CULTURE OF NON-CONVENTIONAL LIVE FEEDS AND THEIR ROLES IN MARICULTURE OF NEW SPECIES

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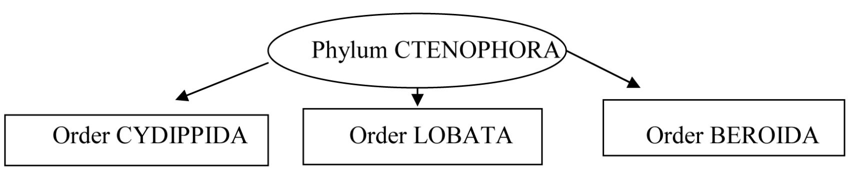
Introduction

Ctenophores, also known as comb jellies, are delicate gelatinous marine zooplankton belonging to the Phylum Ctenophora. Collection of intact specimens and captive rearing are challenging tasks, given the highly fragile nature of these organisms. The ctenophore *Pleurobrachia bachei* commonly found in Indian waters, belongs to the order Cydippida. This species is closely associated with lobster phyllosoma, and advanced phyllosoma of the sand lobster, *Thenus unimaculatus,* have been found to feed very well upon *P. bachei*. As a prerequisite for successful sand lobster larval rearing, captive rearing of *P. bachei* assumes great importance as a source of steady and constant supply of live feed for the developing lobster larvae.



(eg. Pleurobrachia spp.) *(eg. Mnemiopsis spp.)* *(eg. Beroe spp.)*

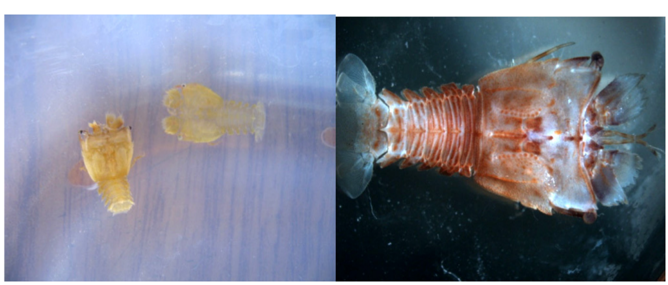
Marine larvae

The larval phase in most lobsters is usually complicated, extended and highly dependent on external factors. Like other crustaceans, lobsters begin life as a developing embryo inside an egg which is carried by the female along with hundreds or thousands of other eggs, on the pleopods. After a rigorous incubation phase (early embryo development inside the eggs) when the eggs are fanned with the help of the pleopods, small, transparent, flattened leaf like larvae called "phyllosoma" hatch out. The incubation period varies from 26-30 days in tropical spiny lobsters to 30-37 days in sand lobsters. Hatching takes place in batches only during the early morning hours and is usually completed in 1-3 days. Larvae are usually small when compared to the adult except in clawed lobsters. These larval stages (phyllososma) undergo progressive molts to complete metamorphosis before settling as the post larval stage, called "puerulus" in spiny lobsters and "nisto" in sand lobsters. The hatchery phase is often the crucial stage in lobster aquaculture, since handling of the delicate phyllosoma is very difficult, and renders the hatchery phase labour intensive. The number of larval stages varies greatly among species, ranging from about 12 stages in spiny lobsters to 4 stages in sand lobsters. Compared to the spiny lobsters, the hatchery phase is of shorter duration in sand lobsters. While larval metamorphoses can extend up to 300 days in spiny lobsters, it is usually completed in 25-60 days in Scyllarid lobsters.

