

First report on rearing of *Octopus cf. vulgaris* type III (Cephalopoda: Octopodidae) from Pakistan (northern Arabian Sea) under laboratory conditions

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ABSTRACT. Present studies provide the first detailed description of the rearing of the common Octopus, *Octopus cf. vulgaris* type III (Cephalopoda: Octopodidae), from Pakistan (Northern Arabian Sea), under laboratory conditions. This data on paralarval development and survival in captivity is essential for understanding sustainable octopus aquaculture practices. The total number of eggs recorded in the studied females was estimated at ~4230 eggs, in 53 strings and each string containing 70±64 eggs. The average egg size was measured as 1.3 mm in length and 0.5 mm in width. Twenty stages were recorded during the study, with hatching occurring from 17–22 (528 h) days after spawning at the temperature about 28–31 °C. The female octopus died after 42 days, when all the young had hatched. This research will help us better understand the best practices for rearing and raising octopuses, as well as identify any potential bottlenecks, which will be valuable in designing proposal for the development of aquaculture potential. The results could serve as a starting point for the development of octopus farming in Pakistan and open up new opportunities for research and innovation within the country.

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KEY WORDS: embryonic development, *Octopus vulgaris*, eggs stages, hatching, paralarvae.

Первое описание выращивания осьминога *Octopus cf. vulgaris* III типа (Cephalopoda: Octopodidae) из Пакистана (северная часть Аравийского моря) в лабораторных условиях

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РЕЗЮМЕ. Настоящее исследование содержит первое подробное описание процесса выращивания обыкновенного осьминога *Octopus cf. vulgaris* III типа (Cephalopoda:

Octopodidae) из Пакистана (северная часть Аравийского моря) в лабораторных условиях. Данные о развитии параличинок и выживании в неволе необходимы для понимания методов устойчивого выращивания осьминогов в аквакультуре. Общее количество икринок, обнаруженных у исследованных самок, составило ~4230 штук в 53 гроздях/кладках, каждая из которых содержала 70 ± 64 яйца. Средний размер яйца составлял 1,3 мм в длину и 0,5 мм в ширину. В ходе исследования зафиксировано 20 стадий, при этом вылупление происходило через 17–22 суток (528 ч) после нереста при температуре около 28–31 °C. Самка осьминога погибла через 42 суток, когда вылупились все личинки. Исследование поможет нам лучше понять методы выращивания осьминогов, а также выявить любые потенциальные наработки и знания, необходимые при разработке предложений по развитию потенциала аквакультуры. Полученные результаты могут послужить отправной точкой для развития практики искусственного разведения осьминогов в Пакистане и открыть новые возможности для исследований и инноваций в стране.

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КЛЮЧЕВЫЕ СЛОВА: эмбриональное развитие, *Octopus vulgaris*, стадии развития яиц, вылупление, параличинка.

Introduction

The common octopus, *Octopus vulgaris* Cuvier, 1797 (Cephalopoda: Octopodidae), a marine carnivorous cephalopod mollusk, was thought to be a cosmopolitan species that is widely exploited by humans. It is an important species economically in the Mediterranean Sea, both the Atlantic and Pacific Oceans along the coasts of Africa, Brazil, Japan and India (Roper *et al.*, 1984; Norman *et al.*, 2014). Almost a century ago, Naef (1928) reported the first description of embryonic development in four species of cephalopods: *Loligo vulgaris*, *Sepia officinalis*, *Octopus vulgaris* and *Argonauta argo*, revealing their potential as model systems in developmental biology.

Recently, the taxonomy of common octopus has been significantly modified. A genomic study confirmed the existence of at least seven species within the *O. vulgaris* species complex: *O. vulgaris sensu stricto* (Northeastern Atlantic and Mediterranean), *Octopus americanus* Monfort, 1802 (formerly known as *O. vulgaris* type II) along the Yucatan continental shelf to Brasil, (Avendaño *et al.*, 2020), *O. maya* and *O. taganga* (Caribbean Sea), *O. insularis* distributed from the north of Argentina to the northwest coast of the USA, *O. vulgaris* type III (South Africa and Indian Ocean), (Avendaño *et al.*, 2020),

O. sinensis (Kermadec), *O. tetricus* (Eastern Australia and New Zealand), and *O. cf. tetricus* (Western Australia) (Amor *et al.*, 2019; Avendaño *et al.*, 2020).

