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EMBRYONIC DEVELOPMENT AND FECUNDITY OF THE PACIFIC PYGMY OCTOPUS, *PAROCTOPUS DIGUETI*

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ABSTRACT The life history including embryonic development of most species of octopus is still poorly understood. This makes the identification of eggs and juveniles difficult, hampering distribution and dispersal studies. The Pacific pygmy octopus, *Paroctopus digueti* (Perrier & Rocheburne 1894), exhibits features, including its direct embryonic development, that make it an ideal candidate for aquaculture. This study provides detailed information on the embryonic development, the morphological characteristics of eggs, and the fecundity of wild female *P. digueti* maintained under laboratory conditions that replicate natural environmental conditions. The Pacific pygmy octopus showed a monocyclic spawning pattern that takes place in time-separated batches, leading to asynchronous embryonic development in the batch. Eggs are between 7 and 10 mm (8.9 ± 0.71 mm) in total length. During the embryonic development, 31 distinct stages were identified with a total duration of 38 days. The distribution of chromatophores showed a specific pattern, with dorsal chromatophores being more abundant and larger than ventral ones. An observed fecundity of 300 eggs per female was twice as high as the value previously reported for this species in Bahia Choya, Sonora. This study contributes to the better understanding of the life cycle of *P. digueti*. Besides being a basic reproductive aspect, fecundity is a key element for studies on the reproductive potential and population dynamics of the species.

KEY WORDS: embryonic development, parental care, spawning, chromatophore pattern, eggs

INTRODUCTION

The embryonic development of cephalopods is considered unique among molluscs (Boletzky 1974). Their limited knowledge makes it difficult to identify eggs and juveniles in the distribution and dispersal studies, and even more, in commercially important species, knowledge of life histories is necessary to propose sustainable management strategies for their populations (Sweeney et al. 1992). In spite of the life histories of most of the octopuses still being unknown, available descriptions of eggs and juveniles have shown that the number and pattern of chromatophores in the skin on the arms, siphon, eyes, head, and mantle during ontogeny are useful to differentiate between species, as well as the number and size of suckers after birth (Naef 1928, Yamamoto 1988, Young et al. 1989, Osborn 1995).

The Pacific pygmy octopus, *Paroctopus digueti* (Perrier & Rocheburne, 1894), formerly classified as *Octopus digueti*, is a species of pigmy octopus that occurs in Mexico in the Gulf of California and adjacent Pacific (Jereb et al. 2016). Being an octopus of small size (74 mm LM) and with a direct development, its conditioning and rearing in captivity and laboratory conditions has been feasible (Hanlon & Forsythe 1985, DeRusha et al. 1987), which has allowed the documentation of the following characteristics: it can live up to 8 mo at 25°C (Hanlon & Forsythe 1985, DeRusha et al. 1987); it exhibits exponential growth, similar to other small sized species such as *Octopus joubini* (Forsythe 1984); it undergoes rapid growth, quadrupling its size during the exponential growth phase (DeRusha et al. 1987); each female lays 50–150 eggs, with embryonic development lasting between 35 and 42 days at 25°C (Hanlon & Forsythe 1985). All these characteristics make *P. digueti* an ideal candidate for its cultivation in spite of the studies on the biology and life history of the Pacific pygmy octopus being scarce. At present, there is no detailed information available about the embryonic development or updated fertility estimate of *P. digueti.* This work provides potentially useful information on the embryonic development and morphological characteristics of the eggs and hatchlings of P. digueti. Knowledge of the early life stages will allow us to have a better understanding of their life cycle and ecology. Additionally, this knowledge will help to document reproductive behavior in captivity and the time of embryonic development. For its part, the estimation of fecundity is important, because it is a parameter of great interest in studies of population balance and represents a basic aspect in the knowledge of the reproductive strategy, constituting an essential element in studies on the potential reproductive performance of the species and on population dynamics.

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MATERIALS AND METHODS

Collection and Rearing of Brooding Females

Twenty brooding females were captured at Ensenada de La Paz (24° 06′N, 24° 11′N, 110° 19′W, 110° 26′W). The specimens were found inside empty bivalve shells, mainly of *Anadara multicostata* and *Megapitaria squalida*. The females were individually placed in 30 × 20 × 20 cm cages made of polyvinyl chloride (PVC) and shade mesh in a 300 L tank supplied with continuous aeration and sea water flow. Sea water was filtered through a closed recirculation system fitted with mechanical and biological filters, and an ultraviolet (UV) lamp. Water quality was monitored daily by recording salinity, pH, oxygen, and nitrogen compounds (ammonium, nitrites, and nitrates). Temperature was kept at 27 ± 1°C by means of automatic heaters and hourly monitoring with a Pendant Temp HOBO logger. This temperature corresponds to those recorded naturally

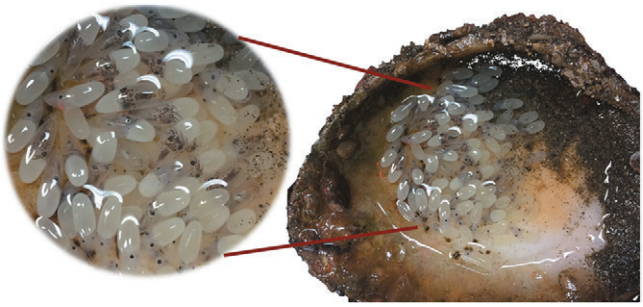

Figure 1. Asynchronous embryonic development of *Paroctopus digueti*. Embryo batches separated in time with marked differences in development.

during the summer (25.5°C–30.4°C) in Ensenada de La Paz (Camacho-Mondragón et al. 2012). Although females in this stage are known to fast, they were initially offered live clams (*Chione californiensis*) as food, but as these were not consumed, they were kept with no food thereafter; this helped to maintain good water quality.

Spawning Pattern, Egg Structure, and Duration of Embryonic Development

The spawning pattern was examined by taking photographs of the entire egg batch and capturing the arrangement and degree of development of eggs during spawning. Two or three eggs were extracted from each brood to examine their structure and degree of development at the time, under an Olympus SZCTV stereo microscope. The dates of the start and end of spawning and first offspring hatching were recorded to determine the spawning pattern (polycyclic or monocyclic) and the duration of embryonic development.

Fecundity

Actual fecundity (number of eggs laid by a female throughout its life) was estimated without inflicting additional stress to the females. Once the development process was completed, the number of eggs extracted to describe the embryonic development stages was counted together with the number of offspring that hatched from eggs laid by each of the 15 brooding females.

