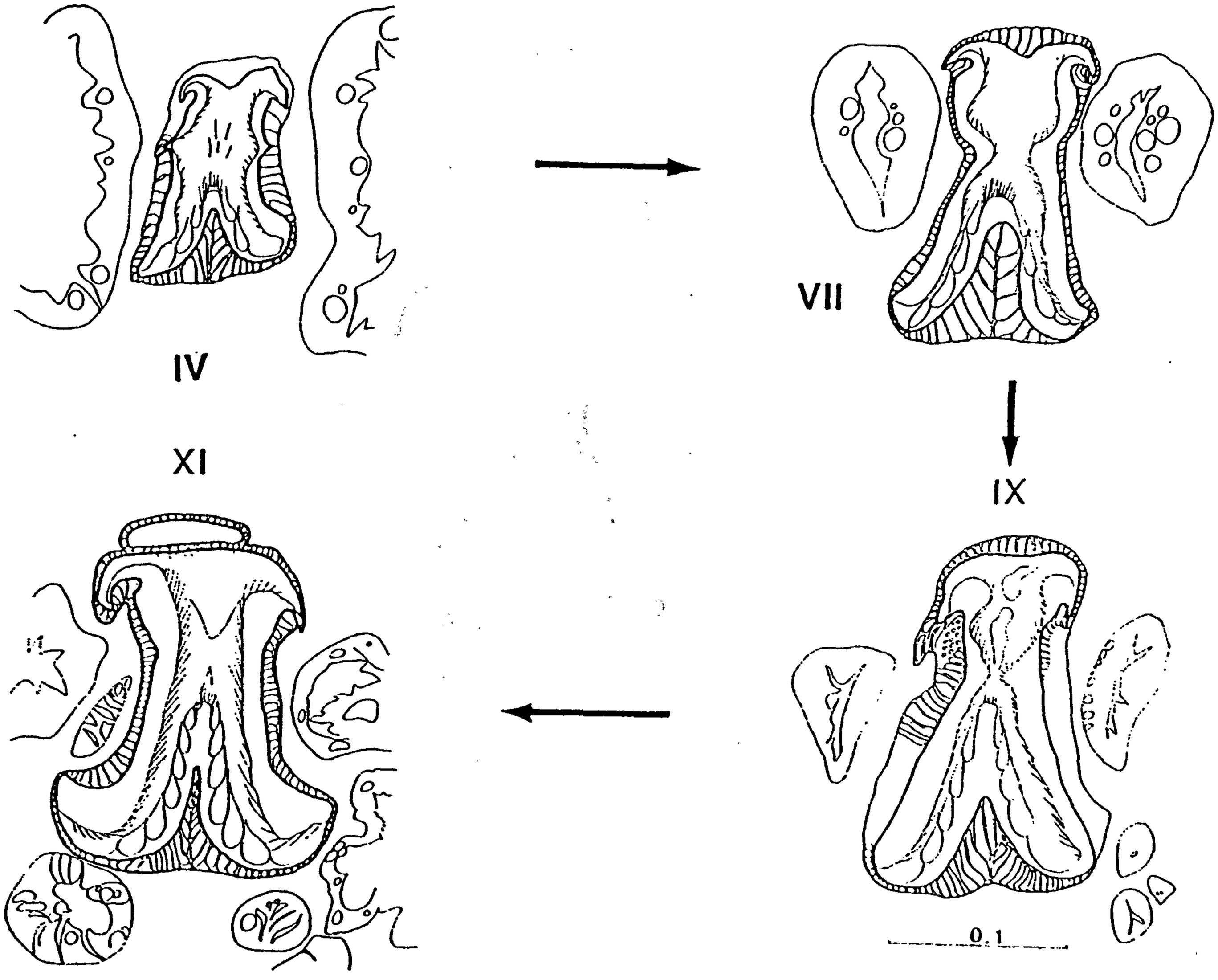


FIGURE 13. Schematic transverse section (fig.1-2, cc') of the pyloric foregut of M. rosenbergii larvae during stages IV, VII, IX and XI (bar = mm)

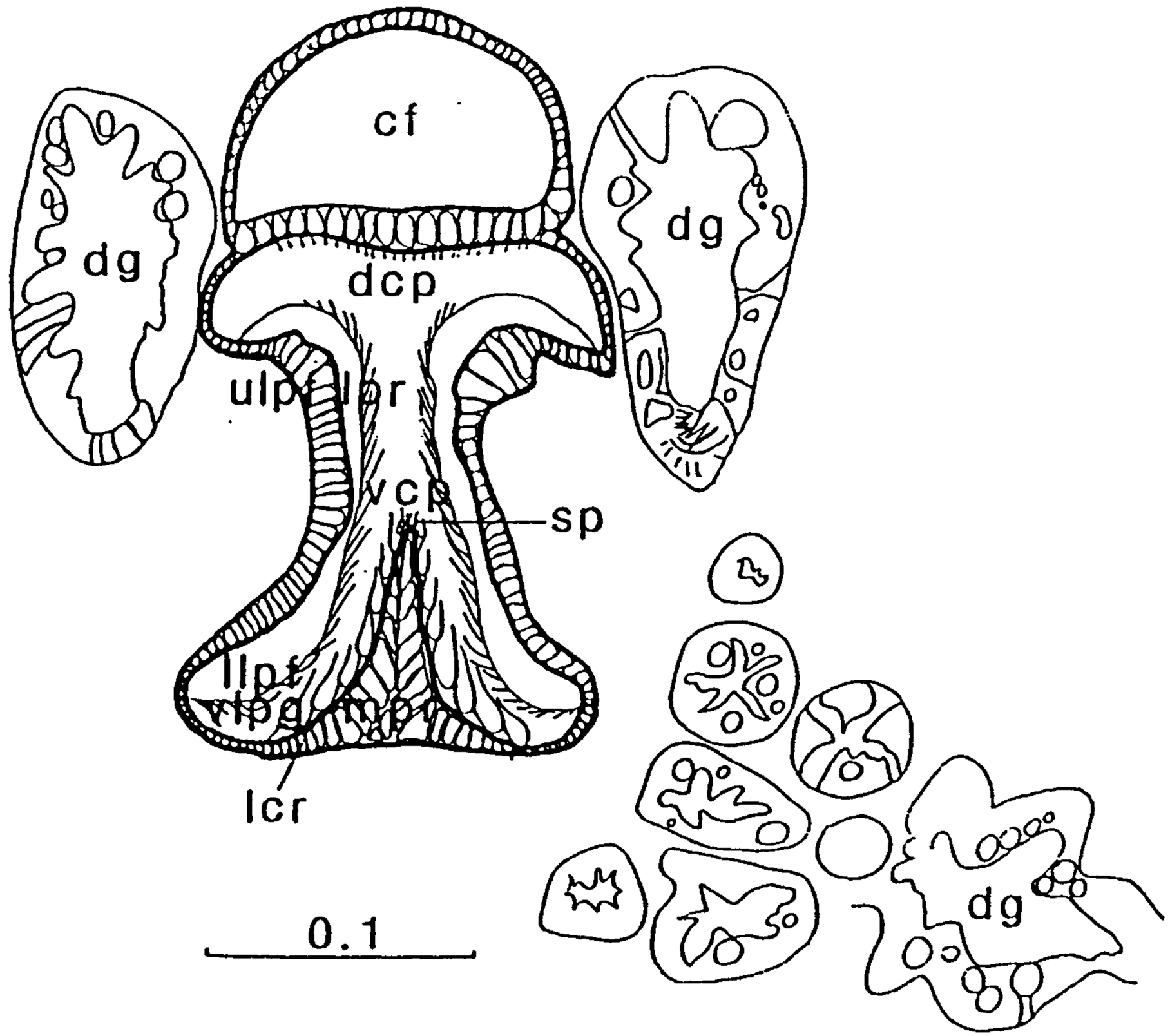
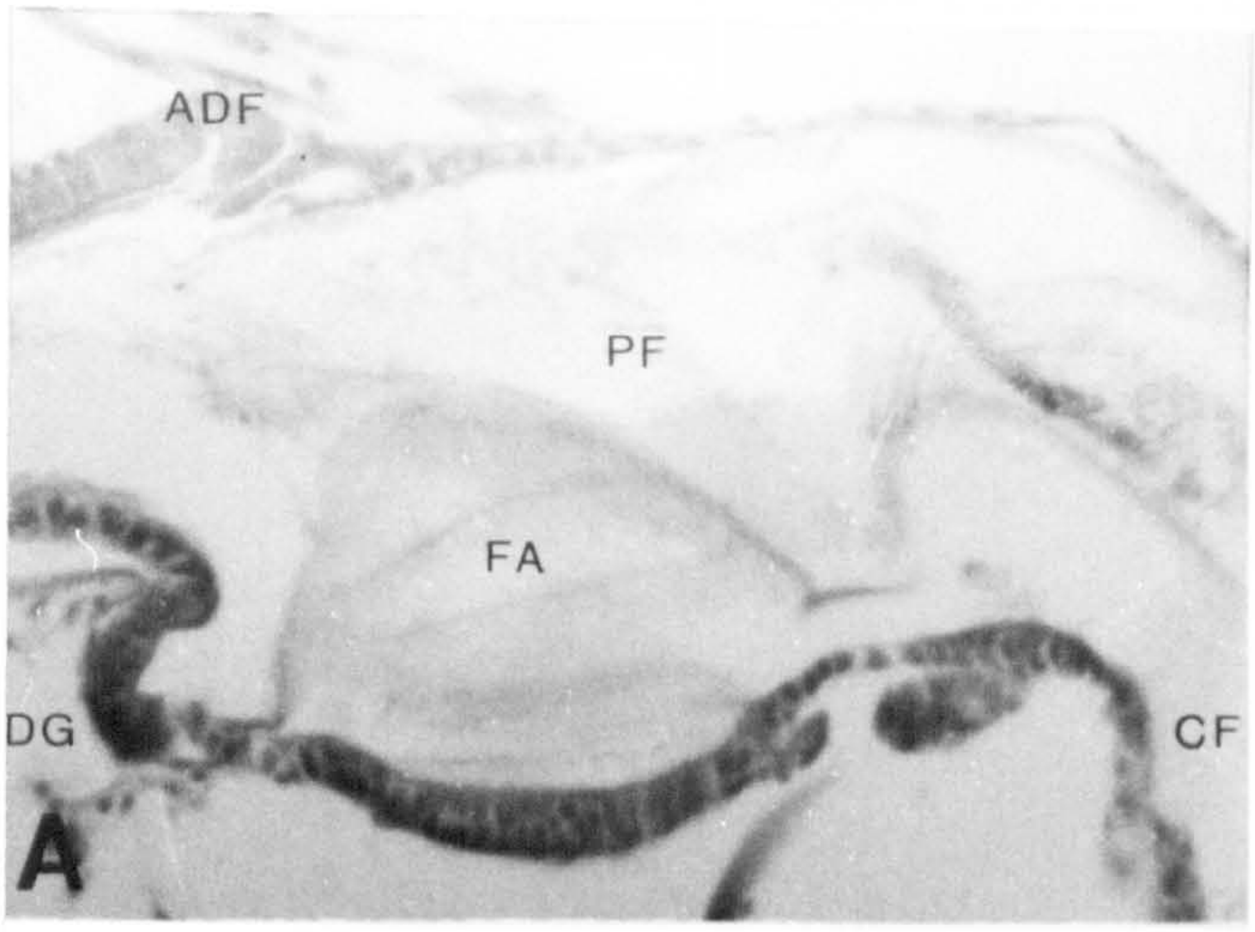


FIGURE 14. Schematic transverse section (fig.3, cc') of the pyloric foregut of M. rosenbergii at PL1 (bar = mm)

FIGURE 15. (A) & (B) longitudinal section of the pyloric filter during stages VIII and XI, respectively (X40); (C) pyloric foregut and digestive gland at PLI (fig.3, cc')(SEM X282); (D) detail of the filter (SEM X2,340)(arrow, median pyloric ridge communication); (E) plan view of the pyloric foregut and digestive gland in adult (X6); (F) & (G) profile and plan view of the dissected filter, respectively (X25)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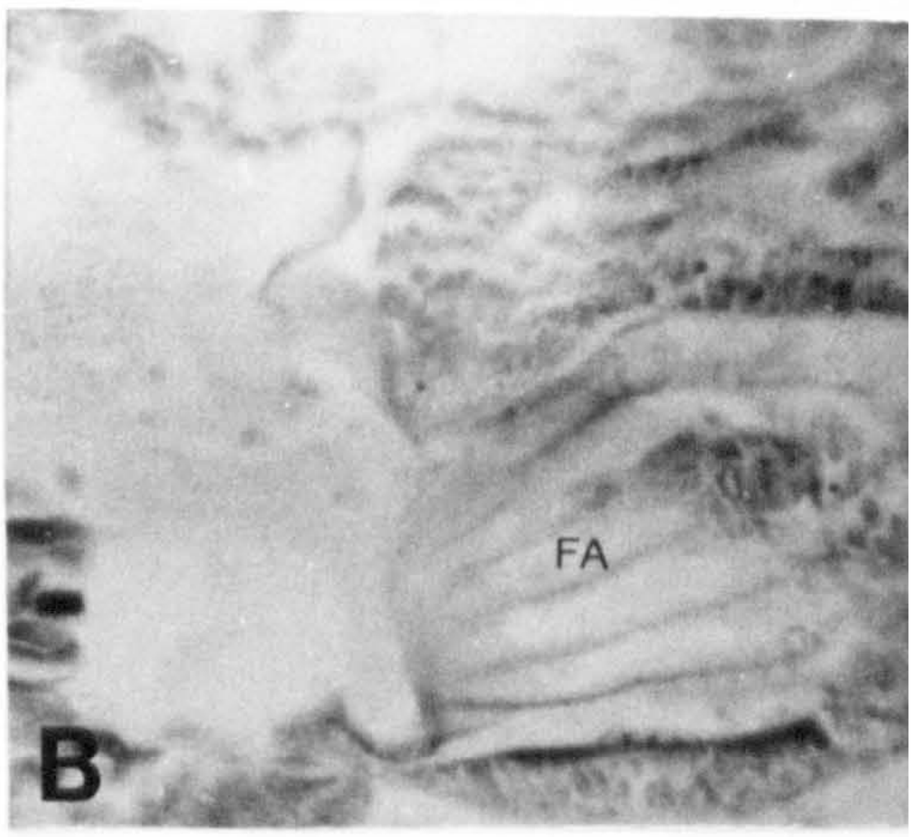
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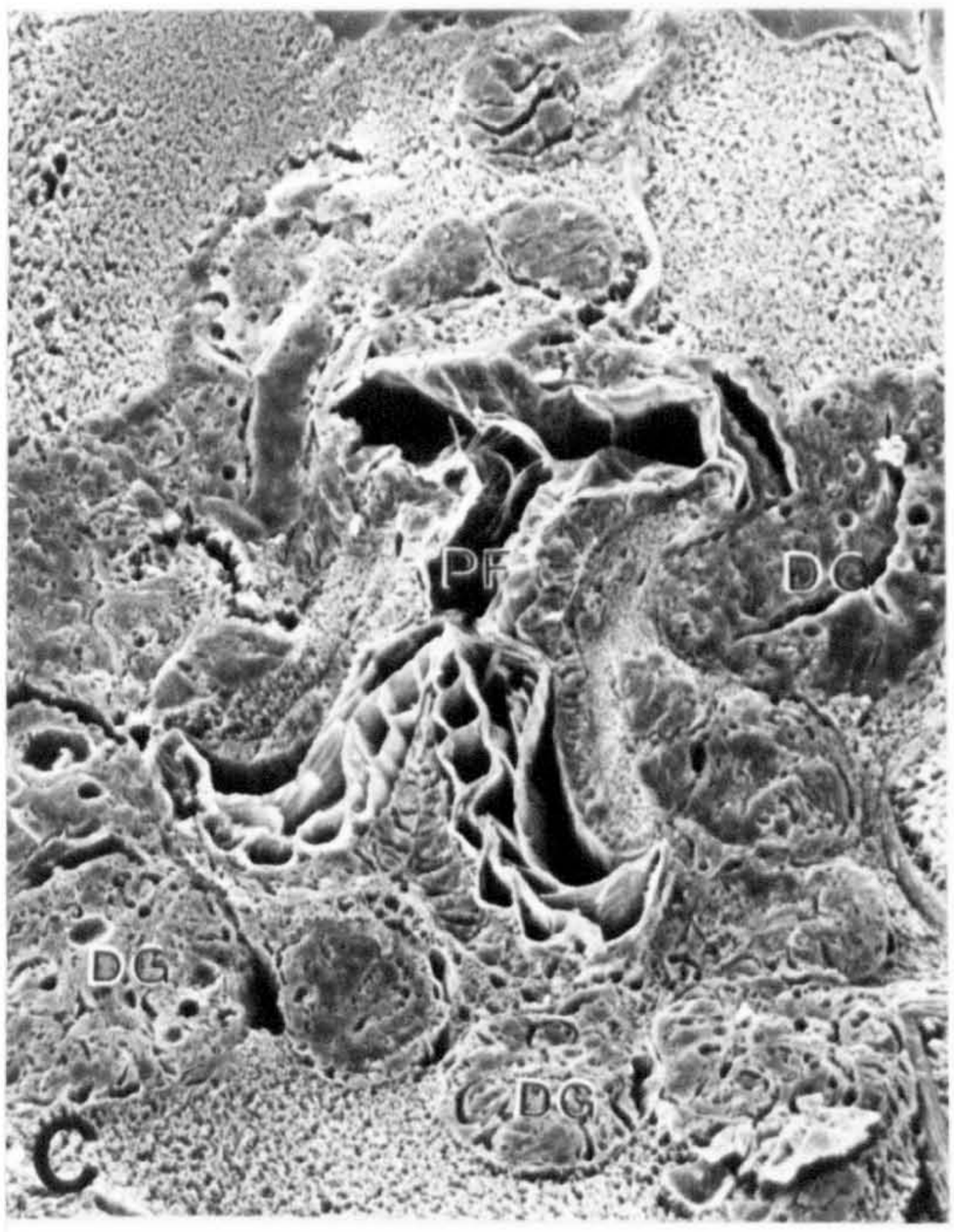
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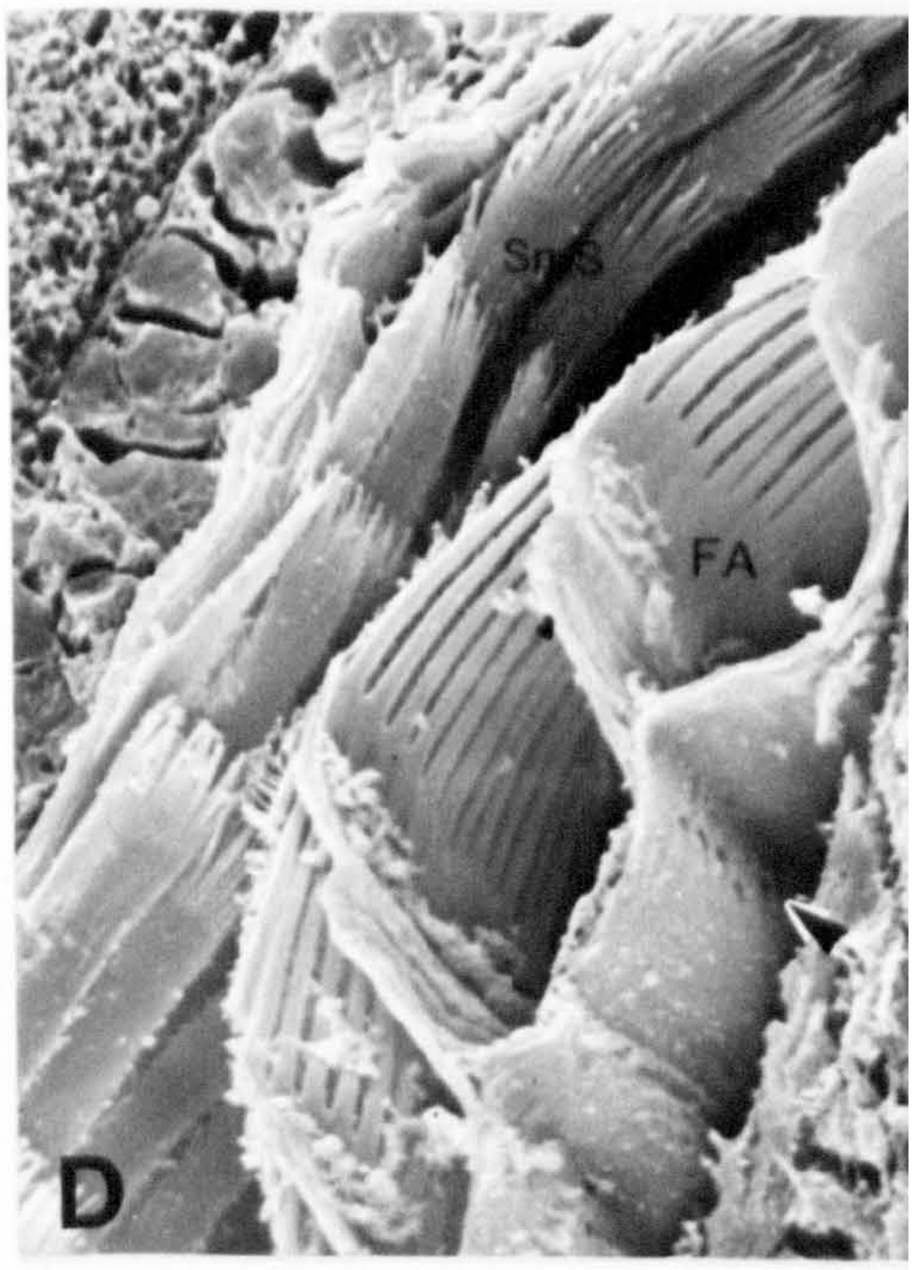
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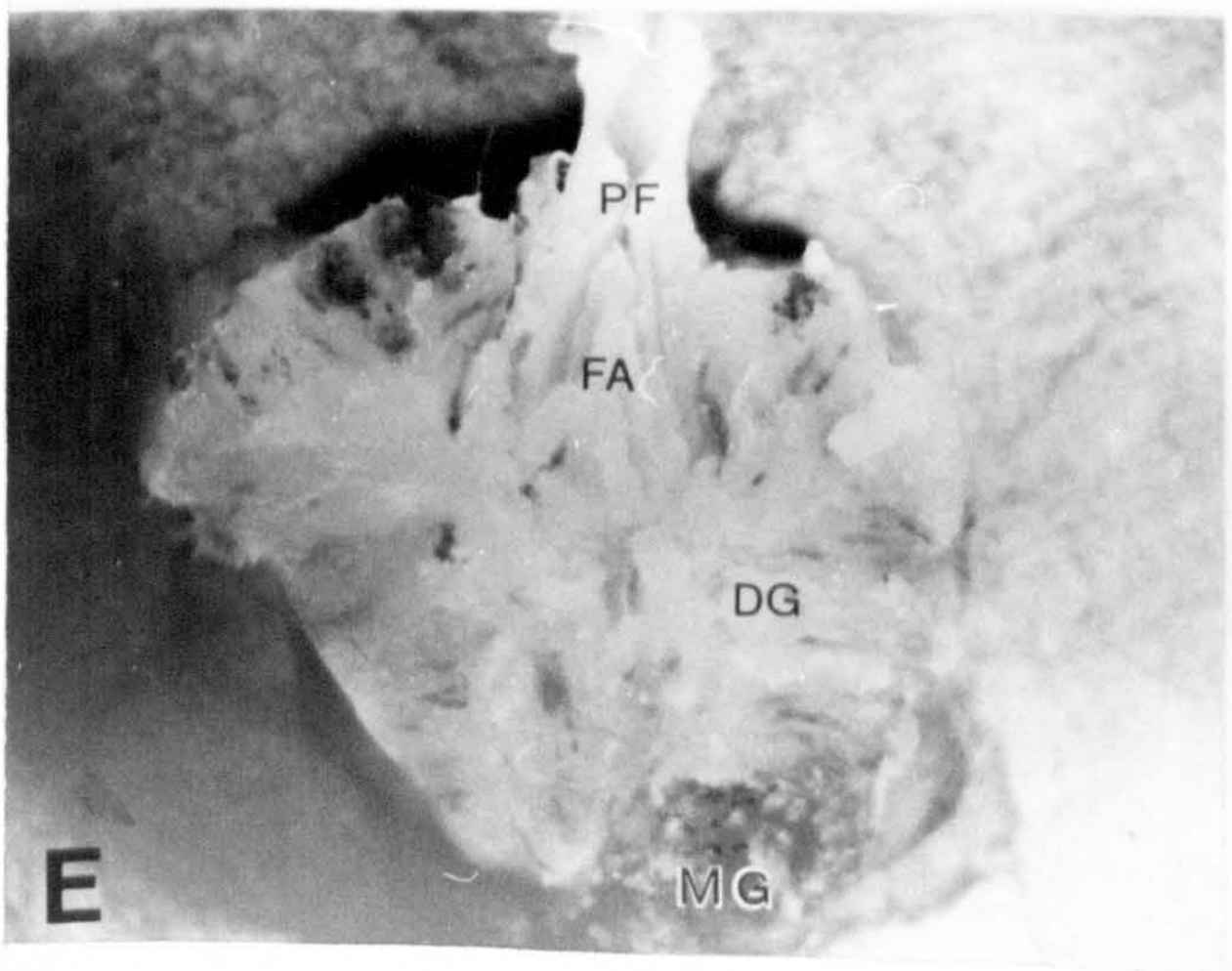
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table 4). This filter is triangular in form, rests on a median pyloric ridge, and is mobile in adult prawns (figures 15-E, F & G). At the bottom of each setal row, a line of holes is found (arrow in figure 15-D) allowing communication with the median pyloric ridge.

