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Research Article

Morphology and biochemical composition of eggs and paralarvae of the two-spotted octopus, *Octopus bimaculatus*

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ABSTRACT. The study highlights the changes in the proximate composition and fatty acid (FA) profile of eggs and paralarvae and describes the chromatophore pattern and morphological changes in paralarvae. Ten gravid females of *Octopus bimaculatus* were collected in Kino Bay, Sonora, Mexico. Paralarvae were obtained from a single female laying event registered 90 days after capturing the organisms. Paralarvae were fed with rotifers and newly hatched enriched *Artemia* nauplii. Morphological description of paralarvae was performed based on Roper & Voss (1983) on 182 organisms collected over 10 days; mean values (mm) for total length, mantle width, head width, mantle length, arms length, funnel length, eye diameter and mouth diameter ranged from 3.66 to 4.86, 1.52 to 1.78, 1.48 to 1.84, 2.33 to 2.85, 1.02 to 1.58, 0.58 to 0.71, 0.44 to 0.63, and 0.10 to 0.14 mm, respectively. Organisms exhibited a characteristic chromatophore pattern of 4+2+2 (dorsal view), in addition to 24-30 and 39-51 chromatophores on the dorsal and ventral views, respectively. The proximate composition of eggs and paralarvae revealed two major components: moisture (78.99 and 82.26%, respectively) and crude protein (15.33 and 11.76%, respectively). The FA profile (mg FA g^{-1} wet tissue) of eggs revealed that 16:0 (3.39), 20:4n-6 (2.67) and DHA (1.67) were the most abundant, whereas in paralarvae 16:0 (2.21), 18:0 (2.37), 20:4n-6 (1.45), 20:5n-3 (1.98) and 22:6n-3 (2.18) were the predominant FA. This information contributes to the knowledge of the morphometric characterization and biochemical composition of *O. bimaculatus* eggs and paralarvae, providing a basis for further research.

Keywords: *Octopus bimaculatus*; eggs; paralarvae; morphological description; fatty acids; chromatophores

INTRODUCTION

In recent years, the global demand for cephalopods, particularly octopuses, has increased due to the rising popularity of Mediterranean and Asian cuisines, leading to a growing interest in their culture (Sauer et al. 2019). Additionally, octopuses are considered excellent candidates for diversifying aquaculture species in many countries, as several studies have demonstrated that they adapt well to captivity and exhibit a high growth rate (Navarro & Villanueva 2003, Spreitzenbarth & Jeffs 2024).

Octopuses are characterized by exhibiting either holobenthic or merobenthic development during early stages of their lifecycle (Braga et al. 2021). Holobenthic larvae typically hatch as large juveniles, and the full cycle occurs on the benthos, which favors their feeding and survival rates under aquaculture conditions (Vidal et al. 2014, Villanueva et al. 2016). In contrast, merobenthic larvae are planktonic (paralarvae) before settling on the benthos, and the absence of appropriate feeding protocols during this planktonic stage has led to high mortality rates, hindering commercial production (Farías et al. 2016, Espinoza et al. 2017, Cerezo-Valverde et al. 2019).

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The two-spotted octopus, *Octopus bimaculatus* (Verrill, 1883), is commonly found in subtidal and intertidal zones, with its distribution generally extending from Catalina Island, California, in the USA, to Panama, including the Gulf of California, Mexico (Alejo-Plata et al. 2012, 2014). However, the exact geographical distribution range remains undetermined (Alejo-Plata et al. 2012). The species is characterized by possessing two large blue ocelli located under each eye, close to the base, between the third and fourth pairs of arms (Roper et al. 1995). Specifically, the ocelli pattern for *O. bimaculatus* resembles the spokes of a wheel, which is a reliable method for distinguishing the species from its sibling species, *O. bimaculoides* (Hofmeister & Voss 2024).

In its natural environment, *O. bimaculatus* feeds on a wide variety of invertebrates, including crustaceans and mollusks. Its predatory nature can result in a significant reduction of several species of gastropods and bivalves (Hofmeister et al. 2018). It is captured by artisanal fishing throughout the Gulf of California, and the fishery has the potential to be performed by fishing cages (Roper et al. 1995). On the other hand, this species is considered a potential candidate for aquaculture in the Pacific coast of Mexico; however, detailed aspects of larval development, particularly those related to morphology, feeding, and nutritional requirements, remain poorly understood (López-Peraza et al. 2018). Although little information is available on *O. bimaculatus*, it is known that the species hatches as a planktonic paralarva (Hofmeister & Voss 2024), with high mortality rates during early larval culture, generally attributed to the failure to feed the juveniles after their yolk reserves are depleted (López-Peraza et al. 2018). Thus, it is evident that further studies are needed to elucidate the key biological aspects of *O. bimaculatus* early development that may contribute to its commercial culture. This study aims to provide the morphological description, proximate composition, and fatty acid (FA) profile of eggs and newly hatched two-spotted octopus, *O. bimaculatus*.