Previous research on the eggs and larvae of benthic octopuses has been conducted under laboratory conditions worldwide to examine their growth, reproductive biology, and lifespan (e.g., Mangold *et al.*, 1973; Overath *et al.*, 1974; Van Heukelom, 1977; Forsythe *et al.*, 1985, 1988; Caverivière *et al.*, 1999; Oosthuizen *et al.*, 2003; Solorzano *et al.*, 2009; Ignatius *et al.*, 2011; Promboon *et al.*, 2011; Regueira *et al.*, 2018; Derycke *et al.*, 2020; Prasopsook *et al.*, 2022). Generally, the embryonic development of *Octopus vulgaris* in captivity, from fertilized egg to paralarvae, is similar to that of other octopod species such as the mimic octopus (*Octopus mimus*) (Naef, 1928; De Castro, Timmis, 2002). The eggs of cephalopods can be classified into small, medium and giant sizes, and the encapsulation processes varies greatly (Boletzky, 1986). Many eggs are interwoven into a string or clusters, which is formed by secretions from the female oviducts. Octopuses that produce planktonic paralarvae often lay thousands of tiny eggs, with some species, such as *O. vulgaris sensu lato*, up to 500,000 eggs (Mangold, Hanlon, 1983). The embryonic development period, from the release of capsules to hatching of paralarvae,

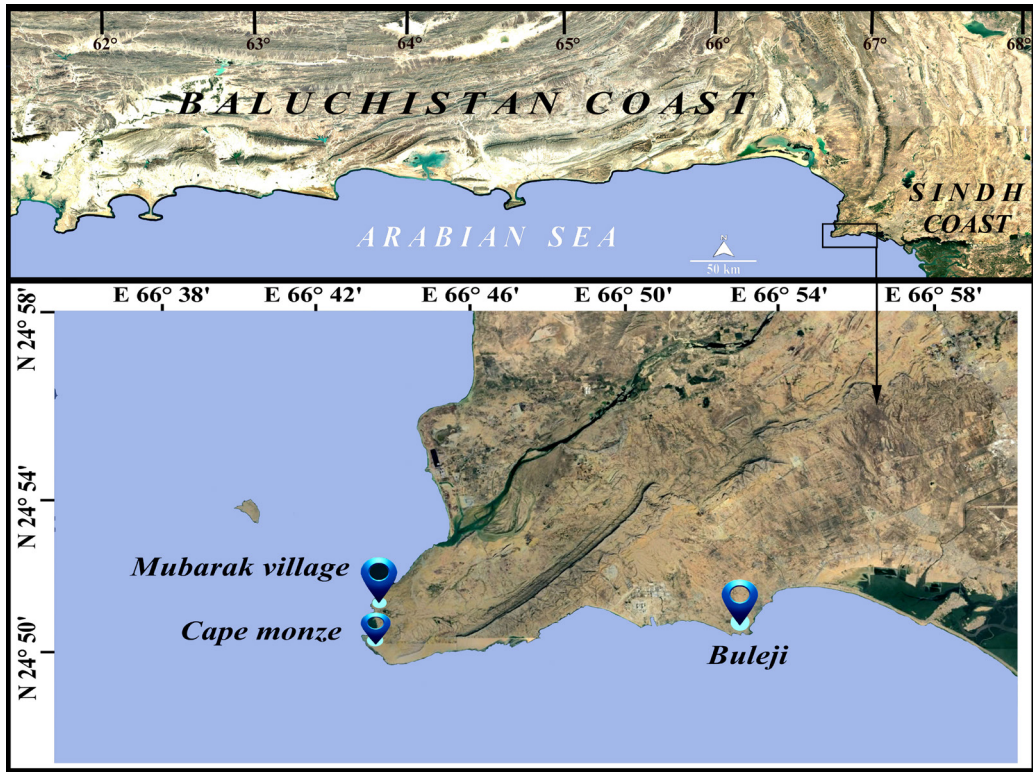


Fig. 1. Maps showing the study site along Pakistan coast: Buleji ($24^{\circ}50'24.1''\text{N}$ $66^{\circ}49'21.2''\text{E}$), Cape Monze ($24^{\circ}50'02''\text{N}$ $66^{\circ}39'24''\text{E}$), Mubarak village ($24^{\circ}50'44''\text{N}$ $66^{\circ}39'35''\text{E}$) during September 2019 and July 2022 (Fig. 1). The samples were collected during low tide, either by hand or using the hand nets. A total of 31 adult *O. cf. vulgaris* type III were collected (18 females, 13 males) were placed in separate plastic bags filled with seawater, and then transferred to containers for transportation to the wet Laboratory at (MRC&RC) UOK. In the laboratory,

is inversely related to water temperature. From fertilization to the end of embryonic development in *O. vulgaris*, the following stages can be identified: meiosis, cleavage, gastrulation and blastoderm displacement, first reversion organogenesis, second reversion, and hatching. The occurrence of these recognizable stages supports early classification (Naef, 1928).

At the same time, many species initial stages of life remain unknown, particularly for numerous commercially valuable species that form the foundation of significant fisheries. There is currently no information available on the embryonic and larval development stages of *O. vulgaris* in Pakistan. Knowledge of these stages is essential for the sustainable management of their populations (Rodhouse *et al.*, 1992; Sweeney *et al.*, 1992). Information about eggs provides valuable insights into the ecological relationships between species (Boletzky, 1998; Barón, 2001).

This research represents the first observations of the development of *O. cf. vulgaris* type III

(from Pakistan coast, Indian Ocean) eggs, embryo, and paralarvae in a controlled laboratory environment condition. The results of this study could serve as a foundation for the development of octopus aquaculture practices in Pakistan and could also open up new avenues for research and innovation.

