





Research Article

Performance Parameters of Paralarvae and Postparalarvae Rearing of Patagonian Red Octopus, *Enteroctopus megalocyathus*, under Experimental Conditions

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Patagonian red octopus, *Enteroctopus megalocyathus*, an interesting species for Chilean aquaculture diversification, requires the improving of their experimental technology to obtain early juveniles. The first objective of the study was to enhance aspects of feeding and temperature management in broodstock, egg incubation, paralarvae rearing, and early juveniles' growth. The results indicated that female weight decreases up to $46.0 \pm 8.6\%$ during the egg incubation. Test of 8, 14, and 18°C during egg incubation shows that at 18°C embryos do not survive. Paralarvae reared under four diet treatments: (1) unfed, (2) enriched *Artemia* (*Nannochloropsis* sp.), (3) copepod (*Acartia* spp.), and (4) juvenile crabs (*Petrolisthes* spp.) showed survival quadruplication when they fed copepods and crabs instead of *Artemia*. Juveniles reared at 11, 13, and 15°C improved feed conversion and protein efficiency ratios at 15°C. The second objective was to analyze batches of paralarvae and early juveniles of two different periods to obtain their performance indicators and to compare them between productive periods. The results of growth rates, the relative weight condition coefficient, and morphometric relationships are discussed in the context of paralarvae culture from 1 to 90 days after hatching (DAH) and early juveniles from 1 to 135 days postsettlement (DPS).

1. Introduction

Enteroctopus megalocyathus, the Patagonian red octopus, is a merobenthic species that inhabits the Pacific and Atlantic coasts of South America, from 42°S to the Strait of Magallanes, including the Malvinas/Falkland Islands. This endemic species accounts for 25%–40% of Chilean octopus landings, making it the second most important octopus species in Chile after *O. mimus*. In 2016, Chile landed 601 tons of Patagonian

red octopus, equivalent to 0.7% of the global octopus production, which reached 86,300 tons [1]. Patagonian red octopus landings are negatively correlated with the success of the *Octopus mimus* fishery [2], which led to high fishing pressure on *E. megalocyathus* that reached its critical point in 2008, resulting in successive bans for this species. The decline of natural *E. megalocyathus* populations in Chile [3], together with the difficulty of finding wild paralarvae [4] or females with clutches in the natural environment, have been critical

facts to advance *E. megalocyathus* reproductive conditioning under controlled conditions to obtain eggs and subsequently paralarvae. The studies based in controlled culture under optimized conditions have led to discover important characteristics of its life cycle including (1) moderate fecundity between 1,000 and 5,000 eggs per female [2]; however, in suboptimal food availability conditions for female breeders (75% of the daily ration), it was observed that the biochemical composition of the clutches was maintained but fecundity decreased to 4.1% in relation to females fed 100% of the daily ration [5]; (2) embryonic development takes between 150 and 176 days depending on the rearing temperature within the range of 10–16°C [6, 7]; (3) the complete paralarvae development takes about 90 days, growing from 10 mm at hatching to 30 mm at settlement [2]; however, it has been documented that *Octopus vulgaris* paralarvae can increase their weight by 2.9% at the end of the paralarval period if the *Artemia* diet is replaced by a zooplankton diet [8]. Research on the *E. megalocyathus* paralarvae rearing has mainly concentrated on the first 6 weeks after hatching due to the high mortality observed afterward, mainly attributed to poor nutrition with *Artemia* [9, 10], and stressful culture conditions [11, 12]. The management of prey to feed paralarvae has been highlighted as a critical point in octopus farming [8, 10, 13, 14]. *E. megalocyathus* paralarvae culture can last 6 weeks with close to 20% survival by feeding exclusively on enriched *Artemia* [9]. At 60 DAH, when presettlement behavior begins, the paralarvae diet includes *Artemia* plus juvenile crabs, which is necessary to reach the juvenile stage [2]. In other species, such as *R. fontaniana*, it has only been possible to obtain settled juveniles from paralarvae fed on king crab, *Lithodes santolla* nauplii, and not on enriched *Artemia* [15]. In *Octopus vulgaris*, the greatest survival and growth has been obtained using exclusively crustacean zoeas as prey or combining them with *Artemia*, as zoeas are difficult to obtain in large quantities [16]. Roura et al. [17] analyzed the gastric content of wild *O. vulgaris* paralarvae with metagenomic tools and found that their intake is composed of 20 different prey species: 17 crustaceans and three fishes. Although *Artemia* is the most widely available paralarvae feed because it is easy to produce in large quantities, its main defect is the unbalanced composition of essential nutrients for octopus paralarvae [18]. For this reason, tests have been carried out to improve *Artemia* quality by using different microalgae as food and different enrichment preparations [19]. Copepods have been suggested as the best prey for carnivorous larvae with immature digestive tubes, like those of paralarvae, but are difficult to culture [20]. Copepods have a higher abundance of free amino acids and proteins than rotifers and *Artemia*, with amino acid values 0.8–4.2 times higher, and proteins on average 0.2 and 0.5 times higher, respectively [20]. The iodine content in copepods is also 109 times higher than in *Artemia* [21]. From this point of view, copepods could be a better food source than *Artemia* during the first days of *E. megalocyathus* megalocyathus paralarvae culture.

