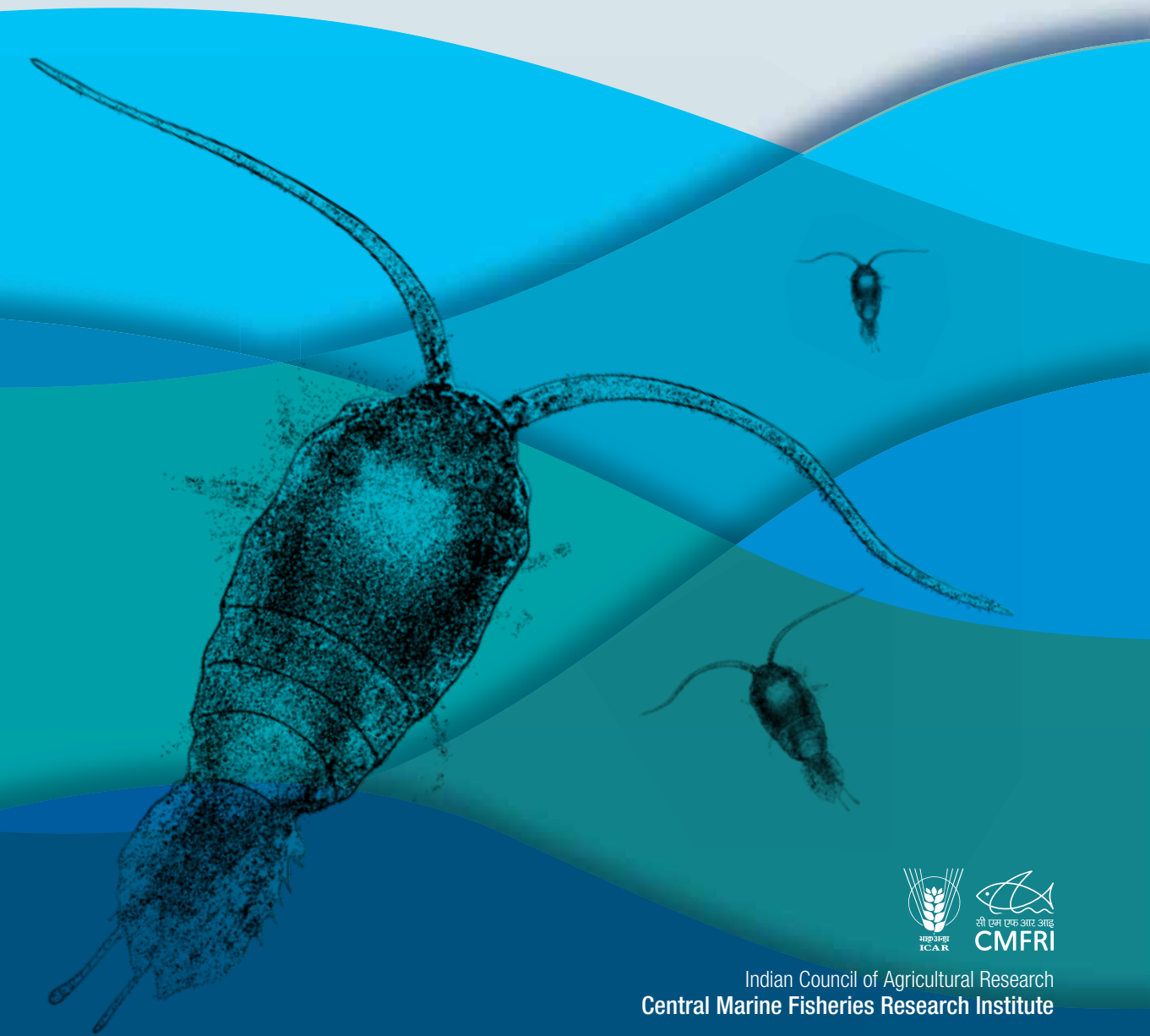
CMFRI Booklet No.9/2018
ISBN 978-93-82263-23-4

Culture Techniques of

Marine Copepods



Indian Council of Agricultural Research
Central Marine Fisheries Research Institute

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Editors

Santhosh B., Anil M. K., Muhammed Anzeer F., Aneesh K. S.,
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Published by
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Design: Blackboard, Kochi
Printed at: St. Francis Press, Kochi

Publication, Production & Co-ordination
Library & Documentation Centre, CMFRI

CMFRI Booklet No.9/2018
ISBN 978-93-82263-23-4

Citation: Santhosh B., Anil M. K., Muhammed Anzeer F., Aneesh K. S., Mijo V. Abraham, Gopakumar G., Rani Mary George, Gopalakrishnan A. and Unnikrishnan C. (Eds.). 2018. Culture techniques of marine copepods. ICAR-Central Marine Fisheries Research Institute, Kochi, Kerala, India, 144pp.

Foreword

In recent years, marine finfish resources have been stagnating or showing a declining trend. It is generally accepted that mariculture of suitable marine finfishes is the only option to meet the increasing demand for fish in the years to come. In this context, the availability of seed is the major issue and the ICAR-Central Marine Fisheries Research Institute has been intensifying its research in the recent past on the seed production of high value finfishes which are suitable for mariculture. Already technologies for commercial seed production of cobia (*Rachycentron canadum*) and silver pompano (*Trachinotus blochii*) have been standardised. One of the major hurdles for the seed production of many lucrative high value finfishes is the lack of proper technologies for mass production of suitable live feeds to initiate the first feeding of the larvae. The larvae of many species of high value food fishes are very small and the conventional live feeds employed in the hatchery such as rotifer and *Artemia* nauplii are not suitable to initiate the larval feeding during the critical stage mainly because of their larger size compared to the mouth size of the concerned fish larvae and also their poor nutritional value especially the fatty acid profile. Copepods are the best live feed due to their small sized nauplii and better fatty acid composition especially the DHA, EPA and ARA combination. But the major bottleneck for employing copepods as live feed is the lack of technologies for their mass culture in hatcheries. Even at a global level, this is a vital issue and even though some technologies were developed, research efforts are now being intensified in this area. In India, not much effort was taken to solve this problem till very recently. In the last few years, the ICAR-Central Marine Fisheries Research Institute has been focusing on this aspect and has come out with technologies for mass production of nine species of copepods. These technologies were successfully applied to seed production of the orange spotted grouper (*Epinephelus coioides*), the Indian pompano (*Trachinotus mookalee*) and the pink ear emperor (*Lethrinus lentjan)*. I congratulate Dr. B. Santhosh and his team for developing this unique technology for mass production of nine species of marine copepods for the first time in India. This publication titled 'Culture techniques of Marine Copepods' details this technology. I hope that the same will be a landmark in the near future which will pave the way for successful seed production of many more species of finfishes in mariculture.

Dr. A. Gopalakrishnan
Director, CMFRI

Contents

Preface

Initiatives taken by ICAR-Central Marine Fisheries Research Institute for the past two decades paved the way for successful farming of many species of marine food fishes and ornamental fishes in India. Availability of hatchery produced seed of cultivable species has been the major bottleneck in the development of the farming sector. To meet the demand for fish seed, research efforts were intensified on breeding and seed production at CMFRI. There have been major breakthroughs in breeding and seed production of cobia, silver pompano and a variety of ornamental fishes. But seed production of many other fishes like groupers, snappers, Indian pompano, anthias and damsels has been a problem because of the very small size of the newly hatched larvae and the consequent difficulties in initiating the first feeding of the larvae by the conventional live feeds employed in hatcheries. In this context, with an objective of finding out the suitable species of copepods as live feed, their effective production, harvest and utilization techniques, research activities have been intensified at Vizhinjam Research Centre of ICAR-CMFRI.

First feeding of fish larvae is the most difficult task in fish larval rearing. The newly hatched larvae of many species of marine fishes are very weak, without proper vision or body functions. Suitable live food is the most critical factor for their survival at this stage. An ideal live feed should be small, easily digestible, nutritionally rich and should be easily available for the larvae. In general, copepod nauplii have all these desirable qualities and in nature they are abundant and form the first food of many species of fishes. Certain fishes have their larvae evolutionarily adapted for feeding copepod nauplii. Reports have stated that the movement of copepod nauplii stimulates feeding instinct even in the weakest larvae. Copepods are nutritionally rich and do not require any enrichment. So copepod culture forms an essential component in marine fin fish hatchery for feeding the fish larvae.

All species of copepods are not ideal for feeding fish larvae. Many of them are predatory or parasitic. Many groups do not have pelagic larvae and may not be available in the water column for the larvae to feed. Only those species which are harmless and easily adapted to hatchery conditions need to be identified, isolated and cultured in a mass scale. Only few species are hardy, have the capacity to multiply fast and reproduce in large quantities within a short time.

With ten years of intensive research, we were able to find out nine suitable species of copepods, their pure stock and mass culture practices, harvest and utilization. Many species are being maintained over the past 8-9 years. All these species are being utilized for seed production of many groups of fishes including groupers, snappers, pompano, anthias, damsels and many other ornamental fishes. With the present level of live feed technologies, CMFRI is in a position to produce seeds of many suitable species of marine finfishes which are needed for farming in India.

This progress has been achieved because of the collective effort of a team of scientists, scholars, technicians, administrative and supporting staffs of CMFRI. There has also been dedicated support of the entire ICAR system in this mission.

Editors

Basic biological aspects of copepods relevant to culture

Santhosh B., Saleela K. N., Muhammed Anzeer F., Aneesh K. S., Mijo V. Abraham, Unnikrishnan C., Jose Kingsley H., Udayakumar A. and Greever Yoyak

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Copepods are small planktonic crustaceans occurring in almost all kinds of water bodies on the earth's surface. There are more than 210 families, 2400 genera and 24000 species identified in this group. Planktonic copepods are considered to be the most abundant metazoans on earth. From Lower Cretaceous period onwards copepods have diversified, adapted and successfully colonized in almost all kinds of aquatic habitats. Copepods are present in all types of water bodies from streams to deep sea and from sea shore to deep hydrothermal vents. Some of them have been adapted to live inside the body cavity of many animals (Razouls *et al.,* 2017).

The name copepod is basically derived from the Greek words meaning "animals with oar shaped foot" - *ie, kope* means 'oar' *podos* means 'foot' (Stottrup, 2003). These form the important secondary producers or primary consumers and ultimately contribute significantly to the food chain in large ecosystems. Almost all types of marine organisms, directly or indirectly depend on these small organisms for their food. Copepods form important food for many marine fishes and invertebrates. Certain fishes and fish larvae were evolutionarily adapted to feed on copepods. Copepods have been found to be nutritionally superior to almost all other live feeds. Many fishes, especially those with altricial fish larvae, totally depend on copepod nauplii for their survival at least for the initial few days. Due to their small sized naupliar stages, nutritional superiority and adaptability to culture conditions, copepod culture became an integral component in marine fin fish hatcheries.

Copepods are very hardy, they can withstand most of the unfavourable conditions and can produce diapause eggs and resting eggs to survive in these conditions. In general, most of these can tolerate wide range of salinity and temperature conditions. Due to its hardy nature, copepods can be easily introduced into all kind of water bodies actively or passively. Even in stock cultures, if enough care is not given, undesirable species of copepods may enter passively. Hence, utmost

care is needed to maintain pure stock culture of copepods. Maintaining pure and single species stock culture is the main strategy behind the production of reliable mass culture of desirable species. Many of the copepods are well adapted to live a parasitic life, some are predators of smaller organisms and some form intermediate hosts for parasites of higher animals.

Classification

Copepods are basically classified under the Phylum Arthropoda, Subphylum Crustacea, Class Hexanauplia and Subclass Copepoda. Most of the free living planktonic copepods feed on phytoplankton. Many benthic copepods feed on other animals and many are parasitic also. Some of them live in association with many invertebrates especially molluscs and echinoderms. Many prefer to graze on living and nonliving substratum.

Basically there are nine orders in the Subclass Copepoda: Calanoida, Cyclopoida, Harpacticoida, Platycopioida, Mormonilloida, Misophrioida, Siphonostomatoida, Monstrilloida and Gelyelloida. The Subclass Poecilostomatoida is mostly considered as a group under Cyclopoida but phylogenetically separated from it. Members of the Subclass Calanoida, Cyclopoida and Harpacticoida form important components in marine plankton. Among the marine pelagic copepods, calanoid copepods dominate (79.2%) the others. The Indian Ocean has the maximum species composition and the Arctic Ocean has the minimum (Boxshall and Halsey, 2004).

Basic body structure

The basic body structure comprises of a large cephalothorax (cephalosome) formed by the fusion of head and thoracic segments and a small segmented abdomen (urosome) (Fig. 1). The thorax has basically six segments. All segments possess a pair of legs or pleopods which are used for swimming. The 5^{th} and 6^{th} legs are considered to be taxonomically very important and often these are modified or reduced. Cephalic region has a rostrum, a pair of median eyes, a pair of antennule, antennae, mandible, maxilla and maxilliped. Most of the appendages except the antennule are generally biramous. The abdominal segments are reduced and without any limbs except for the caudal furca which form a tail fan with long setae. The sixth thoracic segment unites with first abdominal segment to form a genital double somite. The genital double somite together with abdominal somites form a slender tail like portion called urosome.

There are basically two types of articulations in the body. If the articulation of urosome is behind the fifth thoracic segment, it is known as gymnoplean and

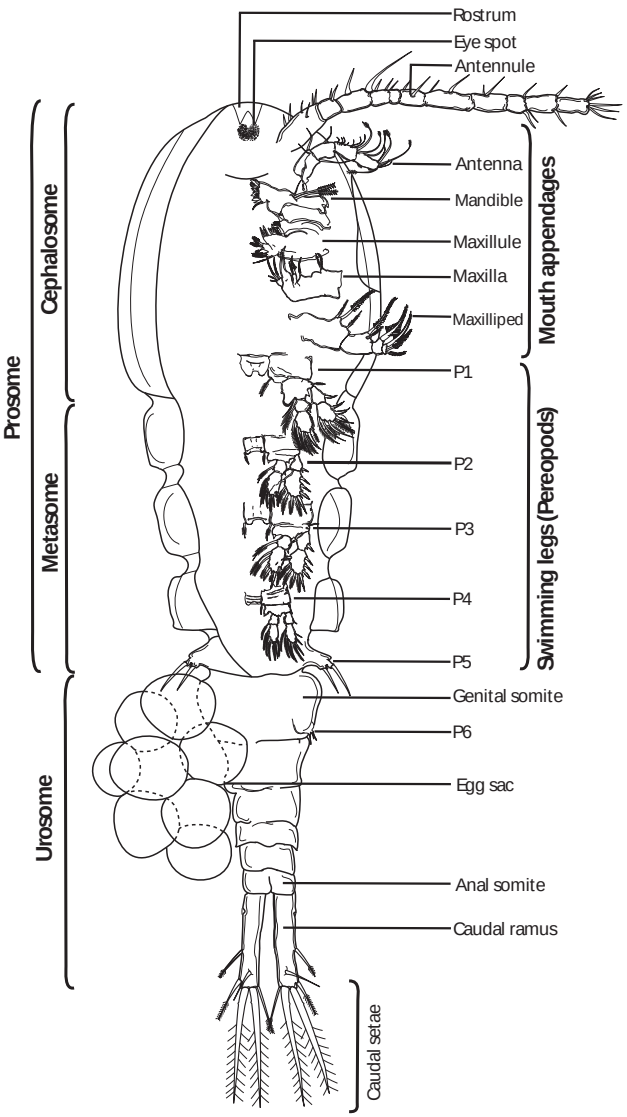

Fig. 1. Diagrammatic representation of body parts and appendages of a copepod (not to scale)

the typical examples are the calanoid copepods. If it is behind the fourth thoracic segment, it is called podoplean and the typical examples are the harpacticoid copepods. Male copepods are usually distinguished from females by their smaller body size and modified antennule. Antennules of males are often modified (geniculate) for holding females during mating.

Copepods have a distinct basic body structure which varies widely even within the groups. They are much modified within their respective groups for adapting to their microhabitats. This modification is clearly visible especially in parasitic forms of cyclopoids and siphonostomatoids. Some of these have their body modified into worm like forms and without any visible segmentation and some are leaf like with many out-growths (Fig. 2a-d). In parasitic forms, often the second antennae are modified (prehensile) for attaching their host. Mouth parts are also modified as cutting, piercing or sucking types. In parasitic forms, males are mostly free living and retain their original basic crustacean features throughout their life.

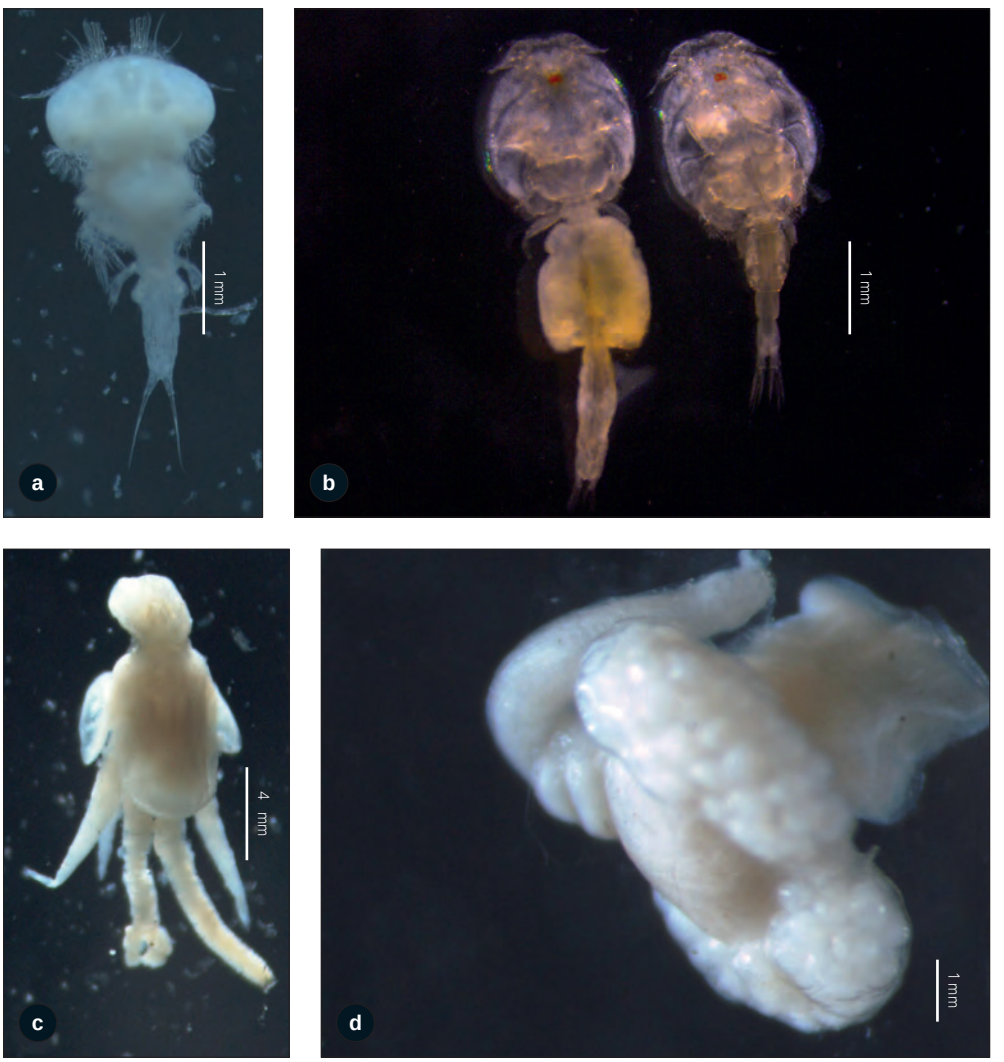

Fig. 2. Parasitic copepods a. *Nothobomolochus* sp., b. *Caligus* sp., c. *Lernanthropus* sp., d. *Naobranchia* sp.

Some copepods carry egg sacs and some are without egg sacs. Many calanoid copepods broadcast their eggs directly to the water. In some genera like that of the *Acartia,* eggs have an additional protective covering with minute spines. Some others have simple and smooth shell. Many of them have a pair of egg sacs. Most of the cyclopoids have a pair of egg sac whereas, calanoids and harpacticoids generally have a single egg sac. There are uniseriate and multiseriate type of egg sacs. Generally, eggs sacs are not considered as taxonomically significant. Within the same genus, there are species with single egg sac and double egg sacs. In general, non egg sac forming species are more prolific in culture and within a short period, these can grow to very high density.

It is essential to have a basic idea about the biology and life cycle of copepods before going into mass production of copepods for larval rearing.

Copepods of aquaculture importance

Copepods of aquaculture importance mainly belong to the orders Calanoida (Fig. 3a&b), Cyclopoida (Fig. 4a&b) and Harpacticoida (Fig. 5a&b). Calanoids can be easily distinguished by their long (20-27 segments) antennules. These are mostly pelagic and filter feeding, rarely these can be carnivores or predatory. Cyclopoids have shorter antennules than calanoids with 6-17 segments. They have a variety of feeding habits from filter feeding to parasitic. Antennules are much reduced in parasitic forms. These are distributed in all depths and more abundant in freshwaters. Harpacticoids are more numerous in species and occupy more than 50% of the total species of copepods. They have a short antennule having less than 10 segments. Generally harpacticoids are benthic with a wide variety of food habits from filter feeding to detritus feeding. There are many predatory and parasitic forms also in this group (Huys and Boxshall, 1991; Dussart and Defaye, 2001).

The measurements of adults and larval copepods are important for choosing appropriate size of live feed in relation to the mouth size of fish larvae. The total length is taken from the tip of cephalothorax to the tip of caudal ramus as shown in the Fig. 6a&b. The width is measured at a point where the animal is maximum wide. Larval mouth size is also important for choosing the live feed. It is difficult to open the mouth of fish larvae to measure the mouth gape. Measurements of mouth gape of fish larvae can be made by measuring the length of upper jaw and lower jaw (Fig. 6c). Mouth gape was calculated using 'Pythagorean theorem', supposing that the jaws represent two sides of a right-angled triangle and the hypotenuse is considered as mouth gape. The optimum size of the live feed is less than half the size of estimated mouth gape (Shirota, 1970; Guma'a, 1978; Jackson and Lenz, 2016).

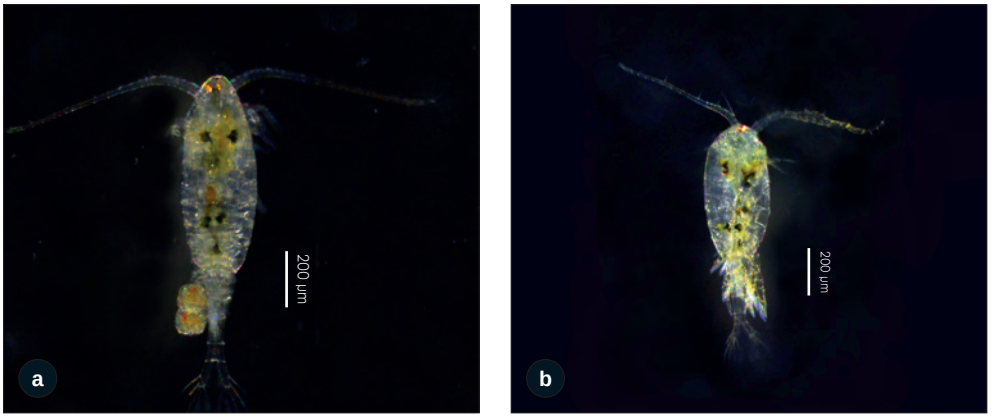

Fig. 3. Calanoid copepod a. Female b. Male

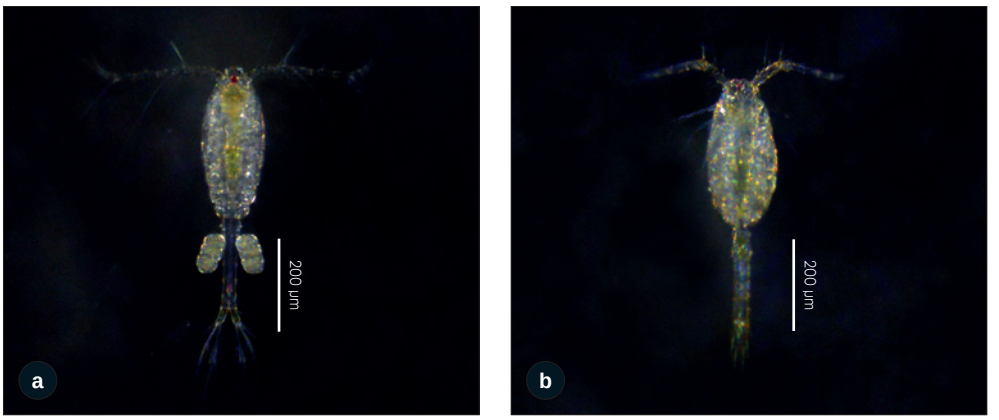

Fig. 4. Cyclopoid copepod a. Female b. Male

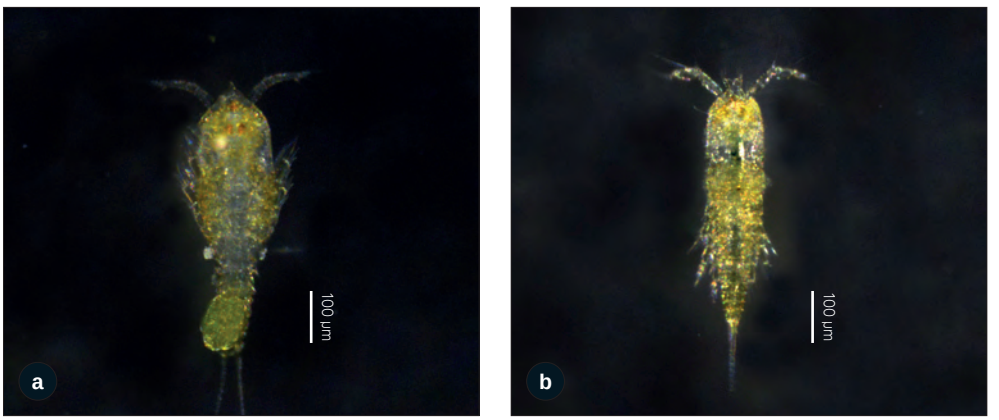

Fig. 5. Harpacticoid copepod a. Female b. Male

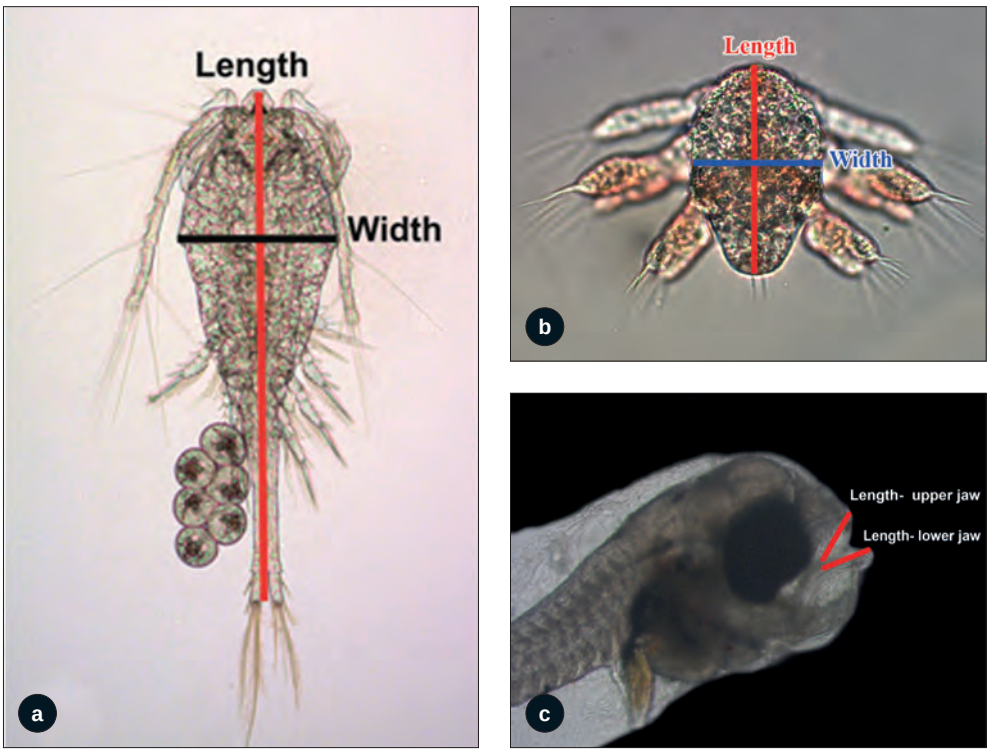

Fig. 6. General measurements: a. Copepod size, b. Naupliar size, c. Larval mouth size

Basic biology of copepods

Reproduction: Sexes are separate and clear sexual dimorphism is there in most of the species. In general, males are smaller and less numerous in any population. In most of the species, male antennule is modified for holding female during courtship. Male generally deposits a sac containing viable spermatozoa near female genital aperture and fertilization is as per the requirement of females. Eggs can either be broadcasted or kept in egg sacs and released after the development. Egg production rate depends on species, climate or season, feed, health status and age of the female. It can vary from 1-230 or more eggs per day (Stottrup, 2003). Eggs are mostly benthic and may hatch within 1-24 h. Resting eggs or diapause eggs are also common which can withstand unfavourable conditions.

Copepods generally lay eggs immediately after fertilization before first cleavage (Fig. 7). Those which carry egg sacs are of two types, either they leave brood pouch fully just before final maturation or they release nauplii directly from the pouch (Fig. 8). Those species which directly release eggs generally have more

Fig. 7. Newly released copepod eggs showing different stages of division

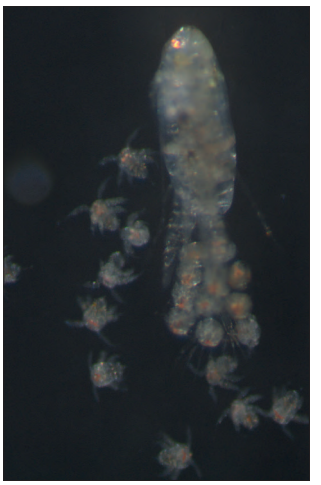

Fig. 8. Nauplii releasing from brood pouch

fecundity. It can be more than 50-60 eggs/day. Paracalanid copepods belongs to this category and these copepods are more important in live feed culture.

Life Cycle: Eggs hatch into nauplii which are morphologically different from the adults. These are mostly round, dorso-ventrally flat and without any visible segmentation. In this stage, cephalic appendages are being used for the movement. These are mostly less than 100 µm in length and in many species, these are even smaller than 50 µm in length. There are six naupliar stages and five copepodite stages. Copepodite stages resemble the adults but may not have full body segmentation or appendages. Sexual dimorphism is clear from 5th copepodite stage. Life cycles of all nine species cultured at CMFRI are incorporated in respective chapters. Feeding generally starts from naupliar stages, but in many cases it starts from second or third stage onwards. It may take few hours to few days to pass through each stage. It mostly depends on the species, feed availability and temperature. For some species, it may take only few days to complete the life cycle and in some cases it may take more than one month. If a species is taking more time to change from nauplii I to nauplii II or III, it is considered as an additional advantage for larval rearing as the nauplii remain small size in the feeding tank for a longer duration and the fish larvae get more chance to consume the smaller nauplii.

Food and feeding: Most of the copepods are filter feeders. They make a current around their mouth and filter desirable particles using their specialized mouth parts. From the mouth parts we usually get a basic idea of its feed preferences. In parasitic or predatory forms, their mouth parts are more chitinised and reduced into cutting blades. In filter feeders, the mouth parts are more setose. Mostly filter

feeding copepods are selective feeders, feed on a particular species or size range of microalge. Copepods are not voracious feeders like rotifers. This usually become a big problem during the initial culture days. If the feed is more, the survival will be affected. If it is less, the entire culture will be down within 2-3 days. Some species are specialized for feeding fine particles of less than 10 µm and some others can feed bigger sized cells or even particulate matter.

Culture

All the three groups form important food for fish and fish larvae in the wild, but only few species have the potential to reproduce in large scale under hatchery conditions. Some representatives from these groups are in Figs. 9, 10 and 11. Calanoids and cyclopoids can multiply (upto 5000-6000 nos/L) in containers of 5-10 t capacity within a short period, but live microalgae are essential as feed. Harpacticoids can reach higher densities than other groups within a short period in small containers and are generally ideal for small scale production sectors like ornamental fish culture units. Some harpacticoids can be cultured using artificial food for short periods. If artificial food is used for longer periods, it may lead to development of contaminants like protozoans and helminths.

Regular close monitoring of subsamples under microscope is essential for understanding growth, composition of life stages, occurrence of contaminants and health status of the culture. It takes close monitoring of several generations of culture to acclimatize a particular species completely to laboratory or hatchery conditions. Initially, the cultures are subjected to some seasonal changes or sudden fluctuations in population. But continuous culture in the hatchery can change the seasonal cycles and increase the stability of the culture.

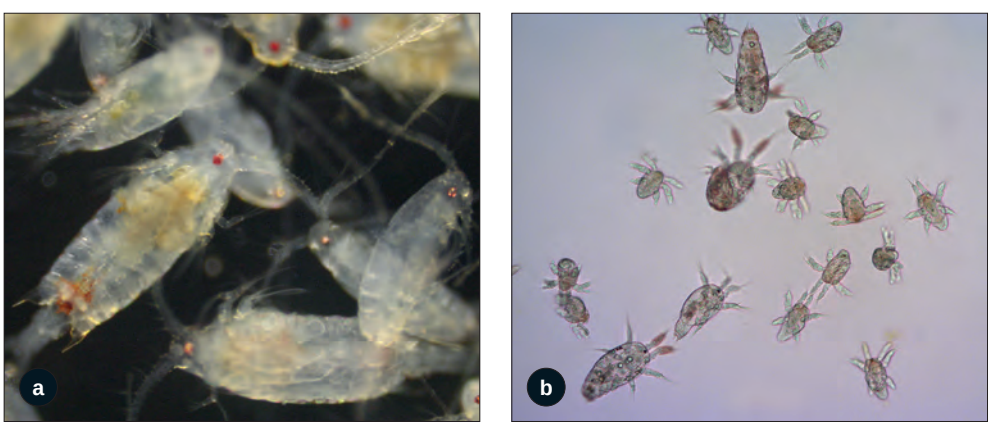

Fig. 9a. Calanoid copepods b. Nauplii of calanoid copepods

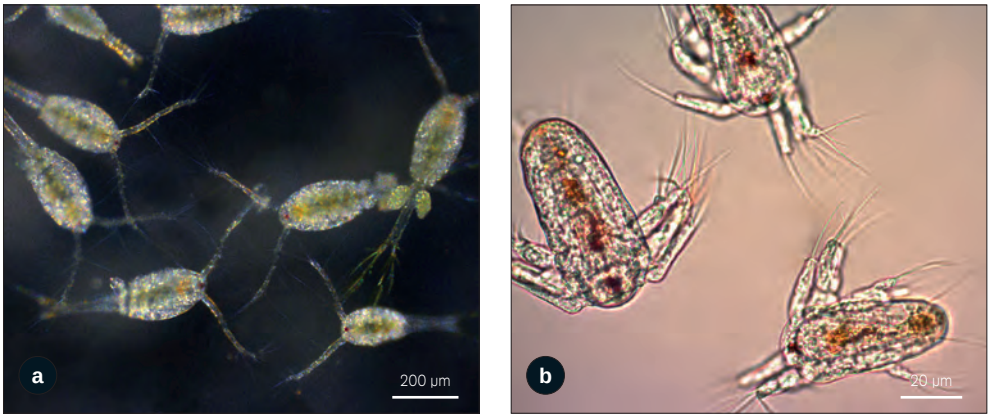

Fig. 10a. Cyclopoid copepods b. Nauplii of cyclopoid copepods

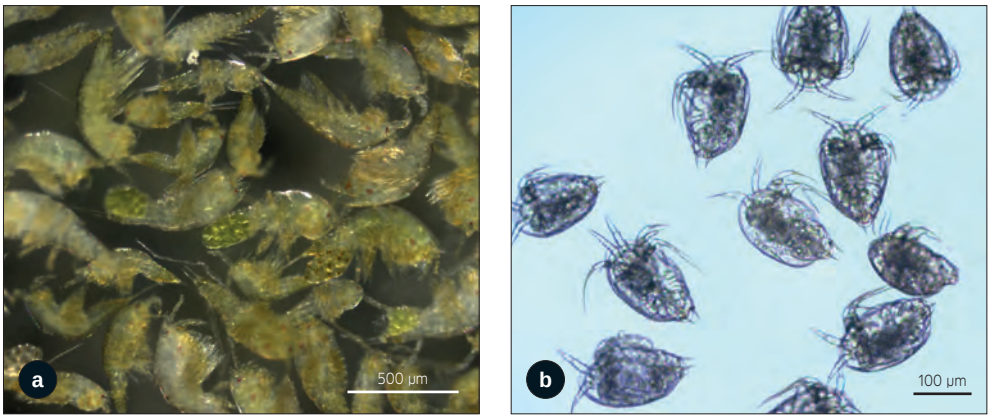

Fig. 11a. Harpacticoid copepods b. Nauplii of harpacticoid copepods

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General methods for stock and mass production of copepods as live feed

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Increased demand for sea food, particularly for finfishes, necessitates their large scale farming. Seed availability is the major bottleneck in fish farming and therefore hatchery production of fish seed is the only way to meet the requirement for seeds. In recent years, a lot of advancements have been made in this regard and technologies for larviculture of many cultivable species have emerged. The conventional live feeds like *Artemia* and rotifers were not sufficient for larval rearing of many species of food fishes and ornamental fishes. Being a reliable, complete, balanced and nutritionally rich live feed, the copepods particularly those with very small nauplii got much attention in the past few decades. Copepods are the only acceptable live feed for many food fish and ornamental fish larvae especially during their first feeding stage. Reports are there stating the suitability of culture of a few species of copepods. Still, commercial production of copepods and their utilization have been achieved only in a few hatcheries of the world. More simple and effective technologies are needed for wide spread acceptance and utilization of copepods as a live feed for feeding larvae of marine food fishes. In India, consistent hatchery production of copepods and utilization of the same for marine fish seed production is being practiced only in ICAR-CMFRI. CMFRI at its Vizhinjam Research Centre achieved large scale production of nine species of copepods which have the potential as effective live feed for larviculture of many fishes with comparatively smaller larvae. The basic practices developed for successful stock and mass production of the nine species of copepods are discussed here.