Embryonic Development

After 2 days of acclimatization and every 2 days thereafter, the sheltering shells of brooding females were carefully opened to extract between three and five eggs in different degrees of development. The eggs extracted were fixed in 5% formaldehyde for subsequent analysis of ontogenetic stages. This procedure was carried out until the entire sequence of embryonic development stages (until hatching) was documented.

Embryonic development stages were described in terms of the development of the mantle, eyes, arms, funnel, and chromatophore pattern, as described for *Octopus vulgaris* by Naef (1928), taking into account the features that are unique to this species. The chromatophore pattern was determined using the orientation patterns described by Sweeney et al. (1992).

Once the development stages were determined, each was documented photographically with an Olympus SZCTV stereo

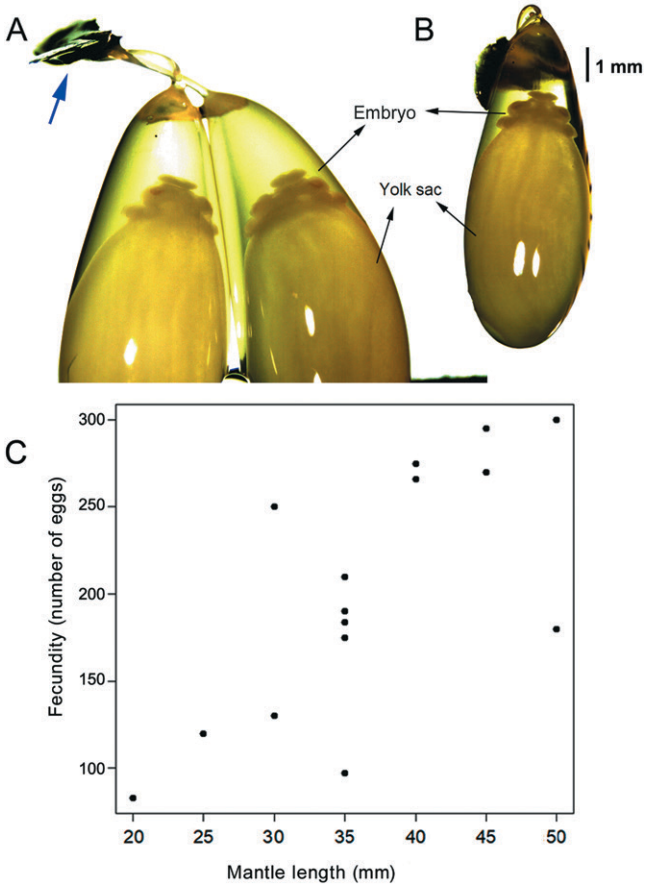

Figure 2. Eggs and fecundity of *Paroctopus digueti*. (A) Peduncles joined with the enlarged free part (blue arrow). (B) Full view of the egg. (C) Dispersion of fecundity values in relation to the female size.

microscope. The Leica Application Suite software was used to measure the total egg length on the digitized images and, in cases where the embryo was already visible, the mantle length (from the distal end of the mantle to the average eye height) was also measured.

Yolk Consumption

Yolk consumption was estimated by measuring the length of the external yolk sac in each embryonic development stage on the digitized images; its reduction relative to mantle length was considered as indicative of consumption.

RESULTS

Spawning Pattern, Egg Structure, and Duration of Embryonic Development

The Pacific pygmy octopus *Paroctopus digueti* showed an intermittent monocyclic spawning pattern with eggs laid in multiple batches. The female lays eggs inside the shell chosen as shelter, in individual batches laid separately over the spawning period of 8–10 days. Embryos in different degree of development were observed, including some small and mostly unpigmented ones, or a few with eye pigmentation, as well as more

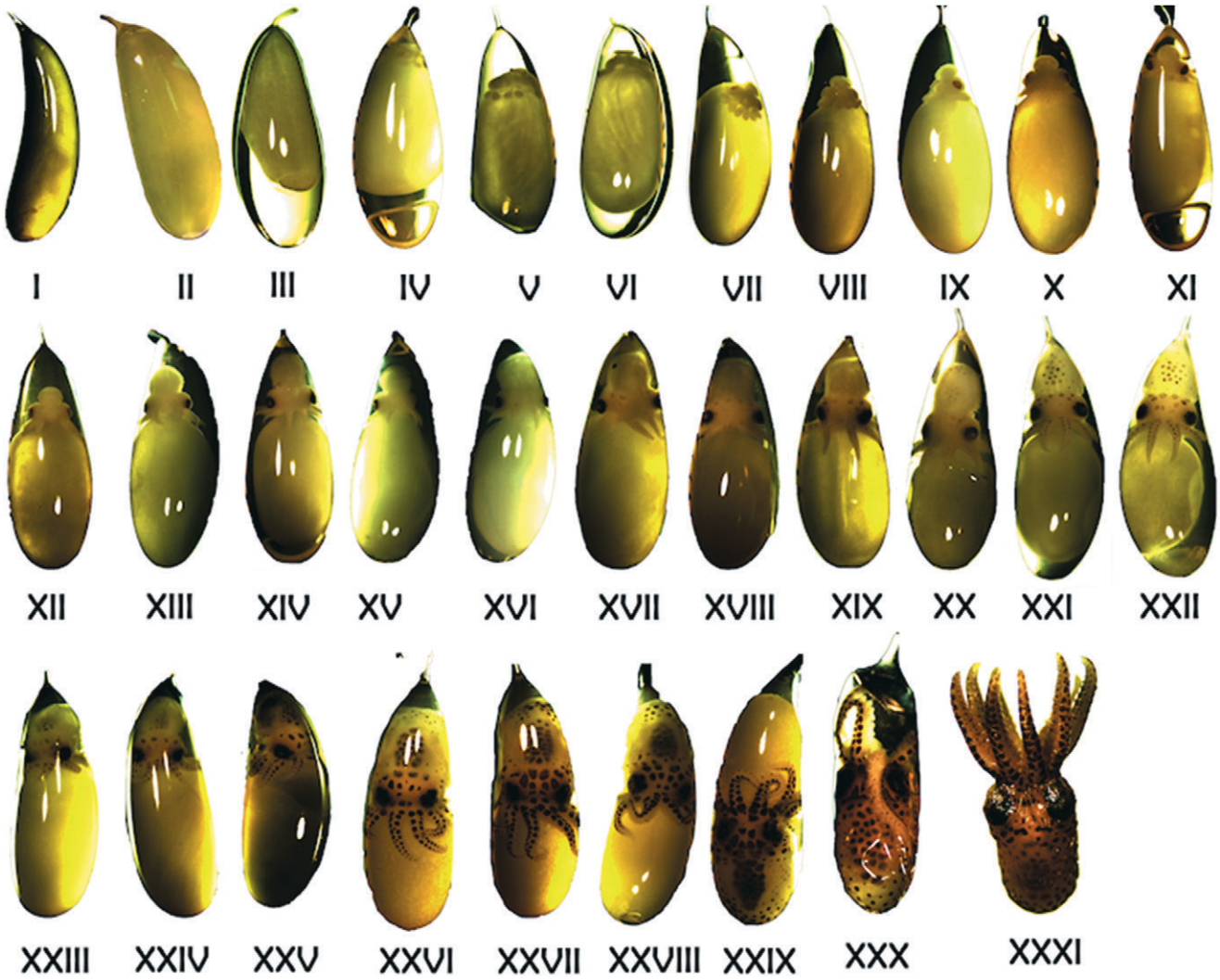

Figure 3. Embryonic development stages of *Paroctopus digueti*. Note the development of chromatophores.