The effect of temperature on *E. megalocyathus* embryos has been studied between 12 and 16°C, demonstrating that the highest growth rate of the embryos is obtained at 15 and

16°C with no difference between these two temperatures, reducing the time hatching in 15% with respect to the culture at 12°C. However, protease activities were higher at 14°C and decreased at 15 and 16°C, while oxygen consumption increased exponentially with temperature, suggesting that higher temperatures could affect the survival capacity of paralarvae after hatching [7]. On the other hand, the effect of temperature on the thermal tolerance of the paralarvae shows that it is possible to acclimatize the paralarvae between 8 and 16°C but not at 6 and 18°C [22]. Another interesting aspect is the thermal tolerance of paralarvae to different temperatures is affected by food availability, observing that the paralarvae subjected to fasting did not present a thermal preferendum, which could be related to a searching for food at warmer temperatures [22]. So far, the effects of temperature on early juveniles of Patagonian red octopus have not been reported. Considering the hypothesis of Pörtner and Peck [23], it is expected that embryos and paralarvae, as well as spawners, show the narrowest range of thermal tolerance. In contrast juveniles and nonreproductive adults would exhibit a widest range, so probably *E. megalocyathus* juveniles could have a greater range of thermal tolerance than those observed in embryos and paralarvae.

The rearing technologies of carnivore mollusks were reviewed by Uriarte et al. [10] and showed that *E. megalocyathus* is a species with high potential to reach commercial aquaculture. The first aim of the current study was to improve aspects of feeding in broodstock and paralarvae, as well as temperature management during egg incubation and the growth of early juveniles. The second objective was to analyze different batches of both paralarvae and early juveniles to update indicators and generate new ones to evaluate and improve the culture of this species in its most critical period, which is the controlled production of paralarvae until the first settled juveniles.

2. Materials and Methods

2.1. Animals. Adult octopuses of 915 ± 115 g were captured by hand by diving near Hueihue (Ancud, Chile, 41°87'S), with permission granted by Chilean Deputy Secretary of Fisheries (SUBPESCA) and transported to the Hatchery of Marine Invertebrates facility at the Universidad Austral de Chile (HIM-UACH, Pelluco, Puerto Montt, Chile, 42°35'S) for reproductive conditioning and mating.

2.2. Broodstock Conditioning. Reproductive conditioning followed the recommendations of Uriarte and Farías [2], with a temperature of $12 \pm 1^\circ\text{C}$, light intensity of 50.1 ± 1.7 lux cm^{-2} , and a photoperiod of 11Light : 13Dark. The experiment tested three different diets for conditioning: 100% crab (*Cancer edwardsii*), 100% squid (*Dosidicus gigas*), and 50% crab/50% squid. The crabs (control diet) were obtained from artisanal fishermen and were supplied alive, the cuttlefish fillets were offered in portioned, thawed pieces following the methodology of Gutiérrez et al. [42]. All diets were dosed at 5% body weight day^{-1} . Diets were tested in triplicate and randomly assigned, with each replica consisting of one female in its own 150 L tank. Males were exclusively conditioned with a 100% crab diet. When the conditioning was completed, each female was bred

with the males following the recommendations of Gutiérrez et al. [24]. Performance during conditioning was measured by specific growth rate (SGR), feed intake, relative fecundity (eggs spawned/kg), clutch size (eggs spawned/female), and the protein and lipid contents of the first day spawned eggs. The newly spawned eggs were analyzed regardless they were fertilized or not.

2.3. Embryo Incubation. Eggs just spawned were incubated at three different temperatures: 8, 14, and 18°C in triplicate. According to Uriarte et al. [7], 14°C provides a good balance between embryo growth and storage reserves during the first days after hatching, while 8 and 18°C are extreme temperatures that represent the temperatures at the latitudinal limits of the species that have not yet been studied. The eggs were distributed randomly between 18 tanks, six at each temperature, and incubated with a precision of $\pm 0.04^\circ\text{C}$ and oxygen saturation of 85%. The eggs were monitored and cleaned routinely with soft paint brushes to avoid the infections. The effect of temperature was compared as days of development, and as accumulated thermal units (ATU), which is the cumulative daily water temperature in degrees Celsius ($^\circ\text{C}$ days) from egg spawning to hatching ($T^\circ\text{C}$ was measured with a YSI Pro2030 multiparameter from the beginning to the end of embryonic development). Besides thermal time was calculated and expressed as degree-days (DD), that were calculated according to Campbell et al. [25], on the assumption that the relationship between experimental temperature (T , $^\circ\text{C}$) and the time (days) to reach stage XX as Naef [26] when is ready to hatch, is a straight line for experimental temperatures:

$$\text{DD}(\text{ }^\circ\text{C day}^{-1}) = (T^\circ\text{C} - \text{TT}) \times t, \quad (1)$$

where t is the time to reach stage XX and T is the experimental temperature in $^\circ\text{C}$. The threshold temperature (TT, $^\circ\text{C}$) was obtained when $\text{TT} = \text{RD} = 0$ (biological zero, temperature without embryo development); $\text{RD} = a + bT^\circ\text{C}$, where a and b are constants. DD was defined as the number of degree-days above TT required by an embryo to complete its development to stage XX.

