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Reproductive biology, salinity tolerance of hatchery-reared mysid *Eurobowmaniella simulans*, W. Tattersall, 1915 (Crustacea, Mysida)

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ABSTRACT

The study was conducted to collect information on reproductive biology, salinity tolerance, and feeding preferences based on rearing experiments with *Eurobowmaniella simulans* in hatchery. Eggs, Eyeless and Eyed larvae can be observed inside the brood pouch of mysids. The embryonic stages E1-E6, the nauplioid stages N1-N4, the post-nauplioid stages P1-P3 and the free-living juvenile stage were observed. The embryonic stages E1- E6 are prolonged for 2-3 days inside the brood pouch. Nauplioid stages N1 - N4 (Early and late nauplioid stages) extended for 3-4 days. The duration of post-nauplioid stages P1-P3 (early and late post-nauplioid stages) was between 2-3 days. The average generation time for mysid *E. simulans* was 39.64 ± 3.77 days in captive conditions. The minimum generation time was 34 days.

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1. Introduction

Mysids are small crustaceans and an important zooplankton component in marine and estuarine waters (Munilla and Vicente, 2005). Mysids are used as feed to rear many difficult species that feed exclusively on live feed. Species identification and detailed studies are required for developing captive production systems. Most mysids are marine species that have adapted to both benthic and pelagic lifestyles. Order Mysidacea has recently been reclassified into Order Lophogastrida and Order Mysida (Mees and Meland, 2012). The order Mysida includes 2 families, 175 genera and 1152 species and these species are found in subterranean, brackish, fresh, coastal and surface to deep-sea habitats (Meland, 2002; Porter *et al.,* 2008; Mees and Meland, 2012).

Mysids of different size ranges are found suitable as the live-feed in the larval rearing of newly hatched squid and cuttlefish larvae (Domingues *et al.,* 2001; Nabhitabhata, 1996 Anil, 2003), nursery rearing of ornamental fishes, food-fishes and serve as live feed for many other marine organisms (Beyst *et al.,* 2001), thus acting as a trophic link between primary producers and secondary consumers (Yamada *et al.,* 2007; Mauchline, 1980; Sardina and Lopez Cazorla, 2005). They show significant variation in their availability in different seasons and occur in all ocean regions. They are grouped under macro-zooplankton since their size ranges from 1.5 to 25 mm in total length. Mysids are considered a common member of the plankton, although they may also burrow in the sand in the surf zones throughout the year (Muller, 1993). Mysids are omnivorous and cannibalistic, filter feeders, feeding on algae, detritus and zooplankton (Odum and Herald, 1972; Mauchline, 1980; Meland, 2002). They are shrimp-like in appearance. Due to the presence of marsupium in the ventral side of females, they are often called “opossum shrimp”. Mysids can be cultured in captivity by providing *Artemia*, Rotifer, copepods, and phytoplankton (Domingues *et al.,*2000; Lussier *et al.,* 1988 and Viherluoto, 2001) and mussel meat suspension (personal observation).

According to Vernberg and Vernberg (1970), the physiological performance of mysids is affected by several environmental factors such as temperature, dissolved oxygen, salinity, currents and suspended particles. As stated in the study of Biju (2009), the chlorophyll concentration in coastal and estuarine waters is also an indicator of phytoplankton abundance and biomass. Environmental factors such as temperature, salinity, dissolved oxygen, suspended particles, and currents influence the seasonal availability, abundance, and distribution of mysid species (Brandt *et al.,*1993; Kohn,1992; DeGreave and Reynolds,1975; McLusky, 1979). Mysids can be used as live feed in aquaculture, especially during the months of their swarming. Laboratory rearing of mysids by previous researchers revealed that they are highly adaptive and can tolerate a wide range of conditions (Domingues *et al.,*1999, 2000, 2001; Lussier *et al.,* 1988).

Information on the culture of mysids is available from the works of Domingues *et al.* (1999, 2000), who described the importance of mysid culture in lowering costs with alternative diets and reared the mysid, *Mysidopsis almyra* in the static water system to examine the effects of density and temperature on production, survival and growth. Marini and Moe (2003) briefed on the method of culture of the mysid shrimp in captive conditions.

