



Original Research Article

Studies on different stages of post embryonic development of Cyclopoid copepod *Apocyclops dengizicus*

P.Anandan^{1*}, R. Krishnamurthy² and K.Altaff³

¹Department of Zoology, Kanchi Shri College of Arts and Science, Kilambi, Kancheepuram - 631 551, Tamil Nadu, India.

²Department of Advance Zoology and Biotechnology, Govt.Arts College, Nandhanam, Chennai - 600 035, Tamil Nadu, India.

³PG and Research Department of Zoology, New College, Chennai - 600 014. Tamil Nadu, India.

*Corresponding author: anandp2211@gmail.com

A B S T R A C T

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Studies on the morphology of the male, female and postembryonic development of estuarine cyclopoid copepod *A. dengizicus* were carried out. Zooplankton samples were collected from Adyar estuary *A. dengizicus* were sorted and subjected to taxonomic and postembryonic development studies. The adult male and female of *A. dengizicus* measure 677 ± 23 mm and $700\pm$ mm in length respectively. In ovigerous female a clutch of eggs undergo embryonic development in the ovisacs and the mean numbers of eggs recorded are 38 ± 5 . Postembryonic development of *A. dengizicus* includes six naupilar and six copepodid stages. The different post embryonic stages are described using photomicrographs and camera lucida diagrams. Morphological details of different stages and structure of their appendages are discussed in comparison with other copepods. The dimensions and durations of different stages of postembryonic development of *A. dengizicus* are documented. Multiple generations of *A. dengizicus* can be raised in the laboratory using yeast (*Saccharomyces cerevisiae*) as food, which suggest that *A. dengizicus* can be mass cultured. Different stages of postembryonic as well as adults of *A. dengizicus* can be used as live food for many commercially important marine cultivable species. Importance of production of indigenous live food aquahatcheries and the role of copepod naupili in promoting the production of marine fish larvae from marine fish culture point are discussed.

Introduction

Copepods are aquatic organism's inhabiting all types of water bodies such as fresh water, estuarine and marine ecosystem.

They are microscopic organisms and mostly exist as free living or parasitic forms. There are three groups of free living copepod (i) Calanoida (ii) Cyclopoida and (iii)

Harpacticoida. These show a remarkable ecological interest, since most species of these orders form the first link of the aquatic food chains. Calanoids dominate in the marine waters; cyclopoids in the fresh water and harpacticoids in the meiobenthic systems. These three orders contribute 5500 species (Bowman and Abele, 1982). With regard to species diversity and abundance of copepods are considered to be the most important group in fresh water zooplankton communities (Hardy, 1943). They occupy an important position in the trophic structure and play a role in the energy transfer (Sarkar *et al.*, 1985). A few species of copepods are used as indicator organisms for assessment of the water quality pollution stress (Dussart and Defaye, 1995).

Copepods are one of the fascinating groups of crustaceans which have attracted many investigators from all over the world. They constitute one of the important subjects of active research. Many aspects of this group such as distribution, taxonomy, population studies, food and feed and reproductive biology and mass culture to serve as live food organisms are current topics of interest. Cyclopoids are considered to be omnivorous as they utilize diverse type of food materials. However, there are many species of cyclopoids, which are predatory and capture moving prey. Sometimes, cyclopoids attack other copepods or exhibit cannibalism by capturing their naupili. The mouth parts of cyclopoids have been modified for seizing and biting. The mechanism of food handling and ingestion of prey by the cyclopoids is provided by number of investigators (Schoeneck *et al.*, 1990; Antony and John, 1990; Adrin and Frost, 1992; Wickham, 1995).

In copepods fertilized eggs are either released to the medium or deposited in an ovisac where development takes place.

Development is the period from extrusion of egg from oviduct to hatching into naupilus. Duration of embryonic and postembryonic development was studied by many authors. In calanoids by Abdullahi (1990) in cyclopoid copepods by Vijverberg (1977) Herzig (1983) and Maier (1989) and harpacticoids (Dahms *et al.*, 1991; Goswami, 1977). Histological investigations on embryonic development has been carried out by Zehra (2000) on *Mesocyclops aspericornis* and by Sujatha (2000) on *Cryptocyclops bicolor*.

Copepods are the main food source of marine fish larvae in nature (Mc Michel and Peten, 1989). In aquaculture, copepods have been proven to be the preferred and most adequate food for fish larvae (Kraul, 1993), and are also used for shrimp larvae and post larvae (Shamsudin and Saad, 1993). Good results in terms of fish larva growth and survival using natural plankton are due to the presence of copepods and their role as the main food component (Bent, 1993). Keeping in this view, the present study was undertaken with the objective of making detailed investigations on the developmental stages of cyclopoid copepod *Apocyclops dengizicus*.

Materials and Methods

Zooplanktons were collected from Adyar estuary using plankton net made up of bolting silk with 50mm mesh size. By towing horizontally for about 40-50 times at a depth of two feet. Zooplanktons were collected during early morning hours or late evening hours of the day. Samples were collected in insulated polyethylene containers after filtering through the plankton net and transported to the laboratory within an hour.

