



Short Communication

Embryonic development of the ornamental shrimp, *Urocaridella arabianensis* Akash *et al.*, 2020

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The ornamental shrimp, *Urocaridella arabianensis* Akash *et al.*, 2020 is a recently discovered colourful species from the Agatti Island, Lakshadweep, India. The present study is a preliminary investigation of embryonic development and fecundity of *U. arabianensis*. The study was conducted at the Germplasm Resource Centre of ICAR-National Bureau of Fish Genetic Resources (NBFGR), Agatti Island, Lakshadweep, India, from January to June 2021. The study documented three reproductive cycles of the species during the study period. Fecundity of the female ranged from 170 – 189 oocytes (mean 182±5.0). The incubation period *i.e.* the period from spawning to hatching of the species, was 14 to 16 days (mean 15±0.75 days), and the embryonic development was completed through nine stages. Study also report synchronous increase in the size of embryo along with morphological changes and continuous reproductive activity during the study period. This information on the reproductive biology of this species will help develop sustainable production of the species for ornamental aquaculture.

[**Keywords:** Egg development, India, Ornamental aquaculture, Reproductive features, *Urocaridella*, Fecundity]

Introduction

The genus *Urocaridella* is generally known as "cleaner shrimp", and is one of the important and popular marine decapods in the aquarium trade due to its attractive colouration and peculiar behaviour patterns¹⁻⁴. They are commonly observed as small assemblages in Indo-Pacific waters⁵⁻⁶, where stocks are heavily harvested from the natural environment for ornamental trade. The *Urocaridella* species have transparent or colourless bodies with a few small patches of white, yellow, red, and brown patterns. Recently, *Urocaridella arabianensis* Akash *et al.*, 2020 was described from the Lakshadweep Islands as a new species having a transparent body with red and

white patches⁷. They are commonly found as small groups in the crevices of live corals. Since the colour patterns and carapace appearance are very similar to *U. antonbruunii* (clear cleaner shrimp), which are highly demanded in the aquarium trade, the introduction of *U. arabianensis* to the ornamental industry will get much attention. However, its biological and ecological characteristics need to be assessed beforehand.

The knowledge of reproductive patterns and embryonic development of a commercially important species will help the establishment of sustainable management regimes and standardization of breeding protocols^{1-2,8}. Among caridean shrimps, a few independent studies have looked at the reproductive characteristics of *Lysmata amboinensis*⁹, *L. boggsi*¹⁰, *L. vittata*¹¹, *Palaemonetes pugio*¹², *Macrobrachium americanum*¹³, and *M. macrobrachion*¹⁴. However, no detailed studies are available on the reproductive patterns of the genus, *Urocaridella*. Hence, the current study aim to examine the fecundity and embryonic developments of *U. arabianensis* in captive conditions, which will help to develop the breeding protocols for the large-scale culture of this species.

Materials and Methods

During an exploratory survey conducted at the reef regions of Agatti Island, captured 55 live specimens of *U. arabianensis* from the bottom of live corals (10°50'03" N, 72°10'85" E; 10°50'04" N, 72°10'51" E) at a depth of 0.5 to 1 m. The captured shrimps were acclimatized with hatchery conditions and transferred to separate 500 l FRP tanks once they became pairs. Among them, three pairs were used for the present study. The animals were maintained at a salinity of 35±1 ppt, temperature of 28±0.5 °C, and a photoperiod of 12 h (optimized conditions). The island water was collected and filtered, which was used for rearing purposes. Subsequently, the animals were fed with boiled mussels, frozen *Artemia*, and commercial pellet feed twice daily. The shrimps were measured (total length, TL, in mm) from the tip of the rostrum to the end of the telson with the aid of a digital calliper and with 0.01 mm precision. They were weighed on a digital scale with a precision of

