

Original Article

Insight into the reproductive biology of euryhaline cyclopoid copepods *Apocyclops dengizicus* and *Apocyclops royi*

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Abstract: The present research work aimed to investigate the unexplored information about mating strategies and reproductive potential of two euryhaline cyclopoid species *Apocyclops dengizicus* and *A. royi*. Laboratory experiments on mating strategy and reproductive aspects such as the efficacy of females under different mating conditions, the reproductive potential of males, mating frequency, and the effect of starvation on survival and reproductive potential, were conducted and documented. The once-mated female and females pairing continuously with males favored higher reproductive potential. In the present study, the mate-pursuing males prefers to copulate with mature virgin females. The older female was unable to produce eggs even after the sperm discharge. The lifespan of both the *Apocyclops* species under different states of mating and unmated conditions showed considerable variation. *Apocyclops dengizicus* produced 7.92 ± 0.57 pairs of spermatophores, while *A. royi* extruded 13.32 ± 0.99 pairs of spermatophores during its lifespan. The results were analyzed and inferred; thus high throughput was applied to reveal the understudied topics of these *Apocyclops* species to add considerable knowledge about its reproductive biology.

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Introduction

Copepods are dominant group of zooplankton enjoying diversified distribution in various aquatic habitats, and play a pivotal role in the energy transfer of the aquatic ecosystem. Copepods contribute more to global ecology by controlling the microbial food web and biogeochemical cycle and are also used as a test organism in ecotoxicological studies (Burriss and Dam, 2014). Despite a large number of outstanding research on copepods, much biological information remains obscure. Copepods transfer spermatozoa to the female through the spermatophore, a highly organized structure with well-stored spermatozoa and a variety of secretory substances (Muthupriya et al., 2004). Successful spermatophores placement by males on a female genital system is a complicated process involving

behavioral and morphological compatibility (Maier, 1992; Lonsdale et al., 1998). Misplacement of spermatophores outside the genital field has been reported for *Centropagid* calanoid copepods by Lee (1972). In copepods, the successful mating and transfer of spermatophore to female is the basic requirement to develop its population growth. But the success of reproduction is not only dependent on female fecundity but also equally shared by male copepods through mating frequency and it is energetic (Kiorboe, 2006).

According to Burriss and Dam (2015), lifetime spermatophore production can provide valuable information about male energy and mating which is not known for many copepod species. Ianora et al. (1989) opine that though it is important to study much about the potential of males in population

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dynamics, very less knowledge is gathered about its reproductive investment. The fecundity rate of the female is extensively studied while information about the spermatophore production rate is lacking (Marshall and Orr, 1958). The spermatophore production and fecundity may also be limited by factors like food and age (Burriss and Dam, 2015) and it is also evident that even sperm quality is affected by these factors (Sichlau and Kiorboe, 2011).

The genus *Apocyclops* includes species found in continental brackish and hypersaline waters worldwide (Cheng et al., 1999). *Apocyclops royi* and *A. dengizicus* are the common dominant species in the Adyar estuary, Chennai, Tamilnadu. Both the species were experimentally studied to reveal the secrets of male mating biology and to explore whether males' reproductive investment limits females' fecundity rate. Thus, the current study was conducted to assess male energetics by conducting experiments on the rate of spermatophore production and reproduction in females related to mating. Further laboratory studies on reproduction in starvation and the status of unmated females were also conducted. The present study is highlighted to address the unanswered questions such as the mating preference of males, determine the frequency of mating, sperm storage, and usage, measuring spermatophore production, factors affecting mating and reproduction, rate of fecundity concerning spermatophore size, and whether remating is necessary to improve reproduction. As little is known about these species, this study will explore more information about their basic biology and energetics.

Materials and Methods

Zooplankton samples were collected from the Adyar estuary using a plankton net made up of Bolten silk of 50 µm mesh size. The collections of zooplankton samples were carried out during the early hours of the day. The collected samples were preserved in 5% buffered formalin. *Apocyclops dengizicus* and *A. royi* were identified based on the minute morphological details and taxonomic key characters

provided by Botelho (1999) and Reid et al. (2002). Live zooplankton samples were transported to the laboratory in a 5 L insulated polyethylene container within an hour. *Apocyclops dengizicus* and *A. royi* were sorted out and maintained in pretreated water (seawater double filtered in 50 µm Bolten silk and allowed to settle in large aquaria at room temperature for 48 hours and aerated for 12-24 hours before use). They were fed baker's yeast (*Saccharomyces cerevisiae*) on alternate days. Both *Apocyclops* species were reared in the laboratory, and male and female Copepodid V (C V) were used for various experimental studies.

Reproduction potential of *A. dengizicus* and *A. royi*: The reproductive potential of female *A. dengizicus* and *A. royi* were carried out with different mating conditions in the laboratory, including Trail I - Male mated with a female once in a lifetime, Trail II - Male mated in regular intervals with female and Trail III - Male continuously presents with female in the culture medium.