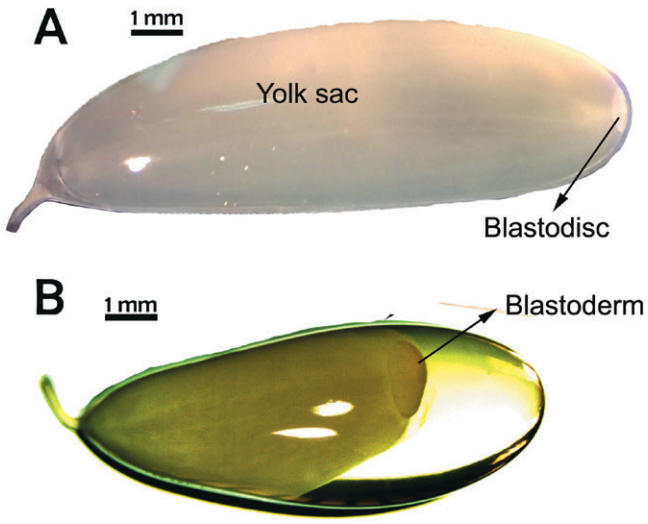

Figure 4. Early embryonic development of *Paroctopus digueti*. (A) Formation of the blastodisc in the animal pole (stage II). (B) Onset of the gastrulation process with the blastoderm expanding over the egg (stage III).

developed embryos with visible chromatophores and arms extended on the surface of the yolk sac (Fig. 1).

The eggs of *Paroctopus digueti* are large, with total length ranging between 7 and 10 mm (mean: 8.9 mm; SD: 0.71 mm). The egg has an elongated chorionic peduncle with the free end enlarged, which the female uses to attach the egg to the shell, either individually or in groups of two to three eggs (Fig. 2A). The chorion capsule is pear shaped, with a translucent surface that allows the observation of the yolk sac and the developing embryo (Fig. 2B).

The embryonic development of *Paroctopus digueti* at 27 ± 1°C lasted 38 days from the onset of spawning until the hatching of juveniles. Because of the nature of samples (brooding females that already came with eggs of which the release date is unknown), it was not possible to accurately determine the duration of each development stage.

Fecundity

Fecundity was estimated to range from 80 to 300 eggs per female. Although the small sample size precluded the use of formal statistical analyses, a trend toward higher fecundity in larger sized females was observed (Fig. 2C).

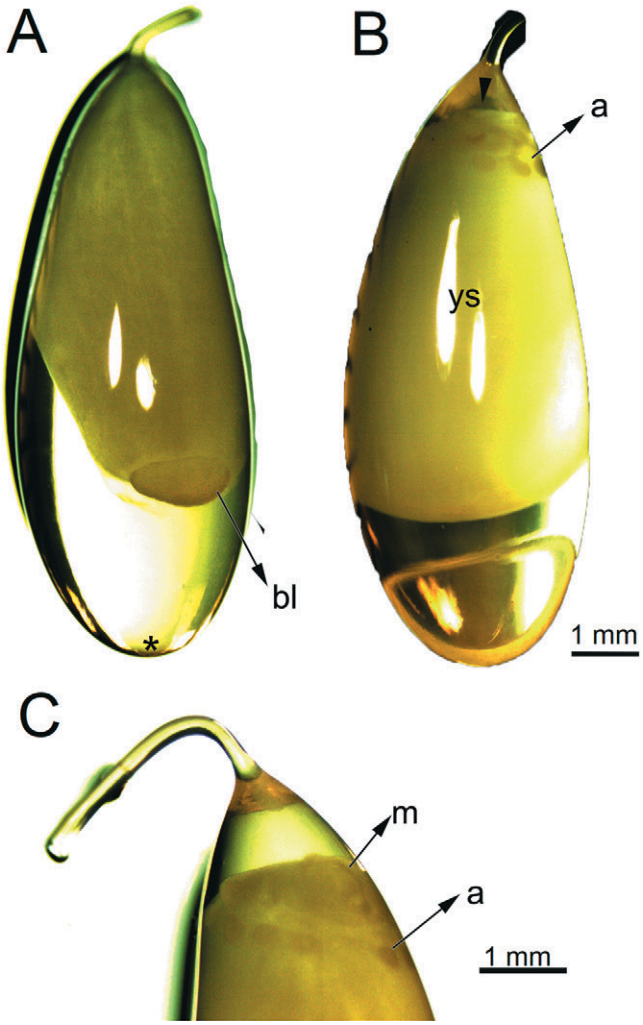

Figure 5. First embryo inversion between stages III and IV and onset of organogenesis in *Paroctopus digueti*. (A) Stage III with embryo still in the animal pole (asterisk). (B) Stage IV with the embryo into the vegetal pole (arrowhead). (C) Stage IV, onset of organogenesis: head and rudimentary arms are easily visible. a, arm; bl, blastoderm; m, mantle; ys, yolk sac.