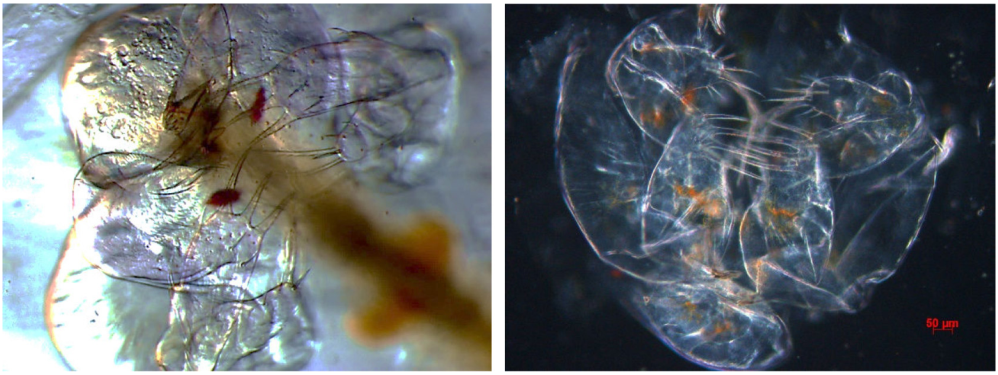
Panulirus homarus **Panulirus ornatus** **Thenus unimaculatus**

Lobster phyllosoma

Final phyllosoma and nisto stages of sand lobster

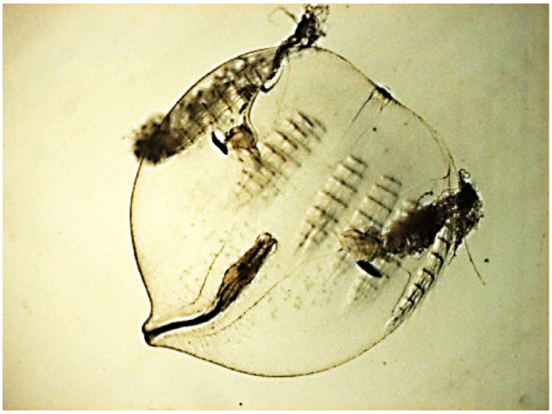


The phyllosoma are mostly phototactic and prefer specific zooplankters as live feed. Spiny lobster larvae ingest arrow worms, fish larvae and small medusae and other live feeds in the early stages. Sand lobster larvae show a preference for ctenophores, small medusae, naupliar stages, copepodites and swimming gastropods and molluscan larvae.

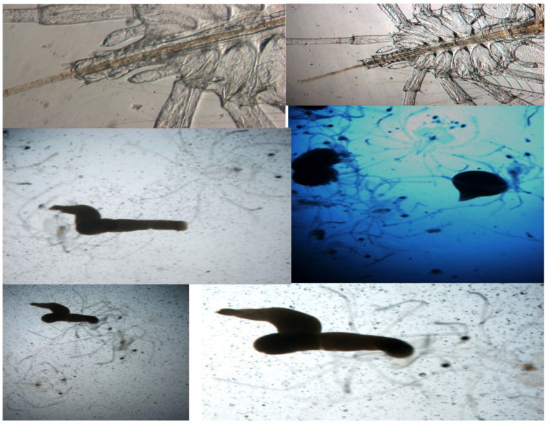


The mandibles and pincers on them - phyllosoma

The mouth parts indicate a soft texture for the diets. Suitable artificial, preferably gel texture, supplementary diets are also essential in lobster hatchery feeding regimes. These diets should be floating and stable in water. Water quality in phyllosoma rearing is of utmost importance as delay in molting attracts too fouling microbes on the shell which render the larvae immobile and obstruct their feeding activity. Organic load and ammonia load should be minimal in the system and tank surfaces should be devoid of biofilm formation to reduce bacterial invasions. Proper feed and health management can improve larval survival and growth to a great extent.



