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Feeding and Reproductive Biology of Two Potential Calanoid Copepod Species of *Pseudodiaptomus* for Mass Culture

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Abstract: Breeding and spawning of many finfish is achieved in captive condition but their larval rearing under hatchery condition is not attained to the desirable level mainly due to non-availability of suitable live feed. Copepods constitute natural food for these larvae and proved to be suitable in terms of nutritional value and size spectrum. Though many copepod species were mass cultured at laboratory level still commercial level production is not achieved. In addition to culture techniques knowledge of food and feeding habits as well as reproductive strategies of the candid species is essential. Present study deals with feeding habits and reproductive biology of two calanoid copepod species, *Pseudodiaptomus annandalei* and *Pseudodiaptomus serricaudatus*. Both these species have well developed feeding appendages and are omnivorous. The setae and setules on maxillae and maxillules show variation denoting filtration of different sized algae. Variation of teeth on the mandibular blade is also observed. Feeding appendages of *P. serricaudatus* suggests that it can consume wide variety of food materials. The different reproductive aspects of both species such as oogenesis, spermatogenesis, spermatophore formation and its transfer to the female are described for the first time. Embryonic and post embryonic development in these species takes 7-9 days. Reproductive phase and fecundity are reported. With continuous breeding, short inter clutch period and high reproductive potential, these species could be ideal for mass culture and use in hatchery rearing of marine finfish larvae.

Keywords: Copepods, Live feed, Feeding, Reproduction, Life cycle

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Introduction

Calanoid copepods are mostly free-living planktonic organisms and their feeding habits vary with species and larvae of different developmental stages. They may be filter feeders, predators or scavengers. They feed on

phytoplankton and microzooplankton and are preyed upon by larger zooplankton and constitute major food of larval fish, small pelagic fishes such as herring and of some whales and seals (Parker, 1982). This group

has vital role to play in successful rearing of marine finfish larvae due to their size spectrum and nutritive suitability (Altaff, 2020). The main factor in the selection of a copepod species for mass production and its application in mariculture is its reproductive potential. The variations pertaining to the fecundity of copepods is mostly related to the reproductive status of the female, food availability and environmental parameters (Vehmaa *et al.*, 2013). Being sexually reproducing organisms with different reproductive strategies, there should be sound knowledge of reproductive process of candid cultivable species. Important contributors on copepod female reproductive system are – Fahrenbach (1962), Park (1966), Razouls *et al.* (1987), Altaff (2003) and Dharani and Altaff (2004 a). Organization of male reproductive system, spermatogenesis and spermatophore formation in different copepods is reported by Blades-Eckelbarger and Youngbluth (1981), Blades Eckelbarger (1986), Altaff (2003) and Dharani and Altaff (2004 a).

Reproductive biology of copepods constituted one of the important areas of research and many aspects such as maturation, fecundity, nature of egg broadcastation, variation in fecundity were reported for many species (Kiorboe and Sabatini, 1995; Uye, 1998; Peterson and Kimmerer, 1994). Despite growing knowledge of copepods on their culture and use in marine finfish larval rearing, their production to the need of commercial level is not achieved (Lavens and Sorgeloos, 1996). Many elements of the life history of these species such as measures of reproductive investments by adult females, egg size, clutch size, mode of reproduction, the rate of release of

eggs and development of ova remain incompletely described (Ohman and Townshed, 1998). Though there are some investigations on the food and feeding habits and reproductive biology of freshwater copepods (Altaff, 2003), such investigations on marine copepods from Indian subcontinent is lacking. Understanding of food and feeding habits and reproductive potentials of female and male copepods is highly essential for determining many vital aspects such as sex ratio, composition of algal diet, reproductive potential, larval size spectrum and nutritive value. This article reports some of these aspects of two marine calanoid copepods, *Pseudodiaptomus annandalei* and *Pseudodiaptomus serricaudatus*.

Materials and Methods

Zooplankton samples were collected from offshore of the Kovalam (13.0827° N, 80.2707° E) station, Chennai (About 5 nautical miles) using a Bongo net made of bolting silk 200 µm mesh size during early hours of the day. The samples were fixed in 5% buffered formalin. The calanoid copepods were separated from zooplankton sample and *Pseudodiaptomus annandalei*, and *Pseudodiaptomus serricaudatus* were identified to species level following the taxonomic descriptions of Kasturirangan (1963) and Conway *et al.* (2003).

Live zooplankton were collected in plastic containers and transported to the laboratory within two hours of collection. Adult females and males of *P. annandalei* and *P. serricaudatus* were separated and transferred into 100 ml beakers containing filtered sea water and maintained with mixed algal diet consisting of *Isochrysis* sp., *Chaetoceros muelleri* and *Chlorella marina*. For life cycle

studies individual ovigerous females of both the species in triplicate were raised with baker's yeast (*Saccharomyces cerevisiae*) and algal diet and development was followed from hatching to ovisac formation in adult female. For the study of postembryonic development, the procedure of Dahms (1990) was adopted. The room temperature was 27 ± 3 C and the photoperiod was 12L: 12D. Every third day 50% of the water was changed. The larval and adult structure was studied under compound microscope and measurements were taken using eyepiece micrometer.