Mating behavior: Each species of virgin male (C V) and female (C V) were transferred to the Petri dish separately, and mating behavior was observed under an inverted microscope. The duration of mating, hatching, number of clutches, clutch interval, number of eggs, egg diameter, and clutch size produced by the mated female during its entire lifespan was recorded.

Reproduction potential of the unmated female of *A. dengizicus* and *A. royi*: Both the species of females in the C V stages were maintained separately in unmated conditions and their reproductive variables were studied.

Spermatophore size variation in males: To study the reproductive potential of male *A. royi* and *A. dengizicus*, experiments were conducted for six hours and six days duration vice versa. In the first experiment: the number of females mated by a single male for six hours duration, and in the second experiment, males were allowed to mate with a single virgin female, once a day regularly. In both experiments, the size of the spermatophore transferred was micro-metrically measured and

photomicrographed.

Effect of starvation on reproduction and survival:

To study the influence of starvation on the reproduction and survival of *A. dengizicus* and *A. royi*, laboratory-reared females and males of C VI stages were exposed to three days of starvation. Using these starved virgin males and females, three types of experiments were conducted, including Trail A - Starved male with starved female, Trail B - Starved male with well-fed female and Trail C - Starved female with well-fed male.

The experiment was conducted in a controlled condition with a temperature of $28\pm 1^{\circ}\text{C}$ and pH of 7.5. The reproductive potential and survival were monitored. The experiments were maintained with the above conditions in 20 ml filtered seawater with *ad libitum* feed. The production of spermatophore number, the total number of clutches produced, interclutch period, number of eggs, egg diameter clutch size, and survival duration were recorded. The well-fed males and females were maintained separately in a yeast-fertilized medium. The number of clutches produced during their lifespan was monitored regularly.

Statistical Analysis: The data of the number of clutches, clutch interval, number of eggs, egg diameter and clutch size, and survival of *A. dengizicus* and *A. royi* were subjected to statistical analysis. Analysis of Variance was used to test significant differences between the variable at $P < 0.05$. The mean difference ($P < 0.05$) of the parameters was analyzed by Duncan Multiple Range's Test.

Results

Mating behavior of *A. dengizicus* and *A. royi*:

Mating and transferring spermatophore in *A. dengizicus* and *A. royi* occur soon after the transfer from copepodid V (C V) to copepodid VI (C VI). Before mating, the male and female show normal leaping, spiraling, and helical swimming movements. The male and female might get approximation through mechano and chemical sensations. When closer to the female, the male



Figure 1. Male capture the female caudal rami—*Apocyclops royi*.

shows the higher activity as a preliminary step for mating; the male captures the caudal rami of the female for mate recognition (Fig. 1). Immediate wriggling movement of the male puts forth both the antennules towards the swimming legs of the female (Fig. 2). In this condition, males actively perceive the female until it becomes passive. Then the male makes a characteristic movement bending its body towards the genital segment of the female and sweeps the genital area with its caudal rami (Fig. 3). After this act, it resumes its mating behavior to bring the genital segment of both sexes together. At this juncture, male and female faces were ventrally. The prehensile distal part of the male antennules firmly holds the female's third and fourth swimming legs. This is happening to be the proper copulatory position, and once the proper copulatory grasp is achieved male continues to show higher activity while the female becomes more and more passive. At this mating posture, the male's urosome shows fluxion, leading to the extrusion of a pair of spermatophores.

During the final phase of spermatophore extrusion, the genital segment fluxes anteriorly to the



Figure 2. Male antennules capture the female pleopods V-*Apocyclops royi*.

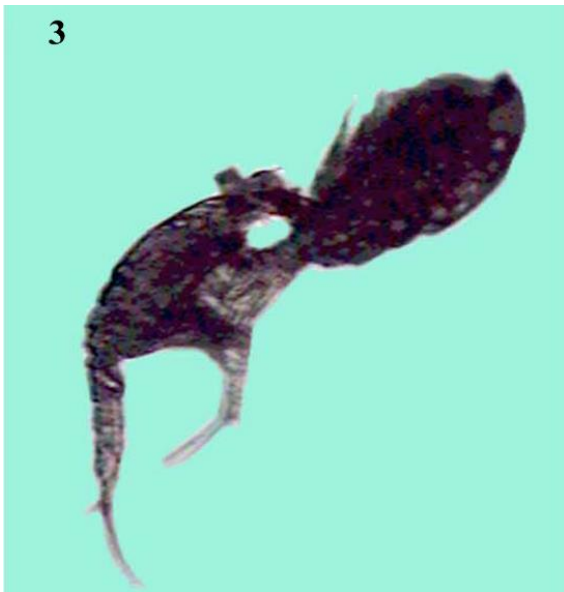


Figure 3. Male animal bending its body into female genital segment-*Apocyclops royi*.

maximum extent, which swiftly transfers the spermatophores onto the setae of swimming legs of the male, which in turn directs forwards and in a quick succession place and attaches the spermatophore closer to the genital pore on the ventral surface of the female genital segment. The whole of this spermatophore extrusion, transfer, and attachment to the genital area is a rapid activity and takes about 15-30 seconds. During the spermatophore transfer and its attachment, females



Figure 4. Male mating with ovigerous female-*Apocyclops royi*.

remain passive. However, the male holds the female's swimming legs either in an upright position or facing the legs more towards the anterior side. Such a copulatory position is maintained for about five minutes. During this male ensures that the genital area of the female where spermatophores are freshly attached is undisturbed either by the male's appendages or the female's swimming legs. Finally, the male loosens its antennular holds and releases the female. Subsequently, the male lies passively, fully stretching its antennules for one or two minutes, and then resumes its normal swimming. After mating and transfer of spermatophores, the female also lies passively. It remains passive for a longer duration compared to males.

