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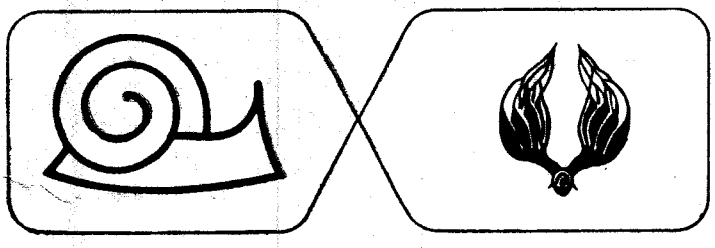

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Development and Culture of Penaeid Larvae A Review

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The larval development of penaeid prawns has attracted the attention of biologists by virtue of the many primitive features exhibited by these larvae. Till recently this interest was purely academic. But, of late, the larvae of this commercially important group of prawns have been studied from the point of view of the management of prawn fishery resources and in the context of large scale production of the seed of desirable species of prawns for coastal aquaculture. The larval development of a number of species of prawns has been worked out in great detail in the course of developing techniques for rearing the larvae under controlled conditions. In this paper our present knowledge of penaeid larval development and the state of the art of larval rearing are reviewed.

LARVAL DEVELOPMENT

Historical

Muller (1863) discovered that penaeid prawns pass through nauplius and protozoea stages during their development from the egg. The proof that the penaeid egg actually gives rise to a nauplius was given by Monticelli and Lo Binaco (1900) and Kishinouye (1900). Since then some of the larval stages of the penaeid prawns have been described from plankton collections by Brooks (1882), Bate (1888), Monticelli and Lo Bianco (1901, 1902), Lo Bianco (1903), Stephensen (1923), Gurney (1924, 1926, 1927, 1942, 1943) Menon (1937, 1940, 1952), Dakin (1938), Pearson (1939), Dakin and Colefax (1940), Morris and Bennett (1952), Heegard (1953, 1966), Cheung (1963), Subrahmaniam (1965, 1971), Paulinose (1967, 1954), Mohamed *et al.* (1968), Kirkegaard (1969, 1972), Al-Kholy and El-Hawary (1970), Subramaniam and Gunther (1970), Hassan (1973), George and Paulinose (1973), Rao (1974) and Hassan and Haq (1975). These investigations were based on plankton collections and consequently the larval series were generally incomplete and the specific identification of the larvae was made on circumstantial evidence.

Side by side with these exercises there have been more serious attempts at rearing the larvae from eggs laid by adult penaeid prawns in the laboratory. Hudinaga (1935, 1942) was the first to rear *Penaeus japonicus* in the laboratory from the egg to the juvenile stage and to describe the larvae and spawning in great detail. This was followed by the excellent work of Heldt (1938) who described the eggs and larvae of *Penaeus trisulcatus* (=*P. Kerathurus*), *Parapenaeus longirostris*, *Sicyonia carinata* and *Solenocera membranacea* from eggs reared in the laboratory. From 1960 onwards there has been a spurt in this direction, thanks to the world-wide awakening of interest in the aquaculture of penaeid prawns. Detailed descriptions of the complete series of laboratory-reared larval stages of the following species have been published:

Name of Species	Authors
Penaeus duorarum	Dobkin (1961); Ewald (1995)
P. aztecus	Cook and Murphy (1971)
P. brasiliensis	Lares (1974)
P. monodon	Villaluz *et al.* (1969); Silas *et al.* (1978); Motoh (1979).
P. esculentus	Fielder, *et al.* (1975)
P. semisulcatus	Devarajan, *et al.* (1978)
P. indicus	Muthu, *et al.* (1978a)
P. merguiensis	Raje and Ranade (1972a); Motoh and Buri (1979)
P. orientalis	Oka (1967a)
Metapenaeus dobsoni	Muthu, *et al.* (1978b)
M. affinis	Muthu, *et al.* (1978c)
M. monoceros	Raje and Ranade (1972b); Mohamed, *et al.* (1978)
M. joyneri	Lee and Lee (1968)
Parapenaeopsis stylifera	Muthu, *et al.* (1978d)
P. acclivirostris	Muthu, *et al.* (1980)
Sicyonia brevirostris	Cook and Murphy (1965)

Although complete larval history has not been traced, some of the larval stages of *Xiphopenaeus kroyeri* (Renfro and Cook, 1963), *M. affinis* (Thomas, *et al.* 1976a), *M. brevicornis* (Rao, 1978) and *Parapenaeopsis acclivirostris* (Thomas *et al.* 1977) have also been described based on actual rearing in the laboratory.

The general pattern of penaeid larval development and the generic characters of the larvae of some penaeid genera have been discussed by Gurney (1942), Cook (1966), Haq and Hassan (1975) and Muthu, *et al.* (1978c).

Penaeid egg:

The demersal eggs of the penaeid prawns are shed free in the water. Fertilization is external and is marked by the development of a perivitelline space, the width of which appears to have some taxonomic significance (Muthu, *et al.* 1978c). The width of the perivitelline space also appears to determine the buoyancy of the egg. A narrow perivitelline space (15 microns) which is characteristic of the genus Penaeus appears to be responsible for the observed fact that, by ordinary aeration, it is very difficult to stir up the eggs of *P. indicus* and *P. monodon* which lie on the bottom of the larval rearing tanks, whereas the eggs of *M. dobsoni* and *P. stylifera* which have a very wide perivitelline space (85 microns and 60 microns respectively) are easily dispersed in the water column by the aerating stones (personal observation). This may also account for the fact that the eggs of these two species have frequently been found in the surface plankton by Menon (1951) and Rao (1974) respectively, while the eggs of the genus

Penaeus have been collected by Heegaard (1953) only in a plankton net which scraped the bottom sand. The less buoyant nature of the eggs of *Penaeus* has also been commented upon by Heedt (1938), Pearson (1939) and Heegaard (1953).

The small size of the penaeid egg (0.3-0.45 mm dia.) with its limited store of yolk results in a short embryonic life inside the egg. The eggs hatch out 8-17 hours after they are laid, depending on the ambient temperature.

The number of eggs laid by penaeid prawns varies from 20,000 to 1,000,000 depending upon the species and the size of the female.

Terminology of the larval stages:

The free swimming nauplius which emerges from the egg metamorphoses successively into the protozoea, mysis and postlarval phases or stages before attaining the adult form. Each stage has a number of sub-stages. There has been some discussion on the terminology used for these various larval stages. Gurney (1942) emphasised that the grouping of the sub-stages into phases or stages should be based on the method of swimming. He recognized the following four phases.

