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Evaluation of Feeding Habit and Reproductive Potential of Calanoid Copepod, *Temora discaudata* for Cultivation and use as Live Feed for Marine Finfish Larvae

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Abstract: The primary food sources for marine finfish larvae in the wild are calanoid copepods, which are also essential to the repopulation of the species. The superiority of copepod live feed over rotifers and *Artemia* nauplii for the growth and survival of fish larvae has also been noted in several recent articles on the hatchery rearing of fish larvae. The goal of the current study was to establish a mass culture procedure by assessing the feeding apparatus and reproductive biology of *Temora discaudata*, a possible clinoid copepod. Well-developed setae, setules, and mandibular teeth adorn the antenna, mandible, maxillule, maxilla, and maxillipede, the feeding appendages of this species, which are used to collect, masticate, and swallow various types of microalgae and other food particles. This species' mature male and female exhibit clear sexual dimorphism, with the male having a geniculate antennule and the female having a modified P5. This copepod's great fecundity and continual breeding are demonstrated by its several oogenetic stages, which culminate in previtellogenic oocytes in the ovary and vitellogenic oocytes in the oviducts. In the mature male spermatogenesis is completed in the testis and spherical spermatozoa occur at the proximal end of the testis. The vas deferens, seminal vesicle, spermatophore sac, and ductus ejaculatoris are the four distinct locations that make up the male single genital duct. Each of these regions produces a unique sort of secretory material that aids in the creation of a tubular spermatophore. It has been observed that this species mates quickly and transfers spermatophores. In the ovisac, fertilised eggs go through embryonic development, and 82% of the eggs hatch. After 7-8 days of post-embryonic growth, copepodites C1 447 µm, C5 female 1180 µm, CV male 1079 µm, and nauplii N1 to N6 range in length from 104 µm to 259 µm. Because of its great reproductive potential and omnivorous diet, *T. discaudata* can be produced in large quantities and used as live feed for fish larvae.

Keywords: Copepoda, Feeding, Reproduction, *Temora discaudata,* Live feed

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Introduction

Copepods are the main consumers in the aquatic ecosystem and are essential for the transfer of energy to higher organisms. Copepods are valuable biological indicators and provide live food for fish and crustacean larvae in aquaculture, in addition to their ecological significance. Copepods are directly obtained from nature and utilised as live food for feeding the larvae of ornamental fishes (Altaff *et al*., 2002). In addition, copepods have an advantage over *Artemia* nauplii in that they have larger concentrations of polyunsaturated fatty acids and digestive enzymes, which improve food digestion and nutritional value in fish larvae. The unique hopping and gliding action of copepods with passive sinking produces visual stimulus for eating in fish larvae and they prefer copepods than rotifers owing to this reason (Lavens and Sorgeloos, 1996). Fish larvae's primary food source in the natural world is the three free-living, planktonic orders of Copepoda are Calanoida, Cyclopoida, and Harpacticoida, along with their larvae. Nauplii, copepodites and adults of various species of calanoid copepods have been reported as suitable live prey for hatchery rearing of marine finfish larvae. Accordingly mass culture technology is developed for *Parvocalanus crassirostris* (Alajmi *et al*., 2015), *Acartia tonsa* (Barroso *et al*., 2013, Drillet *et al*., 2015), *Pseudodiaptomus annandalei* (Kaviyarasan *et al*., 2019), *Nannocalanus minor* (Jothiraj *et al*., 2019), *Eurytemora velox* (Shields *et al*., 1999) and many other calanoid copepods (Altaff and Vijayaraj, 2021).

The reproductive pattern of pelagic copepods determines their population density. There are two different reproductive patterns that arise in calanoid copepods. First, the adult sex ratios of copepods with no seminal receptacle and a need for recurrent mating to remain fertilised are almost equal in field populations. These species have the potential for rapid population increase due to their higher rates of egg production compared to other pelagic copepods. Secondly, other copepods require only one mating to stay fruitful, and populations of these species show substantially female-skewed adult sex-ratios in field populations (Kiørboe, 2006). Further, temperature and phytoplankton community composition have influence on subitaneous and resting egg production rates (Tsunashima *et al*., 2021).

Numerous studies have documented the taxonomy and distribution of freshwater and marine copepods from Indian waterways, providing the ecological significance of these organisms. Sewell (1999) published a thorough description of calanoids and was a pioneer in the taxonomy study of marine copepods. The taxonomy and ecology of marine copepods were published by Krishnaswamy (1957). Additional research on the taxonomy and distribution of marine copepods has been documented by Pillai (1990), Padmanabha Rao (1962), Kasturirangan (1963), Saraswathi (1967), and Wellershaus (1969). Considering their relevance as valued live feed for producing marine finfish larvae currently active research is carried out on copepod food and feeding, reproductive biology, and cultivation. Recent research has revealed that among closely related calanoid copepod species, including *Pseudodiaptomus serricaudatus* and *P. annandalei*, the latter species exhibited greater naupliar size and reproductive potential than the former (Altaff, 2020). Furthermore, very few species' reproductive biology has been documented in comparison to the richness of marine calanoid copepod species that are currently known. As a result, the current study on *Temora discaudata* diet, eating habits, and reproductive biology was conducted to determine whether it may be grown and used as live prey for marine finfish larvae.

