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BREEDING BIOLOGY OF THE FRESHWATER COPEPODA, *HELIODIAPTOMUS VIDUUS* (GURNEY) AND ITS PROSPECTS AS LIVEFOOD ORGANISM

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The tropical freshwater copepoda, *Heliodiaptomus viduus* occur commonly in the peninsular India. This species is comparatively bigger (total mean length of female and male is 2.05 ± 0.09mm and 1.7 ± 0.04mm respectively) than other freshwater diaptomids. Aspects of reproductive biology such as sexual dimorphism, organisation of female and male reproductive system, oogenesis, spermatogenesis and spermatophore formation are described for the first time. Details pertaining to fertilization, embryonic and post embryonic development of this specie is reported. Studies on live span and reproductive potential of this specie indicate continuous breeding with short interclutch period. Importance of the livefood in aquahatcheries and prospects of *H.viduus* as alternate livefood to *Artemia* nauplii is discussed.

Key words: Tropical copepoda, Breeding biology, Livefood, *Heliodiaptomus viduus.*

Introduction

Experimental studies on the rearing of shrimp and fish larvae with live and artificial food clearly indicate that better survival and growth are achieved only with livefood (New 1976; Kahan 1984, 1992; Chen 1991). *Artemia* nauplii are the most common and intensively used livefood in hatcheries. As livefood, *Artemia* has many advantages over other livefood organisms such as occurrence of encysted eggs, suitably sized nauplii, their slow movement and rich and suitable biochemical composition. However, currently available *Artemia* has many constraints such as ever increasing cost of the cyst, depletion in the harvest of cyst, declining percentage of hatching, larger size of nauplii and a decrease in the essential aminoacids and polyunsaturated fatty acids content. The complete dependence on this over exploited animal may pose problems for sustainable aquaculture in the future. Hence other species were also considered as an alternative to *Artemia* nauplii. In this regard, *Brachionus, Daphnia* and *Moina* may also be mass cultured to the required level (Watanabe *et al* 1983).

Copepoda constitute one of the dominant groups of zooplankton and occur in all types of aquatic habitats (Wilson and Yeatman 1959). Recently, there are some reports on the importance of this group as livefood (Kraul *et al* 1991; Vilela 1992; Tawfiq *et al* 1997).

Taxonomy (Rajendran 1973) and distribution (Battish 1992) of this group is reported, however, investigations on biology of this group are scarce. Although many reports were published on the reproductive biology of marine copepoda (Fahrenbach 1962; Park 1966; Hopkins 1978, 1982; Razouls *et al* 1986) but there is no detailed investigation on any tropical freshwater copepoda. Following our earlier studies on biochemical composition (Altaff and Chandran 1989) and food and feeding habits (Altaff and Chandran 1995) on *Heliodiaptomus viduus*, reproductive biology and development of this specie is described in this paper. These studies can be useful in evolving protocol for mass culture of this specie and utilization as livefood for finfish and shellfish larvae.

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Materials and Methods

Zooplankton were collected from the fish pond of the Hydrobiological Station, Tamil Nadu State Fisheries Department, Chetput, Chennai using a plankton net (mesh size 100 μm) and maintained in filtered (mesh size 40 μm) pond water in the laboratory.

The diaptomid *H. viduus* was separated from the plankton sample and identified following the description of Rajendran (1973). For these studies specimens were first narcotised with 20% ethanol and then preserved in 5% formalin. Whole mounts of the animals and appendages were prepared in glycerine or polyvinyl lactophenol.

For viewing the intact reproductive system *in situ,* the borax carmine staining method of Pantin (1964) was adopted. In order to ascertain the correct shape of the gonads and genital duct, the specimens were dissected in glycerine under a stereo-dissection microscope (x30) and the reproductive system was separated and observed under Nikon research microscope (x1000).

For histological studies, narcotised animals were fixed in Bouin's fluid and dehydrated in ascending series of alcohol. The specimens were cleared in methyl salicylate and embedded in paraffin wax. Serial sections, 5-7 μm thick were taken using Errma rotary microtome and stained with Ehrlich's haematoxylin, with aqueous eosin as a counter stain (Patki *et al* 1987).