Collection, isolation and initiation of culture

A series of plankton collections were undertaken using 150 µm mesh plankton net during early hours at Vizhinjam Bay (Fig. 12). The water temperature ranged from 24-26^{o}C and salinity from 33-35 ppt. The collections were further filtered and

Fig. 12. Plankton collection

cleaned. From the subsamples, desired species were identified and isolated into small petri dishes using glass droppers or Pasteur pipettes under a stereo dissecting microscope (SDM) and cultured using microalgae as feed in small containers. Periodic monitoring was done under SDM to confirm purity of the culture. Some of them may again require purification and isolation in similar way. In most of the cases, it is difficult to distinguish species within the genus in live condition especially those from the genera *Paracalanus, Parvocalanus, Oithona* and *Acartia*. Detailed investigation and confirmation of species is necessary to have a pure and reliable culture.

Some species are attracted towards light while others are insensitive to light. Colour, shape, movement and pigmentation pattern seem to be mostly species specific. Some cyclopoids and harpacticoids are predatory or cannibalistic in nature. Maximum care should be taken to maintain purity of the culture during species wise isolation and culture.

Any damage and stress during isolation and purification should be avoided. Temperature, salinity and other physico-chemical conditions should not vary much during transfer. Copepods from wild collections were very sensitive during initial days. Slowly these got acclimatised to laboratory conditions and were able to withstand the handling stress.

After confirmation about the purity of culture, copepods from small containers were serially cultured to larger containers. Stock culture can be maintained in 50 to 500 L tanks. In general, it takes nearly six months to develop protocols for mass culture of each species from plankton samples. The basic initial feed used was a mixture of microalgae *Nannochloropsis oculata* and *Isochrysis galbana*.

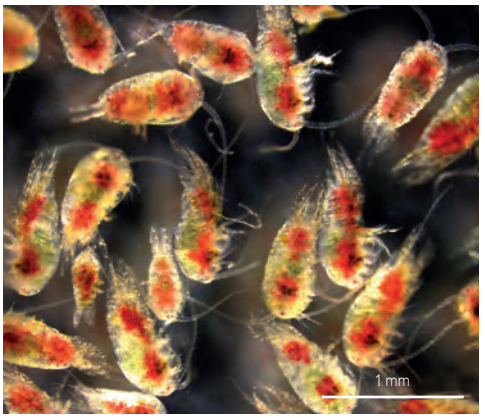


Fig. 13. *Temora turbinata*

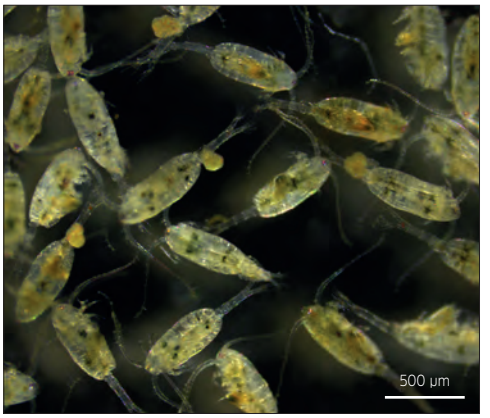


Fig. 14. *Pseudodiaptomus serricaudatus*

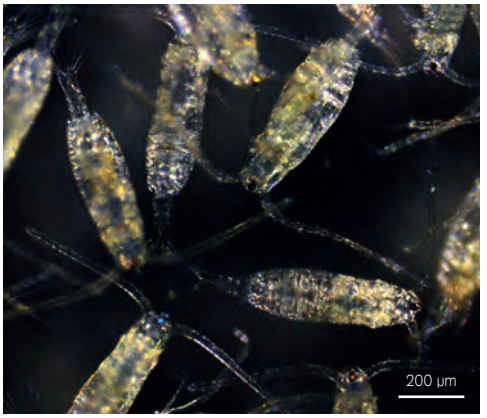


Fig. 15. *Acartia southwelli*

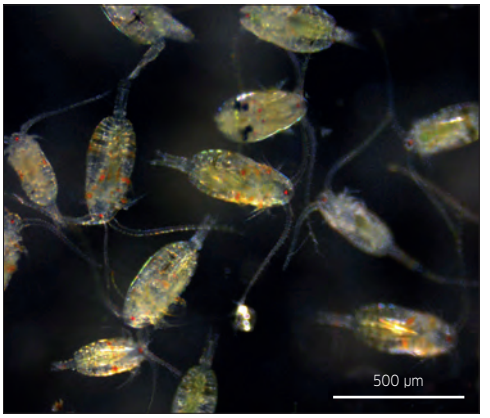


Fig. 16. *Parvocalanus crassirostris* var. *cochinensis*

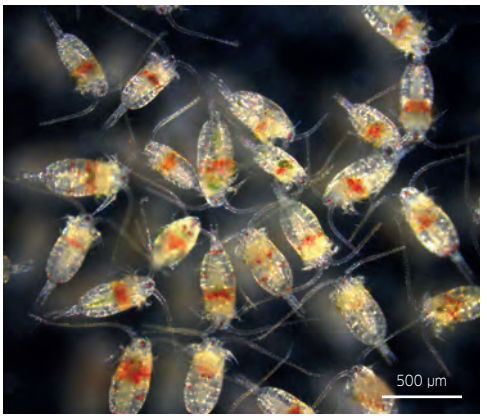


Fig. 17. *Bestiolina similis*

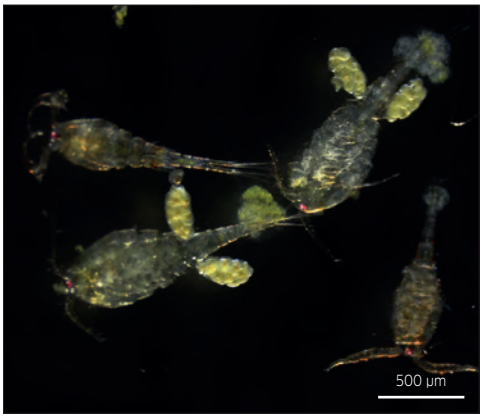


Fig. 18. *Apocyclops cmfri*

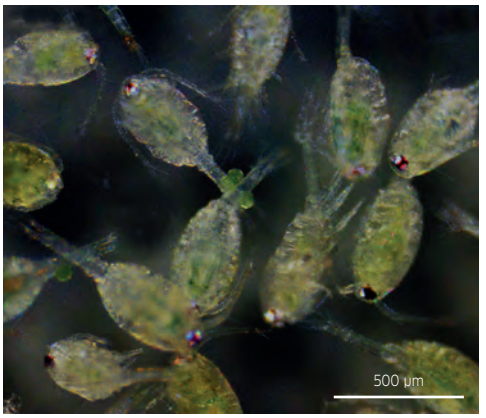


Fig. 19. *Dioithona oculata*

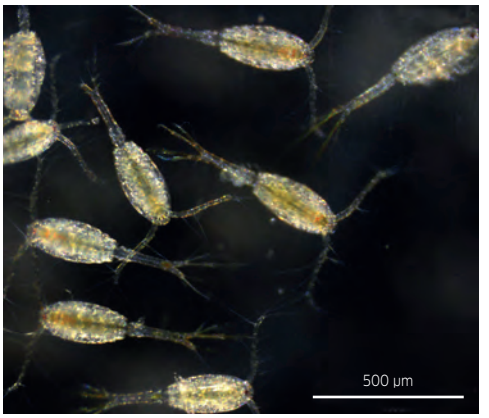


Fig. 20. *Dioithona* sp.

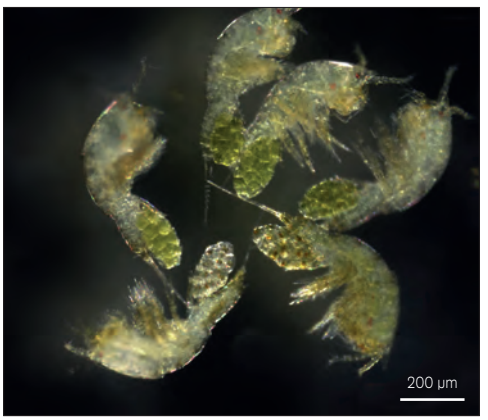


Fig. 21. *Euterpina acutifrons*

Species in culture

Altogether, pure stock and mass cultures of nine species of copepods which have been identified as suitable for larval rearing are being maintained at CMFRI, Vizhinjam. These include calanoid copepods (*Temora turbinata*, *Pseudodiaptomus serricaudatus*, *Acartia southwelli*, *Parvocalanus crassirostris* var. *cochinensis* and *Bestiolina similis*) (Fig. 13-17), Cyclopoid copepods (*Apocyclops cmfri*, *Dioithona oculata* and *Dioithona* sp.) (Fig. 18-20) and Harpacticoid copepod (*Euterpina acutifrons*) (Fig. 21). Details of the basic biology and culture of each species have been included in separate sections in this booklet.

Basic culture protocols

Water quality is one of the most important factors which determines the health of any copepod culture. Most of the copepods can tolerate wide range of salinity and

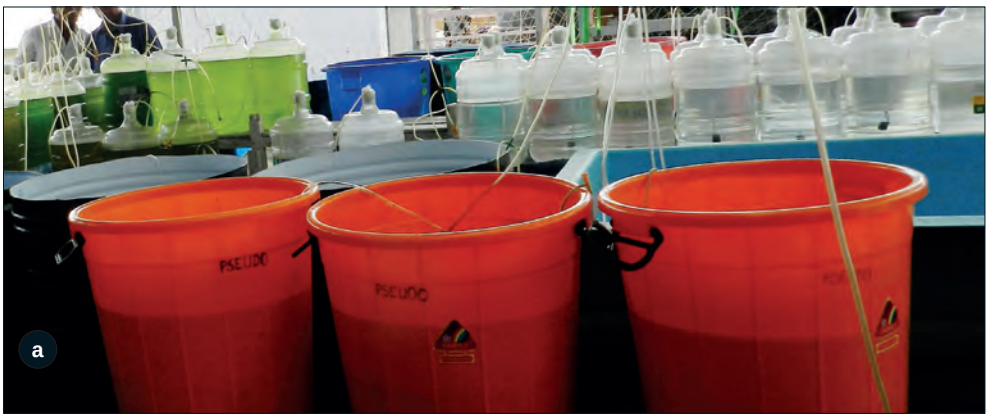
temperature. But sudden fluctuation of any environmental parameters can induce stress to the culture. Water should be chlorine treated and de-chlorinated or ozone treated and filtered through a 5 µm filter bag. Make sure that the water should be clear and free from copepods, rotifers, ciliates and any other contaminants. The tanks should be placed in about 60% shade. Normal 12 h light is ideal. In excess light, algal blooms may occur. If there is marked increase in the phytoplankton cell density, we can regulate the algal density by regulating the light intensity using shade nets. Sometimes at high atmospheric temperature, salinity will increase due to evaporation. This should be monitored using a refractometer and adjusted with diluted sea water. Always check the salinity of the resident seawater periodically and ideally it should be maintained at around 30-35 ppt. The ideal range of temperature for most of the species cultured is 25-29^{o}C. pH range is in between 8-8.5. The dissolved oxygen level should be above 2 mg/L and ammonia should be always less than 1 ppm. It is always necessary to keep a sufficiently large storage tank containing clean and contamination free filtered sea water for meeting emergency requirements.

Copepods can be cultured in batches and can be utilised fully for feeding either in co-culture with larvae or selective feeding with desired size group of copepods. In co-culture with fish larvae, the entire culture will be utilized. In selective feeding, regular harvest of desirable size group of copepods is possible in most of the species without disturbing the stock or mass culture. Many simple naupliar harvesting devices have been already designed for the process of selective harvesting. Details of selective harvest of each species of copepods is explained in respective chapters.

Stock culture

For both batch culture and continuous culture, stock culture is essential. Stock culture can be done in tanks of 50-500 L capacity (Fig. 22a&b). Tanks of PVC, HDPE or fibre are ideal. Cement tanks are not preferred for stock culture. Dark colour tanks are ideal for mass culture. For stock culture, white colour or light colours are desirable because it gives an idea about the population and health of copepods in the first look itself. Cleaning is also easy in white background. In stock culture, long term maintenance of healthy stock is considered as more important than maintaining higher density.

Few hundreds of copepods are sufficient to inoculate stock culture tanks. Tanks attain maximum density within 10-20 days depending on the species cultured. Since production is totally dependent on population, regular harvest is possible even from the stock culture without affecting the total population. Normally the stock culture can continue for 2-3 months with proper maintenance. There is no difference in basic protocols of stock and mass culture.



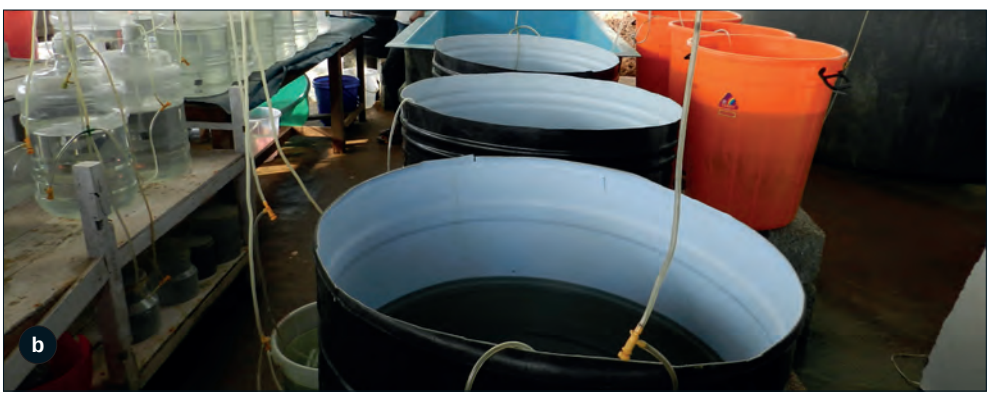


Fig. 22. Stock culture of copepods a. Bins b. Tanks

Mass culture

For mass culture of 1000-5000 L capacity, 50-100 L of inoculum is required. Copepods from one bin can be used to inoculate 3-4 tanks of 1000 L capacity. Inoculum from 1000 L tanks also can be used for inoculating 5 or 10 t tanks (Fig. 23a&b). At a time up to 75% of the stock can be used for mass culture. The inoculum gets ready again within 8-10 days and the tanks will be ready for harvest within 10-25 days period. Thus a series of tanks starting from 100 L, 500 L, 1000 L and 5 or 10 t are necessary for establishing a large scale production system. After inoculation in large systems, water level should be increased slowly with increase in copepod population. All tanks should never be filled beyond 75-80% of its capacity. Round drainable tanks with water depth less than 1 m are ideal. Indoor tanks also can be used with normal lighting. All tanks should be placed in a slightly elevated position so that the bottom sediments can easily be siphoned off. Mild aeration is essential in all tanks.

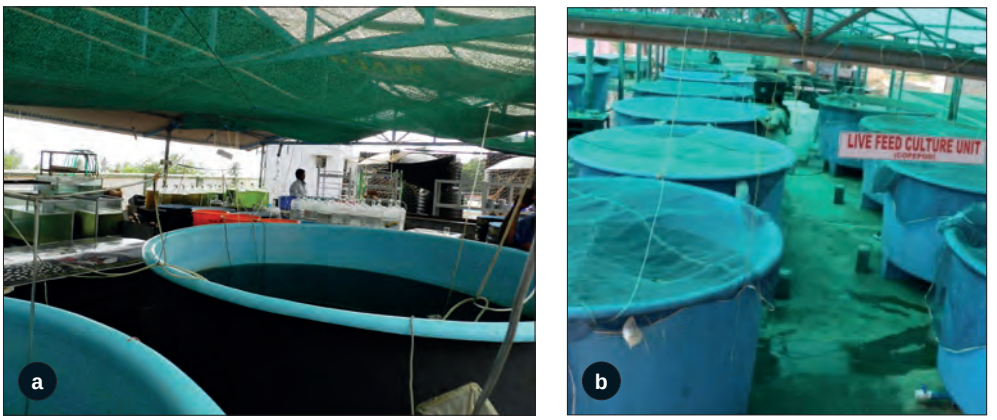


Fig. 23. Mass culture of copepods in large tanks a. Vizhinjam Centre, b. Visakhapatnam Centre

Feeding

Supply of optimal diet is an important factor for maintaining stable culture of copepods. Feed preference of copepods can be determined by conducting short term feeding trials. While selecting food for a species of copepod, the particle size as well as the digestibility of the feed need to be taken into consideration. The chemical composition of the algal feed also needs to be considered as it has some effect on the survival of copepods. Experimental evaluation with different combinations of microalgal diet is essential to identify appropriate feed for each species. Mostly very good survival rates were obtained using a combination of *Isochrysis galbana* and *Nannochloropsis salina. Chlorella marina* in combination with *I. galbana* and *N. salina* is ideal for harpacticoids and cyclopoids. Details of species-wise feed preferences have been included in the following sections of this book.

An independent algal production unit is essential for a copepod culture system. Algal stock and mass cultures need to be maintained as a prerequisite. For stock culture of algae 500 mL conical flasks and 2-4 L Haffkine flasks were used (Fig. 24). Stock culture can be maintained in indoor conditions. Walne's media has been used for algal cultures in stock, carboy and small containers (Fig. 25). Recommended fertilizers can be used for mass culture. Aeration is needed for carboy culture and mass culture of algae and not for the stock culture. Cultured algae should be allowed to reach late exponential phase without any contamination before feeding. If proper care is not given, algae can also be a source of contamination. Algae should be filtered using a bolting silk of suitable size to avoid any contaminations before feeding copepods.

The amount of food required is directly proportional to the copepod biomass present in the culture. By using a compound microscope and haemocytometer, the

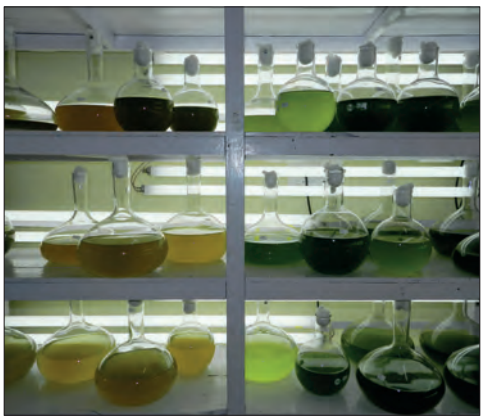


Fig. 24. Stock culture of microalgae

Fig. 25. Carboy culture of microalgae

algal concentration can be quantified and regulated. Ideal density of algal cells may range from 15,000-30000 nos/mL. If the concentration increases beyond 30,000 cells/mL, it may affect the copepod density. Daily assessment of algal cell density and copepod population is essential. After few days of counting and monitoring, it is easy to judge population density approximately by visual methods. If the water appears more turbid, the feed input can be regulated. Both overfeeding and underfeeding will have negative impacts on copepod population. Optimum feeding strategy helps in the effective control of the ciliates in the culture system.

Cleaning and maintenance

Sieves of different mesh sizes are essential for handling copepods in culture. Sieves can be purchased if available in the market or it can be prepared easily using PVC pipes or connectors and bolting silks of desirable measurements (Fig. 26). Bolting silk (filter cloth) of different mesh size can be pasted on one side of the PVC connector or reducer to make a sieve. Sieves ranging from 20 µm to 500 µm are needed for maintaining the culture. If more species need to be reared, use separate set of sieves for each species.

On alternate days, the sediments should be siphoned off from the calanoid tanks to reduce the ciliate growth and to maintain good growth of copepods in the culture system (Fig. 27). For harpacticoids and cyclopoids, cleaning frequency can be once in four days. The siphoned sediment and water should be kept in 20 L buckets with mild aeration for few hours for the settling of debris (Fig. 28). If large volume of water needs to be filtered, use one or more sieves to collect the sediments. In such cases, an open wide flat tray can be used to reduce the pressure of outflowing water. Live copepods, eggs and larval forms accumulated in the clear surface of the buckets can be carefully filtered out by

Fig. 26. Sieves of different mesh sizes

Fig. 27. Bottom siphoning from culture tank

Fig. 28. Bottom sample in bucket to isolate naupliar stages

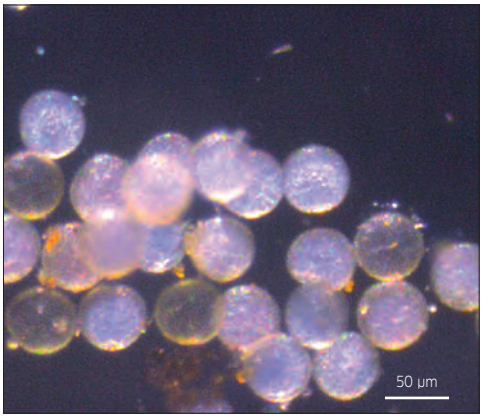


Fig. 29. Sample of eggs and egg shells from bottom sediment

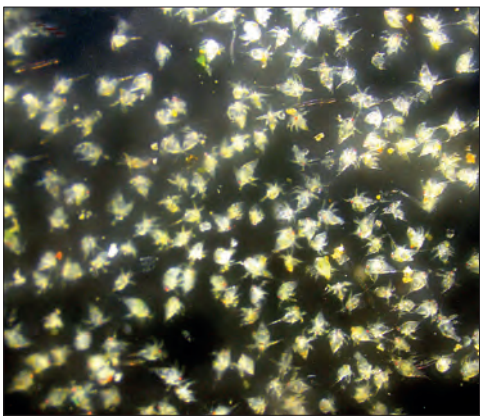


Fig. 30. Harvested nauplii

Fig. 31. Harvested adult copepods

passing it through a filter of desired mesh size. Copepods which are retained in the mesh can be washed and reintroduced in the culture tanks. The sediment can be diluted again and this process can be repeated several times so that all healthy and live copepods are collected and introduced back into culture system. This process is essential for egg-broadcasting species because all the eggs will be settled in the bottom along with faecal pellets, moulted exoskeleton and other wastes (Fig. 29). In this way, ciliates and dead organisms can be regularly removed from the tanks. In case of egg bearing copepods the filtrate should be diluted with clear filtered sea water and kept for one or two days with mild aeration. The freshly hatched nauplii (Fig. 30) can be sieved out regularly using 30 µm sieve and can be introduced back into the culture system. If needed, the adult copepods also can be harvested using suitable sieves (Fig. 31). Water level in the culture tank should be brought back to the original level by adding clean, dechlorinated or ozonised and filtered sea water.

Ciliates are not a major threat to copepod culture. Total removal of ciliates is an impossible task. Through proper cleaning, ciliates can be reduced to a large extent. Removal of accumulated faecal debris and wastes helps to reduce the ciliate density. Every 15 days or whenever it is required, sides of the culture tanks and bottom should be slowly and carefully wiped using appropriate brush without disturbing the water. Aeration should be stopped at least for one hour and all the sediments should be allowed to settle down at the bottom. The sediments can be carefully siphoned off and treated in a similar way as discussed earlier. Use all items like sieves, siphoning tubes, buckets etc separately for each tank. Enough care should be taken to avoid cross contamination, especially if more than one species is being cultured. If severe ciliate infection is noticed, the entire copepods can be collected in a filter of mesh size more than 200 µm and kept in a trough with minimum water turbulence. Allow clean filtered seawater to flow through the filter for 10-15 minutes so that the ciliates adhering on the copepods are also washed off. After washing, copepods collected can be introduced in a fresh tank to start a new culture. If the tank becomes old or if the culture is in a declining phase, new culture can be started in a similar way and the old tank can be completely cleaned using liquid chlorine or any other appropriate disinfectant and sundried.

Renewal of 20-30% resident sea water in every 2 weeks and replacement of tanks in every 2 months can be done for maintaining a healthy stock. Volume of water to be added daily can be adjusted considering the volume of feed added and volume of water reduced while removing debris from the bottom. Care should be given to maintain constant water volume even after the addition of water or feed for continuous culture.

Harvesting

It may take about 15-30 days for a mass culture tank to become ready for harvest. The population should reach a density of approximately 1000 copepods/L. Harvest may include eggs or nauplii or adults or all life stages of copepods. In a continuous culture, 10% of the population can be harvested regularly. Harvesting can be done by siphoning through sieves of desired mesh size. 1-2 million nauplii can be harvested regularly from a continuous culture tank of 1000 L capacity without affecting the total population.

For naupliar collection, special devices are required. Many models have been already developed however, popular design is a modified *Artemia* hatching tank of 50 or 100 L capacity with a bottom frame fitted with 200 µm bolting silk (Fig. 32). Only mature copepods collected from mass culture tanks were kept at higher

Fig. 32. Copepod nauplii harvesting tank used in Visakhapatnam Centre of CMFRI

Fig. 33. Harvested nauplii in sieve

density in the naupliar production tank. Water filtered through the bolting silk in the bottom contain eggs and larvae which can be lifted by air flow using a simple PVC pipe and air stone and allowed to flow back into the tank through a floating filter of 30 µm (Fig. 33). Nauplii collected in the floating filter can be directly used for feeding the fish larvae. Daily harvest of 8-9 million naupliar stages is possible from a tank of 500 L capacity. The naupliar collection tank runs only for 10-15 days and we need to replace the adult copepods after that. Most of the hatcheries use this method of concentrating nauplii for larval rearing.

Regular alternate harvest up to 1 million naupliar stages of *T. turbinata* has been possible from continuous culture tanks of 500 L capacity without affecting the population. Regular daily harvest of 1-1.5 million nauplii of *P. serricaudatus* has been possible from continuous culture tanks of 500 L capacity.

Constraints

Main problem in copepod culture is the growth of unwanted organisms in tanks. Overfeeding, accumulated debris and faecal pellets result in the emergence of ciliates and other organisms in culture system. Ciliate growth can be assessed by the cloudy nature in the bottom samples of the culture tanks. If timely measures are not taken, there will be a decline in the population. Presence of ciliates in the culture tanks and epibionts on the copepods should be evaluated at regular intervals, if possible on alternate days. *Euplotes* spp. is the most common ciliate in the culture system and *Vorticella* spp. is the most common epibiont on the copepods. Generally *Euplotes* spp. does not interfere the culture. Presence of low levels of ciliates can reduce water deterioration by consuming the bacterial

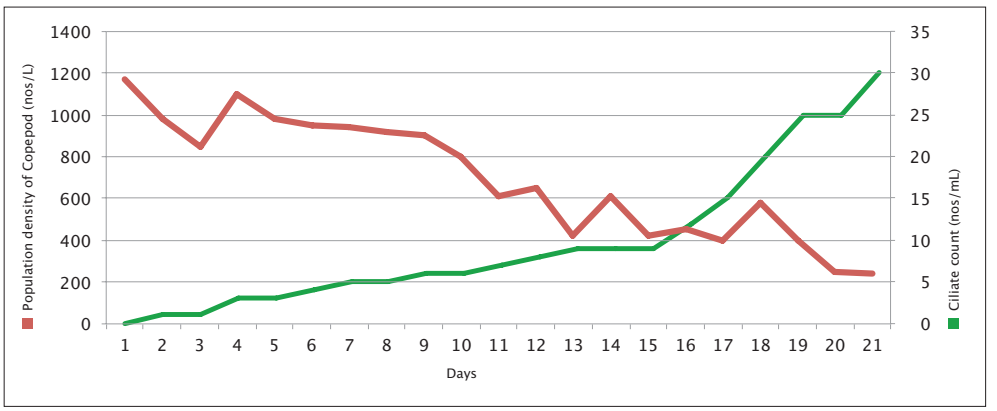


Fig. 34. Variation of copepod *T. turbinata* population in relation to ciliate density in culture tanks

population. Some copepods like *P. serricaudatus* can also consume ciliates. The role of ciliates in the culture is still to be understood clearly.

All the species popular in culture can withstand ciliates up to some extent. If the presence of ciliates exceeds normal limits, the culture can be siphoned out and washed through dechlorinated and filtered sea water using sieves of 70-80 µm and fresh culture can be initiated. Ciliate population will be higher in bottom water. Threshold level of ciliates in the bottom water sample of culture tanks is estimated to be 7-8 nos/mL. If the ciliate level exceeds more than 10 nos/mL in bottom sample, there will be a sharp decline of population of copepods in the culture tanks (Fig. 34).

The deficiency of feed is another reason for the decline of culture population. The feed provided should be proportional to the biomass present. If sufficient feed is not supplied to the culture system, it may reduce the production of eggs and larvae which may lead to total collapse of the culture. The feed provided

should be contamination free, especially of ciliates. Algae in exponential phase only should be used.

The settled debris and accumulated waste in the culture tank also form a suitable substrate for development of ciliates and other organisms. Some of the common contaminant organisms are shown in Fig. 35-42. Renewal of sea water in culture tanks is essential to create a healthy environment for the cultured species. There may be presence of harpacticoid copepods in some culture tanks.

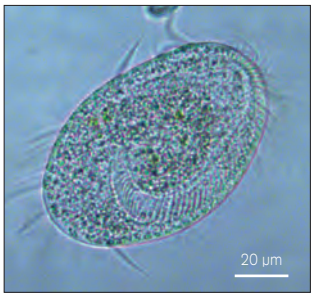


Fig. 35. *Euplotes* sp.

Fig. 36. *Vorticella* sp.

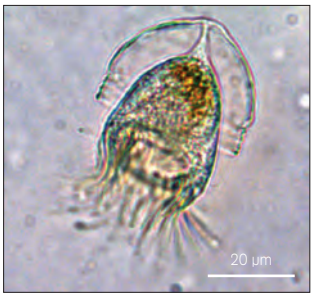


Fig. 37. Tintinnid sp. 1

Fig. 38. Tintinnid sp. 2

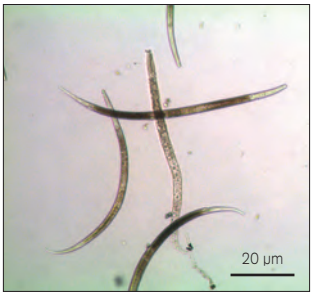


Fig. 39. Nematode


Fig. 40. *Brachionus rotundiformis*

Fig. 41. *Artemia* nauplius

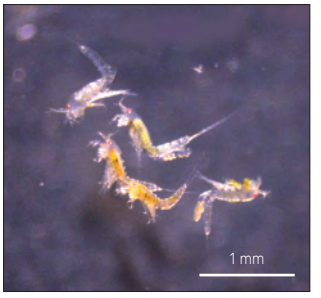


Fig. 42. *Microsetella* sp.

Some harpacticoids are predatory in nature and these should be avoided. If their presence is noticed and a sharp decline in population of cultured species, the entire culture in that tank must be destroyed by adding chlorine. Start fresh culture from uninfected tanks. Harpacticoids of the genus *Microsetella* are generally harmless and can be easily filtered out through siphoning of the bottom water. Nematodes, trematodes, *Zoothamnium* spp. and *Vorticella* spp. also can be contaminants. Sometimes *Vorticella* spp. can infect the copepod (Fig. 43). These can be controlled by increasing the frequency of bottom siphoning. Extreme care should be taken to avoid contamination by tintinnids, rotifers and *Artemia* in the culture.

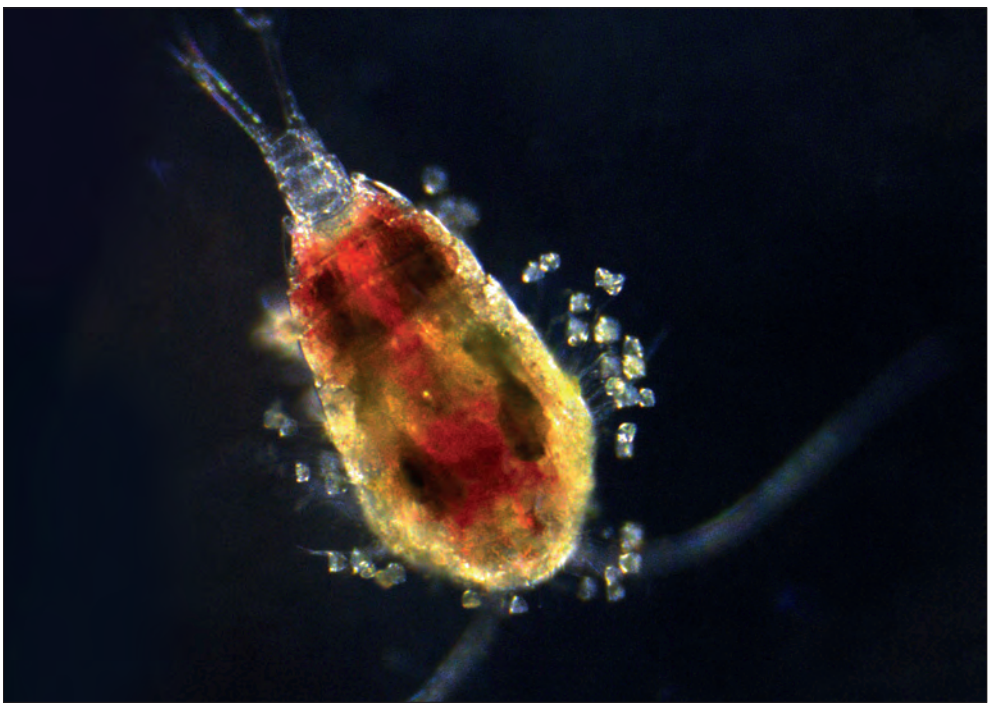


Fig. 43. Copepod infected with *Vorticella* sp.

General remarks: Stock and mass culture methods described here for different species developed at Vizhinjam Research Centre of CMFRI are ideal to meet the requirements of large hatcheries where marine food fish larval rearing has been taken up. The methods are applicable for small scale ornamental fish hatcheries also. This method is ideal for most of the calanoid copepods and cyclopoid copepods. But among harpacticoids studied, only *E. acutifrons* can be cultured using this method. If harpacticoid copepods of the genus *Tisbe* or *Microsetella* or similar genera are to be cultured, smaller containers or trays with substratum can be used. Formulated feeds can be used only for feeding harpacticoid copepods.