Materials and Methods

In the early hours of the day, samples of zooplankton were taken offshore of the Kovalam (13.0827° N, 80.2707° E) station in Chennai (India) using a Bongo net with a 200 µm mesh size made of bolting silk. The samples were fixed in 5% buffered formalin. Following the taxonomic

descriptions of Kasturirangan (1963) and Conway *et al*. (2003), the calanoid copepod *T. discaudata* was recognised to species level. The Borax–carmine staining method of Pantin (1964) was used to observe the mouth parts and reproductive system of *T. discaudata in situ*. Under a stereoscopic dissection microscope, specimens that had been acid-alcohol differentiated and borax-carmine stained were dissected in a glycerol-ethanol mixture to determine the structure of the feeding appendages and the organisation of the male and female reproductive systems. The specimens were then examined under a compound microscope. The terms used to define the reproductive system's components were taken from Dussart and Defaye (1995) and Hopkins (1978).

Specimens were fixed in aqueous Bouin's solution, dehydrated in ethanol, cleared in xylene and embedded in paraffin wax for histology. Haematoxylin and alcoholic eosin were used to stain serial sections (cross sections, longitudinal sections) of 8 µm (Patki *et al*., 1987). The various reproductive system components were examined using a compound microscope and captured on camera at magnifications of 40x, 100x, and 400x. Within an hour of being collected, live zooplankton samples were taken and transported to the laboratory. The male and female *T. discaudata* were separated and placed in separate beakers with 100 ml of filtered seawater. They were then given access to *Isochrysis galbana*, an excellent food source for copepod egg development, on a regular basis. Each pair's daily egg counts, egg viability, and spermatophore production were noted before they were pipetted into a brand-new container filled with fresh medium. A calibrated stage micrometre is used to measure the length of the naupliar, copepodite and adults.

Results and Discussion

T. discaudata has a pear-shaped cephalosome and a big, compact body. This species' male and female are 1.89±0.1 mm and 1.8±0.05 mm long, respectively (Figs. 1, 2). The prosome's anterior most portion is flattened (Fig. 3). The prosome's posterior section gives rise to symmetrical spines in females and asymmetrical spines in males (Fig. 4). In females, the caudal rami are asymmetrical and lengthy, but in males, they are symmetrical (Figs. 5, 6). The male has a severely modified P5 and right antennule. These are basic, uniramous appendages on female P5. There are twenty-four segments on the female antennules. The first segment features an aesthetasc at the outer surface and two lengthy setae (Fig. 7). During mating, the mature male *T. discaudata's* right antennule is highly geniculate and modified to grab the female (Fig. 8). Exopodite and endopodite of the antenna are well developed. The exopodite, which has seven segments decorated with both long and short setae, grows from the base of the basis. Of the two endopod segments, the second is bifid and bears twelve apical setae, while the first segment has two lengthy setae at its inner surface (Fig. 9). The parsmolaris, which forms a broad blade with various types of teeth on its surface, and the corpus mandibulae, which is thick, make up the mandible. The mandibular anatomy of this species indicates omnivorous feeding (Figs. 10, 11). The exopodite and endopodite of maxillule are adorned with lengthy setae and sharp spines. The setae and spines of this appendage might help in establishing a feeding current and in the collection and concentration of food components (Fig. 12). Precoxa, coxa, and basis come together to form a sympodite in the uniramous appendage known as the maxilla. Three to six tiny segments make up the endopodite. It seems that the exopodite is not present. This appendage's inner surface is covered in numerous lengthy setae. These setae seem as a lengthy hair tuft. Except for the apical setae, the setules appear at regular intervals in most setae. The food particles are efficiently captured by the setae and setules (Fig. 13). The precoxa, coxa, and basis of the maxillipede are uniramous appendages that combine to produce a sympodite. Endopodite setae alternate between being long and short (Fig. 14). The swimming legs of P1 through P4 are paired appendages of the copepod type (Figs. 15–18). Female P5 is symmetrical, three-segmented,