Material and Methods

SPECIMEN COLLECTION. Specimens of octopuses were collected from intertidal areas in three different sites: Buleji ($24^{\circ}50'24.1''\text{N}$ $66^{\circ}49'21.2''\text{E}$), Cape Monze ($24^{\circ}50'02''\text{N}$ $66^{\circ}39'24''\text{E}$) and Mubarak village ($24^{\circ}50'44''\text{N}$ $66^{\circ}39'35''\text{E}$) during September 2019 and July 2022 (Fig. 1). The samples were collected during low tide, either by hand or using the hand nets. A total of 31 adult *O. cf. vulgaris* type III were collected (18 females, 13 males) were placed in separate plastic bags filled with seawater, and then transferred to containers for transportation to the wet Laboratory at (MRC&RC) UOK. In the laboratory,

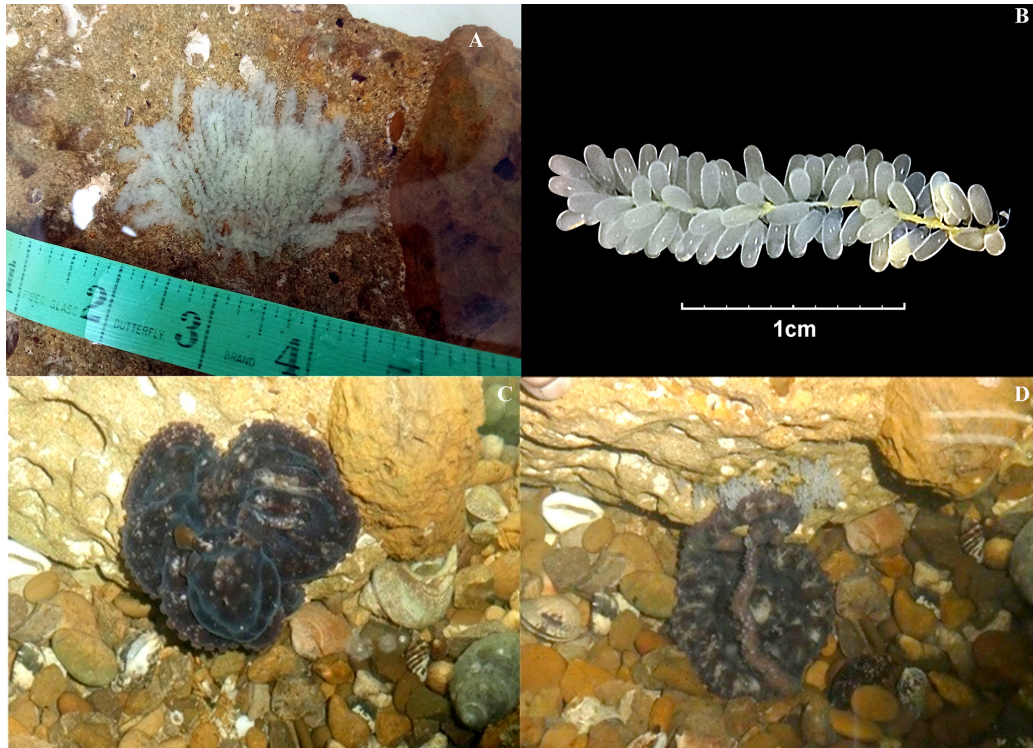


Fig. 2. Egg mass of *Octopus cf. vulgaris* type III: A — egg mass recently laid on the rock; B — single egg string; C — female lying the eggs on the rock; D — female cleaning and oxygenating the egg mass.

the specimens were allowed to acclimate for two days before being moved into designated glass aquaria.

SPECIMEN MAINTENANCE. After two days of acclimatization in the laboratory, octopuses were moved to large, multiple well-aerated glass aquaria measuring (32×18×18 inches, 160 L capacity) to prevent aggression and mimic natural conditions. Each aquarium filled with 40 L of sea water (~25% volume, shallow depth ~20–25 cm) to simulate intertidal habitats, facilitate oxygenation during brooding, and reduce stress. For the substratum, sand was collected from the wild at the selected sites and spread to a depth of 5–10 cm. Rocks were also placed to provide shelter and hiding spaces for the octopus. well-aerated aquaria measuring 32×18×18 inches and filled with 40 L of sea water. The physico-chemical parameters, including water temperature (18.5–36°C), salinity (36–38‰), and pH (7.9–8.2), were maintained according to conditions measured at the collection sites (in the wild).

Totally, 18 females of *Octopus vulgaris* (type III) were kept in captivity about 3 and half month, while only one female successfully brooded during this period of time and laid eggs after 52 days of spawning those hatched to paralarvae (Fig. 2). The female laid eggs in strings and attached them to a rock (mixed natural substrate consisting of fine, well-washed sand

(Fig. 2C) covering the egg mass with her arms and mantle (Fig. 2C). During the incubation, the mother octopus cleaned and oxygenated the eggs by jetting water with the help of a funnel. Female in this study did not eat until the paralarvae hatched and died after 42 days of starvation (Fig. 2D), and when all of the young had hatched. Without disturbing the female octopus, the rock with the attached egg strings was carefully removed from the aquarium. A physical examination was then conducted, including counting the number of strings and eggs on each string, weighing the eggs, strings, and performing other measurements.