Feeding the fish larvae with wild collected copepods is always risky because these carry some unwanted predatory organisms, pathogens and intermediate stages of parasites. Moreover, in wild, the availability of desirable nauplii is always uncertain. Intensive hatchery production of copepod is always better as we completely avoid introduction of unwanted species and parasites. It is stable and production can be synchronised with larval production. It is easy to filter sea water and rear few copepods for few days. But continuous culture and mass production need special care and management.

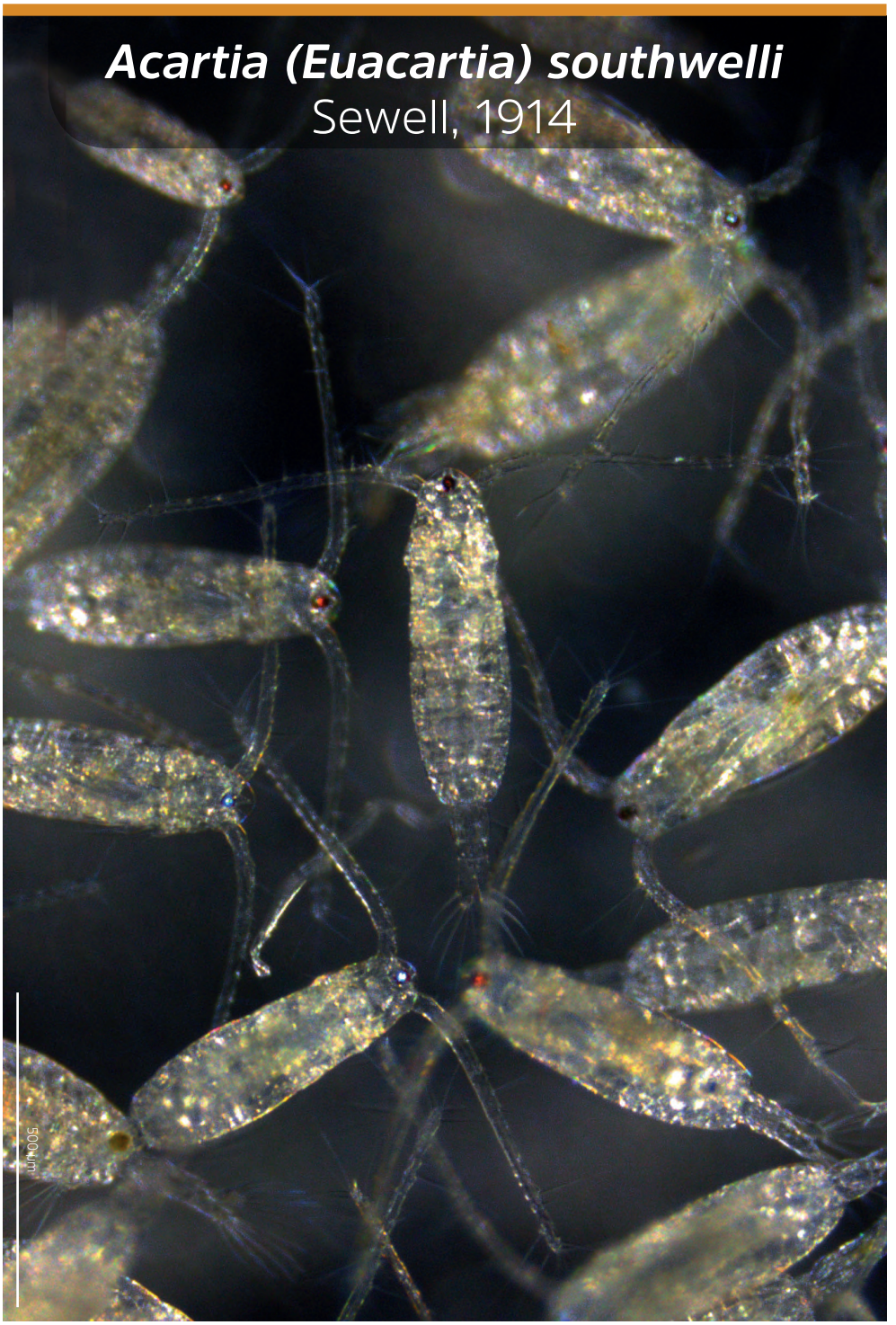
More than 60 species of copepods have been raised in laboratories. For promoting mass culture of copepods in cost-effective way, the development of appropriate culture technique is essential for each species. Copepods can be cultured extensively, intensively and semi-intensively. The extensive cultures are mainly in tanks, outdoor ponds, lagoons or enclosed fjords. By using plankton nets or sieves of appropriate mesh sizes, these cultured copepods can be made available to fish larvae. In extensive systems, culture is done normally by producing microalgal blooms using ordinary agricultural fertilizers. Agricultural fertilizers, both organic and inorganic, were used with or without combination of fishmeal, rice bran, wheat bran and fish feeds as inputs for nutrients. But here the main disadvantage is the unpredictable nature of production. Rotifers if present may easily dominate in the culture. Semi-intensive culture is generally carried out in indoor tanks with regular supply of microalgae in combination with baker's yeast or other feeds. In this system regular harvest is possible and yield a mixed culture of different species of copepods. Here also the culture may not be stable for longer periods. Intensive culture is developed generally by maintaining selected isolated pure culture of copepod species with desired qualities. Basically there are small stock culture units, large mass culture units and modified culture tanks fitted with structures for harvesting naupliar stages on regular basis. Specialised nauplii collection units can be attached to mass culture units also. All important water quality parameters need to be monitored and adjusted regularly.

Though intensive culture may not be economical, most of the hatcheries prefer this because of the assured production of copepods and nauplii of desired size and species. This will help larvae to thrive well during critical periods of larval rearing. Once the critical period is crossed, the larvae can be fed with enriched rotifer and *Artemia* nauplii.

Rotifers can be cultured to a very high density. But in case of most of the copepod species, the density rarely exceeds 2-5 nos/mL for adults and 10 nos/mL for nauplii. Studies conducted on copepod cultivation reveal that, calanoids are comparatively difficult for culture than harpacticoids. Harpacticoid copepods can be produced at higher density for a shorter period. In most of the cases, harpacticoids and their larval forms will be unavailable for fish larvae due to its

epibenthic nature. Calanoids are the most abundant zooplankton which forms a connecting link between phytoplankton and the fish in marine ecosystem. Most of the calanoid copepod species are less than 1.5 mm in total length, some being as small as 0.4 mm. Few species of calanoid copepods especially the temperate forms have already been cultured and utilised in several hatcheries. Calanoids are more ideal for hatchery production. In a short time, these can reach a higher density in tanks of 5-10 t capacity. Larval forms are mostly smaller and distribute evenly in the water column. The stock culture also can be reared easily for years. Many species stocks are being maintained for more than 7 years in CMFRI.

Many species like *Acartia clausi, A. tonsa*, *Centropages hamatus*, *C. typicus, Parvocalanus crassirostris*, *Gladioferens imparipes*, *Tisbe* spp., *Oithona simplex*, *Bestiolina similis*, *Apocyclops* spp. and *Temora stylifera* are being used in fish seed production in many hatcheries. In India, CMFRI is the pioneer in developing techniques for large scale production of copepods. We have used hatchery produced copepods for seed production of ornamental fishes and food fishes including groupers and snappers. At present CMFRI has pure stock and large scale culture of nine species of copepods suitable for larval rearing of almost all types of fish larvae. Now CMFRI is in a position to distribute stock culture and also to impart knowledge on culture techniques to farmers and entrepreneurs.


Acartia (Euacartia) southwelli
Sewell, 1914
500 µm

Biological information and culture techniques of *Acartia (Euacartia) southwelli* Sewell, 1914

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Basic information

Acartia is one of the most important genus of copepod widely reported from different parts of the world (Razouls *et al.,* 2005-2017). This is one of the most extensively studied genus among calanoid copepod with respect to its use as live feed for fish larvae. Many species have already become popular as live feed. *Acartia* spp. are much hardy among calanoid copepods and give very stable culture (Stottrup and Norsker, 1997; Stottrup, 2003, 2006; Marcus, 2005; Toledo *et al.,* 2005). *Acartia* spp. are seen distributed in all continents (Razouls *et al.,* 2005-2017). Important species among these are *A. tonsa, A. erythraeae, A. clausi, A. sinjiensis, A. tsuensis, A. tranteri, A. bilobata, A. grani, A. longirensis, A. pacifica* and *A. plumose* (Santhosh *et al.,* 2016).

Acartia (Euacartia) southwelli is a tropical species with distribution restricted to Asian region particulary from Indian Ocean and Chinese waters. This is one of the common *Acartia* species of Indian waters and particularly reported from brackish waters. Reports are there about the laboratory level culture of *A. tonsa,* A. e*rythraeae, A. clausi, A. gracilis* and *A. centrura* from India (Santhanam, *et al.,* 2015; Santhosh *et al.,* 2016). This is the first report on mass production of A. s*outhwelli* and the use of this species in marine finfish hatchery.

Biological information

Habitus: Body length in adults range from 700-800 µm in females and 650-750 µm in males (Fig. 44a&b, 45a&b). It is difficult to distinguish males and females. The main difference is in the size and segmentation of fifth leg and shape

of the antennule. In males, fifth leg is highly modified and one of the antennules is partially geniculate. Males are slightly smaller than females.

This is a slow swimming species and evenly distributed in the entire water column of the culture tanks. These are partially attracted to light but do not concentrate near the light source as a whole. *A. southwelli* is not a predatory copepod and feed only by filter feeding. Adult life span is about 20-25 days. Females are more in culture and the sex ratio in culture is 1:4.

This species does not have any bright colouration and is very difficult to locate in culture with naked eyes. This species is distributed evenly in the entire water column and hence it is very ideal to culture in higher density. It is not a predatory species but found to consume ciliates and similar smaller protozoans.

Eggs: Small spherical eggs measuring 75-80 µm in size with a spiny eggshell on the surface (Fig. 46a). Eggs are broadcasted in the water and sink to bottom. Eggs alone can be easily harvested by siphoning the bottom sediment and filtering the same using a 60 µm mesh. Most of the eggs hatch in 20-30 h in 25-28°C. Fecundity ranges from 15-30 eggs/day. It is observed that they produce different types of eggs including dormant eggs. Eggs can be temporarily stored for weeks

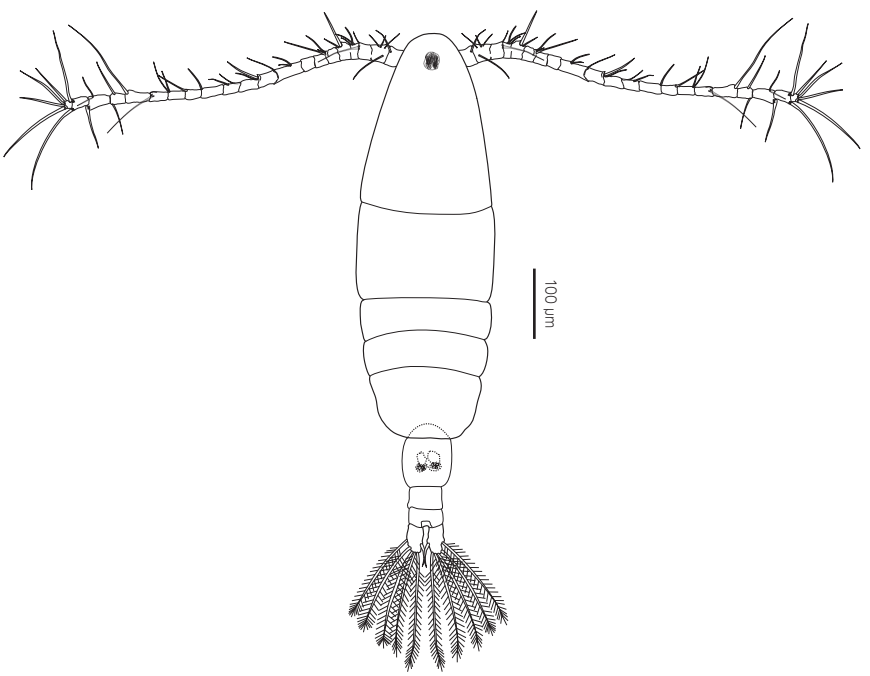


Fig. 44a. *Acartia southwelli* Female

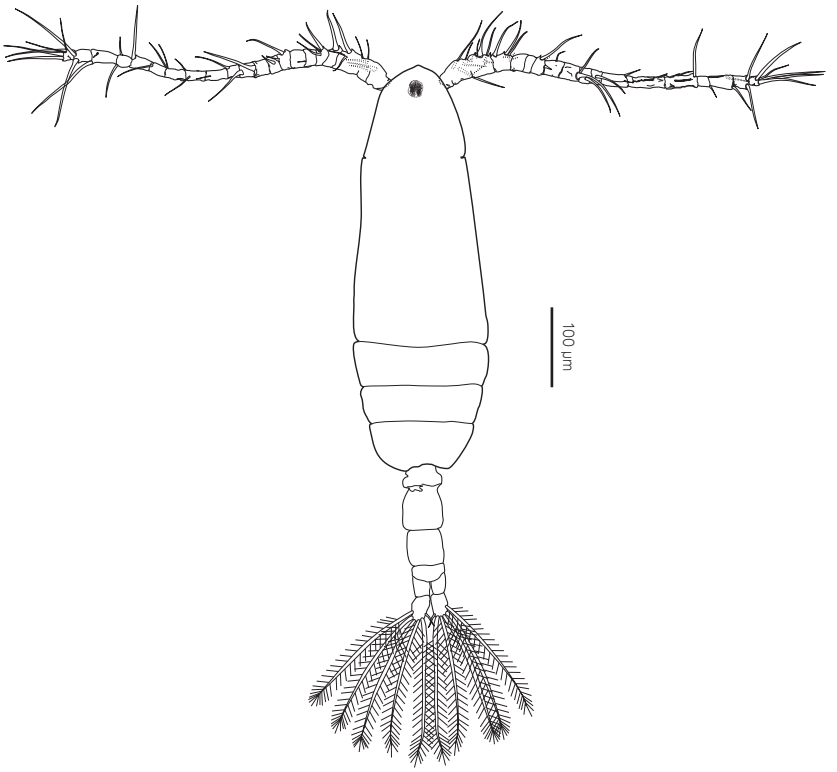


Fig. 44b. *Acartia southwelli* Male

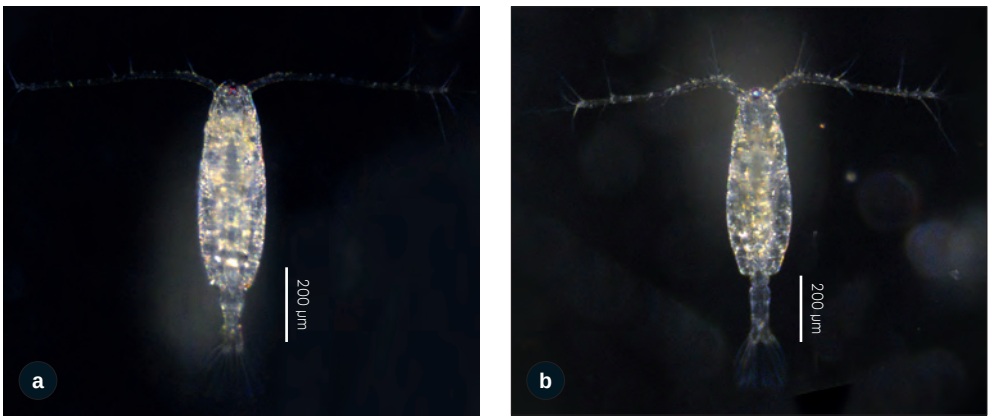


Fig. 45. *A. southwelli* a. Female b. Male

in seawater around 4-5°C without losing their hatchability.

Larval stages: There are 6 naupliar stages and 5 copepodite stages (Fig. 46b-l). The naupliar stages with length range from 80 µm to 200 µm and width from 50 µm to 100 µm. It takes almost 12-14 days to reach adult stage (Fig. 46m&n). The first naupliar stage is about 80 µm in length and 60 µm in width.

Environmental conditions

Acartia southwelli can tolerate wide range of temperature (15-35°C) and the ideal temperature for culture is 25-28°C. It can survive in salinities between 5-45 ppt but optimum salinity range for culture is 30-35 ppt.

Normal diffused day light is needed. Direct sunlight is not preferred. Normal tropical day length is ideal for culture. pH range between 8-8.5 is considered ideal. Ammonia should be below 2 ppm. It can be cultured in 1 L containers to 5 t tanks. Ideal depth of the tank is below 1 m.

Treated seawater (chlorinated and dechlorinated) is ideal for culture. If water quality parameters deteriorate, replace the water using appropriate sieves. Low aeration is needed.

Culture protocols

Food and feeding: This species was found to feed on a variety of algae. Under culture condition, ideally this species can be fed on *Isochrysis galbana* and *Nannochloropsis salina* in the ratio of 3:1. The optimum cell density for culture is between 20000-30000 cells/mL. Algae should be contamination free and in the growing phase.

Density: Maximum density at a sustainable level of culture under normal conditions can be upto 2-3 nos/mL. Stock culture of 20 L with a density of 2000/L is suitable to inoculate tanks of 1000 L capacity and this will reach maximum density within 15-18 days. This can be maintained for 2-3 months in the same containers with proper cleaning and regular harvest. Daily harvest is possible as eggs, nauplii, adults or as mixture of all stages (Table 1).

Single species culture only is possible. This species is comparatively hardy and survive in a mixed culture but never dominates over the other species in culture. It can be used as a feed along with other species in larval rearing tanks.

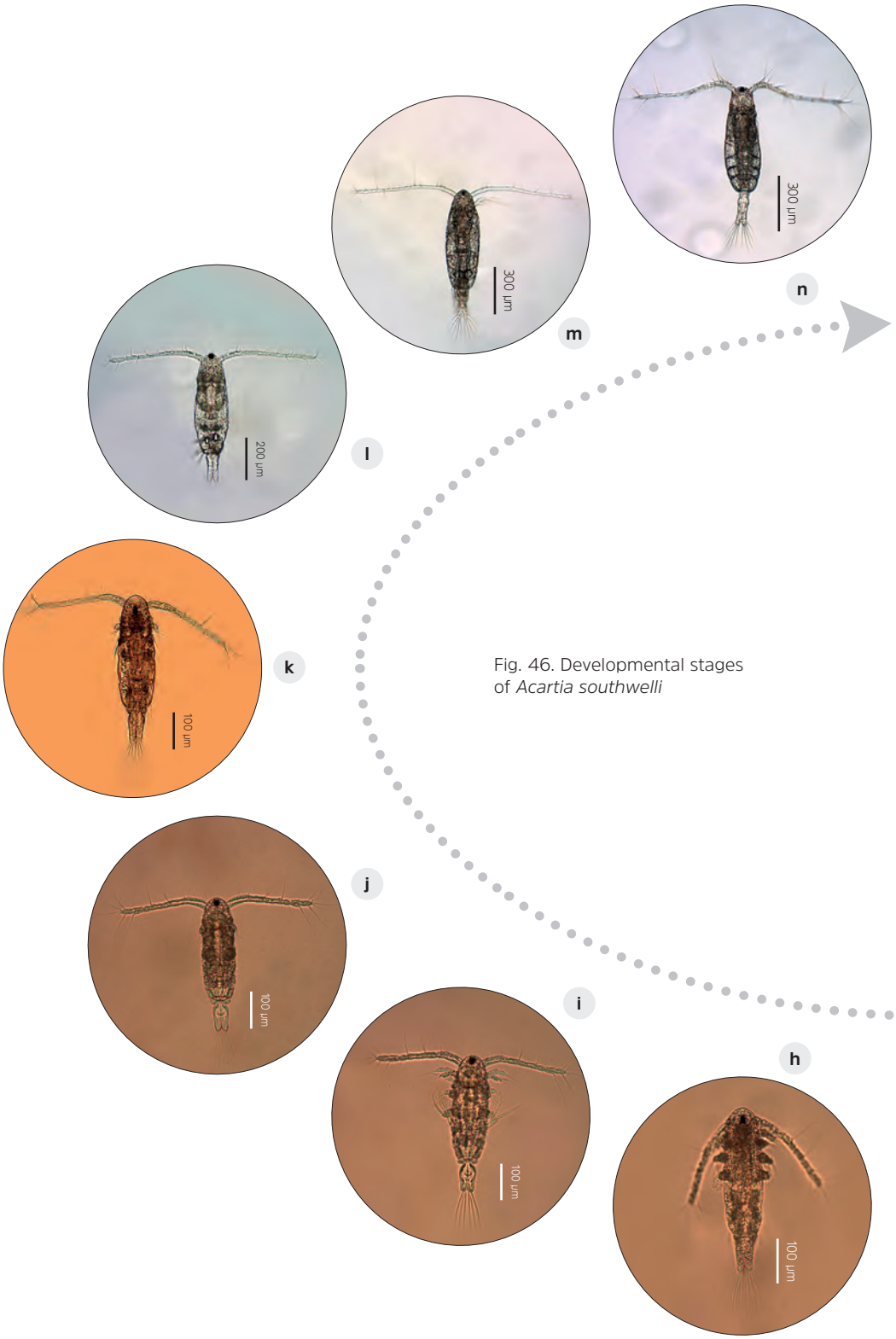
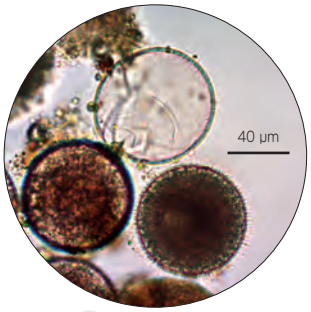


Fig. 46. Developmental stages of *Acartia southwelli*



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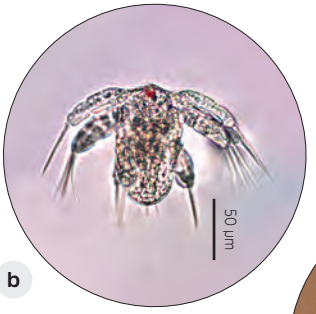

b

c

a. Eggs
b. Nauplius-1
c. Nauplius-2
d. Nauplius-3
e. Nauplius-4
f. Nauplius-5
g. Nauplius-6
h. Copepodite-1
i. Copepodite-2
j. Copepodite-3
k. Copepodite-4
l. Copepodite-5
m. Adult female
n. Adult male

d

e

f

g

Table 1. Mesh size of sieves/bolting silk for filtering different stages of *Acartia southwelli*

Sl. No.	Stages required	Mesh size of sieves (µm)	Filtration pattern
1.	All stages including eggs	45	Single filtration
2.	Nauplii alone	100 and 45	Serial filtration. Filter through 100 µm to remove adults and copepodites and take residue from 45 µm for nauplii
3.	Adult alone	225	Single filtration
4.	Eggs alone	80 and 45	Siphon out the bottom after settling and serially filter through 80 µm and 45 µm, residue in 45 µm will be eggs

Precautions: Stock and mass culture is sensitive and should be fed only with good algae at required level and all parameters need to be maintained at optimum levels. Regular harvest is needed to regulate population level in the culture tank. This species should always be maintained as single species culture and all sorts of contamination should be avoided. If any contamination is noticed, isolate adults and restart the culture. Over feeding and under feeding should be avoided. A sudden drop in population is observed in some mass culture tanks specially if the temperature is low and within a week or two, the species re-establishes its population to maximum density.

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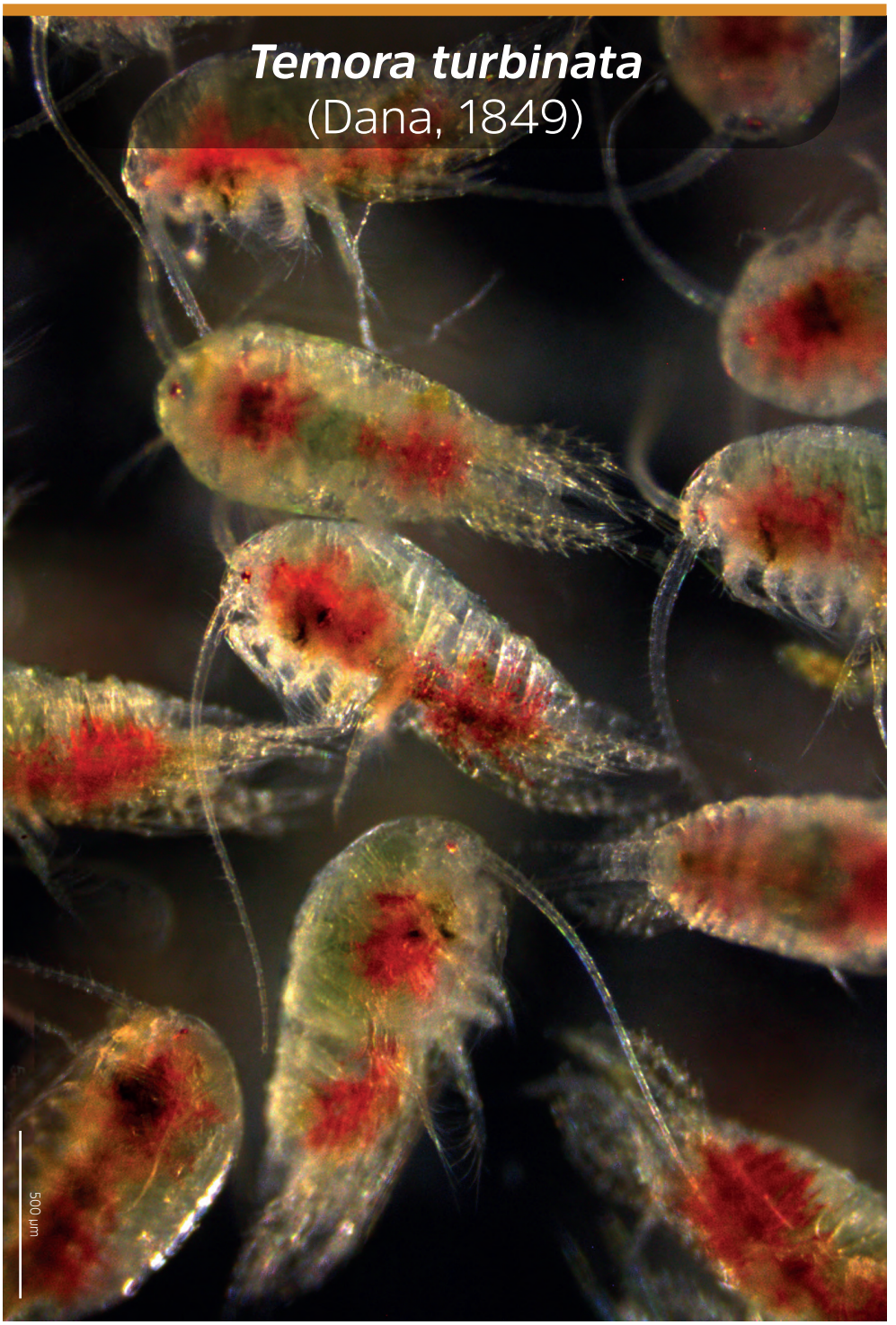
Temora turbinata
(Dana, 1849)
500 µm

Biological information and culture techniques of *Temora turbinata* (Dana, 1849)

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Basic information

Temora turbinata is one of the commonest calanoid copepods in the Indian Ocean. This species is widely distributed in tropical, subtropical and temperate waters of Indian Ocean, Pacific Ocean and Atlantic Ocean (Razouls *et al.,* 2005-217). This is a brilliantly coloured and slow swimming copepod and its abundance in the plankton sample is often correlated with the abundance of certain fishes (Santhakumari and Peter, 1993). This is one of the most widely studied species with respect to their ecology, biology, abundance, feeding and often form a model for various experimental studies (Pillai, 1975; Turner, 1984; Mauchline, 1998; Ara, 2002). This is the slowest moving copepod reported and also forms one of the most vulnerable prey for fishes (Waggett and Buskey, 2008). This is commonly reported from estuarine waters along east and west coast of India.

This species has been cultured for past many years from different parts of the world for various laboratory experiments (Mauchline, 1998; Ara, 2002). But there has been no report on mass production of this species for larval rearing. This is the first report on the mass production of *T. turbinata* and their use for larval rearing in marine finfish hatcheries.

DNA extraction was carried out from the samples preserved in 95% ethanol following standard protocols and a 650 bp region of the Cytochrome C oxidase 1 gene was amplified and sequenced using the universal primers (Folmer *et al*., 1994; Samonte *et al*., 2000). The sequence was submitted to NCBI, GenBank with the accession number MK387707.

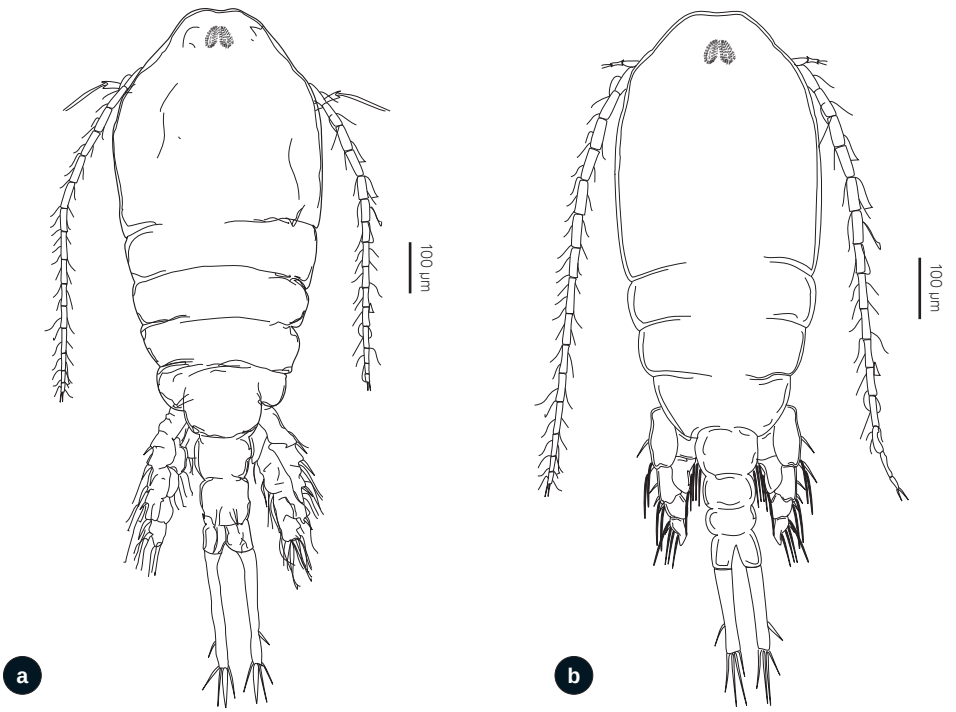


Fig. 47. *T. turbinata* a. Female b. Male

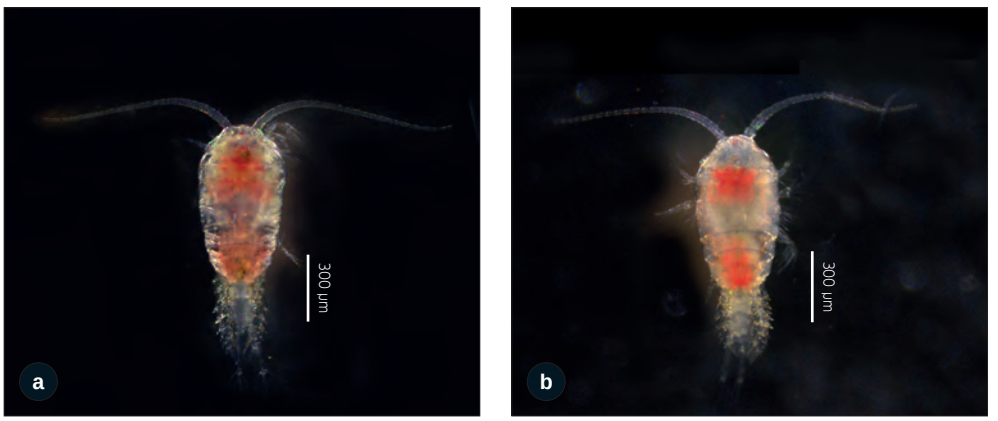


Fig. 48. *T. turbinata* a. Female b. Male

Biological information

Habitus: Adult size ranges from 1070-1350 µm in females and 1010-1150 µm in males (Fig. 47a&b, 48a&b). It is very difficult to distinguish male and female. Females are slightly swollen than males. Caudal ramous of female has a leaf like flattened seta. Fifth leg is highly modified in males.

This is one of the slowest swimming species and form an easy prey even for weak fish larvae. In culture, it gets attracted to light and form a crowd near the surface edge of the tanks. This is not a predatory or cannibalistic species. It feeds on a variety of microalgae by filter feeding. Adult life span is about 20-25 days. Females are more in culture and the sex ratio in culture is 1:5. This species is bright orange in colour and is easy to locate in culture with naked eyes.

Eggs: Small spherical eggs measuring 80-90 µm in size without any special covering (Fig. 49a). Eggs are broadcasted in water and sinks to the bottom. Eggs alone can be easily harvested by siphoning the bottom sediment and filtering the same using a 60 µm sieve. Eggs hatches in 15-20 h at 25-28°C, 80% hatching was observed. Fecundity ranged from 15-30 eggs per day. This species generally do not produce any diapause or dormant eggs.

Larval stages: There are 6 naupliar stages and 5 copepodite stages (Fig. 49b-l). The naupliar stages ranges from 90 µm to 300 µm in length and 75 µm to 150 µm in width. It takes almost 15 days to reach adult stage (Fig. 49m&n). The first naupliar stage is of 90 µm in length and 60 µm in width.

Environmental conditions

T. turbinata can tolerate wide range of temperature (5-35°C) and the ideal temperature for culture is 25-28°C. It can survive in salinities between 20-45 ppt but optimum salinity range for culture is 30-35 ppt.

Normal diffused day light is favourable. Direct sunlight is not preferred. Normal tropical day length and pH range of 8-8.5 are ideal. Ammonia should be below 4 ppm. It can be cultured in 1 L containers to 1 t tanks. Ideal depth of the tank is up to 1 m.

Low aeration is needed throughout day and night. Treated seawater (chlorinated and dechlorinated) is ideal. If water quality parameters deteriorate, replace the water using various sieves and remove all dead copepods without delay.

Culture protocols

Food and feeding: This species basically feed on a variety of algae. Better production was observed when *Isochrysis galbana* and *Nannochloropsis salina* were used in ratio 3:1 in mass production trials. The ideal range of algal cell density for culture is 20000- 30000 cells/mL. Algae should be contamination free and in the growing phase. This is a sensitive species and it is essential to remove faecal pellets and dead copepods regularly from the culture tank.

Density: Maximum density at a sustainable level of culture under normal conditions can be upto 2-3 nos/mL. Under normal conditions, stocking of 20 L stock culture (1000 nos/L) in 1000 L mass culture can reach its maximum density within 22-25 days and the culture can be maintained for 2-3 months in the same containers with proper cleaning and regular harvest. Daily harvest is possible as eggs, nauplii, adults or as a mixture of all stages (Table 2).

Single species culture only is possible. This species is comparatively sensitive and does not survive in a mixed culture. However, it can be used as a mixed feed with other species in larval rearing tanks.