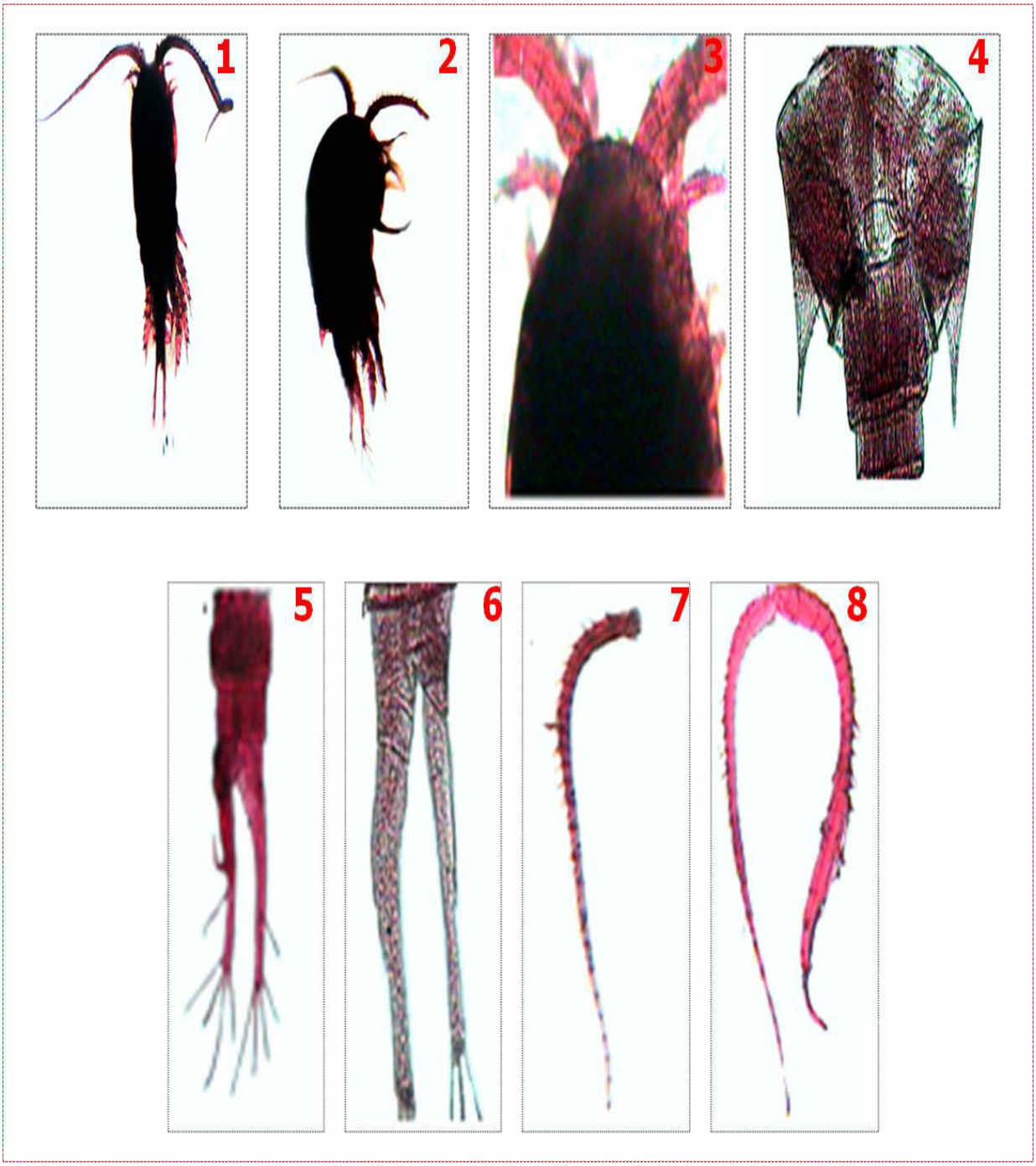


Fig. 1: Adult male; Fig. 2: Adult female; Fig. 3: Anterior part of cephalosome; Fig. 4: Metasomal spines of female; Fig. 5: Caudal rami of male; Fig. 6: Caudal rami of female; Fig. 7: Antennules of female; Fig. 8: Antennules of male.

and uniramous (Fig. 19). The male's P5 is asymmetrical because his left leg is bigger than the right. The inner surface of the second segment of the left leg, which has four segments, resembles a long, curving process similar to a thumb. There are three segments on the right P5, with the second segment being bigger than the other two. This appendage's terminal section forms a structure resembling a hook (Fig. 20).

The zooplankton ecosystem in the open ocean is dominated by copepods, one of the most prevalent groups of metazoans. The majority of calanoids are omnivorous, although certain species are primarily herbivorous. The primary

