

GROWTH OF AN ESTUARINE HARPACTICOID COPEPOD
NITOCRA SPINIPES BOECK CULTURED IN THE LABORATORY

SARAMMA ABRAHAM AND U. K. GOPALAN

National Institute of Oceanography Regional Centre, Cochin - 682018

ABSTRACT

A harpacticoid copepod, *Nitocra spinipes* Boeck occurring in Cochin Backwaters was cultured in the laboratory on a diet of detritus supplemented with phytoplankton. Its life cycle, comprised of six naupliar and five copepodid stages, the sixth following the latter being the adult, has been described in detail. Development from egg to adult took 17-19 days. Number of eggs in a brood varied from 10-20. The possibility of mass culture of the species for feeding larval fish is also discussed.

INTRODUCTION

Copepods are an essential link in the food chain of aquatic systems, particularly as they form food for the early stages of several species of fish and prawn. In view of growing needs for the production of large quantities of larval fish and shrimps for aquacultural purposes, it becomes necessary to evolve methods for mass culture of relevant food organisms. Studies on growth efficiency and life cycle of such organisms as copepods, under laboratory conditions enable us to evaluate their applicability in mass culture.

In a recent review on the cultivation of copepods, Omori (1973) has mentioned that so far only 30 species of copepods, 20 calanoids, 1 cyclopoid and 9 harpacticoids have been reared for one or more filial generations under laboratory conditions. Studies on continuous culture have not been done in India, though some rearing work has been carried out mainly to obtain the sequential stages in the life cycle; (Krishnaswamy, 1950; Rao, 1958; Ummerkutty, 1960 and Goswamy, 1972).

Harpacticoids cultured so far come under three genera, *Euterpina*, *Tigriopus* and *Tisbe* (Omori, 1973). The present paper describes the various growth stages, from egg to adult of *Nitocra spinipes* Boeck collected from Cochin Backwaters and cultured in the laboratory during 1973-'74.

MATERIAL AND METHODS

Zooplankton samples collected from Cochin harbour area were kept alive in the laboratory. Egg bearing females were sorted out and transferred to half filled cylindrical glass vessels of 10 litre capacity with circulation of natural water effected by an air bubble water pump similar to that of planktonkriessel described by Greve (1970). A small quantity of a mixed culture of phytoplankton dominated by *Nitzschia* was inoculated into the medium which was also enriched by the addition of soil extract. About 500 mg. of detritus filtered out from the collection ground following the method adopted by Heald (1971), dried and stored in refrigerator was added to the culture once in a fortnight.

Temperature of the culture medium fluctuated between 27.5°C and 31.5°C but the salinity was steadily maintained at 15‰ \pm 1‰ by the addition of distilled water. Once in every month half the medium was replaced using a siphon fitted with nylon gauze. Excess of food accumulating at the bottom was also removed occasionally.

From the laboratory-acclimatised population subcultures were maintained in glass jars on similar diet. Days required for the development from egg to adult was determined by isolating ovigerous females in small beakers where nauplius hatched out. The morphological development was observed by collecting and examining the moults and instars regularly.

OBSERVATIONS

From a few individuals in the beginning the population in the culture vessel reached a density of 10000 individuals/litre (excluding nauplii) in 40-45 days. Larval forms as well as adults preferred to creep on the bottom and walls of the container. Naupliar stages exhibited a peculiar form of movement resulting in the complete vibration of the body. Occasionally, they were found attached to floating phytoplankters and feeding on them. Claspings or mating in the manner described by Johnson and Olson (1948) in *Tisbe furcata* started from the 5th copepodid stage. Single egg sac contained usually 16-20 eggs and the minimum was ten. Growth from egg to 6th naupliar stage took 6-7 days and that to adult was completed in 17-19 days.

Life cycle includes 6 naupliar (figs. 1-6) and 5 copepodid stages, the sixth following the latter being the adult. Sexes were distinguishable from copepodid I onwards.