MATERIALS AND METHODS

Capture and maintenance of *O. bimaculatus* specimens

Experimental animals in this study were handled and maintained following the Technical Specifications for the Production, Care and Use of Laboratory Animals of the Official Mexican Norm NOM-062-ZOO-1999 from the Ministry of Agriculture, Livestock, Rural Development, Fisheries, and Food. A total of 10 females of *O. bimaculatus* were collected in the coastal zone of Kino Bay, Sonora, Mexico (28°48′59.99″N, 111°55′59.99″W) by SCUBA diving utilizing hand nets in March 2024, during the breeding season.

The organisms were kept in 10-L individual containers and transported alive to the Wet Laboratory of Aquaculture Nutrition at the Kino Bay Experiment Station, University of Sonora. Upon arrival, the organisms were identified as *O. bimaculatus* based on their ring pattern (Hofmeister & Voss 2024) and subsequently placed individually in a 250 L circular tank (0.40 m² bottom area), filled with 200 L of filtered seawater. Each tank was equipped with one air stone to ensure air flows of 8-10 L min^{-1}. Water in the system recirculated through a sand filter, biological filter, cartridge filter (50 µm), and ultraviolet sterilizer at a rate of 1.5 L min^{-1} to provide one full turnover of water exchange in each tank every 2.2 h, with 15% of the total water renewed daily. Seawater used for water renewal was initially collected at ambient temperature and stored for at least 24 h in 250 L containers at a controlled room temperature of 24°C before use. Daily maintenance also included removing uneaten feed and feces. Dissolved oxygen and temperature were recorded daily using a multi-function oxygen meter (YSI, Model Pro2030, Yellow Springs, OH, USA), with mean values (mean ± standard deviation, SD) of 6.4 ± 0.3 mg L^{-1} and 24.25 ± 0.28°C, respectively. Females were fed once daily to satiation with a combination of live crabs and clams in a 75:25 ratio, respectively.

Additionally, an 8" cross-shaped PVC pipe, cut to a length of approximately 25 cm with one end open and one end permanently closed, was placed at the bottom of each tank as a refuge for the gravid females. Females were allowed to incubate the eggs themselves inside the refuges. After an incubation period of approximately three weeks, the eggs spawned by a single *O. bimaculatus* female were hatched. The newly hatched paralarvae were captured manually using a 1-L plastic bowl (to avoid direct contact or excessive manipulation) and placed into a single 45-L tank filled with 40 L of filtered seawater, equipped with one air-stone to ensure gentle aeration. A daily water renewal of 60% of the volume and maintenance was performed manually using a siphon fitted with a 200 µm mesh to prevent siphoning paralarvae. From day one post-hatching (PH), paralarvae were fed rotifers and newly hatched *Artemia* nauplii enriched with SELCO S.presso® following the manufacturer's recommendation (INVE, Dendermonde, Belgium), at a concentration of 10 rotifers mL^{-1} and 1 *Artemia* nauplius mL^{-1}, which

was maintained during the 10 days the experiment lasted.

Morphological description and chromatophore pattern of *O. bimaculatus* paralarvae

The morphological description of the eggs was carried out on a single initial sample of 30 eggs selected at random from the cluster immediately after spawning. Once the eggs hatched, a total of 182 paralarvae of *O. bimaculatus* (Fig. 1a-b) were randomly collected throughout the 10 days of this study. The paralarvae were sacrificed by placing them in Davidson's solution during 24 h, and subsequently, in ethanol (70%) (Fig. 1c). The following morphometric data based on descriptions suggested by Roper & Voss (1983) were individually recorded from day 1 to day 10 PH: total length (Tl), mantle width (Mw), head width (Hw), mantle length (Ml), arms length (Al), funnel length (Fl), and eye diameter (Ed) (Fig. 2a-b). Additionally, the mouth diameter (Md) measurement was recorded (Fig. 2c). In the case of eggs, morphological measurements of egg total length (Etl), egg total width (Etw), yolk total length (Vtl), and yolk total width (Vtw) were registered (Fig. 2d-e). Data obtained from the morphological measurement of eggs were used to calculate the percentage of yolk volume (%) using the ImageJ software for Windows. The chromatophore pattern was established based on the arrangement consistently observed in all paralarvae sampled from day 1 to day 10 PH. Measurements were performed using a Leica Microscope Flexacam i5, equipped with Enersight Software for Desktop. Additionally, representative photos of the organisms were taken and used to create schematic drawings of the paralarvae, which were then used to describe the chromatophore patterns in the mantle, head, arms, and funnel, based on dorsal, lateral, and ventral views (Hochberg et al. 1992, Lenz et al. 2015).