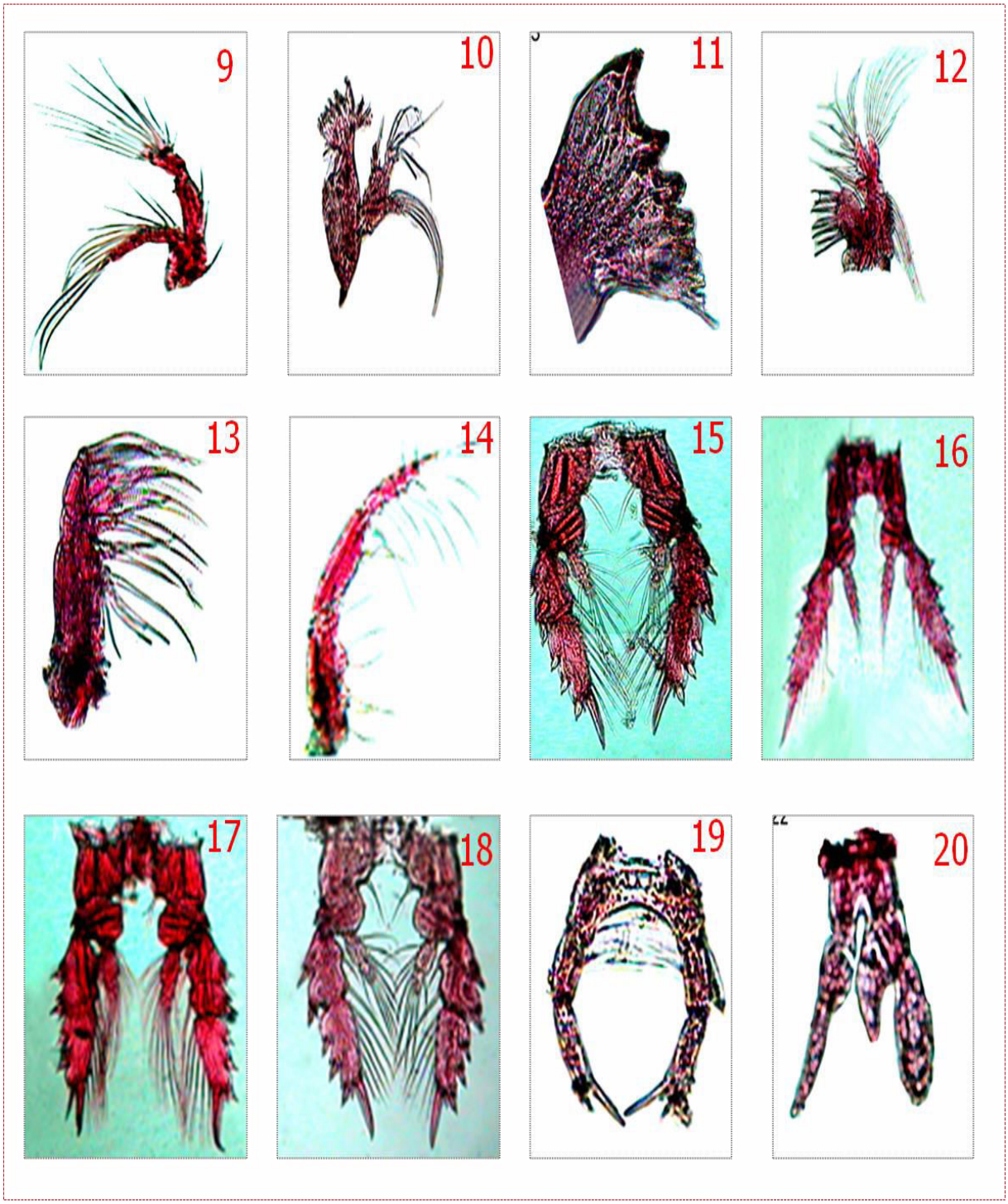


Fig. 9: Antenna; Fig. 10: Mandible; Fig. 11: Higher magnification of mandibular blade; Fig. 12: Maxillule; Fig. 13: Maxilla; Fig. 14: Maxilliped; Fig. 15: P1; Fig. 16: P2; Fig. 17: P3; Fig. 18: P4; Fig. 19: P5 of Adult female; Fig. 20: P5 of Adult male.

factor affecting these copepods' life cycles is probably the presence of phytoplankton (Peterson *et al*., 1990; Kleppel, 1993). Research has shown that there are a wide range of processes for planktonic calanoid copepods to identify, chase, capture, and reject prey, changing the old theory

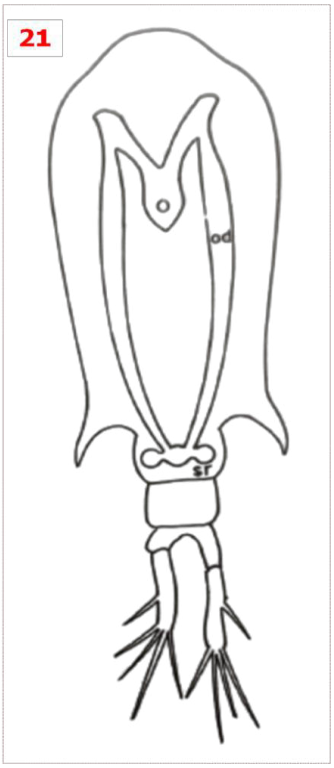


Fig. 21: Female Reproductive System (o – ovary; od – oviduct; sr – seminal receptacles).