For post embryonic development studies, ovigerous females were separated and maintained individually in 100 ml glass containers. After hatching of eggs, the females were removed from the container and the development of nauplii and copepodid stages were monitored. Representative specimens for each developmental stage were fixed in 5% formalin at regular intervals to record their duration. Morphometric measurements of ten larvae were made with ocular micrometer calibrated with a stage micrometer. Baker's yeast was used as larval food at a concentration of 0.1 ppt. For lifespan and egg production studies laboratory reared pair of adults were individually maintained in 250 ml glass beakers and fed with cowdung solution at a concentration of 2 ppt on alternate days. In addition to this mixed algae were also provided. Ovisac formation and hatching was carefully monitored to record interclutch period and number of ovisacs produced. Experiments were continued upto the ceasation of egg production or death of the animal. All the experiments were carried out in triplicate and mean and SD of ten measurements were given in μm.

Results and Discussion

Heliodiaptomus viduus is an elongate, cylindrical and spindle shaped copepod. Mean length of female and male is 2.05 ± 0.09 and 1.7 ± 0.04 mm respectively. The right antennule of the male is geniculate and the fifth legs of both the sexes are modified for mating and spermatophore transfer.

The female reproductive system consists of a single median ovary and a pair of genital ducts which pass through the perivisceral cavity and open to the exterior by a common reproductive pore on the ventral side of the first urosomal segment. The ovary rests on the dorsal surface of the foregut. Oviducts originate from the lateral sides of the ovary and give rise to an anterior diverticulum on either side before running posteriorly. At the end of the prosome, the oviduct leads to an oviducal gland (described in detail elsewhere, Altaff and Chandran 1994) which inturn leads to the distal part of the oviduct (Fig 1). Histologically the wall of the ovary is made up of an external connective layer and an internal syncytial layer of epithelium. The germinal zone is restricted to the posteriormost part of the ovary where germinal cells characterized by a large nucleus occur. The different stages of developing oocytes such as oogonial cells, primary oocytes and secondary oocytes occur in ascending rows from the posterior to the anterior end of the ovary. The anterior most part of the ovary contains oocytes mostly in the germinal vesicle stage (Fig 2). The oviduct contains two batches of oocytes, a batch in vitellogenesis and maturation and another previtellogenic batch. During vitellogenesis of the oocytes in the oviduct, oviducal gland produces secretory material, which occurs as elastic sac in the oviducal gland (Fig 3). Secretory materials are also produced by the distal part of the oviduct. At the end of vitellogenesis mature oocytes are packed with yolk granules and globules (Fig 4).

The male reproductive system consists of a single median testis and a genital duct which passes through the left side of perivisceral cavity and opens to the exterior through a lateral slit in the first urosome segment. The genital duct is distinguishable into a vas deferens, a seminal vesicle, a spermatophore sac and a ductus ejaculatorius (Fig 5).

The testis is an elongate organ broad at the anterior end and narrow at the posterior end. The wall of the testis is non-muscular and consists of outer connective tissue and an in-

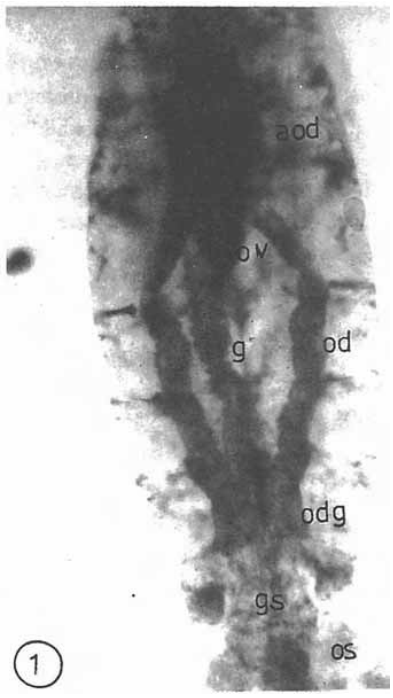

Fig 1. *Heliodiaptomus viduus*. Female reproductive system *in situ*. and, anterior diverticulae of oviduct; g,gut; gs,genital segment; od,oviduct; odg,oviducal gland; os,ovisac; ov,ovary.