Proximate composition and fatty acid analysis

Composite samples of eggs or newly hatched paralarvae of *O. bimaculatus* were analyzed in triplicate; after collecting them, they were kept at -82.0°C until further analysis, four days after the trial ended. The number of eggs or paralarvae used in composite samples varied depending on the specific analysis, with approximately 75 (crude protein) to 300 (crude fat) eggs or paralarvae per composite sample. Official methods were used for the determination of crude protein (Dumas's protocol, factor = 6.25; method 968.06; AOAC, 2005), moisture (930.15; AOAC 2005), ash (942.05; AOAC 2005), and crude fat (Folch et al. 1957). The same composite sample used for crude fat determination was used to perform the FA analysis (Lochmann & Gatlin 1993). Briefly, after evaporation of the solvents with nitrogen, a known amount of heptadecanoic acid (17:0) was added as an internal standard, and the samples were subjected to saponification with KOH in methanol and then methylated by transesterification with BF_3. The samples were then resuspended in HPLC grade hexane to a concentration of 50 mg FA mL^{-1}, and an aliquot of 2.0 µL was injected into a gas chromatograph (Model Varian 3800, Varian, Inc., Palo Alto, CA, USA) equipped with a 30 m × 0.25 mm silica capillary column and a flame ionization detector using helium as the carrier gas. FA identification was performed by comparing retention times using a known standard (Supelco CRM18918 FAME Mix C8-C24; Millipore-Sigma, St. Louis, MO, USA) and quantified using the internal standard. Results were expressed as mg FA g^{-1} wet tissue as well as % of identified fatty acid methyl esters (% FAME).

RESULTS

Morphological description and chromatophore pattern of *O. bimaculatus* eggs and paralarvae

The morphological description of *O. bimaculatus* eggs showed mean values ± SD for Etl, Etw, Vtl, and Vtw of 3.84 ± 1.14, 1.58 ± 0.45, 2.13 ± 0.40, and 1.35 ± 0.40 mm, respectively. Additionally, yolk volume showed a mean value ± SD of 69.7 ± 5.44 %. Meanwhile, for *O. bimaculatus* paralarvae mean values of Tl, Mw, Hw, Ml, Al, Fl, Ed and Md during the 10 days after hatching, ranged from 3.66 to 4.86, 1.52 to 1.78, 1.48 to 1.84, 2.33 to 2.85, 1.02 to 1.58, 0.58 to 0.71, 0.44 to 0.63, and 0.10 to 0.14 mm, respectively (Table 1). The distribution of chromatophores in paralarvae was:

Dorsal view: on the mantle, between 14 and 18 chromatophores were observed covering the visceral mass, following an oval arrangement. Additionally, 2 to 4 chromatophores were consistently located along the posterior edge of the mantle (Fig. 3a).

On the head, a defined pattern of 8 chromatophores (4+2+2) was recorded: 4 situated above the eyes, 2 between the eyes, and 2 posterior to the eyes near the base of the arms (Figs. 1c, 3a). Lateral view: 2 prominent chromatophores were observed along the lateral region of the mantle (Fig. 3b). Ventral view: on the ventral side of the mantle, between 30 and 36 chromatophores were densely distributed. Along the siphon, 4 to 7 small, rounded chromatophores were identified, and each arm exhibited 5 to 8 chromatophores (Fig. 3c).