Table 2. Mesh size of sieves/bolting silk for filtering different stages of *Temora turbinata*

Sl. No.	Stages required	Mesh size of sieves (µm)	Filtration pattern
1.	All stages including eggs	60	Single filtration
2.	Nauplii alone	150 and 60	Serial filtration. Filter through 150 µm to remove adults and copepodites and take residue from 60 µm for nauplii
3.	Adult alone	270	Single filtration
4.	Eggs alone	90 and 50	Siphon out the bottom. After settling, filter serially through 90 µm and 50 µm. Residue in 50 µm contains eggs

Precautions: *T. turbinata* is very sensitive in both stock and mass culture. It needs to be fed only with good algae at required level and all water quality parameters at optimum levels have to be maintained. Regular harvest is needed to regulate population level. This species should always be maintained as single species culture and all sorts of contamination should be avoided. If there is any contamination, isolate the adults and restart the culture. Over feeding and under feeding should be avoided. Ciliate level should be monitored regularly. This species is highly prone

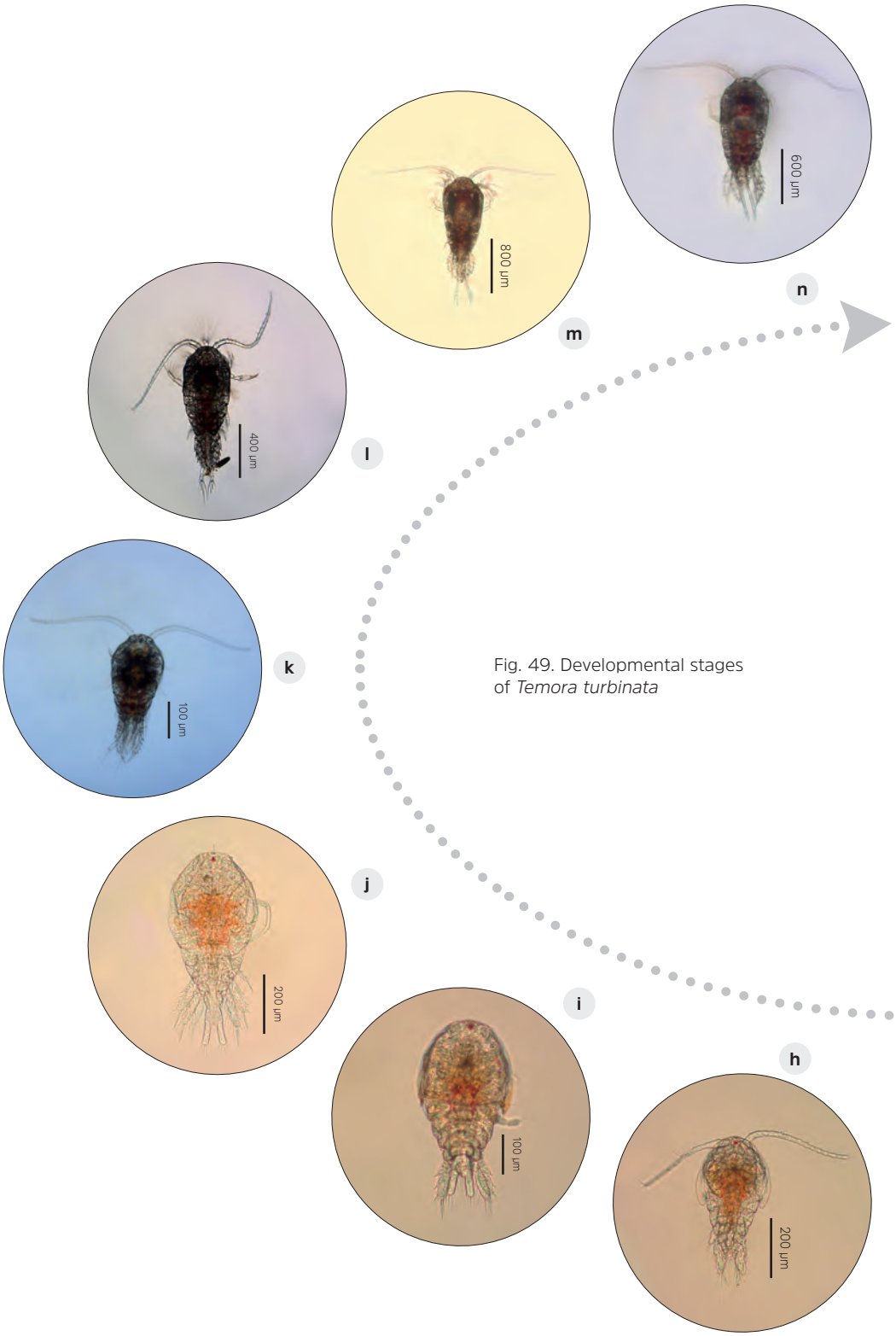
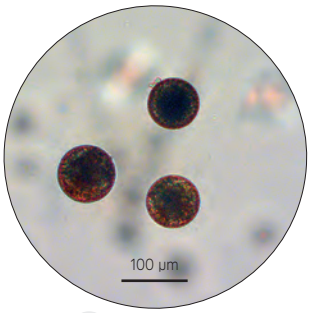


Fig. 49. Developmental stages of *Temora turbinata*



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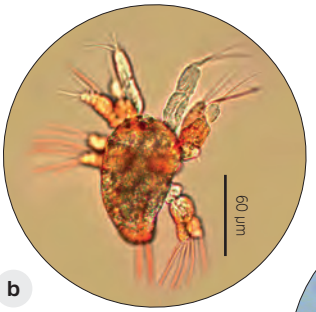

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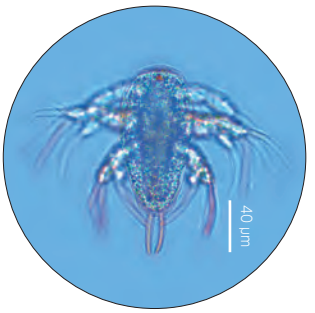

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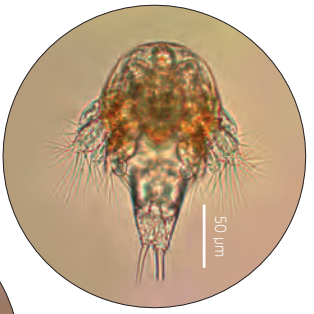

e

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a. Eggs
b. Nauplius-1
c. Nauplius-2
d. Nauplius-3
e. Nauplius-4
f. Nauplius-5
g. Nauplius-6
h. Copepodite-1
i. Copepodite-2
j. Copepodite-3
k. Copepodite-4
l. Copepodite-5
m. Adult female
n. Adult male

to *Zoothamnium* or *Vorticella* infection. Hence regular monitoring and cleaning are essential in both stock and mass culture tanks.

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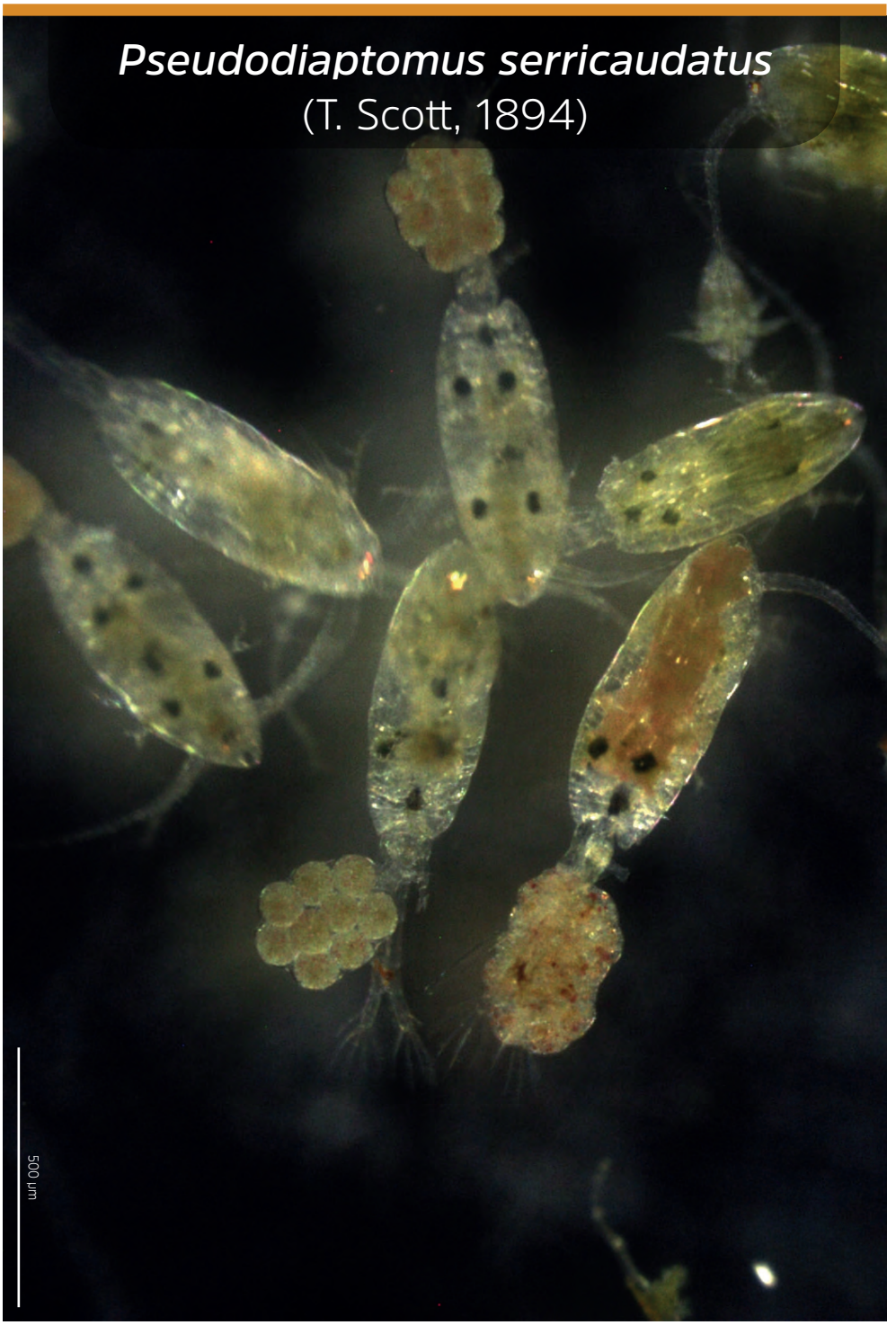
Pseudodiaptomus serricaudatus
(T. Scott, 1894)
500 um

Biological information and culture techniques of *Pseudodiaptomus serricaudatus* (T. Scott, 1894)

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Basic information

Pseudodiaptomus is a major genus of calanoid copepod with more than 80 valid species reported from different parts of the world (Razouls *et al.,* 2005-2017). *P. serricaudatus* is one of the most common species from Indian waters. It is predominantly a coastal species from Indian and African waters. It is very common in estuaries and brackish water lagoons of these areas (Rebello *et al.,* 2014). *P. serricaudatus* is one of the pioneer species cultured in India by CMFRI (Gopakumar and Santhosi, 2009; Gopakumar *et al.,* 2009 a, b).

Many species of this genus are known to occur in Vizhinjam waters and among them, *P. serricaudatus* is the commonest species. This species is light greenish yellow in colour. *P. serricaudatus* is a hardy species, grow well even in smaller containers and it is an ideal species for the beginners.

Biological information

Habitus: Adult size ranges from 950-1250 µm in females and 910-1210 µm in males (Fig. 50a&b, 51a&b). These are light greenish yellow in colour and are active swimmers. Males are smaller than females and it is very easy to distinguish them. The main difference is in the shape and segmentation of antennule. In males, one of the antennules is geniculate. Females carry single large egg sac under the abdomen and this is generally uncommon among calanoids.

This is an actively swimming species and often found drifting in water column. These are strongly attracted towards light and concentrate near the light source.

P. serricaudatus is generally not a predatory copepod and feed only by filter feeding. In case of a scarcity of food, this species may consume its own smaller naupliar stages. Adult life span is about 30-40 days. Females are more in culture and the sex ratio is 1:5. This is a hardy species and can control itself the ciliates and similar smaller protozoans in the culture to some extent.

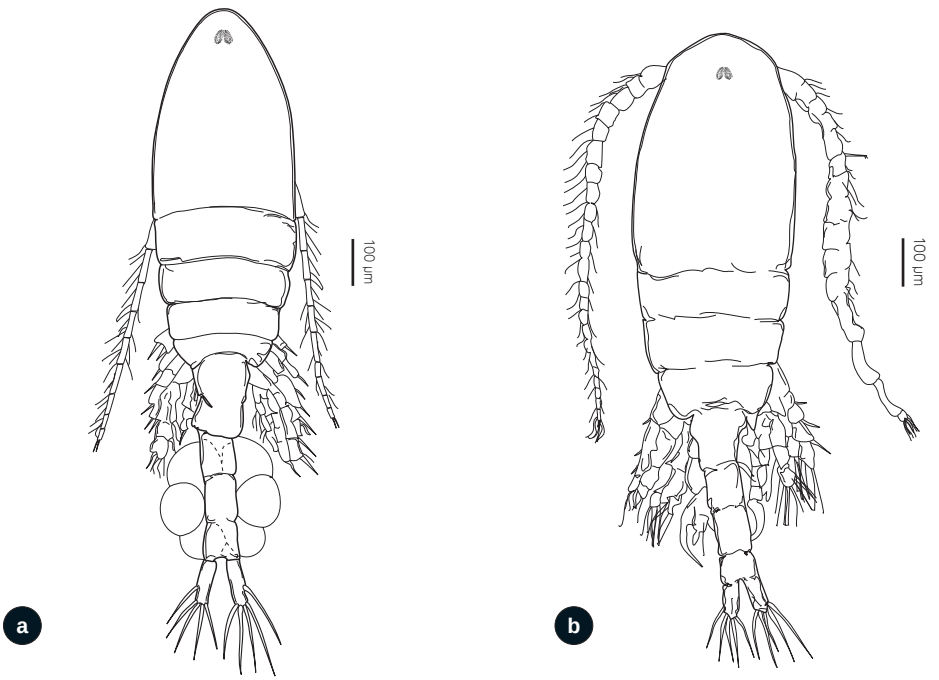


Fig. 50. *P. serricaudatus* a. Female b. Male

Eggs: Small spherical eggs measuring 80-100 µm in size and enclosed in a single egg sac (Fig. 52 and 54a). Number of eggs per egg sac ranges from 3-15. Eggs develop in the brood pouch for 3-5 days and nauplii at N1 stage released into water (Fig. 53). In case of any stress, including stress due to filtration and handling, generally females leave the entire egg sac into the water. If the eggs are mature enough, it may hatch out from the abandoned egg sac also.

Larval stages: There are 6 naupliar stages and 5 copepodite stages (Fig. 54b-l). The first naupliar stage is very short and last only for few minutes. So naupliar stages from 2 to 6 have been commonly reported. The length of naupliar stages ranges from 115 µm to 250 µm and width from 90 µm-140 µm and takes almost

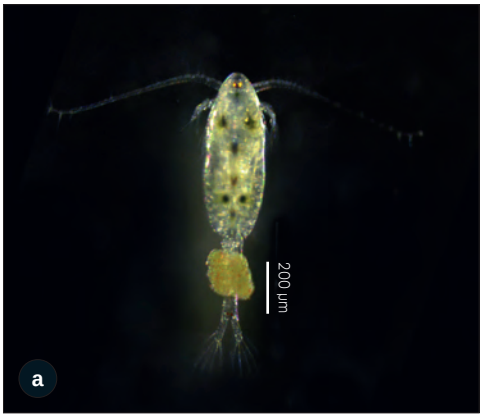


Fig. 51. *P. serricaudatus* a. Female b. Male

Fig. 52. Egg sac showing eggs in different developmental stages

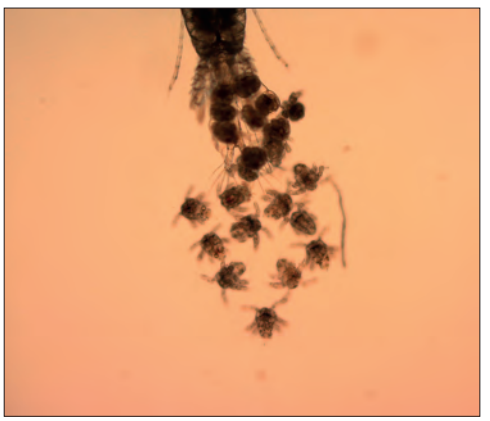


Fig. 53. Nauplii released from egg sac

8-10 days to reach adult stage (Fig. 54m&n). The first naupliar stage is of 115 µm in length and 90 µm in width.

Environmental conditions

P. serricaudatus can tolerate wide range of temperature (5-35°C) and the ideal temperature for culture is 25-28°C. It can survive in salinities between 5-45 ppt but optimum salinity range for culture is from 30-35 ppt. Normal diffused day light is needed for culture and direct sunlight is not ideal. Normal tropical day length and pH range from 8-8.5 is favourable for culture. Ammonia should be below 4 ppm. It can be cultured from small containers to 5 t tanks. Ideal depth of the culture tank is below 1 m.

Treated seawater (chlorinated and dechlorinated) is ideal for culture. If water quality parameters deteriorate, replace the water using various sieves. Using a strong beam of light it can be concentrated to a smaller area of the culture tank and can harvested easily. Low aeration only is needed.

Culture protocols

Food and feeding: This species basically feed on a variety of algae. Under ideal culture condition, this species can be fed with *Isochrysis galbana, Nannochloropsis salina* and *Chlorella marina* in the ratio 1:1:1. The ideal range of cell density for culture is 20000-30000 cells/mL. Algae should be contamination free and in the growing phase.

Density: Maximum density at a sustainable level of culture under normal conditions can be upto 2-3 nos/mL. For mass culture, 20 L stock culture (1000 nos/L) is needed to inoculate in 1000 L mass culture which will reach maximum density within 25-30 days and can be maintained for 2-3 months in the same container with proper cleaning. Harvest is possible in every 3 days as nauplii, adults or as mixture of all stages (Table 3). Single species culture is ideal. This species is comparatively hardy and survive in a mixed culture and dominate over many other species in culture especially *Temora turbinata*. It can also be used as a feed along with other species of copepods in larval rearing tanks.

Table 3. Mesh size of sieves/bolting silk for filtering different stages of *Pseudodiaptomus serricaudatus*

Sl. No.	Stages required	Mesh size of sieves (µm)	Filtration pattern
1.	All stages	55	Single filtration
2.	Nauplii alone	140 and 55	Serial filtration. Filter through 140 µm to remove adults and copepodites and take residue from 55 µm for nauplii
3.	Adult alone	300	Single filtration

Precautions: Both stock and mass culture is sensitive and are difficult to maintain for longer period. It should be fed only with good algae at required level and all parameters at optimum level needs to be maintained. Regular harvest is ideal to regulate population level. This species should always be kept as single species culture. If any contamination is noted, isolate adults and restart the culture. Over feeding and under feeding should be monitored regularly for better production.

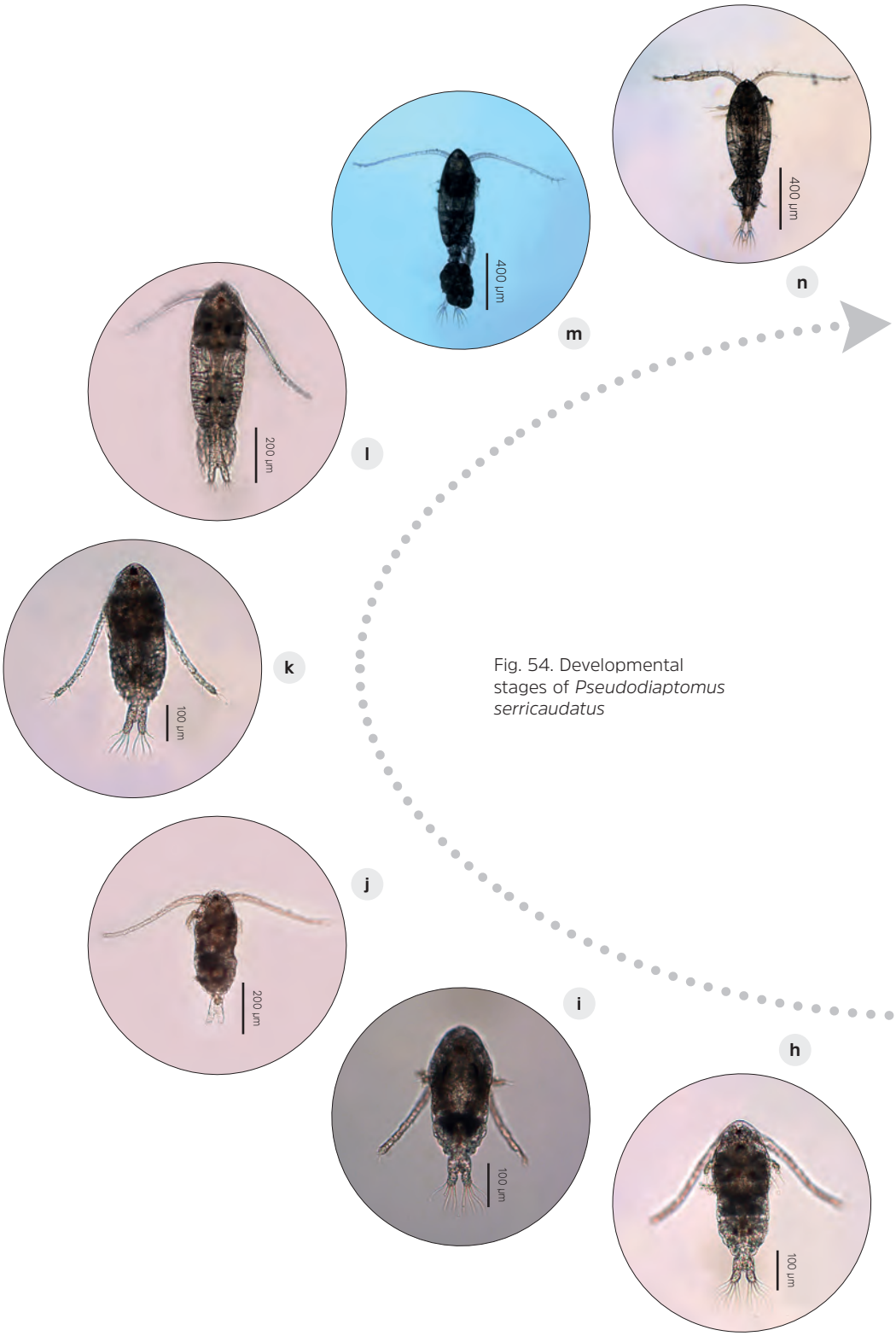
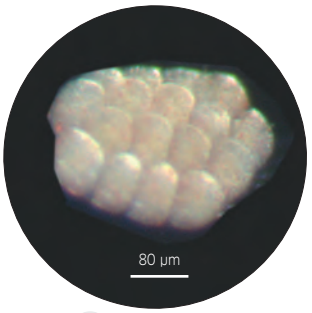
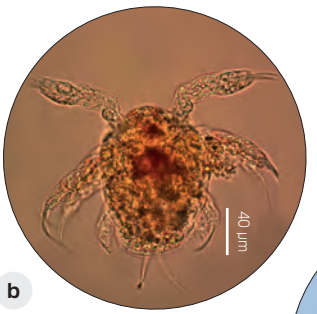


Fig. 54. Developmental stages of *Pseudodiaptomus serricaudatus*



a



b

c

d

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f

g

a. Egg sac
b. Nauplius-1
c. Nauplius-2
d. Nauplius-3
e. Nauplius-4
f. Nauplius-5
g. Nauplius-6
h. Copepodite-1
i. Copepodite-2
j. Copepodite-3
k. Copepodite-4
l. Copepodite-5
m. Adult female
n. Adult male

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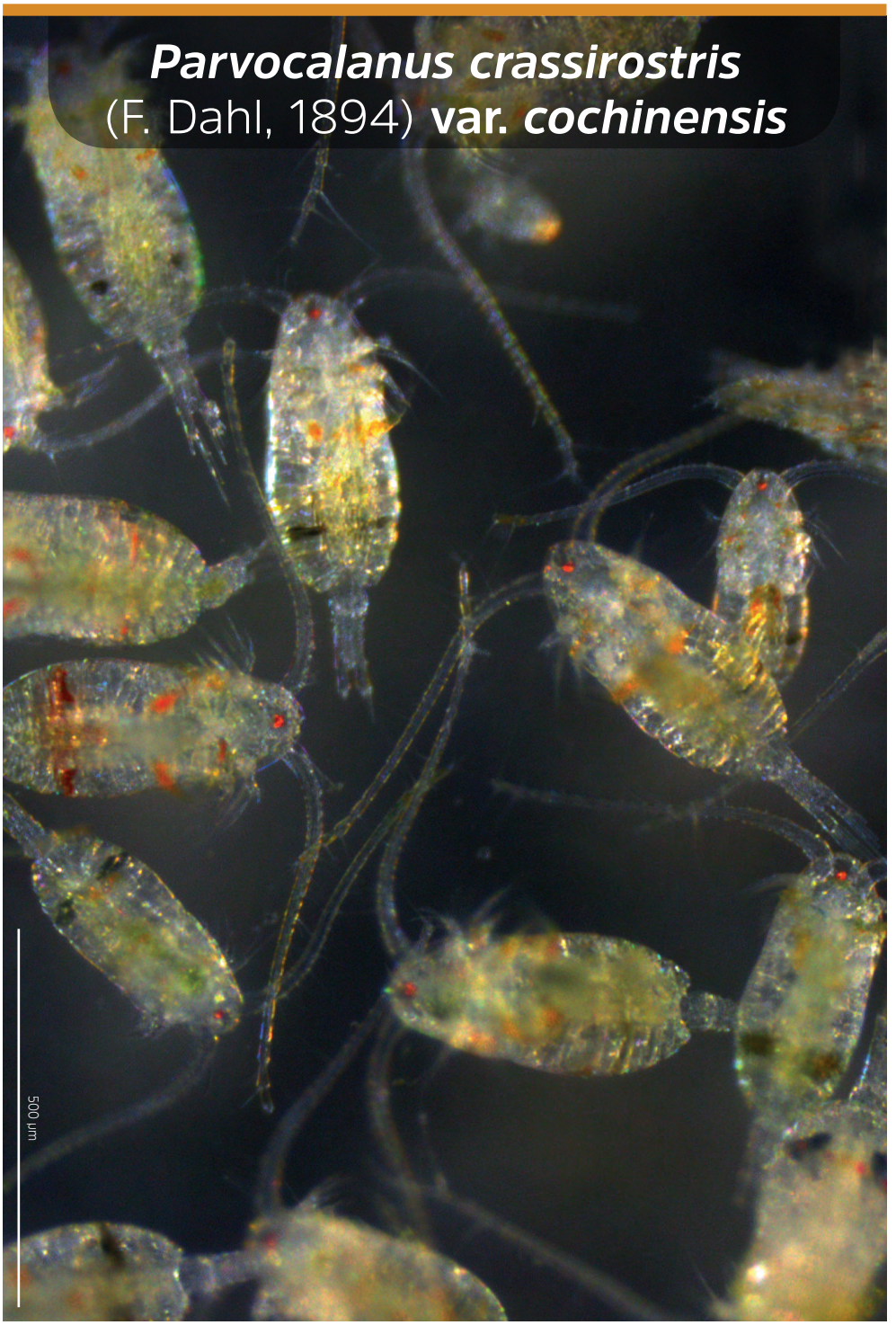
Parvocalanus crassirostris
(F. Dahl, 1894) **var. *cochinensis***
500 µm

Biological information and culture techniques of *Parvocalanus crassirostris* (F. Dahl, 1894) var. *cochinensis*

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Basic information

Parvocalanus is an important genus of planktonic copepod, distributed widely in marine and estuarine habitats throughout the world. *Parvocalanus* spp. are similar to *Paracalanus* spp. morphologically and both come under the family Paracalanidae. Only notable difference between the two genera is the size and segmentation of the 5th leg of both male and female. Even after molecular analyses, taxonomic ambiguity is not yet cleared for this group of copepods (Razouls *et al.,* 2005-2017).

Parvocalanus crassirostris is one the most important species of copepod extensively used in hatcheries throughout the world for commercial cultivation. In India, this has been the first report of successful culture of this species for fin fish larviculture (Stottrup and Norsker, 1997; Stottrup, 2003, 2006; Marcus, 2005; Toledo *et al.,* 2005; Santhosh *et al.,* 2016).

This is a species of copepod widely distributed in all tropical and subtropical waters. Due to its small size and prolific growth, this species is widely used in marine hatcheries for larval rearing of fishes with very small larval stages like groupers, snappers and damsels. This species is very ideal for feeding all types of fish larvae. This is one of the most accepted and established live feed for marine finfish hatchery, reef aquariums and for all other planktivorous species. *Parvocalanus crassirostris cochinensis* is a different strain reported only from Indian waters (Wellershaus, 1969).

DNA extraction was carried out from the samples preserved in 95% ethanol following standard protocols and a 650 bp region of the Cytochrome C oxidase 1 gene was amplified and sequenced using the universal primers (Folmer *et al*., 1994; Samonte *et al*., 2000). The sequence was submitted to NCBI, GenBank with the accession number MK387708.

Biological information

Habitus: Adult size ranges from 500-610 µm for females and 470-550 µm in males. It is difficult to distinguish male and female (Fig. 55a&b, 56a&b). The main difference is in the shape and segmentation of leg 5 (Fig. 57m&n).

This species is a slow swimming copepod, found often drifting in the water column. In culture, this will be evenly distributed in the entire water column. *P. crassirostris* gets partially attracted to light but does not concentrate near the light source. This is not a predatory copepod and feed only by filter feeding. Adult life span is about 15-20 days. Females are seen more in culture and the sex ratio in culture is always above 1:10.

Eggs: Small spherical eggs measuring 55-65 µm in size with smooth external surface (Fig. 57a). Eggs are broadcasted in water and sink to bottom. Eggs alone can be easily harvested by siphoning the bottom sediment and filtering the same using a 40 µm mesh. Eggs hatch in 12-20 h in 25-28°C. Fecundity ranges from 25-50 eggs/day.

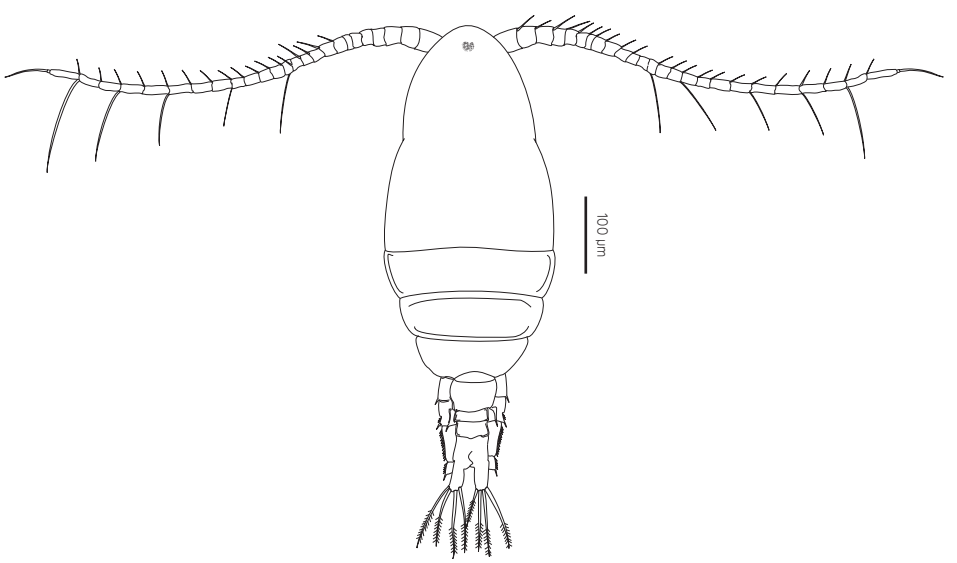


Fig. 55a. *P. crassirostris* Female

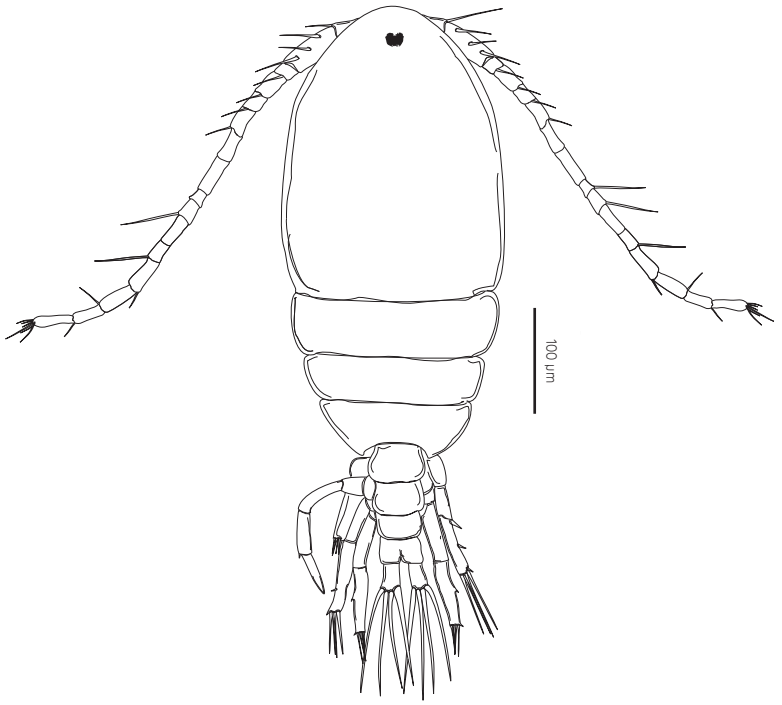


Fig. 55b. *P. crassirostris* Male

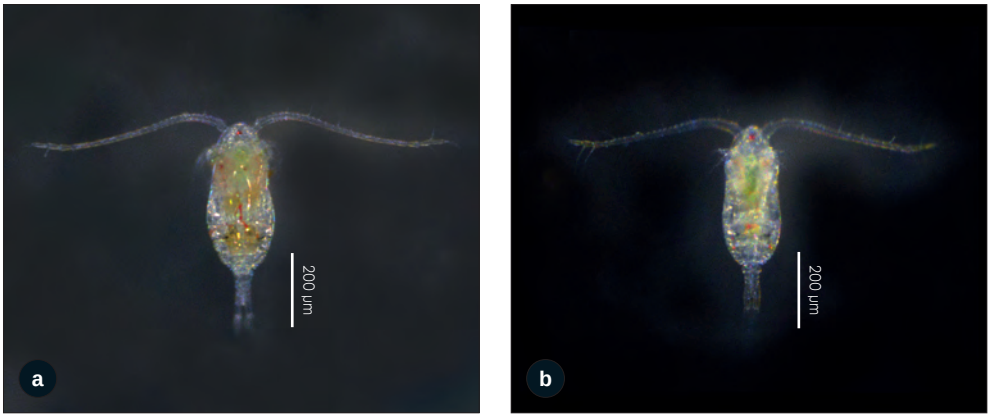


Fig. 56. *P. crassirostris* a. Female b. Male

Larval stages: There are 6 naupliar stages and 5 copepodite stages (Fig. 57b-l). The nauplii takes almost 9 days to reach adult stage (Fig. 57m&n). The first naupliar stage is of 55-65 µm in length and 35-45 µm in width. Naupliar length ranged from 55 µm to 160 µm in length and width from 35 µm to 65 µm.

Environmental conditions

P. crassirostris can tolerate wide range of temperatures (15-35°C) and the ideal temperature for culture is between 25-28°C. It can survive in salinities between 15-45 ppt but optimum salinity range for culture is from 30 to 35 ppt.

Normal diffused day light is preferred. Direct sunlight is not favourable. Normal tropical day length is ideal for culture. pH range between 8-8.5 is ideal. Ammonia level should be below 1 ppm. It can be cultured in 1 L containers to 5 t tanks. Depth of the tank can be upto 1 m.

Low aeration is preferred throughout the day and night. Treated seawater (chlorinated and dechlorinated) is ideal for culture. If water quality parameters deteriorate, replace the water.

Culture protocols

Food and feeding: This species is basically very selective and sensitive in their feeding. In mass culture, good production was obtained when fed with a combination of *Isochrysis galbana* and *Nannochloropsis salina* in the ratio 3:1. The ideal range of algal cell density for culture was 30000-40000 cells/mL. Algae should be contamination free and in the growing phase.

Density: Maximum density at a sustainable level of culture under normal conditions can be up to 4-5 nos/mL. Under normal conditions, stock culture of 20 L with density of 2000/L, if introduced to a 1000 L tank for mass culture, it can reach maximum density within 14-16 days. The same culture can be maintained for 2-3 months in the same containers with proper cleaning and regular harvest. Daily harvest is possible as eggs or nauplii or adults or as mixture of all stages (Table 4).