Nauplius Protozoea	Antennal or cephalic propulsion
Zoea	Thoracic propulsion
Postlarva	Abdominal propulsion

Gurney (1942) advocated the use of the term "zoea" for the mysis stage of the penaeids as the term "mysis implies a false relationship to the Mysidacea. Williamson (1968) used the term "zoea" to cover the protozoea and mysis stages of the penaeids and argued for the adoption of the term "megalopa" for the postlarval stage of panaeids as well. These changes in terminology were proposed mainly for the sake of uniformity and for pointing out homologies. However, in view of the fact that the larval development of the various groups of crustaceans has evolved along different lines following distinct patterns which are strikingly uniform within the group, it is advisable to retain the terminology generally in use for each group so as to highlight the distinctive features of each group. For example, by including the protozoea of penaeids under the term "zoea", a very distinct and well defined phase in the development of penaeids will be obscured. Similarly, only in the mysis stage of penaeids, all the pereopods appear simultaneously and all the 8 thoracic appendages have well developed exopods. By calling this stage a "zoea" we will be equating it with the zoea of Anomura and Brachyura in which only the first two maxillipeds have well developed exopods, the other thoracic appendages remaining rudimentary. In the zoea of Caridea, all the thoracic legs do not appear at the same time; in many cases the 5th pair of legs appear before the fourth pair and are generally without exopods. Again, the postlarval phase of the penaeids is not a single stage; it is made up of a number of sub-stages which merge without marked changes into the juvenile phase. In marked contrast, the megalopa of the Anomura and Brachyura is a distinct phase clearly separable from the crab stage. Hence it is proposed here that the old terminology for the penaeid larval phases viz., nauplius, protozoea, mysis and postlarva may be retained.

Characteristic features of the larval stages:

The larval phases in the development of the *Penaeidae* can be characterised as follows.

Nauplius: Body pear shaped with three pairs of natatory appendages, no spines or processes on the antennae and mandibles for feeding purposes, mouth not formed, dependent on internal yolk for development; pairs of caudal setae of equal length extending straight posteriorly; lateral setae on appendages arise singly or in pairs, not in clusters; carapace only a close-fitting rudiment in later stages.

There are usually six nauplius sub-stages identifiable by the increase in the number of setae on antennal exopods and the caudal lobes. Heldt (1938) has described eight sub-stages, Cook and Murphy (1965, 1969), Dobkin (1961), Renfro and Cook (1963) and Raje and Ranade (1972a and b) five sub-stages and Hudinaga (1942), Muthu *et al.* (1961 a, b, c, d,), Motoh and Buri (1969) and Motoh (1979) six sub-stages. It is likely that there is some variability in the development of the setae on the furcal lobes and the antennal exopods which may account for the differences in the number of sub-stages recognised by the various workers. The normal sequence of development of the setae on the antennules and antennae of the various sub-stages of the nauplius phase is discussed by Muthu *et al.* (1978e).

Protozoea: A large carapace followed by a slender thorax and abdomen; carapace does not cover the thorax completely; uniramous antennules and biramous antennae with fully segmented exopods; abdomen bifurcate posteriorly, each furca with at least 7 setae; biramous 1st and 2nd maxillipeds well developed, the 3rd absent or rudimentary; usually no spines on the posterior half of the carapace, if spines occur, dorsal organ also present.

The protozoea phase is clearly divided into three distinct sub-stages in all the penaeids so far studied.

Protozoea 1 : Eyes sessile; rostrum or supraorbital spines absent; pereopods absent; abdomen unsegmented.

Protozoea II : Eyes stalked, rostrum and supraorbital spines (if any) appear, first five abdominal segments demarcated, telson not separate from last abdominal segment; uropods absent.

Protozoea III : Uropods present, telson separated from last abdominal segment; first 5 abdominal segments with dorsal spines.

The intermoult growth of the protozoea sub-stages first observed by Hudinaga (1942) has also been noted by Muthu *et al.* (1978 a and d). The intermoult increase in length of the larvae and increase in length of the rudimentary appendages is quite marked.

Mysis: Carapace covers the thorax; 3rd maxillipeds and the five pereopods functional, with well developed exopods; first 3 pereopods with rudimentary chela; pleopods, if present, rudimentary, without setae; antennal exopod unsegmented and scale-like; telson narrow and notched medianly; pleura of 1st abdominal segment overlaps the 2nd segment.

Unlike the protozoea sub-stages which are distinguished by clear-cut morphological changes, the mysis sub-stages are separated only by small increases in the size of the larvae, the length of the pleopods and the number of setae on the antennal scale, maxillary exopod and the uropod rami. Although there are only 3 mysis sub-stages in the genus *penaeus* the number appears to be highly variable in the other genera and even in the same species under different environmental conditions (*Muthu et al.* 1978e).

Sometimes the last mysis moults into one or two "intermediate stages" in which the larvae have setose pleopods but still retain the exopods on the thoracic appendages and sometimes also the serrated standing teeth in the mandible; reduction in the endopod of the maxilluie and maxilla and the broadening of the protopod of the 1st maxilliped are also usually observed (Muthu *et al.* 1978 b, c; Silas *et al.* 1971: Devarajan *et al.* 1978). The mysis IV of *P. trisulcatus* described by Heldt (1938) and the mysis V of Raje and Ranade (1972 b) are obviously "intermediate stages".

Postlarva: The pleopods setose and functional; exopods on pereopods and 2nd and 3rd maxillipeds lost, that of first maxilliped reduced; mandibles lose the serrated teeth between the incisor and the molar processes, endopod of maxilluie reduced, without setae; the endopod and endites of maxilla reduced; protopod of first maxilliped broad, with stiff bristles. the endopod of second maxilliped recurved, with stiff bristles.

The postlarval phase gradually merges with the juvenile phase after subsequent moults and it is difficult to pinpoint the transition.

Variability in larval stages: In penaeid larval development there seems to be some variability of the number of sub-stages during the mysis phase (Ewald, 1965), where the transition from one sub-stage to the next is very gradual (Muthu *et al.* 1978e). Variability seems to be a common feature in the larval development of many groups of crustaceans. The most well-known case is that of the furcilia larvae of Euphausiacea where the occurrence of "dominant" stages, variant stages and "skipping" of stages has been reported by a number of authors (Frost, 1935; Fraser, 1936; Einarson, 1945; Boden, 1951; Mathew, 1978; Silas and Mathew, 1977). Skipping of stages and "extra" stages have been recorded among the later zoea stages of Caridea (Broad, 1957; Pike and Williamson, 1961; Dobkin, 1963, 1971; Little, 1968; Ewald, 1969; Hubschman and Broad, 1974; Pillai and Mohamed, 1974 and Pillai, 1976), Anomura (Johnson and Lewis, 1942; Rees 1959; Provenzano, 1962 a, b, 1967; Boyd and Johnson (1963) and Brachyura (Costlow and Bookhout, 1959, 1962; Porter 1960; Nicols and Keney, 1963; Costlow, 1965). Larvae have also been observed to moult repeatedly without changing into the next stage (Pillai and Mohamed, 1974 and Costlow 1965). It is now recognised that the variability of larval stages occurs both in nature as well as under laboratory conditions (Costlow, 1965).