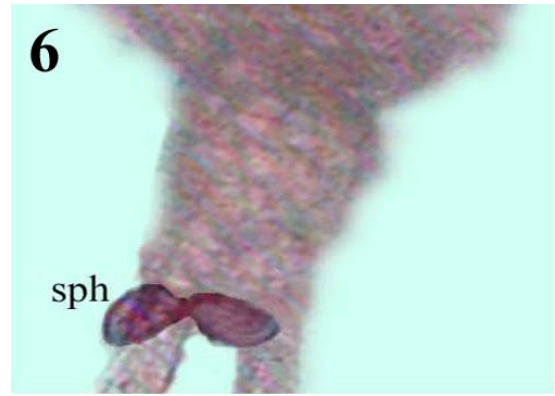
In the present study, the mate-pursuing males prefer to copulate with mature virgin females than with mated females carrying or not carrying fertilized eggs. Mating was also observed in ovigerous females but was not always successful (Fig. 4). Many males attempted to mate with a female and mating with C V females were also observed (Fig. 5). Misplacement of spermatophores around the gonophore, in the urosomal segments, and caudal ramus were also observed in the *A. royi* (Figs, 6, 7). In both species, the entire mating process



Figure 5. Two males attempting to mate with female–*Apocyclops royi*.

takes place in about 15-30 minutes. After mating, a pair of spermatophores is attached to the ventral surface of the female genital segment. Discharge of spermatophores content into the seminal receptacle and removal of exuviae of the spermatophores takes about 2 hours. The females of both species produced a pair of ovisacs containing fertilized eggs about one hour after mating (Fig. 8). However, the duration taken for ovisac formation varies depending upon the maturation of oocytes.

Embryonic development is completed in 24 hours and nauplii are hatched thereafter. Hatching takes about 20-30 minutes. Single nauplii take about five minutes to release from the ovisac (Fig. 9). In spent females, the spermatophore remained attached to the genital pore for even five days. If mature oocytes were available in the oviduct, the female readily produces a new clutch within one-hour duration after hatching. When recently mated females with or without spermatophores were allowed to mate immediately with a virgin male, the females could not mate. It escapes from the male's grip before the spermatophores were transferred. As the female aged, there was a gradual decrease in the egg-laying rate. In the final phase of reproduction, the female



Figures 6 and 7. Misplacement of spermatophore around the female caudal rami and gonophores–*Apocyclops royi*. (sph–spermatophore).

produced empty sacs without eggs. Though the older female mates, it is unable to produce eggs even after the sperm discharge. In a few cases in the late phase of reproduction, a new ovisac emerges before the fall of the older empty sacs and this triovisac condition was observed many times (Fig. 10).

Reproduction potential of *A. dengizicus* and *A. royi*: The reproductive potential of female *A. dengizicus* and *A. royi* in different mating conditions showed variations in the number of clutches, egg numbers, and ovisac length and width in the present study. The numbers of clutch were significantly ($P < 0.05$) highest in females mated at regular intervals with males (Trail III) of 9.08 ± 0.40 and 12.64 ± 0.86 , in *A. dengizicus* and *A. royi*, respectively. However, the number of eggs of 38.84 ± 0.62 and 39.96 ± 1.14 and ovisac length (308.80 ± 1.50 and 288.12 ± 1.86 μm) and width (210.04 ± 2.07 and 146.24 ± 1.61 μm) were recorded



Figure 8. Ovigerous female–*Apocyclops royi*.

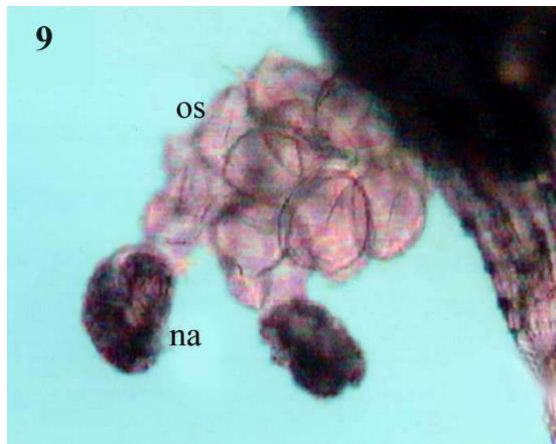


Figure 9. Nauplii release from ovisac–*Apocyclops dengizicus*. (na–nauplii; os–ovisac).