Ctenophore



Larvae of the sand lobster *Thenus* ***orientalis*** were successfully reared in the hatchery at the Kovalam Field Laboratory using ctenophores collected live from the sea. The arrow-worm ***Sagitta enflata*** proved to be a better feed for phyllosomal stages I and IV as the larvae showed a natural preference for this feed over other live feeds, including ***Artemia***. The advanced phyllosomal stages (stages III and IV) showed excellent response to the ctenophore ***Pleurobrachia bachiae***. The common specimen collected were *Pleurobrachia bachiae* (spherical 1mm - 8mm dia) These ctenophores are composed of 96% water, 3% salt and only 1% organic matter (mucopolysaccharide matrix with fibrous collagen matter). They lack a true organ system. Coelenterates (cnidaria and ctenophores) are known as predators in food webs of marine ecosystems but are less considered as prey. May be because they are digested very rapidly and cannot be easily detected. There is also a tendency to assume that gelatinous organisms, with their high water and salt content relative to organic content, are poor food. However, given the high rates of digestion (and presumably of assimilation) coelenterates may provide sources of fast energy, comparable to feeds such as arthropods. It is already becoming known that a number of cnidaria and ctenophores as well as fish (pomfrets, rabbit fishes, sweet lips, leather jackets etc) utilize gelatinous organisms as prey. Data is accumulating more slowly on predation by a wide range of other carnivores such as molluscs, arthropods, reptiles and birds. The carbon-specific growth rates of gelatinous zooplankton were 2.2 times those of other zooplankton, and carbon specific ammonium excretion rates were one tenth. Most of the known planktonic association of the phyllosoma is with the medusae and gelatinous forms and these assist them considerably in transport and continuous supply of food energy.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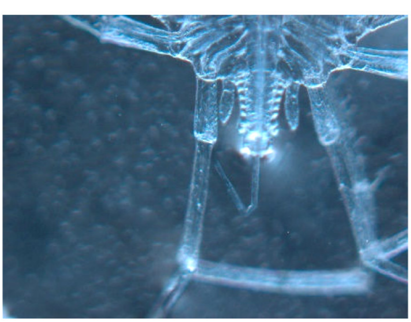
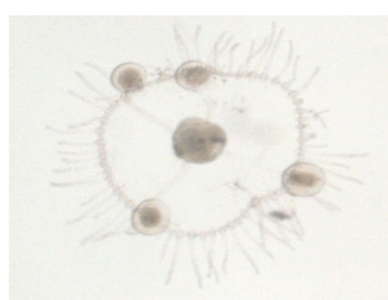