This species survives only for few days in a mixed culture but rarely it can dominate the other species in culture. Sustainable production can be obtained only through single species culture. It can be used as a feed along with other species in larval rearing tanks.

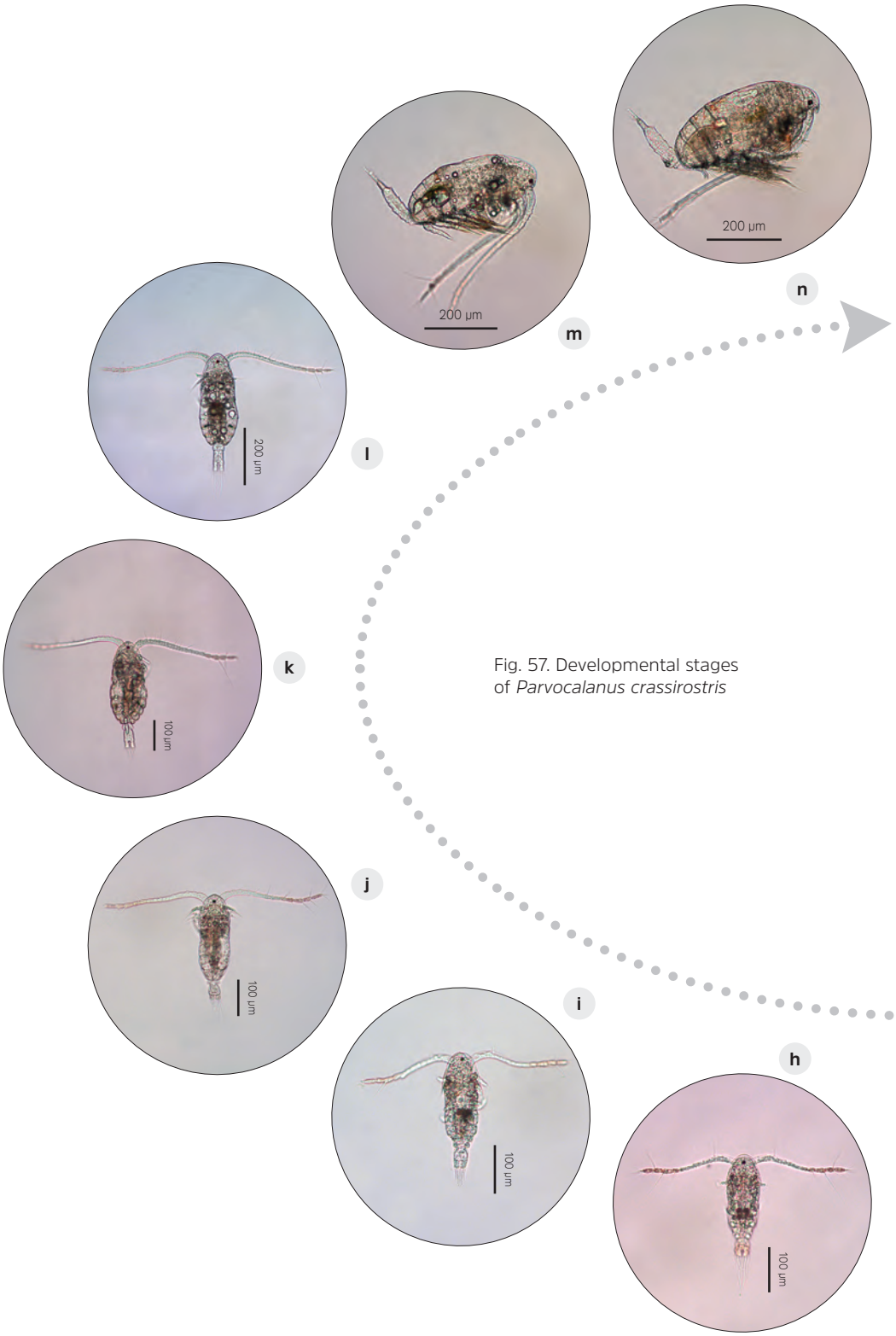
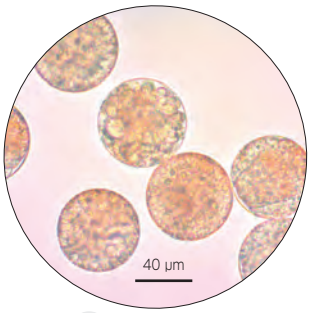


Fig. 57. Developmental stages of *Parvocalanus crassirostris*



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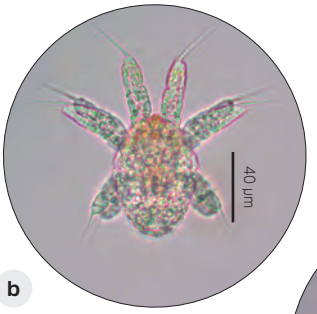

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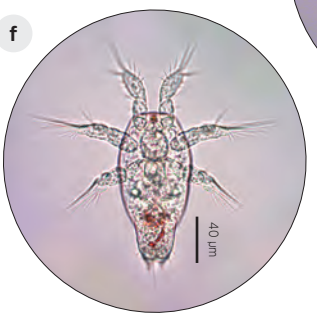

f

g

a. Eggs
b. Nauplius-1
c. Nauplius-2
d. Nauplius-3
e. Nauplius-4
f. Nauplius-5
g. Nauplius-6
h. Copepodite-1
i. Copepodite-2
j. Copepodite-3
k. Copepodite-4
l. Copepodite-5
m. Adult female
n. Adult male

Table. 4. Mesh size of sieves/bolting silk for filtering different stages of *Parvocalanus crassirostris*

Sl. No.	Stages required	Mesh size of sieves (µm)	Filtration pattern
1.	All stages including eggs	35	Single filtration
2.	Nauplii alone	70 and 35	Serial filtration. Filter through 70 µm to remove adults and copepodites and take residue from 35 µm for nauplii.
3.	Adult alone	170	Single filtration
4.	Eggs alone	60 and 35	Siphon out the bottom after settling and serially filter through 60 µm and 35 µm and residue in 35 µm for eggs.

Precautions: It is a sensitive species in culture. It needs to be fed only with good algae at required level and all parameters at optimum levels have to be maintained. Regular harvest is needed to regulate population level. All sorts of contamination should be regularly monitored. Over feeding and under feeding should be avoided.

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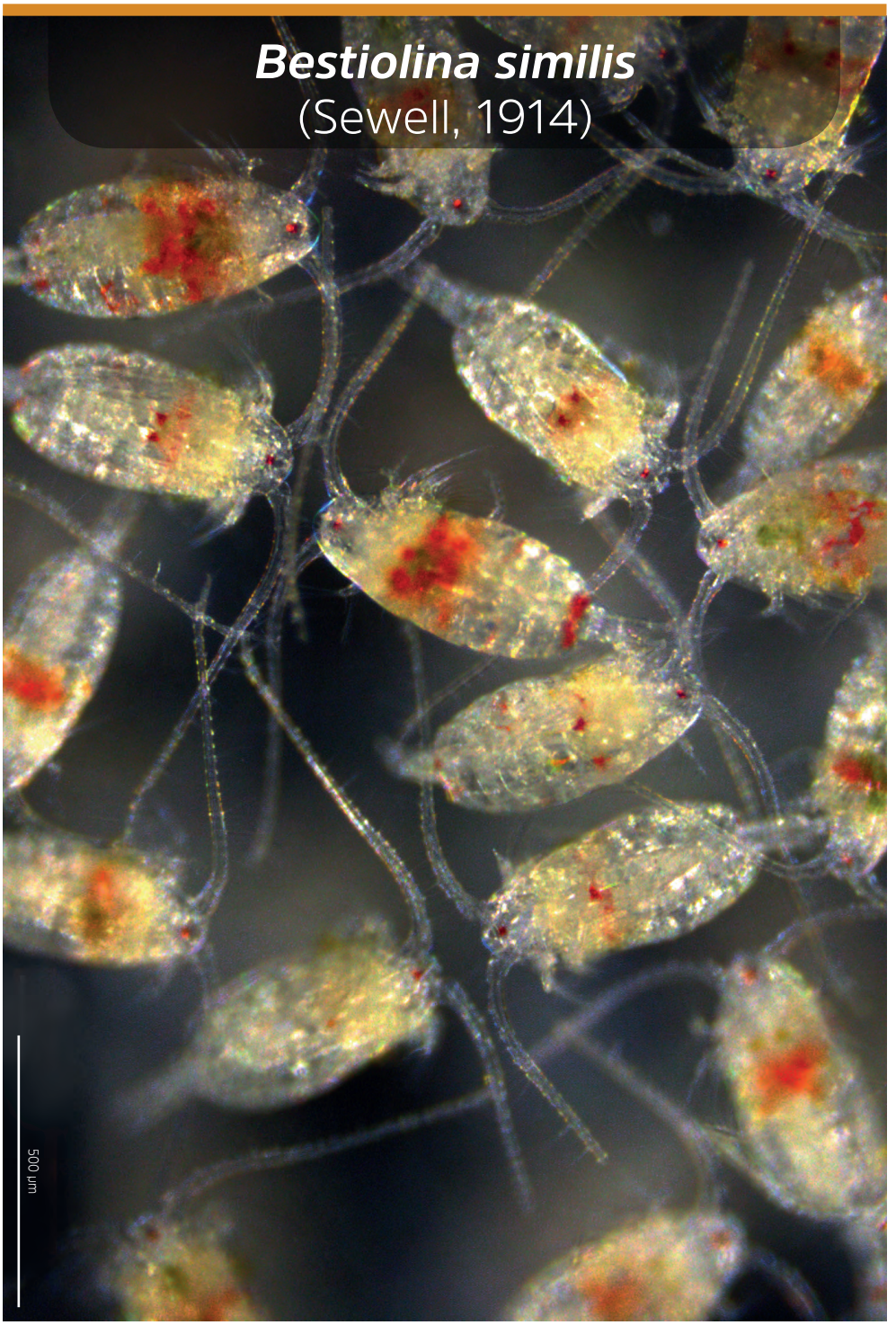
Bestiolina similis
(Sewell, 1914)
500 µm

Biological information and culture techniques of *Bestiolina similis* (Sewell, 1914)

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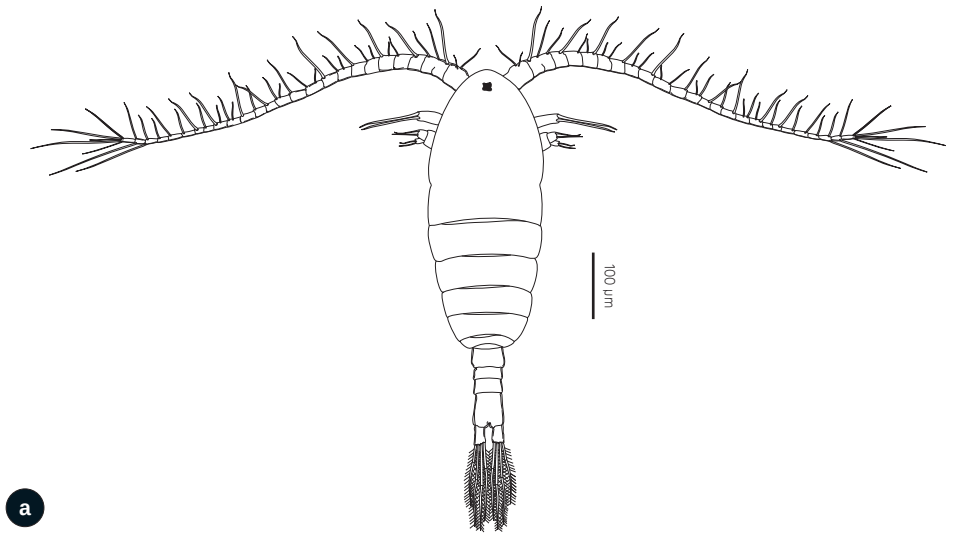
Basic information

Bestiolina Andronov, 1991 is a genus in the family Paracalanidae which share many features with its congeners *Paracalanus, Acrocalanus* and *Parvocalanus.* The family Paracalanidae is very important because most of the popular copepods used as live feed belong to this family (Stottrup, 2003, 2006; Razouls *et al.,* 2005-2017). *Bestiolina similis* (Sewell, 1914) is already popular as a live feed and is popularly used in larval rearing of many species of marine fin fishes. This species is mainly distributed in the tropical waters throughout the world. This is one of the common species in Indian waters. Originally this species was described as *Acrocalanus similis* and later this was transferred to *Bestiolina* Andronov, 1991. According to Moon *et al.,* (2010) the genus *Bestiolina* originated in the Indo-Malayan Region and radiated to all tropical seas during Pleistocene glacial period. At present there are eight valid species in the genus (Morales and Artigas, 2016). *B. similis* is more similar to *P. crassirostris.* Adult body size is slightly larger than *P. crassirostris.* There is strong similarity in size range of naupliar stages between these two species.

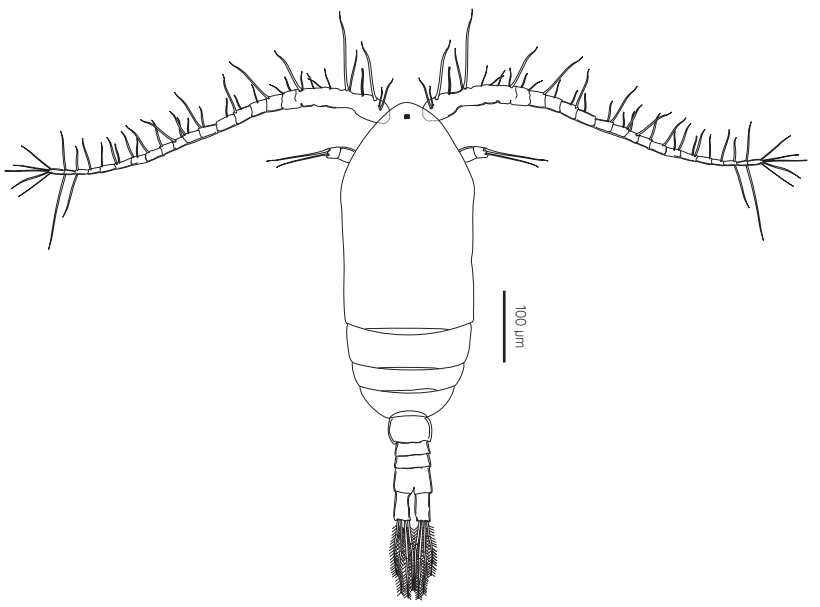
Biological information

Habitus: Adult size of males and females are similar and ranged between 650-750 µm (Fig. 58a&b, 59a&b). It is difficult to distinguish sexes. In females, 5th leg is reduced and knob like. In male, right P5 is reduced as in case of female and the left P5 is elongated and 5 segmented.

This species is a slow swimmer and gets evenly distributed in the culture tanks. It is not a predatory copepod and feed microalgae by filter feeding. This species is



a



b

Fig. 58. *B. similis* a. Female b. Male

attracted towards light. Adult life span is about 20-25 days. Occurrence of females is more in culture compared to males. Male and female sex ratio in culture is 1:7.

Eggs: Small spherical eggs measuring 60-70 µm in size with smooth external surface (Fig. 60a). Eggs are broadcasted in water and sink to the bottom. Eggs alone can be easily harvested by siphoning the bottom sediment and filtering the same using a 40 µm mesh. Eggs hatches in 15-20 h in 25-28°C and the hatching rate observed was 92%. Fecundity ranged from 30-50 eggs per day.

Larval stages: Life cycle is very similar to that of common calanoid copepods comprising of six naupliar stages and five copepodite stages (Fig. 60b-l). Shortest life cycle of captive reared *B. similis* was recorded as 8 days to reach adult (Fig. 60m&n) when fed with *Isochrysis galbana*. Naupliar stages lasted for five days and copepodite stages for three days. It is comparatively hardy and easy to rear in hatchery using live microalgae as feed. The first naupliar stage is of 70 µm in length and 50 µm in width. Naupliar stages length ranged from 70 µm to 182 µm and width ranged from 50 µm to 82 µm.

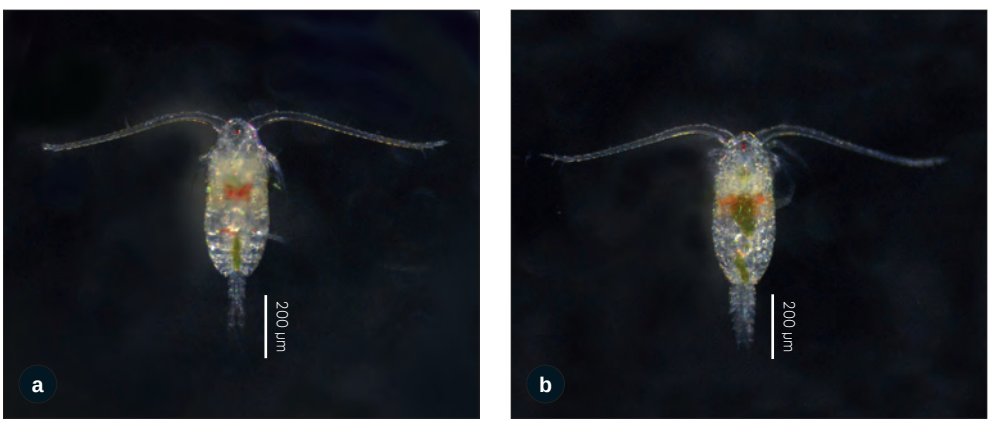


Fig. 59. *B. similis* a. Female b. Male

Environmental conditions

This species can tolerate wide range of temperatures (15-32°C) and the ideal range of temperature for culture is 25-28°C. It can survive in salinities between 20-40 ppt but optimum salinity range for culture is from 30-35 ppt. pH range 7.5-8.2 is ideal. Ammonia should be below 1 ppm. Low aeration is needed for the culture. Normal diffused day light is needed. Direct sunlight is not favourable. Normal tropical day length is ideal for culture.

Culture protocols

Food and feeding: This species prefer *Isochrysis galbana* and *Nannochloropsis salina/Chlorella marina* in the ratio of 3:1 with a cell density of 30000-40000 cells/mL in culture.

Density: Maximum density under normal conditions has been obtained as 4-5 nos/mL. Under normal culture conditions, stock of 20 L with a density of 2000 nos/L can reach maximum density within 18 days and can be maintained for 2-3 months in the same tanks with proper cleaning and regular harvest. It is ideal to culture this species alone. This species can be raised to mass culture in 1 L to 5 t tanks. Continuous aeration is also required. Clean the bottom portion by siphoning in 2 days interval. Daily harvest is possible as eggs or nauplii or adults or as mixture of all stages (Table 5).

Table 5. Mesh size of sieves/bolting silk for filtering different stages of *Bestiolina similis*

Sl. No.	Stages required	Mesh size of sieves (µm)	Filtration pattern
1.	All stages	45	Single filtration
2.	Nauplii alone	90 and 45	Serial filtration. Filter through 90 µm to remove adults and copepodites and take residue from 45 µm for nauplii
3.	Adult alone	225	Single filtration
4.	Eggs alone	80 and 45	Siphon out the bottom and allow to settle. Bottom sediment can be diluted in fresh seawater and serially filter through 80 µm and 45 µm and residue in 45 µm for eggs.

Precautions: This species is hardy, euryhaline and can be cultured in high densities. Regular harvest is essential to have a stable production. This species is hardy and can tolerate ciliate contamination to certain extent.

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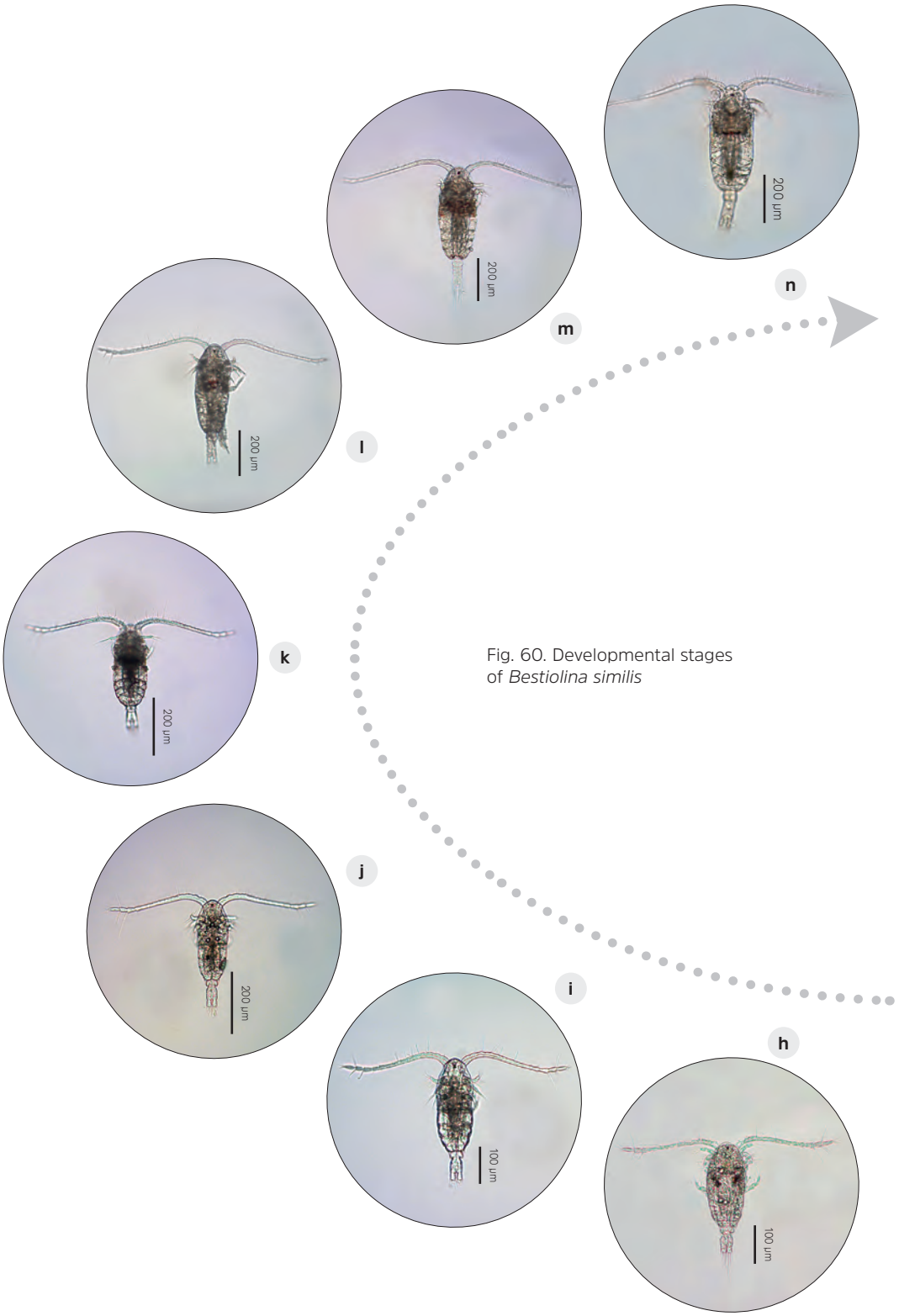
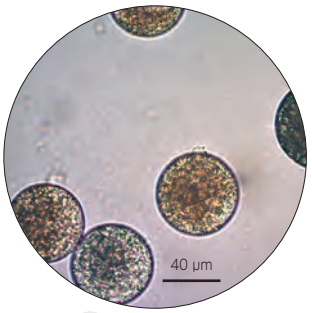


Fig. 60. Developmental stages of *Bestiolina similis*



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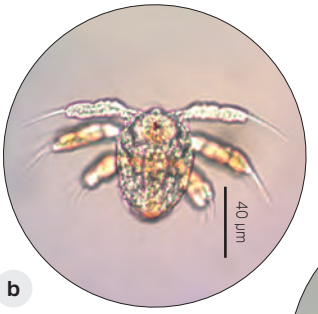

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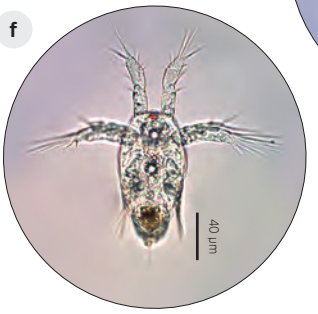

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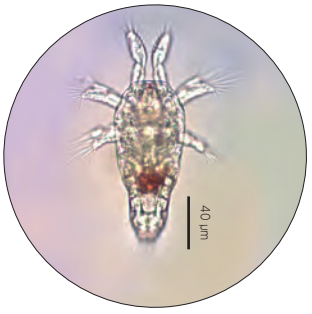

g

a. Eggs
b. Nauplius-1
c. Nauplius-2
d. Nauplius-3
e. Nauplius-4
f. Nauplius-5
g. Nauplius-6
h. Copepodite-1
i. Copepodite-2
j. Copepodite-3
k. Copepodite-4
l. Copepodite-5
m. Adult female
n. Adult male

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Apocyclops cmfri
Loka and Santhosh, 2017
500 µm

Biological information and culture techniques of *Apocyclops cmfri* Loka and Santhosh, 2017

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Basic information

Apocyclops Lindberg, 1942 is one of the most important genus among cyclopoid copepods which mainly inhabit in estuarine and coastal waters throughout the world (Chullasorn *et al.,* 2008; Hołynska *et al*., 2016; Pesce, 2016). The genus *Apocyclops* has previously been included as a subgenus of *Cyclops* and Lindberg (1940) described the two Indian species (*A. dengizicus* and *A. royi)* in the genus *Metacyclops*. At present, there are 11 valid and accepted species in this genus. Six valid species of *Apocyclops* has been reported from the Asian region, including three species from India. *Apocyclops cmfri* Loka and Santhosh (2017) was collected and identified from Karwar waters (Loka *et al.,* 2017) and it is closely related to the previously

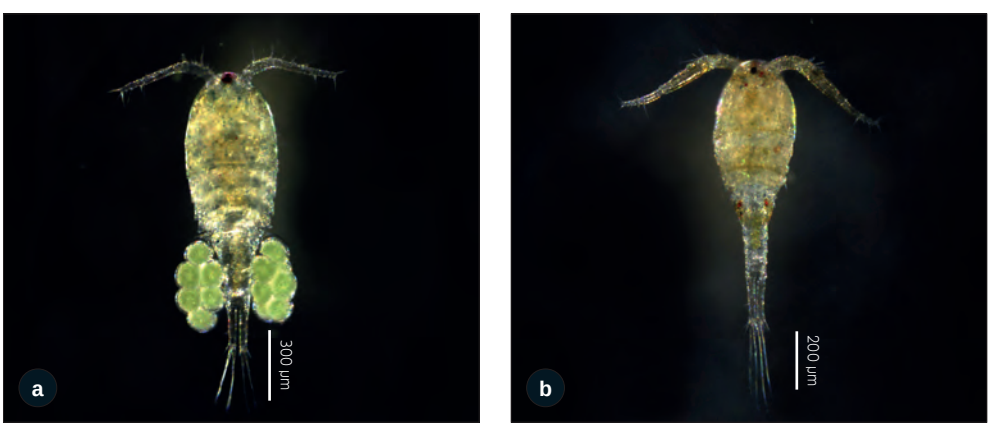
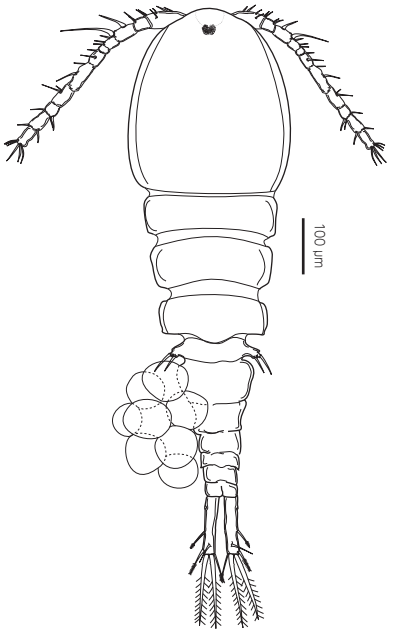
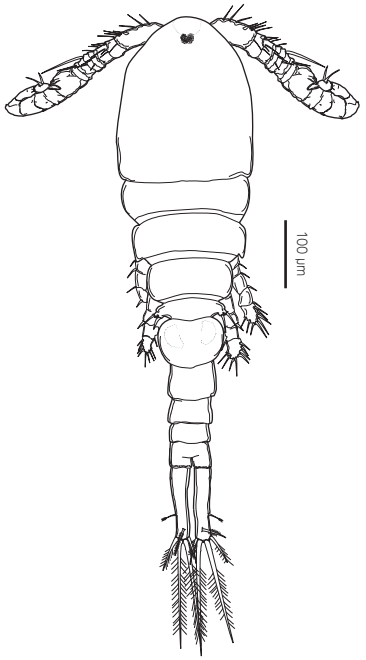


Fig. 61. *A. cmfri* a. Female b. Male



a



b

Fig. 62. *A. cmfri* a. Female b. Male (Figures from Loka *et al., 2017*)

reported Indian species, *A. royi* (Lindberg) and *A. dengizicus dengizicus* (Lepeshkin). *Apocyclops cmfri* can be distinguished from all its congeners in special features of first to fourth pleopods (P1-P4). *A. cmfri* has a characteristic inward projection of disto-medial part of basis and a single broad spinous expansion with a pointed tip in between exopod and endopod of P1-P4. Only one terminal spine is present in the second segment of endopod of P1. In P2 to P4, both exopod and endopod terminate in a spine and a seta of almost equal size. *Apocyclops* spp. are the most widely used cyclopoid copepod in larviculture. Trials on *A. cmfri* has also proved that it is a suitable candidate species for finfish and ornamental fish larval rearing (Loka *et al.,* 2017).

DNA extraction was carried out from the samples preserved in 95% ethanol following standard protocols and a 650 bp region of the Cytochrome C oxidase 1 gene was amplified and sequenced using the universal primers (Folmer *et al*., 1994; Samonte *et al*., 2000). The sequence was submitted to NCBI, GenBank with the accession number KX263726 (Loka *et al.,* 2017).

Biological information

Habitus: Size of the adult copepods ranged between 850-1260 µm (Fig. 61a&b, 62a&b). Females are slow swimming in nature and prefer the bottom portion of the culture tanks. Males are fast swimmers and move fast in the column. It is not a predatory copepod and feed only by filter feeding. Adult life span is about 15-20 days. Occurrence of females is more in culture compared to males. Male and female sex ratio in culture is 1:3.

Life cycle: Life cycle is very similar to that of common cyclopoid copepods comprising of six naupliar stages and five copepodite stages (Fig. 63b-l). Shortest life cycle of captive reared *A. cmfri* sp. nov. was recorded as 8 days when fed with *Chaetoceros calcitrans*. Small spherical eggs measures 70-80 µm in size (Fig. 63a). Eggs hatch within an hour at 26°C and the hatching rate observed was 90%. Fecundity has been estimated to be about 20-30 eggs per female per day when they are fed with *C. calcitrans*. Naupliar stages lasted for two days and the survival was 90%. Further development of copepodite stages into adults (male and female) (Fig. 63m&n) and brooders took four days and the survival rate was 95%. It is comparatively hardy and easy to rear in hatchery using live microalgae as feed. The first naupliar stage is of 105 µm in length and 90 µm in width in size. Naupliar length ranged from 105 µm to 287 µm and width 90 µm to 166 µm.

Environmental conditions

A. cmfri can tolerate wide range of temperatures (15-32°C) and the ideal temperature for culture is 30°C. It can survive in salinities between 0-40 ppt but optimum salinity

range for culture is from 29-35 ppt. pH range 7.2-8.2 is ideal. Ammonia should be below 1 ppm. Low aeration is needed for the culture of this copepod.

Culture protocols

Food and feeding: This species feed on almost all species of marine microalgae. Higher density and hatching rates were recorded when fed with *Chaetoceros calcitrans* with a cell density of 70000-80000 cells/mL in culture.

Table 6. Mesh size of sieves/bolting silk for filtering different stages of *Apocyclops cmfri*

Sl. No.	Stages required	Mesh size of sieves (µm)	Filtration pattern
1.	All stages	60	Single filtration
2.	Nauplii alone	170 and 60	Serial filtration. Filter through 170 µm to remove adults and copepodites and take residue from 60 µm for nauplii
3.	Adult alone	300	Single filtration

Density: Maximum density under normal conditions has been obtained as 2-3 nos/mL. Under normal culture conditions, stock of 20 L with a density 1000/L is needed for inoculation in 1000 L capacity mass culture tank. It can reach maximum density within 15-20 days and can be maintained for 2 months in the same tanks with proper cleaning and regular harvest. This species is compatible and can be cultured with other hardy species of copepods.

This species can be raised to mass culture in 1 L containers to 5 t tanks. Continuous aeration is also required. Clean the bottom portion by siphoning in 3 days interval. Daily harvest is possible from mass culture as nauplii, or adult or both (Table 6).

Precautions: This species is very hardy, tolerate wide range of salinities and it can be cultured in high densities when fed with *C. calcitrans*. After reaching maximum density, regular harvest is essential to have a stable production. This species can tolerate ciliate contamination than many other species.