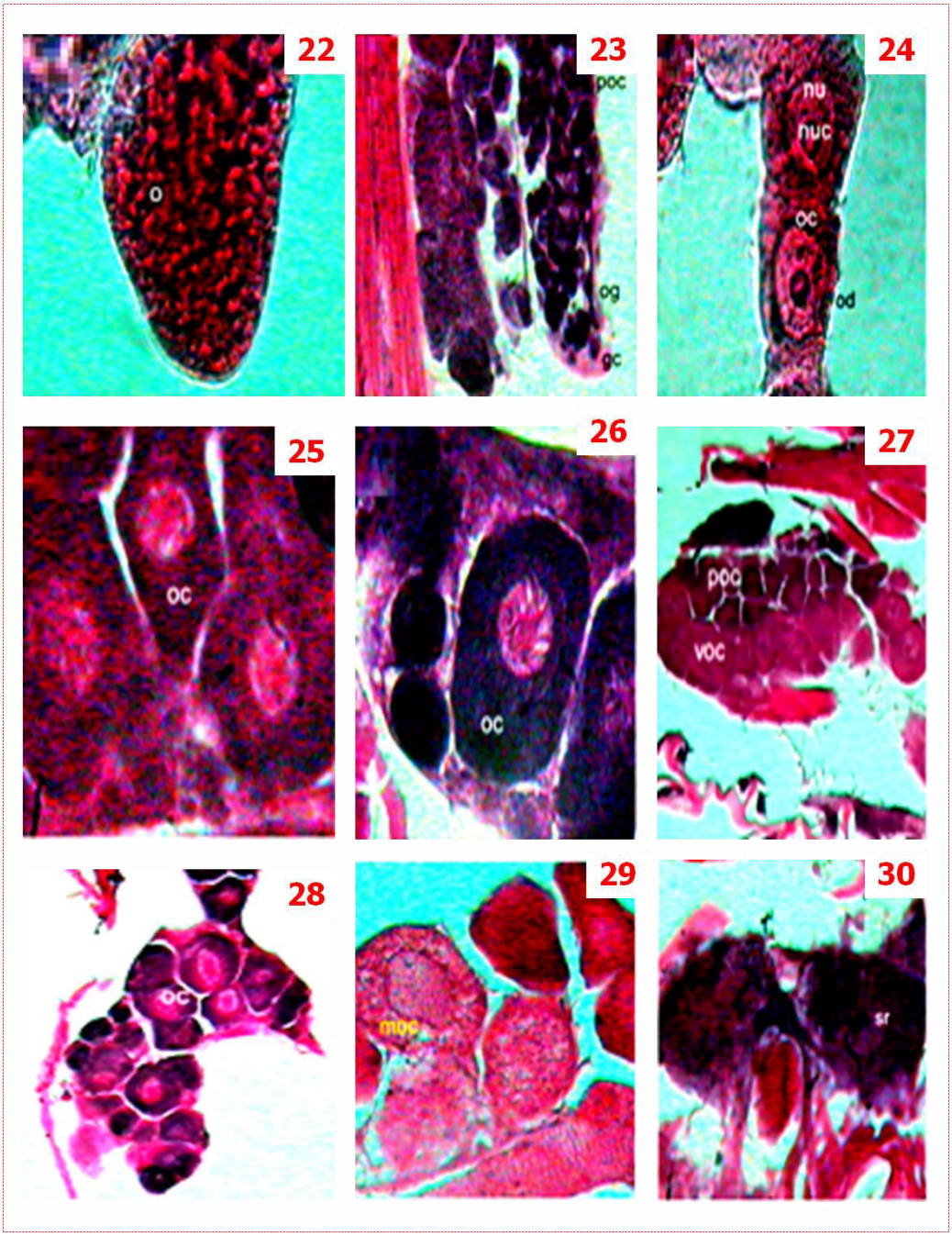
that these organisms are primarily mechanical suspension feeders (Price, 1988). In most of the herbivorous and omnivorous copepods, multiple feeding styles such as passive suspension feeding and the raptorial feeding mode are observed however, many calanoid copepod species can adopt to both kind of feeding based on the type of food available (Kiørboe *et al*., 1996). Depending on the size of the prey, either active or passive filtration or capture is used (Price and Paffenhöfer, 1986). The copepod uses the passive feeding mode to collect cells that are too tiny for it to recognise individually. When employing the active feeding mode, the majority of calanoid copepods often favour bigger microalgal food (Paffenhöfer, 1988). *T. discaudata* uses its feeding appendages, which are of the classic calanoid omnivorous variety, for active feeding. The synchronised action of a pair of antennae, mandible, maxillule, maxilla, and maxillipede allows this species to create feeding currents, drive food towards the mouth cavity, masticate, and ingestion of food. The sensory function of the antennules allows them to detect the motions of the prey. Water currents are produced on the ventral side of *T. discaudata's* body by its feeding tentacles. The food particles are subsequently actively captured by the maxillule and maxilla and sent towards the mouth cavity. Following their capture by the mandibular gnathobases, the food particles are chopped, minced, and ground before being swallowed. Other calanoid copepods have also been observed to engage in similar feeding current production, food particle collection, and mincing processes (Schnack, 1989, Michels and Schnack-Schiel, 2005). The many feeding apparatus appendages of *T. discaudata* demonstrate its capacity to filter and consume nutrient-rich microalgae like *Chloroidium saccharophilum* and *Isochrysis galbana*.