Information on the salinity tolerance of mysid species is available from the works of Carrasco and Perissinotto (2011), who studied the temperature and salinity tolerance of *Mesopodopsis africana* in the freshwater-deprived St. Lucia Estuary, South Africa and the works of Paul and Calliari (2016) on salinity and temperature tolerances of *Neomysis americana* subadults in South America. The distribution and abundance of any coastal species can be influenced by its ability to tolerate wide ranges of salinity and temperatures (Segal and Burbanck, 1963; Galvan *et*

al., 2016). As reported by DeLisle and Roberts (1986), McKenney and Celestial (1995), Ramarn *et al.* (2012) and Paul *et al.* (2013), the spatial distribution and survival of mysid species can be influenced by their tolerance to a wide range of temperatures and salinities. Fluctuations in salinity can influence the survival, population structure and distribution of zooplankton (David *et al.,* 2005; Miyashita and Calliari, 2016).

2. Materials and Methods

The samples of *E. simulans* were collected from the Vizhinjam coast during morning hours from the intertidal areas. Mysids were immediately transferred to plastic buckets containing seawater collected from the sea. Samples were brought to the laboratory for further analysis. Brooders, males, and juveniles were separated from the whole sample. Total body length (TL) was measured from the rostral tip to the posterior border of the telson, and carapace length (CL) from the tip of the rostrum to the posteromedial margin of the carapace (Greenwood *et al.,* 1985). Brood pouch size and the number of embryos were calculated.

2.1. Reproductive biology of *E. simulans.*

Ovigerous females collected from the wild were analyzed under a stereo zoom microscope (Leica DFC 295) to identify different stages of embryonic development. Marsupia of brooders were opened using fine needles to liberate embryos. The developmental stages were distinguished according to Mauchline (1973) and Wittmann (1981). The development of the embryo from the single-celled stage was followed. Mauchline (1971) classified the embryonic stages of mysids inside the brood pouch *viz*., (1) Eggs, (2) Eyeless and (3) Eyed larvae. The developmental stages identified by Wittmann 1981 were, (1) Embryonic stages E1-E6, from oviposition to hatching from the egg membrane, (2) Nauplioid stages N1-N4 (from hatching to the first intra-marsupial larval moult, (3) Post-nauplioid stages P1-P3 until larval moult at which the free-living juvenile stage is attained. Both categorizations were used to classify the developmental stages.

2.2. Generation time

To estimate the generation time, the juveniles released from the brood pouch were reared until they started to produce the next generation of juveniles.

For culture experiments, 20 egg-bearing adults each were kept in perforated baskets of 10L capacity wrapped with 500µm mesh cloth, which was immersed in a basin (20L) containing seawater with aeration. The mesh cloth was provided to prevent cannibalism by separating the newly released juveniles from the adults. These juveniles were kept in different troughs and fed with different feeds to study variations in growth and survival rates. Rotifer (10 nos per ml) + algae were provided for the newly released mysids, *Artemia nauplii* (10-15 nos per ml) and copepods (10-15 nos per ml) and boiled mussel meat suspension were provided sequentially. The uneaten feed, especially mussel meat suspension was removed from the culture tank to maintain the quality of water. Water exchange was done daily to avoid ammonia accumulation and subsequent mortality. Various physicochemical parameters such as temperature, salinity, pH and ammonia were measured using standard techniques. The temperature was kept at 28 ± 2^0 C and salinity was maintained at 30 ± 2 ppt. pH was maintained between 7.9-8.2. The ammonia level was kept very low, 0.01 mg/l.

2.3. Survival (tolerance) of mysid *E. simulans* to different salinity ranges.

The survival (tolerance) of mysids in different salinities (0ppt, 5ppt, 10ppt, 15ppt, 20ppt, 25ppt, 30ppt, 35 ppt) were tested for a period of 80 hours. Water in different salinity was prepared by the Patterson method and salinity was checked by using a 'Master Refractometer (ATAGO). Before the beginning of the experiment, all organisms were acclimated to the ambient salinity of seawater (32 ppt in the present study). Later, the animals were carefully released into the water in different salinity ranges. Stocking density was 10 nos in a 3-litre bucket, and triplicates were maintained for each salinity. The number of mysids that survived in each salinity was counted to estimate the percentage of survival. Sub-adults were chosen for the study. Mysids were fed with the proper diet.