Enrich *Artemia* has been shown to be the best starting food for the growth of paralarvae, as we know that temperature and food availability appear to have the most significant impact on their growth and survivability (Garcia, Gimenez, 2002; Vidal *et al.*, 2002; Leporati *et al.*, 2007; André *et al.*, 2008; Uriarte *et al.*, 2010; Ziga *et al.*, 2013; Ramos *et al.*, 2014). The temperature and salinity were controlled in the aquaria during entire study and not affected the growth rate of juveniles.

OBSERVATIONS AND MEASUREMENTS. During early stages of organogenesis, observations were made every 6h due to the rapid changes, while during later stages of development, observations were

Table 1. Egg stages of *Octopus cf. vulgaris* type III recorded during the experiment.

Embryonic stage	Stage	Observation on embryonic development stages
1	I	Eggs were transparent, fertilization of the oocyte, discoidal meroblastic cleavage to form the blastodisc (Fig. 3 I).
2	II	Formation of the blastula is completed, eggs a transparent cytoplasmic region just adjacent to the micropyle. It maintained as a fine cytoplasmic membrane all around yolk. (Fig. 3 II).
3	III	The cytoplasmic space beneath the micropyle expanded substantially (Fig. 3 III).
4	IV	Embryonic ectoderm continues to envelop the yolk (Fig. 3 IV).
5	V	The cap-like yolk sac gradually grew larger till development (Fig. 3V).
6	VI	First inversion, embryo twists in the egg such that the animal pore's posterior faces the stalk rather than just the micropyle (Fig. 3VI).
7	VII	Organogenesis and primordial development begin. Extra embryonic embryo and yolk envelopes cover one third of the yolk before closing completely so at vegetative pole (Fig. 3VII).
8	VIII	Organogenesis begins, the posterior and anterior sides, bright-field photos of <i>O. vulgaris</i> embryos, The yolk is almost completely covered in additional embryonic ectoderm (Fig. 3VIII).
9	IX	A pair of darker orange eyespots appears, as do the rudiments of arms, mantle, and funnel (Fig. 3 IX). The yolk sac was found to be pulsing.
10	X	darkness Eyespots are well-developed; arms, suckers, and funnel are further developed; and gills are visible (Fig. 3X).
11	XI	Eyespots visible through egg case; arms, suckers and funnel are developed further (Fig. 4XI).
12	XII	Further development of funnel, tentacles and suckers (Fig. 4 XII).
13	XIII	Chromatophores appear on the mantle, head and arms (Fig. 4XIII).
14	XIV	Two chromatophores appear on the ventral head (Fig. 4 XIV).
15	XV	Second inversion, the first chromatophore appears on the posterior mantle at this stage, and it is a light small yellow color Fig. 4 XV).
16	XVI	The chromatophore appears in various locations, with a strong presence in the anterior mantle (Fig. 4 XVI).
17	XVII	Embryos well-developed, outer yolk sac reduced considerably. This stage causes the chromatophore to appear darker and more numerous (Fig. 4 XVII).
18	XVIII	Outer yolk sac disappears. Release of few hatch lings was noticed. The chromatophore appears darker on the posterior and only a few appear near the eyes (Fig. 4 XVIII).
19	XIX	With all chromatophores, pigmentation of the ink sac was seen; the embryo responded to mechanical stimulation and completed its second reversion with minimal outer yolk sac (Fig. 4 XIX).
20	XX	The chromatophore expands, and the contraction becomes more widely distributed. Hatchlings of juvenile with or without yolk sac were released, and the process was repeated (Fig. 4 XX).