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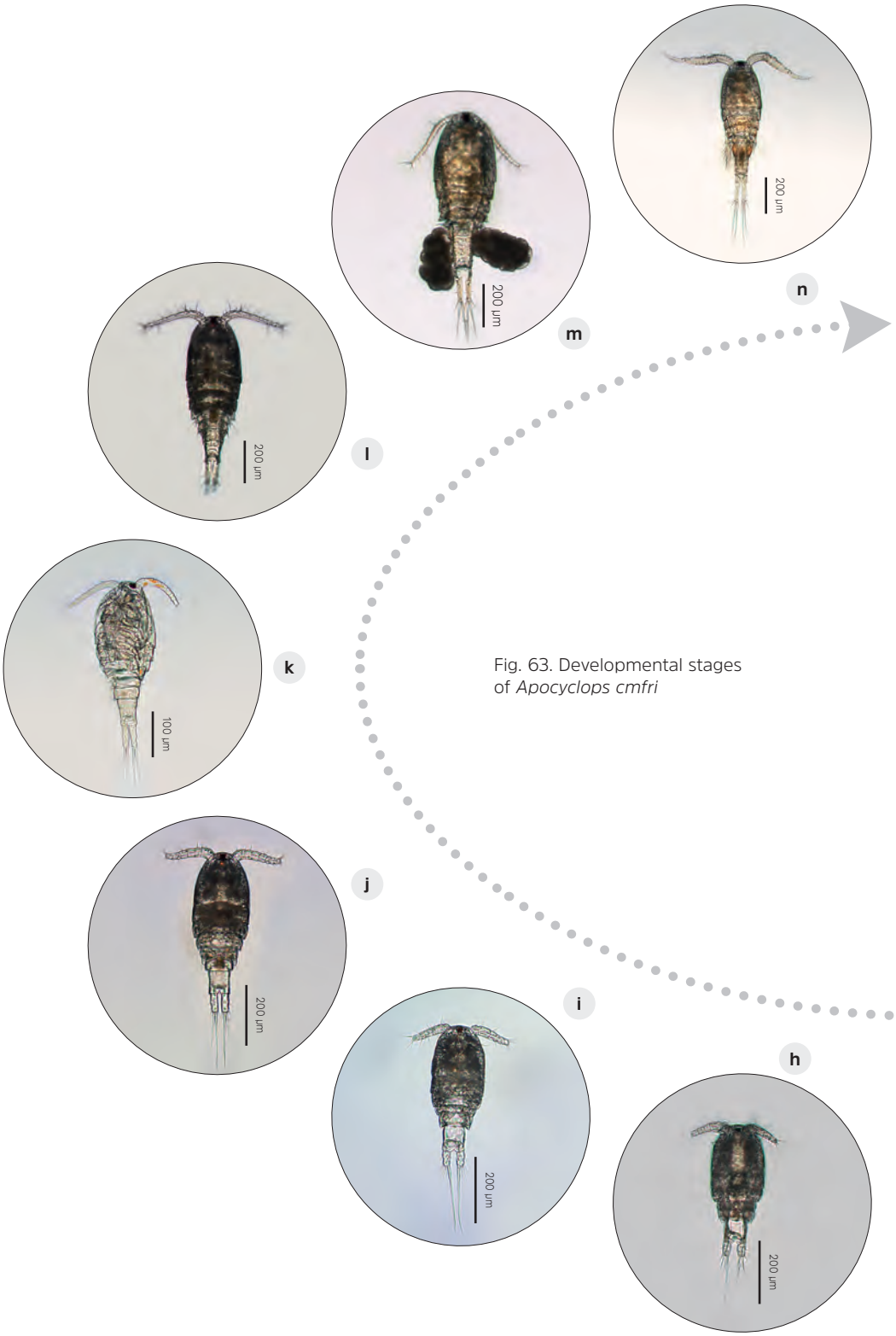
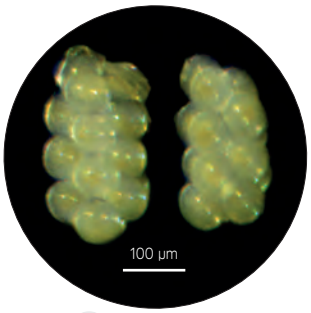
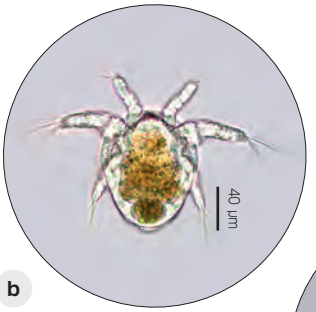


Fig. 63. Developmental stages of *Apocyclops cmfri*



a



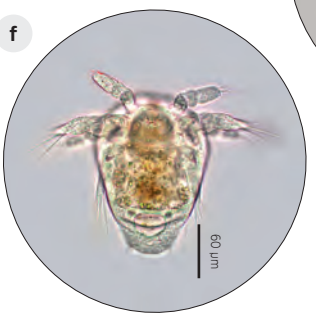
b

c

a. Egg sacs
b. Nauplius-1
c. Nauplius-2
d. Nauplius-3
e. Nauplius-4
f. Nauplius-5
g. Nauplius-6
h. Copepodite-1
i. Copepodite-2
j. Copepodite-3
k. Copepodite-4
l. Copepodite-5
m. Adult female
n. Adult male

d

e



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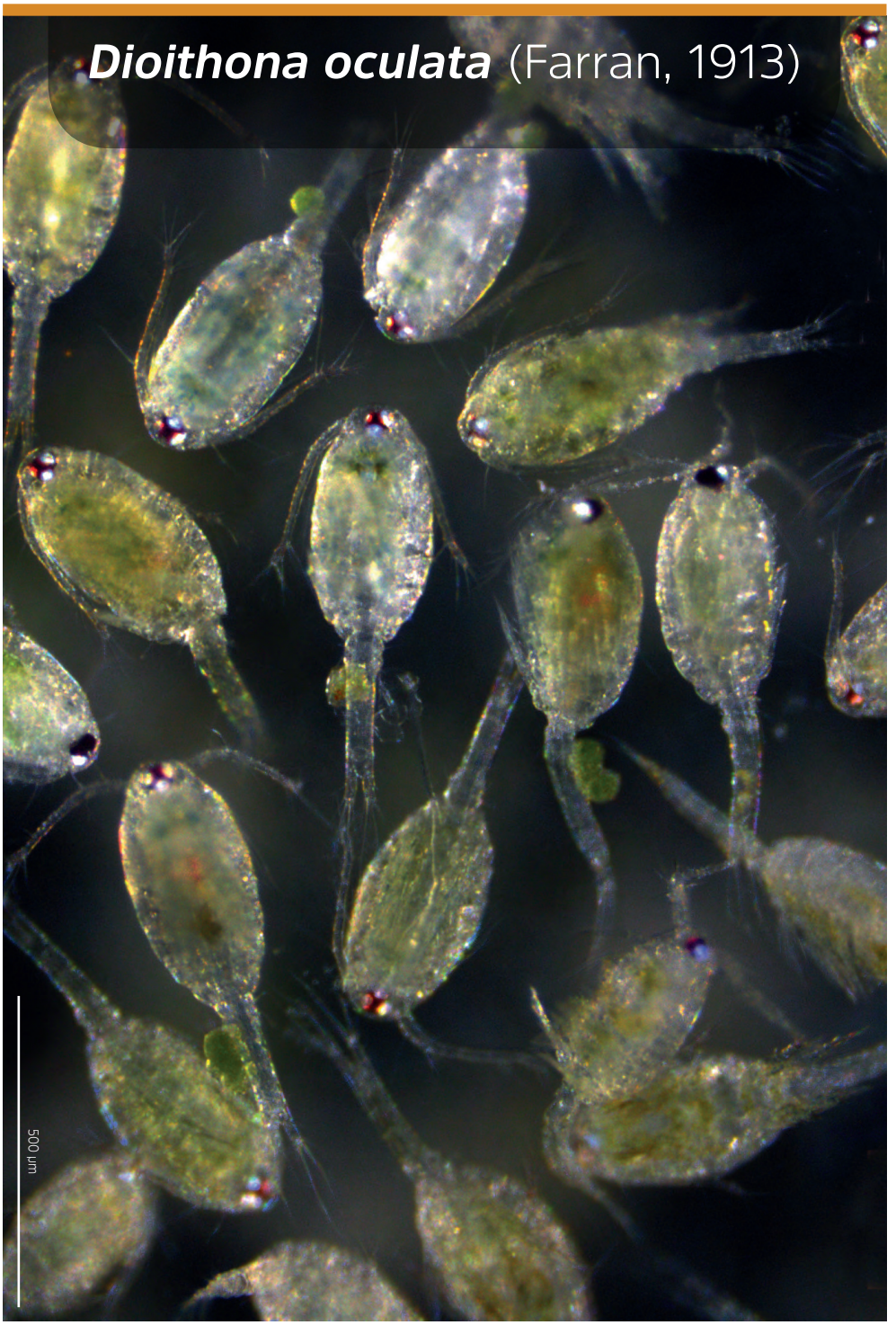
Dioithona oculata (Farran, 1913)
500 µm

Biological information and culture techniques of *Dioithona oculata* (Farran, 1913)

Darsana S., Muhammed Anzeer F., Aneesh K. S., Mijo V. Abraham, Santhosh B., Unnikrishnan C., Jose Kingsly H., Udayakumar, A. and Greever Yoyak

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Basic information

Dioithona oculata is a common copepod of wider distribution. It was originated from Indo-Pacific region. It is commonly reported from almost all parts of the world except Arctic and Antarctic regions (Razouls *et al.,* 2005-2017). It is a common species near mangrove swamps which are ideal breeding ground for many fishes and invertebrates. Due to its hardy nature and wider distribution, many reports are there about its distribution, ecology behaviour and development (Ambler *et al.,* 1991, 1996; Buskey, 1998, 2003). *D. oculata* can be easily distinguished from all other copepods by its large and prominent eye spot in its cephalothorax. No reports are available on the mass production and utilization of this species for larval rearing. Hernandez Molejona and Alvarez-Lajonchere (2003) has conducted few trials in indoor and outdoor production of this species and concluded that

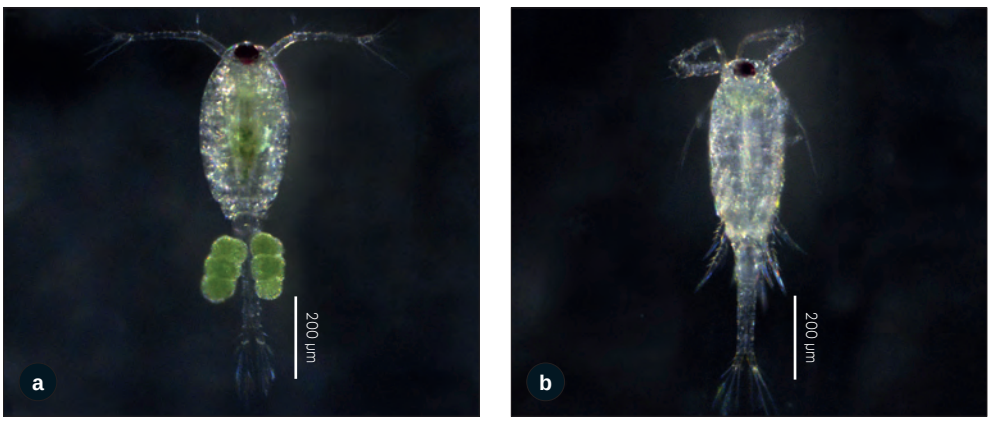
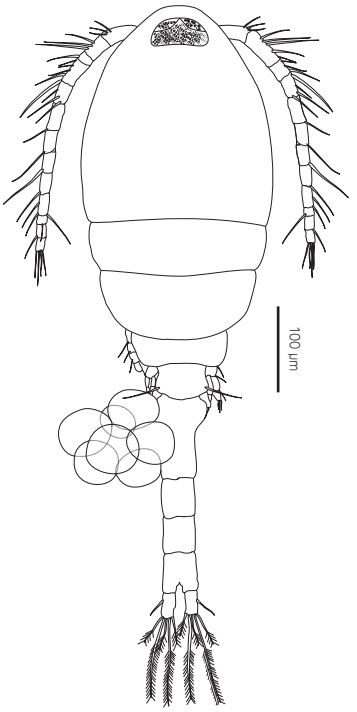
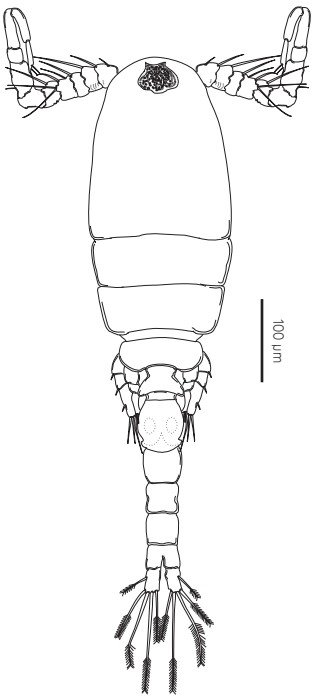


Fig. 64. *D. oculata* a. Female b. Male



a



b

Fig. 65. *D. oculata* a. Female b. Male

this is an excellent species for utilization as larval feed. This species has been frequently reported from almost all coastal water bodies in India.

It is hardy like other cyclopoid copepods and it is easy to maintain a stable culture. It can be cultured in large containers like calanoid copepods. The adults and larvae are pelagic in nature.

Biological information

Habitus: Adult size ranged from 640 µm to 720 µm in females and from 550 µm to 650 µm in males (Fig. 64a&b, 65a&b). It is easy to differentiate male and female. In males, both the antennules are geniculate. Females often carry two egg sacs. Mating or copulating position is also common in culture. These are partially attracted to light but do not concentrate near the light source.

D. oculata is bright greenish yellow in colour and have pigmentation in egg sacs also. This is an active species and is seen evenly distributed in the entire water column of culture tanks. *D. oculata* take 10-12 days to complete the larval cycle.

It is not a predatory copepod and feed only by filter feeding but it can also consume ciliates and similar smaller protozoans. Adult life span is about 20-25 days. Females are more in culture and the sex ratio in culture is 1:6. This species have large bright red eyespots and is easy to locate in culture with naked eyes. This species is very ideal for culture in higher density.

Eggs: Small spherical eggs measuring 65-70 µm in size enclosed in egg sac (Fig. 66a). Number of eggs per egg sac ranges from 1 to 15. Nauplii are released directly from egg sacs into water.

Larval stages: There are 6 naupliar stages and 5 copepodite stages (Fig. 66b-l). The naupliar stages ranged from 90 µm to 180 µm in length and width from 80 µm to 110 µm. It takes almost 10-12 days to reach adult stage (Fig. 66m&n). The first naupliar stage is of 90 µm in length and 60 µm in width.

Environmental conditions

D. oculata can tolerate wide range of temperature (10-35°C) and the ideal temperature for culture is 25-28°C. It can survive in salinities between 20-40 ppt but optimum salinity range for culture is 30-35 ppt.

Normal diffused day light is needed for better growth. Direct sunlight is not advantageous. Normal tropical day length is ideal for culture. pH range between

8-8.5 is ideal. Ammonia level should be below 1 ppm. It can be cultured in 1 L containers to 5 t tanks. Water depth of the tank can go upto 1 m.

Low aeration is required. Treated seawater (chlorinated and dechlorinated) is ideal for culture. If water quality parameters deteriorate, change the water using appropriate sieves.

Culture protocols

Food and feeding: This species basically feed on a variety of algae. A combination of *Isochrysis galbana, Nannochloropsis salina* and *Chlorella marina* in the ratio 1:1:1 was found ideal. The algal cell density for culture is 20000- 30000 cells/mL. Algae should be pure and in the growing phase.

Density: Maximum density at a sustainable level of culture under standard conditions can be upto 2-4 nos/mL. Under normal conditions, culture can reach maximum density within 18-20 days and can be continued for 2-3 months in the same containers with proper cleaning and systematic harvest. Regular harvest is possible as nauplii or adults or as a mixture of all stages (Table 7).

Single species culture is ideal. This species is comparatively hardy and survive in a mixed culture and can also dominate over many other species in culture. It can be used as a feed along with other species in larval rearing tanks.

Table 7. Mesh size of sieves/bolting silk for filtering different stages of *Dioithona oculata*

Sl. No.	Stages required	Mesh size of sieves (µm)	Filtration pattern
1.	All stages	50	Single filtration
2.	Nauplii alone	100 and 50	Serial filtration. Filter through 100 µm to remove adults and copepodites and take residue from 50 µm for nauplii
3.	Adult alone	225	Single filtration

Precautions: It is a hardy species for stock and mass culture. It is to be fed only with good algae at a required level and all parameters need to be retained at optimum levels. Regular harvest is needed to regulate and maintain the population level. If any contamination is seen, restart the culture after isolating the adults.

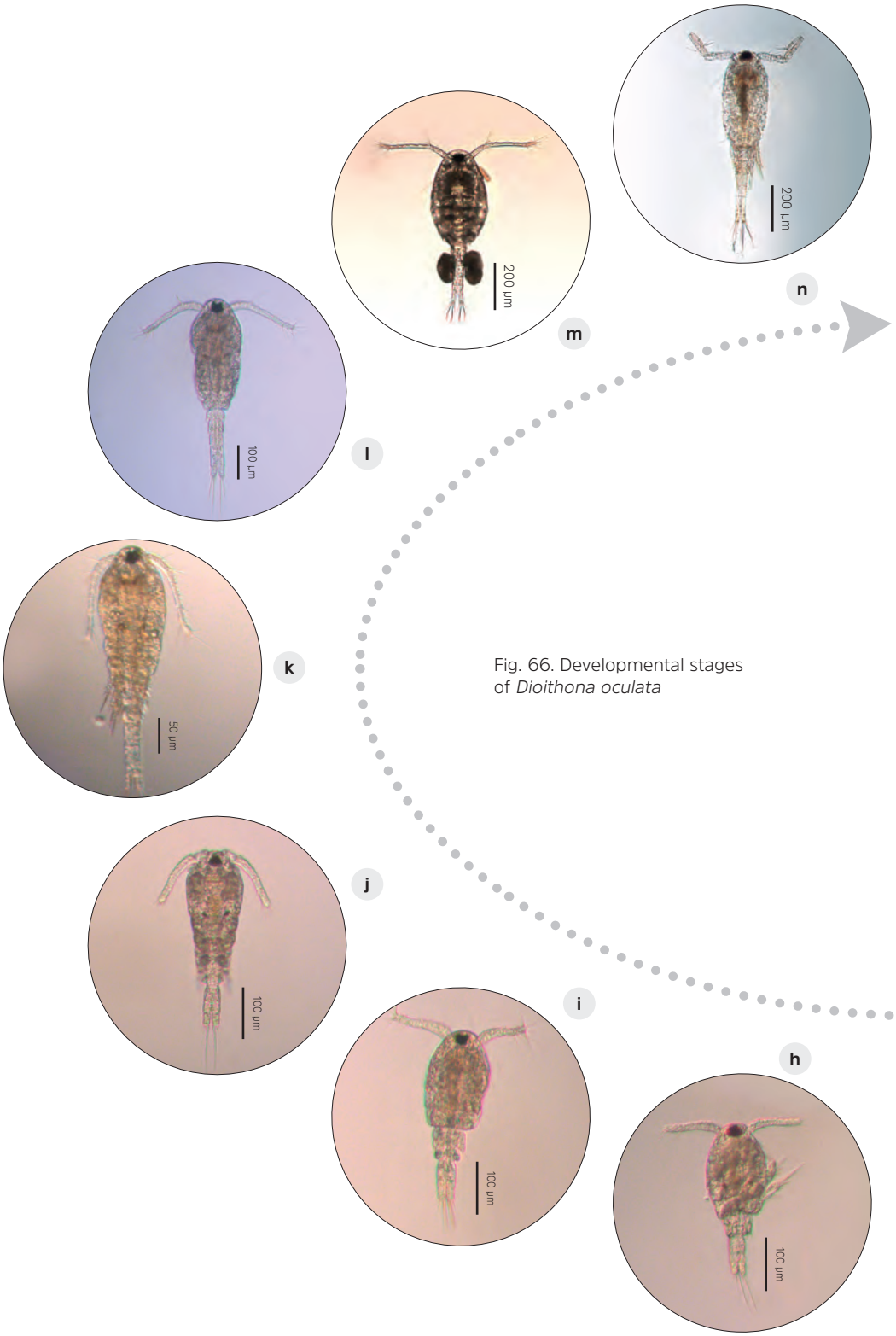
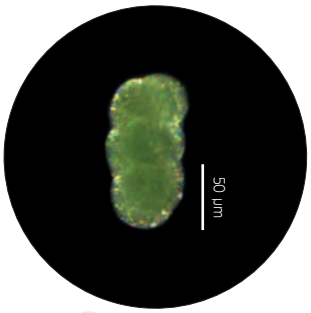


Fig. 66. Developmental stages of *Dioithona oculata*



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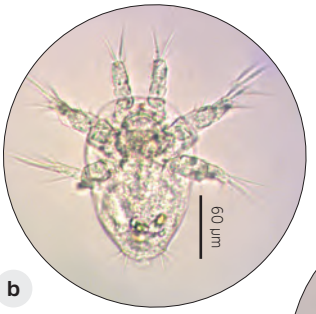

b

c

d

e

f

g

a. Egg sac
b. Nauplius-1
c. Nauplius-2
d. Nauplius-3
e. Nauplius-4
f. Nauplius-5
g. Nauplius-6
h. Copepodite-1
i. Copepodite-2
j. Copepodite-3
k. Copepodite-4
l. Copepodite-5
m. Adult female
n. Adult male

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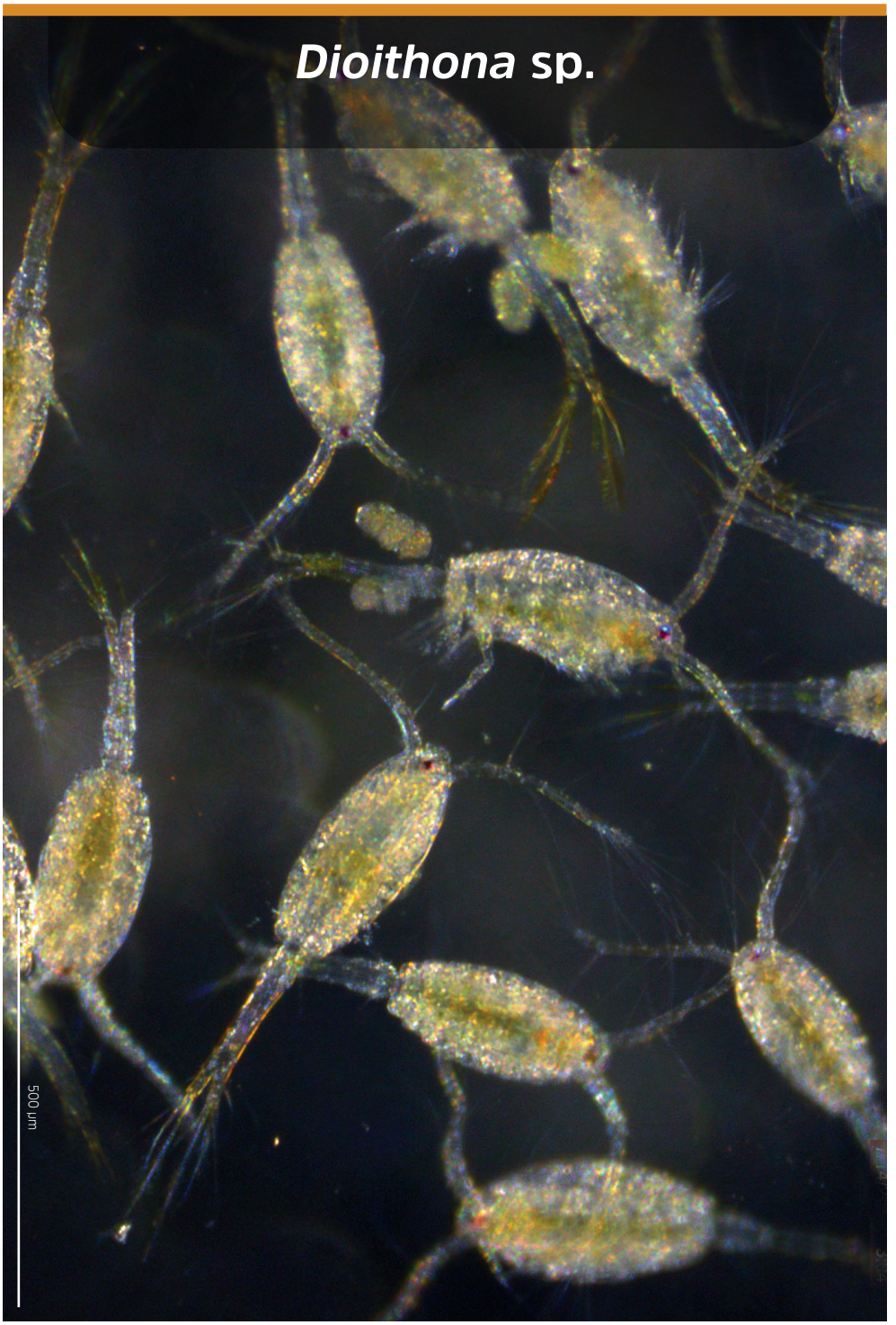
Dioithona sp.
500 µm

Biological information and culture techniques of *Dioithona* sp.

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Basic information

Copepods of the closely related genera *Oithona* and *Dioithona* are widely distributed in marine and estuarine habitats of tropical and subtropical regions (Razouls *et al.,* 2005-2017). Members of the family Oithonidae are hardy and can tolerate wide range of salinity and temperature. Due to their hardy nature, many reports are available describing several species as invasive. Many species are popular as live feeds and are widely used in hatcheries in many parts of the world (Stottrup and Norsker, 1997; Stottrup, 2003, 2006; 2005; Santhanam *et al*., 2015; Santhosh *et al.,* 2016). The genera *Oithona* and *Dioithona* are closely related and it is very difficult to distinguish morphologically. Even after molecular analyses, taxonomic ambiguity still exists in this group of copepods (Razouls *et al.,* 2005-2017).

Dioithona sp. isolated and cultured Vizhinjam Research Centre of CMFRI is a prolific species with very small eggs and larvae. Nauplii retain its small size and

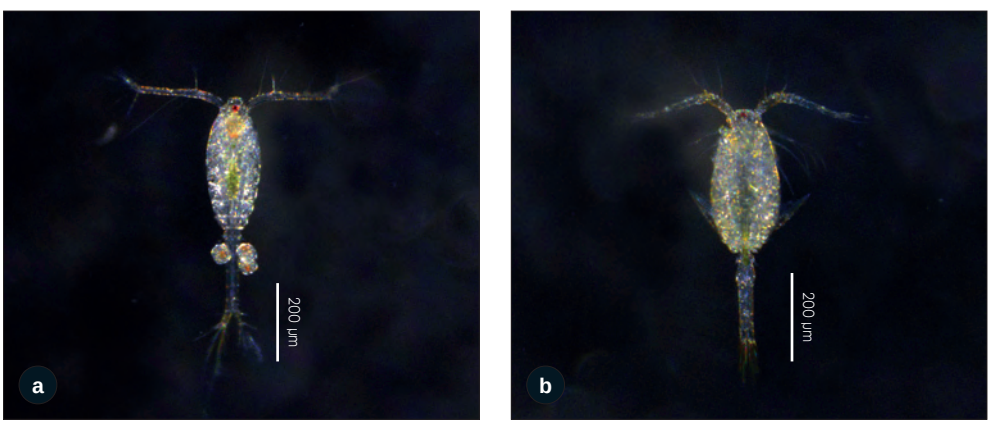
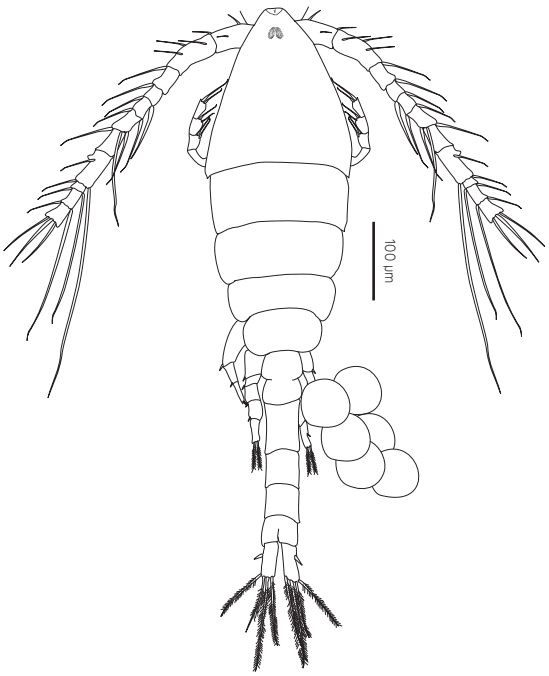
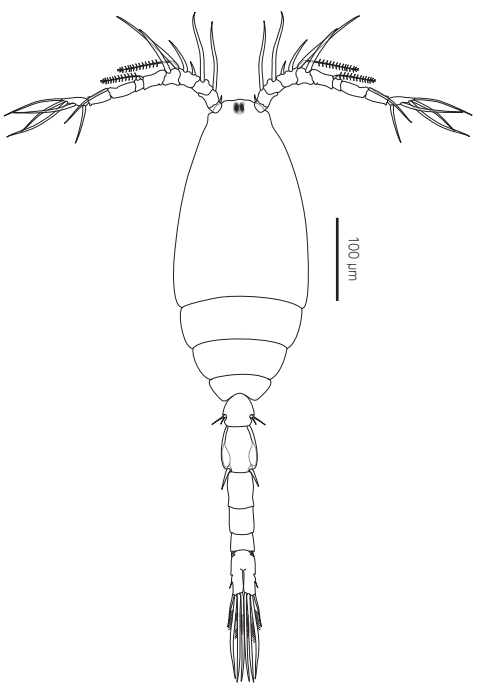


Fig. 67. *Dioithona* sp. a. Female b. Male



a



b

Fig. 68. *Dioithona* sp. a. Female b. Male

remain as it is even after 5-8 days in culture. This character of the species made it popular in CMFRI hatcheries for feeding altricial larvae. Nauplii are hardy and thrive well in larval rearing tanks without much change in their size and hence they are available to fish larvae for a longer period when compared to all other copepods discussed here. This species is ideal for larval rearing of fishes with very small larval stages like groupers, snappers and damsels.

Biological information

Habitus: Adult size ranged from 650 µm to 700 µm for females and from 540 µm to 600 µm in males (Fig. 67a&b, 68a&b). It is difficult to distinguish male and female. The main difference has been in the shape and segmentation of the antennule.

This is a slow swimming species and is seen evenly distributed in the entire water column of the culture tanks. *Dioithona* sp. is easily attracted to light but does not concentrate on surface of the culture. This is not a predatory copepod and feed only by filter feeding. Adult life span is about 25-30 days. Females dominate in culture and the sex ratio in culture is 1:10.

Eggs: Small spherical eggs measuring 50-60 µm in size with smooth external surface and enclosed in a pair of egg sacs (Fig. 69a). Number of eggs per egg sac ranges from 1-10. Eggs are released in water and hatch within 1 h. Fecundity ranged from 1-10 eggs per day.

Larval stages: There are 6 naupliar stages and 5 copepodite stages (Fig. 69b-l). The nauplii takes almost 5-8 days to reach copepodite stage and again takes 7 more days to reach adult stage (Fig. 69m&n). The first naupliar stage is of 65-75 µm in length and 40-50 µm in width. Even in the 6th naupliar stage, it will reach a width of around 80 µm only.

Environmental conditions

Dioithona sp. can tolerate wide range of temperature (15-35°C) and the ideal temperature for culture is 25-28°C. It can survive in salinities between 15-45 ppt but optimum salinity range for culture is between 30-35 ppt.

Normal diffused day light is needed. Direct sunlight is not ideal. Normal tropical day length is suitable for culture. pH range between 8-8.5 is considered ideal. Ammonia should be below 1 ppm. It can be cultured in 1 L containers to 5 t capacity tanks. Depth of the tank can be up to 1 m.

Treated seawater (chlorinated and dechlorinated) is ideal for stable mass culture. If water quality parameters deteriorate, replace the water with treated seawater using appropriate sieves. Low aeration is needed.

Culture protocols

Food and feeding: This species basically feed on a variety of algae. But better production can be achieved using a combination of *Isochrysis galbana, Nannochloropsis salina* and *Chlorella marina* in the ratio 1:2:1. The ideal range of cell density for culture is between 30000-40000 cells/mL. Algae should be contamination free and in the growing phase.

Density: Maximum density at a sustainable level of culture under normal conditions can be up to 4-5 nos/mL. Under normal conditions, 20 L stock culture with 2000 nos/L density can reach maximum density in 1000 L tanks within 25-30 days. The culture can be maintained for 2-3 months in the same containers with proper cleaning and regular harvest. Daily harvest is possible as nauplii or adults or as a mixture of all stages (Table 8).

This species survives only for few days in a mixed culture but never dominate over the other species in culture. So single species culture only possible for this species. It can be used as a feed along with other species in larval rearing tanks.

Table 8. Mesh size of sieves/bolting silk for filtering different stages of *Dioithona* sp.

Sl. No.	Stages required	Mesh size of sieves (µm)	Filtration pattern
1.	All stages including nauplii	35	Single filtration
2.	Nauplii alone	90 and 35	Serial filtration. Filter through 90 µm to remove adults and copepodites and take residue from 35 µm for nauplii
3.	Adult alone	170	Single filtration

Precautions: It is a sensitive species in culture and needs to be fed only with good algae at required levels. All parameters at optimum level have to be maintained. Regular harvest is needed to maintain the population level. Only monoculture is possible and care should be taken to avoid any sort of contamination in the culture. Over feeding and under feeding should be avoided.

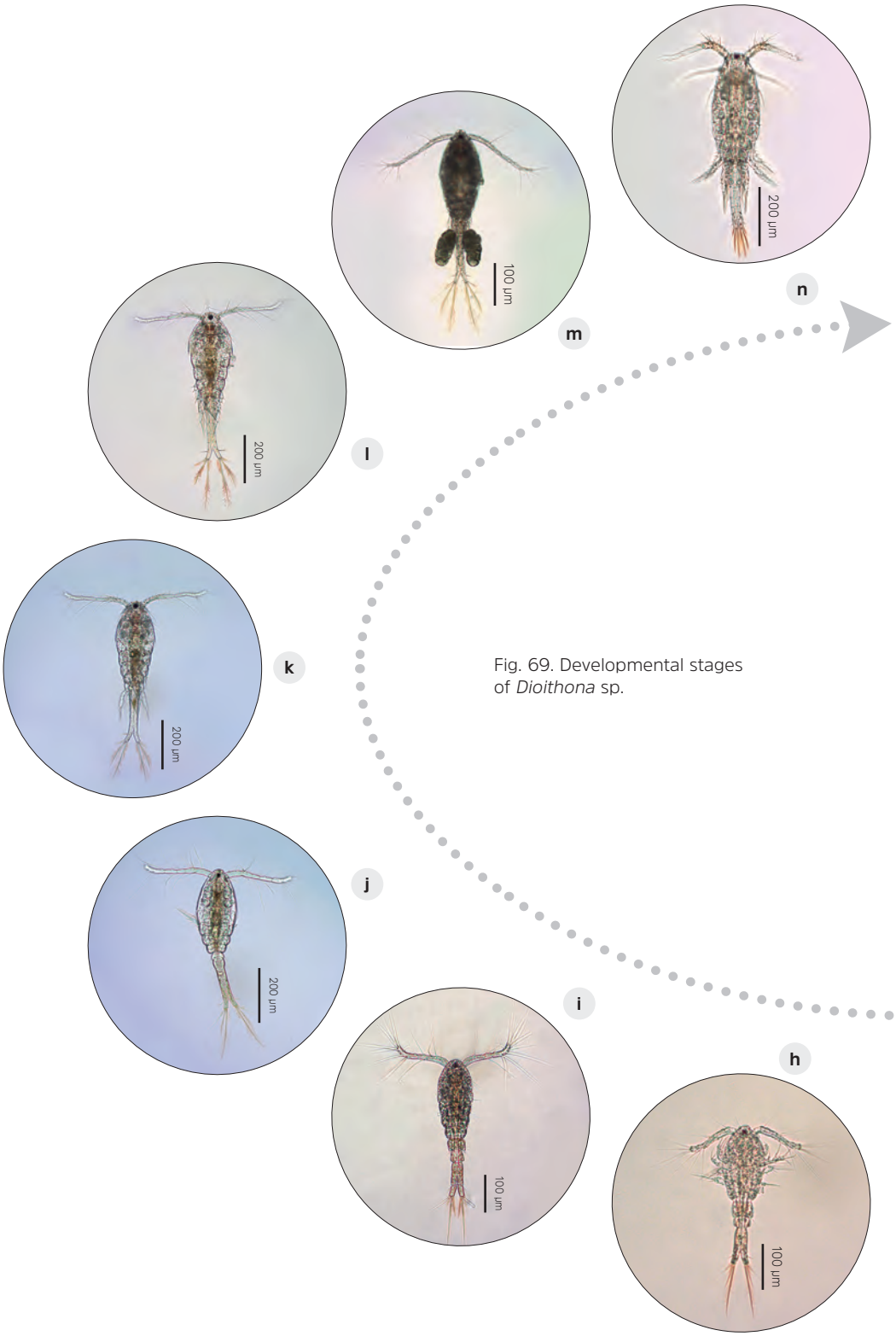
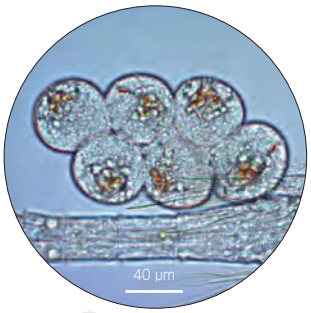
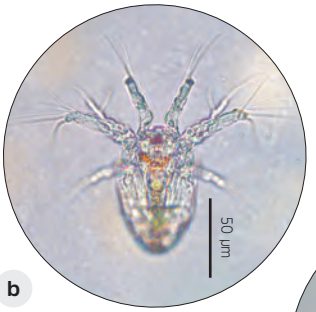


Fig. 69. Developmental stages of *Dioithona* sp.



a



b

c

a. Egg sac
b. Nauplius-1
c. Nauplius-2
d. Nauplius-3
e. Nauplius-4
f. Nauplius-5
g. Nauplius-6
h. Copepodite-1
i. Copepodite-2
j. Copepodite-3
k. Copepodite-4
l. Copepodite-5
m. Adult female
n. Adult male

d

e

f

g

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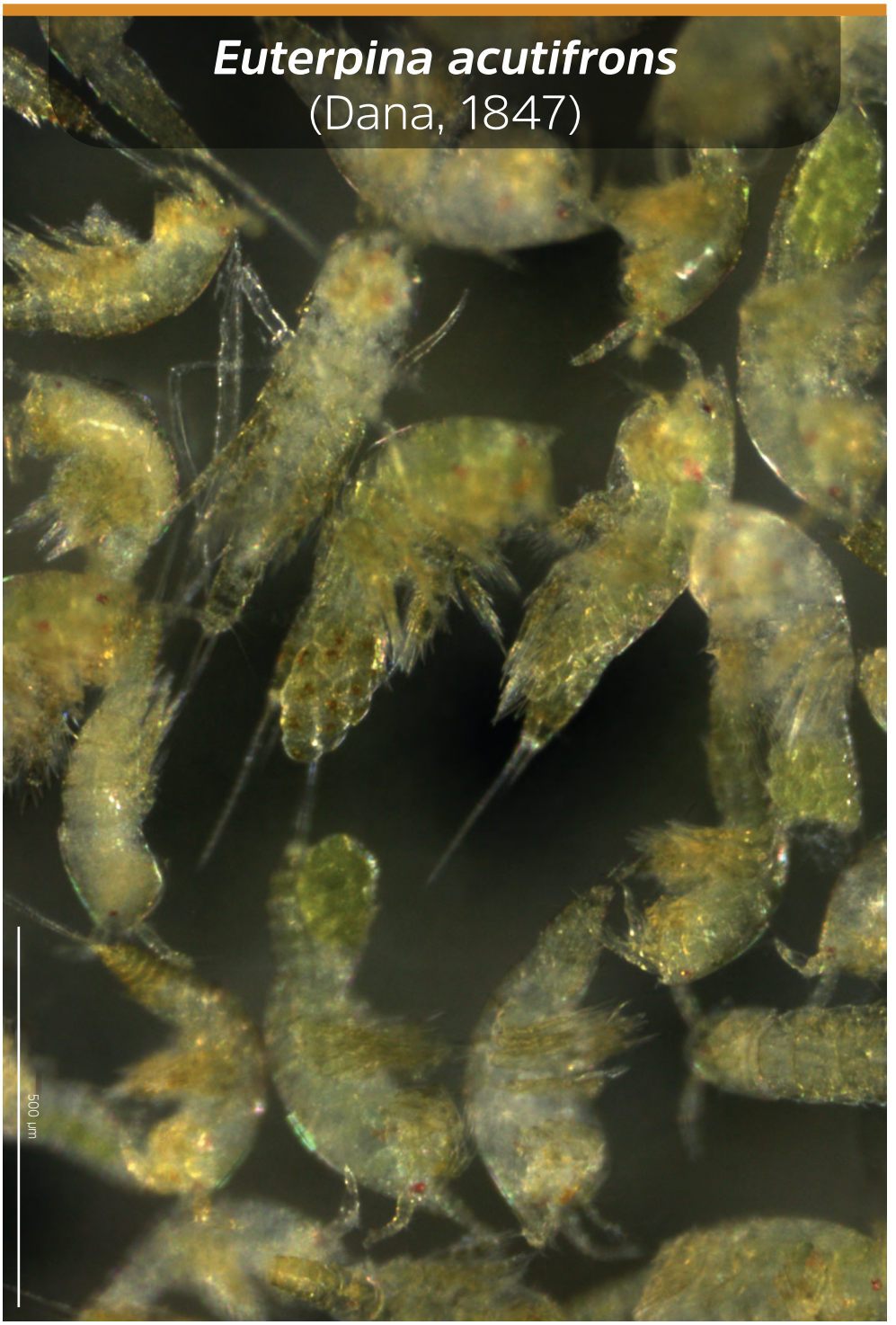

Euterpina acutifrons
(Dana, 1847)

Biological information and culture techniques of *Euterpina acutifrons* (Dana, 1847)

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Basic information

Euterpina acutifrons is one of the common harpacticoid copepod frequently reported from almost all parts of the world except Arctic and Antarctic regions. Due to their hardy nature and wider distribution, there are many reports of culture of this species. *E. acutifrons* can be easily distinguished from all other copepods due to the presence of a spine like projection on the anterior most end of cephalothorax. In India, culture of *E. acutifrons* was started at CMFRI Vizhinjam Centre. Several reports are there on the successful utilization of this species in larval rearing alone or in combination with *Pseudodiaptomus serricaudatus* (Gopakumar and Santhosi, 2009; Gopakumar *et al.,* 2009a&b). Jasmine *et al.,* (2016), had reared this species using artificial feed under laboratory conditions. This is a hardy species which can grow well even in smaller containers.