$$\text{Percentage of survival} = \frac{\text{No of mysid survived}}{\text{Initial no of mysids}} \times 100$$

3. Results

3.1. Reproductive biology of *E. simulans.*

E. simulans attained sexual maturity within 26 - 30 days at a temperature of 28 ± 2^0 C and salinity of 30 ± 2 ppt. The ovigerous females took 6 days for incubation. The brood size of each female mysid varied according to its size and developmental stages; here 766±519 eggs were noted in the E1 stage. However, only 24 ± 10 eggs developed, reached the nauplii stage (eyed larvae) and were released from the brood pouch as juveniles. This indicated the development of the embryo in batches. The average body length of an adult female in this stage was observed to be 8.26 ± 0.92mm. The size of eyed larvae just before hatching was 1.23±0.07mm.

Sex could be first distinguished externally when mysid attains a total length of about 4.1-5mm and in about 10 to 15 days after hatching. Females were observed to attain sexual maturity from a total length of 5.7±0.08mm; in males, it was from 4.9±0.09 mm. The eggs could be seen through the transparent brood pouch as small dark spots in mature females. The liberation of juvenile mysids occurred just after the moulting of the female parent.

3.1.1. Stage I: Embryonic stages E1-E6 (Egg stage) (Fig. 1c to Fig. 1k).

The embryonic stages E1- E6 are prolonged for 2-3 days inside the brood pouch. This stage's duration was from the egg's first appearance inside the brood pouch to its hatching from the egg membrane. At the E1 stage (Fig. 1c-1d), a huge number of small spherical eggs were observed (766 ± 519 numbers), as the development advances few eggs tend to become larger and spherical. The germinal disc was visible from the E3 (Fig.1 g & h) stage of eggs. The appendages of early nauplii began to develop from the E4