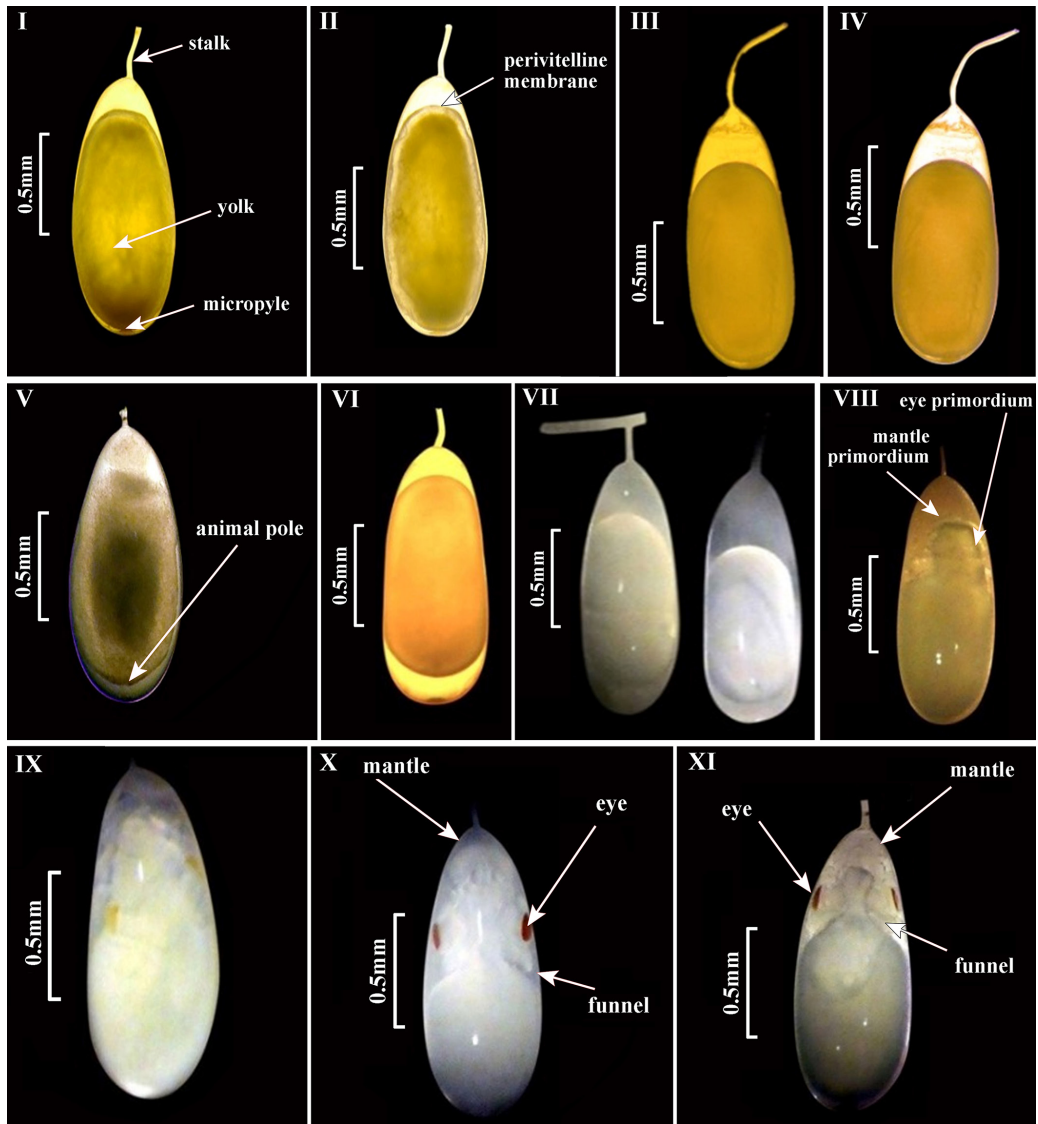


Fig. 3. Embryonic stages of *Octopus cf. vulgaris* type III observed during experiment in the laboratory: I — stage 1; II — stage 2; III — stage 3; IV — stage 4; V — stage 5; VI — stage 6; VII — stage 7; VIII — stage 8; IX — stage 9; X — stage 10; XI — stage 11.

made after every 18 h. Embryonic development stages were examined using a stereo zoom microscope Zeiss Stemi SV6 at a magnification of 10X, and recorded with digital camera. Samples were preserved in a glycerin solution with 5% alcohol and later fixed it into 5% formaldehyde. The morphological characteristics and measurements of octopus hatchlings were noted following the descriptions provided by Youngs *et al.* (1989) and Hochberg *et al.* (1992).

The following morphological characteristics were recorded and described for the hatched paralarvae (in

mm): Total Length (TL), Mantle Length (ML), Mantle Width (MW), Head Length (HL), Head Width (HW), Eyes Diameter (ED), Length of Arms (AZL), Number of Suckers on the arms (SAr).

Results and Discussion

Individual eggs are transparent and surrounded by a grape-shaped chorion. The egg size was 1.3 mm long and 0.5 mm wide. A total