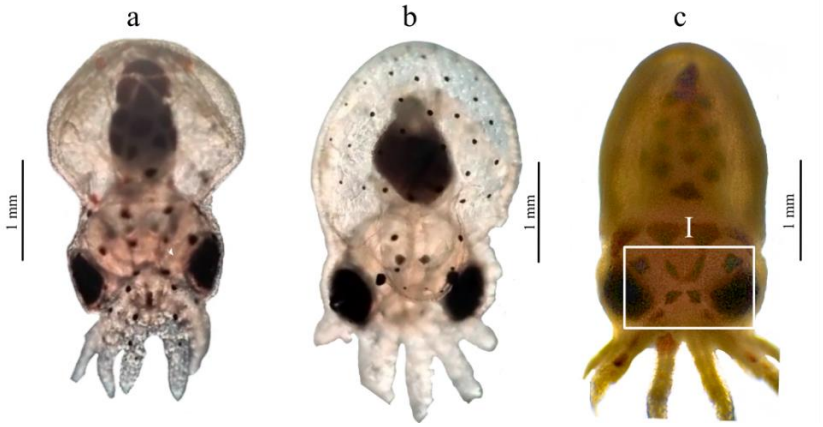

Figure 1. *O. bimaculatus* paralarvae. a) Paralarvae dorsal view, b) paralarvae ventral view, and c) dorsal view of a paralarvae fixed in Davidson's solution. (I) chromatophore pattern 4+2+2. Octopus orientations (dorsal and ventral views) were based on Hochberg et al. (1992) and Lenz et al. (2015).

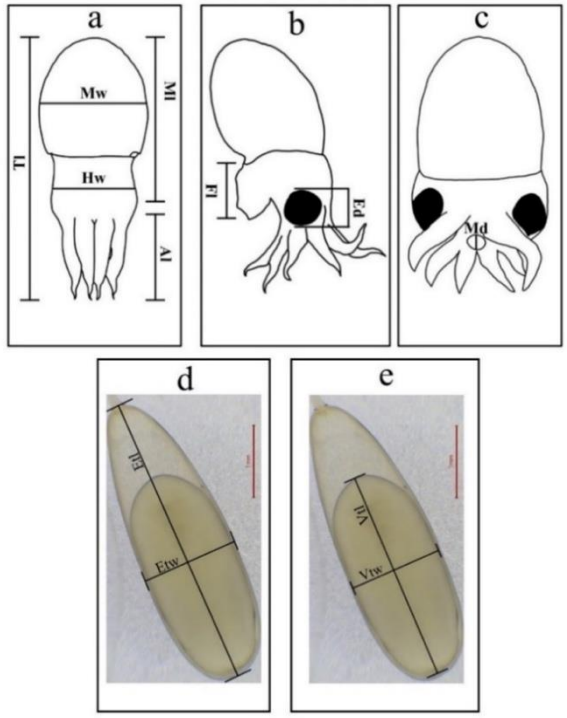

Figure 2. a-c). Schematic illustration based on Roper & Voss (1983) and Lenz et al. (2015) showing the body measurements in *O. bimaculatus* paralarvae. Tl: total length, Mw: mantle width, Hw: head width, Ml: mantle length, Al: arms length, Fl: funnel length, Ed: eye diameter, and Md: mouth diameter. d-e) Egg measurement. Etl: egg total length, Etw: egg total width, Vtl: yolk total length, and Vtw: yolk total width. Scale bar: 1mm.

Proximate composition and fatty acid (FA) profile

The proximate composition analyses of *O. bimaculatus* eggs and paralarvae showed similar mean values (%) ± standard error of the mean (SEM) for moisture and crude protein, together representing 94.32 and 94.02% of the eggs and paralarvae, respectively. Crude fat represented 3.16 and 2.83%, respectively, whereas ash was less relevant in eggs (0.68%) than in paralarvae (3.39%) (Table 2).

The FA analysis of eggs revealed that palmitic acid (16:0: 3.39 mg g^{-1} wet tissue) was the predominant FA, followed by arachidonic acid (ARA; 20:4n-6: 2.66

Table 1. Morphometric data of *Octopus bimaculatus* eggs and paralarvae. Values are means ± standard deviation of the number of eggs and paralarvae analyzed each day.