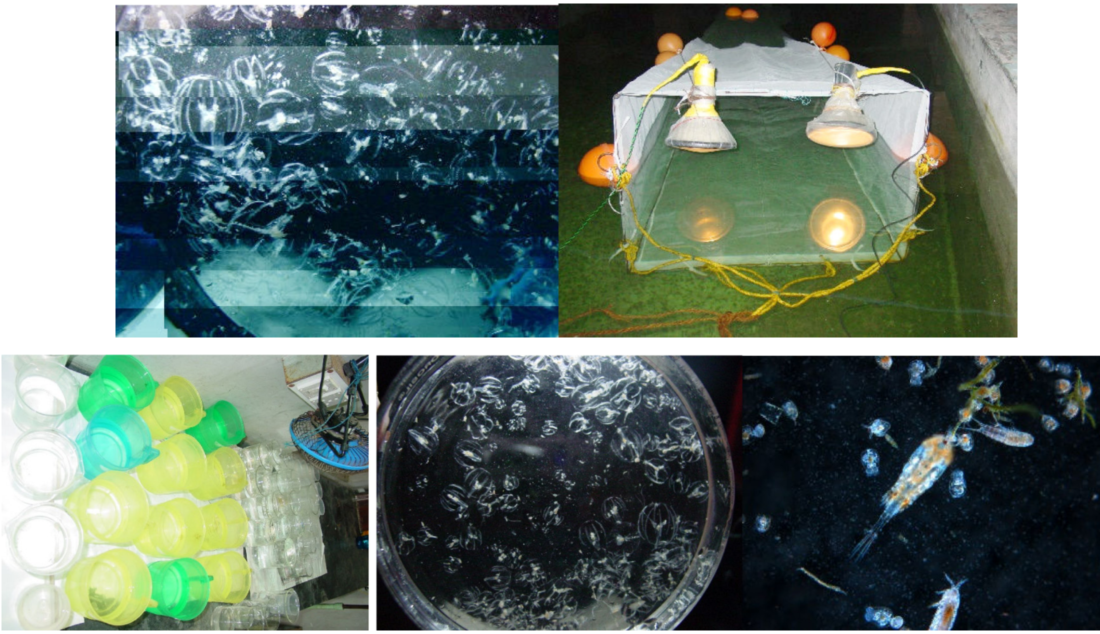
Zooplankton collection

The zooplankton collected with plankton nets (200 microns mesh size) in the nearshore waters and during morning hours were sorted and the sea combs and arrow worms were transferred immediately to preconditioned rearing chambers and aquaria using small siphons, beakers or spoons. Handling with screens and strains cause damages to the surfaces and filaments and lead to retrogressive developments and turn morbid soon. Particular attention must be given to the collection of these delicate animals, the handling and provision of live microzooplankton of suitable size for the larvae, and the provision of food densities for the adults which neither stimulate "wasteful" feeding nor limit their growth. Although these ctenophores will ingest detritus and algal cells in high concentration, they lost weight at the same rate as starved individuals unless provided with living zooplankton.

Ctenophores, also known as comb jellies, are delicate gelatinous marine zooplankton belonging to the phylum Ctenophora. Collection of intact specimens and captive rearing are challenging tasks, given the highly fragile nature of these organisms. The ctenophore *Pleurobrachia bachei*. Has two conspicuous tentacles in a tentacle sheath without oral lobes, body spherical .These are biradially symmetrical and at the aboral end is the prominent sense organ and radiating orally from this are the eight rows of strong ciliary combs .these ar the locomotory organs along with the pair of tentacles .the ciliary combs of each row beat in a coordinated manner –away from the mouth towards the aboral sense organ . The tentacular systems are more used for the food gathering. The mouth leads in to a lobed digestive tract and has anal pores at the aboral end. The mesodermal muscles (specialize) lying between the mesodermal layer of cells is the dif\ference of these animals from that of the coelenterate, commonly found in Indian waters, belongs to the order Cydippida. This species is closely associated with lobster phyllosoma, and advanced phyllosoma of the sand lobster, *Thenus unimaculatus,* have been found to feed very well upon *P. bachei*. As a prerequisite for successful sand lobster larval rearing, captive rearing of *P. bachei* assumes great importance as a source of steady and constant supply of live feed for the developing lobster larvae. These animals are typically devoid of nematocysts. The phyllosoma attack the ctenophores as soon as they are introduced into the rearing system. The pincers on the peraeiopods of the larvae are pierced into the gelatinous tissues and the last longer peraieopods are mostly remaing hooked till the feeding gets over.larvae hold on to the ctenophore and roll around with them for a time before sinking to the bottom. Feeding is completed in thirty minutes to about two and a half hours and faecal strands are extruded. The legs of the larvae are coated with mucous and remnants of the wrinkled gel sheath of the ctenophores. Throughout the process the sea combs transport the phyllosoma and keep rotating them aand swirling until the density and the functional cilial in the combs ceases to operate. The larvae brush this matter away with their legs before resuming their swimming activity. Although there is no cannibalism, competition for food is very high. Lysis of captured feed and ingestion rates are very fast. Hence the point of administration of enriched diets and then encapsulating them for higher level incorporation to phyllosoma becomes a promising possibility. Ctenophores can be easily cut or sliced into small pieces and fed from Phyllosoma II stage onwards in *T. unimaculatus* and from Phyllosoma IV stage onwards in *Petrarctus rugosus*.

Ctenophore rearing:

Low-density rearing experiments were conducted in glass aquaria and glass jars to assess the environmental and feed requirements for successful rearing and propagation of *P. bachei* in captivity. The different diets tried out were clam meat, *Artemia* nauplii, copepods, *Lucifer*, *Nanochloropsis* sp. and *Chlorella* sp. Aquaria set with mild aeration and dripping water exchange proved to be a better rearing system than the steady tank system. Net survival was higher in this system and the animals survived up to 25 days, while in the steady tank system, the mortality rate was very high and the experiment lasted only up to 7 days from the date of stocking. The occurrence of ctenophores in the coastal waters of Kovalam is highly seasonal and is often observed in massive shoals. The collections are usually good during January-May and then the numbers decline and they were grown in glass aquaria using *Chlorella* and *Nannochloropsis sp.* as rearing media and artemia nauplii as the feed. High density rearing trials were carried out in large cement tanks of 40 t capacity.