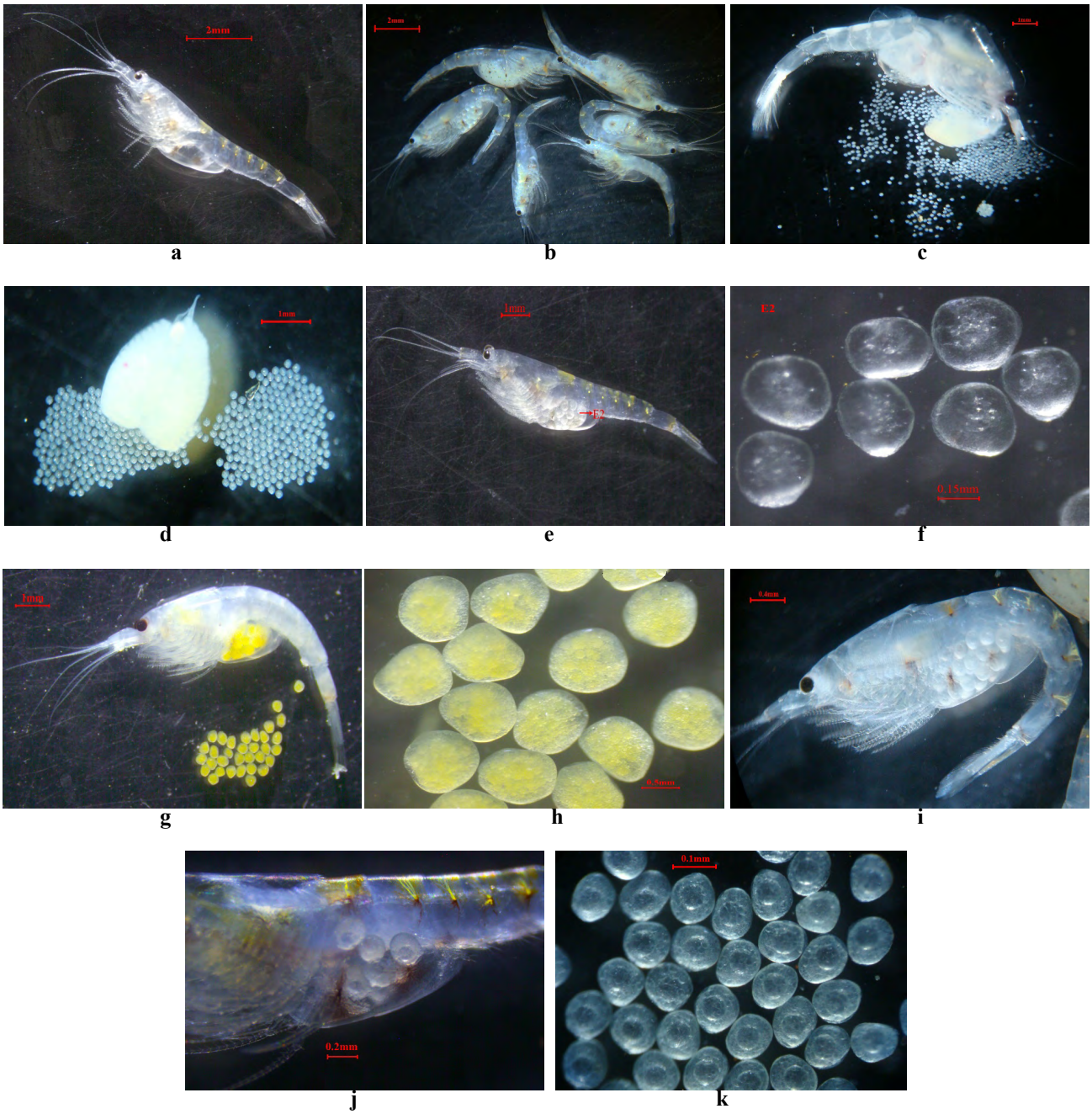

Fig. 1. Embryonic stages of *E.simulans*: **(a).** Female with empty brood pouch; **(b).** Ovigerous females; **(c)&(d).** Females bearing eggs in embryonic stage 1 (E1); **(e).** Ovigerous females with embryonic stage 2 (E2) eggs; **(f).** An enlarged view of brood pouch with E2 stage eggs; **(g).** Ovigerous female with embryonic stage 3 (E3) eggs, yellowish yolk granules could be seen; **(h).** An enlarged view of E3 eggs; **(i).** Ovigerous females with E4 stage eggs; **(j)** Enlarged view of E4 eggs inside the brood pouch. **(k).** Embryonic stage 6 (E6), the embryonic abdomen can be seen folded over the germ.

stage of the egg (Fig.1i&j). At the end of stage E6 (Fig.1k) with the development of naupliar appendages, the egg membrane ruptured, and about 26±10 eggs were observed at this stage. The size of the embryo at the E6 stage was found to be 0.55 ± 0.05mm (Table 1).

3.1.2. Stage II: Nauplioid stages N1 - N4 (Early and late nauplioid stages) (Fig.2 a-f)

This stage was observed from hatching to the first larval moult inside the brood pouch. These stages are prolonged for 3-4 days. The embryo size at this stage was found to be 0.83±0.062 mm. In the early nauplioid stage (Fig.2.a & b), the yolk occupied the major part of the embryo. The eye was not developed and larvae remained immobile. The larva at this stage was 'comma-shaped.

In the later nauplioid stage (Fig.2.e &f), the yolk mass started to shrink, and only the remnants of yolk could be seen on the anterodorsal part of the larvae. Body segmentation could be clearly visible. Eye development progresses in this stage. Round-shaped pigmented spots could be observed in the prospective eye region. Casting off of older cuticles (moulting) was observed at the end of this stage; this could happen due to the constant dorsoventral stretching of the larvae.

Table 1. Details of different stages of developing embryo with respect to the size of the female parent.

Stage of developing egg/ embryo inside the brood pouch	N	Total length of the female parent (mm)		Brood pouch length of the female parent (mm)		Carapace length of the female parent (mm)		Size of egg/ embryo (mm)		Total number	
		MEAN	STDEV	MEAN	STDEV	MEAN	STDEV	MEAN	STDEV	MEAN	STDEV
E1	16	7.55	1.3	2.27	0.47	2.6	0.58	0.11	0.020	766	519
E2	15	7.88	0.42	2.2	0.09	2.43	0.28	0.25	0.05	47	19
E3	18	7.5	0.75	2.12	0.18	2.4	0.35	0.35	0.05	30	8
E4	14	8.42	1.30	2.33	0.40	2.77	0.48	0.45	0.05	35	9
E5	17	8.27	1.13	2.27	0.40	2.63	0.49	0.52	0.04	28	12
E6	15	7.9	1.03	1.98	0.31	2.62	0.46	0.55	0.05	26	10
Eye less	15	8.53	1.02	2.23	0.44	2.89	0.40	0.83	0.062	30	10
Eyed	15	8.26	0.92	2.2	0.39	2.72	0.40	1.23	0.07	24	10