Samplings and biochemical analyses of spawned eggs were performed on the whole egg and besides separating the embryos from the yolk and chorion and analyzing each part separately following the protocols of Uriarte et al. [6, 27]. Briefly, the carbon, hydrogen, and nitrogen contents were analyzed in a LECO CHN autoanalyzer, with 1 mg of lyophilized samples that were previously weighed in a Mettler Toledo microbalance. Crude protein was obtained by multiplying the nitrogen content by 6.25. The total lipid content (TL) was obtained gravimetrically after the extraction of samples, following the protocol of Bligh and Dyer [28]. Whole egg energy values were determined by adiabatic calorimeter in samples of 1 g (Parr Instrument Co., model 1455, Moline, IL, USA).

2.4. Paralarval Rearing. Paralarvae batches cultivated under standard conditions were pooled in two periods: 2012–2016

and 2017–2021. The morphometry (TL = total length, ML = mantle length, AL = arm length, and ED = eye diameter) following Uriarte et al. [15] and relative condition index ($\text{Wr} = \text{final weight}/(a \times L^b)$), as cited by Farías et al. [9], of the paralarvae were compared between the two periods. The standard cultivation conditions in the 2012–2016 period were a temperature of 12°C, a density of 10 paralarvae L^{-1} , and a diet of five *Artemia* enriched with *Nannochloropsis* sp. per paralarvae. In the 2017–2021 period, the same conditions were applied, with the exception that the diet consisted of 10 *Artemia* enriched with the same microalgae per paralarvae during the first 2 weeks after hatching and then switched to Origold (Skretting) until 90 DAH. At 60 DAH, when the presettlement behavior began, the paralarvae were given dens and juvenile crabs were integrated into their diet in a 10:1 *Artemia*:crab ratio. Biometrical measurements of the paralarvae were sampled at 1, 15, 25, 50, and 90 DAH. The mantle length was measured dorsally from the midpoint between the eyes to the posterior end of the mantle. The arm length was measured on the dorsal (sucker-bearing) side, from the tip of the arm to the mouth. The growth in length was calculated by fitting an exponential regression to relate total length (TL) with octopus age (t), $\text{TL} = a \times e^{bt}$, where a and b are the intercept and slope, respectively, and t is the number of days after hatching, the TL-growth rate in length (GRL) was the slope in ln-units per time (day^{-1}). For SGR, the exponential regression was fitted to wet weight (WW) as a function of t , where the slope was multiplied by 100 to obtain the SGR in $\% \text{ day}^{-1}$ [15].

The allometric relationship between paralarvae weight and length was calculated from the power regression between the whole WW and the total length (TL) of paralarvae through their 90-day rearing period, $\text{WW} = a \times \text{TL}^b$, where a is the intercept, and b is the slope and the allometric exponent.

With the aim of testing marine crustaceans in the first 15 days of life of the paralarvae a bioassay was conducted with newly hatched paralarvae under four diet treatments: (1) unfed, (2) *Nannochloropsis*-enriched *Artemia*, (3) *Acartia* spp. copepods, and (4) *Petrolisthes* spp. juvenile crabs. A density of five paralarvae L^{-1} , and 10 prey paralarva $^{-1}$ day $^{-1}$ was used, according to [11]. The experiment ended at 15 DAH and growth rate, survival and whole-body fatty acid composition were evaluated.

2.5. Juvenile Culture. The newly settled juveniles were reared in a recirculated water system with UV sterilization in which the seawater recirculation system operated at $12 \pm 1^\circ\text{C}$, with an O_2 concentration above 85% saturation, and a density of eight octopuses per 30 L. Each octopus received three juvenile crabs daily, supplemented in the first weeks with two daily dosages of adult *Artemia*. The paralarvae of 90 DAH correspond to the beginning of the postparalarvae stage, so they were also considered as 1-day-old juveniles. The following samples were taken at 30- and 135-days postsettlement (DPS). Biometric measurements, growth rate, and allometric relationship calculations followed the same protocol as in paralarvae, with the difference that age was measured in

TABLE 1: Performance indexes during reproductive conditioning from experiment 1 with three different fresh diets.