***P. bachei* collected from the wild were stocked @ 2 to 5 per litre.**

Under high saline conditions ctenophores can grow and multiply very fast when grown in large cement tanks with no direct sunlight, good zooplankton density and water column height (>2 m). However, the foraging plankters have to be introduced in large quantities. The rearing medium (filtered seawater) was fertilized by urea and phosphate, in order to produce thick algal growth. They feed on smaller zooplankton – copepods, krill, eggs and small larvae. Other zooplankton, particularly copepods and mysids obtained from zooplankton collections were also added. Chopped clam meat was also added daily. Feeding was voracious and the bottom of the rearing chambers were easily coated with a film of organic repels from the ctenophores. Maintenance was found to be easiest in large cement tanks holding water at lower salinities of 26-30 ppt, with sufficient shadow areas, algal scum on tank surface and large densities of live feed (*Lucifer*, copepods and *Artemia* nauplii) in order of preference. Several specimens carried developing gonads. Some of the specimens had gonads and released nearly 3-5000 eggs while most of the sexes were separate. Larval development was recorded in 12 hours but survival to the final larval stages was poor. Larval rearing was found to be easier in clear seawater with no adult ctenophores. High levels of Ammonia and nitrates are not tolerated but turbidity and low oxygen levels are easily tolerated. Aeration is not preferred in rearing. Although there is no cannibalism, competition for food is very high. Lysis of captured feed and ingestion rates are very fast. The smaller stages required smaller feed organisms. The hatched out larval stage was with a strong flagellar structure called the Cydippid stage, however they used to mature in this stages also .These specimens were isolated and transferred to glass aquaria. Under optimum conditions, specimens would lay eggs within 10-15 days of their own birth and this would continue if the animals stay healthy for a few days more (a few thousand eggs) The maximum number of eggs laid by a single wild individual within 24 h after being brought into the laboratory was 10,000. Their high fecundity, rapid generation time, and ability to self-fertilize help to explain their sudden appearance in bloom proportions at periods of high food concentration in the environment.

Aquaria (100 L) set with mild aeration and dripping water exchange proved efficient than the steady tank system. Acrylic kreisels and cylindroconical or inverted U jars are being used to create mild upwelling type holding systems for the specimens for longer durations with flow through arrangements to keep agitations at the minimum. Since the temperatures were not regulated during the experiment the water temperatures touched 31°C. It was observed that they tend to settle at the bottom and the combs and the webbings get entangled in the mucus. The ones fed on copepods and artemia nauplii gave better results than the ones grown on rotifers and clam meat specimens carried developing gonads and were stocked in 5 l beakers and 40 l aquaria.

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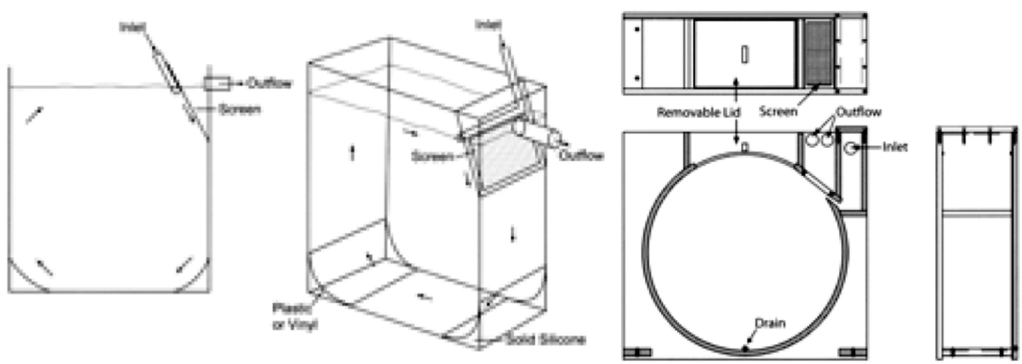
Length-weight relations were worked out for the samples collected as –

log W = -2.88623 + 1.99997 log L

Maturing and breeding spawners were studied in captivity and reproductive behaviour was observed in beakers. The larval development was recorded in 12 hours but survival to the final larval stages was poor. Development of the cydippid larva has been recorded but further studies are required.Larval rearing is easier in clear seawater with no adult ctenophores. High levels of Ammonia and nitrates are not tolerated but turbidity and low oxygen levels are easily tolerated. Aeration is not preferred in rearing.