0.1 g. The total length ranged between 33.10 to 43.12 mm, and the carapace length of 7.23 to 10.25 mm. The total weight ranged from 0.22 to 0.53 g. The fecundity was recorded by counting the number of oocytes present in the abdominal segment of the females (N = 16). Each mating pair was followed for three consecutive reproductive cycles to estimate the mean duration of a reproductive cycle. The reproductive process and embryonic development was recorded for six months from January to June 2021. The incubation period was considered from copulation to embryo hatching. Daily, 5 – 7 eggs were collected from the pleopods of the female without scarification of the animal, observed under a stereo/compound microscope and the diameter of different embryonic stages was documented. The eggs at the embryonic stage were measured for width and length under an optical microscope to calculate their volume by the formula $V = (\pi lh^2/6)$, in which l is the long axis and h is the short axis¹⁵. The difference between egg volumes was tested using Analysis of

Variance (ANOVA). The statistical analysis was performed using GraphPad Prism 9 software. The experiments were conducted at the Germplasm Resource Centre of ICAR-National Bureau of Fish Genetic Resources (NBFGR), Agatti Island, Lakshadweep, India.

Results and Discussion

The spawning and embryonic developments of the shrimps were documented for a period of six months. Embryonic development period ranged from 14 to 16 days (means 15 ± 0.75 days). The average fecundity recorded was 182 ± 5.1 (range: 170 to 189 oocytes). Three reproductive cycles were documented during the study period, which did not differ significantly ($p < 0.5$). Additionally, nine stages of embryonic development were observed during the incubation period (Fig.1). Maximum embryo size (long axis) varied from 0.38 mm on day 1 of incubation to 0.56 mm on day 13 of incubation. In contrast, the maximum value along the short axis ranged from 0.42 mm on