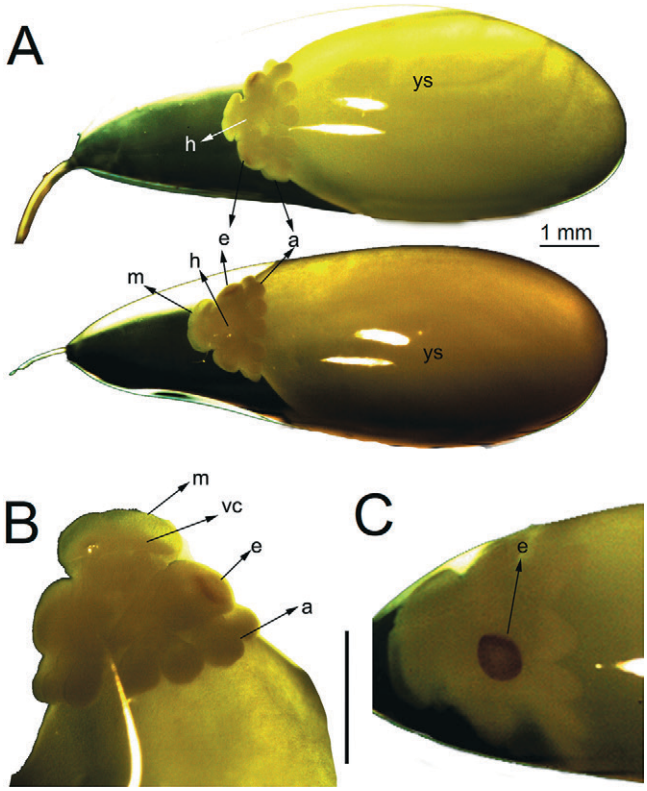

Figure 6. Mantle in process of covering the visceral tissue forming the head in the embryonic development of *Paroctopus digueti*. (A) Stages VII (above) and VIII (below). (B) Stage VIII, detail of the embryo with growing arms. (C) Detail showing the large, brown eyes. a, arm; e, eye; h, head; m, mantle; vc, visceral mass; ys, yolk sac.

Embryonic Development

For *Paroctopus digueti*, 31 distinct stages were identified (Fig. 3). The three earliest stages (I–III) correspond to the blastulation and gastrulation processes (Fig. 4), and the remaining stages (IV–XXIX) encompass the organogenesis process (the embryo reverses its position twice during development). The development of chromatophores goes from stage XVI to stage XXIX, and the XXXI stage corresponds to hatching.

The first inversion of the embryo from the animal to the vegetal pole took place between stages III and IV (Fig. 5A, B) with the onset of organogenesis; the embryo is barely visible, showing the formation of head and arm rudiments (Fig. 5C). In this stage, eggs measure 9.2 mm (±0.16) by 3.54 mm (±0.16), average length and width, respectively.

In stage VIII, the mantle develops toward the ventral part and starts covering the visceral tissue; its average length is 0.67 mm (±0.1) (Fig. 6A). The mouth becomes visible as an elongated tube located in the middle of the eyes; it migrates toward the ventral part as development progresses. The elongated heart-shaped arms appear arranged in pairs (Fig. 6B). The large eyes show an orange coloration (Fig. 6C). Organ development is still barely visible, but an aggregation of proliferating cells can be seen in the central part of the mantle. Eggs in this stage measure 8.95 mm (±0.5) by 3.33 mm (±0.3), average length and width, respectively; the yolk sac covers 64.59% of the egg.

In stage X, the mouth is clearly distinguished (Fig. 7A); the mantle is 0.9 mm (±0.1) long (Fig. 7A, B); funnel development starts and two rows of three or four suckers appear on each arm (Fig. 7C), which will develop along with arms during embryonic development. The average length and width of eggs are 8.8 mm (±0.3) and 3.2 mm (±0.1), respectively; the yolk sac covers 63.44% of the egg.

In stages XI and XII, mantle development continues (Fig. 8A), and the mouth is now located in a ventral position below the eyes (Fig. 8B). A rudimentary, light beige crystalline appears to the center of the eye; well-defined arms can be observed individually (Fig. 8C). Eggs measure 8.02 mm (±0.1) by 3.30 mm (±0.1), average length and width, respectively; the yolk sac covers 61.97% of the egg.

In stage XV, the mouth reaches its final ventral position (Fig. 9A, B), and mantle development is completed (reaching a length of 1.4 ± 0.07 mm) (Fig. 9C). The funnel development is completed when the mantle covers up to its mid part and water is moved and expelled through it, indicating its functionality.

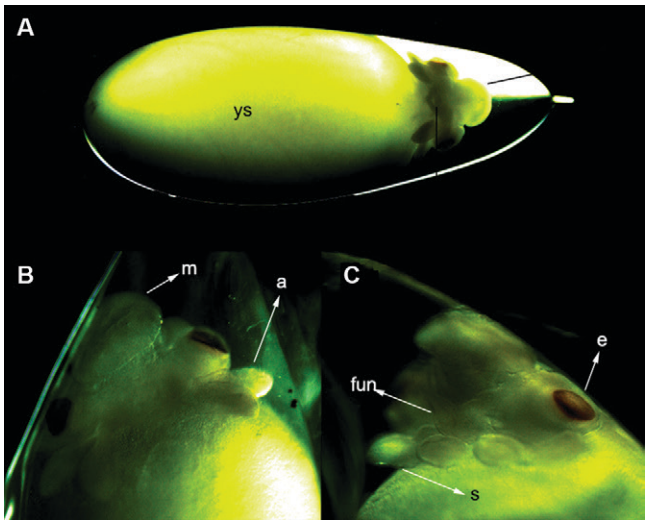

Figure 7. Stage X of the embryonic development of *Paroctopus digueti*. (A) Differentiation of the mouth. (B) Detail of mantle, arms, and suckers. (C) Funnel in development, large brown eye. a, arm; bm, buccal mass; e, eye; fun, funnel; m, mantle; s, sucker; ys, yolk sac.

The average length and width of eggs in this stage are 8.8 mm (±0.07) and 3.4 mm (±0.1), respectively; the yolk sac covers 59.21% of the egg.

In stage XVI, chromatophores start appearing at the base of the arms and on the dorsal part of the head (Figs. 3 and 10). The gills, the three functioning hearts (distinguished by palpitations), and small salivary glands are apparent. Eggs measure 8.5 mm (±0.7) by 3.1 mm (±0.4), average length and width, respectively; the yolk sac covers 58.73%.

In stage XIX, chromatophores appear on the anterior margin of the ventral mantle, the visceral epithelium, and the posterior part of the mantle (Figs. 3 and 11). Two chromatophores can be seen on the dorsal part of the eye. The inner organs can be seen functioning, except for the reproductive system, which could not be seen. The mantle is now 1.7 mm (±0.09) long on average, and eggs are 8.1 mm (±0.2) by 2.9 mm (±0.1), average length and width, respectively; the yolk sac covers 58.63% of the egg.

Starting in stage XX, the embryos continue growing and digesting their external yolk reserves. The digestion process is characterized by marked contractions in the mantle and the external yolk sac. The eyes are brown with a whitish crystalline.