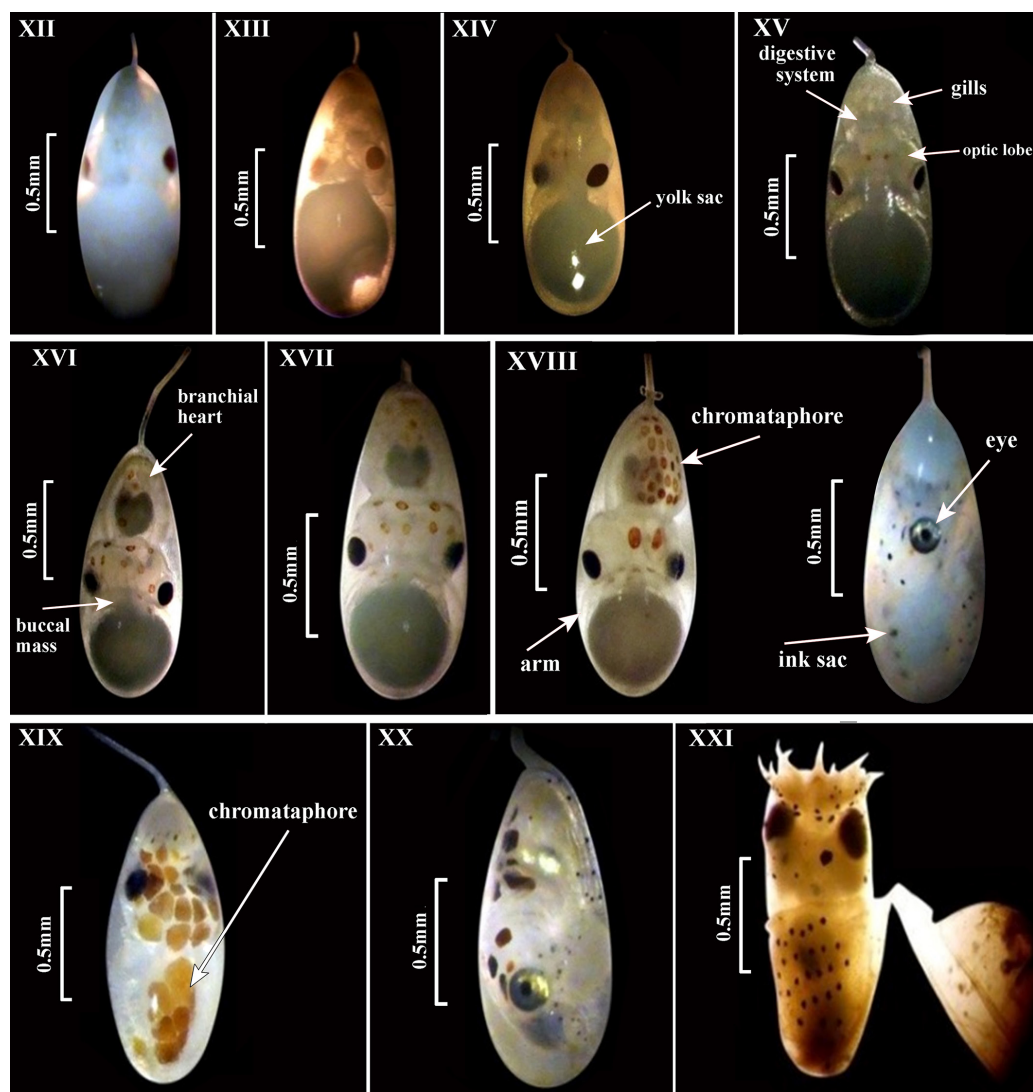


Fig. 4. Embryonic stages of *Octopus cf. vulgaris* type III observed during experiment in the laboratory: XII — stage 12; XIII — stage 13; XIV — stage 14; XV — stage 15; XVI — stage 16; XVII — stage 17; XVIII — stage 18; XIX — stage 19; XX — stage 20; XXI — hatched paralarva.

of ~4230 eggs were recorded in 53 strings, with each string containing from 6 to 134 eggs (in average, ~70 eggs per a string). In this study, we observed 20 embryonic development stages of *Octopus cf. vulgaris* following the description of Naef, which were grouped major developmental phases: cleavage, blastula, gastrula, embryogenesis, organogenesis, and hatching (Table 1, Figs 3–5) with hatchlings occurring 17–22 days (408–528 h) after spawning at 28–31 °C. Clutches reared at ambient controlled

temperatures not showed the growth in mantle length. Paralarvae were 1.3–4.8 mm in total length. The hatchlings were planktonic, and had five suckers arranged in a single row on each juvenile arm. They had eight to ten reddish brown chromatophores arranged in two to three rows on the dorsal head. Paralarvae were not able to survive longer than 27 days.

Paralarvae exhibited typical planktonic behavior after hatching, remaining active in the water column with frequent mantle contractions