Stage 1 or orthonauplius (Fig. 1): Measures about 0.09 mm. Rounded in shape with slightly flat anterior end. Mouth large. 3 pairs of appendages;

antennule, antenna and mandible. Antennule 3 - segmented; terminal segment bearing 4 setae, 3 long at apex and 1 small at mid-length; 1st and 2nd segments each with 1 short marginal seta. Antenna biramous; basipodite 2-jointed, the joint facing the mouth armed with masticatory denticles; exopodite 3-jointed with long terminal and short marginal setae, endopodite consists of a cylindrical joint bearing terminally a strong movably articulated claw. Mandible biramous; basipodite flat, thick with serrated inner margin; exopodite 2-jointed, 1st joint bearing two serrated setae, one at proximal and the other at distal inner margins, second joint with a long hairy apical seta and a very short marginal seta; endopodite widebased bearing 5 finger-shaped setae at the inner margin towards the mouth. posterior margin of the body slightly ovate with a pair of small setae representing caudal armature.

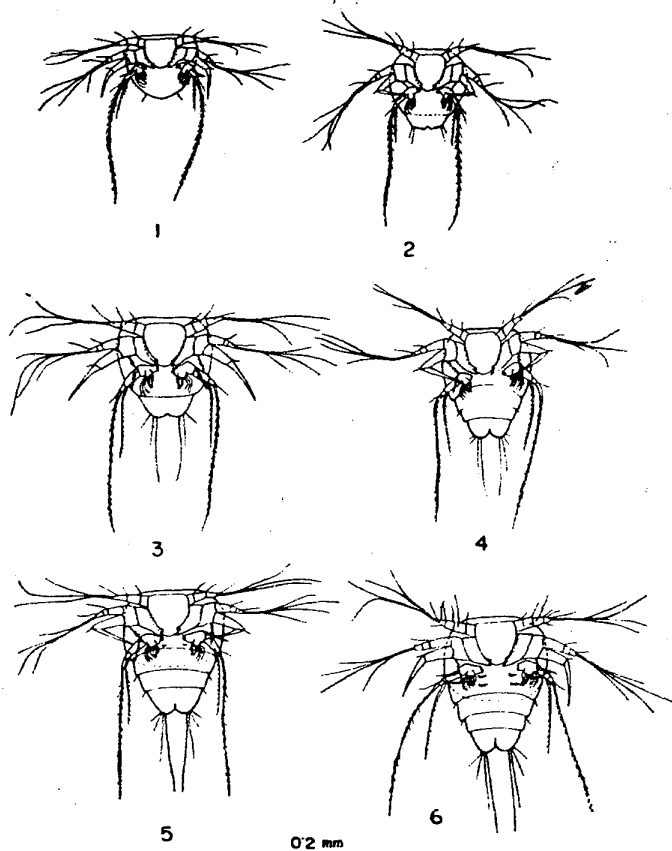
Stage 2 or metanauplius (Fig. 2): 0.11 mm. General structure of appendages remains the same but with more clearly defined shape. Body ovate, posterior margin serrated bearing a pair of setae on either side; postero-lateral margins with constriction that tends to deepen transversely. Anal cleft visible.

Stage 3 (Fig. 3): 0.14 mm. Body gets differentiated into an anterior broad and a posterior narrow portion by segmentation. Rudiments of caudal branches appear as anal cleft deepens. Caudal armature consists of a long inner seta and a short outer seta on either side.

Stage 4 (Fig. 4): 0.17 mm. Size increased and shape changed into a long oval form. Body shows 3 clear divisions, the middle one showing lateral constriction indicating the formation of another segment. A pair of setae present at the lateral constriction of the middle segment seems to be the rudiment of 1st pair of swimming legs. Anal cleft further deepens. Caudal armature shows additional setae, each side having 4.