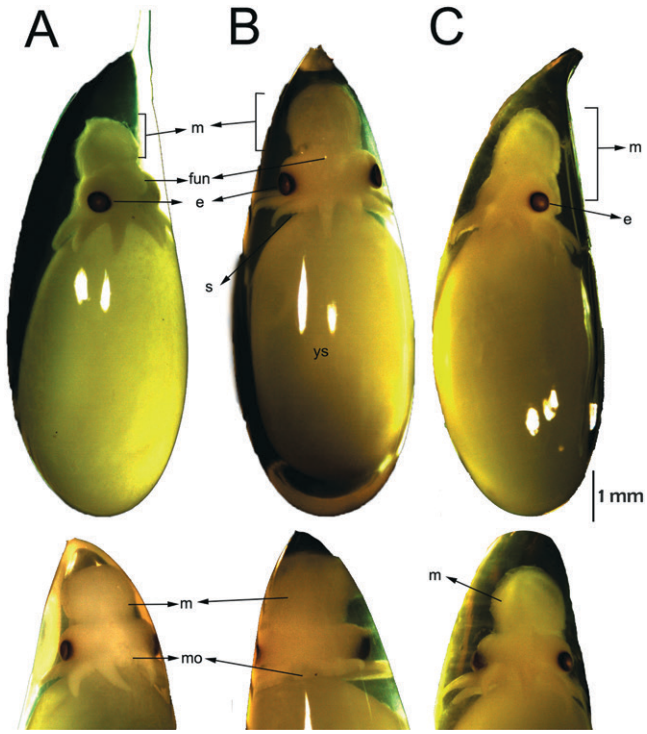

Figure 9. Fully developed funnel and mouth of *Paroctopus digueti*. (A) Stage XIII. (B) Stage XIV. (C) Stage XV. e, eye; fun, funnel; m, mantle; mo, mouth; s, sucker; ys, yolk sac.

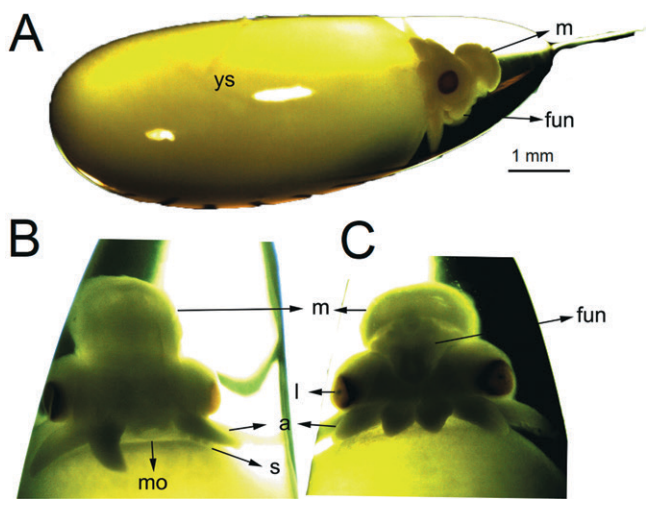

Figure 8. Stages XI and XII of the embryonic development of *Paroctopus digueti*. (A) Stage XI, developing mantle. (B) Stage XII, mouth below the eyes migrating to the ventral part of the embryo. (C) Developing funnel, arms, and suckers. a, arm; fun, funnel; l, lens; m, mantle; mo, mouth; s, sucker; ys, yolk sac.

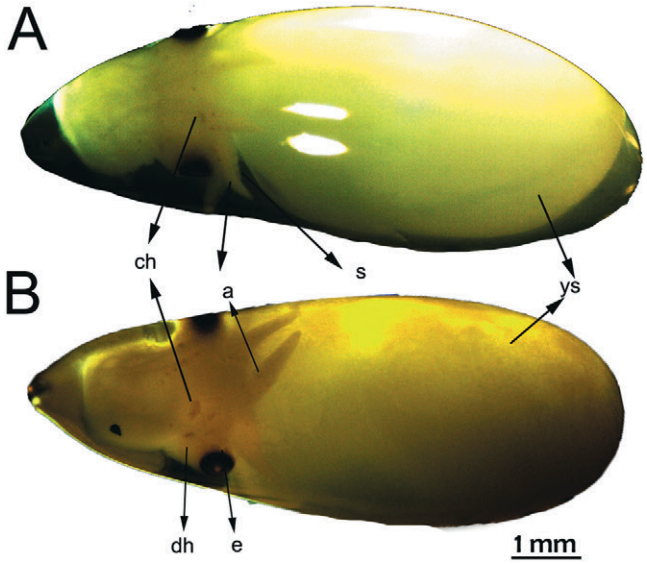

Figure 10. Onset of the chromatophore pattern in the embryonic development of *Paroctopus digueti*, dorsal view. (A) Stage XVI. (B) Stage XVII. a, arm; ch, chromatophores; dh, dorsal head; e, eye; s, sucker; ys, yolk sac.

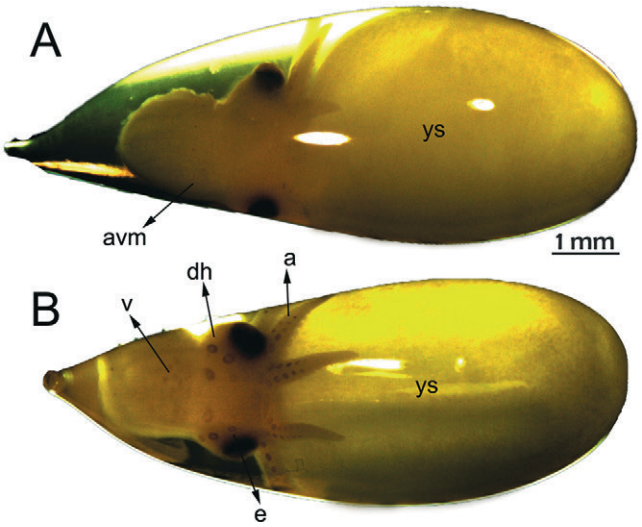

Figure 11. Stage XIX of the embryonic development of *Paroctopus digueti*. (A) Ventral view. (B) Dorsal view. a, arm; avm, anterior margin of ventral mantle; dh, dorsal head; e, eye; v, visceral; ys, yolk sac.

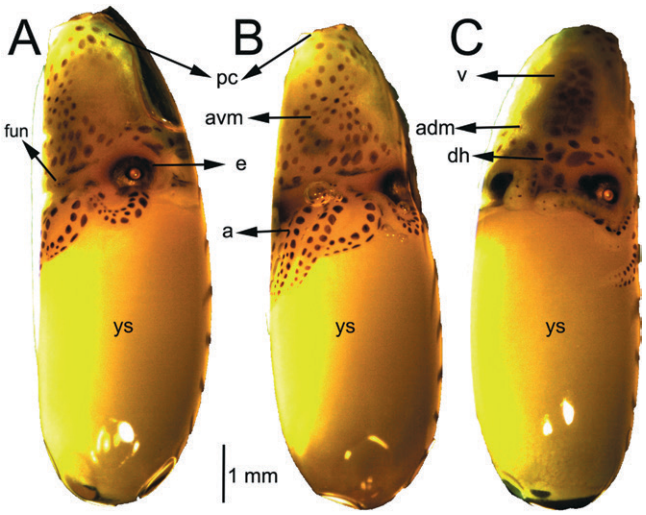

Figure 13. Chromatophore pattern in Stage XXVIII of the embryonic development of *Paroctopus digueti*. (A) Side view. (B) Ventral view. (C) Dorsal view. a, arm; adm, anterior margin of dorsal mantle; avm, anterior margin of ventral mantle; dh, dorsal head; e, eye, fun, funnel; pc, posterior cap; v, visceral; ys, yolk sac.