significantly ($P < 0.05$) high in once mated female (Trail I), in *A. dengizicus* and *A. royi*, respectively. The interclutch period was found to be shorter in the females who were mated at a regular interval (Trail II) than once mated females (Trail I) as well as females continuously paired with males (Trail III) in both the species (Tables 1 and 2). ANOVA for the number of clutches, ovisac length, ovisac width,

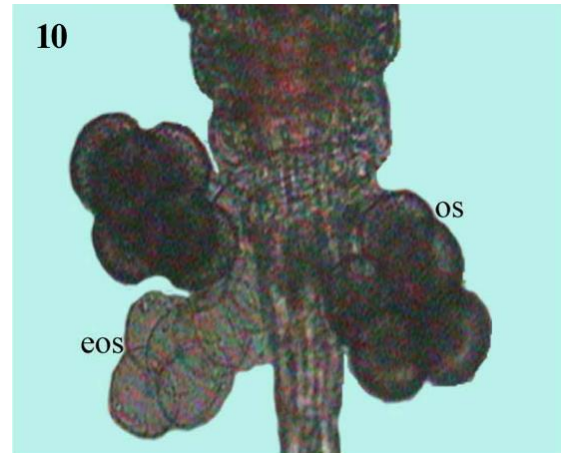


Figure 10. Triovisac–*Apocyclops dengizicus*. (eos–empty ovisac;

number of eggs in the clutch, egg diameter, and clutch interval of both the species in different mating conditions showed significant differences ($P < 0.05$). The gross observation indicated that once mated or pairing females continuously with males favored higher reproductive potential. In the once-mated female of both the species, during the final phase of reproduction, it extruded unfertilized eggs which were shredded or disintegrated. After extruding a few batches of unfertilized eggs, they released granular secretion (egg material) until death.

Unmated females: The female's *A. dengizicus* and *A. royi* were maintained in unmated condition after the final molt extruded unfertilized eggs from the fifth day or one week onwards. It extruded very few infertile eggs (1-5) and rarely a complete batch of eggs. It was observed that there was oocyte production in the unmated females; from the early oocyte stage they transformed into germinal vesicle stage with a conspicuous nucleus and nucleolus. These oocytes remained in the oviduct without undergoing intensive vitellogenesis. Though females without mating for 8 days showed normal production of oocytes, a greater abnormality was observed in the number and size of eggs. After this period, the oviducts of the unmated females contained eggs of normal texture and abnormal eggs in the form of disintegrated secretory material (Fig. 11). Further, advancement in the unmated condition leads to the production of mostly abnormal eggs in the form of

Table 1. Reproductive potential of *Apocyclops dengizicus* in different mating conditions.

	No. of Clutches	Ovisac Length (µm)	Ovisac Width (µm)	No. of Eggs	Egg Diameters (µm)	Clutch Interval
Trail I	8.04±0.45 ^a	308.80±1.50 ^a	210.04±2.07 ^a	38.84±0.62 ^a	84.08±0.64 ^a	2.20±0.41 ^a
Trail II	7.04±0.35 ^b	258.96±1.77 ^b	179.92±2.12 ^b	27.92±0.76 ^b	82.00±0.71 ^b	5.12±0.44 ^b
Trail III	9.08±0.40 ^c	288.88±1.81 ^c	182.92±2.12 ^c	25.16±1.43 ^c	83.96±0.68 ^a	3.08±0.49 ^c

The values are represented as Mean±SD (n=25).

Different superscripts on the column between the Trails showed significant different ($P<0.05$) of either species.

Table 2. Reproductive potential of *Apocyclops royi* in different mating conditions.

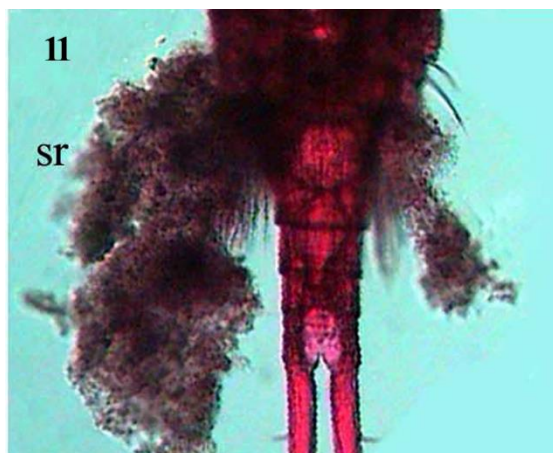
	No. of Clutches	Ovisac Length (µm)	Ovisac Width (µm)	No. of Eggs	Egg Diameters (µm)	Clutch Interval
Trail I	9.56±0.71 ^c	288.12±1.86 ^b	146.24±1.61 ^b	39.96±1.14 ^a	71.04±0.89 ^b	8.00±0.50 ^a
Trail II	8.56±0.77 ^b	210.72±1.59 ^c	116.32±1.28 ^c	29.96±1.24 ^b	68.24±0.83 ^a	1.12±0.33 ^b
Trail III	12.64±0.86 ^a	231.96±1.79 ^a	125.68±0.95 ^a	21.12±1.33 ^c	69.80±0.96 ^c	3.04±0.35 ^c

The values are represented as Mean±SD (n=25).