Index	Broodstock conditioning diet		
	100% crab	100% squid	50% crab + 50% squid
SGR (% day ⁻¹)	0.45 ± 0.04	0.70 ± 0.23	0.65 ± 0.10
Feed intake (% BW day ⁻¹)	1.93 ± 0.42 ^b	0.50 ± 0.10 ^a	1.00 ± 0.17 ^a
Relative fecundity (eggs kg ⁻¹ female)	3,152 ± 896	2,673 ± 576	1,308 ± 123
WW egg/WW female (%)	0.0036 ± 0.00033 ^a	0.0053 ± 0.00067 ^b	0.0033 ± 0.00033 ^a
WW clutch/WW female (%)	18.13 ± 4.50	16.77 ± 4.92	9.17 ± 1.54
Female lost weight (%)	54.79 ± 13.52	37.70 ± 11.28	71.48 ± 11.94
Whole egg protein (% DW)	69.41 ± 0.28	68.68 ± 0.80	71.69 ± 2.14
Protein/energy whole egg (g protein MJ ⁻¹)	28.13 ± 0.13 ^a	28.13 ± 0.23 ^a	29.83 ± 0.52 ^b
Hatching (% of embryo survival)	59.81 ± 21.67	58.22	49.69

Values with different superindexes are significantly different with $P < 0.05$. The values are mean of three different females and their clutches.

DPS. The juveniles from two massive cultures sampled in 2012 and 2017 were compared.

An experiment was carried out with 18 recently settled Patagonian red octopus juveniles (26 ± 4 DPS), with an initial total length of 3.4 ± 0.1 cm and an initial live weight of 0.57 ± 0.04 g to assess the best-growing temperature. The juveniles were individually placed in 24 L tanks and randomly assigned at three experimental temperatures: 11, 13, and 15°C ($\pm 0.5^\circ\text{C}$). Tanks were connected to recirculatory seawater systems, allowing 400% water change daily. Oxygen was maintained at $91.0\% \pm 0.4\%$ saturation and salinity of 30.1 ± 0.0 UPS. The octopuses were fed 15 adult *Origo*-enriched *Artemia* and three juvenile *Petrolisthes* spp. crabs of 156.7 ± 6.9 mg WW daily. After each feeding period, uneaten prey was removed and counted, and the crab carcasses were analyzed for remaining meat content to assess feed intake rate. The dry matter, ash, protein, and lipid content of *Artemia* and crabs were determined. Octopus biometry was performed at 1, 16, 33, and 49 days to determine the WW, biometric relationships (ML/TL, AL/TL, AL/ML), relative weight condition index (W_r), TL-growth rates in length (GRL), SGR, food ingestion rate, protein efficiency ratio, energy efficiency ratio, and feed conversion ratio.

Data are presented as mean \pm SE of three or more replicates. In the experiments, groups or treatments were compared with parametric one-way ANOVAs if the data were homoscedastic and normally distributed. If not a nonparametric Kruskal Wallis test was performed. Tukey's or range analysis test, respectively, was applied to separate significant effects at P value ≤ 0.05 . Spearman's nonparametric correlation coefficient was used to evaluate correlations between independent variables. Statistical analyses were carried out using Statistica software. The slopes of the regression equations were compared for the differences following Zar's [43] study.

2.6. Ethical Statement. *E. megalocyathus* adults, embryos, paralarvae, and early juveniles were used in the experimental procedure and all animal work performed was conducted according to the Universidad Austral de Chile's regulations for the Use of Animals in Experimentation, approved by decree 95 in 2020 (<https://adminvidca.uach.cl/wp-content/>

[uploads/2022/05/Decreto-95-de-2020.pdf](https://adminvidca.uach.cl/wp-content/uploads/2022/05/Decreto-95-de-2020.pdf)) and following ANID 2022 guidelines (<https://adminvidca.uach.cl/wp-content/uploads/2022/05/Lineamientos-Bioeticos-para-la-Investigacion-CC%81n-con-Animales-ANID.pdf>). The specific certificate numbers issued by the Bioethics Committee for work with octopuses correspond to 68-2012 and 401-2020.

3. Results

3.1. Reproductive Conditioning. The SGR of females during conditioning period was not affected by the diet. As a result, a mean value of $0.60\% \pm 0.08\%$ day⁻¹ was calculated (Table 1). Ingestion rate of females was affected by type of diet ($F_{2,6} = 7.40$, $P = 0.02$), with higher consumption of animals fed crab-based diet than females fed squid and squid-crab mix diets (Table 1). Considering that there were no effects of the diet on the spawned eggs a mean value of 2378 ± 415 eggs kg⁻¹ was calculated. (Table 1). However, the ratio egg weight/female weight differed, with the greatest ratio observed for eggs of females fed squid ($F_{2,6} = 7.71$, $P = 0.02$).

The conditioning diet has not influenced the size of egg clutch when expressed as a proportion of the final weight of females (Table 1; $P > 0.05$). During the egg-brooding period, the females did not feed and their weight decreased similarly between diets ($P > 0.05$) with an average of $46.0\% \pm 8.6\%$ by the time the eggs hatched. The females that spawned non-fecundated eggs invested similar weight loss during egg-brooding to those with fertile eggs (Figure S1). The female weight loss was negatively correlated with the egg size as percentage of female weight (Figure S2).

There were no significant differences in protein and energy composition of whole eggs of the first day from females fed different diets ($P > 0.05$) showing averages of $69.93\% \pm 0.80\%$ protein and 24.36 ± 0.28 kJ. But P/E ratio of whole egg varied between conditioning diets ($F_{2,6} = 8.34$, $P = 0.018$) debt to the highest value obtained in eggs from females fed squid-crab mix diets (Table 1).