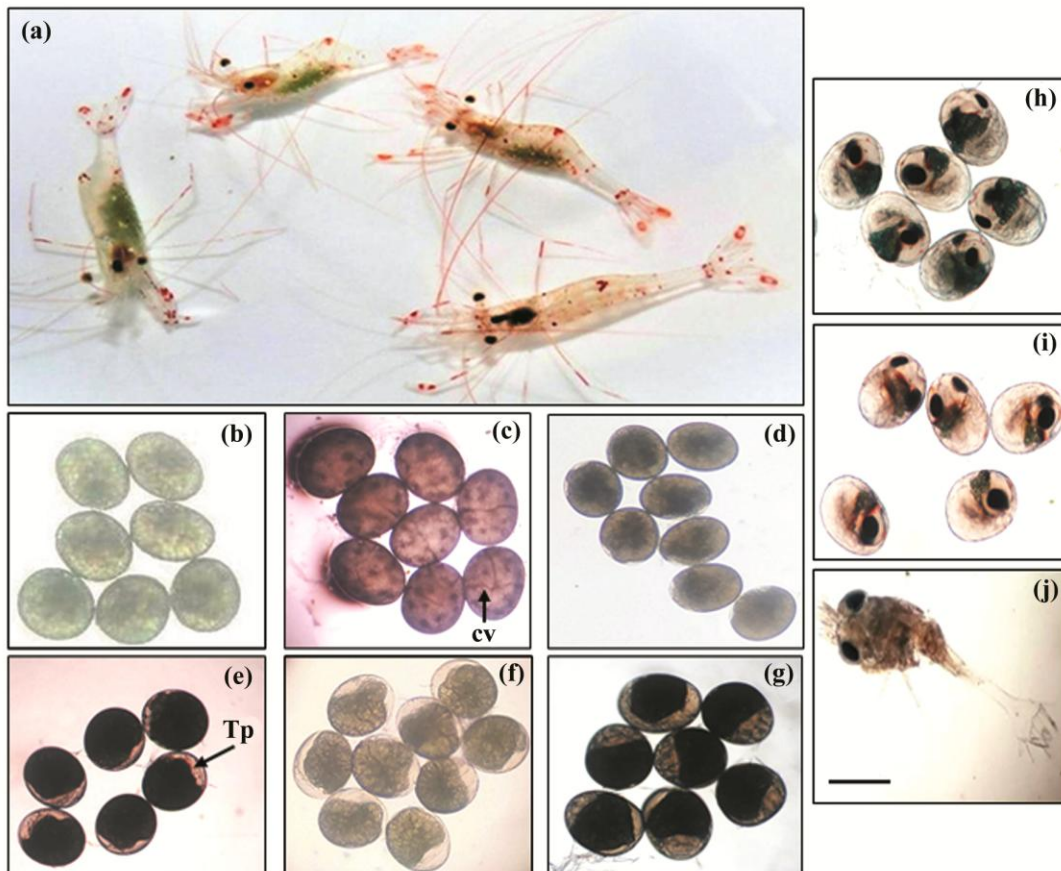


Fig. 1 — Embryonic development of *U. arabianensis*: (a) Brooder animals; (b) Stage I - Fertilized eggs; (c) Stage II - Cleavage (Cv); (d) Stage III - Blastula; (e) Stage IV - Gastrula, Tp - Translucid area; (f) Stage V - Nauplius; (g) Stage VI - Post-Nauplius I; (h) Stage VII - Post-Nauplius II; (i) Stage VIII - Pre-hatching; and (j) Stage IX - Newly hatched larva (Zoea I). Scale bar: 0.5 mm

day 1 to 0.61 mm on day 13. The mean egg volume ranged from $0.17 \pm 0.03 \text{ mm}^3$ on the first day of incubation to $0.36 \pm 0.02 \text{ mm}^3$ on thirteen days of incubation (Table 1 and Fig. 2). The results of the ANOVA showed that the mean egg volumes of *U. arabianensis* were significantly different among the different embryonic developmental stages ($p < 0.0001$). During development, the colour of the egg clutch changed from translucent (unfertilized stage) to bright greenish opaque (fertilized), green (cleavage stage), light green (blastula stage), olive green (gastrula and pre-nauplius stage), deep green (nauplius and post nauplius) and tawny with dark spots (complete embryo stage). The colour change was caused by yolk absorption and the development of pigments.

Stage I (Fertilization): Eggs are attached under the abdomen and pleopods; eggs are oval-shaped, with sizes ranging from 0.38 to 0.40 mm, with 100 % of yolk mass.

Table 1 — Measurements of embryo volume of *U. arabianensis* during different incubation periods (n = 7) of embryonic development

Incubation days	Egg volume (mm^3) (Mean \pm SD)
Day 1	0.17 \pm 0.03
Day 2	0.18 \pm 0.02
Day 3	0.20 \pm 0.01
Day 4	0.22 \pm 0.01
Day 5	0.24 \pm 0.03
Day 6	0.23 \pm 0.01
Day 7	0.26 \pm 0.04
Day 8	0.29 \pm 0.03
Day 9	0.28 \pm 0.04
Day 10	0.32 \pm 0.01
Day 11	0.32 \pm 0.02
Day 12	0.34 \pm 0.02
Day 13	0.36 \pm 0.02

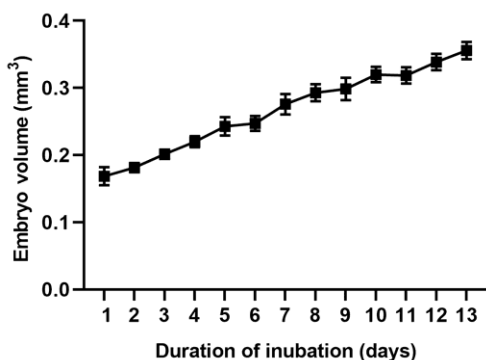


Fig. 2 — Mean embryonic volume at different developmental stages (incubation period) of *U. arabianensis* (n = 7; mean \pm SD), (ANOVA; $F = 177$; $p < 0.0001$)

Stage II (Cleavage): Within a few hours after fertilization, the first cleavage began with the appearance of a shallow cleavage furrow on the egg surface, girdling the egg along the middle region and eventually cleaving the embryo into two equal blastomeres. The yolk mass occupied around 95 to 97 %, which indicates the initiation of embryonic developments. The second cleavage divided the yolk mass into four equal blastomeres. After the formation of around 16 blastomeres, the divisions become irregular. The size of the eggs ranged from 0.39 to 0.41 mm.