Midgut

At larval stage VI, a dorsal fold appears at the junction between the pyloric foregut and the midgut trunk. This remains underdeveloped at postlarval metamorphosis as no special internal structures can be observed (figures 1, 2, 3 and 15-A).

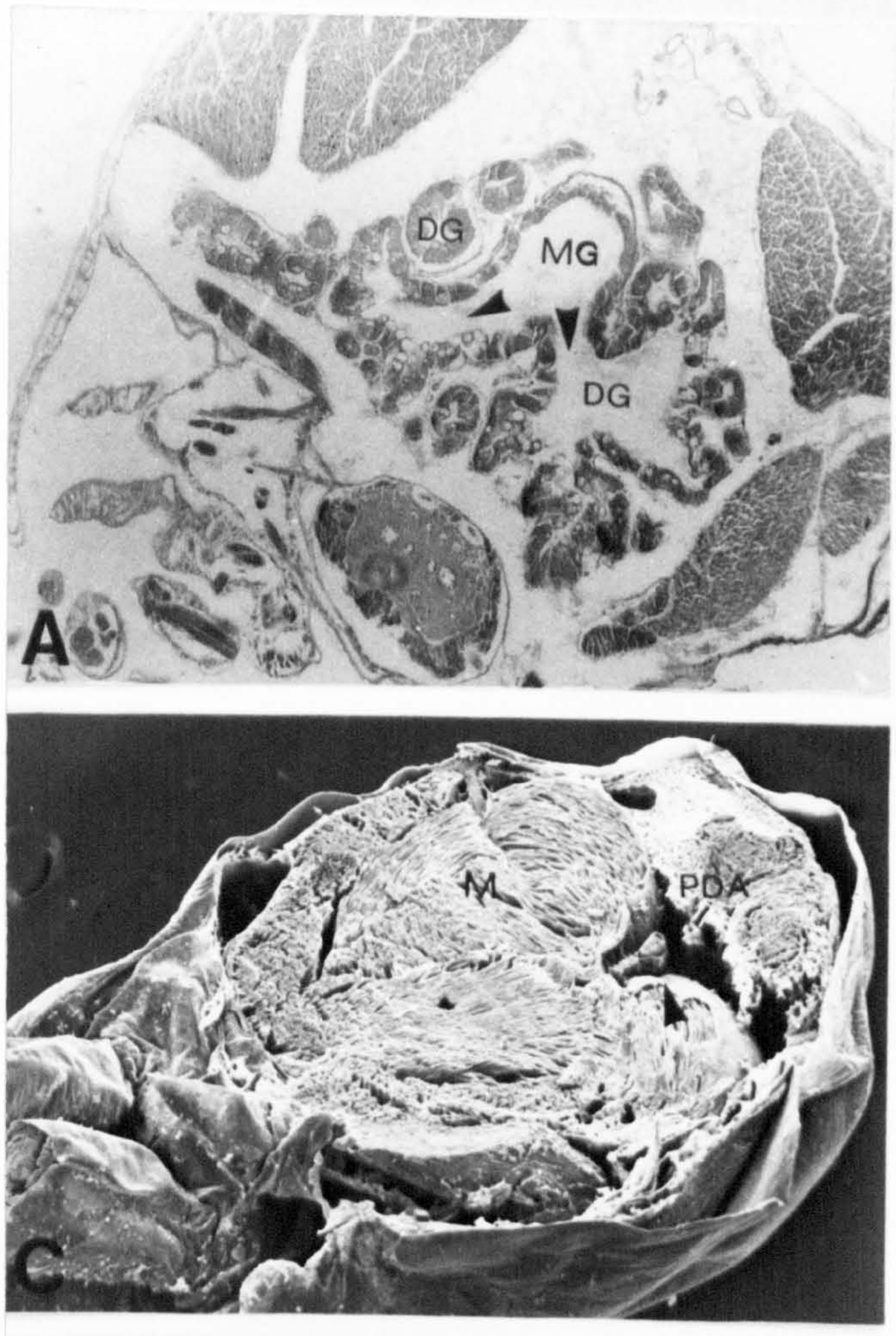
During the early stages, the digestive gland consists of a pair of small lateral caecae filled with embryonic lipid droplets which allow the larva to survive during the first stage without feeding (figure 4-B, C & D). The diameter of each lobe of the midgut gland is generally greater than that of the midgut trunk (figure 1). Each lateral midgut caecum differentiates into three distinct lobes: antero-ventral, antero-dorsal and postero-lateral. The lobes of each caecum share a common ventral opening into the midgut, posterior to the pyloric foregut (figures 10-C, 16-A and 17). As the hepatopancreas develops smaller diameter tubules appear (figures 2, 3, 10-B, 15-B & C and 16-B). All lobes increase

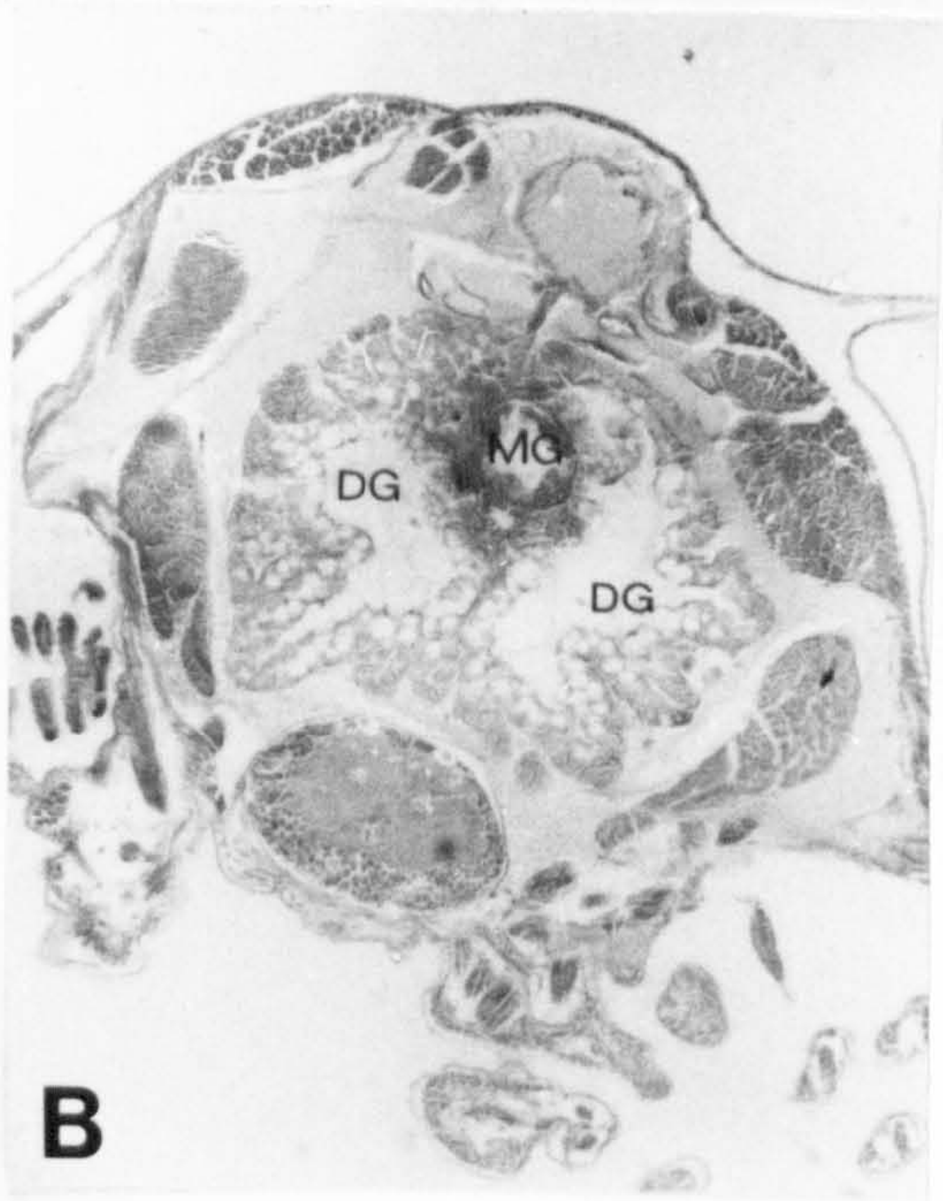
TABLE 4. Number of seta rows on the ventral pyloric fold (filter apparatus) for two caridean decapods during the larval stages

Palaemonetes varians (Leach) (1)		*Macrobrachium rosenbergii* (de Man)	
Stage	row number	Stage	Row number
I	0 (2)	I	0 (2)
II	2	II	2 (3)
III	3	III	2
IV	4	IV	2
PLI	5	V	2
		VI	3
		VII	4
		VIII	4
		IX	4
		X	5
		XI	6
		PLI	7-8

(1) Le Roux, 1971
(2) according to Regnault (1968); this author also recorded the absence of any filter apparatus in the first stage of the caridean decapod *Hippolyte inermis* (Leach)
(3) hardly visible

FIGURE 16. (A) transverse section (fig.3, dd') of the pyloric foregut/midgut junction showing the openings (arrows) into the digestive gland at PLI (X10); (B) transverse section (fig.3, dd') of the midgut, posterior to the pyloric foregut, showing the digestive gland at PLI (X10); (C) transverse section (fig.3,ee') through the abdomen showing the midgut (arrow) at PLI (SEM X62.2); (D) detail of the midgut (SEM X1,160); (E) posterior section of a PL midgut (oil immersion X100); (F) hindgut during stage VII (X40)(arrow, posterior midgut caecum)


DG
MG
DG
A


DG
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B

M
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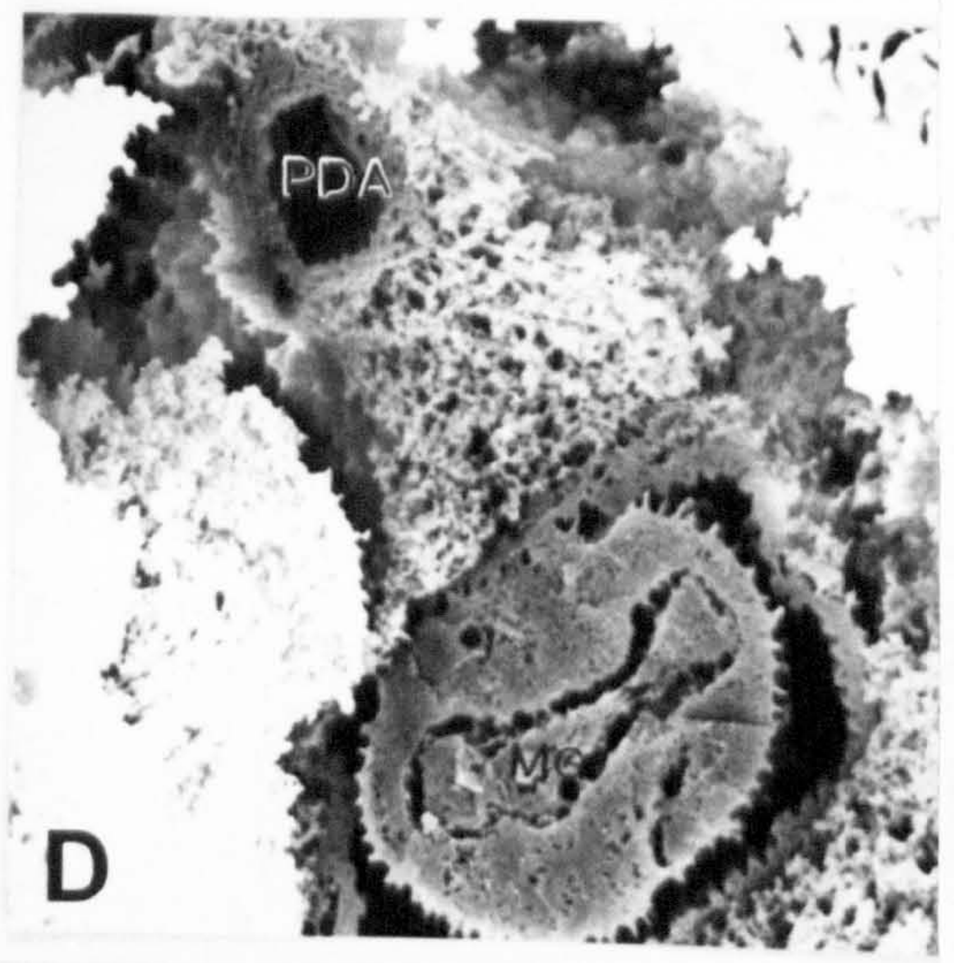
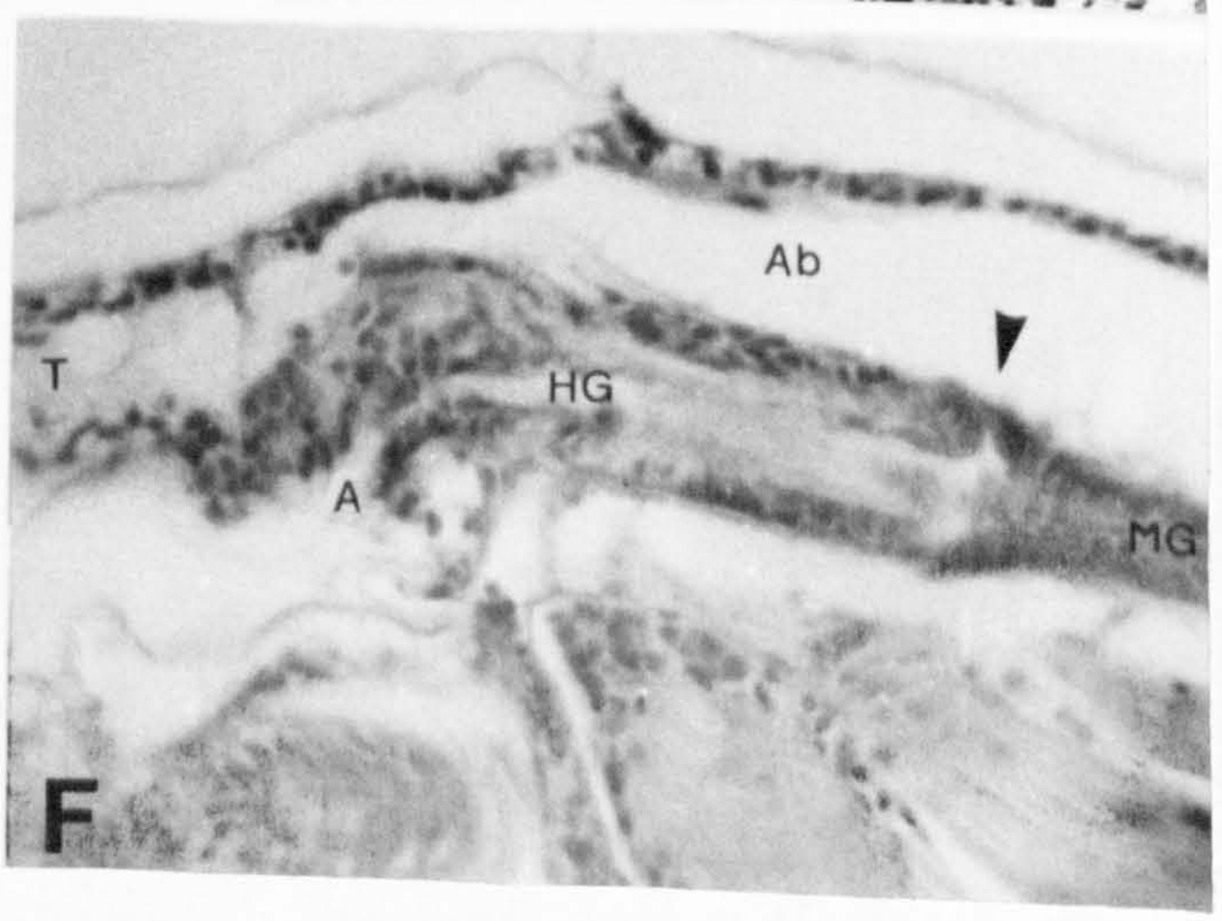
C