Yolk and chorion protein contents were not affected by the female diet, with averages of 68.87 ± 0.90 and 70.07 ± 1.23 , respectively. However, perivitelline fluid showed higher protein content in eggs from crab-fed females

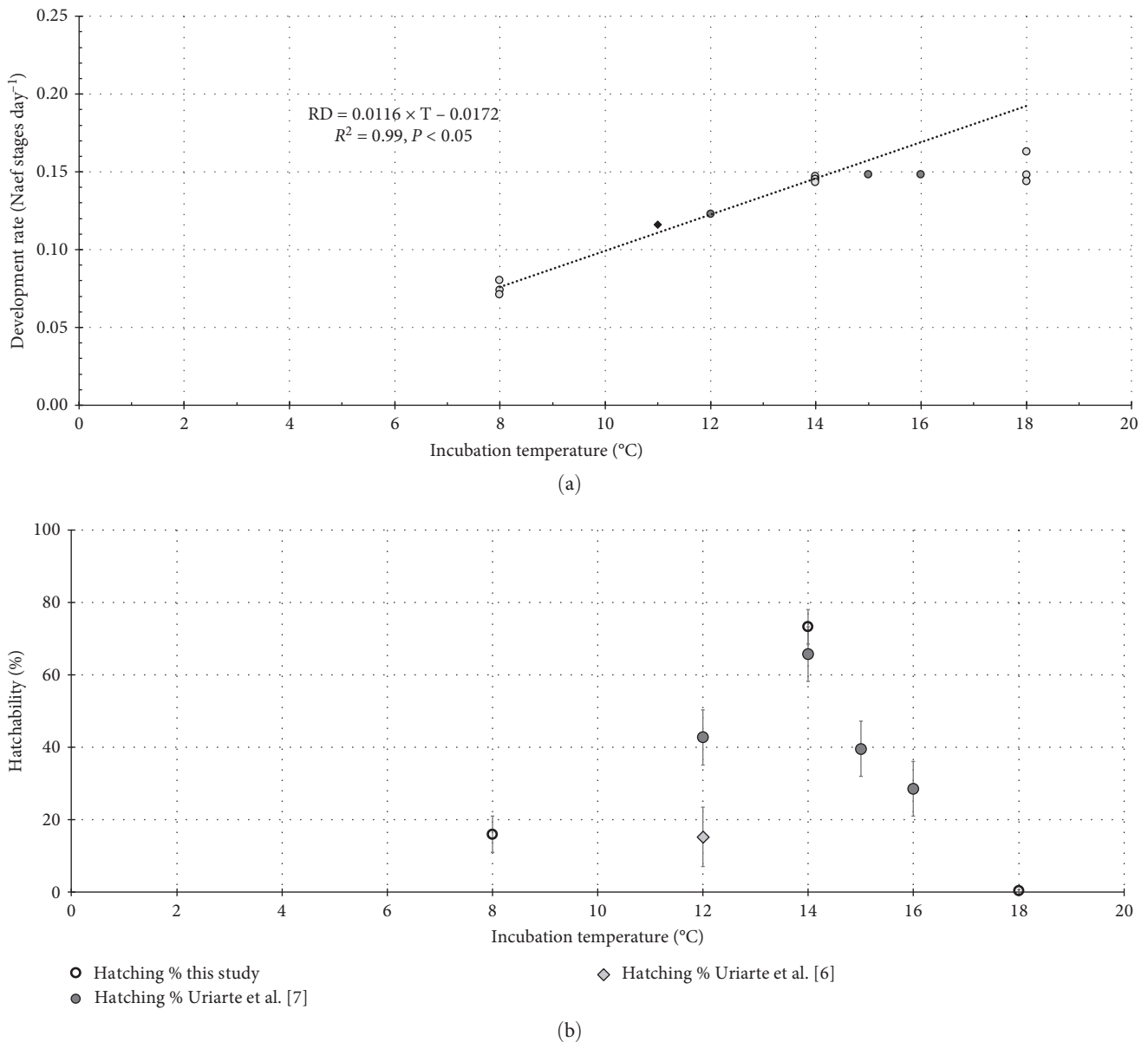


FIGURE 1: Incubation efficiency of *E. megalocyathus* embryo at three temperatures: 8, 14, and 18°C. (a) Embryo development rate (RD) as a function of experimental temperature and (b) embryo survival measured as hatching percentage of total incubated eggs. Each point (dark ring) is the value of three independent clutches of different females. Other data of *E. megalocyathus* were taken from Uriarte et al. [6] (dark rhombus) and Uriarte et al. [7] (light ring).

and lower protein content in eggs from squid-fed females ($F_{2,6} = 5.66, P = 0.04$). Lipids of the first day spawned eggs did not vary due to diet, with average values of 12.89 ± 1.30 , 11.60 ± 2.13 , and 9.55 ± 1.09 for yolk, perivitelline fluid, and chorion, respectively (Figure S3). The components of *Cancer edwardsii* and *Dosidicus gigas* described by Gutiérrez et al. [42] were correlated with the composition of the egg compartments. The results showed only correlation with perivitelline protein. It was observed that perivitelline protein correlates positively with the dietary lipid ($r = 0.87, P = 0.002$) and negatively with the dietary protein ($r = -0.82, P = 0.007$), and with the protein/energy ratio of the diet ($r = -0.82, P = 0.007$) (Figure S4).