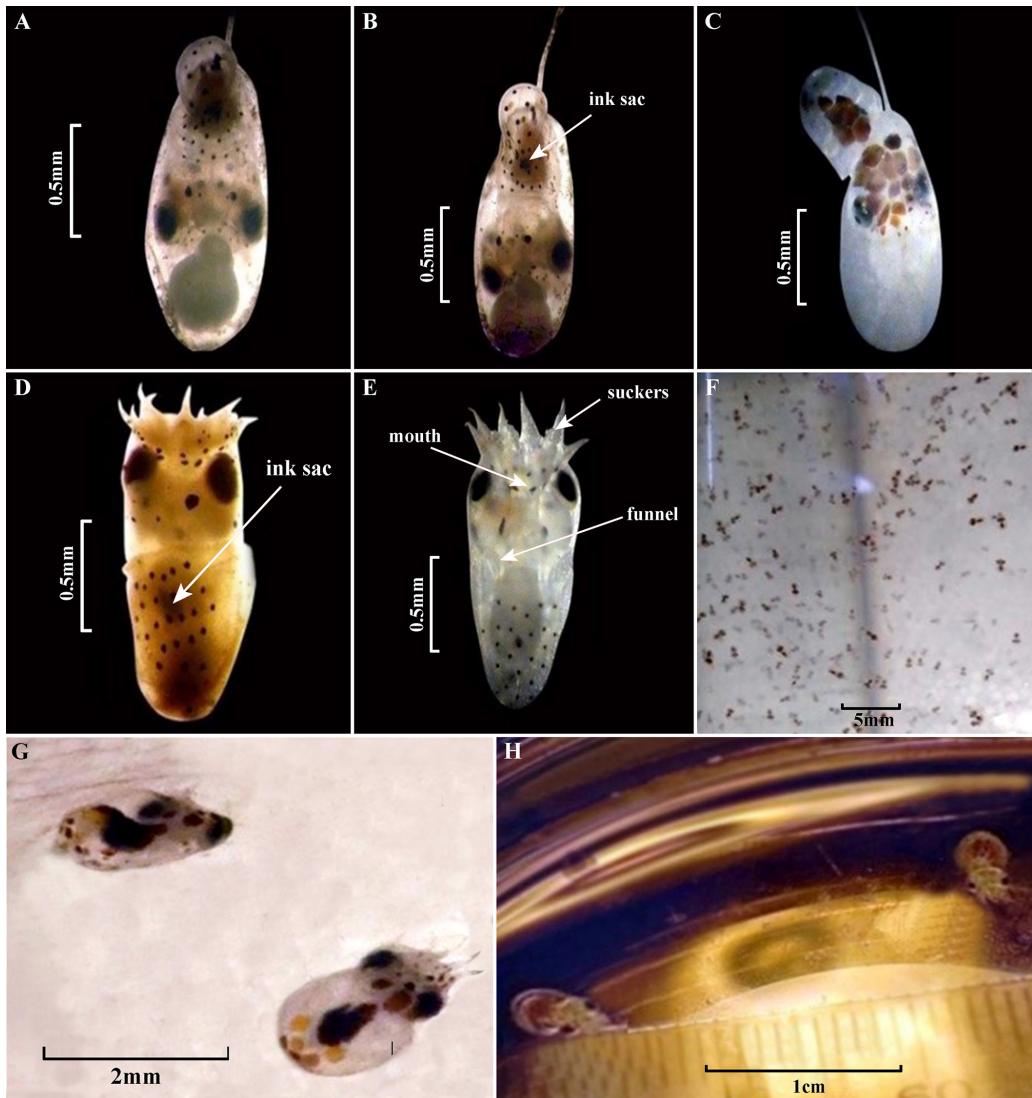


Fig. 5. Hatching paralarvae of *Octopus cf. vulgaris* type III: A — hatching stage 1; B — hatching stage 2; C — hatching stage 3; D — hatching stage 4; E — hatched; F–H — swimming paralarvae.

and jet propulsion via the siphon for swimming and orientation (as illustrated in Figure 5, showing paralarvae in various postures and swimming). They displayed predatory activity, including arm extension toward potential prey, chromatophore-based responses (8–10 reddish-brown chromatophores arranged in 2–3 rows on the dorsal head for camouflage or signaling), and occasional ink expulsion upon disturbance. These behaviors align with observations in related *Octopus* species, where paralarvae actively

hunt small zooplankton in the planktonic phase. Morphology description and measurement of the paralarvae (in captivity) indifferent body dimension mention below, along with the (Tables 2, 3).

The data obtained provides the first insight into the biology of the early life stages, larval development, growth patterns, and survival rates of *O. cf. vulgaris* type III from Pakistan. It allows us to draw some generalized conclusions.

The eggs of incirrate octopuses (Octopodidae) resemble rice or pears in appearance. The egg

Table 2. Survival data of paralarvae of *Octopus cf. vulgaris* type III during the experiment under laboratory conditions.

Days post-hatching	Water temperature	Rate of hatching	Rate of mortality	Percentage % of mortality
1	28 °C	03	00	0.0%
2	27 °C	15	06	0.141%
3	29 °C	60	7	0.165%
4	28 °C	141	10	0.236%
5	27 °C	351	0	0.0%
6	29 °C	112	17	0.401%
7	28 °C	47	163	3.853%
8	29 °C	161	203	4.799%
9	29 °C	1259	135	3.191%
10	29 °C	167	254	6.004%
11	30 °C	228	147	3.47%
12	30 °C	119	330	7.80%
13	30 °C	118	358	8.463%
14	30 °C	73	115	2.718%
15	29.8 °C	36	104	2.458%
16	30 °C	16	264	6.241%
17	28.5 °C	100	0	0.0%
18	30 °C	132	249	5.88%
19	30 °C	66	230	5.437%
20	29.5 °C	03	85	2.0%
21	30 °C	115	100	2.36%
22	31 °C	79	213	5.035%
23	29 °C	57	69	1.631%
24	30 °C	82	08	0.189%
25	30 °C	0	230	5.437%
26	30 °C	0	242	5.721%
27	31 °C	0	01	0.02%

capsules are protected by a chorion membrane. Normally, eggs differ in size according previous observations (Sweeney *et al.*, 1992; Villanueva, 2008; Sukhsangchan, 2015). The size of the egg in our study is very similar to that of *Octopus vulgaris* studied by Boletzky (1984) (2.2×0.95 mm) and other studies (Mangold, 1983; Roper *et al.*, 1984; Vidal *et al.*, 2010). The cephalopod incubation period decreased with increasing temperature, indicating that temperature signifi-

cantly affects embryonic development. Numerous species of squid and octopus such as *O. mimus* (Uriarte *et al.*, 2012), *Sepia officinalis*, *Octopus vulgaris*, *Sepiola robusta*, *Loligo vulgaris*, *Loligo pealei*, and *Illex illecebrosus* (Boletzky, 1994) have been shown to experience prolonged embryonic development periods when temperatures are low.