PDA
MG
D


PDA
MG
L
M
E


Ab
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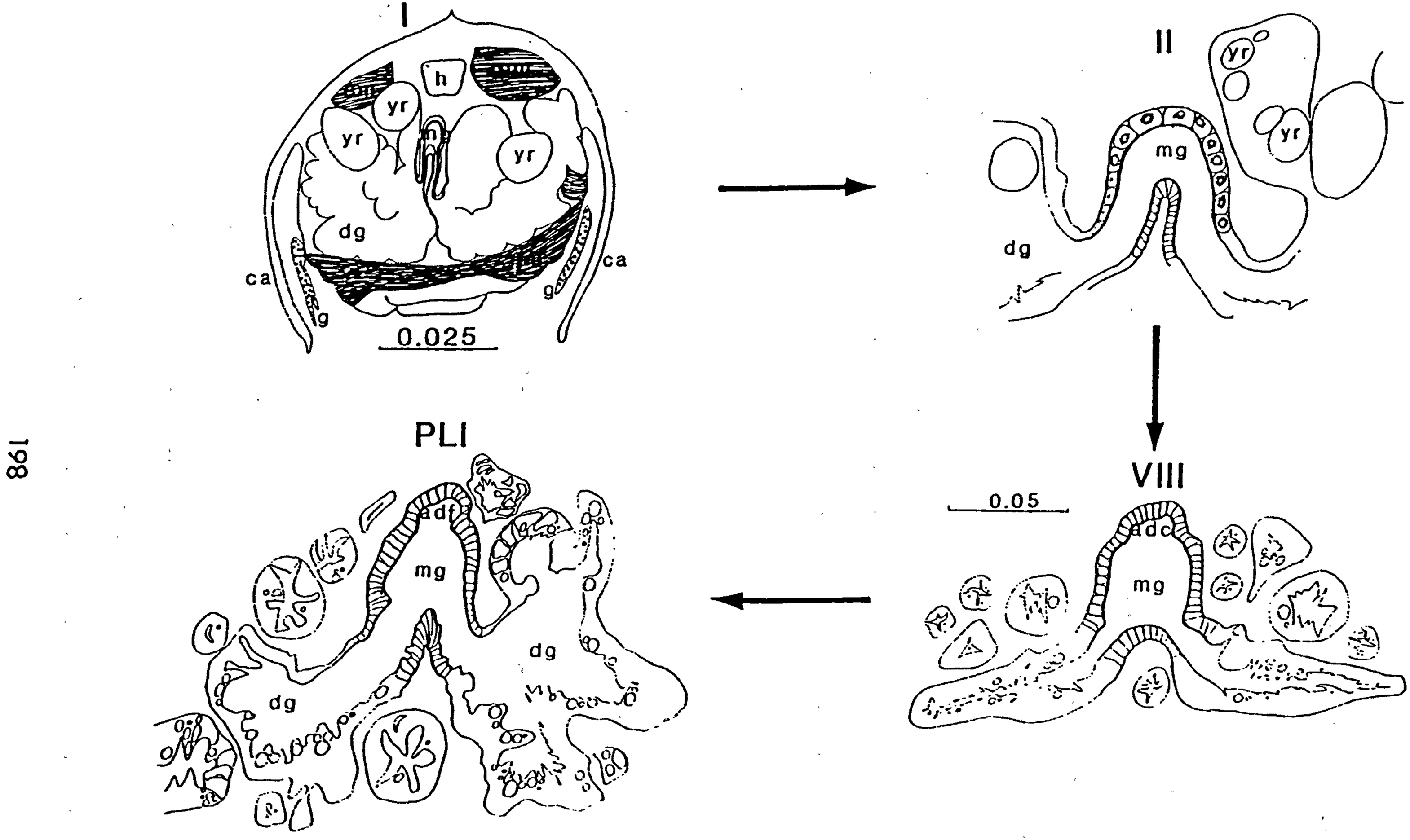


FIGURE 17. Schematic transverse section (fig.1-3, dd') through the pyloric foregut/midgut junction of M. rosenbergii larvae during stages I, II, VIII and at PL1 (bar = mm)

in length and size during the larval cycle. The digestive gland enters into active division around stages VI-VII and these lobes dramatically expand (figure 10-D), which corresponds to a period of high mortality in the larval development of M. rosenbergii. Between stages V and VII, the volume of the hepatopancreas increases by seventeen fold in size (tables 5 & 17.0, in annex). As it can be observed at figure 18, this increase in hepatopancreas volume is by far much greater than the increase in total body length of the larva. At the postlarval metamorphosis, the hepatopancreas is well ramified into small diameter tubules and appears similar to the hepatopancreas of adult prawns (figure 15-C).

Figures 16-B, C & D and 19 show an anterior section of M. rosenbergii midgut, whilst figures 16-E and 20 present the posterior section. The posterior midgut caecum is short during the early stages and still remains a simple evagination at postlarval metamorphosis (figures 16-F, arrow, and 21).

Hindgut

In M. rosenbergii larvae, there is little ontogenetic change in the hindgut. During the larval development, it increases in length. The appearance of rectal ridges is gradual and cannot be assigned to any particular stage (figure 16-F).

TABLE 5. Volume of the hepatopancreas of Macrobrachium rosenbergii at each larval stage compared to total length and dry weight

Stage	Hepatopancreas volume (mm3)	Total length (mm)	Tot. length / Hepat. vol.	Dry weight (µg)	Dry weight / Hepat. vol.
I	0.00393	1.9	483.5	71	18066.2
II	0.00417	2.2	527.6	85	20383.7
III	0.00439	2.6	592.3	96	21867.9
IV	0.00490	3.0	612.2	103	21020.4
V	0.00583	3.6	617.5	117	20068.6
VI	0.03133	4.3	137.2	151	4819.7
VII	0.09844	5.4	54.9	278	2824.1
VIII	0.10534	6.0	57.0	392	3721.3
IX	0.12411	6.7	54.0	515	4149.5
X	0.14071	7.5	53.3	633	4498.6
XI	0.16008	8.3	51.8	796	4972.5
PLI	0.17500	10.4	59.4	844	4822.9

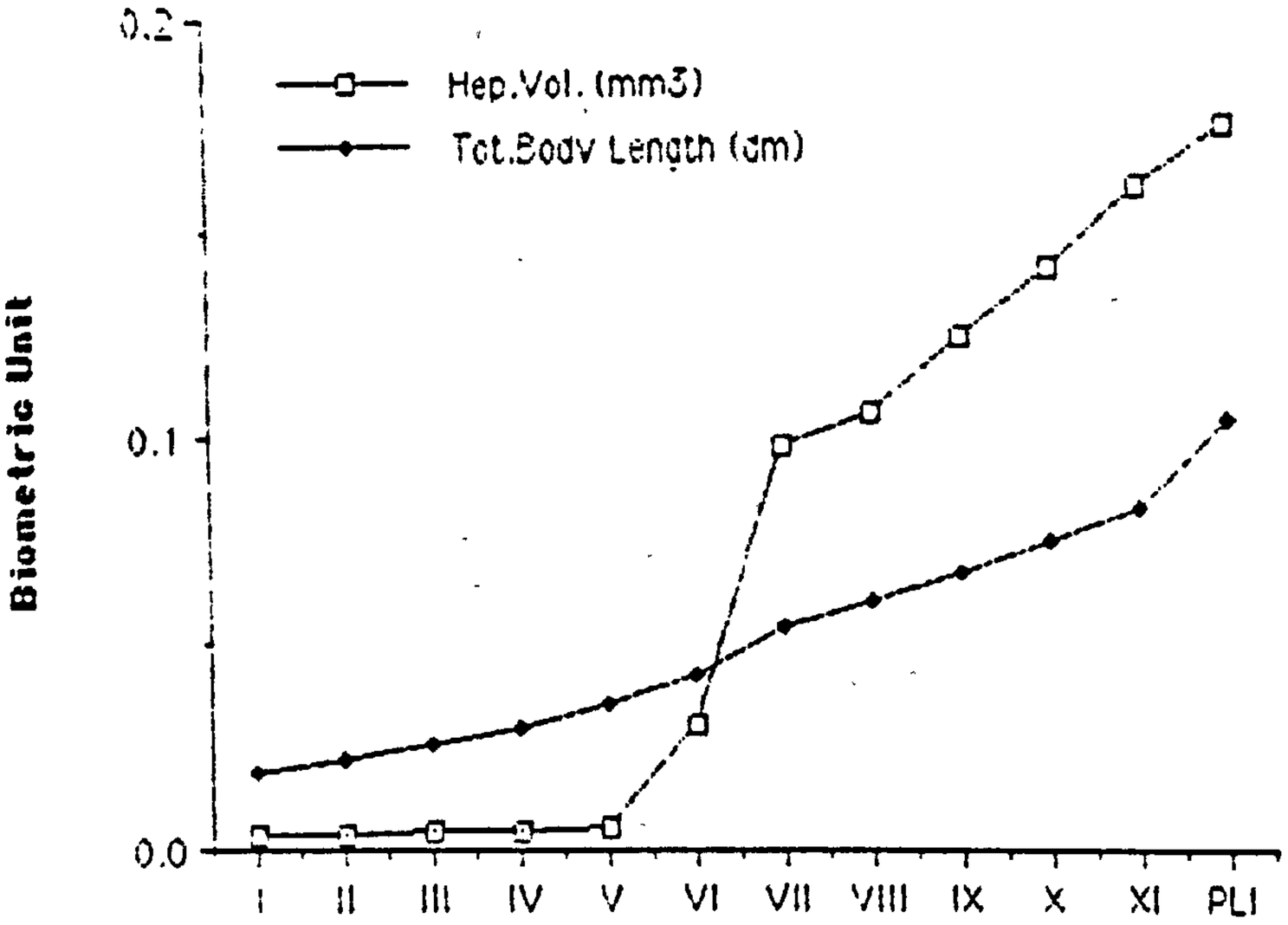
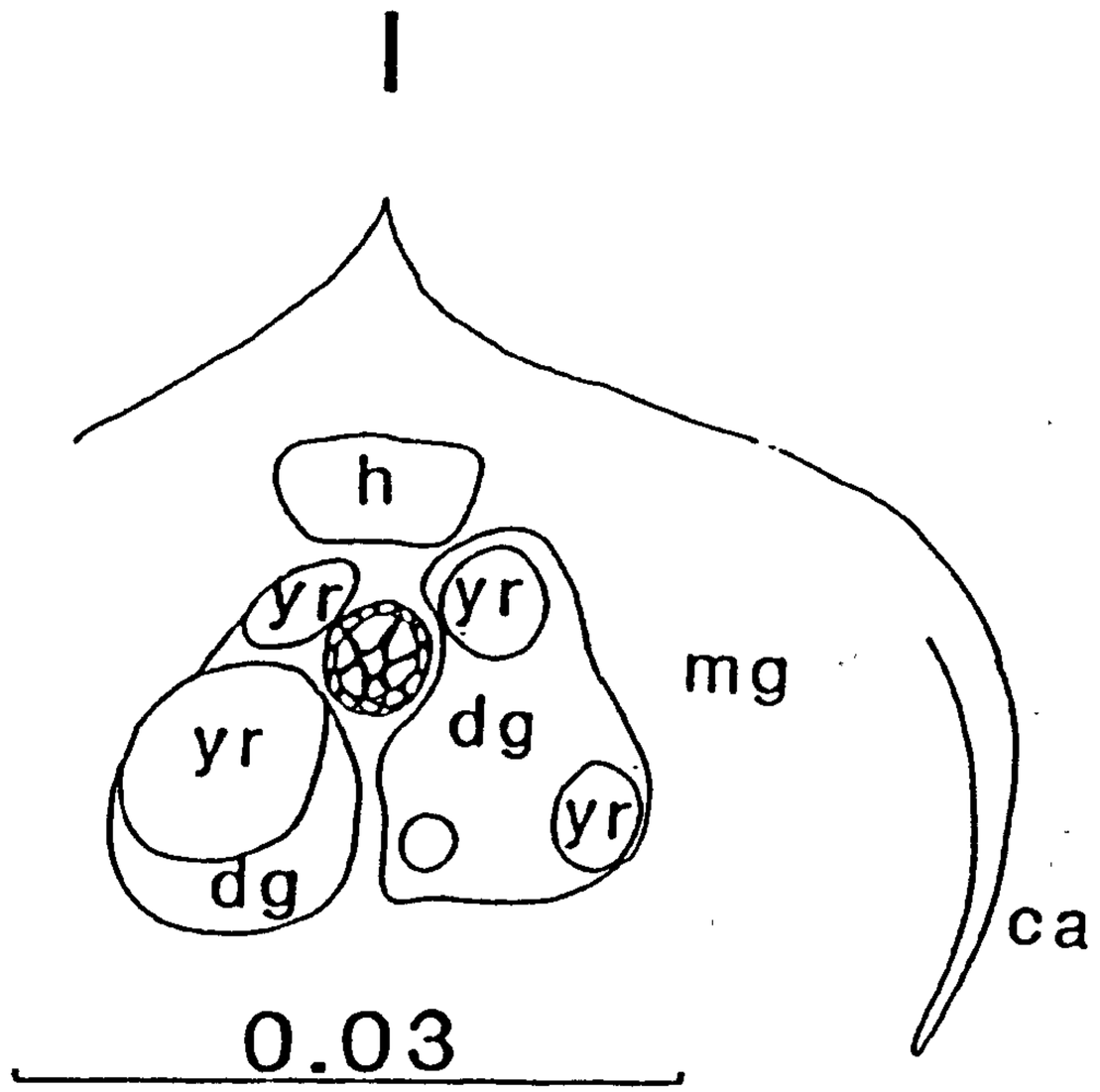


Figure 18. Increase in hepatopancreas volume and total body length during the larval development of M. rosenbergii (Biometric Unit = mm3 or dm)



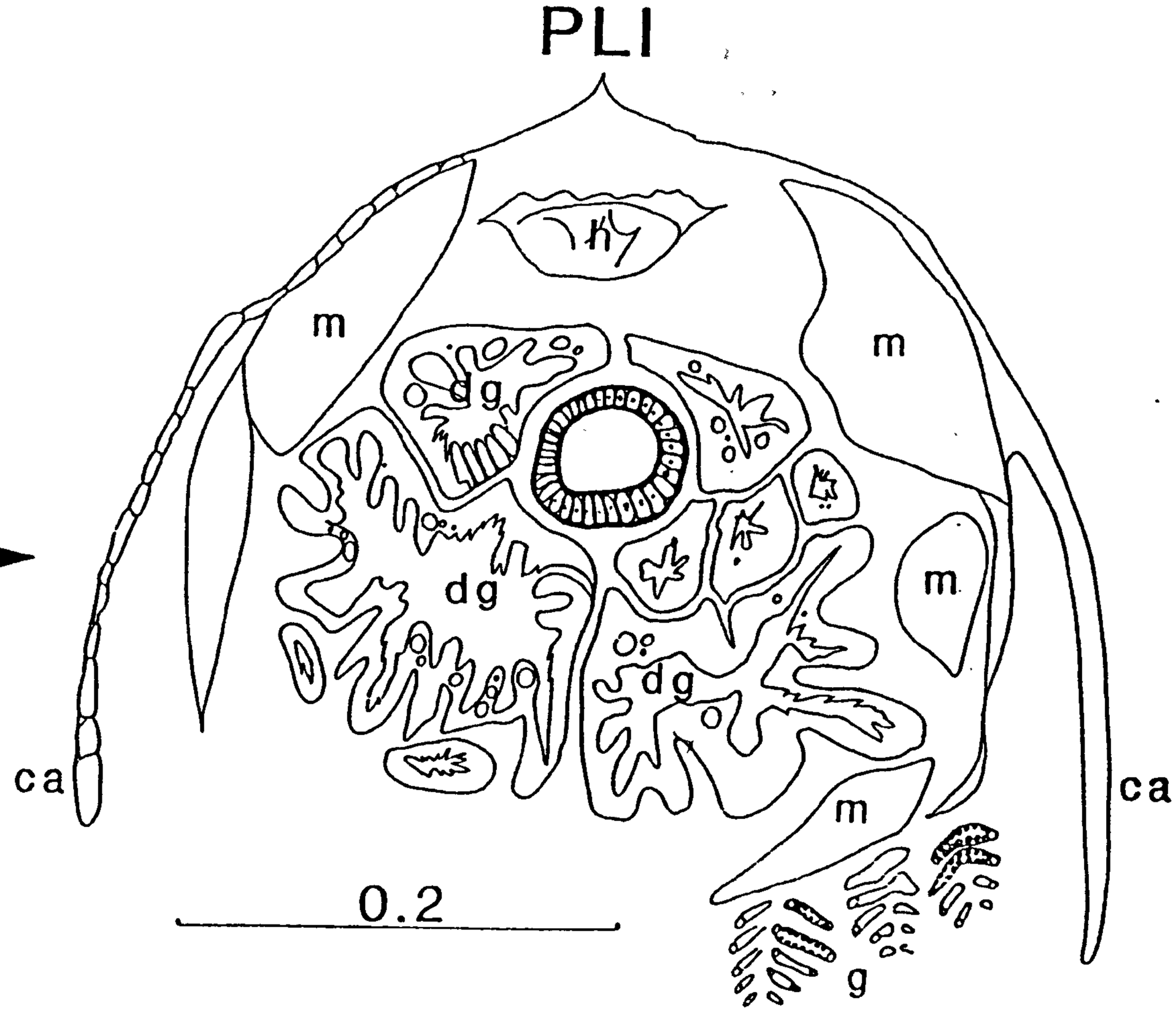


FIGURE 19. Schematic transverse section (fig.1 & 3, ee') of the anterior portion of M. rosenbergii midgut at the beginning and end of its larval development (bar = mm)

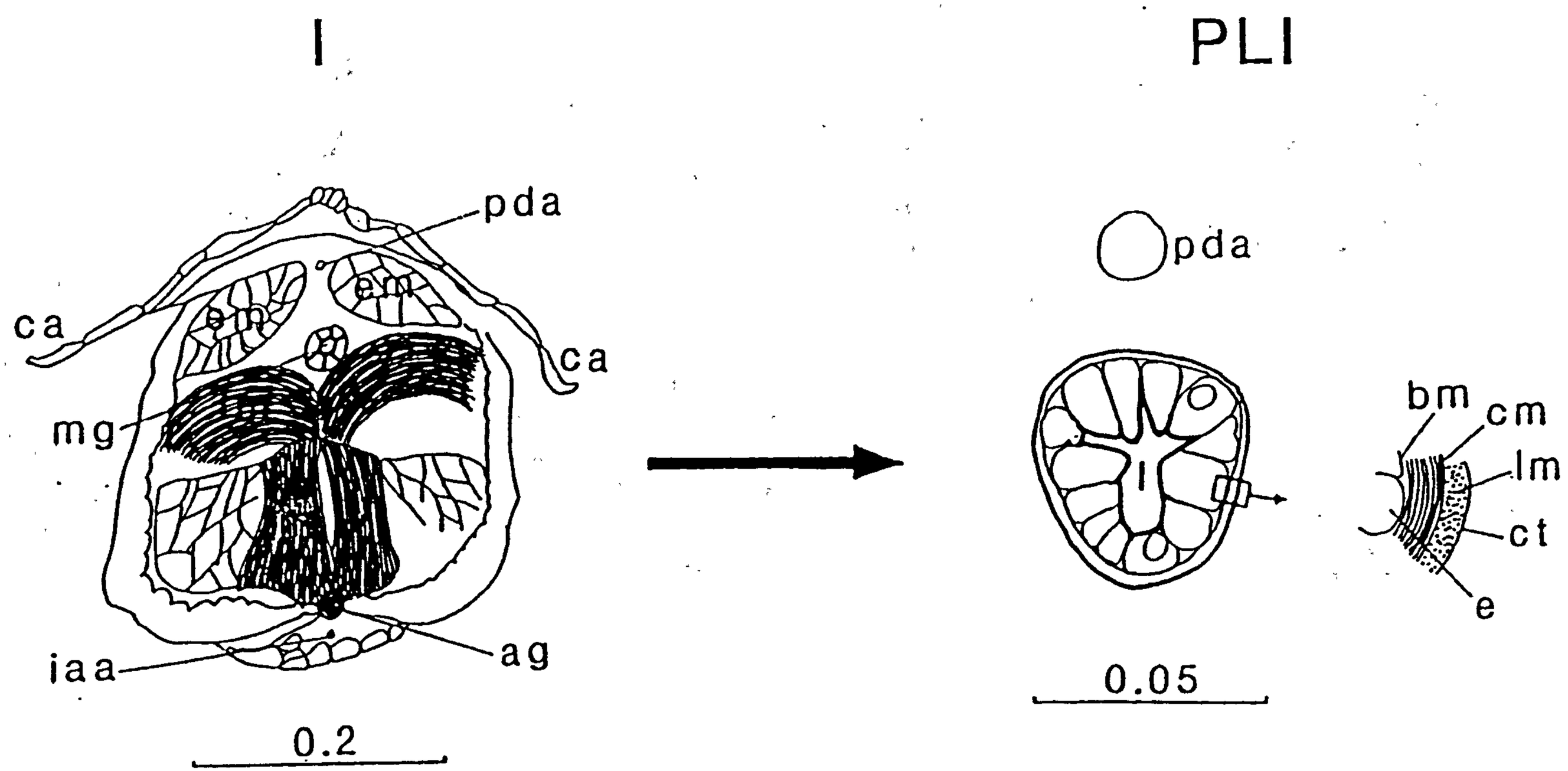


FIGURE 20. Schematic transverse section (fig.I & 3, ff') of the posterior portion of M. rosenbergii midgut at the beginning and end of its larval development (bar = mm)