The variation in hatching percentages (Table 1) could not be associated with diet effects because two-thirds of females in both the 100% squid and 50% crab +50% squid diets started laying eggs before mating with the males, giving rise to unfertilized eggs.

3.2. Embryo Incubation. In the thermal experiment of the embryo, it was observed that the development rate increased linearly from 8 to 14°C and then did not increase when temperature rise to 18°C (Figure 1(a)). At 8°C was observed double development period of the embryos than at 14 and 18°C ($H(2, N=9) = 5.65, P = 0.059$). The equation $RD = -0.0172 + 0.0116 \times T$ (Figure 1(a)) allows to calculate the

TABLE 2: Composition of ash, protein, and lipid in stage VII *E. megalocyathus* embryos (in percentage dry weight), incubated at three temperatures: 8, 14, and 18°C.

Egg compartment	T°C	(%) ash \pm SD	(%) prot \pm SD	(%) lip \pm SD
Yolk	8	8.55 \pm 0.58 ^a	65.39 \pm 1.28	18.73 \pm 0.92
	14	10.22 \pm 0.03 ^b	66.17 \pm 0.48	19.59 \pm 1.22
	18	9.92 \pm 0.39 ^b	65.70 \pm 0.64	19.14 \pm 0.48
Perivitelline liquid	8	4.95 \pm 0.56 ^a	62.26 \pm 0.80	20.43 \pm 4.06
	14	6.08 \pm 2.51 ^a	63.22 \pm 1.99	19.25 \pm 1.45
	18	12.68 \pm 3.24 ^b	61.85 \pm 3.74	18.10 \pm 1.51
Chorion	8	9.02 \pm 1.68	73.46 \pm 1.10 ^b	8.46 \pm 1.86
	14	9.96 \pm 4.45	71.70 \pm 3.03 ^{ab}	10.63 \pm 1.50
	18	10.28 \pm 1.72	68.43 \pm 1.33 ^a	10.39 \pm 0.73

Values with different superindexes are significantly different with $P < 0.05$.

development time for any temperature between the biological zero and 14°C. In accord with this, the biological zero for *E. megalocyathus* embryos is 1.5°C. Regarding accumulated temperature, *E. megalocyathus* embryos require 2,149 \pm 79 ATU to complete their development regardless of temperature (Figure S5). Similarly, the thermal degrees accumulated above the biological zero to reach stage XX are 1,881 \pm 60 degree-days regardless of temperature (Figure S4).

The temperature affected the percentage of paralarvae that hatched ($F_{2,6} = 74.48$, $P < 0.0001$), with 14°C > 8°C > 18°C, with close to zero hatching at 18°C (Figure 1(b), Figure S5).

Temperature only affected the protein content of the chorion ($F_{2,6} = 4.82$, $P = 0.056$), with 8°C \geq 14°C \geq 18°C (Table 2). Lipid content did not vary between temperatures for any of the compartments, obtaining averages of 19.15% \pm 0.29%, 19.26% \pm 0.83%, and 9.8% \pm 0.54% for yolk, perivitelline fluid, and chorion, respectively (Table 2).

Temperature generated differences in yolk ash ($F_{2,5} = 10.89$, $P = 0.015$) with 8°C < 14°C = 18°C, and in perivitelline fluid ($F_{2,5} = 8.02$, $P = 0.03$) with 8°C = 14°C < 18°C (Table 2). No differences were observed in the ash content of the chorion due to temperature.

3.3. Paralarvae Rearing. The paralarvae from two different batches presented similar weights at 1, 25, and 90 DAH, with averages of 106.5 (± 2.2 , $N = 92$), 166.3 (± 5.0 , $N = 62$), and 481.2 (± 22.3 , $N = 42$) mg, respectively. However, paralarvae weights differed at 15 DAH ($H(1, N = 204) = 55.45$; $P < 0.0001$) and 50 DAH ($H(1, N = 127) = 30.61$; $P < 0.0001$) (Figure 2(a)). The SGR adjusted to data of weight vs. paralarvae age were 1.6% and 1.7% DAH⁻¹ for 2012–2016 and 2017–2021 batches, respectively, without differences between them.

The total lengths of the paralarvae were similar between groups at 1 DAH, with an average of 14.3 (± 0.1 , $N = 92$) mm, then there were differences between paralarvae groups at 15, 25, 50, and 90 DAH (Figure 2(b)). The data-adjusted total length growth rates were 0.0076 and 0.0091 DAH⁻¹ for 2012–2016 and 2017–2021 batches, respectively, without differences between them (Figure 2(b)).