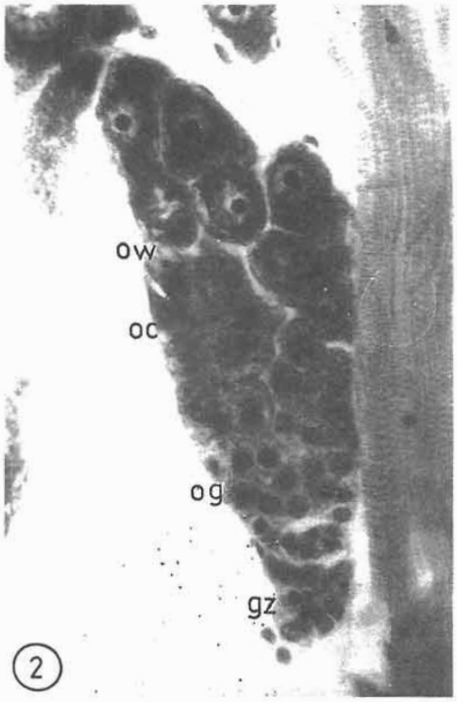

Fig 2. *Heliodiaptomus viduus*. L.S. of ovary. gz,germinal zone; oc,oocytes; og,oogonia; ow,ovarian wall.

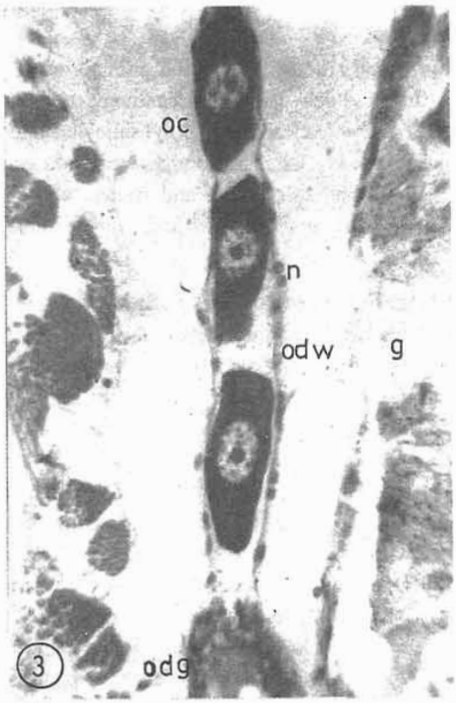

Fig 3. *Heliodiaptomus viduus*. L.S. of oviduct showing vitellogenic oocytes. g, gut; oc, oocyte; odg, oviducal gland; odw, wall of oviduct; n, nucleus.

ner layer of epithelium. Germinal cells and spermatogonial cells occur at the posterior-most part of the testis while spermatozoa are observed in the anterior end. In between, spermatogenic cells of different stages are found in sequential ascending order (Fig 6). The fully mature spermatozoa are found in collection in the central cavity of the testis. The spermatozoa of *H. viduus* are either spherical or slightly oblong in shape and measure 5.5 ± 0.3 μm in diameter (Fig 7).

Histology of the vas deferens reveals highly glandular columnar epithelial cells in its wall which secrete eosin-positive material into its lumen. The seminal vesicle is the part of the male reproductive system where most of the components involved in the formation of spermatophore get accumulated. It is a highly glandular organ and produces secretory material meant for the formation of spermatophore. In the lumen of the seminal vesicle, the core secretion, sperm mass and outer secretory material are arranged in the form of a presumptive spermatophore (Fig 8). Spermatophore formation is completed in the spermatophore sac. The wall of the spermatophore sac is thick and glandular. The spermatophore sac acts like a mould in which a tube shaped spermatophore is formed. Thus, the lumen of the spermatophore sac shows a well formed spermatophore, which has an outer wall, a layer of spermatozoa and a central core secretion. The ductus ejaculatorius is a thin walled non-glandular structure.

During mating, the male grasps the female with the help of its right antennule and holds her abdomen very close to it by the right fifth leg. During this process a spermatophore is extruded and transferred to the female with the help of left fifth leg. The spermatophore is precisely attached to the female reproductive pore (Fig 9). Where a temporary spermatheca is formed and the spermatozoa are discharged into it.

After fertilization, eggs get deposited into the ovisac which is attached to the reproductive pore situated midventrally on the genital segment (Fig 10). Mean clutch size of this specie is 15.73 ± 0.35 (± SD). Mean duration of embryonic development is 18 ± 5 (± SD) hrs. The nauplii are released to the medium by the rupture of the ovisac. Post embryonic development of this specie includes six naupliar and six copepodid stages. Mean duration of each naupliar stage is 16 ± 6 hrs and each copepodid stage is 20 ± 4 hrs. As the sixth copepodid stage is the mature individual, it begins reproduction immediately. The characteristics of the naupliar and copepodid stages are given in Table 1.