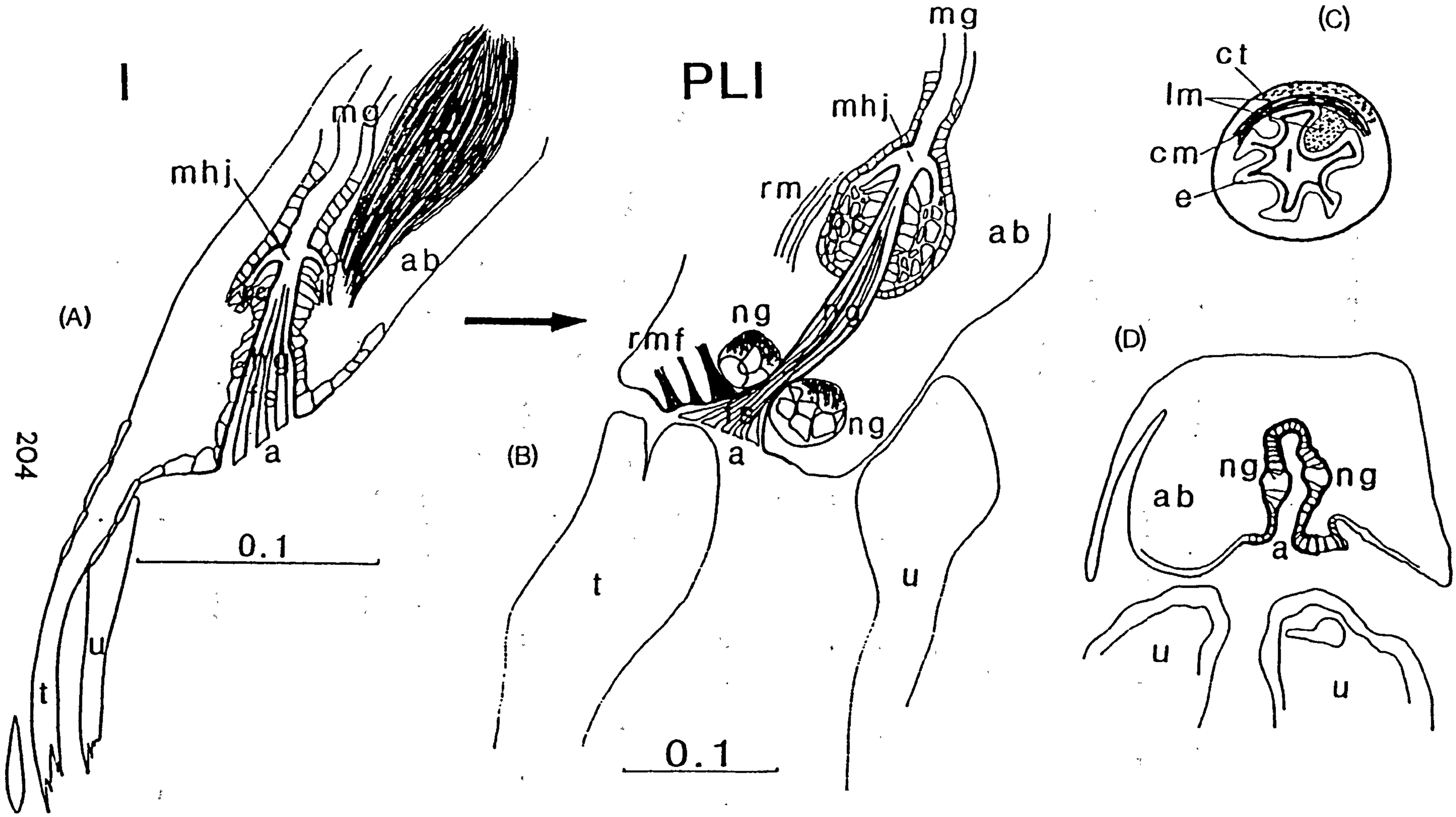


FIGURE 21. Schematic longitudinal (a & b) and transverse (c & d) sections (fig.1 & 3, gg') of M. rosenbergii hindgut at the beginning and end of its larval development (bar = mm)

2. Functional interpretation

During the first larval stages, M. rosenbergii rely on their embryonic lipid droplet reserves contained within the rudimentary hepatopancreas. Recently hatched larvae have been seen to contain these fatty globules for as long as four days (table 1). As the mouth and appendages are not very well developed at stage II, the larvae are only able to ingest small and soft dietary particles found in their environment. Maxillipeds are used for capturing prey whilst the maxillules and maxillae are employed for holding the food. The mandible is adapted for tearing soft animal feed with a poorly developed integument (See section IV). From stages VI onwards, the larval alimentary canal is well developed as the structures shown by sections are similar to those seen in later stages. Larvae are able to process larger particles more efficiently, thus taking advantage of a wider range of feeding opportunities. At the postlarval metamorphosis, the gut is similar to that found in juveniles and adults.

At the beginning of the larval development, M. rosenbergii slowly ingests prey, but as larvae develop the mouth its appendages become chitinous and a strong mandible helps

tearing the harder prey assisting faster ingestion (table 6). Figure 22 represents ingestion and gastroevacuation times at each larval stage. Figures 23a & b reveal the inverse relations between ingestion time and, larval foregut and total body length, respectively.

During the larval development, total length of the gut increases from 1.41 to 6.96 mm. The foregut takes less importance (36.6 to 17.8%) and the midgut expands from 56 to 72.7% (table 7).

Due to differentiation and specialization, the foregut progressively assumes its full function: at postlarval metamorphosis, a huge and muscular cardiac foregut expands, contracts and allows better crushing and partition of food into smaller particles, as well as mixing these particles with the hepatopancreatic juice. A complete filter apparatus in the pyloric foregut ensures that only fine dietary particles enter into the hepatopancreas. Larval gastroevacuation is rapid during the first stages (7.4-18.5 min.), but increasingly slows down so that by stages VI-VII it takes 47.6-51.3 min. This is predictable as the larval gut is simple during the first stages, then as the foregut specializes residence time of the ingested food in this section of the gut increases from stage V and more trituration occurs (table 6; figure 22).

TABLE 6. Ingestion and gastrovacuation time (1) for Macrobrachium rosenbergii at each larval stage

	Ingestion (min)		Gastrovacuation of different gut regions											
			Foregut (min)			Midgut (min)			Hindgut (s)			Total (min)		
Stage	Range	X (S.E.)	Range	X (S.E.)	%	Range	X (S.E.)	%	Range	X (S.E.)	%	Range	X (S.E.)	%
I (2)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II	4.0-6.0	5.0 (1.41)	2.5-5.1	3.8 (1.84)	51.6	2.1-4.9	3.5 (1.98)	47.5	3-5	4 (1.41)	0.9	4.7-10.1	7.4 (3.82)	100
III	3.5-5.2	4.4 (1.20)	4.2-16.6	10.4 (8.77)	56.3	3.0-13.0	8.0 (7.07)	43.3	3-5	4 (1.41)	0.4	7.3-23.7	18.5 (15.84)	100
IV	3.0-5.0	4.0 (1.41)	14.0-21.0	17.5 (4.95)	58.2	5.0-20.0	12.5 (10.61)	41.6	3-5	4 (1.41)	0.2	19.1-41.1	30.1 (15.56)	100
V	3.1-4.7	3.9 (1.13)	16.0-24.0	20.0 (5.66)	55.0	8.9-23.7	16.3 (10.47)	44.8	3-5	4 (1.41)	0.2	25.0-47.8	36.4 (16.12)	100
VI	2.5-4.5	3.5 (1.41)	22.2-31.0	26.6 (6.22)	55.9	12.0-29.8	20.9 (12.59)	43.9	3-5	4 (1.41)	0.1	34.3-60.9	47.6 (18.81)	100
VII	2.5-3.8	3.2 (0.92)	24.7-35.7	30.2 (7.78)	58.9	12.8-29.2	21.0 (11.60)	40.9	3-7	5 (2.83)	0.2	37.6-65.0	51.3 (19.37)	100
VIII	2.0-4.2	3.1 (1.56)	25.0-38.0	31.5 (9.19)	58.0	15.2-30.2	22.7 (10.61)	41.8	3-7	5 (2.83)	0.2	40.3-68.3	54.3 (19.81)	100
IX	1.6-4.1	2.9 (1.77)	27.1-40.5	33.8 (9.43)	58.4	17.9-30.1	24.0 (8.63)	41.5	3-7	5 (2.83)	0.1	46.0-70.7	57.9 (18.17)	100
X	1.2-4.0	2.6 (1.98)	28.1-42.5	35.3 (10.18)	58.1	19.1-31.7	25.4 (8.91)	41.8	3-7	5 (2.83)	0.1	47.3-74.3	60.8 (19.03)	100
XI	0.5-4.0	2.3 (2.47)	30.0-45.0	37.5 (10.61)	57.6	20.0-35.0	27.5 (10.61)	42.2	2-10	6 (5.66)	0.2	50.0-80.2	65.1 (21.36)	100
PLI	0.4-4.0	2.2 (2.55)	31.5-44.9	38.2 (9.43)	57.8	20.4-35.2	27.8 (10.47)	42.1	2-10	6 (5.66)	0.2	51.9-80.3	66.1 (20.08)	100
Increase (%)				1005			794						893	
Decrease (%)		56								22				

(1) determined with Cr203 prefed Artemia (average of 10 larvae)
(2) large fatty globules (yolk reserve) are utilized during this stage and larvae do not feed actively; after this time, food is readily ingested

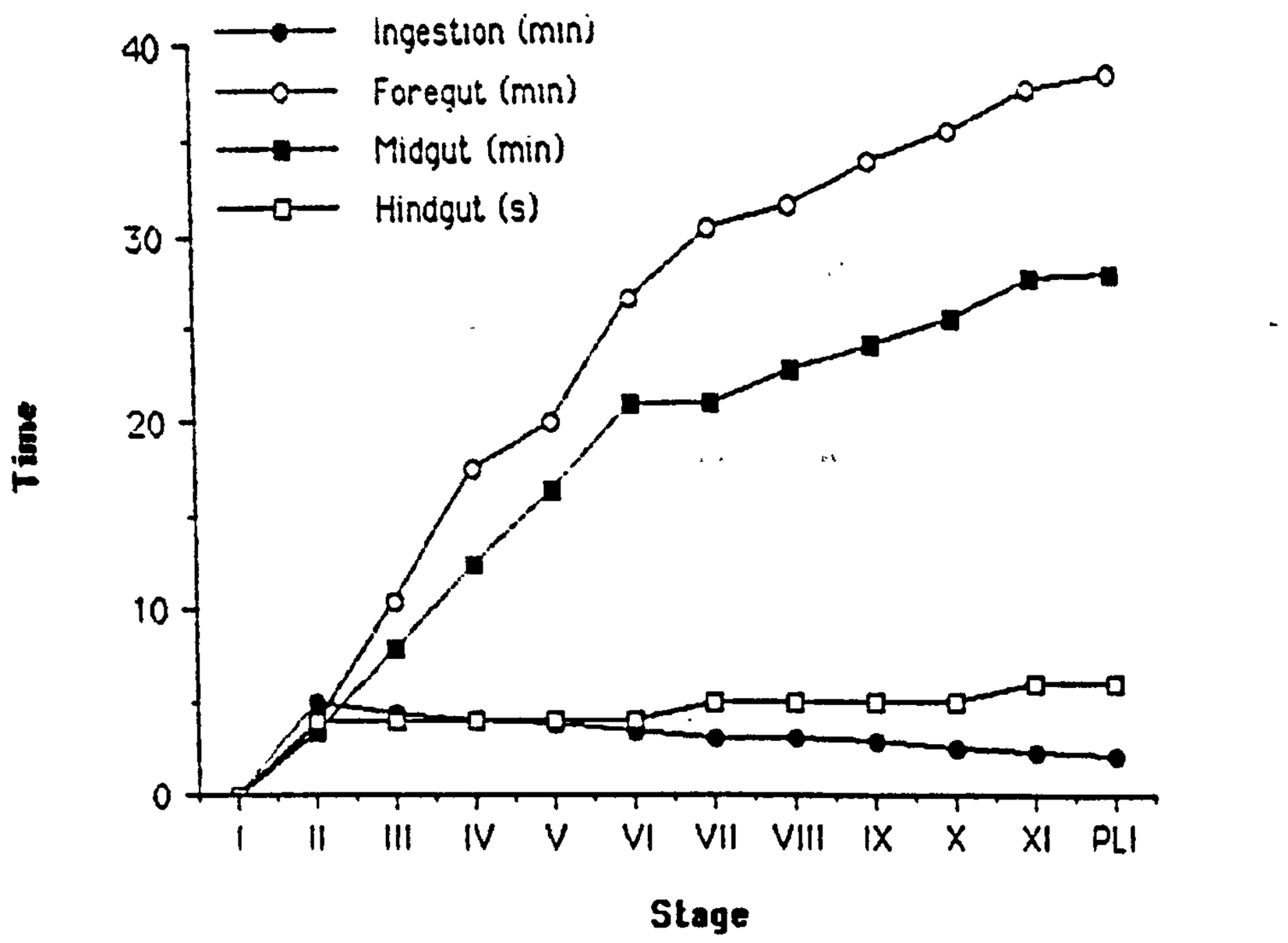
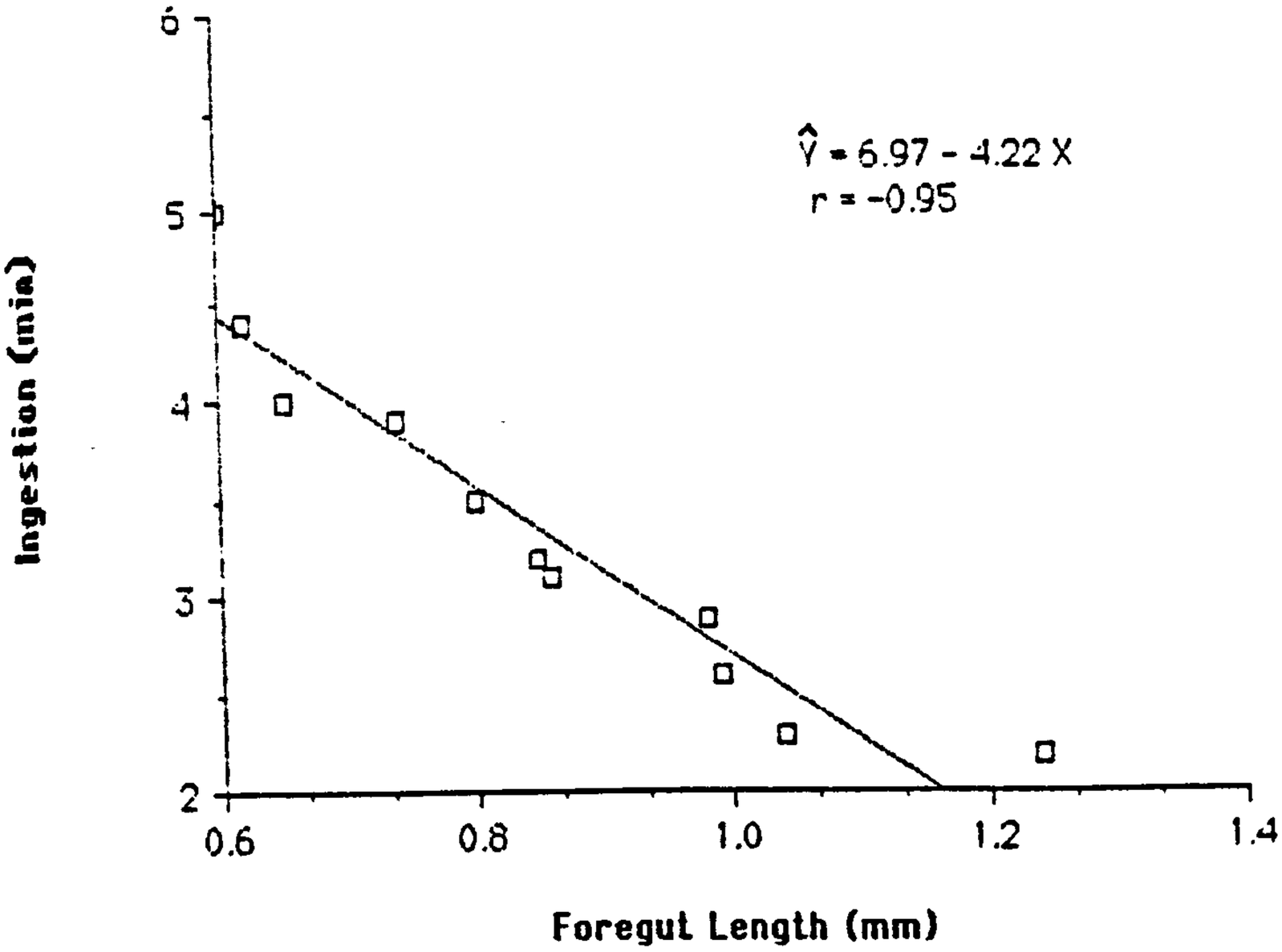


Figure 22. Ingestion and gastroevacuation time during the larval development of M. rosenbergii

a.



b.

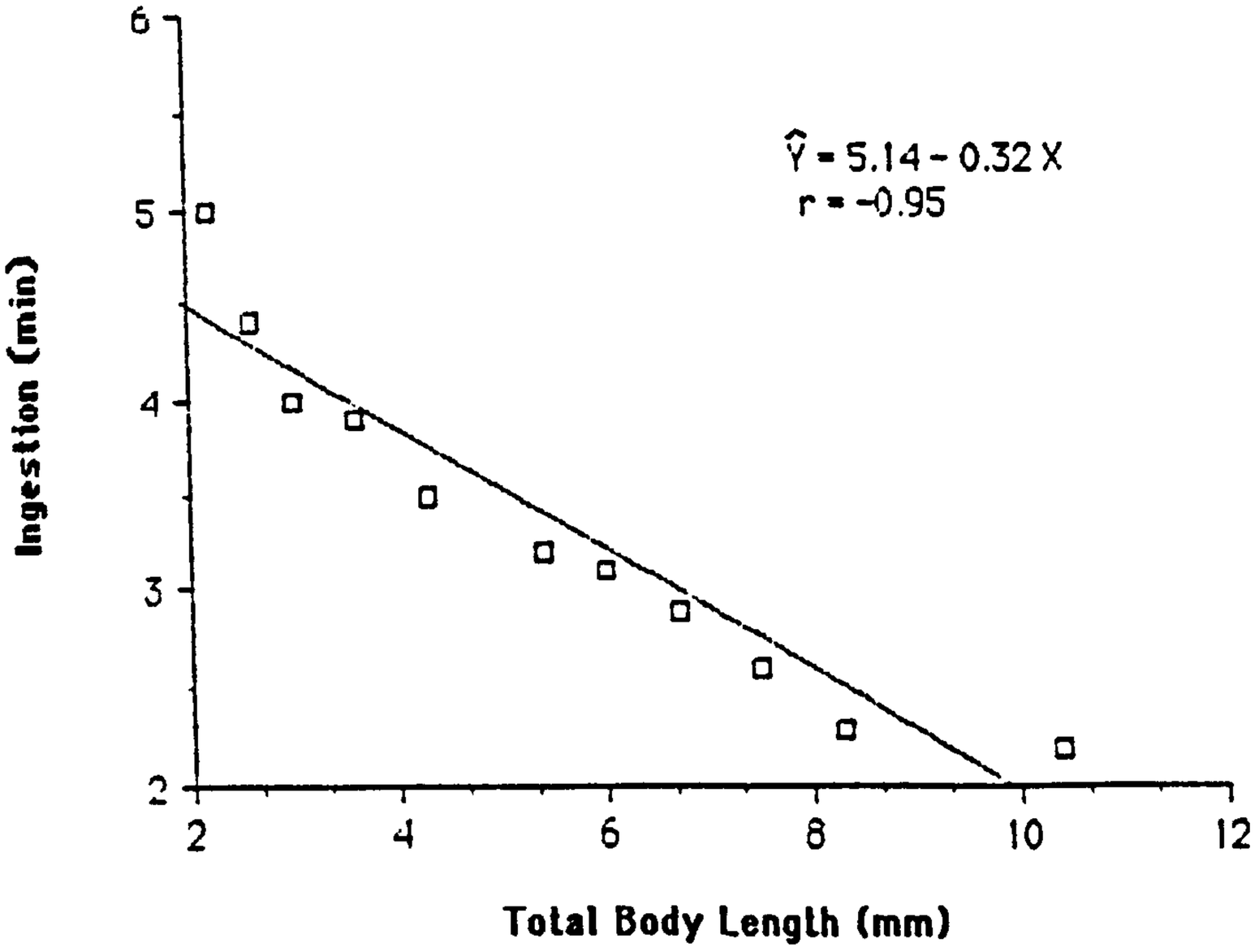


FIGURE 23. Ingestion time related to foregut (a) and total body length (b) in M. rosenbergii during the larval development

TABLE 7. Length of Macrobrachium rosenbergii gut at each larval stage

Stage	Foregut		Midgut		Hindgut		Total
	mm	%	mm	%	mm	%	mm
I	0.46	32.6	0.86	61.0	0.09	6.4	1.41
II	0.60	36.6	0.92	56.1	0.12	7.3	1.64
III	0.62	35.4	0.98	56.0	0.15	8.6	1.75
IV	0.65	30.7	1.27	59.9	0.20	9.4	2.12
V	0.74	28.0	1.65	62.5	0.25	9.5	2.64
VI	0.80	25.2	2.01	63.4	0.36	11.4	3.17
VII	0.85	22.2	2.52	65.8	0.46	12.0	3.83
VIII	0.86	20.0	2.94	68.2	0.51	11.8	4.31
IX	0.98	19.8	3.42	69.3	0.54	10.9	4.94
X	0.99	18.4	3.83	70.9	0.58	10.7	5.40
XI	1.04	18.9	3.87	70.2	0.60	10.9	5.51
PLI	1.24	17.8	5.06	72.7	0.66	9.5	6.96
increase % (I - PLI)	270		590		730		494

The volume of the digestive gland, or hepatopancreas, slowly increases during the first larval stages (0.00393-0.00583 mm^3) (table 5). However, it rapidly increases at stages VI and VII (0.03133 and 0.09844 mm^3, respectively) corresponding to a 17-fold volume increase for only a 1.25-fold increase in total body length but a 2.38-fold increase in body dry weight. The rhythmic contractions and expansions of the hepatopancreas assist in the discharge of digestive gland secretions into the ventral chamber of the pyloric foregut. These secretions dissolve the greater part of the dietary particles and forms the chyme which reenters the digestive gland in its turn for future absorption. From stages VI-VII, the presence of enzymes is clearly indicated by changes in colour of the food in the foregut . Fluid appears in the foregut lumen which confirms that digestion has been initiated. Undigestible feed particles enter the midgut and are evacuated in the form of " faecal pellets ".