Different superscripts on the column between the Trail showed significant different ($P<0.05$) of either species.

Table 3. Survival of *Apocyclops dengizicus* and *A. royi* under different mating conditions.

Parameters	No. of days survived			
	<i>A. dengizicus</i>		<i>A. royi</i>	
	Male	Female	Male	Female
Once mated	21±2	35±1	38±1	36±1
Intensively mated	11±1	27±1	15±1	16±2
Unmated	24±1	63±2	50±2	64±1

**Figure 11.** Secretory materials formed in *Apocyclops dengizicus*.

disintegrating secretory material. The female maintained around 40 days in unmated condition, when allowed to mate with a normal adult male there was no proper sequencing of the mating act, and due to the evasive activity of the female, there was no transfer of spermatophores.

Experiments on the reproductive potential of males: In the mating process, the males of *A. dengizicus* and *A. royi* transfer a pair of bean-

shaped spermatophores. *Apocyclops dengizicus* produced 7.92 ± 0.57 pairs of spermatophores, while *A. royi* extruded 13.32 ± 0.99 pairs of spermatophores during its lifespan. In both the species during the peak reproductive period, after the extrusion of spermatophores from the spermatophore sac, a new pair was formed within 30 minutes (maximum 9 hours). Laboratory experiments indicated that the size ($15\text{--}62\ \mu\text{m}$ in length and $6\text{--}30\ \mu\text{m}$ in width) and shape of the spermatophore varied. The mating capacity was limited by the time taken by the animal to refill the spermatophore. Depending on the duration of refilling the spermatophore, the size of the spermatophore varied. In some cases, instead of a pair, a spermatophore was also extruded. In the case of successive mating, the male was capable of transferring spermatophores to 3-4 females in 2-3 hours. During successive mating, the size ($15\text{--}60\ \mu\text{m}$ in length and $6\text{--}30\ \mu\text{m}$ in width) of the spermatophore gradually decreased. Further, variation in the shape was also observed. After transferring 3-4 pairs of spermatophores in a day,

even though the male initiated mating again by capturing the female, spermatophore transfer was not observed and it takes about 7-9 hours to refill its spermatophore sac.

The size of the spermatophore transferred by a male of *A. royi* during daily mating (45-60 μm) was longer than the male which mated successively (15-60 μm) on 6 hours duration with several females and transferred the spermatophores. The size difference in the spermatophores extruded during six hours duration showed a significant difference ($P<0.05$) while the spermatophore transferred after daily mating for six days did not show a significant difference. The male *A. royi* which was allowed to mate with a female every day extruded spermatophore in normal size (45-60 μm) and the mated females subsequently produced the normal number of clutches (10 ± 2). In successive mating, depending on the size of the spermatophore, the clutches produced by the mated females varied. When the size of the spermatophore transferred was very small (20 μm) the mated female produced only two clutches during its lifespan.

Few males exhibit normal mating with the female and extruded normal size spermatophores. Though females produced a smaller number of clutches and no case a complete egg batch was observed; only a few numbers of eggs were produced. This might be due to the poor viability of sperm.

The lifespan of unmated males and females: The lifespan of *Apocyclops* species under a different state of mating and unmated conditions showed considerable variation. Unmated *A. dengizicus* and *A. royi* male and female survived longer than once mated animals. Compared to these animals, individuals which were intensively mated showed survival for a short period (Table 3).

Effect of starvation on survival and reproduction: The starved adult females of both species survived the longer duration of 10 ± 1 days, while the males were 7 ± 1 days. During starvation, both females and males were active for about two days, and subsequently, their locomotory activity reduced gradually. Experimentation results on the

reproduction of these animals indicated that the males could mate with the female and transfer spermatophores not more than twice under starved conditions. The size of the spermatophores ranged 35-50 μm . Instead of a pair, the extrusion of a single spermatophore was also observed. The female fed to satiation when inseminated by a starved male could produce only one or two clutches during its lifespan. Though the females appeared to be gravid with mature oocytes in oviducts (darkened oviducts), clutch production was not observed. Instead, the oocytes from the oviduct were released to the medium through the reproductive pore. In the case of starved females which were inseminated by well-fed males, only two clutches were produced with the interval of two days.

The development of eggs in the ovisac was arrested and eggs remained in the ovisac for about three days during this period. The eggs turned pale in appearance and the egg content disintegrated leading to its discharge from the sac to the medium (Fig. 12). After the release of aborted eggs, the empty ovisac sometimes remained attached to the genital segment for about two days. The starved female thereafter did not produce normal eggs and ovisac. The oocytes of the oviduct mostly remained in the form of granular secretory material which oozed out of the reproductive pore. In the final phase of the starved period, there was no oocyte production in the female as evidenced by the transparent oviducts.