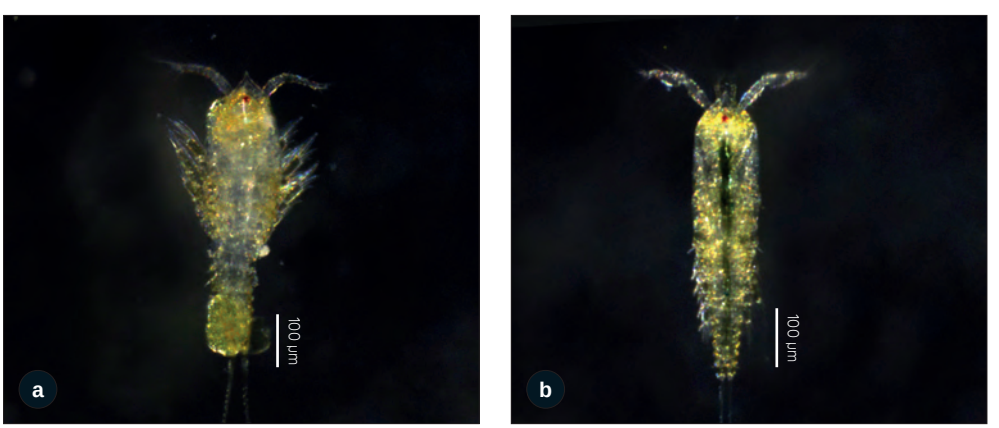


Fig. 70. *E. acutifrons* a. Female b. Male

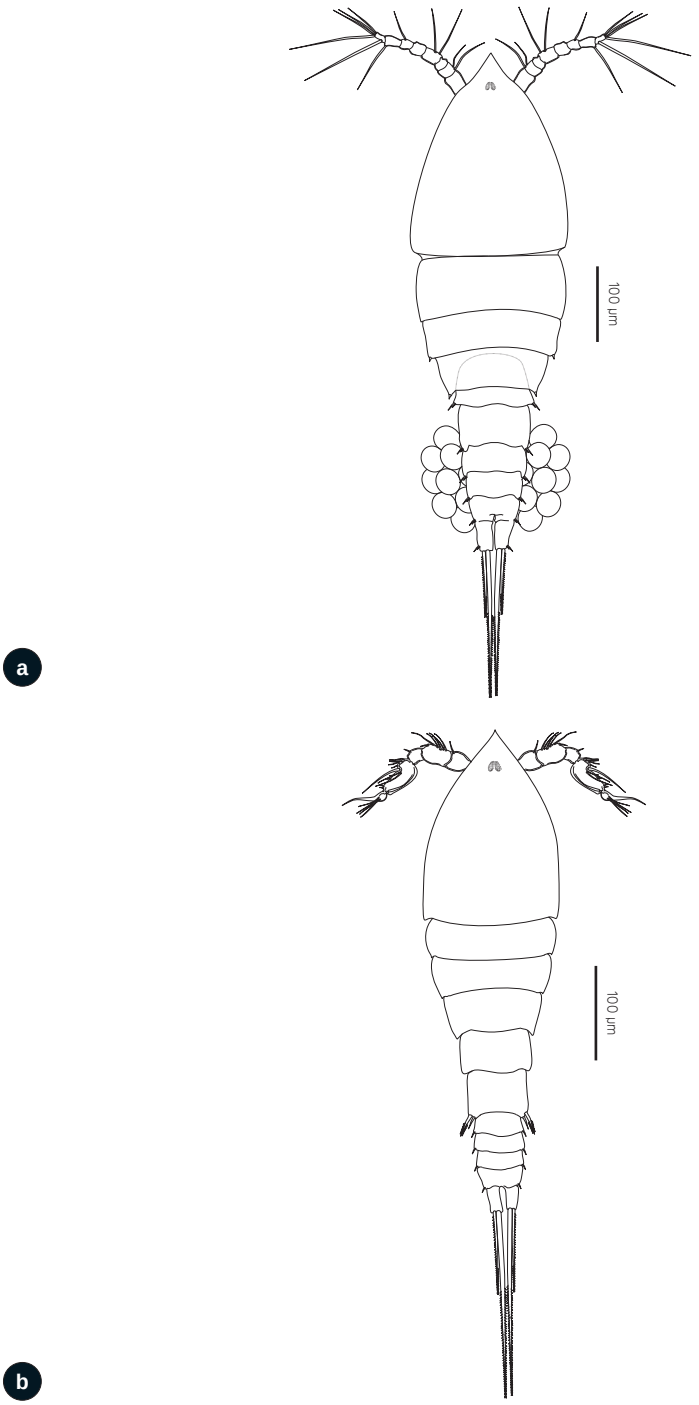


Fig. 71. *E. acutifrons* a. Female b. Male

Like many other harpacticoid copepods it can be maintained as a very stable culture. It is suitable for culture in large containers also. The adults and larvae are pelagic in nature. This is the only valid species in the genus *Euterpina*. The validity of the species *E. gracilis* is still uncertain. Many reports are available on culture of *Euterpina acutifrons* from different parts of the world.

Biological information

Habitus: Adult size ranged from 550 µm to 680 µm in females and from 500 µm to 580 µm in males (Fig. 70a&b, 71a&b). It is easy to distinguish male and female. In males both antennule are geniculate. Females often carry a single egg sac under abdomen. Mating or copulating position is very common in culture. It is a slow moving species and distribute somewhat evenly in the tank. These are partially attracted to light but do not concentrate near the light source.

This is a slow swimming species and is seen evenly distributed in the entire water column of the culture tanks. *E. acutifrons* take 15-16 days to complete its larval cycle.

It is not a predatory copepod and feed mostly by filter feeding. Adult life span is about 20-25 days. Females are more in culture and the sex ratio in culture is 1:4.

This species is light yellowish-green in colour and it is not easy to locate in culture. This species distribute evenly in the entire water column and hence it is very ideal to culture in higher density. It is not a predatory species but found to consume ciliates and similar smaller protozoans.

Eggs: Small spherical eggs measuring 55-65 µm in size (Fig. 72a), enclosed in a single egg sac. Number of eggs per egg sac ranges from 6 to 18. Eggs take 2-3 days to hatch in 25-28°C. Within18-28 h another egg sac will be produced. Egg hatch in the pouch and nauplii are released into water. In case of any stress, including stress due to filtration and handling, females leave the entire egg sac and escape. If the eggs are mature, it may hatch out from the abandoned egg sac also.

Larval stages: There are 6 naupliar stages and 5 copepodite stages (Fig. 72b-l). The naupliar stages ranges from 76 µm to 180 µm in length and from 60 µm to 116 µm in width. It takes almost 12-14 days to reach adult stage (Fig. 72m&n). The first naupliar stage is of 76 µm in length and 60 µm in width.

Environmental conditions

E. acutifrons can tolerate wide range of temperature (1-35°C) and the ideal

temperature for culture is 25-28°C. It can survive in the salinities between 20-45 ppt but optimum salinity range for culture is from 30-35 ppt.

Normal diffused day light is needed. Direct sunlight is not favourable. Normal tropical day length is ideal for culture. pH range between 8-8.5 is considered as ideal. Ammonia level should be below 1 ppm. It can be cultured in 1 L containers to 5 t tanks. Ideal depth of the tank is up to 1 m.

Low aeration is desirable. Treated seawater (chlorinated and dechlorinated) is ideal for mass culture. If water quality parameters deteriorate, replace the water using appropriate sieves.

Culture protocols

Food and feeding: This species basically feed on a variety of algae. Ideally this species can be fed on *Isochrysis galbana*, *Nannochloropsis salina* and *Chlorella marina* in the ratio 1:1:1. The favourable algal cell density for culture is 20000- 30000 cells/mL. Algae should be contamination free and should be in the growing phase.

Density of culture: Maximum density at a sustainable level of culture under normal conditions can be upto 2-3 nos/mL. A stock culture of 20 L with a density of 1000 nos/L can reach maximum density in 1000 L volume within 15-18 days and can be maintained for 2-3 months in the same containers with proper cleaning and regular harvest. Daily harvest is possible as nauplii or adults or as a mixture of all stages (Table 9).

Ideally stock and mass culture of this species is done as pure single species culture. But it is also observed that it is comparatively hardy and survive in a mixed culture but rarely dominate over other species in culture. It can be used as a feed along with other species in larval rearing tanks.

Table 9. Mesh size of sieves/bolting silk for filtering different stages of *Euterpina acutifrons*

Sl. No.	Stages required	Mesh size of sieves (µm)	Filtration pattern
1.	All stages	45	Single filtration
2.	Nauplii alone	110 and 45	Serial filtration. Filter through 110 µm to remove adults and copepodites and take residue from 45 µm for nauplii
3.	Adult alone	170	Single filtration

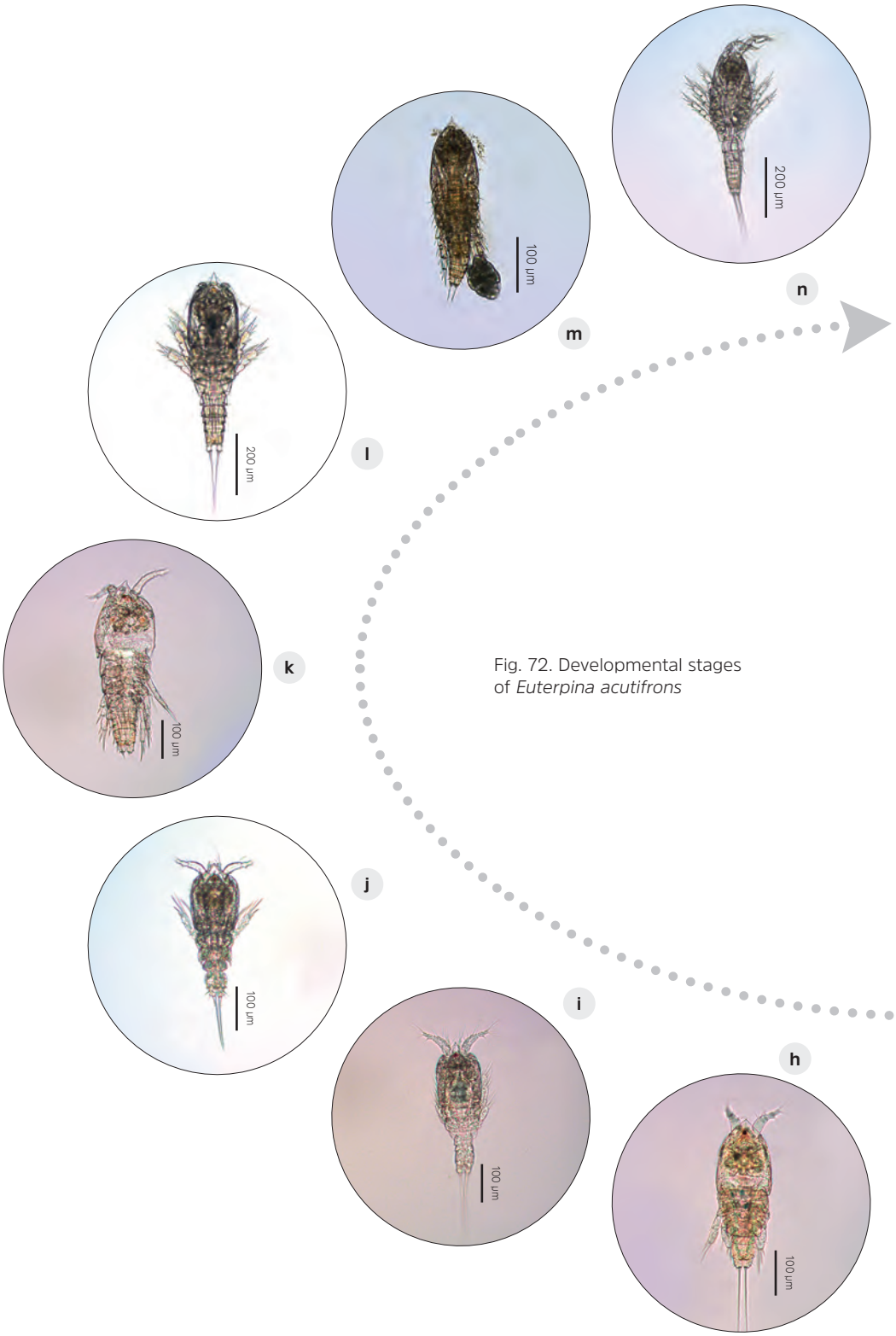
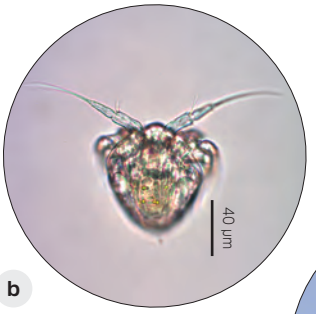


Fig. 72. Developmental stages of *Euterpina acutifrons*

a



b

c

a. Egg sac
b. Nauplius-1
c. Nauplius-2
d. Nauplius-3
e. Nauplius-4
f. Nauplius-5
g. Nauplius-6
h. Copepodite-1
i. Copepodite-2
j. Copepodite-3
k. Copepodite-4
l. Copepodite-5
m. Adult female
n. Adult male

d

e

f

g

Precautions: It is a sensitive species for stock and mass culture. It is to be fed only with good algae at required level and all parameters need to be maintained at optimum levels. Regular harvest is needed to maintain the population level. If any contamination is seen, isolate the adults or sub-adults and restart the culture.

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Fatty acid composition in cultured copepods

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Live food organisms are essential for the survival and growth of fish larvae during critical periods in their life (Lavens and Sorgeloos, 1996). Rotifers are one of the most popular live feed because of their small size and high rate of multiplication (Dhert, 1996). The unique property of *Artemia* to form 'cysts' makes them excellent larval food source (Stappen, 1996). Copepods have a special status among the live feed as they have all essential nutrients required for all types of fish larvae. In most of the cases, copepods are the natural diet for fish larvae. For small fish larvae of many commercially important marine food fishes, copepod nauplii are the only suitable live feed that can be accepted by the first feeding larvae. The superior nutritional quality of copepods especially in the PUFA composition (DHA:EPA) makes them the most suitable live feed during the larviculture. Each species of cultured zooplankton has their specific dietary requirement but almost all species are superior in fatty acid composition (Brown *et al*., 1997).

Reports of biochemical analysis revealed that the copepods are rich in proteins, lipids, essential amino acids (EAA) and essential fatty acids (EFA) (Watanabe *et al.,* 1983; Altaff and Chandran, 1989; Safiullah, 2001). DHA and EPA in copepods can promote proper development of larval fish as the consumption of these essential fatty acids can reduce the chances of morphological abnormalities (Satoh and Takeuchi, 2009; Satoh *et al*., 2009a, b). PUFA content of live feed in marine finfish larval rearing is crucial for maintaining high survival, faster growth, proper development and disease resistance (McEvoy *et al.,* 1998; Shields *et al.,* 1999).

Polyunsaturated fatty acids (PUFA) are almost exclusively synthesized by plants, but very few animals can synthesize PUFA *de novo* (Brett and Muller-Navarra, 1997).

PUFA have very low melting points and they act as membrane lipid anti-freeze. This ability to adjust cell membrane fluidity is advantageous for aquatic poikilotherms including marine and fresh water copepods (Brett and Muller-Navarra, 1997). PUFA, particularly arachidonic acid and EPA are the precursors for eicosanoids which are critical in a variety of physiological functions (Blomquist *et al*., 1991; Brett and Muller-Navarra, 1997). DHA plays an important role in normal physiological functions of brain and eye tissues (Brett and Muller-Navarra, 1997). The survival of marine fish larvae are significantly affected by DHA to EPA ratio (Nanton and Castell, 1998). *Artemia* synthesize or incorporate comparatively less amount of EFA and DHA than copepods and they have lower DHA:EPA ratio even when they were fed DHA rich diet (McEvoy *et al.,* 1995; Nanton and Castell, 1998). Marine copepods have high amounts of n-3 EFA's (Kattner *et al*., 1981; Nanton and Castell, 1998).

Lipid nutrition for fish larvae

Generally lipids are energy sources and particularly phospholipids and essential fatty acids are important for the normal growth, metamorphosis and survival of fish larvae (Cahu and Infante, 2001; Kanazawa, 2003; Tocher 2003; Glencross, 2009). Marine fishes cannot synthesize DHA and it has to be obtained from dietary source (Sargent *et al.,* 1997, 1999). Fatty acid deficiency in the early larval development lead to growth retardation, problems in metamorphosis (Watanabe and Kiron, 1994), problems in pigmentation (McEvoy *et al.,* 1998), abnormalities in development of central nervous systems and eye (Velu and Munuswamy, 2003) reduction in stress tolerance (Vagelli, 2004) and difficulties in swimming, feeding and survival (Olivotto *et al.,* 2006; 2008). Optimum range of EFA requirement varies in different species and ranges from 0.3 to 39 mg/g of DM for green mandarin, *Synchiropus splendidus* (Luchang, 2016) and from 10 to 15 mg/g of DM for gilthead sea bream larva *Sparus aurata* (Rodriguez *et al.,* 1993). Among the essential fatty acids, DHA has higher efficiency than EPA in improving the total health, stress resistance, growth and survival of marine fish larvae (Watanabe *et al.,* 1989; Watanabe and Kiron, 1994). Higher amount of EPA in relation to DHA may lead to imbalance in phospholipid concentration and affect the health and survival of the fish larvae (Rodriguez *et al.,* 1994). Optimum DHA/EPA should be >1 for all marine fish larvae (Gapasin and Duray, 2001; Wu *et al*., 2002, Bell *et al.,* 2003).

The long chain PUFA content particularly the DHA content is considered a critical factor indicating the nutritional quality of feed and the deficiency of which is considered as a primary cause for unsuccessful larval rearing in many marine fish species (Sargent *et al.,* 1999; Glencross, 2009). The three main live feeds used in larval rearing are *Artemia*, rotifer and copepods. By using enrichment media,

DHA/EPA ratio of the rotifer can be improved but it may be difficult in case of *Artemia* because of their ability to reconvert DHA to EPA (Navarro *et al.,* 1999).

Marine fish larvae are unable to synthesize EPA, DHA and ARA from their precursors (Cahu and Infante, 2001; Kanazawa, 2003; Sargent *et al.,* 1999). Copepod naturally contain high amount of DHA (more than 10 times) than *Artemia* and rotifers (Luchang, 2016). Nervous system and sensory organs of vertebrates including fishes contain high levels of DHA and its supply during early stages of life is very critical for the development of these organs (Hamre and Harboe, 2008). Hence, it is possible that the larvae fed with copepods have better brain development and vision thus enabling them to be more successful predators (Luchang, 2016).

Comparative analysis of fatty acids in live feeds

Three species of copepods *Temora turbinata, Pseudodiaptomus serricaudatus* and *Acartia southwelli* and the commonly used live feed *Artemia* nauplii and rotifer (*Brachionus plicatilis*) were analysed for the fatty acid content.

The total saturated fatty acid and total monounsaturated fatty acid contents were very similar in copepods, *Artemia* and rotifer studied (Table 10). Polyunsaturated fatty acid content was highest in *Artemia*, followed by rotifer and copepods. But the highly unsaturated fatty acid (DHA) was very high in all the copepods.

There was a significant difference between the estimated value of EPA between copepods and the other two live feeds (Table 11). EPA estimated in all copepods were less than that of other live feeds which favours a high DHA:EPA ratio. DHA in copepods were significantly higher (19.4 to 21.6) than that of *Artemia* nauplii (0.2) and *B. plicatilis* (3.1). As a result, the ratio of DHA:EPA was found very much higher (8.6-9.7) in all copepods than *Artemia* (0.04) and rotifer (0.08).

Table 10. Total SAFA, MUFA, PUFA and HUFA contents in *Temora turbinata, Pseudodiaptomus serricaudatus, Acartia southwelli, Artemia* nauplii and *Brachionus plicatilis*

Sl. No.	Fatty Acids (Mean)	Copepods			*Artemia*	Rotifer
		T. turbinata	***P. serricaudatus***	***A. southwelli***	**Nauplii**	***B. plicatilis***
1	Total SAFA	50.1	45.2	40.1	44.5	46.6
2	Total MUFA	18.2	9.2	13.4	5.7	17.2
3	Total PUFA	10.2	21.6	22.4	42.4	29.1
4	Total HUFA	21.4	24.1	24	7.2	7.1

Table 11. DHA-EPA ratio of *Temora turbinata, Pseudodiaptomus serricaudatus, Acartia southwelli, Artemia* nauplii and *Brachionus plicatilis*

Fatty Acids (Mean + SD)	**Copepods**			***Artemia***	**Rotifer**
	T. turbinata	***P. serricaudatus***	***A. southwelli***	**Nauplii**	***B. plicatilis***
DHA	19.4	21.6	21.6	0.2	3.1
EP	2.0	2.5	2.4	4.6	4.0
DHA/EPA ratio	9.7	8.6	9	0.04	0.8

The amount of DHA is typically important as it plays an important role in normal physiological functions of brain and eye tissues (Brett and Muller-Navarra, 1997). DHA content was higher in all the copepods, indicating their importance as first feed for fish larvae (van der Meeren *et al.,* 2008). The increased level of DHA and a comparatively decreased EPA level favoured a high DHA-EPA ratio in all copepods which indicated their use as live feed that favours higher survival of marine fish larvae (Nanton and Castell, 1998). The results were consistent with previous studies which explained the high DHA to EPA ratio of copepods (Bell *et al.,* 1985; 2003; Watanabe, 1993; Nanton and Castell, 1998). All these copepods contain much higher DHA:EPA (8.6-9.7) of minimum 2:1 recommended by Sargent *et al*., (1997) as required for marine finfish larvae.

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Use of copepods in marine fin fish larval rearing

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Initial feeding of marine finfish larvae has been one of the major problems in fish seed production. The newly hatched fish larvae have undeveloped digestive system, poor vision, very small mouth gape and a very high nutritional demand especially in terms of highly unsaturated fatty acids. Only live feeds can meet all these requirements. The conventional live feeds used for initial feeding of fish larvae are *Artemia* spp. and rotifers. But both these have their limitations for feeding smaller fish larvae, in terms of size range, nutritional composition, digestive efficiency and feed preference (Cahu and Infante, 2001; Marcus, 2005). Gut content examination of wild caught fish larvae proved that the larvae feed mainly on copepods. Live feed size should be much smaller than the mouth size of fish larvae for successful initial feeding. In terms of size, copepod nauplii are highly suitable when compared to *Artemia* and rotifer. Moreover, copepods do not require any sort of enrichment. They have a highly relevant and rich biochemical profile needed for the proper development of most of the marine fish larvae. Copepods are rich in essential fatty acids which are important for larval fish survival and growth. Highly unsaturated fatty acids such as DHA and EPA are present in the most appropriate ratios in copepods suitable for fish larval development (Sun and Fleeger, 1995; Stotrup and Norsker, 1997; Sargent *et al.,* 1997, 1999; Olivotto *et al.,* 2008). The characteristic moving pattern 'pause and move' of copepod nauplii makes them more vulnerable prey for initial feeding. Copepods can improve health, reduce abnormalities in growth, increase stress tolerance, enhance development and improve pigmentation and growth of fish larvae (Bell *et al.,* 2003; Copeman *et al.,* 2002; Olivotto *et al.,* 2006, 2008; Vagelli, 2004).

Copepods are the best option as initial feed for larvae of a variety of marine food fishes and ornamental fishes. But due to the difficulties in rearing, these are being used as a feed for a critical period when larvae are unable to feed or survive with

Artemia and rotifers. This may be in terms of size, nutritional factors, digestibility, vision or movement. In general, feeding protocols are developed in such a way that the use of copepods/naupliar stages is limited to critical stages mentioned above and a combination of *Artemia* and rotifers are used till the weaning of fish larvae to artificial feeds. The practice of using wild copepods from natural ponds is not advisable as it increases the risk of parasitic infections (Ajiboye, *et al.,* 2010).

The main demerit of copepods as a commercial larval feed is that, these cannot be cultured in high densities. Rotifers are routinely cultured in numbers exceeding 2000 nos/mL and *Artemia* can also be hatched and cultured at higher density. But copepod cultures rarely exceed densities of 2-5 nos/mL for adults and 10 nos/mL for nauplii. Some harpacticoid copepods have been reported to reach densities of more than 100 nos/mL but due to their epibenthic nature, they may not be available to pelagic fish larvae (Stottrup, 2006). So an efficient feeding protocol needs to be developed for each fish species by using combinations of cultured copepods, rotifer, *Artemia* and artificial feed for fish larval production.

Ornamental fishes

Copepods are widely used in larval rearing of many ornamental fishes in CMFRI. Trials on seed production of damsel fishes were successful only after using copepods as initial feed (Table 12). Initial trials were all done using a combination of a calanoid copepod *Pseudodiaptomus serricaudatus* and a harpacticoid copepod *Euterpina acutifrons*. Mostly co-culture method was used initially. Damsel fishes like *Dascyllus trimaculatus* (Three spot damsel), *Dascyllus aruanus* (Humbug damsel), *Pomacentrus caeruleus* (Caerulean damsel), *Chromis viridis* (Blue green damsel), *Neopomacentrus nemurus* (Yellowtail damsel) and *Chrysiptera cyanea* (Sapphire devil damselfish), were successfully bred and reared here (Gopakumar and Santhosi, 2009; Gopakumar *et al*., 2009 a, b). Recently, *Neopomacentrus cyanomos* (Regal demoiselle) (Rohini Krishna *et al.,* 2016) and *Dascyllus carneus* (Cloudy damsel) were also successfully reared here using nauplii of *Parvocalanus crassirostris, Dioithona* sp. and *Acartia southwelli.*

Laboratory trials revealed 70% better survival in frys of *Hippocampus kuda* (Fig. 74a&b) fed with a combination of copepods *Temora turbinata* and *Pseudodiaptomus serricaudatus* than in traditional *Artemia* and rotifer feeding. The frys fed with copepods showed faster growth and brighter colouration. Ornamental fish production trials conducted using copepod naupliar stages of *T. turbinata* to feed the larvae of *Amphiprion frenatus* against the traditional practice of rotifer and artemia nauplii combination gave 24.5% higher survival, better growth and brighter colouration in copepod fed larvae (Fig.73a-d). Copepod fed fish larvae showed better survival, brighter colouration, better growth and

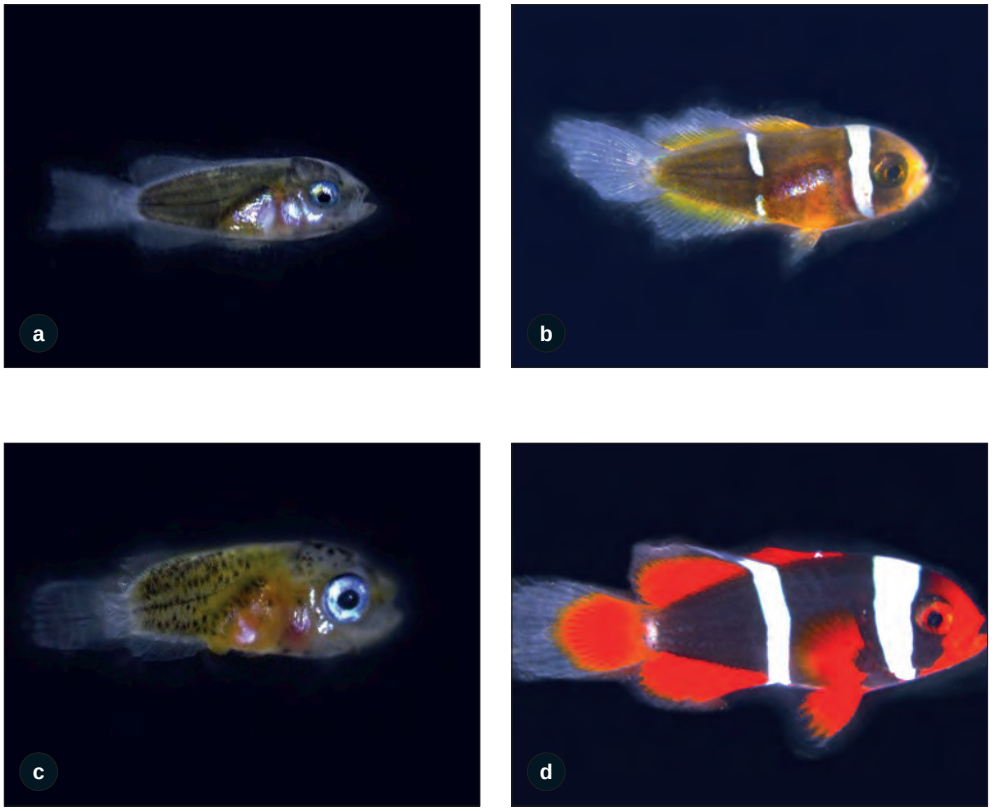

Fig. 73. *Amphiprion frenatus* larva showing higher growth and brighter colouration in an experimental trial using copepod *T. turbinata* a. Larva (control) on 8 dph b. Larva (control) on 30 dph c. Larva fed with copepod on 8 dph d. Larva fed with copepod on 30 dph

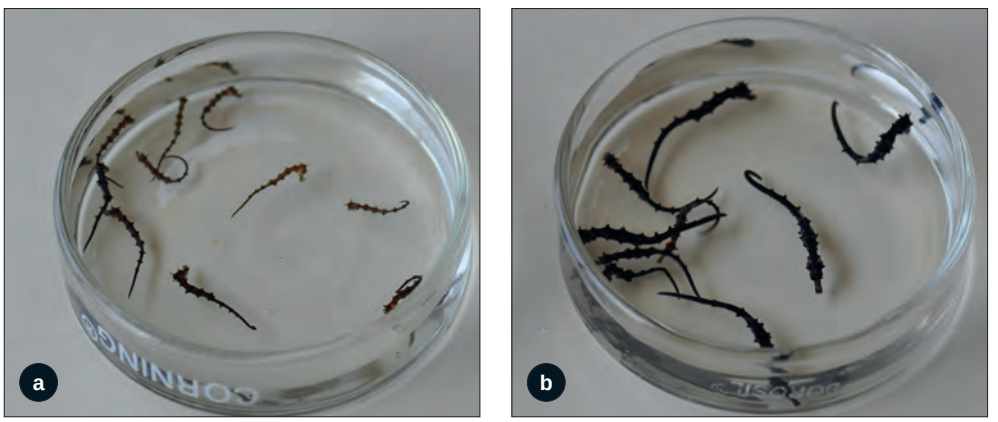

Fig. 74.. *Hippocampus kuda* cultured using a. *Artemia* and rotifer b. Copepods

Table 12. Details of fish larvae reared using copepods at CMFRI

Sl. No.	Common name	Scientific name	Live feed used	Nauplii / copepodite / adult density	Day of metamorphosis	Survival (%)	Source
1.	Three spot damsel	*Dascyllus trimaculatus*	Mixed copepods, *Euterpina acutifrons* and *Pseudodiaptomus serricaudatus*	4/mL(Nauplii) 1.94/mL(Adult)	35-40 dph	10-15%, 3-4%	Gopakumar *et al.*, 2009a, Gopakumar and Santhosi, 2009
2.	Humbug damsel	*Dascyllus aruanus*	mixed copepods	5.76/mL(Nauplii) 2.18/mL(Adult)	25-31 dph	10-15%, 3-8%	Gopakumar *et al.*, 2009a, Gopakumar and Santhosi, 2009
3.	Caerulean damsel	*Pomacentrus caeruleus*	*E. acutifrons* and *P. serricaudatus*	1.64/mL(Nauplii) 0.82/mL(Adult)	17-21 dph	3-4%	Gopakumar *et al.*, 2009a
4.	Blue green damsel	*Chromis viridis*	*E. acutifrons* and *P. serricaudatus*	1-13/mL(Adult), 7-78/mL(Nauplii) 1-31/mL(Copepodites)	30-49 dph	5%.	Gopakumar *et al.*, 2009b
5.	Yellowtail damsel	*Neopomacentrus nemurus*	Wild copepods	_	16-21 dph	_	Gopakumar *et al.*, 2009b
6.	Sapphire devil damselfish	*Chrysiptera cyanea*	*E. acutifrons* and *P. serricaudatus*	_	24-30 dph	5-8%	Gopakumar *et al.*, 2013
7.	Regal demoiselle	*Neopomacentrus cyanomos*	*Acartia spinicauda, P. serricaudatus* and *Temora turbinata.*	0.2-0.3/mL(Nauplii) 0.5-1.0/mL(Nauplii) 0.4/mL(Nauplii)	28-32 dph	12%	Rohini Krishna *et al.*, 2016
8.	Spotted seahorse	*Hippocampus kuda*	*T. turbinata* and *P. serricaudatus*	2/mL(mixture of all stages)	_	70%	Present study
9.	Tomato clownfish	*Amphiprion frenatus*	*T. turbinata*	2/mL(Nauplii)	_	24.5%	Present study
10.	Marcia's anthias,	*Pseudanthias marcia*	*Parvocalanus crassirostris*	2-3/mL(Nauplii) 0.7-1.0/mL(Adult)	32-34 dph	7.3%.	Anil *et al.*, 2018
11.	Cloudy damsel	*Dascyllus carneus*	*P. crassirostris*	_	32-50 dph	6.05%	(Unpublished data)
12.	Orange spotted grouper	*Epinephelus coioides*	*P. crassirostris, Dioithona* sp. and *Acartia southwelli*	_	_	_	Ranjan, 2017
13.	Indian Pompano	*Trachinotus mookalee*	*P. crassirostris, Dioithona* sp. and *A. southwelli*	2/mL(Nauplii)	17-21 dph	21.53%	Ranjan *et al.*, 2018

stress tolerance in similar trials (Hamre *et al.,* 2008; 2013; Imsland *et al.,* 2006; Srivastava *et al.,* 2006; van der Meeren *et al.,* 2008).