To view *in situ*, the mouth parts and reproductive system of *Pseudodiaptomus* species, Borax-carmin staining method of Pantin (1964) was followed. To ascertain the structure of feeding appendages and organization of female and male reproductive system the borax-carmin stained and acid alcohol differentiated specimens were dissected in a glycerol-ethanol mixture under stereo dissection microscope and then observed under compound microscope. The parts of the reproductive system were described following the terminology of Hopkins (1978) and Dussart and Defaye (1995).

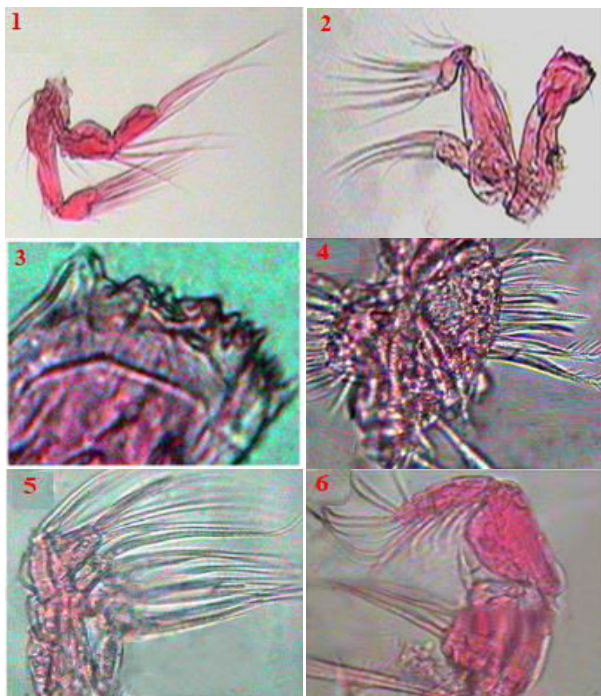
For histology, specimens were fixed in aqueous Bouin's fluid, dehydrated in ethanol, cleared in xylene and embedded in paraffin wax. Serial sections (cross sections, longitudinal sections,) of $8\ \mu\text{m}$ were cut and stained with haematoxylin and counter stained with alcoholic eosin (Patki *et al.*, 1987). The different parts of reproductive system was studied under compound microscope and photomicrographed at magnification of 40x, 100x and 400x.

Results

Feeding Appendages:

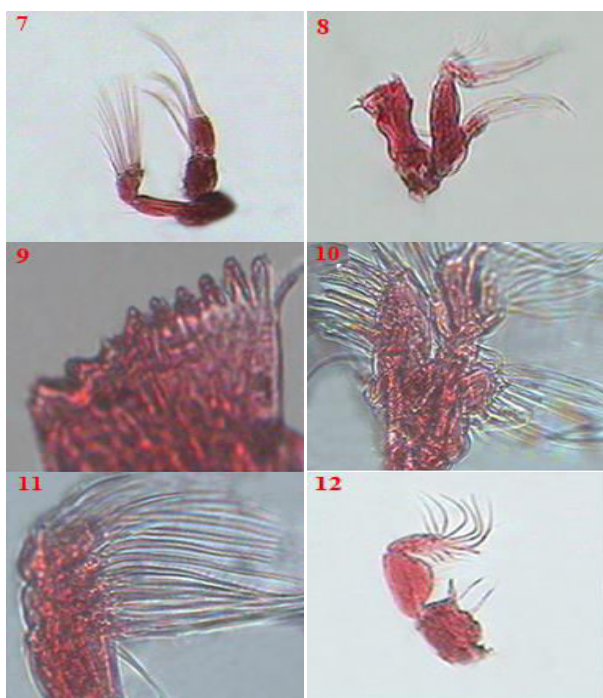
P. annandalei is one of the dominant calanoid copepods of east coast of India. The female and male of this species measure 1.2 ± 0.1 mm and 1.0 ± 0.2 mm, respectively, in their body length. *P. serricaudatus* is also a common calanoid copepod of east coast of India and coexist with *P. annandalei*. The female and male of this species measure 1.1 ± 0.1 mm and 0.91 ± 0.2 mm, respectively, in their total body length. Sexual dimorphism of both the species is evident in the geniculated right antennule of male, number of urosomal somites and distinctly modified P5 in both the sexes. In the female P5 is simple and symmetrical while in male it is asymmetrical and highly modified for transfer of spermatophore to the female.