Different groups of copepods were separated under binocular stereo zoom dissection

microscope using a fine brush and needle. *Apocyclops dengizicus* were identified based on the minute morphological details and taxonomic key characters provided by Mass (1994) Dussart and Defaye (1995) and Huys *et al* (1996).

For taxonomic and morphological studies of animals were narcotised using 40% alcohol. They were then fixed and preserved in 5% formalin. Animals with straight body and undamaged appendages were selected for taxonomic and morphological studies. Whole mounts as well as appendages of taxonomic importance were prepared using Borax carmine or eosin stain. Larval forms were also studied.

Live *A. dengizicus* were maintained in pretreated water (habitat water filtered in 50 mm bolten silk and allowed to stand in large aquaria at room temperature $29\pm 1^{\circ}\text{C}$ for 48 hours and aerated for 12-24 hours prior to use). They were fed with yeast *Saccharomyces cerevisiae* at concentrations of 0.2ppt daily.

Laboratory reared ovigerous females were maintained individually in 10ml of pre-treated habitat water. After hatching and release of nauplii from ovisac, the female and nauplii were separated using 150mm nylon sieves and the development of nauplii was followed by fixing them in 5% formalin at regular intervals of 6-12 hours and copepodid development at intervals of 12-24 hours. The experiments were continued until the copepodid attained adult's stage. They were fed with yeast at a concentration of 0.2ppt daily. The general morphology and characters such as number of appendages, number of segments and number of setae were studied by drawing camera lucida diagrams. The different naupilar and copepodid of *A. dengizicus* were photomicrographed. Durations of the

post-embryonic development is also documented.

Results

Morphology of adult female and male

Prosoma of this species has rounded from anteriorly in dorsal view. From anterolateral view rostrum appears to be blunt. The genital segment is as long as wide. The adult female length and width are $700\pm 28\text{mm}$ and $255\pm 20\text{mm}$ respectively. The genital segment measures $120\pm 10\text{mm}$ in length and $80\pm 5\text{mm}$ in width. Length and width of adult male are $677\pm 23\text{mm}$ and $210\pm 10\text{mm}$ respectively.

Antennule is 11 segmented; it is upto the length of 4th segment of the thorax. Antennae are two segmented and has 7 setae in the 1st segment on the inner surface. The second segment has 3 stout and 3 filamentous setae proximally. Exopodite reduced to setae.

Labrum has rounded teeth. Mandible is simple and has blunt teeth. Maxilla is 2 segmented. The terminal segment of maxilla consists of spinulose long seta and short seta, while maxilliped is 3 segmented, distal segment of maxilliped is 3 segmented, distal segment has 2 spines and terminal segment has 2 long spinulose setae. Maxillule of inner lobe constitute 3 equal spines without denticules. The outer lobe has 4 thinner setae.

Embryonic development

During mating the male *A. dengizicus* holds the female at its caudal region with its geniculate antennules then the male moves forward and holds the female firmly with both the antennules. The female becomes inactive and movement of both the animals is brought about by the activity of the male,

sometimes during this position both the animals remains still. The male then shifts itself to a position where the genitalia of female and the genital pore of the male face ventrally. During this activity the male flexes its genital segment, which extrudes a pair of spermatophores from the spermatophore sac. When an exact mating position is attained the male vigorously flexes its body, which bring the spermatophore closer to the gonophores and attaches the same to the genital segment of the female. The whole process of mating and transfer of spermatophore takes about 30 to 50 minutes.

In *A. dengizicus* fertilized eggs are deposited in two ovisacs, which are attached to the lateral side of the genital segment and the number of eggs are 38 ± 5 in a clutch. Embryonic development takes place in this ovisac. During cleavage the egg undergoes first two divisions to give rise to 4 similar sized cells. Thereafter active cleavage takes place in the peripheral region compared to the central part of the egg. As a result of this, two of the cells, which are smaller in size, are arranged peripherally while cells of central region are fairly bigger in size. After blastulation morphogenesis movement of the blastomeres transform the blastula into an oval shaped gastrula, which further transforms into a naupilius with 3 pairs of appendages. In this species duration of embryonic development is 18 to 24 hours. After embryonic development nauplii hatch out from the ovisac.

Post embryonic development

The life history of *A.dengizicus* includes six naupliar and six copepodid stages, the last being the adult. The nauplii pass through the six stages to become the copepodid and at every stage, they undergo a single moult. The duration of each nauplius stage is about

12-24 hours and the copepodid takes 24 hours, and finally, the adult appears on the 10th day. Generally nauplius is oval in shape with three pairs of appendages. The length and width of nauplius one to six range between 120 to 294 mm and 75 to 168 mm respectively (Tabel.1).

Different stages of Nauplius development

Egg hatch into nauplius I within 24 hours of the female becoming ovigerous. The nauplius I is a typical cyclopoid nauplius having an egg shaped body which is dorso ventrally flattened. The length and width are 120mm and 75mm respectively.