The effect of starvation on the number of clutches produced, length and width of ovisac, number of eggs, and egg diameter of *A. dengizicus* and *A. royi* are presented in Tables 4 and 5. The number of clutches (2.20 ± 0.42 and 2.20 ± 0.79), the number of eggs (29.40 ± 0.84 and 29.40 ± 0.52) and the length and width of the ovisac were significantly ($P<0.05$) higher in starved male with well-fed female (Trail B), starved female with well-fed male (Trail C), and starved male with starved female (Trail A) in *A. dengizicus* and *A. royi*, respectively. DMRT's test showed that the number of clutches between Trail A and Trail C and egg diameter between Trail B and

Table 4. Reproductive potential of *Apocyclops dengizicus* under starvation condition.

	No. of Clutches	Ovisac Length (μm)	Ovisac Width (μm)	No. of Eggs	Egg Diameters (μm)
Trail A	1.60 \pm 0.52 ^a	258.80 \pm 5.94 ^a	129.40 \pm 5.87 ^a	24.40 \pm 0.84 ^a	68.90 \pm 0.88 ^b
Trail B	2.20 \pm 0.42 ^b	231.40 \pm 6.72 ^b	118.00 \pm 3.20 ^b	28.40 \pm 0.84 ^b	67.00 \pm 0.94 ^a
Trail C	1.40 \pm 0.52 ^a	241.60 \pm 7.01 ^c	123.80 \pm 2.86 ^c	29.40 \pm 0.84 ^c	66.80 \pm 0.92 ^a

The values are represented as Mean \pm SD (n=25).

Different superscripts on the column between the Trail showed significant different ($P<0.05$) of either species.

Table 5. Reproductive potential of *Apocyclops royi* under starvation condition.

	No. of Clutches	Ovisac Length (μm)	Ovisac Width (μm)	No. of Eggs	Egg Diameters (μm)
Trail A	1.40 \pm 0.52 ^a	253.80 \pm 5.94 ^a	129.00 \pm 4.85 ^a	23.80 \pm 0.79 ^a	68.20 \pm 0.79 ^a
Trail B	2.20 \pm 0.79 ^b	225.80 \pm 4.85 ^b	117.80 \pm 2.28 ^b	28.00 \pm 0.67 ^b	66.60 \pm 0.52 ^b
Trail C	1.20 \pm 0.42 ^a	235.80 \pm 5.34 ^c	123.80 \pm 3.36 ^c	29.40 \pm 0.52 ^c	66.40 \pm 0.52 ^b

The values are represented as Mean \pm SD (n=25).

Different superscripts on the column between the Trail showed significant different ($P<0.05$) of either species.

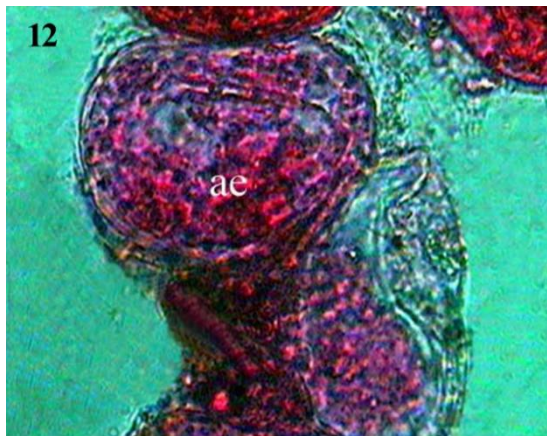


Figure 12. Discharge of egg from ovisac to medium—*Apocyclops dengizicus*. (ae—aborted egg)

Trail C were no significant ($P>0.05$) variation in both the species (Tables 4 and 5).

Discussion

It is well understood that the copepod taxa express an array of mating behavior depending on the life history and mating strategy of each species. In general, mating behavior involves a series of steps (Foltz, 1995; Vacquier et al., 1995). In the present study based on the observation, the sequential events of the mating process and spermatophore transfer was reconstructed and reported for the first time in both *Apocyclops* species. It is interesting to note that in the current experimental studies males of both the *Apocyclops* species prefer to mate with newly molted

virgin females than mated females. Similar to the present result the males of *Epischura lacustris* (Chow-Fraser and Maly, 1991) *Acartia hudsonica*, and *A. tonsa* (Burriss and Dam, 2015) also reported mating exclusively with virgin females of comparable size. Uchima (1985) and Uchima and Murano (1988) suggested that a sex-attractant pheromone is present with mature virgin females and absent in immature or mated females, which enhances the males to prefer virgin females for copulation. Leeuwen and Maly (1991) opined that the chemical signal released by the female varies in strength with its reproductive status.

Although the general features of cyclopoid reproductive biology are known, many details, for example, the reproductive cycle or a male's mating capacity are still unclear (Maier, 1992). Defaye et al. (2003) stated that single mating is sufficient to fertilize several clutches in *M. albidus* as is usually observed in Cyclopidae. Muthupriya et al. (2004) observed that the freshwater cyclopoid species *Mesocyclops thermocyclopoides* and *Thermocyclops decipines* produced a maximum of five to nine clutches after a single mating. Uchima (1985) found that in *O. davisae* one copulation is usually enough to fertilize all eggs produced by the female in its entire lifespan. Similar to all these reports, in the present study, the reproduction of *A. dengizicus* and *A. royi* showed normal fecundity and clutch size after a single mating.