3.1.3. Stage III: Post-nauplioid stages P1-P3 (Early and late post nauplioid stages) (Fig.3a-d)

The average size of the larvae at this stage was observed to be 1.23±0.07mm. The duration of this stage was between 2-3 days. In the early post- nauplioid stage, thoracic and abdominal segmentation was more clearly visible than that of the previous stages. The pleopods, carapace, thoracic limb, uropods, maxillae and maxillules were well developed. Chromatophore pigments could be seen in the thoracic and abdominal regions. The carapace was well-developed. Still, small spots of yolk could be seen. The eyes were almost developed and were stalked (eyed stage).

At the late post-nauplioid stage the appendages were well-developed and elongated. Segmentation in appendages could be clearly observed. Antennules and antennae could also be observed clearly. Young ones were ready to be liberated from the brood pouch. Heartbeats could also be observed at this stage.

3.1.4. The juvenile stage (Fig.3e)

After the incubation period mysids were released from the brood pouch and the young ones underwent a second larval moulting and reached a size of 1.6-1.8 mm in total length. Sex cannot be differentiated at this stage because it lacks secondary sexual characteristics. An ecdysis of the female parent usually follows the release of the completely developed young ones.

The number of embryos in the brood pouch and the size of the embryo varied with the size of the female (Table.1).