AL/ML morphometric ratios were similar between paralarvae at 1, 25, and 90 DAH with means of 0.93 (± 0.01 , $N = 92$), 1.00 (± 0.03 , $N = 62$), and 1.51 (± 0.03 , $N = 43$), respectively. Differences were observed in paralarvae

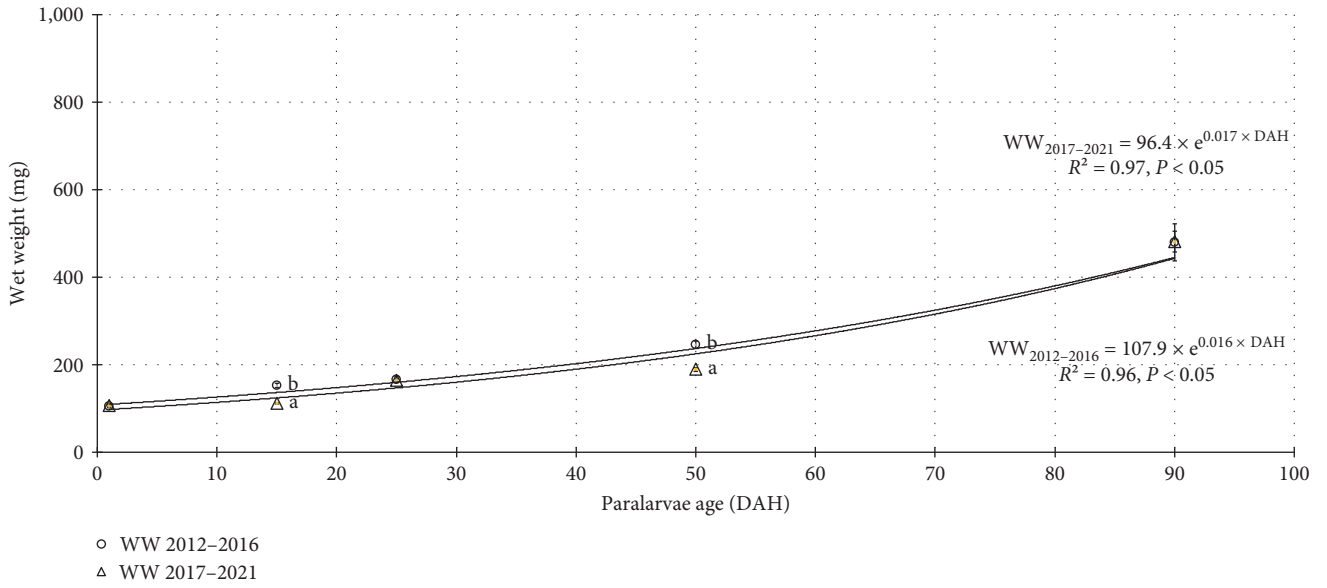
between groups at 15 and 50 DAH (Figure 2(c)). The AL/ML ratio showed a trend to increase during the first 10 days of paralarva life and after 50 DAH. In between, it remained almost constant (Figure 2(c)).

The allometric equation between weight and total length of paralarvae showed different allometry coefficient of 2.07 and 1.87 for 2012–2016 and 2017–2021 batches ($F_{1,525} = 78.05$, $P < 0.05$) (Figure 2(d)). Therefore, the weight condition index in paralarvae were $Wr = WW/0.53 \times TL^{2.07}$ and $Wr = WW/0.67 \times TL^{1.87}$, respectively.