Between stages XXVI (Figs. 3 and 12) and XXVIII (Figs. 3 and 13), chromatophores of the head, eyes, visceral epithelium, and arms become larger and abundant, adopting a circular distribution. Three chromatophores are apparent in the funnel on the ventral mantle (Fig. 13). The 35–40 chromatophores of the visceral epithelium exhibit a compact oval arrangement. The anterior margin of the dorsal mantle exhibits yellow and brown chromatophores surrounding the chromatophore pattern of the visceral epithelium. Nine or 10 large chromatophores are observed on the dorsal part of the head (Figs. 3 and 13). The mantle is now 3.04 mm (±0.4) long on average, and the yolk sac covers 48.74% of the egg.

In stage XXIX, the chromatophore pattern is fully developed (Fig. 14). In addition, the reversion takes place, in which the embryo adopts a position more suitable in preparation for hatching. A marked embryonic growth with an ensuing great decrease in external yolk reserves is evident, leading to a fully developed embryo ready for hatching (stage XXX). In stage XXX,

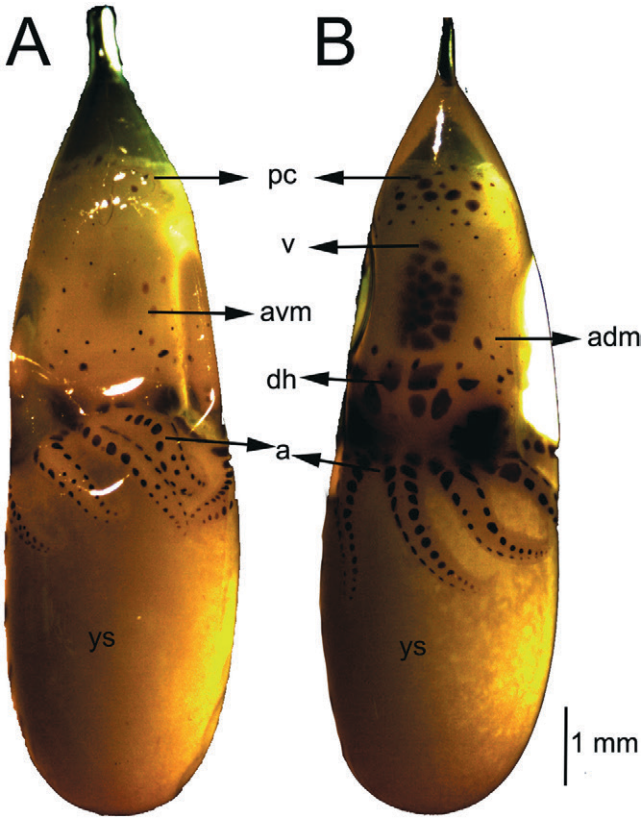

Figure 12. Chromatophore pattern in Stage XXVI of the embryonic development of *Paroctopus digueti*. (A) Ventral view. (B) Dorsal view. a, arm; adm, anterior margin of dorsal mantle; avm, anterior margin of ventral mantle; dh, dorsal head; pc, posterior cap; v, visceral; ys, yolk sac.

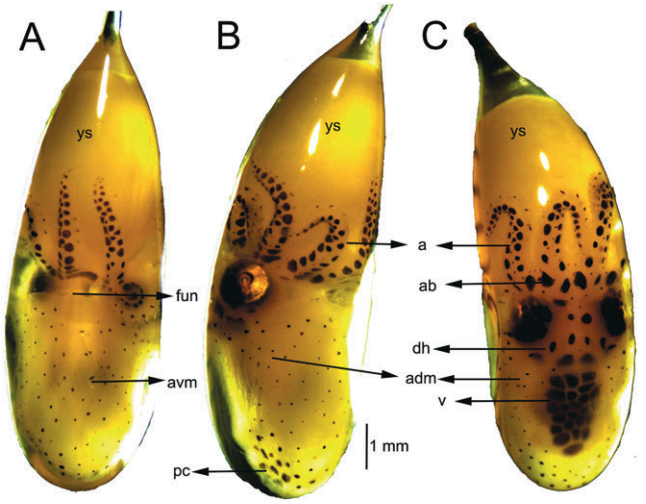

Figure 14. Fully developed chromatophore pattern in stage XXIX of the embryonic development of *Paroctopus digueti*. (A) Ventral view. (B) Side view. (C) Dorsal view. a, arm; ab, arm base; adm, anterior margin of dorsal mantle; avm, anterior margin of ventral mantle; dh, dorsal head; e, eye; fun, funnel; pc, posterior cap; v, visceral; ys, yolk sac.

Figure 15. Hatching and loss of the yolk sac. Newborn juvenile of *Paroctopus digueti*. (A) Side view. (B) Dorsal view. (C) Ventral view.

the average mantle length is 5.6 mm (±0.1), and all the yolk has been consumed.

Stage XXXI corresponds to the newly hatched, fully developed organism exhibiting features similar to those of an adult (Fig. 15) that make it fully able to inhabit the benthic environment.

Yolk Consumption

During the embryonic development of *Paroctopus digueti* at 27 ± 1°C, most of the yolk was consumed during the penultimate development stage prior to hatching (Fig. 16). Yolk consumption was imperceptible in the first two stages of embryonic development, followed by accelerated consumption (from 10.4% to 34%) between stages III and VI and then by a slower but steady yolk consumption (from 35.4% to 51.5%) from stage VII to stage XXIX, when the embryo reached its full development. A large (39.4%) consumption occurred between stages XXIX and XXX (from 51.5% to 90.9%), coinciding with the accelerated growth of the embryo.

DISCUSSION

Octopuses use a reproductive tactic consisting of simultaneous terminal spawning, with synchronous ovulation and no oocyte maturation during the spawning period (semelparity). In this strategy, the spawning pattern is monocyclic and eggs are laid over a brief period at the end of the lifetime (Rocha et al. 2001). It was confirmed that *Paroctopus digueti* is semelparous (female dies after the birth of their offspring) with intermittent monocyclic spawning in which eggs are laid in multiple batches separated over time. As a result of this lag, embryonic development within any given batch is asynchronous.