The feeding appendages of the free living copepods viz. antenna, mandibles, maxillules, maxillae and maxillipedes gather food and aid in concentrating and ingestion of the same. The mouthparts of *P. annandalei* and *P. serricaudatus* in particular mandibular blade and the distance between setae and setules of their other mouth parts show variations. The mandibular blade of *P. annandalei* has rounded structures might be capable of crushing algal mass to open the cell wall while that of *P. serricaudatus* has sharp teeth capable of immobilizing active prey. In both the species maxillules and maxillae are main filtering structures with well developed setae and setules. The setae and setules on maxillules and maxillae occur more intense in *P. serricaudatus* than in *P. annandalei*. The size of mesh formed by these appendages ranges from $7-16\ \mu\text{m}$ and $3.5-9\ \mu\text{m}$, respectively. The structure of mouth parts of these species suggest *P. serricaudatus* could filter much



Figures 1-6: Feeding appendages of *P. annandalei*

Fig. 1: Antenna; Fig. 2: Mandible; Fig. 3: Higher magnification of mandibular blade; Fig. 4: Maxillule; Fig. 5: Maxilla; Fig. 6: Maxillipede



Figures 7-12: Feeding appendages of *P. serricaudatus*
 Fig. 7: Antenna; Fig. 8: Mandible; Fig. 9: Higher magnification of mandibular blade; Fig. 10: Maxillule; Fig. 11: Maxilla; Fig. 12: Maxillipede

smaller algae than those filtered by *P. annandalei* (Figs. 1-12).

Female Reproductive System:

The female reproductive system of *P. annandalei* and *P. serricaudatus* consists of a median ovary, a pair of oviducts, antrum, seminal receptacle and reproductive pore. The oviducts enlarge at the end of the prosome to give rise to oviducal glands which extends into the genital segment and opens into the antrum. The antrum in turn opens to the exterior ventrally through a median female reproductive pore. A pair of spherical seminal receptacle occurs in the genital segment (Figs. 13, 14). In these species fertilized eggs are held in the ovisac where embryonic development to naupliar stage takes place.

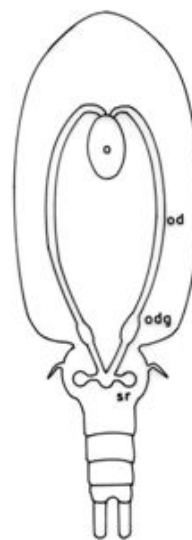


Figure 13: Female Reproductive system of *P. annandalei* (o-ovary; od-oviduct; odg-oviducal gland; sr-seminal receptacle)

Histology of the ovary of *P. annandalei* and *P. serricaudatus* shows germinal cells at the posterior most regions and the next anterior region is occupied by oogonial cells. The oogonial cells are smaller in diameter compared to germinal cells and give rise to primary oocytes with relatively higher volume

of nucleus. The anterior most part of the ovary is occupied by secondary oocytes and previtellogenic oocytes which are spherical, elongate or triangular in shape. (Figs. 15, 16).

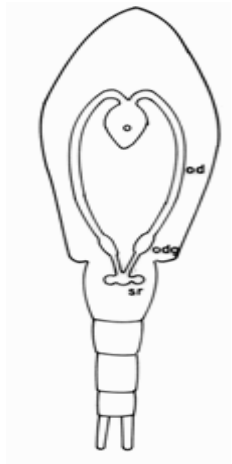
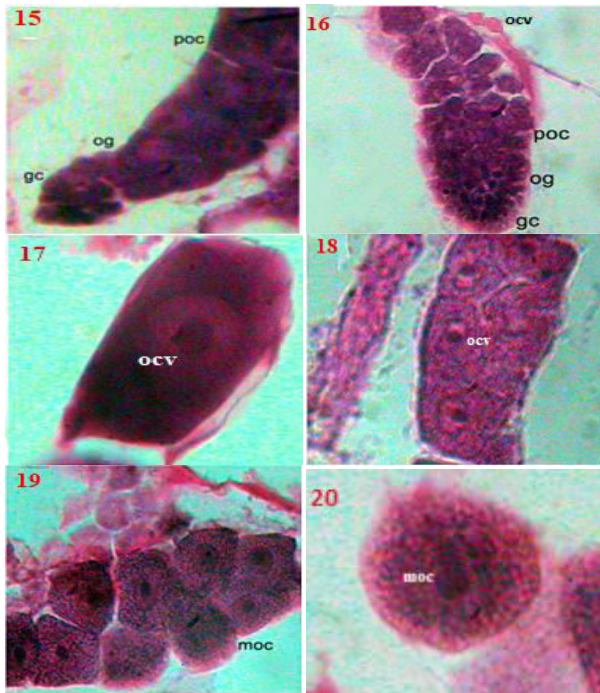


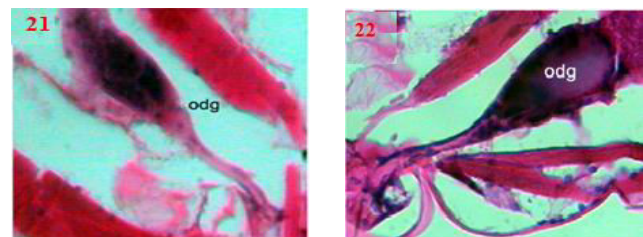
Figure 14: Female Reproductive System of *P. serricaudatus* (o-ovary; od-oviduct; odg-oviducal gland; sr-seminal receptacle)