The median ovary, two oviducts, and an antrum make up *T. discaudata*'s female reproductive system. A pair of oviducts arises antero laterally from the ovary and takes an anterior route dorsally up to the anterior end of cephalosome and takes a ventral course to bend

towards the posterior side of the cephalosome. It proceeds along its ventral path until it reaches the end of the metasome, at which point it enters the genital segment and proceeds into the median antrum. Through the female reproductive pore, the antrum connects with the outside world (Fig. 21). Histologically, the ovary of *T. discaudata* is composed of a thin wall and has ascending rows of oocytes from the posterior to the anterior end, each at a different stage of oogenesis. Only the posterior most portion of the ovary contains the spherical germinal cells with a big nucleus (Fig. 22). The oogonial cells, which multiply into primary oocytes through mitotic division, are produced by the germinal cells. In comparison to the oogonial cells, the primary oocytes are marginally smaller. At this point, it is easy to distinguish between the cytoplasm and the nucleus. Secondary oocytes, which are bigger than primary oocytes, occupy the next anterior region of the ovary (Fig. 23). The secondary oocytes then develop into germinal vesicle stage oocytes, which have a big, chromophobic nucleus. At this point, the nucleus's centre has a noticeable nucleolus. Most of the oocytes in the anterior portion of the ovary are in the previtellogenic stage. The syncytial epithelium and connective tissue layer found on the walls of the oviducts and the ovary exhibit histological similarities. Nonetheless, the thick wall of the oviduct's posterior end is made of glandular epithelial cells. After being moved to the oviduct, the previtellogenic oocytes mature and go through vitellogenesis. The volume of the oocytes rises several times during vitellogenesis. Yolk granules are actively deposited in the cytoplasm throughout this step, and yolk globules are also deposited during the last stage of vitellogenesis. Yolk granules and yolk globules abound in mature oocytes (Figs. 24–29). Mature oocytes have caused the oviducts to become greatly enlarged. They take up the majority of the prosome's perivisceral cavity. The secretory material produced by the posterior part of the oviduct is clearly different from that of the other parts, and it transforms into the membrane ovisac, which is where fertilised eggs go through embryonic development. Spermatozoa and secretory materials are found within the thin-walled seminal receptacles (Fig. 30).

Most of the calanoid copepod females have a single median ovary located dorsally to the gut in the posterior part of the cephalosome. Paired oviducts, with large diverticula, originate antero-laterally and extend forwards as diverticula into the anterior cephalosome and backwards on either side of the gut to the urosome. The oviducts open into a single chamber, the genital atrium (Blades-Eckelbarger, 1991). The genital atrium has paired dorso-laterally directed spermathecae. The organization of the female reproductive system of *T. discaudata* shows similarity with other calanoid copepods (Blades-Eckelbarger and Youngbluth, 1984; Batchelder, 1986; Razouls *et al*., 1986, 1987; Norrbin, 1994). As in the case of other calanoid copepods (Park 1966; Blades-Eckelbarger and Youngbluth, 1984), oogenesis in *T. discaudata* up to the previtellogenic stage takes place in the ovary, and vitellogenesis and maturation of oocytes happen in the oviducts and their diverticulae. However, some differences in the mechanisms of vitellogenesis are reported in different calanoid species (Mauchline *et al*.,1998).

T. discaudata has a single genital duct and a median testis as its male reproductive organs. The left genital duct emerges from the anterior region of the testis, travels ventrolaterally in the perivisceral cavity, and exits the body through the genital segment. The right genital duct is nonexistent. The vas deferens, seminal vesicle, spermatophore sac, and ductus ejaculatoris can be identified within the vaginal duct. The ductus ejaculatoris then opens to the outside through a horizontal lateral slit (Figs. 31, 32). The testis's wall is made up of two thin layers: syncytial epithelium on the inside and connective tissue on the outside. The posterior portion of the testis is where the germinal cells are located. The various stages of spermatogenic cells are grouped in ascending rows, and the anterior portion of the testis is where mature spermatozoa are found. The germinal cells divide mitotically to produce more spermatogonial cells. The next anterior region of



Figs. 22 and 23: Ovary; Fig. 24: Oocytes in oviduct; Figs. 25 - 28: Oocytes in different stages of vitellogenesis; Fig. 29: Mature oocytes; Fig. 30: Seminal receptacles (od – oviduct; o- ovary; poc – primary oocytes; voc – vitellogenic oocytes; og – oogonial cells; gc – germinal cells; nu – nucleus; nuc – nucleolus; sr – seminal receptacle; moc – mature oocytes).

the testis is occupied by spermatogonial cells, which develop into primary spermatocytes. Meiotic division is the process by which the primary spermatocyte divides into secondary spermatocytes. There are multiple rows of spermatids oriented towards the front portion of the testis. Differently from the secondary spermatocytes, these cells are smaller. These cells have strong haematoxylin staining in the periphery of their nuclei. These cells turn into spermatozoa by enduring alterations in their nucleus and cytoplasm. The mature spermatozoa

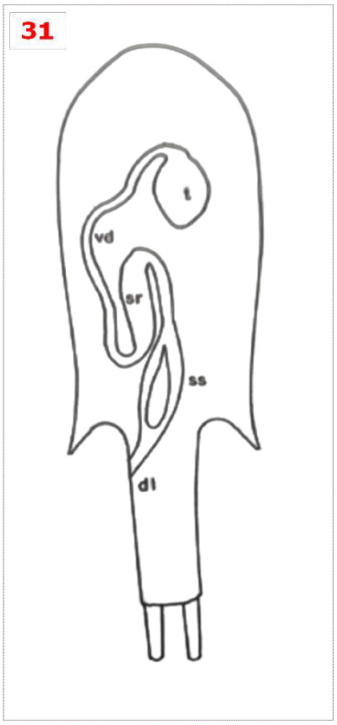


Fig. 31: Male Reproductive System (t – testis vd- vas deferens sv – seminal vesicle ss - spermatophore sac de - ductus ejaculatoris).