The estimated fecundity of up to 300 eggs per female is twice as high as previous reports for this same species (DeRusha et al. 1987, Voight 1990, Boletzky 1994). Voight (1990) pointed out that the low fecundity (50–150) observed in this species in Bahia Choya, Sonora, may be related to the high intertidal temperature (36°C) that occurs in the northern part of the Gulf of California, which might have stressed the individuals, adversely affecting both eggs and developing embryos. Another contributing factor may have been the stress caused by the handling of females during spawning; two instances of this sort of adverse effect were observed. One female died before completing embryonic development, with eggs in oviducts ready for spawning, indicating incomplete spawning; the second case was a female that started eating all its eggs after being subjected to excessive manipulation.

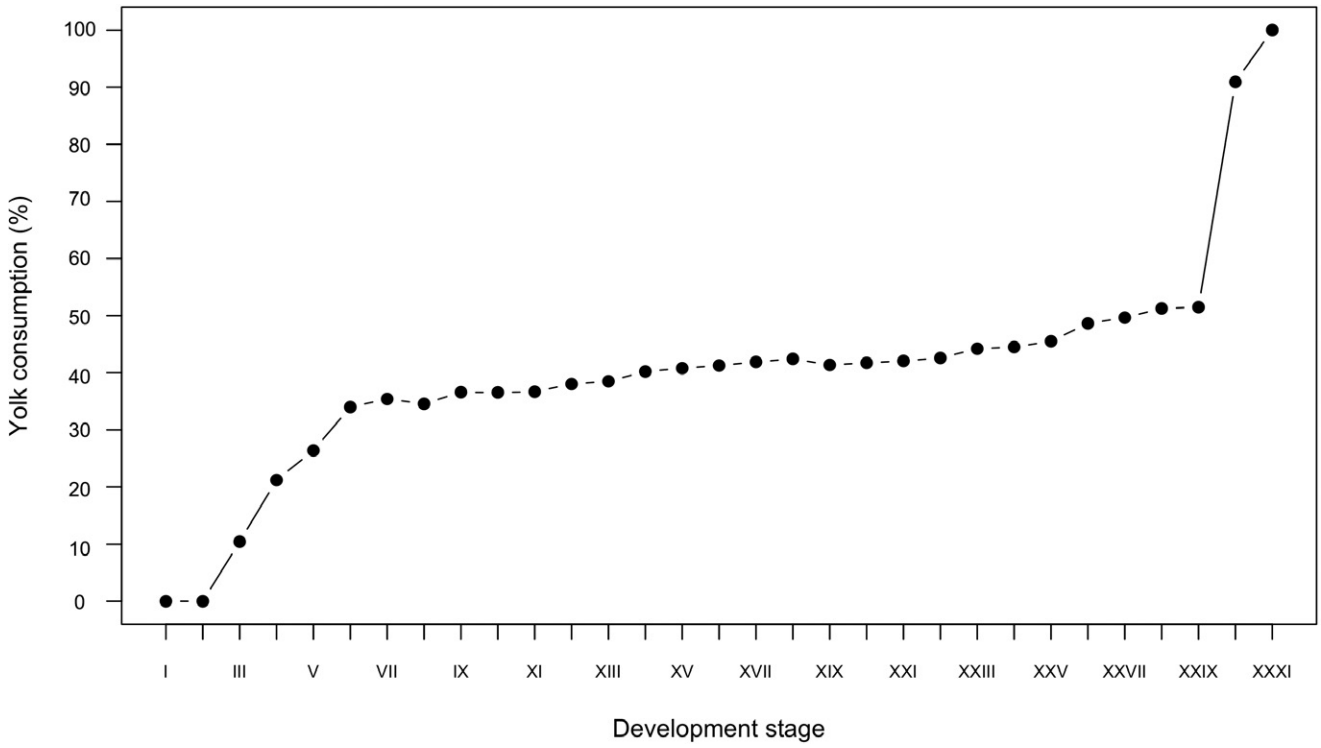

Figure 16. Yolk consumption during embryonic development of *Paroctopus digueti*.

TABLE 1.

Morphological characteristics, duration of embryonic development, and fecundity in octopuses with direct and indirect development.

Species	Adult size ML (cm)	Egg size (mm)	Birth size ML (mm)	Duration of embryonic development days/ temperature (°C)	Fecundity (Number of eggs)	Source
			Direct development			
Paroctopus digueti	4–5	7–10 7–8	5.5 4.5–6	38/27°C 42/25°C	50–300 50–150	Present study Hanlon and Forsythe (1985)
Octopus joubini	4.5 3	6–7 6–10 2.5	5.8 5.5 2.5	35–40/25°C 35–42/25°C 32–42/22–24°C	50–200 25–300 136–2,400	Hanlon (1983) Forsythe (1984) Forsythe and Ronald (1991)
Octopus fitchi	4.5	4–10	4	30–45/25°C	150–300	Sweeney et al. (1992)
Hapalochlaena maculosa	5.7	6–7 6–9	4	40–50/20°C 60/20.8–22.5°C	150 100–200	Tranter and Augustine (1973) Reynolds (1983)
Octopus tehuelchus	4.95	9–12	6.64	120–150/4-19°C	80+	Iribarne (1991)
Octopus minor	8	21–22	8.5–11.5	72–89/21–25°C	50–200	Iglesias et al. (2014)
Octopus bimaculoides	8.5	10–17	6–7 6.5	55/24°C and 85/18°C 46–50/23°C	250–750	Hanlon and Forsythe (1985) Forsythe and Hanlon (1988)
Octopus briareus	12	12–13 10–14	7	60–70/25°C	300–700 150–950	Hanlon (1983) Sweeney et al. (1992)
Octopus maya	12	11–17	7 4–9	45/25°C 50–65/wild	300–500 500–5,000	Van Heukelem (1983) Moguel et al. (2010)
Octopus californicus	14	14–17			50–100	Sweeney et al. (1992)
			Indirect development			
Octopus rubecens	8–10	3–4	1.7–2	52/17.7°C 91/14.8°C	4,000–45,000 1,000–19,000	Sweeney et al. (1992) Osborn (1995)
Octopus insularis	12	2.13–2.29	1.68	30–38/26°C	85,000	Lenz et al. (2015)
Octopus vulgaris	25	1.5–2	2.18 2.4 2–3	29–49/22–23°C 87/17°C, 15– 28/27°C	100,000–600,000 100,000–500,000	Mangold (1983) Sweeney et al. (1992) Caveriviére et al. (1999)
Octopus bimaculatus	20	2.5–4	2.6	31/16°C, 50/19°C	20,000	Ambrose (1981)
Octopus dofleini	36	6–8	3–3.5	548/5°C	30,000–180,000	Sweeney et al. (1992)
Octopus hubbsorum	11	1.6	1.22	45/24–26°C	105,000–144,000	Alejo-Plata and Herrera-Alejo (2014)
Hapalochlaena lunulata	5	2.5–3.5	2.3	35/23–24°C 25/26°C	50+	Overath and Boletzky (1974)
Octopus defilippi	9	1.5–2.1	1.3–1.5		10,000+	Sweeney et al. (1992)