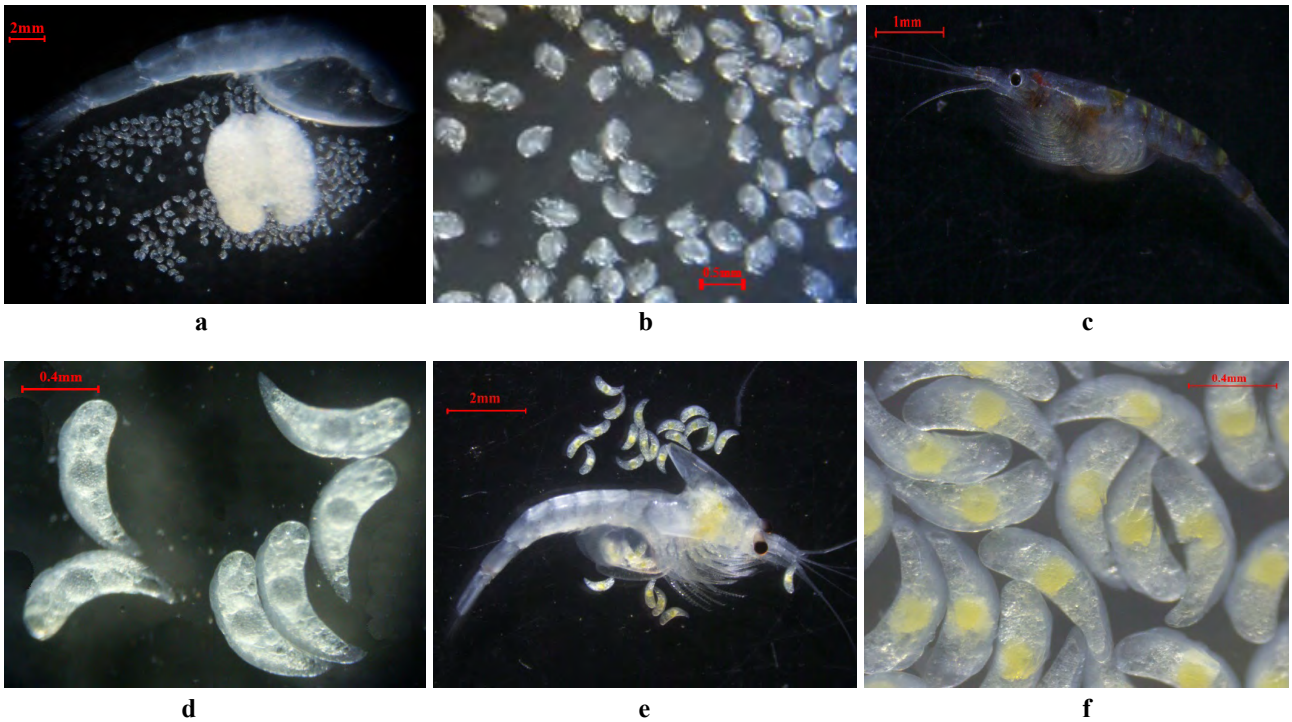

Fig. 2. Nauplioid stages of *E. simulans*: **(a).** Hatched out eggs (Nauplioid stage 1(N1)); **(b).** An enlarged view of the embryo in N1; **(c).** Female with late nauplioid stage 2 embryo (late N2) in the brood pouch; **(d).** Enlarged view late nauplioid stage 2 embryo, the invaginated yolk gave the embryo its characteristic comma shape; **(e).** Female with nauplioid stage 3 embryo (N3) in the brood pouch; **(f).** An enlarged view of N3 stage embryo.

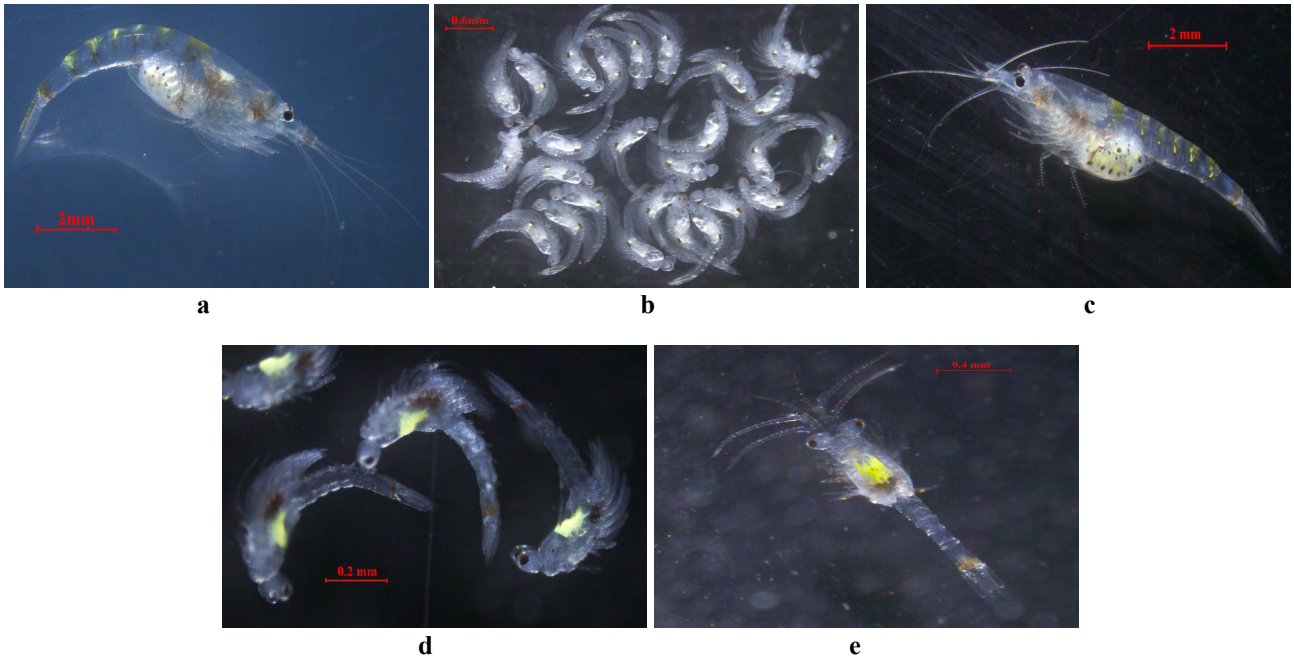

Fig. 3. Post-nauplioid stages of *E. simulans*: **(a).** Female with post nauplioid stage 2 embryo (p2) in the brood pouch; **(b).** An enlarged view of P2 stage embryo; **(c).** Female with post-nauplioid stage 3 embryo (P3) in the brood pouch; **(d).** An enlarged view of P3 stage embryo; **(e).** Juvenile.