Egg size also affects development time of embryos, with smaller eggs typically develop-

Table 3. Growth of *Octopus cf. vulgaris* type III paralarvae after hatching recorded during the experiment.

Morphometric parameter	Age of paralarvae (days post-hatching)							
	0 (newly hatched)	4	6	10	15	17	20	27
Total length (TL, mm)	1.3 ± 0.1	1.57 ± 0.12	1.85 ± 0.15	2.2 ± 0.18	3.0 ± 0.25	3.6 ± 0.30	4.0 ± 0.35	4.8 ± 0.40
Mantle length (ML, mm)	0.63 ± 0.05	0.77 ± 0.06	1.0 ± 0.08	1.2 ± 0.10	1.5 ± 0.12	1.8 ± 0.15	2.16 ± 0.18	2.3 ± 0.20
Mantle width (MW, mm)	0.48 ± 0.04	1.0 ± 0.08	1.2 ± 0.10	1.1 ± 0.09	1.3 ± 0.11	1.4 ± 0.12	1.7 ± 0.15	2.0 ± 0.18
Head length (HL, mm)	0.37 ± 0.03	0.55 ± 0.05	0.37 ± 0.04	0.5 ± 0.05	0.7 ± 0.06	0.7 ± 0.06	0.84 ± 0.07	1.0 ± 0.09
Head width (HW, mm)	0.55 ± 0.05	1.1 ± 0.09	1.1 ± 0.09	1.2 ± 0.10	1.1 ± 0.09	1.2 ± 0.10	1.6 ± 0.14	1.78 ± 0.16
Eyes diameter (ED, mm)	0.3 ± 0.02	0.3 ± 0.02	0.3 ± 0.02	0.3 ± 0.02	0.35 ± 0.03	0.4 ± 0.03	0.5 ± 0.04	0.6 ± 0.05
Length of arms (AZL, mm)	0.3 ± 0.03	0.25 ± 0.03	0.48 ± 0.05	0.5 ± 0.05	0.8 ± 0.07	1.01 ± 0.09	1.0 ± 0.09	1.5 ± 0.13
No. of suckers on arms (SAr)	3	3	3–4	3–5	4–7	5–7	5–8	7–9
Number of specimens measured	~3540	~3000	~2500	~1800	~800	~400	~100	1

ing faster than larger eggs incubated at the same temperature (Boletzky, 1994). This has been observed in various species, including *Sepiola*, which eggs developed slower (2.5 months) at 13 °C than at 15 °C (2 months) (Boletzky, 1983). *Octopus mimus* reared at 21 °C also reached stage XV 24, 58, and 75% faster than at 18, 15, and 12 °C, respectively (Uriarte *et al.*, 2012). However, it is still unclear how this difference in temperature and developmental time affects the health and survivability of the paralarvae.

Octopus female in this study brooded eggs in her arms during entire experiment on 22 March 2021 when eggs laid and female started holding them till 17 April 2021, while hatching were recorded on 7 April, when 17 of egg were hatched, while all remaining eggs hatched on 17 April (total 528 h for hatching). The gestation period *Octopus aegina* from India was 18–20 days at a temperature range of 28–30 °C (Ignatius, 2006), and *Octopus vulgaris* from Spain hatches in 25 days (Repolho, 2014). The *O. vulgaris* embryos developed highly synchronous within the string and hatched after approximately 1 month at 19 °C and 2.5 mm long with 1 mm wide from Tenerife, Spain (Deryckere *et al.*, 2020). Paralarvae of *Am-*

phioctopus aegina from Thailand hatched after 18–22 days at 28.0 °C and swam to the surface; the egg size was 2.64±0.13 mm in length and 0.94±0.06 mm in width (Prasopsook *et al.*, 2022). The development period in *Octopus oliveri* was 38 days at 25.140± 4 °C (Ylitalo *et al.*, 2014). Paralarvae of *Haplochaena maculosa* hatched in 25–35 days at 21.0–22.5 °C (Overath, 1974), whereas cold-adapted species have a much longer embryonic development *Octopus bimaculoides* (from Mexico), which paralarvae hatched in 65 days at 18.0 °C (Ibarra-García *et al.*, 2018). The development of *Eledone cirrhosa* takes 90–120 days at 14.0–18.0 °C (Mangold, 1971). The embryonic development period in *Enteroctopus dofleini* was estimated for about 161 days at 9.2–13.9 °C (Gabe, 1975; Villanueva, Norman, 2008). It cannot be determined if this increase in survivability was due to a longer incubation time or modification of an aquarium set up, so further studies are needed to be certain.

Generally, we could conclude that the embryonic development of our studies octopus did not seem to differ significantly from that in the other *Octopus* species, such as *Haplochaena* sp. (Overath, Boletzky, 1974), *Octopus mimus*

(Uriarte *et al.*, 2012), and *O. vulgaris* (Naef, 1928), and was consistent with other studies on *O. huttoni* (Carrasco, 2014).

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Data Availability: No data additional to the sources cited were used for the research described in the article.

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