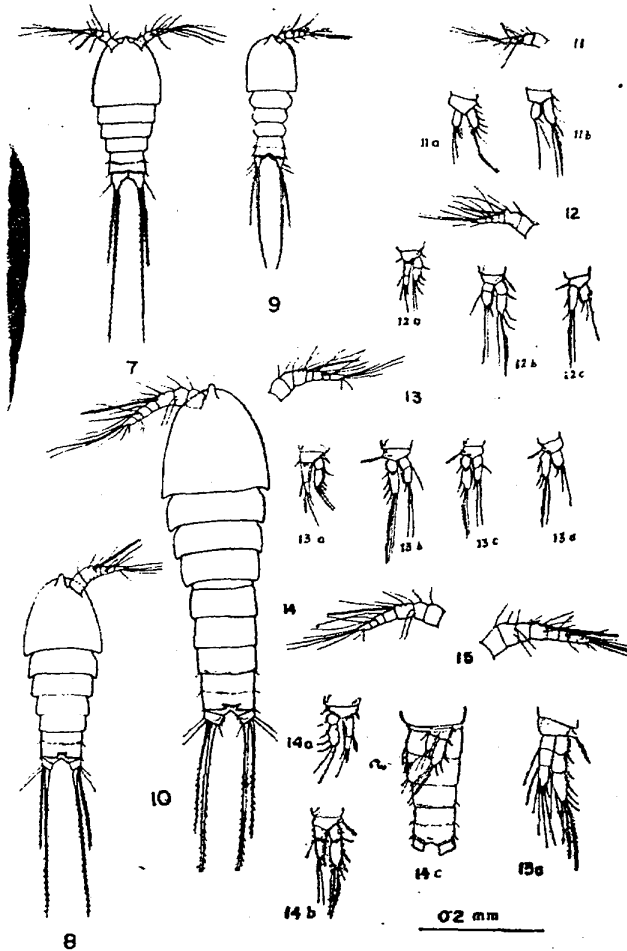
Stage 5 (Fig. 5): 0.18 mm. Body longer, consists of 4 clear segments, 2nd and 3rd segments bear a pair of short setae on lateral margins. Rudiments of maxillules appear as small pinnate processes just behind mandible. Caudal armature well developed.

Stage 6 (Fig. 6) 0.2 mm. Antennule with an additional segment, making it 4-segmented; first 3 segments bear a small seta at dorso-distal end of each joint while 2nd bears an additional seta at anterior distal end, the last segment carries 4 long apical setae and 3 short marginal setae. Maxillules, maxillae, maxilliped and first 2 pairs of swimming legs make their appearance as rudiments. Body segments 5 in number, with division between 1st and 2nd segments clearly seen only on lateral margins just behind the mandible. Caudal branches large and prominent with deep middle cleft and well developed caudal armature.



Figs 1-6. *Niocra spinipes* Boeck Naupliar stages, 1st - 6th

Copepodid-Stage-1 (Fig. 7): 0.23 mm. Rostrum well developed. Body consists of 5 segments; 3-segmented prosome and 2-segmented urosome. Caudal fork resembles that of almost sexually mature specimens in form and armature but slender carrying abnormally developed setae. Antennule with 5 joints; 2nd joint with a stout strong seta, probably the one to form the sensory cylinder, (fig. 11). Antenna 3-jointed. All mouth parts present and resemble those of adult in their basic pattern. 2 pairs of legs; each leg with exopod and endopod of one joint only. Head segment carries 1st swimming leg as 1st thoracic segment gets fused with head. 2nd leg on 1st of 2 free thoracic segments; 3rd represented by a pair of setae on 2nd thoracic segment.