In the midgut, an extremely thin and transparent peritrophic membrane invests the faecal pellet. Antiperistaltic movements help move the pellets towards the hindgut. Residence time of the food in the midgut rapidly increases at the beginning of the larval development, then remains the same for stages VI-VII, and only slightly increases during the later larval stages (table 6; figure 22).

The posterior midgut caecum is a small protuberance at the midgut/hindgut junction and remains simple during the whole larval development of M. rosenbergii.

The larval hindgut is extremely short and is restricted by the formation of rectal pads (figures 16-F). It has a similar form to that of M. rosenbergii adult. The faecal pellet only remains in this part of the larval gut for 4-6 sec.

The whole gastroevacuation time varies from 7.4 min., at stage II, to 126 min., at postlarval metamorphosis. Table 8 compares ingestion and gastroevacuation times for several diets presented to M. rosenbergii larvae. These appear to process live diets more rapidly than artificial diets: both larvae and postlarvae ingest Artemia (5 and 2.2 min., respectively) and B. plicatilis (6.2 and 2.5 min., respectively) more rapidly than Frippak encapsulated diets (10.2 and 3.1-3.6 min., respectively). Gastroevacuation is also faster when Artemia and B. plicatilis are given to M. rosenbergii larvae in comparison to Frippak diets (for larvae at stage II: 7.4 & 7.2 min. vs 7.7 min., respectively; for PL1: 66.1 & 68.5 min. vs 82.6-83.2 min., respectively). Microencapsulated diets apparently spend more time in the foregut than live Artemia and rotifers, as they

TABLE 8. Ingestion and gastroevacuation for *Macrobrachium rosenbergii* larvae when fed on several diets

	Feed			Ingestion		Gastroevacuation							
						Foregut		Midgut		Hindgut		Total	
Stage	Type	Size (um)	Density	min	S.E.	min	%	min	%	s	%	min	%
II	*Artemia* instar I	390-425	12.ml-1	5.0	1.41	3.8	51.6	3.5	47.2	4	0.9	7.4	100
	rotifer *Brachionus plicatilis* Muller	65-160	70.ml-1	6.2	1.43	3.6	50.2	3.5	48.8	4	0.9	7.2	100
	microencapsulated diet	0-20	8mg.l-1	10.2	1.65	4.1	53.5	3.5	45.7	4	0.9	7.7	100
PLI	*Artemia* instar I	390-425	12.ml-1	2.2	2.55	38.2	57.8	27.8	42.1	6	0.2	66.1	100
	rotifer *Brachionus plicatilis* Muller	65-160	70.ml-1	2.5	1.96	39.9	58.2	28.5	41.6	6	0.1	68.5	100
	microencapsulated diet	90-150	8mg.l-1	3.6	2.06	53.8	64.7	29.3	35.2	6	0.1	83.2	100
	microencapsulated diet	150-250	8mg.l-1	3.1	3.55	54.0	65.4	28.5	34.5	6	0.1	82.6	100

respectively remain 4.1, 3.8 and 3.6 min. at stage II and 53.8-54, 38.2 and 39.9 at PLI (table 8).

DISCUSSION

Macrobrachium rosenbergii is similar to other caridean decapods (Alpheus, Brooks & Herrick, 1891; Crangon, Regnault, 1972; Palaemonetes, Le Roux, 1971a,b), and penaeids (Penaeus setiferus, Lovett & Felder, 1989), in that it has no gastric mill present during any larval stage. These carideans also lack a gastric mill in the adult. In M. rosenbergii, as in Crangon and Palaemonetes, a complex arrangement of setae and spines is present during the later larval stages, but spines are lost at the postlarval metamorphosis as a functional filter apparatus more efficiently sorts the food (Khan, 1976). In other decapod larvae (Homarus, Panulirus, Pagurus, Cancer, Portunus and Menippe), the median and lateral teeth of the gastric mill are present at or before metamorphosis and in most genera the adult form of the foregut is attained one or two moults later (Lovett & Felder, 1989). In M. rosenbergii, the chitinous lining of the postlarval foregut does not have any

deposition of calcium which forms the complex food-crushing apparatus known as the gastric mill in some other groups of natantia decapods (Khan, 1976). Thus, it appears that the gut is not specialised for grinding large food particles with a hard coating. This deficiency appears to be compensated for by the development of a highly efficient mandible which undertakes the preliminary mastication. During larval stages VI and VII, cardiac and pyloric regions are well separated, the former becoming a large extensive bag, the latter being furnished with a more complete filter apparatus. Lovett and Felder (1990a) also observed that the filter apparatus is not present until the second mysis stage in P. setiferus. Like Khan's observations (1976), three distinct types of movement have been identified in the foregut during the feeding and digestion of M. rosenbergii: breakdown of food, compression for extraction of feed juices, and mixing of digestive enzymes by back- and forwards movements allowing prolonged enzymatic action on the feed. During the first larval stages, animals are unable to crush their feed due to the poorly chitinized mandible and oesophagus (figures 1, 4-E and 5). The breakdown of feed occurs more efficiently later in the larval development, and after the postlarval metamorphosis (figures 2, 3, 4-F, 6 and 10-A & B). Compression is achieved by alternate expansion and contraction of the oesophagus and foregut. This was also observed by Khan (1976) and, for other palaemonid prawns, by

Le Roux (1971b). M. rosenbergii shows a capacity for considerable foregut dilatation during feed ingestion. Early in its larval development, there is no major functional difference between the cardiac and the pyloric foregut because there is no filtration of feed in the latter due to the lack of a functional filter apparatus. This is also reported by Khan (1976) and for the protozoeal stages of Penaeus setiferus (Lovett & Felder, 1989). These different modes of functional operation of the larval and adult foregut may be correlated with the type of diet. The simple larval gastric armature is limited to the compression and churning of soft feed rather than grinding, and this appears to be sufficient to deal with the tissues of small prey such as nauplii of Artemia and rotifers which have a thin integument. In contrast, the adult is omnivorous (Ling, 1967) with a strong, differentiated and muscular gastric armature. A cardio-pyloric valve develops between stages III and VI and divides the foregut into cardiac and pyloric chambers (figures 2 & 11). This was also observed by Lovett & Felder (1989) during the mysis stages of Penaeus setiferus. Furthermore, the filter apparatus of M. rosenbergii is absent at stage I and clearly appears at stage III. It starts to be functional at stages VI-VII and is well developed at the postlarval metamorphosis. Lovett and Felder (1989) also observed that this filter appears at the mysis stage (M2) for P. setiferus and only consists of

two pairs of longitudinal channels. At the postlarval metamorphosis (PL1), the filter has 7-8 pairs of channels in M. rosenbergii compared to only 3-4 pairs in P. setiferus. At this stage, the function of the pyloric foregut appears solely to act as a filter and mixing apparatus. The median pyloric ridge is extremely simple and is equipped with a single apical rod-like projection (figure 15-C, D, E, F & G).

Several investigators clearly demonstrated in adult decapods that this filter acts as a sieve that excludes particles larger than 1 µm in diameter from the hepatopancreas (Bayer et al., 1979; Powell, 1974; Hopkin & Nott, 1980). It also appears to act as a pump that moves the chyme between cardiac chamber, dorsal pyloric chamber, and midgut and agitates this chyme to mix enzymes and emulsifiers. This was also noted by Lovett and Felder (1990a) working on the larvae and postlarvae of P. setiferus. In the adult prawn, the filter apparatus of M. rosenbergii is mobile and the chyme can flow under this filter between the openings of the digestive gland (figure 10-C, arrow) and the pyloric foregut (figures 15-E, F & G). Small holes also apparently allow the chyme to communicate directly with the longitudinal canals of the filter, between the setal rows on each side of the median pyloric ridge (figure 15-D, arrow). Numerous brushed setae cover the walls of the pyloric foregut on each side of the filter and form a complex structure which allows this

chyme to move to and from preventing the entry of bigger particles in the digestive glands (figure 15-C & D).

From larval stage V, the hepatopancreas of M. rosenbergii enormously increases in volume, passing from 0.00583 mm^3 at stage V to 0.09844 mm^3 at stage VII, a 17-fold increase in size (table 5). This important change in hepatopancreas volume is much greater than changes in body size (figure 18). Hence, presumably a much greater volume of enzyme secretion becomes available. Contractions and expansions of the hepatopancreas assist in the discharge of these enzymes into the foregut where food now spends more time allowing longer enzymatic action. In contrast, residence time of food in the midgut remains the same during larval stages VI and VII and only slightly increases after this period. Hence, efficiency in digestion must be improved otherwise a similar increase in residence time might be expected. The only explanation for these observations is that enzyme production increases. Increase in food retention time probably reflects an increase in the efficiency of digestion and assimilation in M. rosenbergii larva from stage V. In Penaeus spp. (Abubakr, 1987; Lovett & Felder, 1989), the well developed anterior diverticulae present at Z1 progressively decrease during the later larval stages, giving way to an expanding hepatopancreas which takes its full size only after the postlarval metamorphosis. The

increase in size of the hepatopancreas in the caridean M. rosenbergii and in penaeids, with higher enzyme production must be greatly responsible for the successful survival on artificial diets. In P. monodon, the anterior diverticulae play a major role in the acceptance of artificial diet in early larval stages as they release enzymes at the beginning of larval development (Abubakr, in press). In M. rosenbergii, a similar organ or anterior diverticulum is absent, and before larval stage V, the hepatopancreas is undeveloped, hence there is no or little endogenous enzymes to breakdown the food. This fact alone may be the reason why such caridean larvae do not survive on artificial feeds.

At stages VI-VII, the drastic increase in the hepatopancreas volume corresponds to a delicate period during which rates of mortality are often very high (Ling, 1969a; Wickins, 1976a; New & Singholka, 1985). During this period, the larvae are probably under stress due to these morphological changes, but do not appear to have any problem feeding. However, they can be more susceptible to external constraints such as water quality and agressivity due to high density, as mortality more heavily occurs under intensive rearing (personal observations). Lovett & Felder (1989) also found that a critical period of high mortality in P. setiferus, between PL_1 and PL_{10} corresponds to an increase in the

hepatopancreas volume which is not isometric with growth of the body as intensive ramification of the organ occurs. Perhaps, this suggests that osmotic stress would be much higher as the gut volume increases greatly and water loss could be rapid if salinity changed.

For M. rosenbergii, the early carnivorous larval stages which lack efficient methods of mechanical breakdown of food and enzymatic activity, are adapted to ingest easily degradable feeds, such as live Artemia and rotifers. These already contain their own enzymes which aid digestion in the larval M. rosenbergii gut. Munilla-Moran et al. (1990) recently demonstrated the significance of exogenous enzymes obtained from the digestion of live diets in the alimentary system of carnivorous fish larvae. They also mention that the potential enzymatic supply from Artemia nauplii depends on its nutritional state since enzyme activity levels of starved Artemia nauplii are lower than those in fed nauplii. This explains why M. rosenbergii fed Artemia which have ingested algae produce better growth at the beginning of the larval development. Lovett and Felder (1990b,c) also observed a strong increase in enzyme levels with increasing age in Artemia. Similar findings have been reported by Marco et al. (1980) and Samain et al. (1980). All these authors agree that from the start of the first feeding, exogenous digestive enzymes appear to play an important role in larval

digestion. Lovett and Felder (1990b,c) demonstrated that peak activities for all enzymes occurs during late protozoeal or mysis stages in P. setiferus. Low activity occurs at postlarval metamorphosis for this marine prawn. The same authors also noticed that diet does not appear to be the primary factor causing change in digestive enzymatic activity. They suggest that change in such activity reflects either a developmental change in enzyme synthesis, or a secondary effect of change in the function and relative size of the midgut during its differentiation.

It would be interesting to identify the major enzymes (proteases, esterases, amylase) and their occurrence and activity in relation to the larval development in M. rosenbergii.

No anterior midgut caecum is found in M. rosenbergii larvae but a small antero-dorsal fold develops at stage VI and remains underdeveloped at postlarval metamorphosis. This is also the case in the caridean Palaemonetes varians (Le Roux, 1971a) which has a well developed dorsal fold at larval stage IV. However, it contrasts with the brachyuran Carcinus maenas which already has two short and narrow tubular blind ending antero-tubular midgut caeca during the zoeal stages (Khan, 1976; Brewster, 1987). This also contrasts with Penaeus monodon (Abubakr, 1987) and P. setiferus (Lovett &

Felder, 1989), where well developed anterior caecae are present at protozoeal stages, and degenerate into a small protuberance by the end of mysis stages. By the fifth day, M. rosenbergii larvae have completely utilised their lipid reserves (table 1), whilst the digestive gland has increased in volume (table 17.0, appendix). This growth is similar to that of the digestive gland for larvae of P. monodon (Abubakr, 1987) (table 9) and P. setiferus (Lovett & Felder, 1989) which differentiates into several distinct lobes, ramifies into small-diameter tubules, and increases substantially in length during the mysis stages. However, there is no sign of a lipid reserve in Penaeus species where anterior dorsal diverticae (caecae) appear to act as a functional hepatopancreas from the first larval stage.

Table 10 summarizes the presence of important regions in the alimentary canal for several larval and postlarval decapods. From this table, it can be deduced that Palaemon elegans, Procambarus clarkii and Macrobrachium rosenbergii belong to the most primitive stage in the evolution as far as the gut is concerned. Penaeus monodon and Homarus gammarus represent a more advanced stage in which trituration of feed occurs in the cardiac foregut, and Carcinus maenas represents the most advanced condition with a fully functional gastric mill, a well developed digestive gland and extended anterior caecae.

TABLE 9. Comparison between the hepatopancreas volumes of Penaeus monodon and Macrobrachium rosenbergii at (proto)zoeal I and post-larval stages

	Penaeus monodon (1)		Macrobrachium rosenbergii	
Stage	Total body length (mm)	Hepatopancreas volume (mm3)	Total body length (mm)	Hepatopancreas volume (mm3)
(Proto)Zoea I	1.66	0.00104	1.9	0.00393
PLI	4.33	0.03360	10.4	0.17500
Increase %	261	3231	547	4453

(1) Abubakr, 1987

TABLE 10. Comparison of different regions of the alimentary canal in several larval and post-larval decapods

Species	Stage	Length (mm)	Large yolk reserve	Strong external masticatory appendages	Foregut		Hepatopancreas	Midgut		diet
					Gastric mill	Filter		Anterior caecum (caeca)	Posterior caecum	
Penaeus monodon	PZI	1.07	-	-	-	(+)	(+)	+	+	phytoplanctivorous
(1)	PLI	4.99	-	-	+	+	+	(+)	+	carnivorous
Carcinus maenas	PZI	1.45	(+)	-	(+)	(+)	(+)	(+)	+	omnivorous
(2)	PLI	2.03	-	+	+	+	+	+	+	carnivorous
Palaemon elegans	ZI	3.50	+	+	-	(+)	+	-	?	omnivorous ?
(3)	PLI	7.50	-	(+)	-	+	+	-	?	carnivorous
Macrobrachium rosenbergii	II	2.20	+	(+)	-	(+)	+	-	+	omnivorous
	PLI	10.40	-	+	-	+	+	(+)	+	carnivorous
Homarus gammarus	I	6.40	+	+	(+)	(+)	+	+	?	carnivorous ?
(4)	PLI	14.30	-	+	+	+	+	(+)	+	carnivorous
Procambarus clarkii	Instar I	4.50	+	-	(+)	(+)	+	?	-	omnivorous
(5)	PLI	7.60 ?	-	+	+	+	+	+	-	omnivorous

+ present (+) intermediate, underdeveloped or reduced - absent

(1) Patsy & McLaughlin, 1985; Abubakr, 1987; Bell & Lightner, 1988
(2) Khan, 1976; Rice & Ingle, 1975; Bauchau, 1966
(3) Hoglund, 1943; Le Roux, 1971
(4) Patsy & McLaughlin, 1985; Brewster, 1987; Barker & Gibson, 1977
(5) Huner & Barr, 1984

When ingestion and gastroevacuation are compared at the beginning and end of the larval development for several decapods, it is apparent that these processes are most rapid in M. rosenbergii (table 11). The larvae continually ingest food if the foregut is not full, and food apparently does not stay as long in the foregut of M. rosenbergii as in other crustaceans. Larvae feed and defaecate virtually continually at the beginning of their development, but meals are more spaced out towards the end of larval development. Intensive observation reveals that food is processed for a much longer time in the foregut than in the other sections of the gut. As larvae develop, the gastroevacuation increases in time as the total retention period increases from 7.4 min., at stage II, to 66.1 min., at PLI. Increase in retention time of food in the foregut is mostly marked as the tubules of the hepatopancreas differentiate and the foregut reaches a well structures organ around stages VI-VII. Working on P. setiferus, Lovett and Felder (1990a) noticed that increase in feed retention time, decrease in gut motility and increase in time between successive feedings in postlarval stages which may reflect an increase in efficiency of digestion and assimilation. M. rosenbergii juveniles and adults apparently ingest no more than what they can effectively assimilate at one time (Condrey et al., 1972).

TABLE 11. Ingestion and gastroevacuation for several larval crustacean decapods

Species	Diet	Ingestion (min)	Gastroevacuation of different regions: Foregut (min)	Foregut %	Midgut (min)	Midgut %	Hindgut (min)	Hindgut %	Total (h:min:s)
Macrobrachium rosenbergii									
Larvae stage II	*Artemia*	5.0	3.8	51.6	3.5	47.5	0.1	0.9	00:07:24
Post-larvae stage I	*Artemia*	2.2	38.2	57.8	27.8	42.1	0.1	0.2	01:06:06
Homarus gammarus (1)									
Larvae stage I (2)	Frozen mysids	6.7	101.0	42.0	134.5	56.0	4.7	2.0	04:00:12
Post-larvae	Frozen mysids	-	-	-	-	-	-	-	14:00:00
Palaemon elegans (1)									
Larvae	*Artemia*	-	-	-	-	-	-	-	02:00:00
Post-larvae	Frozen mysids	2.7	261.0	74.0	85.0	24.1	6.7	1.9	05:52:42
Penaeus monodon (3)									
Larvae stage I	Algae	-	-	-	-	-	-	-	00:12:00
Post-larvae stage I	Frozen mysids	-	-	-	-	-	-	-	03:00:00

(1) Brewster, 1987
(2) Feeding occurs only 12 hours after hatching
(3) Jones, 1988

From stage II, larvae readily ingest live prey. The plasticity of Artemia and rotifers allows such feed to enter the gut orifice easily and to be ingested without any difficulty. Exogenous enzymes coming from Artemia help the breakdown of food in M. rosenbergii. However, Frippak microencapsulated diets are handled for a longer time by the mandible, maxilla, maxillipeds and first pereiopods before ingestion. Close observation reveals apparent difficulties in breaking down the microcapsules, to allow entry into the alimentary canal, despite initial size selection of microcapsules at each stage (ref. table 12.0, appendix). Difficulty in breakdown and digestion of microcapsules may be the reason for their longer residence period in the foregut when compared to Artemia and rotifers. This is not seen in larval Penaeus monodon which ingest the same artificial diets without difficulty, due apparently to a greater oesophageal dilatation. However, as food reaches the foregut of M. rosenbergii, breakdown of the food takes place more efficiently: at the beginning of the larval development, food churning is secured by a huge and non-differentiated pyloric foregut, but as postlarval metamorphosis approaches grinding and mixing are secured by a bigger and muscular cardiac foregut. At each larval stage, breakdown of the feed inside the foregut occurs as compressed faecal pellets are produced. This was clearly seen under binocular microscope as the foregut strongly

contracts and extends to reduce the dietary bulk to a compacted volume.

From stage VII, digestion of artificial feeds also occurs as gastric juices and chyme are formed. Changes in colour inside the foregut and digestive gland indicate further digestive interactions between the juices and the microcapsules. This could be clearly observed, but strong pigmentation appearing on the integument of the larvae made observation more difficult towards the end of larval development. However, M. rosenbergii larvae never survived on Frippak diets during the early larval stages (ref. table 2, section III). Almost certainly the 17-fold increase in size of the hepatopancreas during stages V-VII is directly correlated with the ability of larvae to digest and assimilate artificial diets successfully. In contrast, P. monodon and setiferus have large anterior caecae actively producing enzyme secretion from Z_1 which enables these larvae to digest artificial diets. As the larvae of Homarus, Palaemonetes, Palaemon and Carcinus are also unable to survive on artificial feeds during early larval stages, it is proposed that this may be due to the absence of functional anterior caecae and or the relatively small size of the hepatopancreas. The absence of this structure in M. rosenbergii could be well responsible for the inability of this animal to digest and assimilate microcapsules

during early larval stages. Support for this view is also demonstrated by the fact that both C. maenas and H. gammarus larvae also failed to survive when fed on encapsulated feed presumably due to the non functional nature of their anterior caecae. The absence of a gastric mill in carideans may also mean that ingested prey must be high in readily assimilable nutrients and energy content. Perhaps the diet has to be autodigestible, at least at the beginning of the larval development (Munilla-Moran et al., 1990). It is also possible that M. rosenbergii may not find enough energy for its requirements from encapsulated feeds although these provide all the requirements of P. monodon larvae (Jones & Kurmaly, 1987).