Days after hatching	Number of paralarvae	Total length (mm)	Mantle width (mm)	Head width (mm)	Mantle length (mm)	Arm length (mm)	Funnel length (mm)	Eye diameter (mm)	Mouth diameter (mm)
1	11	3.66 ± 0.30	1.61 ± 0.14	1.48 ± 0.18	2.44 ± 0.25	1.08 ± 0.19	0.68 ± 0.09	0.51 ± 0.12	0.13 ± 0.03
2	29	4.17 ± 0.48	1.66 ± 0.11	1.68 ± 0.07	2.38 ± 0.14	1.40 ± 0.13	0.58 ± 0.06	0.58 ± 0.03	0.11 ± 0.01
3	33	4.39 ± 0.38	1.78 ± 0.19	1.84 ± 0.05	2.84 ± 0.29	1.30 ± 0.28	0.66 ± 0.08	0.60 ± 0.07	0.13 ± 0.01
4	19	4.86 ± 0.35	1.69 ± 0.17	1.79 ± 0.10	2.79 ± 0.22	1.58 ± 0.24	0.71 ± 0.10	0.63 ± 0.04	0.10 ± 0.03
5	22	3.87 ± 0.25	1.63 ± 0.08	1.55 ± 0.08	2.60 ± 0.14	1.27 ± 0.16	0.66 ± 0.10	0.52 ± 0.05	0.10 ± 0.01
6	20	4.13 ± 0.24	1.72 ± 0.08	1.63 ± 0.06	2.74 ± 0.13	1.23 ± 0.14	0.71 ± 0.15	0.59 ± 0.03	0.13 ± 0.02
7	11	3.84 ± 0.48	1.52 ± 0.15	1.60 ± 0.06	2.85 ± 0.24	1.14 ± 0.13	0.65 ± 0.13	0.47 ± 0.02	0.13 ± 0.01
8	16	3.90 ± 0.18	1.60 ± 0.12	1.60 ± 0.04	2.60 ± 0.12	1.31 ± 0.11	0.65 ± 0.07	0.56 ± 0.07	0.12 ± 0.02
9	12	3.73 ± 0.18	1.62 ± 0.11	1.67 ± 0.04	2.49 ± 0.10	1.02 ± 0.14	0.65 ± 0.09	0.44 ± 0.05	0.11 ± 0.01
10	9	3.71 ± 0.46	1.53 ± 0.13	1.65± 0.06	2.33 ± 0.16	1.17 ± 0.14	0.65 ± 0.08	0.59 ± 0.05	0.14 ± 0.01

Number of eggs	Egg total length (mm)	Egg total width (mm)	Yolk total length (mm)	Yolk total width (mm)	Yolk volumen (%)
30	3.84 ± 1.14	1.58 ± 0.45	2.13 ± 0.40	1.35 ± 0.40	69.7 ± 5.44

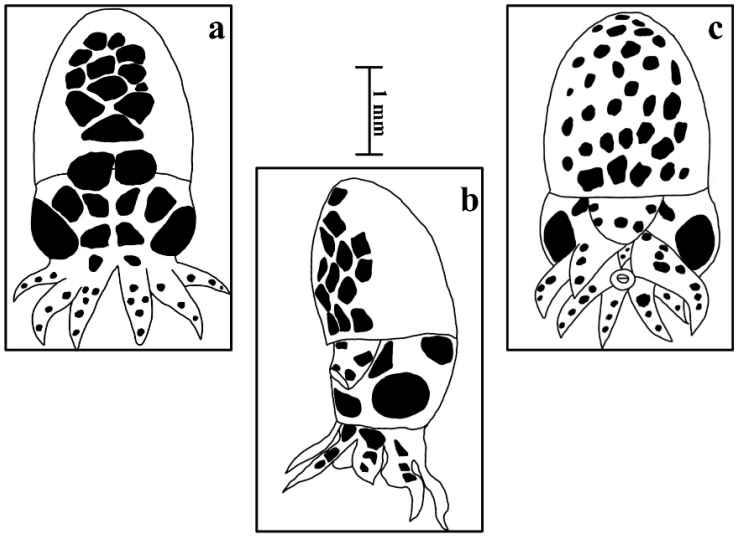

Figure 3. Schematic illustration of the chromatophore pattern of newly hatched *O. bimaculatus* paralarvae. a) Dorsal view, b) lateral view, and c) ventral view. Octopus orientations (dorsal, ventral, and lateral views) were based on Hochberg et al. (1992) and Lenz et al. (2015).

Table 2. Proximate composition (%) of eggs and paralarvae (10 days post-hatching) of *O. bimaculatus*. Values are means ± standard error of the mean of three replicate composite samples.