Stage III (Blastula): After one day of incubation of eggs, size ranged from 0.41 to 0.43 mm. Cell division preceded without any morphological changes during the blastula period. The cleaved blastomeres exhibited a high degree of compaction and intense merging, resulting in the formation of irregular cells resembling a solid aggregate ball. Yolk mass was present in 85 – 90 %.

Stage IV (Gastrula): Gastrulation occurred after cleavage and blastulation and lasted for around 3 – 4 days. Embryo size ranged from 0.42 to 0.44 mm. There was a small invagination with cell migration resulting formation of a translucent area in the pole. During the process of continuous cell migration, the transparent area expanded. Gastrulation was followed by organogenesis where cells started differentiating into abdominal and cephalothorax regions. The yolk mass occupied 80 to 86 % of the total volume. Abdominal and cephalothorax segment sizes increased and antennal and mouthparts became visible.

Stage V (Nauplius): Five to seven days after incubation, the embryo size ranged from 0.44 to 0.49 mm. The yolk mass was present in 70 to 81 % of the egg. The cephalothorax and abdominal parts are extended, and the dark region appeared on the anterior region of the embryo at the end of day 7, representing the differentiation of the ocular region of the animal.

Stage VI (Post-Nauplius I): The size of embryos ranged from 0.50 to 0.52 mm after eight to nine days of incubation. The yolk mass reduced to 35 – 40 % of the total egg volume. Body of the post-nauplius was transparent with the formation of optic rudiments, which later became dark colour eyes. The size and colour of the eyes increase, and they apparently become more visible. Heartbeat was observed during this stage.

Stage VII (Post-Nauplius II): After ten to eleven days of incubation, the yolk mass occupied around

20 – 25 % of the egg and size ranged from 0.52 to 0.54 mm. Eyes were completely developed, and the cornea and retina were differentiated. Telson and uropodal segments developed and were clearly visible.

Stage VIII (Pre-hatching): After twelve to thirteen days, embryos became translucent with a size of 0.54 to 0.55 mm. A few granules of yolk attached in dorsal regions of the cephalothorax, occupying 5 – 8 % of egg volume. Irregular movements of the abdomen were observed. The blood vessels were visible, and the heart rate increased. The embryonic development was complete for hatching.

Stage IX (Hatching): After 14 – 15 days of incubation, the embryos hatched out into new larvae, Zoea I stage, during the late evening. The larvae had sessile eyes and developed appendages and telson with functional swimming capacity.

Research on embryonic development in ornamental shrimps was predominantly concentrated on selected families, notably Alpheidae, Atyidae and Palaemonidae¹⁶. Embryonic development unfolds as a continuous process, with the pre-naupliar and naupliar stages being relatively brief due to their simpler and less complex features. In contrast, the post-naupliar stage extends over a longer duration, attributed to the intricate organization required for various structures to become functional at eclosion¹⁷. The incubation time is temperature-dependent, and a decrease in water temperature increases the incubation time¹⁸. The size of eggs and fecundity of shrimps are also negatively affected by temperature variations¹⁹. The fecundity of the species is comparatively lower than other caridean shrimps. In the current study, the eggs hatched in 14 – 16 days at 29 – 30 °C. The observed incubation period and embryonic stages were similar to other studies on crustaceans, which have also been examined by the presence of distinct morphological features such as eyes, heartbeat, and main appendages^{9,20-23}. The embryonic development of *Urocaridella arabianensis* was unknown until the present study. The pattern of embryonic development in *U. arabianensis* is comparable with that of caridean shrimps, as larvae hatch by means of zoea^{16,24}. The current study represents the first report on the embryonic development of the genus *Urocaridella* in captive conditions. Overall, the results provide preliminary data on the reproductive and embryonic developmental characteristics of *U. arabianensis*, which might be favourable for the establishment and sustainable production of the animal for ornamental

aquaculture. However, further research is needed on sexual maturation, larval cycle and tolerance to various environmental factors in captivity.

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Conflict of Interest

The authors declare that they do not have a conflict of interest in this manuscript.

Author Contributions

SB: Specimen collection, experimentation, and manuscript preparation; TKTJ: Revision of accepted manuscript; TTAK: Manuscript editing and review; and KKL: Overall concept and guidance.

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