The variability of crustacean larvae has been attributed to various causes. Temperature appears to be one of the factors. Ewald (1969) showed that the zoea of *Tozeuma carolinense* passes through a greater number of stages when reacted at a temperature of 15°C than at 25°C, while Boyd and Johnson (1963) found that the variability of larval *Pleuroncodes planipes* increased with increase in temperature. Broad (1957) suggested that the number of larval stages of *Palaemonetes pugio* and *P. vulgaris* was related to the amount and nature of food available. An interesting case of variability was recorded by Ewald (1969) in *Tozeuma carolinensis*, where the larvae from adults living on the sea

grass *Cymodocea* passed through a lesser number of zoeal stages than from those living on Alcyonarians. Among Euphausids variability was greater in the neritic species than in the oceanic species and is apparently related to the greater variability of environmental factors in the neritic region (Silas and Mathew, 1977).

There has been some discussion about the mechanisms involved in this larval variability. Broad (1957) suggested that the rate of development of the larvae and the frequency of moulting were independent and that the variability was caused if the rate of development did not synchronise with the moulting rate which was assumed to be constant. Working with the zoea of crabs Costlow, Bookhout and Monroe (1960, 1962) emphasised the regularity in moulting frequency of the zoea if environmental factors are optimal. Studying the effects of eyestalk ablation on the megalopa of *Callinectes sapidus*, Costlow (1963a) came to the conclusion that moulting and growth were indeed independent and apparently controlled by separate mechanisms. According to Costlow (1965) any factor which altered the rate of morphological development without affecting the mechanisms controlling moulting would be observed as "skipping" of stages (rate of development accelerated) or as "extra" stages (rate of development retarded). If the mechanisms controlling morphological development were completely inhibited while the frequency of moulting was normal the larvae would "mark time" in the same stage. The "intermediate stages" could be accounted for by a constant moulting rate preceded by normal or accelerated development in some parts of the body while in some other parts the development was inhibited or normal.

Costlow (1963b) and Little (1968), who found that eyestalk removal in the late zoea stages, resulted in the production of "extra" zoeal stages, suggested that at least part of the control mechanism for normal development is centred in the eyestalks and may be endocrine in nature. Costlow (1963a) suggested that early in the development of larvae only the moult accelerating hormone of the Y-organ is functional activating the regular sequence of moults and that late in larval development the X-organ sinus gland complex of the eyestalk becomes functional prolonging the intermoult period and eliminating the established frequency of moulting. So larval variability may be linked to to the malfunction of endocrine systems which do not allow the development of morphological features to keep pace with the frequency of larval moulting which is controlled by separate endocrine systems. Insufficient food, dietary deficiencies or the presence or absence of inorganic or organic compounds in sea water or any other environmental change could lead to malfunction of the endocrine systems which would manifest as variability in the number of larval stages or in minor differences in the morphological characters of the larvae (Costlow, 1965). This hypothesis should be tested by further experiments. The term "malfunction of the endocrine systems" may suggest that the variant larvae are in some way abnormal. But as Little (1968) states "the occurrence of 5, 6 or 7 larval stages need not be considered abnormal, for the end result of all three of the developmental sequences is a normal postlarval animal".

Flexibility in the functioning of the endocrine systems which is perhaps genetically determined may be responsible for larval variability. The resultant flexibility in larval development may have survival value as suggested by Muthu *et al* (1978 c) who found that, in rearing the larvae of *Penaeus indicus* and *P. monodon* which had only 3 mysis sub-stages, mass mortalirty was more frequent, if the conditions were not optimal, than

in the case of *Metapenaeus dobsononi*, *M. affinis*, *M. monoceros* and *Parapenaeopsis stylifera* which had 5 to 7 mysis sub-stages. Even in nature, when the conditions are unfavourable, the larvae of these later species may linger for a longer time in the plankton by moulting into these 5 to 7 mysis sub-stages and thus gain time till the conditions improve.

Functional morphology of the appendages

The structure and setation of the larval appendages is related to their function. The antennules with long terminal seate in the nauplius and protozoea stages serve as swimming organs. To improve their flexibility, the basal segment of the antennule is subdivided into 4-5 sub segments in protozoea I and II. As the mysis stage is approached the antennule loses its mobility when these subsegments fuse into a single basal segment in protozoea III. In the mysis phase the antennule altogether loses its natatory function and serve as a sensory organ. Similarly the natatory antenna of the protozoea has a fully segmented exopod for greater flexibility; in the mysis stage the exopod loses its segmentation and is transformed into a scale which perhaps acts as a stabilizing organ. The natatory function is taken over by the setose exopods of the thoracic appendages during the mysis stage. The endopods of the pereopods which bear long terminal setae during the mysis phase are not suitable for grasping the prey. The uropods which are fully developed in the mysis stage form, along with the telson, the tail fan which, when flicked by the flexure of the abdomen, suddenly jerks the larva backwards and enables it to escape from predators. The appearance of setae on the pleopods coincides with the disappearance of the thoracic exopods during the post-larval phase and the natatory function is taken over by the pleopods.

The structure of the feeding appendages of the larvae gives us an idea about their food requirements. The present author closely studied the mouth parts of the larvae of various species of penaeids and compared them with those of caridean larvae from published descriptions and illustrations and found that the mouth parts of the penaeids and carideans fall into three broad categories :

1. The filter feeding type
2. The mixed feeding type
3. The carnivorous type

The filter feeding type is found in the protozoea and mysis stages of all penaeids. They have a well developed maxillary filter (Fig. 1b) with numerous close-set filtering setae on the endites of the protopod; among the filtering setae which have fine setules pinnately arranged on either side, a few stronger setae with stiff bristles are interspersed, perhaps providing reinforcement to the delicate filter. The space between the setules of the filtering setae are small enough to retain the phytoplankton on which they feed. The maxillary filter is supplemented by a coarser filter made up of numerous barbed setae on the protopod of the first maxilliped (Fig. 1c). The mandible (Fig. 1a) is weak, with serrated teeth in between the incisor and molar processes. The endopods of the thoracic legs (Fig. 1d) are tipped with long weak setae which are unsuitable for holding the prey.