3.2. Generation time

The juveniles coming out of the net-wrapped perforated buckets (as given in materials and methods) were transferred to troughs (10L capacity) for rearing for estimating the generation time. The experiment was done in triplicates. Stocking density was 5 numbers per litre and 40 juveniles were reared in 8 litres of seawater contained in the trough. They were fed at regular intervals to avoid cannibalism. The development was observed at regular intervals. The juveniles were grown to the next generation by feeding them with microalgae and rotifer for 20 days. They were later fed with newly hatched artemia, copepods or boiled mussel meat suspension. The average generation time for mysid *E.simulans* was 39.64 ± 3.77 days in captive conditions. The minimum generation time was 34 days.

3.3. Salinity tolerance of mysid *E. simulans*.

During the experiment, subadult mysids (size: 3-4mm) were exposed to different salinities (0ppt, 5ppt, 10ppt, 15ppt, 20ppt, 25ppt, 30ppt, 35 ppt) for 80 hours. The maximum survival of *E. simulans* was recorded at 30ppt (97%) and 35ppt (95%). The mysids didn't survive at 0ppt salinity; total mortality was observed within an hour. In all other treatments, from 5ppt to 35ppt, survival was more than 95% until 9 hours. After 9 hours the mortality began in the case of 5 and 10 ppt and a few numbers survived even after 80 hours in these salinities. Survival was about 50% in the case of animals subjected to 15ppt of salinity. Mysids showed above 85% survival in 20,25, 30 and 35ppt salinities. The percentage survival of *E. simulans* to varying salinities is shown in Fig. 4.

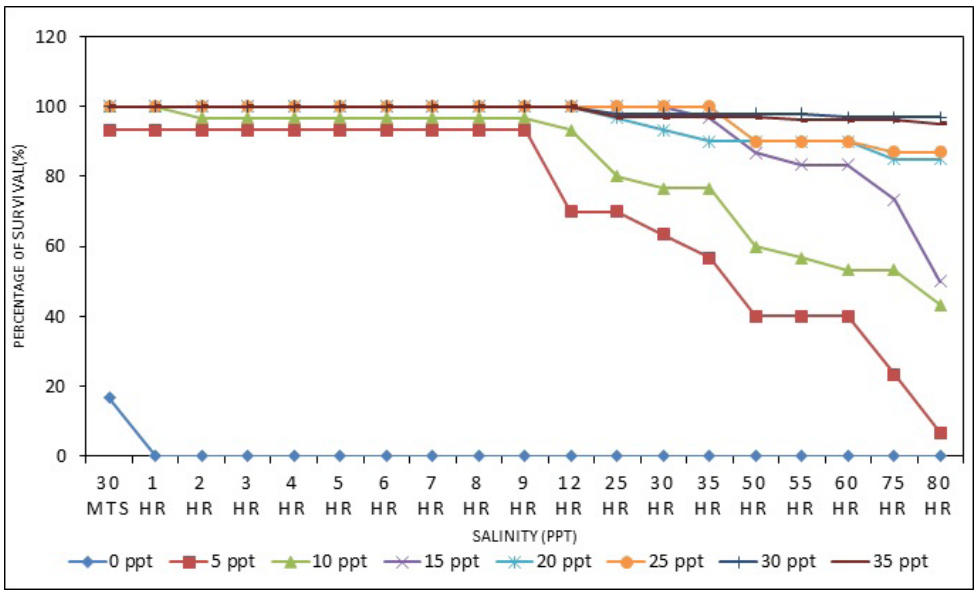

Fig.4. Percentage survival of *E. Simulans* to varying salinities

4. Discussion

The present study was aimed at getting information regarding the reproductive biology, generation time, salinity tolerance and feed preferences of *E. simulans* so that their culture techniques could be developed as mysid is one of the important live feeds used for the larviculture of cephalopods.