Figures 15 – 20: Histology of ovary and oocytes
 Fig. 15: Ovary of *P. annandalei*; Fig. 16: Ovary of *P. serricaudatus*; Fig. 17: Vitellogenic oocytes of *P. annandalei*; Fig. 18: Vitellogenic oocytes of *P. serricaudatus*; Fig. 19: Mature oocytes of *P. annandalei*; Fig. 20: Mature oocyte of *P. serricaudatus* (ocv- vitellogenic oocytes; og – oogonial cells; gc – germinal cells; moc – mature oocytes; poc – primary oocytes; og – oogonial cells; gc – germinal cell)

The previtellogenic oocytes are transferred from the anterior part of the ovary into the oviducts where vitellogenesis and maturation of these oocytes takes place. Vitellogenesis begins with the deposition of small granules of yolk and during the course of maturation bigger yolk granules and globules are formed leading to enlargement of the oocytes (Figs. 17-18). A batch of mature oocytes in the oviducts occupies most of the perivisceral cavity of the metasome. The shape of vitellogenic oocytes of *P. annandalei* is rectangular (Fig. 19) while those of *P. serricaudatus* are mostly spherical (Fig. 20).

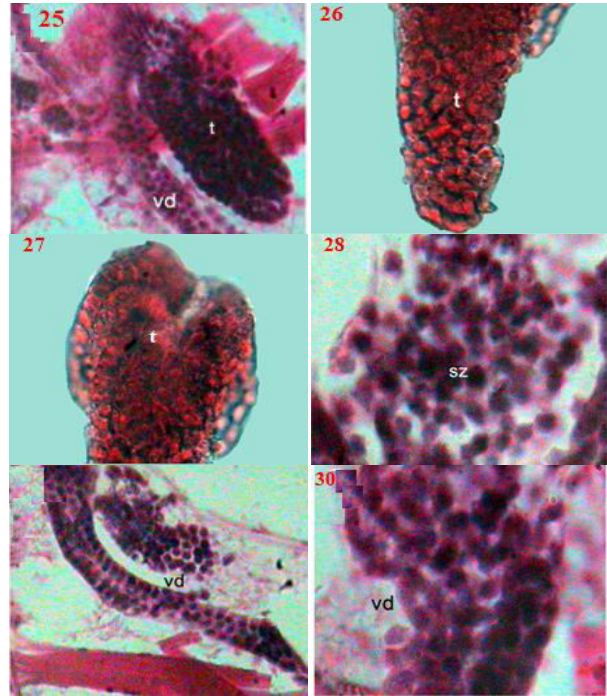
The wall of the oviducal glands is thick and glandular (Figs. 21, 22). During the process of maturation of oocytes in the oviducts, the oviducal glands produce large quantity of secretory material. The secretory activity of the oviducal glands synchronizes with the vitellogenesis and maturation of oocytes in the oviduct. During the reproductive phase of the female, ovary produce previtellogenic oocytes continuously. Batches of oocytes undergo maturation in the oviduct and are released to get fertilized as they pass through antrum with the sperm from seminal receptacle. Prior to the release of the mature eggs secretory material of the oviducal gland is released which forms membranous ovisac in which fertilized eggs are deposited. The number of clutches produced by a species during the reproductive phase constitutes the reproductive potential of the species.



Histology of oviducal gland (odg) Fig. 21: *P. annandalei*; Fig. 22: *P. serricaudatus*

Male Reproductive System:

In both the species of *Pseudodiaptomus* male reproductive system consists of a median testis and a single genital duct. The genital duct is differentiated into anterior vas deferens, mid vas deferens, seminal vesicle, spermatophoric sac and ductus ejaculatorius (Figs. 23, 24). The testicular cells are compactly arranged except at the anterior cavity of the testis (Figs. 25-27). The posterior most region of the testis is occupied by germinal cells which are the biggest cells of the testis. The spermatogonial cells, primary spermatocytes, secondary spermatocytes and spermatids are arranged in ascending series from posterior to the anterior region of the testis. They show slight decrease in size from early to late stage of spermatogenesis. The spermatids undergo metamorphosis to become spherical immotile mature sperms which are accumulated in the anterior cavity of the testis (Fig. 28). The anterior most part of the testis has a glandular mass of tissue which produces secretory material into the anterior cavity of the testis.