A sudden transformation in the mouth parts takes place when the mysis metamorphoses into the postlarval stage. The mandibles (Fig. 1e) lose the serrated

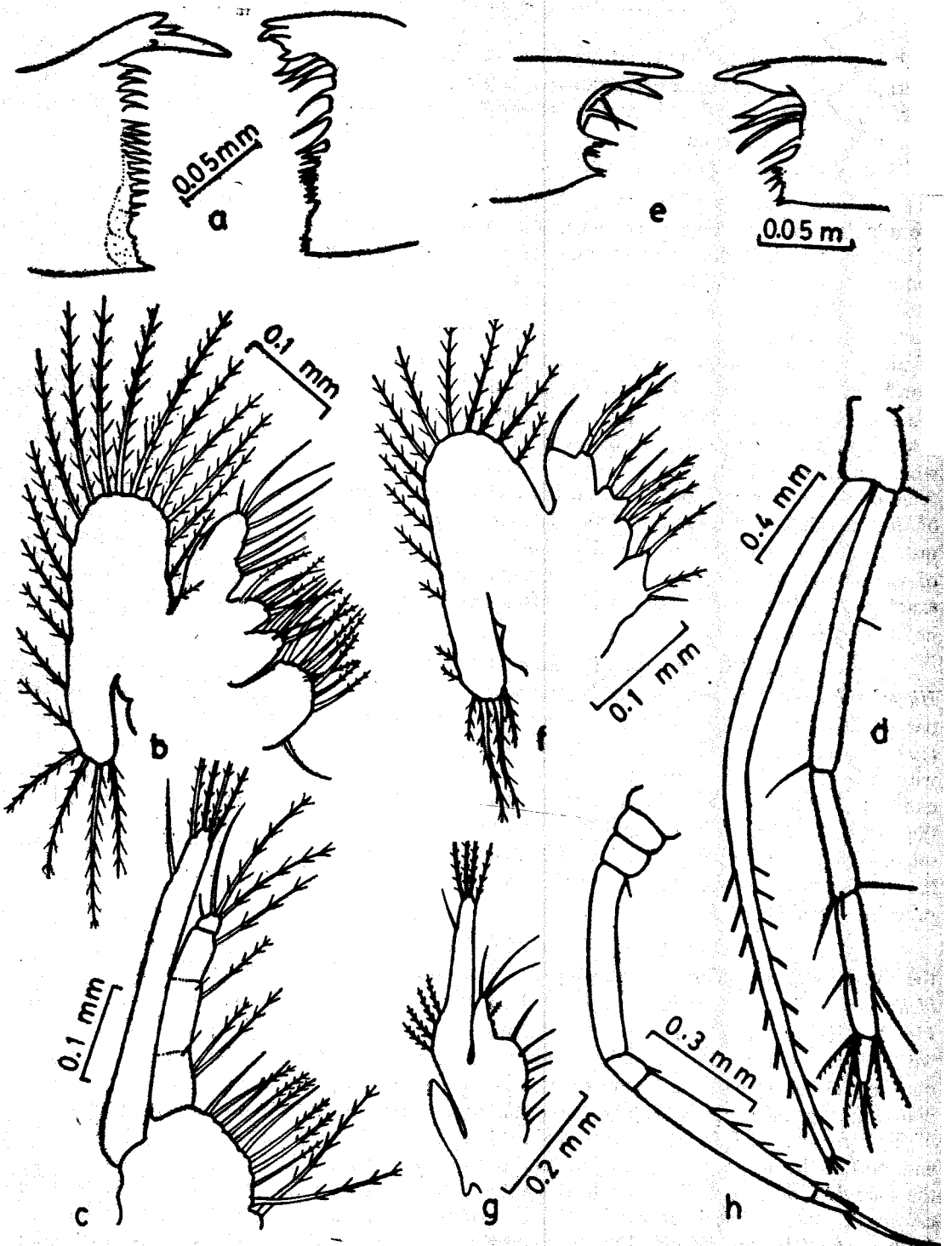

Fig. 2. *Hippolysmata* (*Exhippolysmata*) *ensirostris*. Zoea VII; a-mandible; b-Maxilla; c–First maxilliped; d-First pereiopod

Macrobrachium idella Zoea VII : e-mandible; f-Maxilla; g-First maxilliped; h-Fifth pereiopod

The above examples show that it is possible to predict the feeding habits of the larvae by studying their appendages.

Larval behaviour

Some published information on the behaviour of the penaeid larvae is available. It is supplemented here by personal observation. The penaeid nauplii are attracted towards a weak source of light but bright sunlight is harmful to them. The protozoea stages are also very photopositive to weak light. Attraction to low intensities becomes less pronounced in the mysis stage; the third mysis and postlarva are not attracted to low light intensities (Cook, 1968). The nauplii at rest remain suspended with the ventral side up in the water. They swim in short spurts. The protozoea are very active and swim swiftly in a horizontal position with the dorsal side up. The protozoea of *Parapenaeopsis* with their longer antennular peduncle and very long apical setae swim at a slower pace than the protozoea of *Metapenaeus* and *Penaeus* which have shorter antennular peduncles and apical setae. The swimming speeds determined for *P. aztecus* (Cook, 1968) are given below:

Larval stage	*Rate of movement* cm/min.
Nauplius II	2.3
Nanplius V	6.2
Protozoea I	14.0
Protozoea II	30.0
Protozoea III	60.0

The rate of movement doubles with each moult. The protozoea swim ceaselessly and can be seen trailing a long "tail" of fecal matter if they are well fed and healthy. If they become listless and are not attracted towards a beam of weak light it is a sure sign that all is not well with them. When the aeration is stopped the healthy nauplii and protozoea form swirling swarms reminiscent of a swarm of bees. There seems to be some attraction between the members of a swarm (Gurney, 1942).

The moult to the first mysis transforms a very active protozoea of *Penaeus* into relatively sluggish animal which hangs with the anterior end pointing obliquely downwards. It hovers around like a helicopter and jumps back suddenly by flexing the abdomen. The mysis of *Metapenaeus* on the other hand is oriented in a horizontal position and is more active than that of *Penaeus*. The late stage mysis of *Parapenaeopsis* are strong swimmers because the exopods of pereopods acquire additional pairs of setae with each moult of the mysis. The exopod setae remain constant in number in the other two genera.

With the acquisition of plumose pleopods the postlarvae become horizontally oriented while swimming. The first postlarvae of *Metapenaeus* and *Parapenaeopsis* promptly settle to the bottom of the container but the early postlarvae of *Penaeus* continue to be pelagic for 4–5 days. Among the later stage postlarvae, those of *P. indicus* are the most active while those of *P. monodon* and *P. semisulcatus* tend to rest on the sides of the container in a vertical position.