Abubakr and Jones (in press) suggest that the presence of the large anterior caecae in penaeid larvae are an adaptation for phytoplankton feeding. This adaptation may not be necessary in the carnivorous larvae of M. rosenbergii larvae which rely on prey autolysis for digestion during early larval life.

As larvae of M. rosenbergii do survive and reach their postlarval metamorphosis when fed solely on artificial diets from stage VI (ref. tables 4 & 5, section III) efficient digestion and assimilation must occur and exogenous enzymes from the diet are no longer required. Similarly, artificial

diets capable of sustaining good growth for penaeid larvae are ineffective when fed to recently hatched Homarus and Palaemon larvae. Final confirmation of the cause requires evaluation of enzyme activity during development for these species.

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APPENDIX

TABLE 1.0. Pellet composition (1)

Ingredient	%
Squid	26.0
Shrimp meal	30.0
Wheat flour	28.0
Rice starch	11.0
Cod liver oil	2.0
Tapioca	1.0
Premix (microcapsules)	2.0
Protein	35.0
Fat	6.4

(1) Prepared at the Marine Science Laboratories, U.C.N.W., Menai Bridge

TABLE 2.0. Comparison of corresponding morphological larval stages for Penaeus monodon and Macrobrachium rosenbergii

Penaeus monodon stage (1)	Macrobrachium rosenbergii stage (2)	Prominent characteristic
Egg - NI-VI	Egg	-
PZI	I	Sessile eyes
PZII	II	Stalked eyes
	III	Two uropods
PZIII	IV	Two dorsal teeth on the rostrum
	V	Four uropods
MI	VI	Pleopod buds appear
MII	VII	Pleopods appear (biramous on Macrobrachium)
MIII	VIII	Longer pleopods
	IX	Endopods of pleopods with appendices internae
	X	Three or four dorsal teeth on rostrum
	XI	Teeth on half of upper dorsal margin
PLI	PLI	Straight line body swimming

(1) Gurney, 1960; Motoh, 1984
(2) Ling, 1967; Uno & Kwon, 1969; New & Singholka, 1985

TABLE 3.0. Final stage of *Artemia* (1) fed *Macrobrachium rosenbergii* larvae after 8 days when *Chlorella vulgaris* (Beijer) was added to the rearing water

	Algal Concentration (Cells per µl)								
Larva #	295	456	461	537	545	630	739	781	1533
1	III	II	III	IV	V	V	V	IV	III
2	II	III	III	IV	V	V	IV	IV	III
3	III	III	IV	V	IV	V	IV	IV	III
4	III	III	IV	IV	IV	V	V	III	II
5	III	II	IV	V	V	V	V	III	II
6	III	III	IV	V	V	IV	V	III	II
7	III	IV	IV	V	V	V	V	IV	III
8	III	IV	IV	V	V	V	V	V	III
9	IV	III	IV	V	V	IV	IV	V	IV
10	III	III	IV	V	V	V	V	IV	III
11	III	III	V	V	V	IV	IV	IV	IV
12	III	IV	IV	V	V	V	V	IV	III
13	II	III	IV	V	V	V	IV	IV	III
14	IV	III	IV	V	V	V	V	IV	III
15	III	III	IV	V	V	IV	IV	IV	III
16	III	III	IV	VI	IV	V	V	IV	III
17	III	III	IV	V	V	V	V	IV	III
18	III	III	V	V	V	V	IV	IV	III
19	II	II	IV	V	V	IV	V	III	II
20	III	III	IV	V	V	V	V	III	III
21	III	IV	V	V	V	V	V	IV	II
22	III	III	III	V	V	V	V	V	III
23	IV	III	IV	V	V	V	V	IV	III
24	III	III	IV	V	V	V	V	IV	III
25	III	IV	IV	V	V	V	V	IV	III
X	3.00	3.08	4.00	4.92	4.88	4.80	4.72	3.92	2.88

(1) 12 *Artemia* per ml

TABLE 4.0. Total length (in mm) of Macrobrachium rosenbergii post-larvae (PLI) reared (1) and fed (2) in light (3)

PL #	1	2	3	X
1	7.50	7.75	7.25	7.50
2	8.25	9.00	7.50	8.25
3	9.50	11.00	10.25	10.25
4	9.00	10.00	8.00	9.00
5	7.25	7.25	8.00	7.50
6	8.75	8.00	8.00	8.25
7	8.00	8.00	8.00	8.00
8	8.25	8.00	9.25	8.50
9	8.50	9.00	8.00	8.50
10	10.50	10.25	10.00	10.25
11	8.50	9.50	9.00	9.00
12	10.00	9.50	9.75	9.75
13	9.50	9.50	9.50	9.50
14	8.75	8.00	8.00	8.25
15	10.50	10.25	10.75	10.50
16	7.75	8.00	7.50	7.75
17	7.50	7.25	7.75	7.50
18	10.75	11.00	11.25	11.00
19	10.50	10.75	10.25	10.50
20	10.25	9.50	9.50	9.75
x	8.98	9.08	8.88	8.96
S.E.	1.14	1.23	1.22	1.12
range	7.25-10.75	7.25-11.00	7.25-11.25	7.50-11.00
Survival % (S.E.)	23	18	22	21 (2.65)
Days to reach PL's (S.E.)	33	42	33	36 (5.20)

(1) In triplicate
(2) 12 Artemia per ml with Chlorella vulgaris at 553 + 71 µl-1 (temperature: 29 + 0.5oC; pH: 7.76 + 0.39; larval density: 75.ml-1; light intensities measured with an irradiance collector, model no QSL-100)
(3) 12.044 x 1014 µe.s-1.cm-2 or 2.0 x 1014 quanta.s-1.cm-2 or 2.89 x 1014 W.cm-2 or 1.927 x 1021 lx

TABLE 4.1. Total length (in mm) of Macrobrachium rosenbergii post-larvae (PLI) reared (1) and fed (2) in dark (3)

PL #	1	2	3	N
1	9.00	11.00	10.00	10.00
2	9.50	10.25	9.50	9.75
3	10.75	11.00	11.25	11.00
4	11.25	11.50	11.75	11.50
5	11.00	10.50	10.00	10.50
6	9.00	9.50	10.00	9.50
7	9.75	8.25	8.25	8.75
8	9.75	9.75	10.50	10.00
9	12.00	12.25	11.75	12.00
10	12.25	12.00	12.50	12.25
11	9.00	9.75	9.75	9.50
12	10.50	10.00	11.00	10.50
13	11.50	12.25	12.25	12.00
14	9.00	9.00	8.25	8.75
15	9.50	9.50	9.50	9.50
16	9.75	8.75	9.25	9.25
17	10.25	10.75	10.50	10.50
18	8.75	9.50	8.75	9.00
19	11.75	11.50	11.25	11.50
20	10.25	11.25	10.75	10.75
x	10.23	10.41	10.34	10.33
S.E.	1.10	1.17	1.24	1.11
range	8.75-12.25	8.25-12.25	8.25-12.50	8.75-12.25
Survival % (S.E.)	56	52	51	53 (2.65)
Days to reach PL's (S.E.)	23	24	25	24 (5.20)

(1) In triplicate
(2) 12 Artemia per ml with Chlorella vulgaris at 553 + 71 µl-1 (temperature: 29 + 0.5oC; pH: 7.76 + 0.39; larval density: 75.ml-1; light intensities measured with an irradiance collector, model no QSL-100
(3) 4.8176 x 1014 µe.s-1.cm-2 or 0.8 x 1014 quanta.s-1.cm-2 or 1.156224 x 1014 W.cm-2 or 0.771 x 1021 lx

TABLE 5.0. Fatty acid composition and total lipid content of a low ω3 PUFA *Artemia* (1) unfed and fed on Frippak Boost microcapsules

Fatty acid	Unfed *Artemia*				Fed *Artemia*			
	1	2	3	x	1	2	3	x
14:0	0.7	0.7	t (2)	0.5	3.3	1.2	2.0	2.2
16:0	13.9	18.6	15.5	16.0	15.7	13.7	15.8	15.1
16:1ω7	15.4	18.0	16.0	16.4	10.2	11.4	9.1	10.2
18:0	4.6	5.5	5.1	5.0	5.3	5.2	6.8	5.8
18:1ω9	26.6	27.4	28.0	27.4	29.5	29.5	33.5	30.8
18:1ω7	17.7	18.1	17.9	17.9	18.2	19.7	20.5	19.5
18:2ω6	6.1	4.5	5.4	5.3	5.0	5.6	4.1	4.9
18:3ω3	5.2	2.5	3.8	3.8	3.1	3.5	2.3	3.0
18:4ω3	0.5	t	t	0.2	0.4	0.3	t	0.2
20:1ω9	0.6	0.5	t	0.4	0.6	0.4	1.4	0.8
20:4ω6	1.5	0.8	1.8	1.3	1.2	1.1	t	0.8
20:3ω3	nd (3)	nd	nd	nd	nd	nd	nd	nd
20:4ω3	nd	nd	nd	nd	nd	nd	nd	nd
20:5ω3	5.9	2.4	4.3	4.2	7.2	7.8	3.2	6.1
20:1ω11	nd	nd	nd	nd	nd	nd	nd	nd
22:5ω3	t	t	t	t	t	t	t	t
22:6ω3	1.5	1.2	2.3	1.6	0.4	0.6	1.4	0.8
SAT	19.2	24.8	20.6	21.5	24.3	20.1	24.6	23.1
MUFA	60.3	64.0	61.9	62.1	58.5	61.0	64.5	61.3
SAT + MUFA	79.5	88.8	82.5	83.6	82.8	81.1	89.1	84.4
PUFA	20.5	11.2	17.5	16.4	17.2	18.9	10.9	15.6
20:5ω3 + 22:6ω3	7.4	3.6	6.6	5.8	7.6	8.4	4.6	6.9
ω6	7.5	5.3	7.2	6.6	6.1	6.8	4.1	5.7
ω3	13.0	6.1	10.4	9.8	11.1	12.2	6.8	10.1
ω6/ω3	0.58	0.87	0.69	0.67	0.55	0.55	0.60	0.56
% total lipid in dry wt *Artemia*	15.2	9.7	2.9	9.3	25.0	21.1	12.4	19.5
Survival %	35	39	42	38	38	43	44	41
(S.E.)				(3.51)				(3.21)
Days to PL	43	36	36	38	29	33	35	32
(S.E.)				(4.04)				(3.06)

(1) from San Francisco Bay Brand Inc., 8239 Enterprise Drive, Newark, California 94560 U.S.A.
(2) < 0.02 %
(3) not determined

TABLE 5.1. Fatty acid composition and total lipid content of a medio-low ω3 PUFA *Artemia* (1) unfed and fed on Frippak Boost microcapsules

Fatty acid	Unfed *Artemia*				Fed *Artemia*			
	1	2	3	x	1	2	3	x
14:0	1.0	t (2)	1.2	0.7	1.1	t	0.6	0.6
16:0	18.3	25.5	26.6	23.5	24.7	16.4	15.2	18.8
16:1ω7	3.1	3.8	3.1	3.3	2.0	2.9	2.0	2.3
18:0	10.9	14.9	15.2	13.7	16.0	11.2	12.8	13.3
18:1ω9	20.0	21.8	20.5	20.8	15.5	28.4	27.5	23.8
18:1ω7	8.2	11.7	10.7	10.2	7.2	14.6	14.9	12.2
18:2ω6	10.1	7.5	7.1	8.2	2.7	4.8	5.8	4.4
18:3ω3	15.3	9.8	8.9	11.3	7.3	16.2	15.1	12.9
18:4ω3	4.1	2.5	1.9	2.8	2.8	5.5	0.7	3.0
20:1ω9	1.0	t	0.4	0.5	2.1	t	1.1	1.1
20:4ω6	0.7	t	0.4	0.4	1.6	nd (3)	2.3	1.3
20:3ω3	0.7	nd	0.6	0.4	11.1	t	1.4	4.2
20:4ω3	0.7	nd	0.5	0.4	2.5	t	0.4	1.0
20:5ω3	4.5	2.6	2.2	3.1	1.6	t	0.1	0.6
20:1ω11	0.4	nd	t	0.1	nd	nd	nd	nd
22:5ω3	0.2	nd	nd	0.1	nd	nd	nd	nd
22:6ω3	1.0	t	0.7	0.6	2.0	t	0.1	0.7
SAT	30.2	40.4	43.0	37.9	41.8	27.6	28.6	32.7
MUFA	32.6	37.3	34.7	34.9	26.7	45.9	45.5	39.4
SAT + MUFA	62.8	77.7	77.7	72.8	68.5	73.5	74.1	72.1
PUFA	37.2	22.3	22.3	27.2	31.6	26.5	25.9	27.9
20:5ω3 + 22:6ω3	5.5	2.6	2.9	3.7	3.6	t	0.2	1.3
ω6	10.7	7.7	7.6	8.6	4.3	4.8	8.1	5.7
ω3	26.5	15.0	14.8	18.7	27.3	21.7	17.9	22.4
ω6/ω3	0.41	0.51	0.51	0.46	0.16	0.22	0.45	0.25
% total lipid in dry wt *Artemia*	9.6	13.1	11.8	11.5	16.4	16.0	15.1	15.8
Survival % (S.E.)	25	11	29	21 (9.45)	38	41	34	37 (3.51)
Days to PL (S.E.)	53	46	51	50 (3.61)	28	37	32	32 (4.51)

(1) from Bio-Marine Brand-Aquafauna, P.O. Box 5, Hawthorne, California 90250 U.S.A.
(2) < 0.02 %
(3) not detected

TABLE 5.2. Fatty acid composition and total lipid content of a medio-high ω3 PUFA *Artemia* (1) unfed and fed on Frippak Boost microcapsules

Fatty acid	Unfed *Artemia*				Fed *Artemia*			
	1	2	3	x	1	2	3	x
14:0	1.8	1.1	0.7	1.2	0.6	0.6	0.3	0.5
16:0	14.5	14.3	13.0	13.9	11.7	10.4	12.6	11.6
16:1ω7	4.5	2.9	3.1	3.5	3.3	3.4	3.5	3.4
18:0	4.0	3.2	2.6	3.3	6.5	6.0	7.2	6.6
18:1ω9	26.8	29.5	29.4	28.6	26.2	26.2	29.4	27.2
18:1ω7	9.9	8.8	8.5	9.1	13.3	14.4	14.0	13.9
18:2ω6	8.4	7.6	7.6	7.9	7.8	8.1	8.0	8.0
18:3ω3	24.9	27.7	30.8	27.8	20.1	20.3	19.3	19.9
18:4ω3	2.0	1.4	1.7	1.7	1.9	1.9	1.1	1.7
20:1ω9	0.3	0.2	0.3	0.3	1.0	1.1	0.5	0.9
20:4ω6	1.2	1.1	1.0	1.1	3.1	3.1	1.7	2.6
20:3ω3	nd (2)	nd	nd	nd	nd	nd	nd	nd
20:4ω3	nd	nd	nd	nd	nd	nd	nd	nd
20:5ω3	1.8	1.6	1.4	1.6	4.6	4.5	2.5	3.8
20:1ω11	nd	nd	nd	nd	nd	nd	nd	nd
22:5ω3	t (3)	t	t	t	t	t	t	t
22:6ω3	0.1	0.6	t	0.2	t	t	0.5	t
SAT	20.3	18.6	16.3	18.4	18.8	17.0	20.1	18.7
MUFA	41.5	41.4	41.3	41.5	43.8	45.1	47.4	45.4
SAT + MUFA	61.8	60.0	57.6	59.9	62.6	62.1	67.5	64.1
PUFA	38.2	40.0	42.4	40.1	37.4	37.9	32.5	35.9
20:5ω3 + 22:6ω3	1.9	2.2	1.4	1.8	4.6	4.5	3.0	3.8
ω6	9.6	8.6	8.6	9.0	10.9	11.2	9.7	10.6
ω3	28.7	31.3	34.0	31.3	26.6	26.8	22.9	25.4
ω6/ω3	0.33	0.28	0.25	0.29	0.41	0.42	0.42	0.42
% total lipid in dry wt *Artemia*	18.9	23.0	18.1	18.3	22.1	20.8	33.1	25.3
Survival %	35	34	29	32	38	37	44	39
(S.E.)				(3.21)				(3.79)
Days to PL	30	33	33	32	33	41	38	37
(S.E.)				(1.73)				(4.04)

(1) from Bio-Marine Brand-Aquafauna, P.O.Box 5, Hawthorne, California 90250 U.S.A.
(2) not determined
(3) < 0.02 %

TABLE 5.3. Fatty acid composition and total lipid content of a high ω3 PUFA *Artemia* (1) unfed and fed on Frippak Boost microcapsules

Fatty acid	Unfed *Artemia*				Fed *Artemia*			
	1	2	3	x	1	2	3	x
14:0	1.2	0.8	1.0	1.0	4.3	6.8	3.4	4.8
16:0	12.6	11.3	12.3	12.1	15.8	19.1	15.5	16.8
16:1ω7	1.5	2.0	2.2	1.9	2.8	1.7	1.9	2.1
18:0	5.7	6.0	6.4	6.0	9.4	9.3	6.5	8.4
18:1ω9	22.2	21.6	22.0	21.9	25.1	33.7	29.3	29.4
18:1ω7	7.1	7.9	7.8	7.6	10.7	10.0	8.7	9.8
18:2ω6	6.9	7.5	7.3	7.3	4.9	3.7	4.4	4.3
18:3ω3	35.2	34.2	32.8	34.1	21.4	11.4	24.0	18.5
18:4ω3	6.7	7.4	6.9	7.0	4.1	1.1	2.6	2.3
20:1ω9	0.4	0.6	0.6	0.6	1.3	1.2	1.0	1.2
20:4ω6	0.2	0.3	0.3	0.3	1.0	t	t	0.3
20:3ω3	nd (3)	nd	nd	nd	nd	nd	nd	nd
20:4ω3	nd	nd	nd	nd	nd	nd	nd	nd
20:5ω3	0.3	0.5	0.4	0.4	1.6	0.8	1.1	1.2
20:1ω11	nd	nd	nd	nd	nd	nd	nd	nd
22:5ω3	t	t	t	t	0.1	t	t	t
22:6ω3	t	t	t	t	0.1	1.3	1.5	1.0
SAT	19.5	18.1	19.7	19.1	29.5	35.2	25.4	30.0
MUFA	31.2	32.1	32.6	32.0	39.9	46.6	40.9	42.5
SAT + MUFA	50.7	50.2	52.3	51.1	69.4	81.8	66.3	72.5
PUFA	49.3	49.8	47.7	48.9	30.6	18.2	33.7	27.5
20:5ω3 + 22:6ω3	0.3	0.5	0.4	0.4	1.7	2.1	2.6	2.2
ω6	7.1	7.8	7.6	7.6	5.9	3.7	4.4	4.6
ω3	42.3	42.0	40.1	41.5	25.0	14.6	29.2	23.0
ω6/ω3	0.16	0.19	0.19	0.18	0.24	0.25	0.15	0.20
% total lipid in dry wt *Artemia*	18.4	25.0	15.1	19.5	43.8	25.0	14.7	27.8
Survival % (S.E.)	48	52	51	50 (2.08)	50	57	58	55 (4.36)
Days to PL (S.E.)	37	35	31	34 (3.06)	31	40	38	36 (4.93)

(1) From Artemis Inc., P.O.Box 485, Newark, California 94560 U.S.A.
(2) < 0.02 %
(3) not determined

TABLE 6.0. Survival and days to reach post-larval stages for *Macrobrachium rosenbergii* when reared at 29oC on a low ω3 PUFA *Artemia*

Day number	Unfed				Prefed (1)			
	1	2	3	X	1	2	3	X
1	100	100	100	100	100	100	100	100
10	79	79	81	79	68	93	83	81
20	62	59	58	59	58	70	66	64
30	39	41	45	41	38	47	45	43
40	37	39	42	39	38	43	44	41
50	35	39	42	38	38	43	44	41
55	35	39	42	38	38	43	44	41
(S.E.)(2)	-	-	-	(3.51)	-	-	-	(3.21)
Days to reach PL's	43	36	36	38	29	33	35	32
(S.E.)	-	-	-	(4.04)	-	-	-	(3.06)

(1) with Frippak microencapsulated diet
(2) standard error of the final day number

TABLE 6.1. Survival and days to reach post-larval stages for *Macrobrachium rosenbergii* when reared at 29oC on a medio-low ω3 PUFA *Artemia*

Day number	Unfed				Prefed (1)			
	1	2	3	X	1	2	3	X
1	100	100	100	100	100	100	100	100
5	96	97	98	97	100	96	98	98
10	85	74	85	81	87	85	88	86
15	66	43	67	58	69	81	71	73
20	48	29	49	42	51	67	50	56
25	39	28	41	36	48	55	46	49
30	29	19	36	28	38	49	35	40
35	27	17	35	26	38	42	34	38
40	27	16	32	25	38	41	34	37
45	26	15	32	24	38	41	34	37
50	25	11	30	22	38	41	34	37
55	25	11	29	21	38	41	34	37
(S.E.)(2)	-	-	-	(9.45)	-	-	-	(3.51)
Days to reach PL's	53	46	51	50	28	37	32	32
(S.E.)	-	-	-	(3.61)	-	-	-	(4.51)