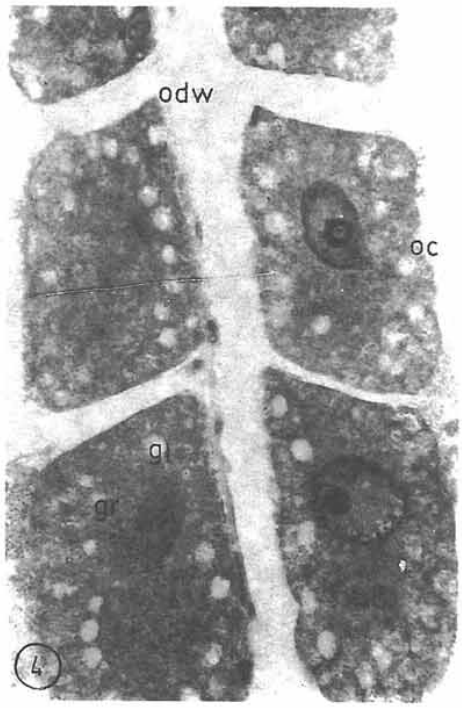

Fig 4. *Heliodiaptomus viduus*. L.S. of oviduct showing mature oocytes. oc, oocytes; odw, wall of oviduct; gl, yolk globule; gr, york granule.

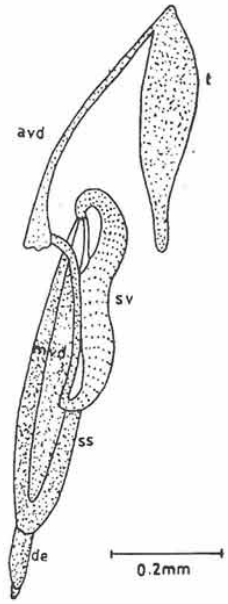

Fig 5. *Heliodiaptomus viduus*. Line Diagram of reproductive system of adult male viewed from the left side. avd, anterior vas deferens; de, ductus ejaculatorius; mvd, mid vas deferens; ss, spermatophore sac; sv, seminal vesicle; t, testis.

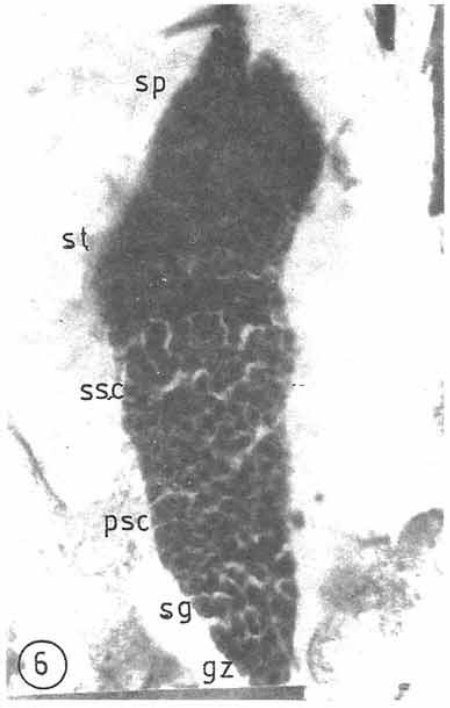

Fig 6. *Heliodiaptomus viduus*. L.S. of Testis. gz, germinal zone; psc, primary spermatocytes; sg, spermatogonia; sp, spermatozoa; ssc, secondary spermatocytes; st, spermatids.

Laboratory experiments indicate that the normal growth and development of nauplii and copepodid stages are recorded with baker's yeast and cowdung at a concentration of 2 ppt. In addition to this ingredients, culture medium of adults was provided with algae, which has given better survival and reproduction of this specie. The lifespan (from naupliar hatching to death) of this specie at 33 ± 1°C with cowdung + algal food has a mean and SD of 75 ± 5 days. The female produces a batch of eggs (clutch) on alternate days. The egg production continued regularly for a mean and SD of 20 ± 2 days and thereafter interclutch period was increased to three to five days. Egg production with longer interclutch period was observed for a mean and SD of 14 ± 3 days and thereafter egg production was ceased.