Larval development and taxonomic relationships

Dobkin (1971) states that "one of the most valuable results of descriptive work on the larval stages of any group of organisms is the information this can provide on the relationship between species, genera, etc". This has indeed been proved correct in the case of penaeids by Cook (1966), Haq and Hassan (1975) and Muthu *et al.* (1978e) who have pointed out the similarity in the structure, segmentation and setation of the appendages of the larvae of penaeid species belonging to the same genus. This has enabled them to delineate the morphological features of the larvae that are characteristic of various genera. Indeed the larvae of various species belonging to the same genus look so much alike that it is practically impossible to distinguish between them, especially in the nauplius and protozoea stages. But in the mysis and postlarval stages the chromatophore pattern on the tail fan and the 6th abdominal segment have been found to be species specific and very useful for identification of the species (Muthu, 1978). It is likely that when the larval development of species belonging to more penaeid genera are worked out the generic characters referred to by these authors may prove to be shared by closely related genera, thereby revealing the affinities at the suprageneric level. The close similarity in the setation of the antennule of the late nauplius stage of *Parapenaeopsis, Trachepenaeus* and *Xiphopenaeus,* has already been pointed out by Muthu *et al.* (1978e). These three genera also exhibit close similarity in the basic structure of the petasma and thelycum.

That the larval characters may throw light on taxonomic relationships within the family has been indicated by Gurney (1942) who has recognised the three following types of mysis larvae which appear to be characteristic of three sub-families.

Penaeinae: Body slender; carapace smooth, rostrum not longer than antennules; abdomen with small dorsal spine on three or more somites; telson parallel sided or widening distally, sometimes with median spine; gills delayed in appearance and very small; pleopods appearing all together.

Solenocerinae: Body stout, carapace and abdomen covered with numerous spines; carapace with dorsal organ and series of antero-lateral spines; abdomen with postero-dorsal spine on all segments; telson deeply forked, arms diverging; gills larger, but not in full adult number; pleopods appearing all together.

Aristaeinae: Body stout; carapace not spiny; abdomen bent at right angles to thorax; rostrum very long; abdomen with very long spine on second segment; fifth pereopod rudimentary in mysis I; gills appearing early, very large, and in some species in full adult number in last mysis stage; telson narrowed behind, ending either in a small fork or deep cleft, the arms parallel; pleopod I appears before the rest.

In this connection it is necessary to mention that larvae of the genus *Sicyonia* described so far, closely resemble those of *Trachypenaeus, Metapenaeus* and *Parapenaeopsis* in many respects and would fit into the first category of larvae (Penaeinae) recognised by Gurney (1942). In fact, Burkenroad (1934) believed on the basis of larval development that *Sicyoninae* is closer to *Penaeinae* than to *Aristaeinae*. Kubo (1949) also felt that *Sysioninae* stands close to *Penaeinae* in the features of the petasma, thelycum and appendix masculina of the adult. It may be suggested here that the peculiar features of *Sycioninae* such as the absence of pleurobrachia and exopods on the thoracic segments posterior to the third segment and the absence of endopods on the pleopods and the heavily armou-

red cuticle are related to the sedentary and burrowing habits of these prawns and may not be of much taxonomic significance. The larval characters, in fact, show the real affinities of this monogeric sub-family which should perhaps be merged with the *Penaeinae*.

PENAEID LARVAL CULTURE

Historical:

The pioneering efforts of Hudinaga and his associates in Japan (Hudinaga, 1935, 1942; Hudinaga and Miyamura, 1962; Hudinaga and Kittaka, 1966, 1967) who demostrated that *Penaeus japonicus* could be made to spawn in the laboratory and the larvae reared under controlled conditions upto the juvenile stage, gave an impetus to the mass culturing of the larvae of penaeid prawns for aquaculture purposes. By adopting Hudinaga's method or modifications thereof, artificial propagation of the following species of penaeid prawns has been achieved with varying degrees of success.

Species	Country	Authors
Penaeus japonicus	Taiwan	Huang *et al.* (1969)
P. teraoi	Taiwan	Liao and Huang (1973)
P. latisulcatus	Japan	Shokita (1970)
	Australia	Pownall (1974)
P. aztecus	U.S.A.	Cook and Murphy (1969) Cook (1969)
	Tahiti	Aquacop (1977)
P. duorarum	U.S.A.	Ewald (1965); Cook and Murphy (1969); Tabb *et al.* (1972); Krantz and Norris (1976)
P. kerathurus	Italy	Lumare *et al.* (1971);
	Spain	Rodriguez (1975)
P. marginatus	Hawaii	Gopalakrishnan (1977)
P. stylirostris	Tahiti	Aquacop (1977)
P. schmiti	Venezuela	Pinto and Ewald (1974)
	Cuba	Perez and Saurez (1979)
P. vannamei	Tahiti	Aquacop (1977)
P. orientalis	Japan	Oka (1967a and b)
	Korea	Kim (1967)
P. indicus	Philippines	Anon (1976 b)
	India	Muthu *et al.* (1977, 1978a)
P. merguiensis	India	Raje and Ranade (1972a)
	Thailand	Ruangpanit *et al.* (1971)
	Tahiti	Aquacop (1975, 1977)
	U.K.	Beard *et al.* (1977)
	Indonesia	Hameed Ali (1980)
	Philippines	Platon (1978), Motoh and Buri (1979)

P. monodon	Taiwan	Liao *et al.* (1969a)
	Tahiti	Aquacop (1975, 1977)
	Philippines	Villaluz *et al.* (1969); Anon (1976a); Platon (1968); Motoh (1979)
	Thailand	Kungvankij (1976)
	India	Silas *et al.* (1978)
P. semisulcatus	Taiwan	Liao and Huang (1973)
	Israel	Samocha and Lewinsohn (1977)
	Thailand	Kungvankij (1972)
	India	Devarajan *et al.* (1978)
P. esculentus	Australia	Fielder *et al.* (1975)
Metapenaeus dobsoni	India	Thomas *et al.* (1976b); Silas and Muthu (1977); Muthn *et al.* (1978b)
	Kuwait	Enomoto (1971)
M. affinis	India	Thomas *et al.* (1976a); Silas and Muthn (1977); Muthu *et al.* (1978c)
M. monoceros	India	Raje and Ranade (1972b); Silas and Muthu (1977); Mohamed *et al.* (1978)
M. ensis	Japan	Funada (1966)
	Taiwan	Liao *et al.* (1969b)
M. joyneri	Korea	Lee and Lee (1968)
	Taiwan	Liao and Huang (1973)
M. burkenroadi	Japan	Kurata and Pusadee (1974)
M. stebbingi	Israel	Samocha and Leweinsohn (1977)
Parapenaeopsis stylifera	India	Thomas *et al.* (1975); Silas and Muthu (1977); Muthu *et al.* (1978d)
Artemesia longinaris	Argentina	Boschi and Scelzo (1974)
Hymenopenaeus mulleri	Argentina	Sceizo and Boschi (1975)

Culture systems used for larval rearing:

The culture techniques used for mass production of post-larvae fall into two major categories. (1) The "community culture" system or the fertilized system and (2) the feeding system or unfertilized system.