Figures 25 – 30 Histology testis and vas deferens
 Fig. 25: Testis of *P. annandalei*; Fig. 26-28: Testis of *P. serricaudatus*; Fig. 29: Vas deferens of *P. annandalei*;
 Fig. 30: Vas deferens of *P. serricaudatus* (t – testis; sz – spermatozoa; vd – vas deferens)

Spermatogenesis and Spermatophore Formation:

In both the species spermatogenesis is completed in the testis and formation of spermatophore commences in the anterior part of the vas deferens. The secretory material of the anterior vas deferens glues the spermatozoa and carries them to the mid vas deferens (Figs. 29, 30). The spermatozoa and secretory material of the mid vas deferens are transferred to the seminal vesicle which acts as organ of storage of spermatophoric content (Figs. 31-33). The seminal vesicle produces secretory material which contributes for the formation of wall of the spermatophore. From seminal vesicle spermatophoric contents required for the formation of a single spermatophore is transferred to the spermatophore sac where a tube shaped spermatophore is molded to its final shape (Figs. 34-36). It has a tubular body, a narrow

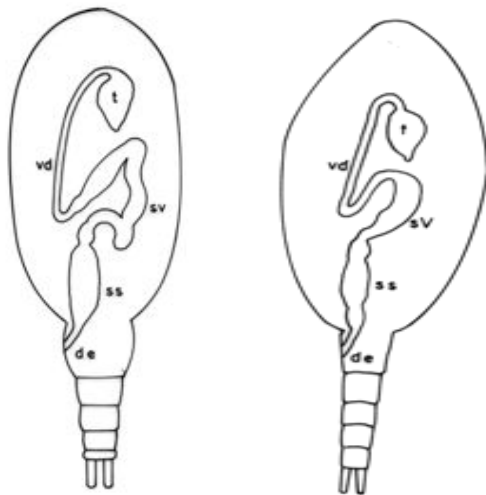
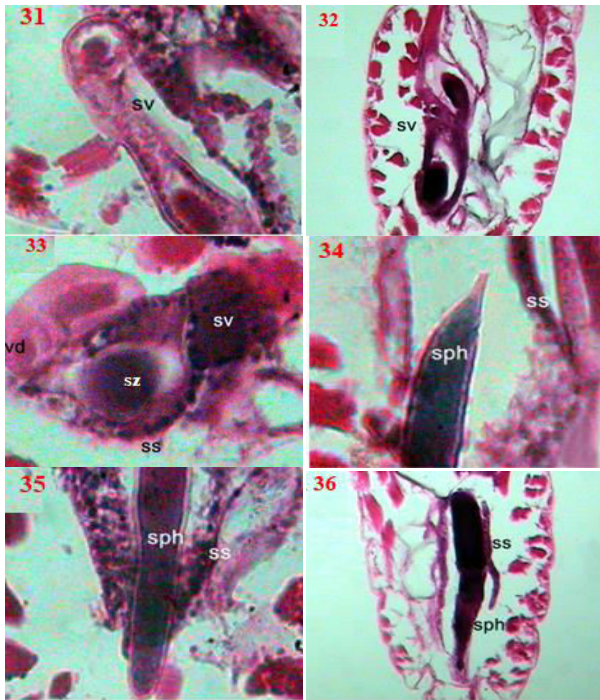


Fig. 23

Fig. 24

Male reproductive system Fig. 23: *P. annandalei*; Fig. 24: *P. serricaudatus* (t- testis; vd- vas deferens; sv- seminal vesicle; ss- spermatophore sac; de- ductus ejaculatorius)



Figures 31–36: Histology of male genital duct

Fig. 31: Seminal vesicle of *P. annandalei*; Fig. 32: Seminal vesicle of *P. serricaudatus*; Fig. 33: C.S of seminal vesicle and spermatophore sac of *P. serricaudatus*; Fig. 34, 35: spermatophore sac and spermatophore of *P. annandalei*, Fig. 36: spermatophore sac and spermatophore of *P. serricaudatus* (sv - seminal vesicle; ss - spermatophoric sac; sph - spermatophore; sz - spermatozoa)

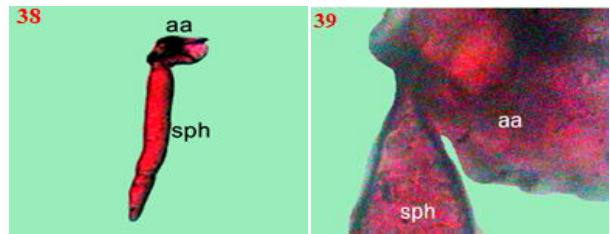
neck and an anterior apparatus. The anterior apparatus appears to be species specific with regard to its size and shape. (Figs. 37-39). The spermatophore sac produces secretory material which is extruded along with the anterior apparatus and helps in the attachment of spermatophore to the female genitalia. The length of the spermatophore of *P. annandalei* and *P. serricaudatus* is 278 ± 22 and 316 ± 31 μm , respectively.