Table.1 Development and durations of Postembryonic development of *Apocyclops dengizicus*

S. No	Stage	Duration (Hours)	Length (mm)	Width (mm)
1.	N1	12	120	75
2.	N2	12	190	110
3.	N3	12	210	130
4.	N4	12	230	142
5.	N5	24	248	154
6.	N6	24	280	160
7.	C1	24	400	160
8.	C2	24	440	168
9.	C3	24	540	200
10.	C4	24	600	210
11.	C5	24	630	210
12.	C6 (Female)	24	700	231
13.	C6 Male)	24	677	210

It consists of three pairs of appendages, antennae, antennules and mandible. The average length and width of nauplius II is 190mm in length and 110mm in width. This stage resembles nauplii I all the characters.

The nauplius II moult into nauplius III which measures about 210mm in length and 130mm in width respectively. The antenna bears additional terminal setae. The fourth stage measures about 230mm in length and 142 mm in width and the body is broader and narrow posteriorly. It measures about

248mm in length and 154mm in width. Rudiments of maxilla and maxillipeds are noticed. The length and width are 28mm and 160mm respectively. The antennule bears 13 setae terminally. The swimming leg protrudes in the form of lobes. Additional segment or setae are observed in the cephalic appendages.

Different stages of Copepodid development

The Copepodid I measures about 400mm in length and 160mm in width respectively. The body shape is similar to an adult with broader four segmented anterior prosome and a posteriorly narrower unsegmented urosome. The antennule is 6 segmented. Two pairs of biramous swimming legs are present. Copepodid II stage has an average length of 440mm and width 168mm and urosome becomes 2 segmented. The antennules are 7 segmented. Three pairs of swimming legs are seen.

The third Copepodid stage measures an average length 540mm and 200 in width. The urosome is three segmented. The antennule is 9 segmented. The fourth swimming leg makes its appearance in this stage. Copepodid IV stages the Copepodid measures about 600mm in length and 210mm in width. Antennule is further developed with 10 segments. Copepodid V stage measures about 630mm in length and 210mm in width. The antennules become 10 segmented. The cephalic appendages are well developed. The sixth legs are represented by a pair of spine. The length and width of females Copepodid measures 700mm and 231 mm and male measures 677mm and 210 mm respectively. This is the adult stage and sexual differentiation is noticed by the geniculation of male antennules. The fifth and sixth cephalic

appendages are well developed. The antennules bear eleven segments.

Discussion

Zooplankton are irregularly distributed vertically in the surface 1000-1500 m of the oceanic column, there being layers of increased concentration and decreases exponentially towards the depth and normally dominates the offshore plankton with numerical abundance (Koppedelmann and Wekert, 1992). Of all the marine zooplankton, copepods are the most familiar and comprise at least 70% of the planktonic fauna. Copepods are considered to be the world's most abundant metazoans (Mauchline, 1998). Cyclopoid copepods include free-living, associates and parasitic form. Due to their various modes of life, habitats they live in, life cycle strategies and food, feeding habits there has been greater variations in their body and structure of their appendages.

With regard to the taxonomy of cyclopoids characters such as number of segments in the antennules, type of processes, structure of antenna, mandible, maxillipeds, structure of segment of exopod of swimming legs. Based on these characters five families and seven genera are reported from Indian waters (Kasturirangan, 1963). A strong interest in marine cyclopoid copepods has developed in recent years, along with the increasing awareness of their high abundance in almost all oceanic environments and often numerical dominance within the metazoan plankton (Paffenhofer, 1983; Paffenhofer *et al.*, 1987; Peterson *et al.*, 1988; Bottger-Schnack *et al.*, 1989; Hay *et al.*, 1991; Nielson *et al.*, 1993). Post embryonic development of some of the harpacticoids such as *E.acutifrons* (Tesch, 1915; Rao, 1958; Haq, 1965; Fanta, 1972; Goswami, 1976) *Tisbe* species

(Johnson and Olson, 1948; Vilela,1969; Chua,1975; Park,1976; Lopez, 1980, Dahms *et al.*, 1991) and some other species have shown species specific variations and also variations pertaining to different geographic locations.

As a preliminary step towards their culture results of experiments of present study on the postembryonic development of *A. dengizicus* are encouraging as this species shows, higher survival rate and short durations of post embryonic development. Due to their high nutritive value of copepod naupli than those of *Artemia* and rotifers, *A. dengizicus* naupli can promote higher growth and survival of the larvae of many commercial important cultivable species. Perhaps, naupli of *A. dengizicus* can be an ideal starter food for many marine fish larvae. Development of such as indigenous live food might help in the replacement of the import of *Artemia* cyst. This kind of aquaculture practice might also help increase the number of cultivable species, in general and marine fishes in particular. Thus, there can be a sustain development of aquaculture which is cost effective, mass oriented and eco-friendly.

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