In general, anatomy of female reproductive system calanoids shows similar pattern, the enlargement of oviducts into conspicuous oviducal glands in *H. viduus* at the posterior end is absent in the oviducts of other copepoda like *Pseudocalanus*, *Calanus finmarcricus*, *Temora stylifera* and *Epilabidocera amphitrites*. The lateral diverticulae of the oviducts of *E.amphitrites* and *T. stylifera* are not found in the *H.viduus*. Contrary to the other crustaceans in *H. viduus* follicle cells are

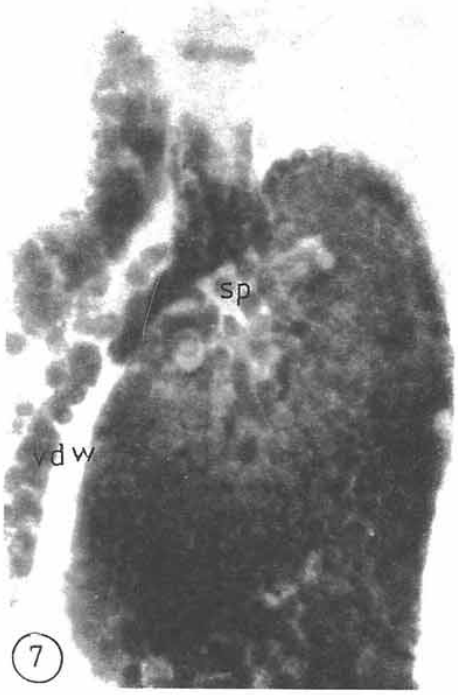

Fig 7. *Heliodiaptomus viduus*. L.S. of testis and vas deferens. sp, spermatozoa; st, spermatids; vdw, wall of vas deferens.

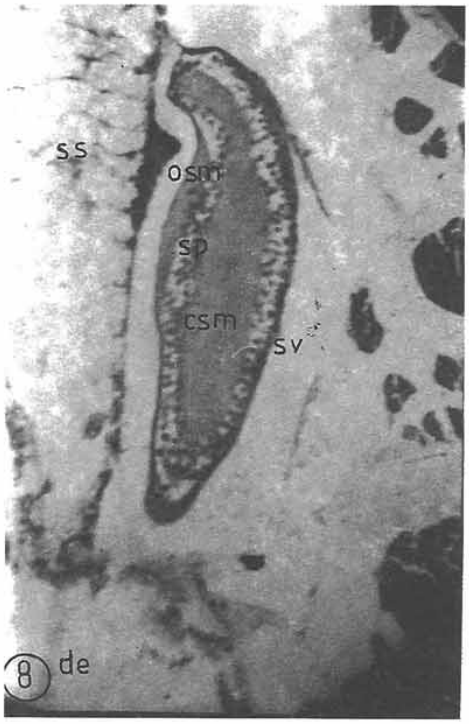

Fig 8. *Heliodiaptomus viduus*. L.S. of seminal vesicle ,spermatophore sac and ductus ejaculatorius. csm, core secretory material; de, ductus ejaculatorius; osm, outer secretory material; sp, spermatozoa; ss, spermatophore sac.

entirely absent in the ovary, the probably reason for this may be their small size with a relatively greater diffusion surface as well as the thin wall of oviduct which facilitate easy squestration of yolk material into the oocytes from haemolymph.

The male reproductive system of *H. viduus* appears to deviate considerably from those of other copepoda mainly in the shape of the testis, the course of vasdeferens and the structure of spermatophore. Testis of this specie does not contain any somatic cells. Spermatogenesis gets completed at the anterior end of the testis where mature sperms occur. The vasdeferens and seminal versicle are highly glandular structures and produce material required for the formation of spermatophore. The spermatozoa of *H. viduus* differ from the spermatozoa of other species in the shape and a whorl of spherical vacoules. In many copepoda, "Q" spermatoza and "B" spermatozoa type of spermatozoa were reported. However, in *H. viduus* only one type of spermatozoa are observed. Unlike male of *C.finmarchicus* where shorter life span and few spermatozoa are produced, in *H. viduus* higher life span and reproductive potential is observed in males.