Proximate composition (%)	Eggs	Paralarvae
Crude protein	15.33 ± 2.36	11.76 ± 1.37
Crude fat	3.16 ± 0.95	2.83 ± 0.13
Moisture	78.99 ± 0.61	82.26 ± 0.40
Ash	0.68 ± 0.02	3.39 ± 0.16

mg g^{-1} wet tissue), which exhibited a higher concentration compared to docosahexaenoic acid (DHA; 22:6n-3: 1.67 mg g^{-1} wet tissue) and eicosapentaenoic acid (EPA; 20:5n-3: 0.89 mg g^{-1} wet tissue). Total concentrations of n-3 and n-6 FAs were 3.70 and 3.12 mg g^{-1} wet tissue, respectively, and the n-3/n-6 ratio was 1.19. In contrast, the paralarvae's FA profile showed high concentrations of palmitic (16:0), stearic (18:0), and oleic acid (18:1n-9), with concentrations of 2.21, 2.37, and 0.87 mg g^{-1} wet tissue, respectively. Additionally, higher concentrations of DHA and EPA were observed compared to ARA, with concentrations of 2.18, 1.98, and 1.45 mg g^{-1} wet tissue, respectively. Total n-3 and n-6 FAs in paralarvae were 4.79 and 1.76 mg g^{-1} wet tissue, respectively, with a larger n-3/n-6 ratio of 2.72 (Table 3).

DISCUSSION

The present study represents the first to provide a morphological description of *O. bimaculatus* paralarvae based on criteria established by Roper & Voss (1983), allowing comparisons with other studies. Morphometric data for *O. bimaculatus* eggs showed higher values of Etl and Etw (3.84 and 1.58 mm, respectively) when compared to other octopus species such as *Octopus insularis* (Etl: 2.25 mm; Etw: 0.91 mm), and *Octopus vulgaris* (Etl: 2.50 mm; Etw: 1.00 mm) (Lenz et al. 2015, Deryckere et al. 2020). Meanwhile, morphometric data for Tl, Mw, Hw, Ml, Al, and Ed showed the largest values between days 3 and 4 PH of this study, decreasing by days 9 and 10. Mortality was observed from day 1 PH, increasing as the study progressed.

Morphometric data of paralarvae obtained in the present study may be compared with values reported for *O. insularis* by Lenz et al. (2015), which showed a smaller mean Tl (2.34 mm) for newly hatched organisms, compared to those observed in the present study (3.66 mm). Since *O. bimaculatus* hatched into slightly larger organisms, the morphological measurements of Ml, Mw, Hw, Fl, and Ed are also larger than those of *O. insularis*. Alejo-Plata et al. (2012) reported Ml for paralarvae of *O. bimaculatus* ranging from 0.7 to 2.7 mm, the upper range is quite similar to the range reported in this study, of 2.33 to 2.85 mm, while the

Table 3. Fatty acid (mg g^{-1} wet tissue and fatty acid methyl esters (% FAME)) profile of eggs and paralarvae of *O. bimaculatus*. Values are means ± standard error of the mean of three replicate composite samples. Saturates: 12:0, 14:0, 16:0, 18:0, 20:0. Monounsaturated fatty acids (MUFA): 12:1, 14:1, 16:1, 18:1n-9, 18:1n-5, 20:1, 22:1, 24:1. Polyunsaturated fatty acids (PUFA): 16:2, 16:3, 18:2n-6, 18:3n-6, 18:3n-3, 20:3n-6, 20:3n-3. Highly unsaturated fatty acids (HUFA): 18:4n-3, 20:4n-6, 20:4n-3, 20:5n-3, 21:5n-3, 22:4n-6, 22:5n-6, 22:5n-3, 22:6n-3, 24:4n-6, 24:5n-6, 24:5n-3, 24:6n-3. Total n-6: 18:2n-6, 18:3n-6, 20:3n-6, 20:4n-6, 22:4n-6, 22:5n-6, 24:4n-6, 24:5n-6. Total n-3: 18:3n-3, 18:4n-3, 20:3n-3, 20:4n-3, 20:5n-3, 21:5n-3, 22:5n-3, 22:6n-3, 24:5n-3, 24:6n-3. N.D.: not detected.