Community culture system:—In this system the prawn larvae and their phytoplankton and zooplankton food organisms are cultured together in large concrete tanks (Hudinaga and Cittaka, 1967; Eujinaga, 1969; Shigueno, 1975; Yang, 1975). The spawning of the eggs and the growth of the resultant larvae upto the fry stage take place in the same tank. After the nauplii hatch out the tank water is fertilized daily with nitrates (K NO_3 2 ppm) and phosphates (KH PO_4 0.2 ppm) to maintain a good growth of naturally occurring diatoms (5,000 to 20,000 cells/ml) on which the protozoea feed. By the time the mysis stage is reached a good population of zooplankton organisms will have grown up in the tank. Thus a feeding environment closely similar to the

conditions obtaining in the sea is created. Anaerobic decay of the dead organisms is prevented by vigorously aerating the water, by air stones or by slowly rotating vanes to which perforated air line pipes are attached. Eggs of Artemia are added to the tank water as additional food for the mysis and postlarval stages. During the later postlarval stages crushed and washed clam meat is added as food. If the diatom bloom does not develop due to some reason, finely ground soybean waste is used to feed the larvae. From the first day of mysis to the fourth day of postlarvae, clean sea water is added to the tank every day until the water level is increased from the original 40 cm to 2 metres. Once feeding with clam meat is started 1/5 to 1/3 of the water in the tank is changed daily.

By this procedure about 1 million prawn fry (P_{82}) are obtained from each 10 m x 2 m tank. The tanks vary in size from 60 to 200 tons water capacity; the smaller ones are built inside enclosures with transparent roofing to protect the larvae from changes in weather conditions. The large 200 ton out-door tanks are not affected much by the weather. In Taiwan, Liao and Huang (1973) added oyster larvae produced by artificial fertilization as food if the phytoplankton bloom did not develop well. In the Philippines (Anon, 1976 a, b) bread yeast at @2 gm/tonne per day has been used along with mixed diatoms to feed the protozoea and mysis stages; the rotifer *Brachionus* cultured separately is also used at a concentration of 10-25 cells/ml. for the mysis and early postlarval stages.

A careful study of the results published by Fujinaga (1969), Shigueno (1975), Liao and Huang (1973) and Anon (1976 a, b) shows that high initial concentration of nauplii invariably results in poor survival rate, while low initial larval density ensures good survival. The Philippine workers recommend that the concentration of the larvae in the large concrete tanks should not exceed 6000 larvae/ton of sea water and they use only 6 spawners of *P. monodon* even in a 200 tonne concrete tank (Anon, 1976c). The high mortality in crowded tanks appears to be due to the accumulation of metabolites produced by the larvae themselves. Hence the trend in the Japanese method of larval rearing is towards employing larger and larger tanks so that the metabolites released by the larvae are diluted by the large volumes of water used. But the method is wasteful of food and sea water owing to the following reasons. The larvae do not hunt for food but filter the food particles that happen to be in their vicinity and so a good amount of food added will remain unutilized by the relatively low density of the larval population and the poor circulation inside the tank. The other disadvantages of this method are the high initial cost of construction, the lack of control over the intensity of the phytoplankton bloom and the frequent growth of undesirable species of organisms such as dinoflagellates and *Noctiluca* which lead to mass mortality of larvae. Hence in Japan and the Philippines, although large concrete tanks are still used, the food organisms are separately cultured on a very large scale, concentrated and fed to the larvae.

The feeding system or unfertilised system

This system developed at Galveston, Texas (Cook and Murphy, 1966, 1969; Cook, 1969; Mock and Murphy, 1971; Mock and Neal, 1974; Salser and Mock, 1974) is more sophisticated and consists of a number of independent processes which involves higher technical skills.

1. Mass production and storage of pure algal cultures.
2. Mass production of freshly hatched *Artemia* nauplii.
3. Larval rearing operations.

The use of desirable species of food organisms and the greater control over the water quality facilitated by the use of smaller containers make this method more dependable.

For small scale work Cook (1969) used a fibre glass tank (900 litres) for spawning, and inverted 11-litres polyurethane carbuoys for rearing the nauplii to the postlarval stage. In the latter case the larvae and the phytoplankton that is added for feeding them are not allowed to settle down by the action of an air stone that is placed in the neck of the inverted carbuoy. The efficient circulation prevents the larvae from getting entangled in the debris at the bottom and dying and also helps to bring the larvae and the phytoplankton cells into contact with each other more frequently so that the feed is utilised effectively. Half the volume of water in the carbuoy is also replaced every day with fresh filtered sea water which in effect prevents the accumalation of metabolites in the medium. This system is especially suitable in places where there are no facilities for getting large volumes of good sea water. By this method Cook (1969) has been able to produce, 2000 postlarvae in 15 litres of water in the carbuoy (i.e., 133 postlarvae/litre) starting with an initial concentration of 266 nauplii/litre. In comparison, the Japanese method yields only 5-10 postlarvae per litre of sea water.

Later Cook and Murphy (1969) used 1890 litre cylindrical polyethylene containers fitted with a system in which the sea water is recirculated through a crushed oyster shell filter for both spawning and larval rearing. The recirculation is stopped as soon as pure cultures of desirable species of diatoms and unicellular algae are added to the tank water for feeding the protozoeal stages. The concentration of phytoplankton is maintained at a density of 10,000 to 15,000 cells per ml by an automatic dispensing system which controls a peristalic pump that delivers fixed amounts of pure algal cultures from a reservoir kept in a refrigerator. Later the algal cultures were shifted to an attic over the rearing tanks so that the algae flowed by gravity into the lower tanks. From the mysis stage onwards the algal food is stopped and the larvae are given freshly hatched brine shrimp nauplii at a concentration of 3-5 nauplii per ml of water. Salser and Mock (1974) have reported the improvements that were made in this system. The major change is in the shape and size of the container. The fibre glass containers with a capacity of 2 M³ are cylindro-conical in shape to facilitate cleaning, draining and efficient dispersal of the food particles and larvae throughout the water column. Aerating stones at the tip of the cone and suitably constructed air-lifts keep the water in constant vertical motion so that the food particles are not allowed to settle down. A system of hatching, separating and collecting the brine shrimp nauplii on a large scale is also described.