have a diameter of 4 to 5 µm and are primarily spherical in shape (Figs. 34, 35). Males of several calanoid copepod species have a single testis and a developed left genital duct as part of their male reproductive system, like that of *T. discaudata*. At the testis' anterior end, spermatozoa are produced when the process of spermiogenesis is finished (Park, 1966; Raymont *et al*.,1974; Hopkins, 1978; Blades and Youngbluth, 1981).

Histologically, the vas deferens wall is composed of an outer layer of connective tissue and several layers of glandular epithelial cells. Secretory material is produced by the vas deferens wall and adheres to the spermatozoa in its lumen. The posterior part of vas deferens collects significant number of spermatozoa and secretory material compared to the anterior region (Fig. 36). There are ascending and descending limbs on the seminal vesicle. In comparison to the descending limb, the ascending limb is thicker. Its thick wall is made up of numerous layers of cuboidal and elongated cells. It is lined with epithelial cells on the inside and has a thin layer of connective tissue and muscle on the outside. It yields a lot of secretory material with fine granular material in addition to resembling vas deferens secretory material. Comparatively thin-walled and less glandular is the wall of the seminal vesicle's descending leg (Figs. 37-39). The seminal vesicle serves as a storage organ for spermatophore material in the ascending limb in addition to generating secretory material needed for the production of various spermatophore components. Different spermatophoric layers, including core secretory material, a layer of spermatozoa, secretory material exterior to the spermatozoa, and material intended for the creation of spermatophore wall, are visible in the longitudinal section of the ascending limb of the seminal vesicle (Figs. 40, 41). The descending limb of the seminal vesicle transfers spermatophoric material from ascending limb to the spermatophore sac. As the spermatophore takes on its final shape and form, the spermatophore sac functions as a mould (Fig. 42). The thick wall of the spermatophore sac is made up of both