Fatty acid	Eggs (mg g^{-1} wet tissue)	Eggs (% FAME)	Paralarvae (mg g^{-1} wet tissue)	Paralarvae (% FAME)
14:0	0.31 ± 0.05	2.11 ± 0.20	0.29 ± 0.16	1.89 ± 0.89
14:1	0.14 ± 0.08	0.98 ± 0.63	0.08 ± 0.06	0.46 ± 0.35
16:0	3.39 ± 0.14	23.69 ± 2.62	2.21 ± 0.29	16.43 ± 0.45
16:1	0.19 ± 0.12	1.39 ± 0.96	0.12 ± 0.06	0.83 ± 0.28
16:3	0.20 ± 0.11	1.48 ± 0.86	0.13 ± 0.03	1.04 ± 0.27
18:0	1.34 ± 0.09	9.27 ± 0.03	2.37 ± 0.30	17.69 ± 0.38
18:1n-9	1.22 ± 0.01	8.50 ± 0.67	0.87 ± 0.11	6.47 ± 0.21
18:2n-6	0.27 ± 0.12	1.97 ± 0.99	0.14 ± 0.03	0.97 ± 0.09
18:3n-3	0.15 ± 0.12	1.12 ± 0.88	0.01 ± 0.01	0.04 ± 0.04
18:4n-3	0.44 ± 0.33	2.87 ± 2.10	0.10 ± 0.02	0.69 ± 0.04
20:0	0.72 ± 0.12	4.98 ± 0.50	0.73 ± 0.10	5.45 ± 0.14
20:1	0.08 ± 0.01	0.60 ± 0.11	0.12 ± 0.02	0.86 ± 0.10
20:4n-6	2.66 ± 1.06	18.15 ± 6.10	1.45 ± 0.18	10.88 ± 0.41
20:5n-3	0.89 ± 0.12	6.15 ± 0.41	1.98 ± 0.23	14.86 ± 0.49
22:4n-6	0.09 ± 0.01	0.61 ± 0.04	0.11 ± 0.02	0.85 ± 0.11
22:5n-6	N.D.	N.D.	0.05 ± 0.02	0.34 ± 0.12
22:5n-3	0.17 ± 0.03	1.19 ± 0.10	0.30 ± 0.04	2.25 ± 0.12
22:6n-3	1.67 ± 0.01	11.62 ± 0.87	2.18 ± 0.25	16.37 ± 0.59
Saturates	5.76 ± 0.12	40.05 ± 1.95	5.60 ± 0.79	41.46 ± 0.41
MUFA	1.63 ± 0.23	11.46 ± 2.36	1.18 ± 0.21	8.62 ± 0.46
PUFA+HUFA	7.04 ± 1.11	48.49 ± 4.31	6.69 ± 0.84	49.92 ± 0.76
Total n-3	3.70 ± 0.36	25.61 ± 0.73	4.79 ± 0.61	35.68 ± 0.66
Total n-6	3.12 ± 0.86	21.44 ± 4.44	1.76 ± 0.23	13.13 ± 0.20
n-3/n-6	1.19 ± 0.22	1.19 ± 0.22	2.72 ± 0.07	2.72 ± 0.07

smaller end of this range may be explained by the origin of the paralarvae, with paralarvae directly collected from the ocean *vs*. paralarvae obtained from eggs hatched under controlled conditions in the present study. It is possible that Alejo-Plata et al. (2012) inadvertently collected paralarvae from other species, therefore measurements of captive-bred *O. bimaculatus* paralarvae help clarify their results.

Chromatophore patterns are considered a reliable taxonomic feature for identifying cephalopod paralarvae (Young et al. 1989). In the present study, the paralarvae exhibited a characteristic chromatophore pattern distribution (4+2+2) in the head region (dorsal view), which differs from that reported by Alejo-Plata et al. (2012) for the same species. Differences may be attributed to either a misidentification of the species, as identification of wild paralarvae captured directly from the ocean is extremely difficult, and no identification guide for the paralarvae of *O. bimaculatus* is available. Alternatively, paralarvae of the same species could exhibit different chromatophore patterns.

Nevertheless, the chromatophore head pattern observed in the present study is similar to that reported by Vidal et al. (2010) for *O. vulgaris* paralarvae. Still, the distribution of chromatophores in the rest of the body is different. Interestingly, Vidal et al. (2010) also observed a different distribution pattern of chromatophores between two geographically separated populations of *O. vulgaris*, suggesting the presence of different populations of a supposedly cosmopolitan species or the existence of a cryptic *O. vulgaris* species along the southern Brazilian coast. Further research on the chromatophores distribution pattern is required for *O. bimaculatus* to determine if these features are truly a reliable method to distinguish among octopus paralarvae from different species.