(1) with Frippak microencapsulated diet
(2) standard error of the final day number

TABLE 6.2. Survival and days to reach post-larval stages for *Macrobrachium rosenbergii* when reared at 29oC on a medio-high ω3 PUFA *Artemia*

Day number	Unfed				Prefed (1)			
	1	2	3	X	1	2	3	X
1	100	100	100	100	100	100	100	100
10	80	74	66	73	74	82	79	78
20	71	63	53	62	55	61	63	59
30	35	40	37	37	44	50	51	48
40	35	34	29	32	38	46	44	42
50	35	34	29	32	38	37	44	39
55	35	34	29	32	38	37	44	39
(S.E.)(2)	-	-	-	(3.21)	-	-	-	(3.79)
Days to reach PL's	30	33	33	32	33	41	38	37
(S.E.)	-	-	-	(1.73)	-	-	-	(4.04)

(1) with Frippak microencapsulated diet
(2) standard error of the final day number

TABLE 6.3. Survival and days to reach post-larval stages for Macrobrachium rosenbergii when reared at 29oC on a high ω3 PUFA Artemia

Day number	Unfed				Prefed (1)			
	1	2	3	X	1	2	3	X
1	100	100	100	100	100	100	100	100
10	84	83	78	81	86	85	85	85
20	65	67	73	68	81	79	78	79
30	51	57	60	56	63	66	65	64
40	48	52	51	50	50	57	58	55
50	48	52	51	50	50	57	58	55
55	48	52	51	50	50	57	58	55
(S.E.)(2)	-	-	-	(2.08)	-	-	-	(4.36)
Days to reach PL's	37	35	31	34	31	40	39	36
(S.E.)	-	-	-	(3.06)	-	-	-	(4.93)

(1) with Frippak microencapsulated diet
(2) standard error of the final day number

TABLE 7.0. Water total ammonia concentrations (in mg NH4-N.l-1) during Macrobrachium rosenbergii larval rearing (1) when fed on a medio-low ω3 PUFA Artemia (2) unfed and fed on Frippak Boost microcapsules (3)

	Unfed Artemia				Fed Artemia			
Day	1	2	3	X	1	2	3	X
1	0.02	0.02	0.06	0.03	0.05	0.02	0.04	0.04
5	0.06	0.06	0.04	0.05	0.05	0.06	0.06	0.06
10	0.04	0.06	0.08	0.06	0.04	0.04	0.03	0.04
15	0.03	0.12	0.13	0.10	0.02	0.03	0.06	0.04
20	0.14	0.07	0.15	0.12	0.14	0.14	0.15	0.14
25	0.15	0.07	0.11	0.11	0.15	0.13	0.10	0.13
30	0.12	0.04	0.13	0.10	-	0.15	0.14	0.15
35	0.08	0.03	0.16	0.09	-	0.16	-	0.16
40	0.11	0.02	0.09	0.07	-	-	-	-
45	0.10	0.01	0.13	0.08	-	-	-	-
50	0.09	0.01	0.06	0.05	-	-	-	-
55	0.07	0.01	0.06	0.05	-	-	-	-
X				0.08				0.10
Survival %	25	11	29	21	38	41	34	37
(S.E.)				(9.45)				(3.51)
Days to PL	53	46	51	50	28	37	32	32
(S.E.)				(3.61)				(4.51)

(1) temperature: 29oC
(2) from Bio-Marine Brand-Aquafauna, P.O. Box 5, Hawthorne, California 90250 U.S.A.
(3) an average pH of 7.75 was assumed for all calculations as no pH values were recorded during this experiment

TABLE 7.1. Water un-ionized ammonia concentrations (in µg NH3-N.l-1) during Macrobrachium rosenbergii larval rearing (1) when fed on a medio-low ω3 PUFA Artemia (2) unfed and fed on Frippak Boost microcapsules (3)

	Unfed Artemia				Fed Artemia			
Day	1	2	3	X	1	2	3	X
1	1.14	0.80	2.98	1.64	2.68	1.14	2.04	1.95
5	2.98	2.68	1.89	2.58	2.63	2.98	2.88	2.83
10	2.04	2.98	4.13	3.05	2.19	2.04	1.44	1.89
15	1.54	6.06	6.51	4.70	0.80	1.54	3.18	1.84
20	7.16	3.33	7.60	6.03	7.16	7.16	7.60	7.31
25	7.50	3.68	5.52	5.57	7.60	6.96	5.02	6.36
30	6.11	1.99	6.41	4.84	-	7.55	6.71	7.13
35	3.98	1.64	7.70	4.44	-	7.95	-	7.95
40	5.47	0.75	4.47	3.56	-	-	-	-
45	4.77	0.60	6.31	3.89	-	-	-	-
50	4.22	0.40	2.73	2.45	-	-	-	-
55	3.50	0.35	2.98	2.30	-	-	-	-
X				3.75				4.66
Survival % (S.E.)	25	11	29	21 (9.45)	38	41	34	37 (3.51)
Days to PL (S.E.)	53	46	51	50 (3.61)	28	37	32	32 (4.51)

(1) temperature: 29oC
(2) from Bio-Marine Brand-flquafauna, P.O. Box 5, Hawthorne, California 90250 U.S.A.
(3) an average pH of 7.75 was assumed for all calculations as no pH values were recorded during this experiment

TABLE 7.2. Water nitrite concentrations (in µg NO2-N.l-1) during Macrobrachium rosenbergii larval rearing (1) when fed on a medio-low ω3 PUFA Artemia (2) unfed and fed on Frippak Boost microcapsules (3)

	Unfed Artemia				Fed Artemia			
Day	1	2	3	X	1	2	3	X
1	99	58	45	67	64	46	86	65
5	87	37	19	48	25	37	122	61
10	128	99	18	82	18	25	102	48
15	25	35	24	28	25	30	74	43
20	28	16	44	29	97	78	30	68
25	74	13	41	43	64	55	51	57
30	28	122	31	60	-	237	80	159
35	30	28	29	29	-	258	-	258
40	28	29	27	28	-	-	-	-
45	30	11	31	24	-	-	-	-
50	30	10	24	21	-	-	-	-
55	47	18	18	28	-	-	-	-
X				41				95
Survival %	25	11	29	21	38	41	34	37
(S.E.)				(9.45)				(3.51)
Days to PL	53	46	51	50	28	37	32	32
(S.E.)				(3.61)				(4.51)

(1) temperature: 29oC
(2) from Bio-Marine Brand-Aquafauna, P.O. Box 5, Hawthorne, California 90250 U.S.A.
(3) an average pH of 7.75 was assumed for all calculations as no pH values were recorded during this experiment

TABLE 8.0. Fatty acid composition and total lipid content of unfed *Artemia* (1)

Fatty acid	1	2	3	x
14:0	0.5	0.3	0.5	0.4
16:0	11.8	13.8	14.9	13.5
16:1ω7	2.7	2.3	1.8	2.3
18:0	5.0	5.1	8.0	6.0
18:1ω9	19.7	19.7	19.3	19.6
18:1ω7	7.4	7.7	7.2	7.4
18:2ω6	8.4	7.2	7.0	7.5
18:3ω3	34.3	33.9	31.4	33.2
18:4ω3	7.1	6.9	6.9	7.0
20:1ω9	0.5	0.6	0.4	0.5
20:4ω6	0.1	0.1	0.1	0.1
20:3ω3	1.3	1.2	1.2	1.2
20:4ω3	1.4	1.1	1.2	1.2
20:5ω3	0.1	0.1	0.2	0.1
20:1ω11	t (2)	t	t	t
22:5ω3	nd (3)	nd	nd	nd
22:6ω3	t	0.1	t	0.1
SAT	17.2	19.1	23.4	19.9
MUFA	30.2	30.3	28.6	29.7
SAT + MUFA	47.4	49.4	52.0	49.6
PUFA	52.6	50.6	48.0	50.4
20:5ω3 + 22:6ω3	0.1	0.2	0.2	0.2
ω6	8.5	7.3	7.1	7.6
ω3	44.2	43.3	40.9	42.8
ω6/ω3	0.19	0.17	0.17	0.18
Total lipid dry wt *Artemia*	15.5	13.8	14.9	14.7

(1) from San Francisco Bay Brand Inc., 8239 Enterprise Drive, Newark, California 94560 U.S.A.
(2) < 0.02 %
(3) not detected

TABLE 8.1. Fatty acid composition and total lipid content of Chlorella vulgaris (Beijer) (1)

Fatty acid	1	2	3	X
14:0	2.1	2.1	t (2)	1.4
16:0	27.7	16.8	27.1	23.9
16:1ω7	8.5	14.7	11.5	11.6
18:0	2.1	3.2	4.2	3.2
18:1ω9 + ω7	2.1	31.6	18.8	17.5
18:2ω6	36.2	27.4	37.5	33.7
18:3ω3	21.3	4.2	1.0	8.8
18:4ω3	nd (3)	nd	nd	nd
20:1ω9	nd	nd	nd	nd
20:4ω6	nd	nd	nd	nd
20:3ω3	nd	nd	nd	nd
20:4ω3	nd	nd	nd	nd
20:5ω3	nd	nd	nd	nd
20:1ω11	nd	nd	nd	nd
22:5ω3	nd	nd	nd	nd
22:6ω3	nd	nd	nd	nd
SAT	31.9	22.1	31.3	28.4
MUFA	10.6	46.3	30.2	29.1
SAT + MUFA	42.6	68.4	61.5	57.5
PUFA	57.5	31.6	38.5	42.5
20:5ω3 + 22:6ω3	nd	nd	nd	nd
ω6	36.2	27.4	37.5	33.7
ω3	21.3	4.2	1.0	8.8
ω6/ω3	1.70	6.50	37.50	3.83
Total lipid (dry wt)	1.5	1.6	1.5	1.5

(1) from Stewart, W.D.P. 1974. Algal physiology and biochemistry, Botanical Monographs, Vol.10, Blackwell Scientific Publications, University of Dundee, p.?-?
(2) < 0.02 %
(3) not detected

TABLE 8.2. Fatty acid composition and total lipid content of Dunsliella viridis (Teodor) (1)

Fatty acid	1	2	3	X
14:0	6.8	7.1	7.3	7.1
16:0	26.4	15.7	15.9	19.3
16:1ω7	4.4	14.3	12.2	10.3
18:0	4.2	1.0	nd (3)	1.7
18:1ω9 + ω7	20.3	8.6	9.8	12.9
18:2ω6	2.1	8.6	7.3	6.0
18:3ω3	3.4	17.1	11.0	10.5
18:4ω3	5.6	10.0	9.8	8.5
20:1ω9	8.2	1.0	nd	3.1
20:4ω6	1.6	nd	nd	0.5
20:3ω3	t (2)	1.4	2.4	1.3
20:4ω3	t	2.9	4.9	2.6
20:5ω3	17.2	14.3	12.2	14.6
20:1ω11	nd	nd	nd	nd
22:5ω3	t	1.0	7.3	2.8
22:6ω3	nd	nd	nd	nd
SAT	37.3	22.9	23.2	27.8
MUFA	32.9	22.9	22.0	25.9
SAT + MUFA	70.2	45.7	45.1	53.7
PUFA	29.8	54.3	54.9	46.3
20:5ω3 + 22:6ω3	17.2	14.3	12.2	14.6
ω6	3.7	8.6	7.3	6.5
ω3	26.2	45.7	47.6	40.2
ω6/ω3	0.14	0.19	0.15	0.16
Total lipid (dry wt)	1.6	1.7	1.7	1.7

(1) from Stewart, W.D.P. 1974. Algal physiology and biochemistry, Botanical Monographs, Vol.10, Blackwell Scientific Publications, University of Dundee, p.247
(2) < 0.02 %
(3) not detected

TABLE 8.3. Fatty acid composition and total lipid content of *Chlorella* prefed *Artemia* (1)

Fatty acid	1	2	3	X
14:0	0.2	t	0.2	0.1
16:0	14.0	14.4	12.4	13.6
16:1ω7	1.7	2.6	1.7	2.0
18:0	9.9	12.3	10.1	10.7
18:1ω9	26.3	27.9	24.3	26.1
18:1ω7	10.7	13.6	13.1	12.4
18:2ω6	5.8	6.3	6.0	6.1
18:3ω3	22.0	16.4	24.6	21.0
18:4ω3	4.3	5.1	4.9	4.8
20:1ω9	0.7	0.9	0.9	0.8
20:4ω6	0.4	t (2)	0.1	0.2
20:3ω3	1.6	0.7	0.5	0.9
20:4ω3	1.2	t	1.4	0.9
20:5ω3	1.3	t	nd (3)	0.4
20:1ω11	nd	nd	nd	nd
22:5ω3	nd	nd	nd	nd
22:6ω3	0.1	nd	nd	t
SAT	24.1	26.7	22.6	24.5
MUFA	39.3	44.9	40.0	41.4
SAT + MUFA	63.4	71.5	62.6	65.8
PUFA	36.6	28.5	37.4	34.2
20:5ω3 + 22:6ω3	1.4	t	nd	0.5
ω6	6.2	6.3	6.1	6.2
ω3	30.4	22.1	31.3	27.9
ω6/ω3	0.20	0.29	0.19	0.23
Total lipid dry wt *Artemia*	17.9	21.8	16.7	18.8

(1) from San Francisco Bay Brand Inc., 8239 Enterprise Drive, Newark, California 94560 U.S.A.
(2) < 0.02 %
(3) not detected

TABLE 8.4. Fatty acid composition and total lipid content of *Dunaliella* prefed *Artemia* (1)

Fatty acid	1	2	3	X
14:0	0.2	0.3	0.5	0.3
16:0	10.3	10.8	11.7	10.9
16:1ω7	1.4	2.1	2.3	1.9
18:0	9.5	10.6	11.4	10.5
18:1ω9	26.8	24.2	27.5	26.1
18:1ω7	13.4	16.5	17.2	15.7
18:2ω6	6.3	6.0	4.1	5.5
18:3ω3	23.8	20.0	15.8	19.9
18:4ω3	5.6	4.7	5.2	5.2
20:1ω9	0.5	0.9	1.1	0.8
20:4ω6	0.3	0.3	0.4	0.3
20:3ω3	0.9	1.3	1.1	1.1
20:4ω3	0.6	1.0	1.0	0.9
20:5ω3	0.5	1.3	1.0	0.9
20:1ω11	nd (2)	nd	nd	nd
22:5ω3	nd	nd	nd	nd
22:6ω3	nd	nd	t (3)	t
SAT	20.0	21.8	23.5	21.8
MUFA	42.0	43.7	48.1	44.6
SAT + MUFA	62.0	65.5	71.6	66.4
PUFA	38.0	34.5	28.4	33.6
20:5ω3 + 22:6ω3	0.5	1.3	1.0	0.9
ω6	6.6	6.3	4.4	5.8
ω3	31.4	28.3	24.0	27.9
ω6/ω3	0.21	0.22	0.18	0.20
Total lipid dry wt *Artemia*	18.6	18.8	20.1	19.2

(1) from San Fransisco Bay Brand Inc., 8239 Enterprise Drive, Newark, California 94560 U.S.A.
(2) not detected
(3) < 0.02 %

TABLE 9.0. Fatty acid composition of 3 weak groups of unfed *Macrobrachium rosenbergii* larvae at stage I

Fatty acid	A	B	C	x	S.E.	range
14:0	1.6	1.4	1.4	1.5	0.14	1.4-1.6
16:0	28.7	29.6	30.0	29.4	0.65	28.7-30.0
16:1ω7	8.4	8.8	11.3	9.5	1.57	8.4-11.3
18:0	9.1	9.0	8.3	8.8	0.43	8.3-9.1
18:1ω9	26.0	26.0	24.5	25.5	0.88	24.5-26.0
18:1ω7	10.9	8.6	7.4	9.0	1.75	7.4-10.9
18:2ω6	1.0	0.9	1.2	1.0	0.11	0.9-1.2
18:3ω3	0.3	0.3	0.5	0.4	0.12	0.3-0.5
18:4ω3	0.2	0.1	0.3	0.2	0.10	0.1-0.3
20:1ω9	1.3	1.4	2.2	1.6	0.50	1.3-2.2
20:4ω6	1.7	1.9	1.6	1.7	0.13	1.6-1.9
20:3ω3	t(1)	t	0.1	t	0.03	t-0.1
20:4ω3	0.1	0.2	0.1	0.1	0.05	0.1-0.2
20:5ω3	6.9	7.6	6.1	6.9	0.75	6.1-7.6
20:1ω11	0.2	t	0.1	0.1	0.09	t-0.2
22:5ω3	0.2	0.1	0.3	0.2	0.10	0.1-0.3
22:6ω3	3.3	4.0	4.8	4.0	0.76	3.3-4.8
SAT	39.5	40.0	39.7	39.7	0.28	39.5-40.0
MUFA	46.7	44.9	45.5	45.7	0.94	44.9-46.7
SAT + MUFA	86.2	84.9	85.1	85.4	0.68	84.9-86.2
PUFA	13.8	15.1	14.9	14.6	0.70	13.8-15.1
20:5ω3 + 22:6ω3	10.2	11.7	10.9	10.9	0.76	10.2-11.7
ω6	2.7	2.8	2.8	2.7	0.06	2.7-2.8
ω3	11.1	12.3	12.2	11.8	0.67	11.1-12.3
ω6/ω3	0.24	0.23	0.23	0.23	0.01	0.23-0.24
Stage (2)	2.50	3.00	2.50	2.67	0.29	2.50-3.00

(1) < 0.02 % (2) after 3 48-day larval rearing

TABLE 9.1. Fatty acid composition of 3 strong groups of unfed *Macrobrachium rosenbergii* larvae at stage I

Fatty acid	D	E	F		x	S.E.	range
14:0	1.5	2.1	1.8		1.8	0.30	1.5-2.1
16:0	30.2	28.7	31.3		30.1	1.29	28.7-31.3
16:1ω7	10.2	8.7	8.0		9.0	1.12	8.0-10.2
18:0	7.3	9.7	9.9		9.0	1.42	7.3-9.9
18:1ω9	26.4	26.4	25.0		25.9	0.84	25.0-26.4
18:1ω7	10.1	8.8	9.5		9.5	0.69	8.8-10.1
18:2ω6	1.7	2.5	2.4		2.2	0.41	1.7-2.5
18:3ω3	0.2	0.5	0.5		0.4	0.21	0.2-0.5
18:4ω3	0.1	0.3	0.2		0.2	0.10	0.1-0.3
20:1ω9	1.2	2.1	1.8		1.7	0.47	1.2-2.1
20:4ω6	1.3	1.7	1.9		1.6	0.27	1.3-1.9
20:3ω3	0.1	0.0	nd	(1)	0.1	0.06	nd-0.1
20:4ω3	0.1	0.1	nd		0.1	0.05	nd-0.1
20:5ω3	5.9	5.0	5.1		5.3	0.49	5.0-5.9
20:1ω11	0.1	0.3	0.3		0.2	0.14	0.1-0.3
22:5ω3	0.2	0.2	0.4		0.3	0.09	0.2-0.4
22:6ω3	3.3	2.8	1.9		2.7	0.70	1.9-3.3
SAT	39.1	40.5	42.9		40.9	1.95	39.1-42.9
MUFA	48.0	46.4	44.7		46.3	1.67	44.7-48.0
SAT + MUFA	87.1	86.9	87.6		87.2	0.37	86.9-87.6
PUFA	12.9	13.1	12.4		12.8	0.38	12.4-13.1
20:5ω3 + 22:6ω3	9.2	7.8	7.0		8.0	1.10	7.0-9.2
ω6	3.1	4.2	4.3		3.8	0.66	3.1-4.3
ω3	9.8	9.0	8.1		9.1	0.86	8.1-9.8
ω6/ω3	0.32	0.47	0.53		0.44	0.11	0.32-0.53
Stage (2)	5.00	4.00	4.50		4.50	0.50	4.00-5.00

(1) not detected (2) after 5 days rearing

TABLE 10.0. Fatty acid composition of unfed *Macrobrachium rosenbergii* larvae at stage I

Fatty acid	A	B	C	x	S.E.	range
14:0	1.6	1.4	1.9	1.6	0.23	1.4-1.9
16:0	28.7	29.6	30.2	29.5	0.75	28.7-30.2
16:1ω7	8.4	8.8	8.2	8.5	0.32	8.2-8.8
18:0	9.1	9.0	9.7	9.3	0.35	9.0-9.7
18:1ω9	26.0	26.0	24.2	25.4	1.06	24.2-26.0
18:1ω7	10.9	8.6	8.5	9.3	1.34	8.5-10.9
18:2ω6	1.0	0.9	1.6	1.2	0.35	0.9-1.6
18:3ω3	0.3	0.3	0.3	0.3	0.04	0.3-0.3
18:4ω3	0.2	0.1	0.3	0.2	0.08	0.1-0.3
20:1ω9	1.3	1.4	1.4	1.4	0.08	1.3-1.4
20:4ω6	1.7	1.9	1.8	1.8	0.07	1.7-1.9
20:3ω3	0.0	0.0	0.1	0.0	0.03	0.0-0.1
20:4ω3	0.1	0.2	0.3	0.2	0.09	0.1-0.3
20:5ω3	6.9	7.6	6.7	7.1	0.50	6.7-7.6
22:1ω11	0.2	0.0	0.0	0.1	0.10	0.0-0.2
22:5ω3	0.2	0.1	0.3	0.2	0.10	0.1-0.3
22:6ω3	3.3	4.0	4.6	4.0	0.68	3.3-4.6
SAT	39.5	40.0	41.7	40.4	1.17	39.5-41.7
MUFA	46.7	44.9	42.3	44.6	2.22	42.3-46.7
SAT + MUFA	86.2	84.9	84.0	85.0	1.09	84.0-86.2
PUFA	13.8	15.1	16.0	15.0	1.09	13.8-16.0
20:5ω3 + 22:6ω3	10.2	11.7	11.3	11.0	0.79	10.2-11.7
ω6	2.7	2.8	3.4	3.0	0.36	2.7-3.4
ω3	11.1	12.9	12.6	12.0	0.81	11.1-12.7
ω6/ω3	0.25	0.23	0.27	0.25	0.02	0.23-0.27