Some tested designs of acrylic kreisels and upwelling jars

Problems :

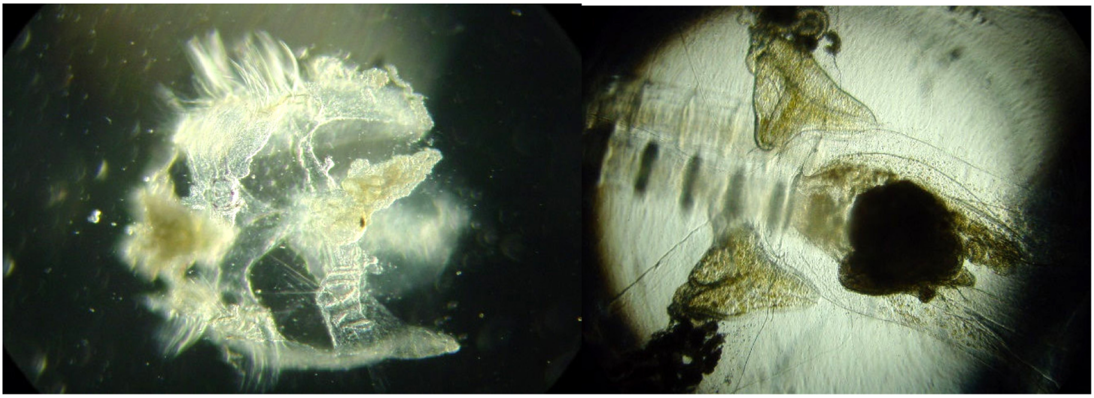
- Collection of larvae / observing a spawning adult and maintenance of the same is not easy
- Suitable larval feed is yet to be identified.

Salient findings :

- Rearing can be done in large tanks

- Voracious feeders – feeding on different live plankton
- Alternate feeds are also accepted
- Breeding can be achieved in captivity
- Bioenrichment / bioencapsulation is easy and therefore, of advantage to phyllosomal rearing

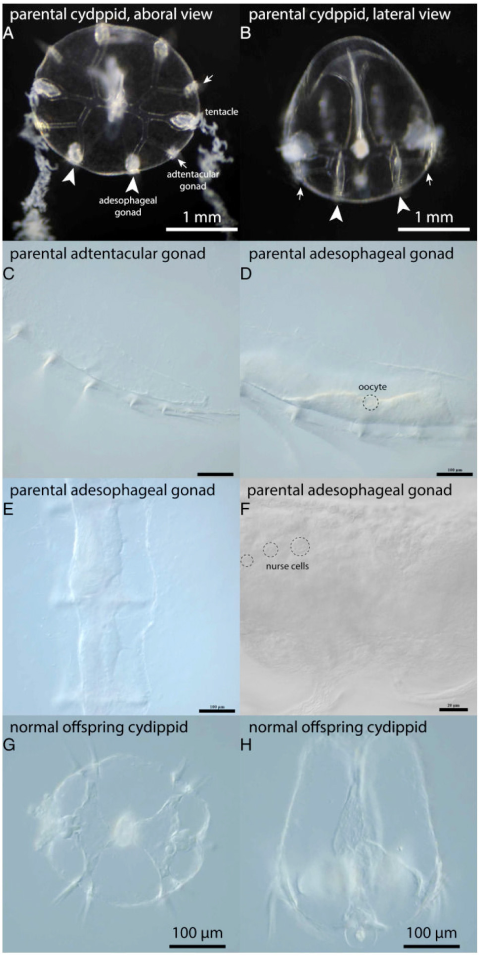
Tanks kept in total darkness (2 m depth) maintained a high density of ctenophores. *Sagitta* and ctenophores were found to co-exist, with ctenophores hardly feeding on *Sagitta*. Certain ctenophores were found to be loaded with thousands of developing egss inside their body as planarian worms. When the ctenophore is fully ready the gonads spread out and release the eggs through their external canals. The ripe ones can be identified by the naked eye as fully spotted individuals.