In general the fish food schedule used in seed production is diatoms for the first few days and rotifers from the third day onwards upto 20 days; from 18 to 32 days copepods are fed and thereafter minced food or other formulated feeds are used. For prawns and shrimps larvae, suitable sized livefood are also used (Watanabe *et al* 1983). After the use of diatoms as initial livefood, shrimp larvae require zooplankton of about 400 μm, at a desirable density. Just hatched nauplii of *Artemia* are of this size and also contain the nutrients required for commercial shrimp seed production. However, due to over-exploitation and a decline in the fatty acid content of *Artemia* nauplii, many problems are caused in the hatchery. The larval sizes of *H. viduus* (Table 1) are smaller than those of *Artemia* nauplii and hence more suitable as initial livefood.

A number of authors have recently commented on the suitability of copepods as potential livefood organisms for hatchery seed production. Kraul *et al* (1991) reported that Mahimahi (*Coryphaena hippurus)* could be raised from the egg stage using a copepod or enriched *Artemia* diet . Further they opined that Mahimahi survives better when cultured with the copepod, *Euterpina acutifrons* particularly when the larvae are under stress due to factors like high stocking density, cold weather or metamorphosis. This was possibly due to the oc-

Table 1
Heliodiaptomus viduus post embryonic development

Day No.	Stage	Length (μm) Mean ± SD	Width (μm) Mean ± SD	Prominent
1	N_1	136.8 ± 0.37	68.4 ± 0.18	Oval in shape with 3 pairs of cephalic appendages
2	N_2	201.6 ± 0.42	86.4 ± 0.21	Caudal region bilobed with single seta
3	N_3	262.8 ± 0.46	108.0 ± 0.24	Body elongated and shows constriction; posteriorly 2 pairs of unequal caudal setae
4	N_4	309.6 ± 0.51	117.0 ± 0.22	Body more elongated, two equal caudal seta, maxillule rudimentary
5	N_5	327.6 ± 0.53	129.0 ± 0.25	Body further elongated 2 segmented, with Maxillule and Maxilla; 3 dissimilar caudal setae
6	N_6	370.8 ± 0.57	135.0 ± 0.24	Body much elongated spindle shaped 3 segmented; urosome segment distinct. 3 unequal setae rudiment of swimming leg 1 & 2
7	C_1	489.6 ± 0.60	144.0 ± 0.29	Body elongated cylindrical; prosome 5 segmented; urosome unsegmented; 2 pairs of developed swimming legs.
8	C_2	612.0 ± 0.72	180.0 ± 0.32	Prosome 5 segmented; urosome2 segmented; 3 pairs of swimming legs
9	C_3	795.6 ± 0.77	216.0 ± 0.31	Prosome 5 segmented; urosome 2 segmented; 4 pairs of swimming legs
10	C_4	936.0 ± 0.86	252.0 ± 0.34	Prosome 6 segmented; urosome3 segmented; 4 pair of swimming legs; rudiment of 5th legs
11	C_5 Female	1386 ± 1.26	281.0 ± 0.35	Urosome 3 segmented; first segmented is larger, 5th leg is short and has a pointed claw terminally
	C_5 Male	1260 ± 0.92	270.0 ± 0.31	Urosome 5 segmented; 5th leg asymmetrical and A1 asymmetrical
12	C_6 Female	1805 ± 0.98	312.0 ± 0.31	5+3 segmented; 11 pair of fullydeveloped appendages; 5th leg symmetrical
	C_6 Male	1642 ± 0.95	292.0 ± 0.33	6+5 segmented; P5 & A1 asymmetrical

currence of higher levels of essential amino acids, in addition to a higher level of docosahexaenoic acid (DHA) compared with unenriched brine shrimp diet (Kraul *et al* 1993). High poly-unsaturated fatty acid (PUFA) content is also reported in the copepoda *Oithona nana* (Shamsudin and Saad 1993) and nauplii and copepodids of *Eurytemora affinis* (Meeran 1993). Faster growth of hatchery-raised post-larvae of *Penaeus latisulcatus* (Kathirvel and Selvaraj 1987) and *Macrobrachium rosenbergii (*Paulraj and Altaff 1999) were also reported on copepod diets.