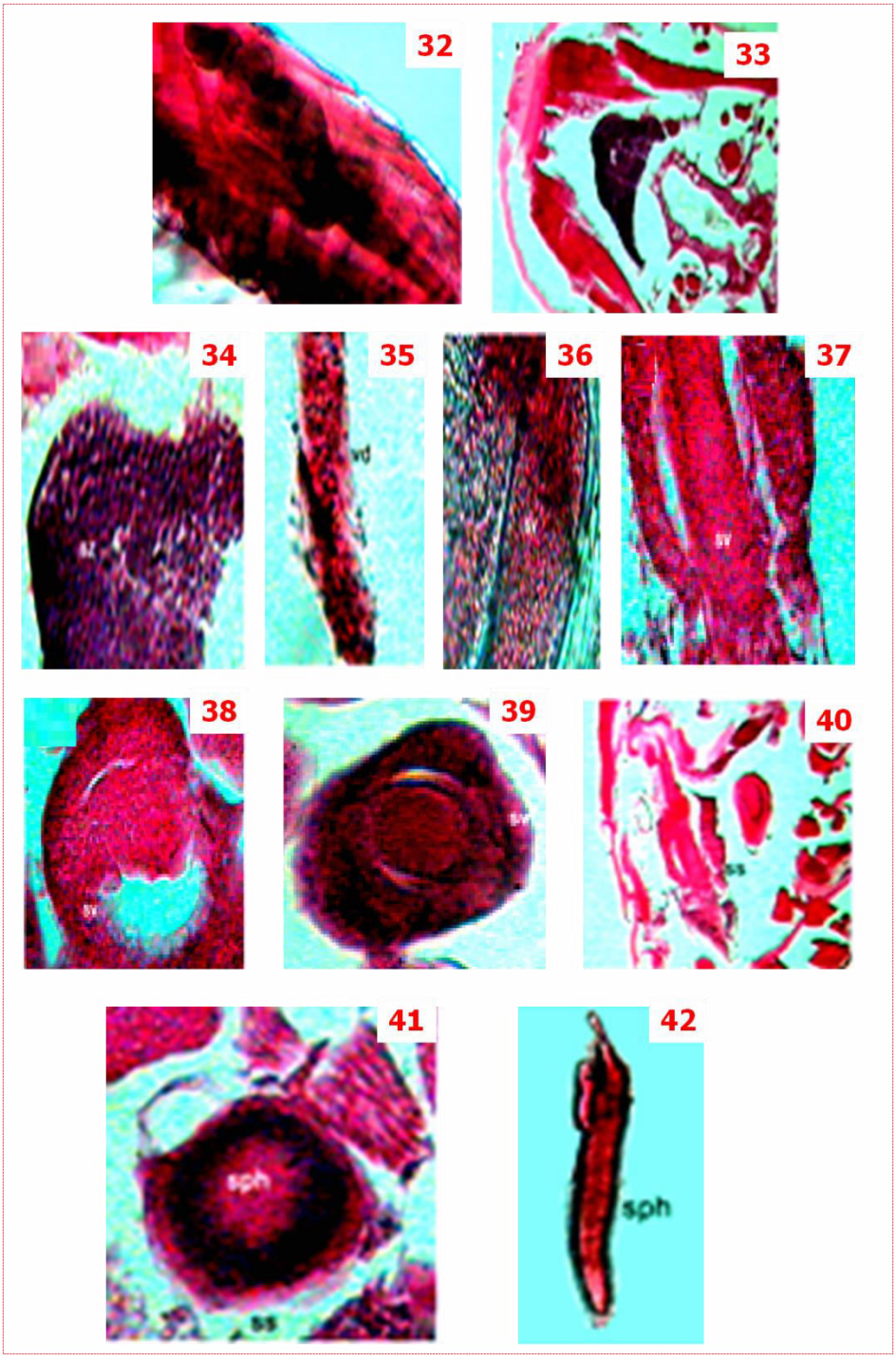


Fig. 32: Male Reproductive System *in situ*; Fig. 33: Testis; Fig. 34: Anterior region of testis; Fig. 35: Vas deferens; Figs. 36 and 37: L.S. of seminal vesicle; Figs. 38 and 39: C.S. of seminal vesicle; Fig. 40: L.S. of spermatophore sac; Fig. 41: C.S. of spermatophore sac; Fig. 42: Spermatophore (t – testis, vd – vas deferens, sv – seminal vesicles – spermatophore sacsz- spermatozoa sph – spermatophore).

vacuolated and spongy cells. It generates granular secretory material that helps the spermatophore smoothly extrude from the spermatophore sac during mating by lubricating its exterior. The ductus ejaculatoris has a thin, non-glandular wall. It connects the external medium to the

spermatophoric sac. The calanoid copepod's male genital duct is composed of five separate areas. It can take two unique forms, depending on whether it produces a complex or simple spermatophore (Mauchline *et al*., 1998). Numerous species' spermatophore formation has been thoroughly described, including Park (1966), Hopkins (1978), Raymont *et al*. (1974), and Blades and Youngbluth (1981). The genial duct of *T. discaudata* creates simple kind of tubular spermatophore using secretory components produced by the vas deference, seminal vesicle, and spermatophoric sac. Once fully developed, the male *T. discaudata* detects the female by using its geniculate right antennule to detect her presence and approaches her. The mating process then occurs, in which the male's modified right P5 holds the female close while the extruded spermatophore is precisely linked to the female vaginal pore through the male's left P5. The seminal receptacle houses the spermatozoa. The ovisac, which is where embryonic development occurs, is where fertilised eggs are kept. Similar to *Calanus finmarchicus* females (Marshall and Orr, 1972), *T. discaudata* females need only one insemination to generate several clutches, whereas *Eurytemora affinis* females require mating for each clutch to be produced (Heinle, 1970). In a clutch, the female *T. discaudata* produces 48±6 eggs. Under laboratory conditions, *T. discaudata* is fed an *Isochrysis galbana* diet and is observed to breed continuously and produce eggs. This species exhibited typical calanoid mating behaviour, including the transfer of spermatozoa and storage of spermatozoa in seminal receptacles. Hatching success of 82% eggs was found in this species. The recorded post-embryonic time was 7-8 days. The average length of copepodites/adults in µm is CI 447, CII 482, CIII 726, CIV 814, CV female 1180, CV male 1079, Adult female 1360, and Adult male 1210. The average length of nauplii reported in µm is NI 104, NII 126, NIII 148, NIV 197, NV 225, and NVI 259. The range of sizes exhibited by *T. discaudata's* nauplii, copepodites, and adults makes them appropriate live prey for the various stage larvae of numerous marine finfishes that are commercially significant. Being natural and preferred diet, many calanoid copepod species *like P. annandalei*, *P. serricaudatus* (Altaff, 2020), *Centrophagus furcatus* (Sheriff *et al*., 2023) and *Eucalanus monachus* (Sivakumar *et al*., 2023) have been recently reported for mass production and use as live feed for fish larvae.

Conclusion

The utilisation of planktonic copepods as live prey in the hatchery raising of marine finfish larvae has resulted in the need to expand the species diversity for mass production. Crucial factors to consider when developing a copepod high density culture procedure are the medium's temperature, the cultivable species' capacity for reproduction, and their food and feeding sources. From this angle, the tropical calanoid copepod *T. discaudata's* diet, feeding, and reproductive tactics were assessed in this study. This species' feeding appendages, which include its mandible, antenna, maxillule, maxilla, and maxillipede, are well-equipped with features that allow for omnivorous feeding. The regular presence of vitellogenic and previtellogenic oocytes in the oviducts and ovary, respectively, indicates that this species breeds continuously. Males have the capacity to produce spermatophores continuously when they are actively reproducing. Because of its high fecundity, appropriate nauplii, copepodites, and adult sizes, as well as its distinctive swimming motion, *T. discaudata* is a perfect live feed for large-scale production and the rearing of several marine fin fish larval stages.

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