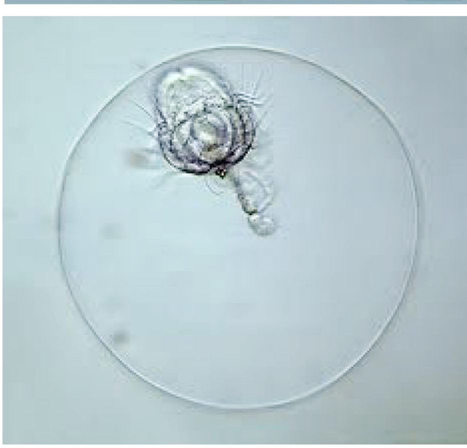


Feeding Cydipid and adult ctenophores

Sagitta Collections and rearing:

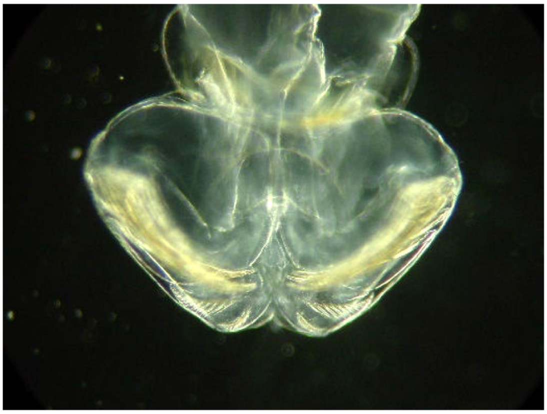
Arrow worms have been found to be suitable for rearing the first and second stages of sand lobster larvae and second stage onwards in the spiny lobster larvae.These are preffered choice of most of the tropical spiny lobster larval stages from 1-6 (*P. ornatus, P homarus, P polyphagus* and *P versicolor*) The predominant forms collected from Kovalam coast includes *Sagitta enflata*. Low salinity conditions are preferred. *Sagitta* can be easily maintained in captivity on a diet of small copepods and nauplii. S. enflata fed most actively at night. Small copepods with a mandible width <0.07 mm (prosome length <1 mm) were its predominant prey.





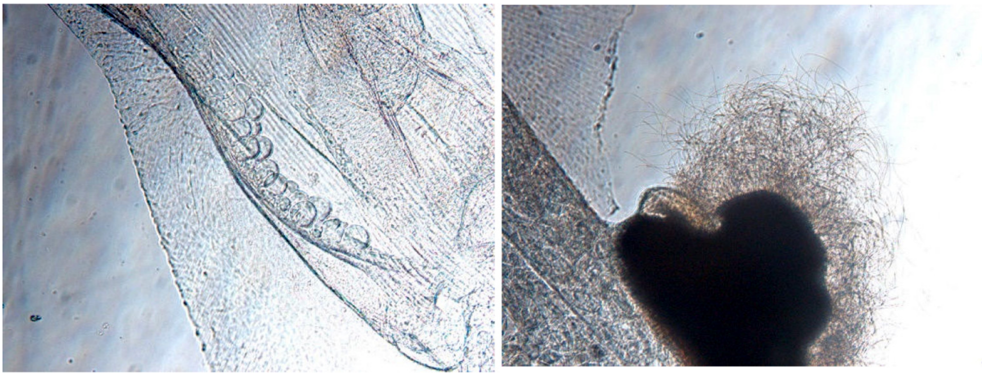
Maturation,gonad developments and larval stages in the Ctenophores