Concerning the biochemical composition, the main differences observed were a decrease in crude protein and fat content, and an increase in moisture and ash content in paralarvae compared to eggs. Important changes in nutrient composition have been reported during egg development and in newly hatched organisms of other cephalopod species, attributed to the utilization of fat and protein for organogenesis, the formation of structural components such as membranes, and energy used for metabolism (Uriarte et al. 2014, Ibarra-García et al. 2018). For the common octopus, *O. vulgaris*, Quintana et al. (2015) reported similar trends in the development from egg to paralarvae to those observed in the present study, with a decrease in crude protein (321-250 μg ind^{-1} in eggs to 161-128 μg ind^{-1} in paralarvae, corresponding to estimated values of 24.18-18.80% of crude protein in eggs to 11.43-9.37% in paralarvae) and fat content (30.4-23.8 μg ind^{-1} in eggs to 26.9-16.7 μg ind^{-1} in paralarvae, corresponding to estimated values of 2.29-1.79% of crude fat in eggs to 1.93-1.22% in paralarvae), in addition to an increment in moisture content (66.5-63.4% in eggs to 83.9-77.0% in paralarvae). Additionally, Estefanell et al. (2017) reported similar trends of decreasing protein (72.7% in eggs to 71.6 and 72% in paralarvae) and increasing moisture (70.4% in eggs to 85.4-86.3% in paralarvae) in *O. vulgaris*; nevertheless, contrary to observations in the present study, they reported an increment in crude fat (11.5% in eggs to 13.3-13.5% in paralarvae) and a decrement in ash (1.6% in eggs to 1.0 and 1.3% in paralarvae). The FA composition of eggs of *O. bimaculatus* was characterized by the high contents of 16:0, 18:0, 18:1n-9, as well as ARA (2.66 mg g^{-1} wet tissue), which was in higher concentration than DHA or EPA (1.67 or 0.89 mg g^{-1} wet tissue). However, in newly hatched paralarvae, the concentrations of DHA and EPA (2.18 and 1.98 mg g^{-1} wet tissue, respectively) were higher than ARA (1.45 mg g^{-1} wet tissue), while those of 16:0 and 18:0 remained consistently high. For *O. vulgaris*, Estefanell et al. (2017) reported somewhat similar FA profiles as % FAME in the total lipid fraction of eggs and paralarvae; for wild eggs, considerable proportions of 16:0 (21.6%) and 18:1n-9 (5.8%), and a higher content of ARA (13.0%) than EPA (9.6%), but not higher than DHA (24.7%) were observed; for hatchlings obtained in captivity, the proportions of ARA (4.5%) and 16:0 (17.3%) decreased, while the concentrations of EPA (15.6%) and DHA (31.9%) increased comparared to their content in eggs. *Octopus maya* showed a very similar FA profile to that of *O. bimaculatus*; egg yolk showed 16:0 (28.71%) as the main FA, followed by ARA (17.32%), the content was greater than EPA (6.95%) and DHA (12.52%). In contrast, the FA profile of the hatchlings also showed the same most abundant FA, but a decrease in 16:0 (16.05%) and ARA (15.81%) content, while EPA (11.71%) and DHA (15.75%) increased (Tercero et al. 2015). Differences in FA profiles between eggs and paralarvae in both the present and previous studies suggest the utilization of saturated FAs, such as 16:0 and 18:0, as well as ARA, during embryonic development, as proposed by Estefanell et al. (2017). In contrast, highly unsaturated fatty acids (HUFA) such as EPA and DHA, for which low biosynthetic capacity has been reported in octopuses like *O. vulgaris*, appear to be selectively retained, most likely as a result of their critical role in neural development and incorporation into the polar lipid fraction of new cell membranes of paralarvae (Monroig et al. 2013, Estefanell et al. 2017).

Information related to nutritional aspects during the early life stages of *O. bimaculatus* is scarce, and determining the mouth diameter, which was obtained in this study (0.10-0.14 mm), is crucial for estimating the possible prey size at first feeding. López-Peraza et al. (2018) reported results of an isolated study in which enriched *Artemia franciscana* was used to feed newly hatched paralarvae of *O. bimaculatus*, including its effect on the FA profile, although eggs were not evaluated. They reported similar trends to those observed in the present study, with 16:0 as the primary FA, followed by considerable amounts of 18:0 and 18:1n-9, and higher contents of DHA and EPA compared to ARA. The two-spotted octopus, *O. bimaculatus*, represents a promising candidate for aquaculture in the northwestern region of Mexico. Therefore, gathering information on biological aspects, such as morphometric data and biochemical composition, during early developmental stages should be the focus of further research, as it provides the basis for establishing protocols during the first feeding and identifying its nutritional requirements to aid in the formulation of balanced feeds and promote its commercial culture.

Author contribution

C.A. Maldonado-Othón: methodology, writing original draft, review, and editing; M. Perez-Velazquez: resources, writing, review, and editing; M.L. González-Félix: resources, writing, review, and editing; C. Minjarez-Osorio: methodology, resources, writing, review, and editing.

Conflict of interest

The authors have no conflict of interest related to this work.

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