Figs. 7-15a - *Nitocra spinipes* Boeck. 7, Copepodid I; 8, Copepodid II; 9, male copepodid I; 10, Copepodid IV; 11-11b, appendages of copepodid I. 11, antennule; 11a, 1st swimming leg; 11b, 2nd swimming leg; 12-12c, appendages of copepodid II. 12, antennule; 12a, 1st swimming leg; 12b, 2nd swimming leg; 12c, 3rd swimming leg; 13-13d, appendages of copepodid III. 13, antennule; 13a, 1st swimming leg; 13b, 2nd swimming leg; 13c, 3rd swimming leg; 13d, 4th swimming leg; 14-14c, appendages of copepodid IV. 14, antennule; 14a, 1st swimming leg; 14b, 2nd swimming leg; 14c, female urosome, lateral view showing rudimentary 5th leg as 2 small lobes; 15 and 15a appendages of copepodid V. 15, antennule; 15a, 2nd swimming leg.

Male copepodid 1. (Fig. 9) with the same structural details but with a different body form and shape. Body slender and long, lateral margins of segments bulged; 2nd urosome segment long. The longest caudal setae as long as body. Antennules bear very strong thick setae. General appearance makes the the male copepodids easily distinguishable from female copepodids.

In the advanced stage of 1st copepodid, the internal formation of the 3rd thoracic segment is visible as a division of the 2nd thoracic segment.

Stage 2 (Fig. 8): 0.28 mm. Body with 6 segments, 4 prosomal and 2 urosomal. General appearance of body the same as in copepodid I, but thicker. Antennule 6-segmented, 2nd segment rather big with lateral constrictions (but not transverse) (Fig. 12). The sensory cylinder makes its appearance on this segment for the first time; 3rd segment very narrow and the last, long and slender bearing long apical setae and short marginal setae. All mouth parts show adult structures. 3 pairs of swimming legs, rami of first two legs 2-segmented and those of third, one segmented. Protopodites of these legs vaguely 2-segmented with a few spines. Rudiments of 4th swimming leg represented by a pair of setae on last thoracic segment.

In male Copepodid II also, sensory cylinder appears on second antennular segment but more thick and strong.

Stage 3 (Fig. 13): 0.33 mm. Body composed of 7 segments, the number of prosomal segments increased to 5. Thoracic segments develop lateral epimeral plates, wider than urosome segments. Antennule 7-segmented sensory cylinder on 3rd segment resulted from deepening of the constriction on 2nd antennular segment of previous stage. 4 pairs of swimming legs and rudiment of fifth; 1st pair rather well developed; 2nd pair with an additional spine on distal joint of exopodite. Rami of first three pairs of legs 2-segmented, those of 4th only one segmented. Rudiment of 5th on last thoracic segment, in the form of a pair of setae on a small peg-like projection. Protopodite of all 4 pairs of legs clearly 2-segmented, with spination as shown in figures 13a to 13d.

Stage 4 (Fig. 10) 0.41 mm. Body 8-segmented clearly demarcated into prosome, and urosome, prosome 5-segmented (including head) and urosome 3-segmented. 5 pairs of swimming legs; rami of 1-4 still 2-segmented, 5th leg consists of a pair of lobes representing exopodite and basoendopodite (Fig. 14c); the former ovate bearing 5 short apical setae, 2nd from outer margin and the inner most longer. Basoendopodite stumpy with 2 small apical setae near outer margin. External lobe small bearing a short nude seta. A pair of setae on a small peg-like projection on 1st urosomal segment represents the 6th leg. Antennule 7-segmented, 3rd segment large bearing lateral constrictions and sensory cylinder, (Fig. 14).

In corresponding male stage 1st urosomal segment bears a single seta representing 6th leg.

Stage 5 (Fig. 15): 0.52 mm. A segment gets added to urosome making the number 4. Thoracic segments wider. Antennule 8-segmented, proximal constriction of 3rd segment of previous stage deepened adding one more segment behind the segment carrying sensory cylinder. Legs 6-pairs; rami of 1-4 pairs 3-segmented with the same setation of adult but without adult development, 5th pair rather well-developed. 6th leg disappears after the next moult.