In the current study, the brood size of mysid carrying E1-E6 (egg stage) stage eggs was found to have the largest number of embryos (766±519 numbers of eggs in E1 Stage). However, a decline in number was noted as it proceeded towards further developmental stages. At the final stage (E6), 24 ± 10 numbers of hatchlings adult $^{-1}$ were observed. Biswas (2005) reported 14-44 hatchlings adult $^{-1}$ in *E simulans*. Domingues (2000) noticed 2-14 hatchlings in *Leptomysis spp.* Reitsema and Neff (1980) observed 3-44 hatchlings in *Mysidopsis almyra.* According to Biju (2009), the number of embryos in the brood pouch varies according to the species, the size of the female, temperature and the time of the year. *E simulans* is a competent species in live feed culture since it is endowed with higher reproductive potential.

Biju (2009) observed 37-eyed larvae in the marsupium of a female with an 8.4 mm length. In the present study, 24±10 numbers of eyed larvae were observed and the average body length in this stage was 8.26±0.92mm. Biju (2009) observed the size variation in mysids and found the species that live in warmer waters tend to mature more rapidly and undergo various growth stages at a much smaller size than those inhabiting the colder region; hence he opined that the actual size of the mysids cannot be exactly determined. In the present study, several large spent females with large marsupia covered with chromatophore pigments were observed. Greenwood *et al.* (1985) also observed the same in their studies and they also observed the absence of juveniles in the marsupium and in many cases damaged marsupia (which was found to be due to the release of young) and in their observations, some spent females were found to have mature ovaries.

In the present study, overcrowding (high density) of the mysids in the culture tanks was avoided as it was one of the limiting factors concerning the population's growth rate; it may also lead to intraspecific competition and exclusion.

According to Reitsema and Neff (1980), mysids usually have a short reproductive cycle (14-21 days) which makes them a potential organism for rearing on a large scale. In the present study, the average generation time for mysid *E.simulans* was 39.64 ± 3.77 days in captive conditions and the minimum generation time was 34 days.

In the present study, *E simulans* survived in 10ppt to 35ppt salinity for 80 hours (the percentage of survival varied). However, the optimum range for survival was found to be 30ppt. Biju (2009) observed that this species could tolerate the temperature and salinity range of 29.2 to 31.1°C and 32.5 to 35 psu. Paul and Calliari (2016) observed the salinity tolerance in mysids and exposed mysid *Neomysis americana* to salinities 5, 10, 15, 20, 25, and 30 at different experimental temperatures for 72 h and noted that the survival was lowest in salinity 5 and survival was high in salinity 15 and in higher salinities such as 25, 30 mysids struggled to survive. Their results indicated that the recruits of *N. americana* can only withstand intermediate salinities and temperatures. Ramarn *et al.* (2012) studied the population structure and reproduction of the mysid shrimp *Acanthomysis thailandica* (Crustacea: Mysidae) in a tropical mangrove estuary, in Malaysia; their results revealed that these species showed large spatial and temporal variations in population density and a huge number of mysids occurred on the mudflat where the mean salinity was 25 ppt but rapidly declined towards the upper estuary (14 km upstream) where the salinity fell to 15 ppt.

According to Mauchline (1980), most mysids are filter feeders, and omnivores, feeding on detritus, algae and zooplankton. Fossa (1986) observed that the pelagic mysids filter particles during swimming, while benthic species collect the particles with their carapace and legs. Tattersall and Tattersall (1951) observed a strict carnivorous habit in some mysid species, and also observed the scavenger mysids (feed on dead remains of polychaetes, amphipods and copepods) and the true predators on zooplankton. In the present study, ecdysis or moulting was observed in the female parents just before the release of juveniles. Some researchers observed an increase in the body size associated with moulting and the continuation of growth even after attaining sexual maturity in many species (Wittmann,1981; Biswas, 2005; Biju, 2009).

Mysids are found to be the potential live feed species for rearing marine fin fishes, cephalopod larvae and some other economically important species. Mariculture parallel with mysid culture could ensure, to some extent, the necessity of live feed for the rearing of high-value marine animals, this may also reduce the higher cost of rearing practices in hatchery conditions.

5. Conclusion

The present study gives information on reproductive biology, generation time, salinity tolerance, and feed preferences of the mysid *E. simulans*, which is an essential live feed for the larviculture of cephalopods and other high-value fish. The results obtained in the present study prove the feasibility of this species in captive-rearing systems. The study also gives information on the optimum salinity ranges and feeds needed for their survival in the captive rearing systems.

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