TABLE 10.1. Fatty acid composition of unfed *Macrobrachium rosenbergii* larvae at stage II

Fatty acid	A	B	C	x	S.E.	range
14:0	1.7	1.0	1.3	1.3	0.37	1.0-1.7
16:0	26.4	31.2	28.8	28.8	2.37	26.4-31.2
16:1ω7	2.2	5.3	6.6	6.7	1.48	5.3-8.2
18:0	7.5	12.1	10.3	10.0	2.34	7.5-12.1
18:1ω9	25.4	21.7	22.5	23.2	1.96	21.7-25.4
18:1ω7	11.5	11.1	8.7	10.4	1.51	8.7-11.5
18:2ω6	1.1	0.9	1.5	1.1	0.30	0.9-1.5
18:3ω3	0.4	0.1	0.5	0.3	0.20	0.1-0.5
18:4ω3	0.3	nd(1)	0.2	0.2	0.15	nd-0.3
20:1ω9	0.8	1.4	1.5	1.3	0.37	0.8-1.5
20:4ω6	2.3	2.6	2.8	2.6	0.29	2.3-2.8
20:3ω3	0.1	nd	0.0	0.0	0.04	nd-0.1
20:4ω3	0.2	nd	0.1	0.1	0.09	nd-0.2
20:5ω3	9.3	7.9	9.6	8.9	0.91	7.9-9.6
22:1ω11	0.1	0.2	0.1	0.2	0.06	0.1-0.2
22:5ω3	0.4	nd	0.1	0.2	0.22	nd-0.4
22:6ω3	4.5	4.6	5.2	4.7	0.38	4.5-5.2
SAT	35.6	44.2	40.4	40.1	4.33	35.6-44.2
MUFA	46.1	39.8	39.5	41.8	3.73	39.5-46.1
SAT + MUFA	81.7	84.0	79.9	81.8	2.04	79.9-84.0
PUFA	18.4	16.1	20.0	18.1	1.98	16.1-20.0
20:5ω3 + 22:6ω3	13.7	12.5	14.8	13.7	1.15	12.5-14.8
ω6	3.3	3.5	4.3	3.7	0.51	3.3-4.3
ω3	15.1	12.6	15.7	14.5	1.66	12.6-15.7
ω6/ω3	0.22	0.28	0.27	0.26	0.03	0.22-0.28

(1) not detected

TABLE 11.0. Fatty acid composition and total lipid content of shrimp diet (1) given to *Macrobrachium rosenbergii* females (in triplicate)

Fatty acid	1	2	3	X
14:0	1.0	1.0	0.7	0.9
16:0	18.3	17.8	16.9	17.7
16:1ω7	8.8	10.4	6.1	8.4
18:0	4.4	3.5	3.8	3.9
18:1ω9	15.4	14.4	14.4	14.7
18:1ω7	8.6	9.0	9.6	9.1
18:2ω6	1.5	0.6	0.8	0.9
18:3ω3	0.7	0.4	0.5	0.6
18:4ω3	2.0	0.5	0.4	0.9
20:1ω9	1.3	1.4	1.4	1.4
20:4ω6	2.7	3.6	3.2	3.2
20:3ω3	0.8	0.1	0.1	0.3
20:4ω3	0.3	0.5	0.4	0.4
20:5ω3	21.1	23.9	26.4	23.8
20:1ω11	0.2	0.4	0.3	0.3
22:5ω3	1.2	0.6	1.1	1.0
22:6ω3	11.8	12.0	14.0	12.6
SAT	23.7	22.3	21.4	22.5
MUFA	34.3	35.5	31.8	33.9
SAT + MUFA	58.0	57.8	53.2	56.3
PUFA	42.0	42.2	46.8	43.7
20:5ω3 + 22:6ω3	32.9	35.9	40.4	36.4
ω6	4.2	4.2	4.0	4.1
ω3	37.9	38.0	42.8	39.6
ω6/ω3	0.11	0.11	0.09	0.10
Total lipid (dry wt)	4.0	4.0	4.4	4.1

(1) Frozen *Pandalus borealis* from Kimfish Co.

TABLE 11.1. Fatty acid composition and total lipid content of squid diet (1) given to Macrobrachium rosenbergii females (in triplicate)

Fatty acid	1	2	3	X
14:0	2.1	2.1	2.9	2.4
16:0	29.2	27.9	31.0	29.4
16:1ω7	1.2	1.2	1.2	1.2
18:0	2.9	2.3	2.7	2.6
18:1ω9	3.5	2.9	3.5	3.3
18:1ω7	2.2	2.0	2.4	2.2
18:2ω6	0.3	0.3	0.3	0.3
18:3ω3	0.2	0.1	0.1	0.1
18:4ω3	0.2	0.1	0.2	0.2
20:1ω9	4.4	3.5	4.3	4.1
20:4ω6	0.8	0.8	0.8	0.8
20:3ω3	0.2	0.2	0.2	0.2
20:4ω3	0.1	0.1	0.1	0.1
20:5ω3	16.0	16.5	17.8	16.8
20:1ω11	0.1	0.3	0.2	0.2
22:5ω3	0.1	0.1	0.3	0.2
22:6ω3	36.6	39.6	35.2	37.1
SAT	34.2	32.3	36.7	34.4
MUFA	11.3	9.8	11.6	10.9
SAT + MUFA	45.6	42.2	48.2	45.3
PUFA	54.5	57.8	51.8	54.7
20:5ω3 + 22:6ω3	52.6	56.1	53.0	53.9
ω6	1.1	1.1	1.0	1.1
ω3	53.4	56.7	53.9	54.7
ω6/ω3	0.02	0.02	0.02	0.02
Total lipid (dry wt)	4.6	5.1	4.0	4.6

(1) Frozen Loligo vulgaris from Kimfish Co.

TABLE 11.2. Fatty acid composition and total lipid content of mussel diet (1) given to Macrobrachium rosenbergii females (in triplicate)

Fatty acid	1	2	3	x
14:0	1.1	1.0	0.3	0.8
16:0	22.3	8.4	17.9	16.2
16:1ω7	3.6	23.8	2.5	10.0
18:0	10.2	8.8	8.8	9.2
18:1ω9	2.0	5.5	1.8	3.1
18:1ω7	0.3	3.3	2.4	2.0
18:2ω6	0.8	1.8	0.6	1.1
18:3ω3	0.6	0.5	0.5	0.5
18:4ω3	0.6	0.4	0.8	0.6
20:1ω9	10.1	8.0	5.9	8.0
20:4ω6	9.0	6.0	7.2	7.4
20:3ω3	t (2)	nd (3)	0.1	0.1
20:4ω3	9.1	nd	1.3	3.5
20:5ω3	13.6	12.0	20.7	15.5
20:1ω11	t	0.3	0.4	0.2
22:5ω3	1.7	1.5	1.4	1.5
22:6ω3	15.1	18.7	27.9	20.5
SAT	33.7	18.2	26.7	26.2
MUFA	16.0	40.8	12.8	23.2
SAT + MUFA	49.7	59.0	39.6	49.4
PUFA	50.4	41.0	60.5	50.6
20:5ω3 + 22:6ω3	28.7	30.7	48.6	36.0
ω6	9.8	7.8	7.9	8.5
ω3	40.7	33.1	52.7	42.2
ω6/ω3	0.24	0.19	0.15	0.19
Total lipid (dry wt)	1.6	1.3	1.7	1.5

(1) Fresh Mytilus edulis from Beaumaris (1 & 2) and Menai Bridge (3)
(2) < 0.02 %
(3) not detected

TABLE 11.3. Fatty acid composition and total lipid content of pellet diet (1) given to *Macrobrachium rosenbergii* females (in triplicate)

Fatty acid	1	2	3	X
14:0	2.7	2.7	2.5	2.6
16:0	19.7	17.5	18.2	18.5
16:1ω7	5.5	4.6	4.8	5.0
18:0	11.8	12.0	13.4	12.4
18:1ω9	17.5	16.5	17.7	17.2
18:1ω7	4.1	3.5	3.8	3.8
18:2ω6	9.5	9.3	9.6	9.5
18:3ω3	1.5	1.3	1.3	1.4
18:4ω3	1.3	1.1	0.9	1.1
20:1ω9	4.3	6.0	7.2	5.8
20:4ω6	2.4	2.5	2.3	2.4
20:3ω3	0.1	0.1	0.1	0.1
20:4ω3	0.5	0.8	0.5	0.6
20:5ω3	7.1	6.3	4.4	5.9
20:1ω11	4.0	7.3	5.5	5.6
22:5ω3	1.8	2.2	1.9	2.0
22:6ω3	6.3	6.4	5.9	6.2
SAT	34.2	32.1	34.2	33.5
MUFA	35.3	38.0	39.0	37.4
SAT + MUFA	69.5	70.1	73.2	70.9
PUFA	30.5	30.0	26.8	29.1
20:5ω3 + 22:6ω3	13.4	12.7	10.3	12.1
ω6	11.9	11.9	11.9	11.9
ω3	18.5	18.1	15.0	17.2
ω6/ω3	0.65	0.65	0.79	0.70
Total lipid (dry wt)	6.6	6.5	6.2	6.4

(1) Made in the Marine Science Laboratories, U.C.N.W., Menai Bridge

TABLE 12.0. Starving resistance (survival %) of recently hatched Macrobrachium rosenbergii larvae fed on encapsulated diets at 29oC

	Larval group #																				
Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	X
1	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100.0
2	100	100	100	100	100	100	100	100	100	100	100	100	100	96	100	100	98	100	100	100	100	97.7
3	100	100	100	62	100	94	100	100	98	98	98	98	94	96	98	100	98	100	96	96	96	96.3
4	96	100	88	46	96	70	90	90	92	98	96	98	88	94	98	100	94	96	96	92	70	89.9
5	94	100	74	6	90	68	88	72	90	92	92	98	84	88	98	92	94	96	90	54	42	81.0
6	92	100	10	6	52	64	80	36	78	80	86	96	60	68	72	56	92	88	78	38	24	64.6
7	48	100	4	4	24	64	50	12	38	50	82	86	58	54	8	38	92	44	14	10	8	42.3
8	32	100	0	2	0	44	4	4	34	26	60	72	50	32	0	22	76	4	4	4	0	27.1
9	0	12	0	0	0	10	0	0	22	4	26	60	30	10	0	2	22	0	0	0	0	9.4
10	0	0	0	0	0	2	0	0	4	2	4	24	8	8	0	0	0	0	0	0	0	2.5
11	0	0	0	0	0	0	0	0	0	0	0	8	0	4	0	0	0	0	0	0	0	0.6
12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.0

TABLE 13.0. Size of microencapsulated diets recommended for Macrobrachium rosenbergii based on rate of ingestion (1)

Stage	Duration (accumulated days)	Total body length (mm)	Carapace length (mm)	Recommended microcapsule diameter (µm)	
				Present experiment	Khan (1976)
I	1-2	1.9	0.57	-	-
II	2-3	2.2	0.63	5-30	600
III	4-5	2.6	0.64	5-30	600
IV	6-7	3.0	0.73	5-30/40-90	600
V	7-9	3.6	0.86	40-90	600/750
VI	9-12	4.3	1.06	40-90	750
VII	12-16	5.4	1.05	40-90/90-150	750
VIII	16-19	6.0	1.12	40-90/90-150	750
IX	18-24	6.7	1.20	90-150	750
X	20-27	7.5	1.33	90-150	750/1260
XI	23-35	8.3	1.47	90-150/150-250	750/1260
PLI	22-47	10.4	1.85	150-250	1260

(1) for each stage, 10 larvae were observed individually in a watch-glass with a binocular

TABLE 14.0. Survival (%) of recentlyhatched Macrobrachium rosenbergii larvae starved at 29oC

Trial #	Larvae #	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12	D13	D14
1	100	100 (1)(a)	99 (2)	98 (2)	87 (2)	79 (2)	68 (2)	46 (2)	29 (2)	11 (2)	1 (3)	0	0	0	0
2	100	100 (1)	99 (2)	96 (2)	89 (2)	81 (2)	64 (2)	42 (2)	27 (2)	9 (2)	3 (3)	1 (3)	0	0	0
3	100	100 (1)	98 (2)	95 (2)	81 (2)	63 (2)	54 (2)	38 (2)	15 (2)	3 (3)	0	0	0	0	0
X	100	100 (1.00)	98.7 (2.00)	96.3 (2.00)	85.7 (2.00)	74.3 (2.00)	62.0 (2.00)	42.0 (2.00)	23.7 (2.00)	7.7 (2.33)	1.3 (3.00)	0.3 (3.00)	0	0	0

(a) stage

TABLE 14.1. Survival (%) of recent hatched Macrobrachium rosenbergii larvae fed on Artemia (a) at 29oC

Trial #	Larvae #	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12	D13	D14
1	100	100 (1)(b)	99 (2)	98 (2)	98 (3)	97 (3)	96 (4)	93 (4)	92 (5)	90 (6)	88 (6)	85 (6)	76 (7)	74 (7)	72 (7)
2	100	100 (1)	99 (2)	98 (2)	96 (2)	96 (3)	94 (3)	90 (4)	89 (5)	86 (5)	83 (6)	81 (6)	78 (6)	69 (7)	67 (7)
3	100	100 (1)	100 (2)	99 (2)	99 (3)	98 (3)	97 (4)	96 (5)	95 (5)	91 (6)	90 (6)	88 (6)	79 (7)	77 (7)	75 (7)
%	100	100 (1.00)	99.3 (2.00)	98.3 (2.00)	97.7 (2.67)	97.0 (3.00)	95.7 (3.67)	93.0 (4.33)	92.0 (5.00)	89.0 (5.67)	87.0 (6.00)	84.7 (6.00)	77.7 (6.67)	73.3 (7.00)	71.3 (7.00)

(a) 12 Artemis per ml; 553 ± 71 cells of Chlorella vulgaris per µl; pH = 7.76 ±0.39
(b) stage

TABLE 14.2. Survival (%) of recently hatched Macrobrachium rosenbergii larvae fed at 29oC on Frippak microencapsulated diets (a)

Trial t	Larvae t	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12	D13	D14
1	100	100 (1) (b)	95 (2)	92 (2)	84 (2)	78 (2)	73 (2)	66 (2)	48 (3)	40 (3)	39 (3)	11 (3)	3 (4)	0	0
2	100	100 (1)	94 (2)	89 (2)	82 (2)	74 (2)	69 (2)	60 (2)	44 (2)	25 (2)	13 (3)	2 (3)	0	0	0
3	100	100 (1)	98 (2)	94 (2)	88 (2)	81 (2)	78 (2)	67 (3)	50 (3)	39 (3)	33 (3)	12 (3)	7 (4)	5 (4)	0
X	100	100 (1.00)	95.7 (2.00)	91.7 (2.00)	84.7 (2.00)	77.7 (2.00)	73.3 (2.00)	64.3 (2.33)	47.3 (2.67)	34.7 (2.67)	28.3 (3.00)	8.3 (3.00)	3.3 (4.00)	1.7 (4.00)	0

(a) 0-30µm capsules
(b) stage

TABLE 15.0. Strassburger-Flemming solution

Alcohol	1/3
Glycerol	1/3
Distilled water	1/3

TABLE 15.1. Bouin's fluid

Picric acid (saturated aqueous solution - brackish water)	75 ml
Formaline (40% aqueous)	25 ml
Glacial acetic acid	5 ml

TABLE 15.2. Davidson solution

95% Ethyl alcohol	330 ml
100% Formalin (sat. aqueous solution of formaldehyde gas, 37-39% sol.)	220 ml
Glacial acetic acid	115 ml
Tap water (distilled)	335 ml

Storage at room temperature (transfer to 50% ethyl alcohol)

TABLE 15.3. Kristensen fluid

Formic acid (sp.gr.1.2)	18 ml
Sodium formate	3.5 g
Distilled water	82 ml

TABLE 15.4. Heidenhaim's azan modified technique

Xylene (dewaxing)	3 min.
Absolute ethanol	1 min.
90% ethanol	1 min.
70% ethanol	1 min.
50% ethanol	1 min.
30% ethanol	1 min.
Distilled water	2 min.
Azocarmine at 56oC (oven) in a stoppered jar	30-40 s.
Wash in tap water	2 min.
Differentiate in Aniline alcohol (under microscope)	10-15 s.
Stop differentiation in acetic alcohol	30 s.
5% Phosphotungstic acid	15 min.
Wash briefly in distilled water	
Stain in Aniline Blue-Orange G	3 min.
Wash	5 s.
Quick dehydration (70%, 90%, 100%, 100%)	
Xylene	3 min.
Mount in a drop of D.P.X. (Raymond A. Lamb, London)	

Stains were prepared according to Ratcliffe (1983), but concentrations for M. rosenbergii larvae had to be modified as followed:

Azocarmine:

(C.I. 50085 - Raymond A. Lamb, Middx)

- Azocarmine B.....0.1 g
- Glacial acetic acid.....12 ml
- Distilled water.....100 ml

Analine alcohol:

(Biological Reagent-Michrome, Edward Gurr, London)

- Aniline.....0.1 g
- 90% ethanol.....100 ml

Acetic alcohol:

- Glacial acetic acid.....1 ml
- 90-95% ethanol.....100 ml

Phosphotungstic acid:

$H_3PO_4.12WO_3 + xH_2O$
(Anala R Analytical Reagent Product No 10287,
BDH Chemicals Ltd., London)

- Phosphotungstic acid.....10 g
- Distilled water.....100 ml

Aniline Blue-Orange G:

- Aniline Blue WS.....0.5 g
  (C.I. 42755, Product No 34003, BDH, England)
- Orange G.....2 g
  (C.I. 16230, Product No 34062, BDH, England)
- Glacial acetic acid.....8 ml
- Distilled water.....100 ml

TABLE 16.0. Meristic characteristics of three important decapod crustaceans for all larval stages

Macrobrachium rosenbergii					*Penaeus monodon* (1)					*Homarus gammarus* (2)				
Stage	Duration (days)	Dry Weight (µg)	Total Length (mm)	Carapace Length (mm)	Stage	Duration (days)	Dry Weight (µg)	Total Length (mm)	Carapace Length (mm)	Stage (3)	Duration (days)	Dry Weight (µg)	Total Length (mm)	Carapace Length (mm)
egg	18-23	35.3	0.509	-	egg	0.5-1	-	0.25	-	egg	14-21	1700	1.62	-
I	1-2	71	1.9	0.57	NI-VI	1.5-2	-	0.48	-	I	5	2190	6.40	2.65
II	1-2	85	2.2	0.63	PZI	1.5-2	5.64	1.07	0.52	II	5	3380	8.30	3.62
III	2	96	2.6	0.64	PZII	1.5-2	14.35	1.71	0.65	III	6	4370	10.90	4.02
IV	2	103	3.0	0.74	PZIII	1.5-2	22.80	2.70	0.77	Megalopa	8	8270	12.80	4.74
V	1-3	117	3.6	0.86	MI	1.5-2	38.67	3.35	0.91	PLI	-	13330	14.30	5.44
VI	2-4	151	4.3	1.06	MII	1.5-2	46.33	3.86	0.99					
VII	3	278	5.4	1.05	MIII	1.5-2	55.48	4.62	1.13					
VIII	2-4	515	6.0	1.20	PLI	-	71.12	4.99	1.37					
IX	2-4	515	6.7	1.20										
X	2-8	633	7.5	1.33										
XI	1-12	796	8.3	1.47										
PLI	-	844	10.4	1.85										

(1) Motoh, H. 1979; Kurmaly et al., 1988
(2) Cobb & Phillips, 1980; Kurmaly et al., 1988
(3) Although lobsters of fourth stage technically are postlarvae, in literature, the term "larval lobster" almost always includes the fourth stage (Cobb & Phillips, 1980)

TABLE 17.0. Dimension and volume of the hepatopancreas of Macrobrachium rosenbergii at each larval stage

Stage	Length (mm)	Width (mm)	Depth (mm)	Volume (mm3)
I	0.200	0.190	0.155	0.00393
II	0.213	0.260	0.113	0.00417
III	0.208	0.280	0.113	0.00439
IV	0.206	0.290	0.123	0.00490
V	0.200	0.350	0.125	0.00583
VI	0.625	0.400	0.188	0.03133
VII	0.875	0.450	0.375	0.09844
VIII	0.895	0.455	0.388	0.10534
IX	0.920	0.475	0.426	0.12411
X	0.950	0.483	0.460	0.14071
XI	0.980	0.492	0.498	0.16008
PLI	1.000	0.500	0.525	0.17500
Increase % (I - PLI)	500	260	340	4450