Food fishes

Copepods are the most appropriate food for fish larvae compared to *Artemia* and rotifers. Nielsen *et al.,* (2017) compared 12 important aspects of live feed with respect to larval rearing and concluded that copepods are the most ideal feed even though, expenditure for production was found higher. When compared to traditional practices, use of copepods in fish larval rearing reported consistent improvement in growth, better survival, reduction in deformities and resistance to stress conditions (Nanton and Castell, 1998; 1999; Drillet *et al.,* 2006; 2011; Ajiboye *et al.,* 2010; Nelsen *et al.,* 2017). Normal larval pigmentation is an indication of health of the larvae. Higher survival and normal pigmentation is generally due to high levels of DHA in live feed organisms. DHA contents of copepods are estimated to be more than 10 times than that of enriched *Artemia* which favours the use of copepods for increasing normal pigmentation and survival (McEvoy *et al.,* 1998; Stottrup, 2003. Nelsen *et al.,* 2017). Fish larvae require a minimum of 0.5 to 1% dry weight of n-3 HUFA in their diet (McEvoy *et. al.,* 1998). DHA is essential for proper development of brain, cell membranes, retinal development and vision (Bell and Sergent, 1996). If sufficient quantity of DHA is not obtained through live feed, it may lead to poor larval survival (Reitan *et al*., 1994).

Copepods are excellent source of DHA compared to *Artemia* and rotifers. Copepods are also a rich source of polar lipids which can be easily digested and utilized by the fish larvae. Copepods are rich source of carotenoid astaxanthin-a precursor to Vitamin A and exogenous digestive enzymes which has an important role in fish larval digestion (Munilla Moran *et al.,* 1990; Stottrup 2003.). High astaxanthin concentration may reduce the oxidative stress that forms in copepods under low temperatures and food shortage. Some of the free astaxanthins get esterified on lipid accumulation. (Schneider *et al.,* 2016). Carotenoids have photo protective roles. Ringelberg *et al.,* (1981) had shown that the carotenoid rich copepods tolerate higher levels of UV radiation compared to unpigmented ones.

It is also confirmed that in turbot *Scophthalmus maximus* and Atlantic herring *Clupea harengus* larvae, copepods not only donate their digestive enzymes (protease and trypsin) but also activate zymogens in the larval gut (Pedersen and Hjelmel, 1988; Munilla-Moran *et al.,* 1990; Sun *et al.,* 2013; Rasdi *et al.,* 2016). Use of copepods as live feed helps to decrease malpigmentation and deformities in fishes (Stottrup, 2000). Hamre *et al.,* (2005) reported significant improvement of both eye migration and pigmentation by using copepods in Atlantic halibut, *Hippoglossus hippoglossus*.

Copepods are being used in larval rearing of many species of food fishes including Turbot *Scophthalmus maximus* (Kuhlmann *et al.,* 1981*), Psetta maxima* (Stottrup *et al.*, 1997) Herring *Clupea harengus* (Hjelmel *et al.,* 1988), Red seabream *Pagrus major* (Ohno, 1992), Mahi mahi *Coryphaena hippurus* (Kraul, 1993; Schipp, 2006) Grouper *Epinephelus coioides* (Toledo *et al.,* 1999; 2005) Flatfish *Scophthalmus maximus* (Bell *et al.,* 2003) Barramundi *Lates calcarifer,* Almaco jack *Seriola rivoliana,* Giant Trevally *Caranx ignobilis* (Schipp, 2006), Japanese Flounder *Paralichthys olivaceus* (Liu and Xu, 2009), Florida Pompano *Trachinotus carolinus* (Cassiano and Ohs, 2011) and Atlantic Cod *Gadus morhua* (Karlsen *et al.,* 2015).

Copepods developed at Vizhinjam Research Centre of CMFRI gave promising results in all trials conducted in larviculture (Table 12). Orange spotted grouper, *Epinephelus coioides* (Fig. 75a&b) and Indian Pompano, *Trachinotus mookalee* (Fig. 76a&b) were successfully bred and reared at Visakhapatnam Regional Centre of CMFRI (Ranjan *et al.*, 2018). A combination of nauplii *Parvocalanus crassirostris, Dioithona* sp. and *Acartia southwelli* are being used as the first larval feed. Very good survival was obtained for both the species.

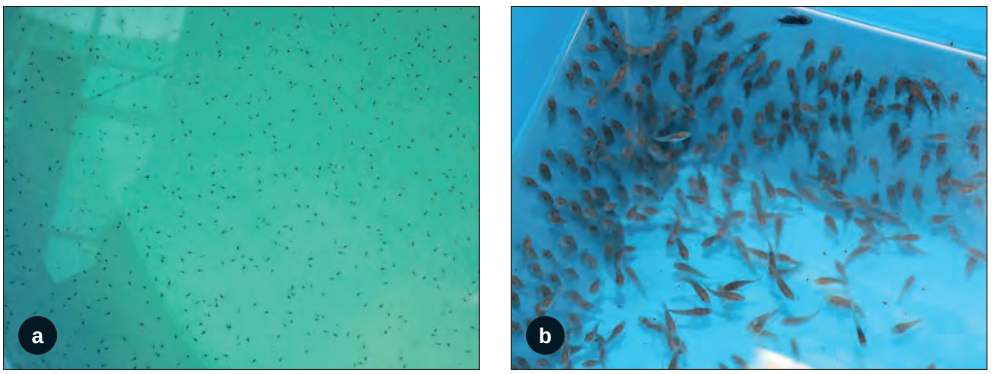

Fig. 75. Orange spotted grouper larvae cultured on copepod nauplii as first feed a. 8 dph b. 32 dph

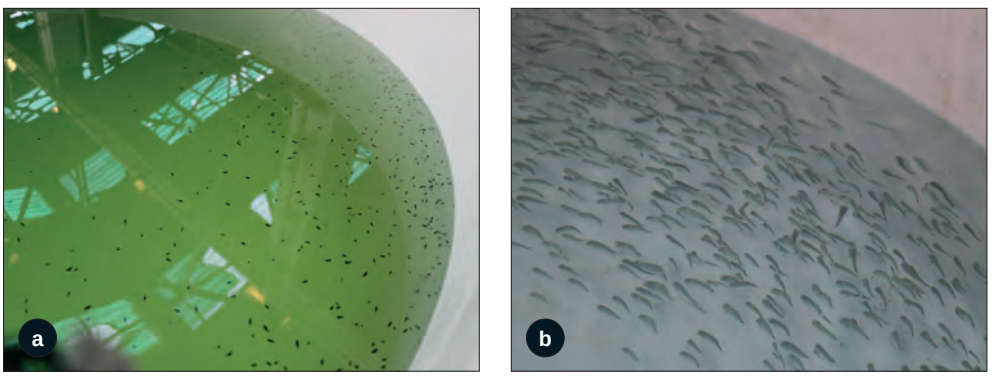

Fig. 76. Indian pompano larvae cultured on copepod nauplii as first feed a. 10 dph b. 24 dph

All the nine copepod species for which culture techniques have been developed by CMFRI are being maintained at different centers located at Visakhapatnam, Mandapam, Tuticorin, Vizhinjam and Karwar. Large scale production of copepods has been already initiated at all centres and the copepods are being utilized for larval rearing of food fishes and ornamental fishes. Many private hatcheries also started utilizing some of the species for improving the production from their hatchery. Copepod nauplii are mainly utilized as the first feed and the mixed stages of copepods are mostly being utilized for feeding the later larval stages whenever a critical phase is expected or experienced. Copepods form a well-balanced diet even for the later phase in larval development and also an ideal feed to overcome nutritional deficiency.

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Cost estimate and financial analysis of a medium scale copepod culture unit

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Aquaculture has been the fastest growing animal food producing sector in the world with 5.8% annual growth rate since 2010. In recent years at the global level, a rapid growth in marine finfish culture has been noted at an average annual growth rate of 9.3% from 1990 onwards. The production was 6.6 million tonnes in 2016 which contributed to 23.6% of the total mariculture production. The total number of farmed species has increased by 26.7% mostly because of the advancement of hatchery production of fish seeds (FAO, 2018). Live feed plays a vital role in larval rearing and inappropriate live feed forms the most important bottleneck in fish seed production. Many marine finfish larvae are very small and require special care to initiate first feeding. Certain finfishes and ornamental fishes need copepod nauplii as first feed for larval survival. Copepods are more nutritious and ideal for the growth and survival of marine fish larvae. Now developing profitable methods of copepod culture has become a major sector in live feed research. ICAR-CMFRI has been focusing on developing technologies for the production and utilization of copepods in finfish larval rearing.

Basic concept and assumptions

Among live feeds, copepod culture has been the most recent initiative and still it is in the developing stage. There are only very few reports explaining the economic feasibility of copepod culture because it has been only a part of hatchery production of fish seed and there is no independent cultivation of copepods. Moreover, there has been no study or report of larval rearing of a fish exclusively using copepods as feed. Often the use of copepod is limited to only specific periods of larval rearing to support survival at certain critical stages in culture where no other feed could replace it. Unlike microalgae, rotifers or *Artemia*, the species and production methods of

copepods has not yet been standardized uniformly throughout the world. Often the reports are diverse, incomplete and fragmentary to delineate a common protocol. There are only a few reports on culture of copepods, that too with minimum description of the monetary aspects involved here (Alvarez-Lajonchere and Taylor, 2003; Molejon and Alvarez-Lajonchere, 2003). Abate *et al.,* (2015) has explained the theoretical level economic feasibility of a prototype RAS copepod culture facility set up at Roskilde University, Denmark. But the biggest challenge in such a system, which is to provide continuous feeding with microalgae, has not been explained in this publication. On the basis of the existing practices in CMFRI, an attempt has been made here to make an assessment of the cost involved in a medium scale copepod culture unit.

The basic assumption followed is that the culture explained here has been exclusively a part of a small-scale larval rearing unit for food fish or ornamental fish, where there is already sufficient supply of clean and filtered seawater. The basic facilities already existed in the hatchery and an extension of the space and facilities were taken in account for calculations. Algal culture, being the major activity in copepod culture, consumed a major share of the cost involved. The method itself is labour intensive and almost 85% of the recurring expenditure has been on the labour component. Skilled support or training for the staff was found essential to identify the critical stages and also to take precautionary or remedial measures against crashing or poor growth that occurred both in algal culture and copepod culture. Hatcheries which already had a well-developed algal production unit can go for copepod culture without much investment. The copepod *Acartia southwelli* has been used as a model organism for this study. Indian rupee (₹) is taken as basic currency for all calculations (1 USD = INR 71.26).

Model set up

A shed of 6 m x 12 m area is sufficient for a basic copepod culture unit. An area of 2 m x 6 m can be delineated as an air conditioned cabin with a sliding door and roofing for developing an algal stock culture unit where carboy level mass cultures could also be accommodated. Algal culture is the most essential component in larval rearing and utmost care should be given for maintaining uninterrupted supply of algae for ensuring copepod production. An area of 3 m x 6 m can be used for algal mass culture and 7 m x 6 m area can be used for copepod culture. Both algal mass culture and copepod mass culture can be undertaken in FRP tanks of 0.75 t capacity tank. Copepod culture can be done only upto 500 L level.

Algal culture with stock and mass cultures of 3 species viz. *Isochrysis galbana, Nannochloropsis salina* and *Chlorella marina* are the prerequisite for culture of *A. southwelli*. Standard method using Walne's media was used upto carboy

level culture and fertilizers were used for mass culture in FRP tanks. Sufficient illumination should be given for producing maximum cell density. Algae with late exponential phase has been found ideal for feeding copepods. Daily harvest is possible after 5 days of inoculation. Total resetting of tanks is essential after every three months. So there will not be production for 20 days in a year for all the tanks. Since the eggs settle at the bottom, bottom-siphoning method has been used for harvesting and cleaning. *Acartia southwelli* is a comparatively small sized copepod in the genus *Acartia* and this species is not very sensitive to light. A culture density > 2000 numbers/L with a higher proportion of females is very common for this species. Average density of 1000 fecund female/L and egg production rate of 10 numbers/female (10,000/L) were taken in to account for all calculations.

Production

Daily harvestable egg production from a single tank was estimated as 5 million eggs/day. Total production from 24 tanks was 120 million eggs/day. Total annual production from the copepod unit has been estimated to be 41400 million eggs/year. Total expenditure for capital investment was estimated as ₹984660, (Table 13) and the yearly recurring expenditure was ₹643039. Total expenditure for first year without considering bank interest and depreciation was estimated as ₹1627700. From available market information, the cost of 100g of good quality *Artemia* cyst has been ₹800 and 5 g of *Artemia* is required to produce 1 million nauplii and this may cost ₹40. Considering the present value of *Artemia* as ₹40/million and selling rate of copepod nauplii as the same, the price for yearly production of 41400 million copepod nauplii has been estimated as ₹1656000.

Table 13. Production estimates of a medium scale copepod (*Acartia southwelli*) culture unit

Sl. No.	Items	Details
1.	Total capital expenditure in the first year for a copepod live feed unit with 24 tanks	₹984660
2.	Total yearly recurring expenditure for a copepod live feed unit with 24 tanks	₹643039
3.	Total expenditure for first year (excluding bank interest and depreciation)	₹1627700
4.	Copepod production tanks	24 tanks of 500 L capacity
5.	Density of *Acartia southwelli*	2000/L
6.	Density of adult female	1000/ L
7.	Rate of production of viable eggs or nauplii/L/ day	10000
8.	Total eggs or nauplii production from a tank/day	5 million

9.	Total production from 24 tanks/day	120 million
10.	Total production/year	41400 million
11.	Cost of *Artemia* for 100 g	₹800
12.	Cost of 1 g of *Artemia*	₹8
13.	Cost of 5 g *Artemia* that is required to produce 1 million nauplii (200000 nos of *Artemia* nauplii from 1 g of egg)	₹40
14.	If we take cost as equivalent to *Artemia*, ₹40/million, the selling price of 1 million copepod nauplii	₹40
15.	Total price for yearly production of 41400 million copepod nauplii	₹1656000
16.	Rate as per international market for 1 million *Acartia tonsa* eggs as €150 (Drillet *et al.,* 2011; Abate *et al.,* 2015), price of 1 million *A. southwelli* eggs	₹12040

Financial analysis

To analyse the potential economic return from a copepod culture unit, we have evaluated the economic performance of a medium scale copepod culture unit at ICAR-CMFRI. The average initial investment on nonrecurring items accounted to ₹9,84,660 (Table 14). The FRP tanks accounted for the maximum share of investment (41%) followed by the prefabricated basic shed (30%), Haffkine flasks (10%), digital balance (3%), stereo zoom microscope (3%) and others (13%).

The annual total cost of production was estimated to be ₹7,91,121, comprising a fixed cost of ₹1,48,082 (18.72%) and variable cost of ₹6,43,039 (81.28%). The annual fixed cost included depreciation on investment, interest on investment @ 7% per annum while variable costs included charges for labour, chemicals, electricity and miscellaneous items. The cost of production for 1 million copepod eggs work out to be ₹19.11 which is far below than the average market price of same quantity of *Artemia* in India (₹40). When compared to the international market, rates for 1 million *Acartia tonsa* eggs which is €150 (₹12040) (Drillet *et al.,* 2011; Abate *et al.,* 2015), the profitability of copepod culture is much higher. If we consider the superiority of copepods in terms of higher nutrition, savings for enrichment media and higher larval survival expected using copepods, the culture seems to be highly economical. Since we have conducted all trials in prototype facilities, further field level trials are essential for validation of the results explained here.

The annual gross returns has been estimated to be ₹16,56,000, leading to an

Table 14. Annual costs and returns of a medium scale copepod culture unit

	Unit	Quantity	Price per unit ₹	Total Value ₹	Share (%)	Economic Life (in years)	Depreciation ₹
I. Initial investment							
Prefabricated basic shed with cement flooring, basic electric and plumbing facilities (area 12m x 6m – 775 square feet @ ₹200/square feet-₹155000 and for flooring and cabin – 145000)	Number	1	300000	300000	30.47	10	30000
Air conditioner (1.5 t)	Number	1	38,490	38,490	3.91	10	3849
Blower/aerator (1 H.P.)	Number	2	10,000	20,000	2.03	7	2857
Gas burner with cylinder	Number	1	5,000	5,000	0.51	10	500
Autoclave	Number	1	11,000	11,000	1.12	20	550
Compound microscope	Number	1	10000	10000	1.02	10	1000
Stereo zoom microscope	Number	1	25,000	25,000	2.54	10	2500
Digital balance	Number	1	30,000	30,000	3.05	10	3000
Refrigerator	Number	1	15,000	15,000	1.52	10	1500
FRP tanks 750 L	Number	30	13500	405000	41.13	20	20250
Haemocytometer	Number	1	1480	1480	0.15	15	99
PVC bins 100 L capacity	Number	12	300	3600	0.37	10	360
Carboy	Number	50	125	6250	0.63	10	625
Haffkine flask (4 L)	Number	20	4986	99720	10.13	10	9972
Conical flask (500 mL)	Number	20	125	2500	0.25	10	250
Conical flask (100 mL)	Number	20	100	2000	0.20	10	200
Ceiling fan	Number	2	1400	2800	0.28	10	280
Filter bag (1 µm)	Sq. m	2	2160	4320	0.44	5	864

	Unit	Quantity	Price per unit ₹	Total Value ₹	Share (%)	Economic Life (in years)	Depreciation ₹
Air tube (50 m)	Number	100	25	2500	0.25	5	500
Total initial investment				**984660**	**100.00**		**79156**
II. Fixed costs							
Depreciation	₹			79156	53.45		
Interest on investment @7% per annum	₹			68926	46.55		
Total fixed costs	₹			**148082**	**100.00**		
III. Variable costs							
Labour Charges	Man days	1095	500	547500	85.14		
Chemicals	Days	365	81.36	29696	4.62		
Electricity–per unit @ ₹2 (Agriculture) (62.8 unit/day)	Days	365	125.6	45844	7.13		
Miscellaneous items including Nylon mesh (20–500 µm), air stone, air controller, Cotton roll, Glass slide, Coverslip, LED lamp, LED tube lights (20 watt), gas, basic glasswares, Plasticwares, fertilizers etc.	₹	365	54.79	19998	3.11		
Total variable costs	₹			**643039**	**100**		
IV. Total cost of production	₹	**(II + III)**		**791121**			
Gross revenue of medium scale copepod culture unit							
Annual yield of eggs	million numbers	41400	40	1656000			
Cost of production for producing 1 million eggs				19.11			

Table 15. Economic and financial indicators for the medium scale copepod culture unit

Indicators	Unit	Year I	Year II	Year III	Year IV	Year V	Average
Gross Investment	₹	984660	0	0	0	0	196932
Total Cost of Production	₹	791121	791121	791121	791121	791121	791121
Gross returns	₹	1656000	1656000	1656000	1656000	1656000	1656000
Net income	₹	864879	864879	864879	864879	864879	864879
NPV @ 20% DR	₹						1601847
BCR@ 20% DR	Ratio						1.48
Return on investment	Per cent						87.84
Pay back period	Years						1.14
IRR	Per cent						>100

Table 16. Sensitivity analysis of the copepod (*Acartia southwelli*) production unit

Year	Cost	Benefit	Discount factor (20%)	Discounted cost at 20%	Discounted benefit at 20%	Reduction in benefit of 10%	Discounted benefit	Reduction in benefit of 20%	Discounted benefit	Reduction in benefit of 30%	Discounted benefit
0	984660	0	1	984660	0	0	0	0	0	0	0
1	791121	1656000	0.8333	659241	1379945	1490400	1241950	1324800	1103956	1159200	965961.36
2	791121	1656000	0.6944	549354	1149926	1490400	1034934	1324800	919941	1159200	804948
3	791121	1656000	0.5787	457822	958327	1490400	862494	1324800	766662	1159200	670829
4	791121	1656000	0.4823	381558	798689	1490400	718820	1324800	638951	1159200	559082
5	791121	1656000	0.4019	317952	665546	1490400	598992	1324800	532437	1159200	465882
				3350586	4952434	7452000	4457190	6624000	3961947	5796000	3466704
				NPV	1601847	NPV	1106604	NPV	611360	NPV	116117
				BCR	1.48	BCR	1.33	BCR	1.18	BCR	1.03

annual net income of ₹8,64,879. For all estimates, the selling price of copepod eggs/nauplii was assumed at a minimum cost equivalent to the market rate of *Artemia* required to produce same quantity of nauplii. Hence, the estimates of gross revenue of copepod eggs/nauplii at a production rate of 41,400 million per annum was calculated at a market price for 1 million eggs as ₹40.

The average annual net income for 5 years in the copepod culture unit (₹8,64,879) is lower than the initial investment (₹9,84,660), suggesting a payback period of 1.48 years (Table 15). The estimated Net Present Value (NPV) at 20% discount rate has been found to be ₹16,01,847 (implying an Internal Rate of Return (IRR) more than 100%) while the Benefit Cost Ratio (BCR) at 20% discount rate is 1.48. All these indicators provide strong evidence of the economic and financial feasibility of a medium scale copepod culture unit.

Sensitivity analysis for different benefit receivables

Uncertainties in a medium scale copepod culture unit arise due to variations in yield, technology used, climatic conditions, nature of institutions involved in culture etc. In countering these uncertainties, the production benefit stream can be sensitized by ex-ante approach of reducing the anticipated project benefit stream at 10%, 20% and 30%, keeping the project cost unchanged. The calculated Net Present Value (NPV) and Benefit Cost Ratio (BCR) indicated that the system could withstand risk even at 30% reduction in production due to the different uncertainties. The NPV and BCR at 30 per cent reduction in production in the project benefit stream were found to be ₹1,16,117 and 1.03 respectively (Table 16).

Conclusion

The economic analyses of a medium scale copepod production unit for the species, *Acartia southwelli* indicated a profitable system of production with an average annual net income for 5 years (₹8,64,879) which is lower than the initial investment (₹9,84,660), suggesting a payback period of 1.48 years. The selling price of copepod eggs/nauplii was considered as equivalent to *Artemia* for all calculations which is far below than the international market rates of copepod eggs. The estimated NPV at 20% discount rate has been found to be ₹16,01,847 (implying an IRR more than 100%) while the BCR at 20% discount rate was 1.48. All these indicators provide strong evidence for the economic and financial feasibility of the medium scale copepod culture unit. The analysis also proved that the production system can withstand risk even to the tune of 30% reduction in production. The profitability of copepod culture is much higher, if we consider the superiority of copepods in terms of higher nutrition, savings for enrichment media and higher larval survival expected by using copepods.

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Acronyms

°C	-	Degree Celcius
ARA	-	Arachidonic acid
BCR	-	Benefit Cost Ratio
C1 – C5	-	Copepodite 1 to Copepodite 5
DHA	-	Docosahexaenoic acid
DM	-	Dry matter
dph	-	Day of post hatch
EAA	-	Essential amino acids
EFA	-	Essential fatty acids
EPA	-	Eicosapentaenoic acid
Fig.	-	Figure
FRP	-	Fibre reinforced plastic
h	-	Hour
HDPE	-	High density polyethylene
HUFA	-	Highly unsaturated fatty acid
IRR	-	Internal Rate of Return
L	-	Litre
m	-	Meter
mg/L	-	Milligram per litre
mm	-	Millimetre
MUFA	-	Monounsaturated fatty acids
N1 – N6	-	Nauplius 1 to Nauplius 6
nos/L	-	Numbers per litre
nos/mL	-	Numbers per millilitre
NPV	-	Net Present Value
P1 - P5	-	Pereopod 1 to Pereopod 5
ppm	-	Parts per million
ppt	-	Parts per thousand
PUFA	-	Polyunsaturated fatty acids
PVC	-	Polyvinyl chloride
SAFA	-	Saturated fatty acid
SD	-	Standard deviation
SDM	-	Stereo dissection microscope
Sl. No.	-	Serial number
sp.	-	Species
spp.		Species (plural)
t	-	Tonne
UV	-	Ultraviolet
var.	-	Variety
µm	-	Micrometre

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f. Nauplius-5
g. Nauplius-6
h. Copepodite-1
i. Copepodite-2
j. Copepodite-3
k. Copepodite-4
l. Copepodite-5
m. Adult female
n. Adult male

Fig. 50. *P. serricaudatus*

a. Female b. Male

Fig. 51. *P. serricaudatus*

a. Female b. Male

Fig. 52. Egg sacs showing different developmental stages

Fig. 53. Nauplii released from egg sac

Fig. 54. Developmental stages of *Pseudodiaptomus serricaudatus*

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d. Nauplius-3
e. Nauplius-4
f. Nauplius-5
g. Nauplius-6
h. Copepodite-1
i. Copepodite-2
j. Copepodite-3
k. Copepodite-4
l. Copepodite-5
m. Adult female
n. Adult male

Fig. 55a. *P. crassirostris* Female

Fig. 55b. *P. crassirostris* Male

Fig. 56. *P. crassirostris*

a. Female b. Male

Fig. 57. Developmental stages of *Parvocalanus crassirostris*

a. Eggs
b. Nauplius-1
c. Nauplius-2
d. Nauplius-3
e. Nauplius-4
f. Nauplius-5
g. Nauplius-6
h. Copepodite-1
i. Copepodite-2
j. Copepodite-3
k. Copepodite-4
l. Copepodite-5
m. Adult female
n. Adult male

Fig. 58 *B. similis*

a. Female b. Male

Fig. 59. *B. similis*

a. Female b. Male

Fig. 60. Developmental stages of *Bestiolina similis*

a. Eggs
b. Nauplius-1
c. Nauplius-2
d. Nauplius-3
e. Nauplius-4
f. Nauplius-5
g. Nauplius-6
h. Copepodite-1
i. Copepodite-2
j. Copepodite-3
k. Copepodite-4
l. Copepodite-5
m. Adult female
n. Adult male

Fig. 61. *A. cmfri*

a. Female b. Male

Fig. 62. *A. cmfri* (Figures from Loka *et al.*, 2017)

a. Female b. Male

Fig. 63. Developmental stages of *Apocyclops cmfri*

a. Eggs
b. Nauplius-1
c. Nauplius-2
d. Nauplius-3
e. Nauplius-4
f. Nauplius-5
g. Nauplius-6
h. Copepodite-1
i. Copepodite-2
j. Copepodite-3
k. Copepodite-4
l. Copepodite-5
m. Adult female
n. Adult male

Fig. 64. *D. oculata*

a. Female b. Male

Fig. 65. *D. oculata*

a. Female b. Male

Fig. 66. Developmental stages of *Dioithona oculata*

a. Eggs
b. Nauplius-1
c. Nauplius-2
d. Nauplius-3
e. Nauplius-4
f. Nauplius-5
g. Nauplius-6
h. Copepodite-1
i. Copepodite-2
j. Copepodite-3
k. Copepodite-4
l. Copepodite-5
m. Adult female
n. Adult male

Fig. 67. *Dioithona* sp.

a. Female b. Male

Fig. 68. *Dioithona* sp.

a. Female b. Male

Fig. 69. Developmental stages of *Dioithona* sp.

a. Eggs
b. Nauplius-1
c. Nauplius-2
d. Nauplius-3
e. Nauplius-4
f. Nauplius-5
g. Nauplius-6
h. Copepodite-1
i. Copepodite-2
j. Copepodite-3
k. Copepodite-4
l. Copepodite-5
m. Adult female
n. Adult male

Fig. 70. *E. acutifrons*

a. Female b. Male

Fig. 71. *E. acutifrons*

a. Female b. Male

Fig. 72. Developmental stages of *Euterpina acutifrons*

a. Eggs
b. Nauplius-1
c. Nauplius-2
d. Nauplius-3
e. Nauplius-4
f. Nauplius-5
g. Nauplius-6
h. Copepodite-1
i. Copepodite-2
j. Copepodite-3
k. Copepodite-4
l. Copepodite-5
m. Adult female
n. Adult male

Fig. 73. *Amphiprion frenatus* larvae showing higher growth and brighter colouration in an experimental trial using copepod *T. turbinata*

a. Larva (control) on 8 dph
b. Larva (Control) on 30 dph
c. Larva fed with copepod on 8 dph
d. Larva fed with copepod on 30 dph

Fig. 74. *Hippocampus kuda* cultured using

a. Artemia and rotifer b. Copepods

Fig. 75. Orange spotted grouper larvae cultured on copepod nauplii as first feed

a. 8 dph b. 32 dph

Fig. 76. Indian pompano larvae cultured on copepod nauplii as first feed

a. 10 dph b. 24 dph

Acknowledgments

My sincere gratitude goes to Dr. A. Gopalakrishnan, Director, ICAR-CMFRI, Kochi for his great inspiration and constant persuasion which lead to the timely completion of this work. Dr. G. Syda Rao, former Director of CMFRI was a constant source of encouragement. This work would never have grown to the present scale, had it not been for the vision and dream of Dr. G. Gopakumar, former Head of Mariculture Division and Emeritus Scientist of CMFRI. Our previous Scientist-in-Charge, of Vizhinjam Research Centre of CMFRI, Dr. Rani Mary George was always around with her guidance and blessings. The unstinting support of Scientist-in-Charge, Dr. M. K. Anil, helped us to dive deep into the subject.

This work is the outcome of the efforts of many in CMFRI. Thanks are also due to Dr. Imelda Joseph, Head, Mariculture Division, Dr. P. Vijayagopal, Head, Marine Biotechnology Division and Dr. K. Sunil Mohamed, Head, Molluscan Fisheries Division, CMFRI, Kochi for their support throughout the course of this work. Dr. George Ninan, Principal Scientist of ICAR-CIFT, Kochi and his team, Dr. Kajal Chakraborty Principal Scientist, CMFRI and Dr. Sandhya Sukumaran, Senior Scientist, CMFRI have again proved their excellence in analysis works through their contributions here.

My sincere gratitude to Dr. Boby Ignatius, Principal Scientist and Scientist-in-Charge, HRD Cell, CMFRI, Kochi who has given his valuable suggestions in the manuscript and also for his constant support for popularizing the techniques developed at CMFRI, Vizhinjam. I also place on record of my gratitude to Dr. Shoji Joseph, Principal Scientist, CMFRI, Kochi who was always there with stock cultures of microalgae facilitating our research and also to Dr. K. Madhu and Dr. Rema Madhu, Principal Scientists, CMFRI, Kochi for their support and encouragements.

Had it not been for the effective field trials conducted by Dr. Shubhadeep Ghosh, Scientist-in-Charge and Scientists, Dr. Ritesh Ranjan, Dr. Sekar Megarajan, Dr. Biji Xavier and team from Visakhapatnam Centre of CMFRI, we would never have reached the present conclusions regarding the effective utilization of these copepod species in culture. The team consisting Dr. K. K. Philipose, former Scientist-in-Charge, Dr. Jayasree Loka, Scientist-in-Charge and their team from

CMFRI Karwar Centre, Dr. A. K. Abdul Nazar, Principal Scientist, Dr. R. Jayakumar, Scientist-in-Charge, Dr. K. K. Anikuttan, Scientist and team from CMFRI, Mandapam, were pillars of support throughout our work. Dr. Joe K. Kizhakudan Principal Scientist, CMFRI, Chennai and Shri. C. Kalidas, Scientist, CMFRI, Tuticorin Centre were always a part of our team. Dr. B. O. Prasad, Assistant Professor, Sree Narayana College, Kannur and Shri. S. Vinod, Kuwait Scientific Centre, Kuwait who were associated with the project during the years of its Initial conceptualisation also deserves a word of appreciation.

The support given by my colleagues Dr. S. Jasmine, Principal Scientist and Dr. K. N. Saleela Senior Scientist, my seniors Dr. A. P. Lipton, former Scientist-in-Charge and Emeritus Scientist and Dr. N. Ramachandran, Principal Scientist need special mention here. Technical officers, Shri, C. Unnikrishnan, Dr. H. Jose Kingsly, Shri. A. Udayakumar, Shri. P. Hillari, Shri. V. P. Benziger and Shri. S. Ramachandran Nair and Supporting staffs, Shri. V. Greever Yoyak, Shri. S. Satheesh Kumar and Shri. P. T. Jithesh of CMFRI, Vizhinjam were the part of this work from the beginning itself. My sincere gratitude goes to our fellow Scientists, Dr. P. S. Swathilakshmi, Smt. P. Gomathi, Smt. S. Surya and Sri. Ambareesh P. Gop for their contributions here. All Technical officers, Administrative staffs, Supporting staffs, Research scholars and Contractual staffs at Vizhinjam Research Centre of CMFRI helped us steer the project in the right direction even during tough times.

Research fellows associated with the project, Shri. F. Muhammed Anzeer, Shri. K. S. Aneesh, Shri. Mijo V. Abraham and Ms. S. Darsana, were the torch bearers in research on copepod culture.

My heartfelt gratitude to all the Scientists, Technical officers, Librarian and Library staffs, Administrative staffs, Supporting staffs, Research scholars and Contractual staffs of ICAR-CMFRI, Kochi for their constant support and encouragements.

My sincere gratefulness to Shri. Shibu Prabhakaran, Blackboard, Kochi for the splendid design and layout of this book.

My heart goes to Dr. S. Radhakrishnan, former Professor, Department of Aquatic Biology and Fisheries, University of Kerala, who has trained me in handling microscopic crustaceans which has given me confidence to identify and culture copepods. Lastly I bow to the Grace of Providence which was amply showered to strengthen us all.

B. Santhosh

Culture Techniques of

Marine Copepods

Copepods are the most important natural food for many fish and fish larvae. They are superior than many other live feeds for larviculture due to their small sized nauplii and better fatty acid composition. Copepods are complete with respect to nutritional requirements of the larvae and do not need any enrichment. Certain fish larvae are evolutionarily adapted for feeding copepod nauplii. So copepod culture forms an essential component in marine fin fish hatchery especially for initiating the first feeding of fish larvae.

But the major bottleneck for employing copepods as live feed is the lack of simple and reliable technologies for their mass culture in hatcheries. For the past ten years, the ICAR-Central Marine Fisheries Research Institute has been focusing on this aspect and has come out with simple and reliable technologies for mass production of nine species of copepods including the popular species from the genera *Parvocalanus, Bestiolina* and *Acartia*. These technologies have been described here which can be applied for the production of copepods in marine finfish hatcheries for successful larviculture.

भाकृअनुप
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ISBN 978-93-82263-23-4