Mating Behaviour:

During mating, male of these species grabs the caudal rami of female with its geniculate antennule to recognize its mate and the partners move in clockwise and anticlockwise direction to get proximity. The male extrudes the spermatophore and holds it in the right P5



Figure 37: Spermatophore of *P. annandalei*



Figures 38 and 39: Spermatophore of *P. serricaudatus* (sph- spermatophore aa- anterior apparatus of spermatophore)

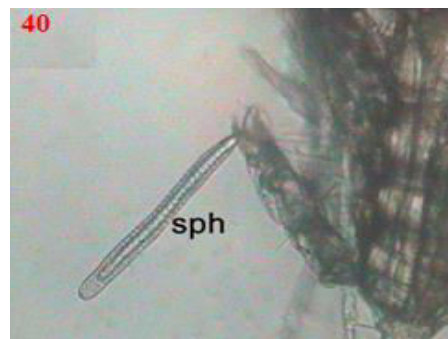


Figure 40: Spermatophore held by the P5 of *P. annandalei* (sph- spermatophore)

(Fig. 40). Then the male makes approaches to reach the genital field of the female and proximity of genital pore to the spermatophore apparatus. Once it is attained then the P5 of the male slowly moves forward so that the spermatophore complex is precisely brought to the female genital pore. With the help of the adhesive material on the handle and plate-like structure, the spermatophore is appropriately fixed to the corresponding microstructure on the genital field of the female by which placement and adhesion of anterior spermatophore complex

to the reproductive pore of the female is accomplished. Once the spermatophore complex is attached, the P5 of the male loses its grip from the urosome of the female gradually and passively to withdraws itself without causing any disturbance to the positioning of the spermatophore. Subsequently, the left P5 releases the female enabling separation of the copulating partners. The whole process of mating and transfer of spermatophore takes about 20 minutes. The spermatophore of these species takes four hours for discharging its contents into the seminal receptacles and then gets detached. The female produces about 3-4 clutches after insemination and thereafter remating is essential for continuation of egg production. The female becomes ovigerous 4 to 5 h after insemination and embryonic development of the fertilized eggs takes place in the ovisac (Fig. 41). Bilobed ovisac is also observed in *Pseudodiaptomus* species (Fig. 42). Nauplii hatch out at a time interval of 20-24 h and the interclutch period is 48 h. Multispermatophoric condition is observed both in non-ovigerous and ovigerous females of both the species. In the case of single spermatophore condition, it is precisely attached to the female reproductive pore while in multispermatophoric condition the spermatophores are not precisely attached to the genital pore. In the multispermatophoric condition two to fifteen spermatophore were observed on the female genital field.

Embryonic Development:

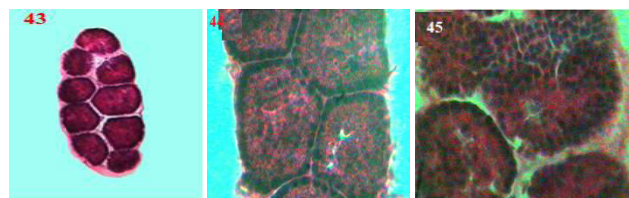
Embryonic development commences with peripheral cleavage of fertilized egg resulting in the formation of the single layer of blastomeres peripherally and then proceeds internally to form blastula. The morphogenetic movements of the blastomeres



Figure 41: Multispermatophoric condition in ovigerous females of *P.annandalei* (sph- spermatophore)



Figure 42: Bilobed ovisac of *P. annandalei*



Figures 43-45: Different stages of Embryonic Development of *P. annandalei*

transform the blastula into an oval shaped gastrula (Figs. 43-45). Subsequently, appendages appear leading to the formation of nauplius which hatches out by rupturing the egg membrane and ovisac membrane. The duration of embryonic development is 24 h. Under laboratory conditions multiple mating of a female by many males is observed and non-ovigerous females are more prone for multiple mating than ovigerous females.

Post-embryonic Development:

The post-embryonic development of these species includes six naupliar and six

copepodid stages, the last stage being the adult. The naupliar and copepodid stages pass to the next stage after a moult. The freshly hatched nauplii are non-feeding larvae and feeding commences after the first moult. The nauplii show smooth swimming by the action of the setae of their appendages. The copepodids show smooth swimming and propelling movement. The length and width of the different larvae of *P. annandalei* and *P. serricaudatus* are presented in Table 1. In both the species duration of post-embryonic development is about 7-9 days. Unbiased sex ratio of male and female is observed in these species.

Reproductive Potential:

Reproduction in these species commences as soon as copepodid VI stage is attained. Males show high reproductive potential and in a day it is capable of mating and transferring spermatophores to 4-5 females. After a single mating the female is capable of producing 4-5 ovisacs and the interclutch period is about 40 ± 4 h. The clutch of *P. annandalei* showed 18 ± 3 eggs while that of *P. serricaudatus* contained 21 ± 4 eggs. The egg diameter of *P. annandalei* and *P. serricaudatus* is 96 ± 3 μm and 91 ± 4 μm , respectively. The life span of *P. annandalei* and *P. serricaudatus* ranges 66-74 days and 64-82 days, respectively. In both the species reproductive phase recorded was 54-58 days. Interclutch period was 24-28 h and *P. annandalei* produced 32 clutches while *P. serricaudatus* produced 36 clutches.