This system has been adopted in a modified form in Tahiti (Aquacop, 1975, 1977) in the Philippines (Platon, 1978) and in the U. K. (Beard *et al*, 1977) to mass produce the postlarvae of penaeid prawns. Higher concentrations of algae are used by these workers (30,000–1,00,000 cells/ml) and *Brachionus* 5-10 rotifers/ml is used for the

mysis stage and Artemia nauplii (5 Nos./ml) for the postlarvae P1-P5. The initial density of nauplii stocked in the tanks is 50–100/litre with a preference for the lower stocking density.

In India, Silas and Muthu (1977) have reared the penaeid larvae in 50 litre plastic basins and fed them with the phytoplankton film collected from the surface of the brackishwater ponds. This film contained a mixture of diatoms, tintinnids, rotifers and copepod nauplii and proved very effective in the case of *P. dobsoni* and *P. affiinis*. Presently, we are using 6′ diameter plastic lined pools for rearing the larvae of *P. indicus*.

Environmental factors in larval rearing

The duration of the larval stages is dependent on temperature and the availability of suitable food organisms for the larvae. Temperature is the only factor influencing the rate of development during the embryonic stage. At a temperature of 27°-28°C the nauplii hatch out 14-15 hrs after the eggs are laid (Muthu *et al.* 1978a, b, c, d). But at a temperature of 30° – 31°C the nauplii of *P. stylifera* and *P. affinis* hatch out in 8-9 hrs (Thomas *et al.* 1976). Lee and Lee (1968) suggested that at a given temperature the eggs belonging to almost all the species of penaeid prawns take more or less the same time to hatch out. This is because the amount of yolk stored in the eggs of the various species is more or less the same, the variations noted in the diameter of the eggs of the different species being due mainly to differences in the size of the perivitelline space (Muthu *et al.* 1978e). During the nauplius stage also the rate of development is mainly controlled by temperature alone as the larvae do not feed at this stage.

Once the protozoea stage is reached they start feeding and the rate of development is not controlled solely by the temperature of the water. The abundance of suitable food organisms accelerates growth while insufficient or poor quality food retards growth rate and the larvae take a longer time for metamorphosis, (Lee and Lee, 1968, 1969; Shigueno, 1975; Hirata *et al.* 1975).

Temperatures ranging from 24° C to 32° C and salinities ranging from 27 to 34 ppt have been found to be suitable for the development of penaeid larvae (Hudinaga, 1942 and Cook and Murphy, 1969). Survival of the larvae of *P. Aztecus* was best at 28 to 30 ppt; at 34 ppt the survival was poorer (Cook 1970). Beard *et al.* (1977), while rearing the larvae of *P. merguiensis*, also found that a gradual reduction of salinity to 25 ppt by the time the postlarval stage is reached was beneficial.

Lumare *et al.* (1971) found the light intensities below 1000 lux inhibited the normal development of the protozoea of *P. kerathurus* and prevented metamorphosis to the mysis stage. But, Cook and Murphy (1966) reported that "the larvae can also be reared in the dark; light is not essential to their development". Kurata and Shigueno (1979) state that adequate light intensity is necessary for successful rearing and that the postlarvae reared outdoors had a healthier appearance. The role of light in the development of the larvae is yet to be understood.

Furukawa (1969) found that a pH higher than 8.5 was inimical to the protozoea of *P. japinicus* and caused large scale mortality and abnormalities. In the community culture method of larval rearing the pH may shoot up to 9.0 when there is a dense bloom of

phytoplankton and lead to heavy mortality. We have also noted the lethal effect of high pH in our larval rearing work.

It is well known that the accumulation of ammonia and nitrites in the rearing medium is toxic to aquatic animals. But very little experimental data on the acute and chronic toxicity levels of these metabolites are available for penaeid larvae. Preliminary work done in the Philippines on the tolerance of *P. monodon* larvae to nitrite and ammonia concentrations are summarised below:

Larval Stage	Tolerance level		Authors
	Nitrite as Na No 2	Ammonia as NH_4 Cl	
Zoea	3 ppm	10 ppm	Cathedral *et al*; (1977)
Mysis	10 ppm	60 ppm	
Postlarvae			
P3	50-80 ppm	50 ppm	Cathedral *et al*; (1977)
P11	100 ppm	50 ppm	

Tolerance to nitrite and ammonia increases as the larvae grow older. However, more precise data on the long term and short term effects of nitrite and ammonia levels on penaeid larvae are urgently needed.

Cook and Murphy (1969) reported that by adding 1 gm. of the sodium salt of EDTA to every 100 litres of the rearing medium unexplained catastrophic mortalities could be avoided. Cook (1970) also found that the larvae of *P. aztecus* survived well in 24 ppt salinity with EDTA, but suffered complete mortality when EDTA was not added at this salinity. Anon (1976 a & b) also added EDTA @ 0.5 gm/1000 litres/day in the large outdoor concrete tanks for large scale production of prawn fry. Seawater used for larval cultures by Beard and Wickins (1981) was treated with EDTA (@ 0. 1gm per 100 litres) and vigorously aerated for 24 hrs before use. EDTA is a metal chelator and has been used successfully in culturing phytoplankton also. The beneficial effects of the addition of EDTA have not been explained.

Hudinaga and Kittaka (1975) have found that the hatching and survival rates of the larvae were affected by biological differences between the seawater from different regions. Ewald (1965) also reported differences between the survival of the pink shrimp larvae cultured in bay and oceanic waters, the survival being greater in oceanic waters. We found that the water in which the ctenophore *Pleurobrachia* abounds is unsuitable for larval rearing even when such water is filtered to remove the ctenopores.

It is evident that proper attention should be paid to these physical, chemical and biological properties of the seawater that is used for rearing the larvae and that these parameters should be monitored regularly during rearing operations.