The secretory material of the seminal epithelium would preserve the sperm and might constitute a sort of “nutritive medium” for the sperm giving the spermatozoa maximum chances for optimal fertilization efficiency (Defaye et al., 2003). However, it remains unclear whether one mating is sufficient to permit the realization of a female's total reproductive potential (Maier, 1992). Supporting this statement, in the present research, in once-mated females the extrusion of unfertilized eggs and granular secretion was observed in the last phase of clutch production, which might be due to the insufficiency of sperms to fertilize more number of clutches.

The investigations of Maier (1992) on the reproductive biology of *C. vicinus* revealed that females that mate only once, show a similar reproductive pattern (clutch size and clutch succession) to those which remain combined with males and thus have the opportunity to remate but tend to produce fewer clutches. However, the extent of sperm storage and clutch formation after a single mate is not known in this group. Under different mating conditions, in *A. dengizicus* and *A. royi*, the diameter of the egg remained similar, while variation was observed in the production of clutches, clutch size, and length and width of the ovisac. Though the results indicated that once-mated females of both species could produce several clutches, there appears to be a wide variation concerning the interclutch period.

Apocyclops dengizicus and *A. royi* females are allowed to remain continuously with males showing a higher number of clutches and moderate inter-clutch period advocating higher reproductive performance with multiple mating. This suggests that there should be remating for optimizing the reproductive output in this species. It is interesting to note that a very short interclutch period was recorded in females mated at regular intervals. Perhaps, regular mating may induce higher gonadal activity leading to increased reproductive potential. Thus, even though the males and females of cyclopoids may mate more than once (Wyngaard and

Chinnappa, 1982), the remating seems to be of little significance for egg production. Willey et al. (1990) opined that remating is most likely to have no importance for egg production and hence for population growth. Such a reason can be attributed to the less reproductive performance of females of *A. dengizicus* and *A. royi* which are mated at regular intervals; in addition to this it might be due to different qualities of insemination (Maier, 1992).

The data reported for egg number in successive broods of *A. viridis* suggested an initial increase followed by a decline during the last days (Abdullahi, 1992). This report was similar to the present observation that in both the cyclopoids there was a gradual decline in eggs production during the last phase of reproduction. Sciandra et al. (1990) opined that the synthesis of matter necessary for the maintenance of oocytes is a biochemical process whose efficiency decreases with age. The information provided by Maier (1992) on the mating duration, spermatophore refilling time of *C. vicinus* is similar to the duration recorded in the present study. A pair of spermatophores discharged sperms into the female's seminal receptacle and detached within two hours in both the *Apocyclops* species. In the case of *O. davisae*, the discharge of spermatophore content takes 24 hours to 2 days (Uchima and Murano, 1988). In the present study, the long-time attachment of spermatophores to the female genital segment is observed in the spent females. However, in aged females, it took longer durations (up to 5 days) to completely discharge spermatophores' content to the seminal receptacles.

Spermatophore production is habitat related and in the marine media, they appear to have evolved to minimize sperm loss (Subramoniam, 1993). Spermatophore protects the delicate sperm cells from drying (Schaller, 1980). Well formed spermatophore layer and a variety of secretory substances enable spermatophore attachment and sperm expulsion after mating. Maier (1992) stated that the mating capacity of males is possibly limited by the time needed to fill a new spermatophore. In the present study, males showed high reproductive potential by producing and

transferring many pairs of spermatophores during their lifespan. Compared to *A. dengizicus* higher number of spermatophores were produced by *A. royi* suggesting that more frequent remating is required in *A. royi* than *A. dengizicus*. The production of spermatophores depends on the sperm storage facility available in the female body. If the seminal receptacle can store a large quantity of seminal content, consequently large spermatophores are produced and transferred by males, and females can store the spermatozoa for a longer duration and hence require less frequent mating. Whereas in females, storing seminal content for a shorter duration might require frequent mating with small spermatophores. Thus, the spermatophore production in cyclopoid copepods might be based on the capacity for sperm storage in the female and their reproductive strategy. This strategy in the case of *A. dengizicus* is towards the adaptation of less frequent mating with a longer duration of sperm storage, while in *A. royi* frequent mating facility with a short duration of storage of spermatozoa. Nevertheless, these strategies are highly suitable for maintaining the high-density population of these species in nature. The extrusion of a spermatophore in the present study was observed during successive mating and unfavorable condition such as starvation.

Laboratory experiments on the life cycle of unmated females suggest that mating and spermatophore transfer is necessary for the normal reproductive activity of the female. In the absence of mating and spermatophore transfer, the female is incapable of normal reproductive processes such as the production of previtellogenic oocytes, vitellogenic oocytes, secretory material meant for the formation of ovisac, and also normal embryonic development of fertilized eggs in the ovisac. There may be involvement of an intricate hormonal mechanism in regulating aspects such as oogenesis, vitellogenesis, fertilization, and embryogenesis. These processes may be regulated in a coordinated or well-directed direction. Nevertheless, mating, spermatophore transfer, and discharge of spermatophore content into the seminal receptacle

might act as the triggering factor for the successive female activity. In spite of the availability of ideal reproductive conditions, the absence of male stimulatory factors can be attributed as the most vital factor leading to the abnormal reproductive activity of the unmated female. Uchima (1985) reported that the unmated females of *O. davisae* lay unfertilized eggs in the same mode as that of mated females. Maier (1992) reported that unmated females of *C. vicinus* extruded a few (1-5) infertile eggs; in no case, a complete egg batch was observed.