This stage resembles the adult female in the number of body segments and swimming legs but is distinguishable by the under developed nature of swimming legs, presence of rudimentary sixth pair of legs and transverse row of spinules along the ventral side of last urosomal segment.

At the next moult the male gains a fifth urosome segment, the female loses the sixth leg and its genital segment becomes large and prominent.

General features of both sexes of adult *N. spinipes* have been described by Boeck (1864), and recently by Borustskii (1964). The present specimens agree with *N. spinipes* var. *orientalis* recorded from Chilka Lake by Sewell (1924) except in minor Variations such as the presence of a peg-like projection on the fourth segment of male antennule, longer endopodal first joint of leg I and a markedly clear demarcation for the caudal fork other than by anal plate which is also well-defined.

Adult female: Total length 0.75 mm. Head bears a very conspicuous eye spot. Cephalothorax about the same size as abdomen (Proportion about 1:1). Relative length of head and body 1:3. Thoracic segments with epimeral plates. 5th thoracic segment articulates with the preceeding segment and very distinct in dorsal and lateral margins with dorso-lateral rows of spinules on both sides. Genital segment large bearing completely closed row of spinules at middle and distal regions. Such closed rows of spinules occur in mid-region of 2nd and 3rd abdominal segments. Anal segment shorter than preceeding one, deeply cleft between furcal rami and bears a pair of stout hairs at the cleft. Anal flap curved, set with strong spinules (8 on the middle curve and 10 each on either side). Furcal rami wider than long with 5 setae.

Adult male: Total length 0.45 - 0.56 mm. Cephalothorax slightly shorter than abdomen, proportion being 1:1.25, General shape of body almost as in female but slender. Urosome with an additional segment. Anal segment less distinct with small spinules.

In the general morphological features of various stages *N. spinipes* is a typical harpacticoid. This species has only one orthonauplius which passes directly to metanauplius unlike as observed in primitive families (Borutskii, 1964).

Developmental stages of the present species closely resemble those of *Epactophanes richardi* (Borutskii, 1964) but vary from those of *Tisbe furcata* (Johnson and Olson, 1948); *Macrosetella gracilis* (Krishnaswamy, 1950) and *Tisbintra janesi* (Ummerkuty, 1960). In *N. spinipes*, a gradation can be traced in the development of antennular segments and sensory cylinder. Second segment of the antennule is the proliferating segment. Both sexes possess the same number of antennular segments; the male having a peg-like projection on the fourth segment. The number of antennular segments differs with sexes (♂ and ♀) both in *Tisbintra janesi* and *Macrosetella gracilis*. The position of the peg-like projection on antennule differs with sexes in *T. janesi*; on the third segment in female and on the fourth segment in male. In *T. janesi* the sensory cylinder of the antennule appears on the third segment in the first copepodid but goes back to the second in the subsequent stage and finally reaches the fourth in the adult.

In *N. spinipes*, segmentation of body of the nauplius starts with second stage and continues in sequence up to sixth stage. In every moult, the nauplius showed gradual development in size and other morphological features, but the three oral appendages remain unchanged in their basic pattern. Maxillule, maxilla and maxilliped develop at 5th and 6th naupliar stages. In *T. janesi*, segmentation of the naupliar body starts at a later stage while maxillule and maxilla appear at an early stage.

Results of the culture experiments indicate that *N. spinipes* is comparatively easy to rear in the laboratory. The rate of multiplication of the species (10000 individuals/litre in 40-45 days) compares well with one of the species used for mass culture, *Tigriopus japonicus*. (Hanaoka, 1973), the copepodid II and older stages of which increased to about 12500 individuals within 40 days on a diet of dry foods. It has been observed in the laboratory that the present species could tolerate salinities 5-40‰. In nature maximum number of ovigerous females occur in the post monsoon months though they are found to breed throughout the year.

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