The duration of embryonic development in octopuses depends mainly on whether the species has direct or indirect development, the egg size, and the temperature (see Table 1). The Pacific pygmy octopus has direct development and lays large eggs. This study describes, for the first time, its embryonic development, which lasted 38 days at 27 ± 1°C. Previous studies on this species reported a duration of 42 days at 25°C and 130 days at 16°C (Hanlon & Forsythe 1985, DeRusha et al. 1987), 2°C and 11°C below the temperature compared with this study. This confirms that embryos develop more slowly at lower temperatures (DeRusha et al. 1987, Boletzky 1994, Caamal-Monsreal et al. 2016).

Several studies have shown that the incubation time of the embryos of octopuses is shorter at optimal (usually high) temperatures and have identified the temperature limits of embryonic development based on physiological and morphological characteristics of developing embryos (Boletzky & Hanlon 1983, Repolho et al. 2014). It has also been reported that incubating embryos at extreme (low or high) temperatures can cause alterations in the development time, survival, and biochemical composition of the yolk, thus affecting embryonic development (Uriarte et al. 2014; Caamal-Monsreal et al. 2016).

The eggs of *Paroctopus digueti* from Ensenada de la Paz are larger (7–10 mm) than those reported (7–8 mm) for this same species in Puerto Peñasco, Sonora, Mexico (DeRusha et al. 1987, Boletzky 1994). This difference in egg size may be related to microenvironmental differences between the localities, as

well as the temperature and conditions under which specimens were kept in captivity (DeRusha et al. 1987, Boletzky 1994). The most important driver of egg size is the amount and quality of food consumed by the female throughout gonad maturation.

As in other octopod species (Naef 1928), organogenesis in *Paroctopus digueti* began with the first inversion of the embryo within the egg, from the animal to the vegetal pole. The differentiation of the head and the appearance of arm rudiments in *P. digueti* were observed, which was one of the most outstanding features in the early development of octopuses (Naef 1928, Yamamoto 1988).

The most noticeable difference in embryogenesis between species with direct or indirect development is the distribution pattern of chromatophores (Osborn 1995). Descriptions of octopus paralarvae and juveniles have shown that the number and pattern of chromatophores on the arms, funnel, eyes, head, mantle, and visceral epithelium are species-specific (Young et al. 1989) and can be used as diagnostic characters.

Chromatophores in *Paroctopus digueti* are first evident in stage XVI. They are brown and appear at the base of the arms and dorsal head, similar to its relatives *Octopus joubini* (Naef 1923, Forsythe & Ronald 1991), *Enteroctopus megalocyathus* (Ortiz et al. 2006), and *Hapalochlaena maculosa* (Tranter & Augustine 1973). Forsythe and Ronald (1991) pointed out that brown chromatophores are the first to appear, being the largest and deepest of the body. The full development of the chromatophore pattern in *P. digueti* took place in stage XXIX, along with the second inversion of the embryo. A total of 484 chromatophores were recorded, exceeding the figures reported for *O. joubini* (420) (Forsythe & Ronald 1991), *E. megalocyathus* (463) (Ortiz et al. 2006), and *Octopus insularis* (112) (Lenz et al. 2015). The number and distribution of chromatophores covering the funnel, mantle, head, and arms are species-specific, thus explaining the difference in the total number of chromatophores (Boletzky 2003). Chromatophores have been reported to assume their definitive functions upon hatching (Messenger 2001). In the case of *P. digueti*, functioning chromatophores were present earlier than that, that is, in the late-stage (XXV–XXX) embryos. In addition, ink was expelled in the late stages prior to hatching. This contrasts with *H. maculosa*, a species that uses its ink 4 wk after hatching (Tranter & Augustine 1973).

The Pacific pygmy octopus showed an irregular yolk consumption during embryonic development. Consumption was scarce in the early stages but increased in the late stages. Boletzky (1989, 1993) reported that the early processes of blastulation and onset of gastrulation does not seem to affect the actual amount of yolk used by cephalopods. In addition, the external yolk functions as transient "gills and heart," enclosing nutrient reserves and continuously circulating in the perivitelline fluid, thus supporting oxygen diffusion and absorption by the embryo (Boletzky 2003).

Consumption of external yolk reserves in *Paroctopus digueti* increased in stage XXIX, when the internal organs of the embryo were fully developed, and reserves were presumably used for embryonic growth. Slight but marked contractions were observed in the mantle and the external yolk sac during this process. According to Tanabe et al. (1991), under normal embryonic development, the mantle experiences progressive contractions caused by the partial extrusion of the external yolk sac as a result of a more compact arrangement of the organs, which gradually reach their final position in the developing animal (Bouchaud & Galois 1990). Consumption of external yolk reserves in *P. digueti* increased rapidly in stages II–VI and continued afterward at a low but steady rate until reaching stage XXIX; the remaining yolk was consumed in stage XXX, just before hatching. The volume of internal yolk after hatching is one of the main factors influencing the initial food conditions of the newborn juvenile (O'Dor et al. 1986).

CONCLUSIONS

This study provides basic biological information on the early life stages of *Paroctopus digueti.* This information deepens the knowledge of this species and is useful as a reference for identification and comparative studies between species. The information on its size and chromatophore pattern is potentially valuable as well. Notably, although these features have not been extensively studied in direct development species yet, they are used as diagnostic characters in indirect development species.

Females of *Paroctopus digueti* from Bahía de la Paz lay large eggs in small batches (300 eggs), which give rise to benthic juveniles. Such strategy is characterized by heavy investment per egg and a low yield per batch.

Embryonic development in *Paroctopus digueti* takes longer to complete relative to species with indirect development (under similar temperature conditions), and the size, body proportion, and life form of the offspring have been shown to be influenced by egg size. Based on this analysis and the classification of morphological characteristics of *P. digueti* embryos, 31 distinct embryonic development stages were identified.

Embryos of *Paroctopus digueti* were fully developed prior to hatching, as demonstrated by their use of chromatophores and ink expulsion within the egg and their feeding immediately upon hatching. These attributes of *P. digueti* make it a suitable species for both experimental biological studies and aquaculture.

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