There are many reports on the longevity of many starved copepods, *A. tonsa* (Dagg, 1977), *Paracalanus parvus* (Checkley, 1980), and *Calanus typicus* (Nival et al., 1990) which did not exceed more than a few days suggesting, the stored energy is rapidly spent. Sciandra et al. (1990) showed that for periodicities more than 9 days of copepods die because their resources are insufficient for more than 5 days of starvation. Similar to the previous reports, in the present study, both species survived for about 10 days under starvation conditions. In these animals, the depletion of food sources is well exhibited in their locomotor activity. In the first few days, the animals were active, while the locomotor activity declined progressively. During the last phase of survival under starved conditions either they became sedentary or showed feeble activity. The survival days in starved males are much shorter than those in starved females (Marshall and Orr, 1972; Uchima and Hirano, 1988). Like previous reports, the present results also suggest that there is higher tolerance of starvation by females than the male.

The experiments of Preetha and Altuff (1996) showed that starvation results in lower fecundity in freshwater calanoid copepods *Sinodiaptomus (Rhinediaptomus) indicus*. Durbin et al. (1983) suggested that each species has the ability for accumulating large stores of energy (especially lipids) and the rate of egg-laying is strongly dependent on the amount of food ingested during the previous 6-48 hours. Experiments on *P. parvus* (Checkley, 1980) and *C. typicus* (Nival et al., 1990) suggested that reproduction is stopped during starvation to reduce

the energetic expenditures involved in the maturation of gametes and hence to permit copepods to survive for a longer time. Thus, it is considered that the maturation of gametes is possible when the level of energetic storage remains higher than a given threshold (Runge, 1985). Such a reason can be attributed to the sharp decline in the reproductive potential of starved males and females of both *A. dengizicus* and *A. royi*.

Pandian (1994) stated that the interruptions of food supply lead to regression of ovaries. Thus, the percentage of females producing eggs was greatly reduced (Jayanthi, 2001) and substantially lowered the hatching success (Ederington et al., 1995). The data of Sciandra et al. (1990) reveals that the egg-laying during starvation in *T. stylifera* originates from already matured oocytes whose vitellogenesis was nearly achieved after which due to starvation, the maturation is completely inhibited. Such a process is observed concerning the egg and spermatophore production by the female and male of *A. royi* and *A. dengizicus*, respectively. During the first two days of starvation, the mature oocytes and spermatophores contents might have formed which was evident in the reproductive activity. As nutrition depleted under starved conditions, the reproductive activity was affected resulting either in the production of abnormal eggs or caesation of egg production.

Misplacement of spermatophores outside the genital field has been reported for many species of copepods (Pandian, 1994). According to Lonsdale et al. (1998) male spermatophore placement is likely to be guided at least partially by chemical signals on the surface of females. The present laboratory studies revealed that the misplacement of spermatophores occurred in a male biased population where more males compete for a single female, males copulate with inappropriate mate such as CV female and rarely due to the abnormal genital segment of females. Thus, these incidences may result in improper placement of spermatophores. Fleminger (1967) has reported that *Labidocera jollae*, postulated that sexual swarming coupled with a high

male and less female sex ratio could be the basis for a large number of misplaced spermatophores. Cases of several males attempting to copulate at the same time with one female have been reported earlier for several copepods such as *Cyclops americanus* and *Pseudodiaptomus coronatus* (Hill and Coker, 1930; Jacobs, 1961). Thus, the slightly misplaced spermatophore may significantly reduce the number of sperm transferred and these mating mistakes would result in wastage of gametes and energy (Blades-Eckelbarger, 1991).

The present study may add more important information for the first time about the reproduction of the *Apocyclops* species which may provide a key for ecologists to understand the basic biology, copepod behavior, and life history. Despite the fact that this study examines the reproductive strategies of both species with remarkable insight, much more research into its biology remains to be investigated. Our finding has concluded that the successful copulation and reproduction in cyclopoids are influenced by various factors and reproductive potential of females of *A. dengizicus* and *A. royi* revealed remating is necessary for the continued reproduction of these species. The experiments on unmated females clearly indicated that the mating and spermatophore transfer is necessary for normal reproductive activity of female. The present attempt has documented that food and aging are the two important limiting factors of normal reproduction. The higher mating frequency of male with virgin females divulges the male mate-choice. The variation in reproduction between two species might be attributed to their genetic variation. Thus, the investment of energetic male and utility of the sperm by female producing viable eggs disclose the equal contribution of both sexes are necessary for successful population growth.

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