The present study on the reproductive biology of *H. viduus* obviously indicates that it has a wide range of different sized developmental stages in its life cycle which could form suitable food for fish and prawn larvae. The slow movement and behavior of the larvae makes them an excellent food for the larvae of commercially important fish and prawn species. Further, their desirable biochemical composition (Altaff and Chandran 1989) and different feeding habits allow survival on a variety of food items ranging through detritus, bacteria to different types of algae (Altaff and Chandran 1995; Preetha and Altaff 1996), makes them an ideal species for mass culture.

The tropical species are more suitable for mass culture due to their shorter development time as recorded in *H. viduus* (9-12

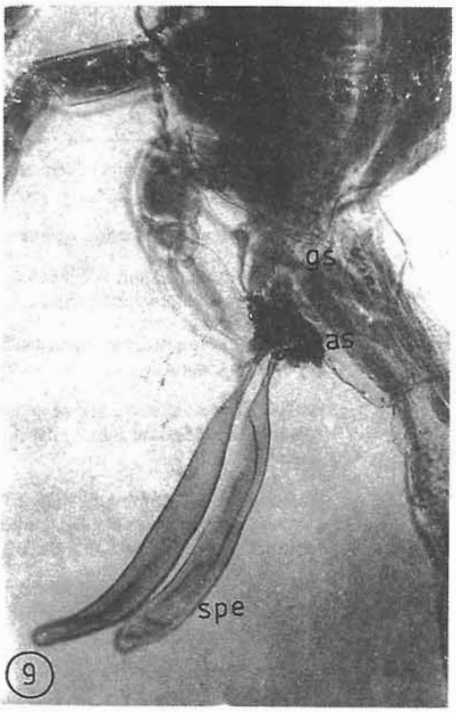

Fig 9. *Heliodiaptomus viduus*. Female with two spermatophore attached. am, adhesive material; gs, genital segment; spe, spermatophore.

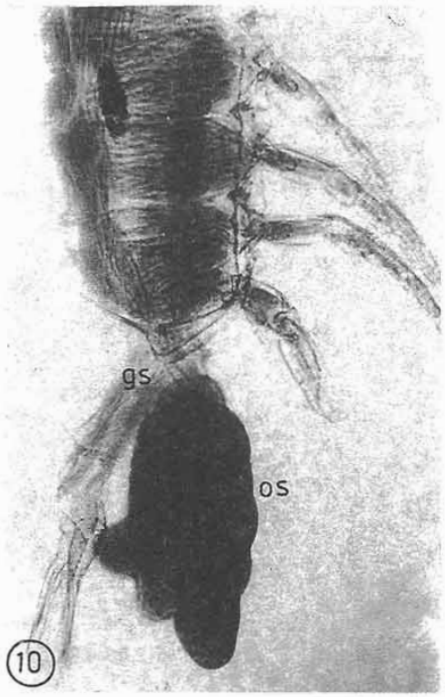

Fig 10. *Heliodiaptomus viduus*. Ovigerous female. gs, genital segment; os, ovisac.

days) when compared to temperate species such as *Acartia californiensis* (17-75 days) (Trujillo - Ortiz 1990) and *Calanus finmarchicus* (1-2 months) (Marshall and Orr 1972). However Sunyoto and Diani (1975) reported adequate culture of *Acartia plumosa* for marine fish hatcheries. Adult *H. viduus* is capable of producing eggs at regular intervals of 48 hrs for more than 30 days. As egg production is mostly related to food and temperature (Lavens and Sorgeloos 1996), production of this specie can be further enhanced by optimising these environmental variables. Such results have been reported in *Temora stylifera* (Razouls *et al* 1986) and *Acartia tonsa* (White and Roman 1992).

The desirable qualities of *H. viduus* such as higher nutritive value, easy digestibility, different sized developmental stages, rapid life cycle, high reproductive potential and good survival, on a variety of food substances, makes it suitable for mass culture. This is the case despite the fact that it has to remate for the production of each clutch (Sheriff and Altaff 1993).

Many species of copepoda belonging to the groups - Calanoida, Harpacticoida and Cyclopoida occur in the freshwater, estuarine and marine habitats of India. Recently, it is reported that many of these copepods have rich biochemical profiles high essential amino acids and polyunsaturated fatty acids (Safiullah 2001). If these species are mass cultured and a hatchery technology for the seed production of economically important fish and shrimp is developed, it will provide commercially viable, ecofriendly as well as sustainable resource.

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