Larval feed:

The nauplius is a nonfeeding stage as it has no mouth; it survives on the internal yolk. The larvae start feeding from protozoea I onwards. Hudinaga (1942) fed the protozoea

with pure cultures of *Skeletonema costatum* which remained the classical food for penaeid protozoea for a long time. Later Hudinaga and Kittaka (1967) and Fujinaga (1969) showed that the larvae survived equally well with the mixed cultures of diatoms that were induced to bloom in the concrete rearing tanks by fertilizing. Cook and Murphy (1969) fed the protozoea with pure cultures of *Skeletonema costatum, Thalassiosira* sp,, *Cyclotella nana, Phaeodactylm tricornutum, Dunaliella, Exuviella, Gymnodinium splendens* and *Isocrysis galbana* and found that only the last was unsuitable for the larvae. When fed as mono cultures *Thalassiosira* proved to be the most effective. But a mixture of *Skeletonema, Dunaliella, Cyclotella* and *Thalassiosira* gave the best results. More recently *Skeletonema* has been replaced by *Chaetoceros* for rearing the larvae upto the post larval stage in Japan (Kurata and Shigueno, 1979) and the Philippines (Anon, 1977). Simon (1978) found high survival rate in the protozoea of *P. stylirostris* and *P. vannamei* fed with *Chaetoceros gracilis.* At the prawn culture laboratory of the CMFRI, *P. indicus* larvae are reared upto the postlarval stage exclusively on a diet of mixed cultures of phytoplankton dominated by *Chaetoceros* and developed by adding fertilizers to raw seawater passed through organdie cloth and kept in white, one ton, fibreglass tanks in a glass roofed shed. In Italy, the protozoea of *P. kerathurus* were grown successfully upto the late mysis stage on a diet of *Coscinodiscus grandi* and *C. centralis* alone (FAO, 1974). *Tetraselmis* has been used successfully by Planton (1978) in the Phillippines for rearing the larvae of *P. monodon* and by Beard *et al* (1977) in the U. K. for *P. merguiensis* larvae. In Tahiti *Cylindrotheca* and *Tetraselmis* have proved effective for feeding the protozoea of penaeid prawns (Aquacop, 1975, 1977). In the Philippines *Chaetoceros* is concentrated by sand filtration and backwashing before it is added to the larval tanks to avoid introducing the algal culture water which is belived to be toxic to the larvae (Planton, 1978). Further, to delink the production schedule of the algae from that of the larvae, the algae are concentrated either by flocculation or by using a cream separator and then kept frozen for feeding the larvae (Salser and Mock, 1974; Anon, 1977).

Although bread yeast was used along with diatoms to feed the larval stages (Anon, 1976a, b) in the Philippines, Kurata and Shigueno (1979) clearly state that bread yeast is not digested. Finely powdered Soycake (Hirata *et al.*, 1975) and a formula feed (Kurata and Shigueno, 1979) have proved effective for rearing the protozoea stages. Other non-conventional feeds found to be of some use in larval rearing are powdered fat-free rice bran (Ishida, 1967), activated sludge (Imamura and Sugita, 1972), marine yeast (Furukawa, 1973) washings of filamentous algae and juice of Sargassum (Anon, 1976a), fermented extract of vegetable refuse from kitchens and egg yolk (Anon, 1967). The latest development is the use of microencapsulated feed 15-25 microns in size (Jones *et al.* 1979) to feed the larvae. Hameed Ali (1980) has stated that he has reared the larvae from the protozoea stage to the postarval stage exclusively on a diet of blended tissues of *Acetes* and *Mesopodopsis.* But it would appear from his account that a good growth of diatoms was present in the rearing tanks and the larvae would have certainly fed on them too.

In the mysis and postlarval stages the larvae are conventionally fed with brine shrimp nauplii. But recently there is a trend towards replacing the costly *Artemia* with the more easily cultured *Brachionus.* Finely chopped and washed mussel or clam meat or formula feeds have also been tried (Shigueno, 1975). *Brachionus* has been found

to be effective whether they are offered to the larvae in live or frozen condition (Platon, 1978 and personal observation). A novel item that has been added to the list of food organisms is the free living nematode *Panagrellus* which has been used in Israel for rearing the mysis stages of *P. semisulcatus* and *M. stebbingii* (Samocha and Leweinsohn, 1977)

In order to avoid wastage of food, the feeding schedule should be reexamined in the light of the evidence presented in this paper that the mouthparts of the penaeid larvae are adopted for filter feeding during the protozoea and mysis stages and become capable of handling larger particulate food only after the metamorphosis to the postlarval stage. The size of the food particles (both live and artificial) offered to the larvae should also be studied in relation to the fine structure of the filtering setae on the maxillary endites during the various larval stages.

Rearing of the postlarvae :

In small tank cultures the postlarvae are generally harvested in the P4-P5 stage and reared in larger outdoor tanks. They are highly cannibalistic at this stage and overcrowding in a small tank results in heavy mortality. A closed system raceway described by Mock *et al.* (1973) has proved efficient for rearing postlarvae at high densities of 2300/m^2 (22 mm), 12,500/M^2 (12 mm) and 26,000/M^2 (6 mm) with survival rates 90-95%. In the prawn culture laboratory of the CMFRI the postlarvae are transferred to 24' diameter plastic lined outdoor pools and fed with frozen *Miona* and pelletised formula feeds.

Diseases of larvae:

Very little is known about diseases of larval shrimp. Larvae of *P. setiferus* reared in the laboratory have been observed to die of fungal infection caused by a species of *Lagenidium* (Lightener and Fantoine, 1973). The "whiteturbid-liver" disease of the larvae of *P. japonicus* which was reported to cause heavy mortality (Momoyema, 1974) is attributed to a *Vibrio* sp.; the infected hepatopancreas are highly corroded. Another larval disease attributed to a *Vibro* sp. initially causes loss of some parts of the appendages and in two days the larvae are annihilated (Shigueno, 1975). In the initial stages of this disease the larvae have yellowish vermillion and red colour along the nervous system throughout the body. At the SEAFDEC hatchery the fungus *Lagenidium* affects the larvae frequently (Anon, 1977); formalin at 1 ppm is used to treat the affected larvae. In Tahiti "Treflan" is used to prevent fungal infection of larvae; bacterial infection prevent fungal infection of larval; bacterial infection which lead to necrosis of the larval appendages is controlled by adding gallimycin to the rearing water (Aquacop, 1977).

CONCLUSION

An attempt has been made here to highlight the importance of studies on penaeid larval development in understanding taxonomic relationships and their usefulness in perfecting the techniques of large scale culturing of the larvae for aquaculture

purposes. A more detailed description of the larval appendages especially the nature of the setulation on the setae will be helpful in understanding their functions in relation to feeding, swimming, etc. Further work on the endocrine mechanism of larval metamorphosis which is at present not fully understood is desirable and will be useful in improving the survival of the larvae in mass cultures, where synchronous moulting of all the larvae has distinct advantages. Rationalising the feeding procedures in the light of the functional morphology of the larval appendages and behaviour will make larval rearing more efficient and economical.

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