During the final phase of reproduction they produce fewer numbers of eggs which are deposited in a smaller and rounded ovisac (Fig. 46). In some of the females ovisac are devoid of oocytes (Fig. 47). Under laboratory conditions non-proper placement of

spermatophore is observed in ovigerous females (Fig. 48). In both the species of *Pseudodiaptomus*, females maintained with varying temperature and feeding regimes produce diapausing eggs. The structure of diapausing eggs and ovisac wall is thick and non-membranous.



Figure 46: Small visac showing single egg of *P. serricaudatus*

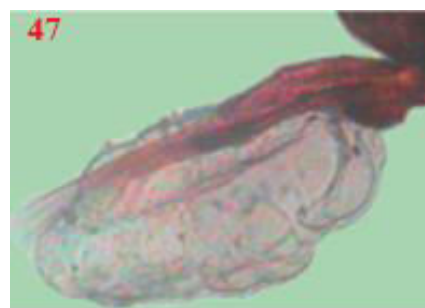


Figure 47: Ovisac devoid of eggs of *P. serricaudatus*



Figure 48: Non-proper placement of spermatophore of *P. serricaudatus* (sph- spermatophore)

Discussion

There is considerable variation among different genera of calanoid copepods with regard to occurrence of mouth parts and feeding in their life span. Males of many genera showed reduced appendages and do

Table 1: Length and Width of larvae of *P. annandalei* and *P. serricaudatus*

Post-Embryonic Development	<i>P. annandalei</i>		<i>P. serricaudatus</i>	
	Length (µm)	Width (µm)	Length (µm)	Width (µm)
Larval stage				
Nauplius I	102 ± 30	64±12	96±27	57±09
Nauplius II	142±38	86±16	114±38	76±14
Nauplius III	194±42	112±14	220±121	82±07
Nauplius IV	236±26	127±17	252±135	117±12
Nauplius V	264±32	134±23	274±138	125±11
Nauplius VI	300±45	148±26	292±135	132±23
Copepodid I	356±63	154±21	346±142	146±13
Copepodid II	420±116	168±34	409±166	163±17
Copepodid III	546±105	181±39	517±159	179±11
Copepodid IV	653±142	204±27	630±220	196±23
Copepodid V Female	768±133	233±31	778±183	221±32
Copepodid V Male	713±214	214±26	723±244	192±26
Copepodid VI Female	849±328	267±42	869±308	248±37
Copepodid V Male	797±263	228±34	826±241	226±23

not feed and in few cases both adult males and females have reduced mandibles and do not feed (Mauchline *et al.*, 1998). Unlike these copepods, adult females and males of *P. annandale* and *P. serricaudaatus*, have well developed feeding appendages throughout their life and create a feeding current which effects gathering and ingestion of food. Mouthparts of these species have well developed exopods, endopods and long setae occurring on their surfaces and also the gnathobase indicate their omnivorous feeding habit.

Most of the calanoids are herbivores and feed on algae, flagellates and bacteria. They collect their food by filtration (Dussart and Defaye, 1995).The feeding appendages of *P. annandalei* and *P. serricaudatus* even though show generalized pattern there are species specific variations evidencing utilization of different type of food material. In the case of *P. annandalei* the mandibular blade is very compact more or less rounded structure on its surface. The mandibular palp, maxillule and maxilla have long setae. Compared to this species the mandibular blade of *P.*

serricaudatus is broader and has ten sharp teeth. The mandibular structure maxillule and maxilla suggest that even though both the species might feed on the algae predominantly, *P. serricaudatus* can use smaller algae than *P. annandalei*. Further *P. serricaudatus* can also feed small moving prey such as rotifers and other micro crustaceans. The food and feeding habits of these species could utilize varied type of food such as nanoplankton, algae of moderate size and other filamentous algae.

Organization of female reproductive system of *P. annandalei* and *P. serricaudatus* shows similarity with other calanoid species (Blades-Eckelbarger and Youngbluth, 1984; Razouls *et al.*, 1986, 1987; Norrbin, 1994). However, variation is observed with regard to the size and shape of the ovary, the diverticula of the oviduct, structure of the antrum and structure of the seminal receptacles. The oviducal gland of *P. annandalei* and *P. serricaudatus* is unique and such a gland is not described in any other marine calanoids copepod (Altaff, 2018). Nevertheless occurrence of such an oviducal gland is reported in the freshwater calanoids copepods (Cuoc *et al.*, 1989 a, b; Altaff and Chandran, 1994; Dharani and Altaff, 2004 a). Autosynthetic vitellogenesis of oocytes occurs in the oviduct of *P. annandalei* and *P. serricaudatus*. The yolk components are directly absorbed by the oocytes from the haemolymph. The thin wall of the oviduct facilitates passage of yolk components from the haemolymph to the oocytes.