Large volume tanks / shadow (no direct sunlight) with only wind action – no aeration, can support dense *Sagitta* populations for several weeks. When *Artemia* nauplii and other nauplii are introduced into the rearing chamber, along with algal fertilization, the *Sagitta* goes into maturation phase and reproduction could thus be recorded. Gonads are seen symmetrically along the central gut near the posterior fins and as they mature the seminal vesicle gets filled with the sperm and ovaries with eggs, gamete release and fertilization were observed; however, the offspring released could not be identified. *Sagitta enflata* were sorted from the samples, measured (head to tail, excluding the caudal fin) to the nearest 0.2 mm. The arrow worms are to be collected using siphons and transferred to smaller upwelling glass bottomed vessels and loaded with copepodites and artemia nauplii. A slow flow in the system will sustain them in a suspended state.



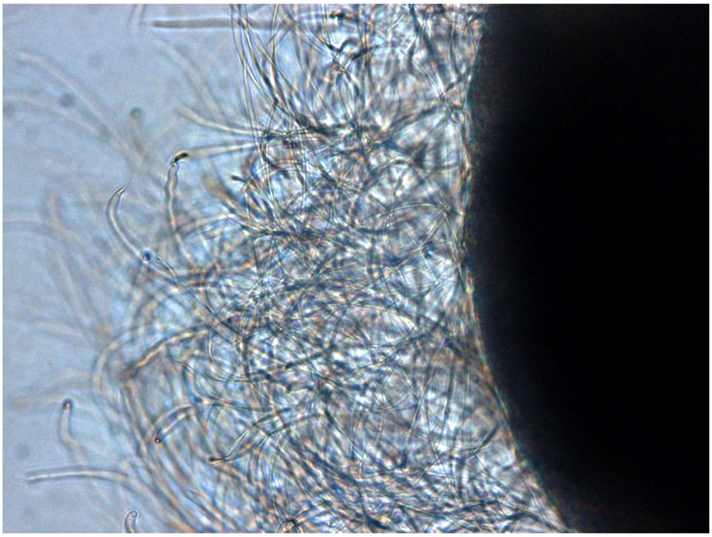
Chaetognath **mandibles of the arrow worm**

The arrowworms are taken by the spiny lobster phyllosoma using their pincers at the tip of the peraeiopods on the Pi-PIII legs hold them like a straw and mostly from the ventral side they take into the clawed mandibular and the palps press through the fluids into the buccal canal. Each healthy phyllosoma can consume 2-6 numbers in a day. The studies elsewhere suggest the arrow worms ingesting maturing copepods concentrate the MUFA and lipids which in turn help the arrow worms to mature and breed quickly. Thus the delivery to phyllosoma nutrition opens up another option.



Mature eggs released into the seminal chamber and the young ones coming out of the pore

In a series of experiments, phyllosoma were fed single and mixed species diets of relatively abundant potential prey items (chaetognaths, salps, and krill).



The release of hundreds of juveniles of the arrowworms from an adult

Chaetognaths were consumed in 2–8 times higher numbers than the other prey, and the rate of consumption of chaetognaths increased with increasing concentration of prey. The highly variable lipid content of the phyllosoma, and the fatty acid profiles of the phyllosoma and chaetognaths. Phyllosoma fed chaetognaths over 6 days showed significant changes in some fatty acids and tended to accumulate lipid, indicating an improvement in overall nutritional condition. The abundance of other soft-bodied potential prey items, such as siphonophores, heteropods , medusa ,squid larvae and fish larvae) were generally recorded at much lower abundances. Including all the potential prey items that we enumerated, chaetognaths comprised up to 49% of the potential prey field. Interestingly chaetognaths tended to have a greater proportion of their lipid as monosaturated fatty acids.

Conclusion

The raising of these gelatinous forms is a challenge but equally interesting as they are fast growing ,multiplying and are deliver good nutrient sources to the delicate larval forms in a sustained manner taking care of their buoyancy and swimming habits .But these are definitely voracious planktivorous and require supporting live feed system such as the copepod and artemia and rotifer culture systems advisable in green waters ,to negotiate the excess ammonia and nitrogenous wastes .the culture media should be microbe and ciliate free to the best extend possible to get the recruitment and multiplication in the systems used .

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