Like other invertebrates, the testis and accessory glands of male reproductive system of crustaceans produces spermatozoa and secretions which contribute to internal and external components of spermatophore

(Adiyodi and Adiyodi, 1975). The organization of male reproductive system of *P. annandalei* and *P. serricaudatus*, in general conform to the basic calanoids pattern. However, variation is observed in the size and shape of the testis and nature of the different region and course of the genital duct. Most of the calanoids produce single and simple spermatophore however, complex ones occur primarily in the Centropagidae and Pontellidae (Blades-Eckelbarger and Youngbluth, 1991). The simple spermatophores consist of flask-like container with a thin neck for attachment to the female (Altaff, 2003).

The spermatozoa are suspended in two different secretory materials in the proximal and distal part of the spermatophore and refractive index of these two secretions differs. The spermatozoa at the proximal end of the spermatophore are suspended in a secretion that allows them to resist swelling while those at the distal end are suspended in a different secretion which once the spermatophore is transferred to the female, swell up forcing the proximal spermatozoa pass into the female's seminal receptacle (Hopkins, 1978). The spermatophores of marine calanoid copepods are tubular in shape and have small narrow neck anteriorly and rounded posterior end (Blades-Eckelbarger and Youngbluth, 1991). The spermatophores of *P. serricaudatus* and *P. annadalei* show occurrence of a distinct anterior apparatus. In both these species the narrow anterior neck of the spermatophore leads into a coupling apparatus usually formed of three plates. In the case of *P. annandalei* the three plates form a more or less rounded structure while in *P. serricaudatus* the apparatus is elongate in structure. Further occurrence of a distinct

notch at the posterior end of the spermatophore of *P. serricaucatus* distinguishes from the spermatophore of *P. annandalei*.

In the present study the females of both the species of *Pseudodiaptomus* showed multiple spermatophore attachments in the laboratory populations. The spermatophores are attached to the genital segment mostly on the lateral aspects of the genital field rather than anterior and posterior sides. Usually one or two spermatophores were properly placed and their content is discharged into the female, when the spermatophores are improperly placed the content is not discharged. Higher number of spermatophore is carried by *P. annandalei* (15 spermatophores) than the multispermatophoric females of *P. serricaudatus* (10 spermatophores). The mating and spermatophore transfer leading to multispermatophoric condition usually takes place during darker period. Such a process indirectly indicates higher reproductive potential of the males. It is interesting to note that multispermatophoric condition is observed even in ovigerous female. However, such condition usually results in the mortality of the female.

The laboratory studies on *P. annandalei* and *P. serricaudatus* with regard to their reproductive biology have shown that these species produce subitaneous and diapausing eggs. The subitaneous eggs are mostly transparent and have thin layer of outer covering, usually the previtelline covering compared to the subitaneous eggs, diapausing eggs are dark and have a very thick external covering. It appears that as suggested by Mauchline *et al.* (1998) these eggs have a

previtelline, a chorion and a cuticular layer which protect the egg from the adversities of the environment. Ultrastructural studies on the subitaneous and diapausing eggs of planktonic diaptomid, *Sinodiaptomus (Rhinediaptomus) indicus* indicated marked difference in their outer covering (Dharani and Altaff, 2004 b).

There are six naupliar stages (NI-NVI) in most of the calanoids copepods. The first three naupliar stages are true nauplii with three pairs of appendages and fourth to sixth stages are similar to the metanauplii of other crustaceans because they have signs of urosomal somites (Sazhina, 1982). The successive naupliar stages within a species are identified by the progressive development of the armature of the posterior end of the body. The NVI moults to the first of six copepodid stages that resemble miniature adult. It successively moults to reach CVI which is accomplished by the progressive development of the segmentation of the body, the increasing differentiation of the appendages and successive increase in body size (Mauchline *et al.*, 1998).

The development of copepodids of *P. serricaudatus* and *P. annandalei* conform to those of other species described earlier. The males complete the postembryonic development faster than the females. In laboratory raised populations usually unbiased sex ratio is resulted in both the species. The naupliar and adults of these species could form ideal prey for the finfish larvae at different developmental stages (Sheriff *et al.*, 2018). The review of Conover (1998) of copepod life cycle strategies concluded that the life cycle characteristics are set primarily by metabolic constraints of

temperature and adequate food resources so long as both variables are within the necessary range individuals pass through the life cycle stage by stage. Even though *P. annandalei* and *P. serricaudatus* happen to be closely related and coexisting species, there is marked differences in their feeding habits and reproductive potential.

Conclusion

For marine finfish larval rearing one of the inevitable factors is availability of copepod live feed. It is essential to know the food and feeding habits as well as reproductive potential of candid species of copepods for mass culture. With the knowledge of feeding and reproductive aspects of *P. annandalei* and *P. serricaudatus* gained in the present study, their sustainable mass culture and use as live feed might improve the growth and survival of finfish larvae in the hatcheries. Considering the naupliar and copepodids size spectrum, fecundity and feeding habits the present study suggests *P. serricaudatus* is better option for mass culture than *P. annandalei*.

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