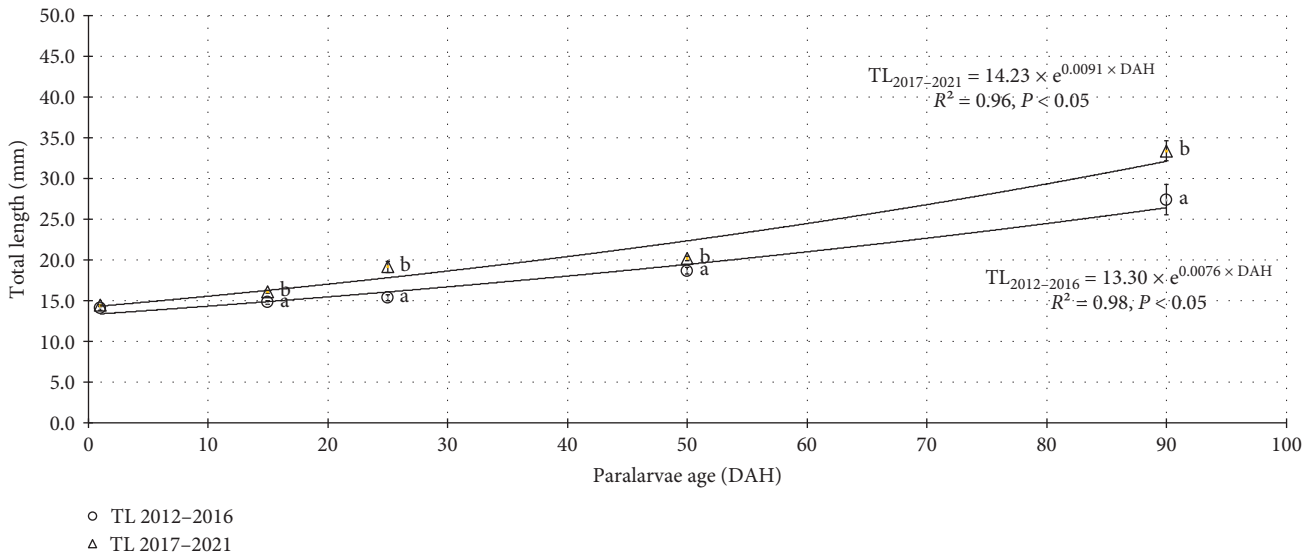
Paralarvae from the experiment with different diets showed a tendency toward lower survival when fed only *Artemia* ($F_{3,8} = 3.38$, $P = 0.07$), with 80% survival when paralarvae fed copepods and crabs and intermediate values in fasted paralarvae (Table 3). On the other hand, fasted and crab-fed paralarvae had negative growth rates ($H(3, N = 11) = 6.62$, $P = 0.08$). The paralarvae that showed growth were those fed with copepods or *Artemia*, with no differences between them (Table 3). Nutrient retention per paralarva showed great variability between replicates, exception was fasting paralarvae (Table 3). The paralarvae fed with copepods showed positive protein retention, unlike the negative retentions observed with the rest of the treatments. Contrarily, a trend toward lipid retention was observed in all treatments (Table 3). The SGR of paralarvae was positively correlated with protein ($r = 0.95$, $P = 0.000005$), lipid ($r = 0.92$, $P = 0.00007$), and energy retention ($r = 0.95$, $P = 0.000005$).

It was observed that fatty acids such as palmitic (hexadecanoic), eicosapentaenoic, and docosahexaenoic acid constituted on average 64.5% of all fatty acids in paralarvae, with PUFAs generally the most abundant, followed by SFA, then MUFAs (Table 3), with no differences between diets or fasting. Fatty acid contents had little correlation with paralarvae performance parameters. There was only a negative correlation between 18:1n-7 and paralarvae survival ($r = -0.84$, $P = 0.0085$) and a positive correlation between DHA/EPA and survival ($r = 0.75$, $P = 0.03$).

3.4. Juvenile Culture. No initial differences were observed when juveniles produced in the years 2012 and 2017 were compared. However, at 30 DPS the juveniles produced in the year 2012 tended to weigh less than in 2017 ($P = 0.08$), and

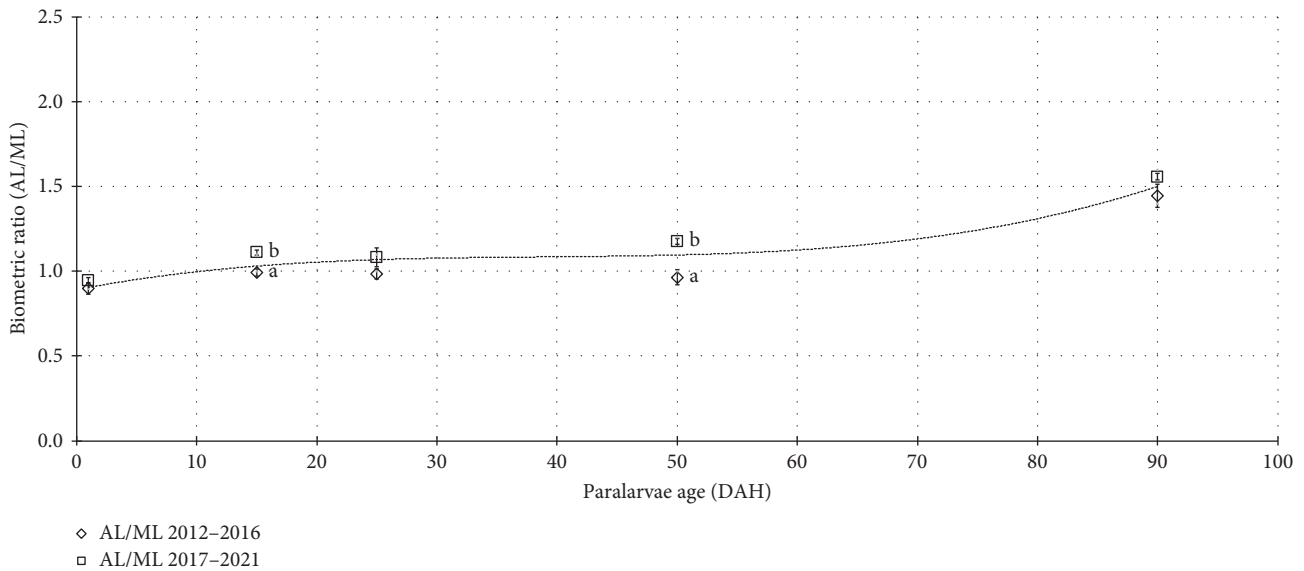


(a)

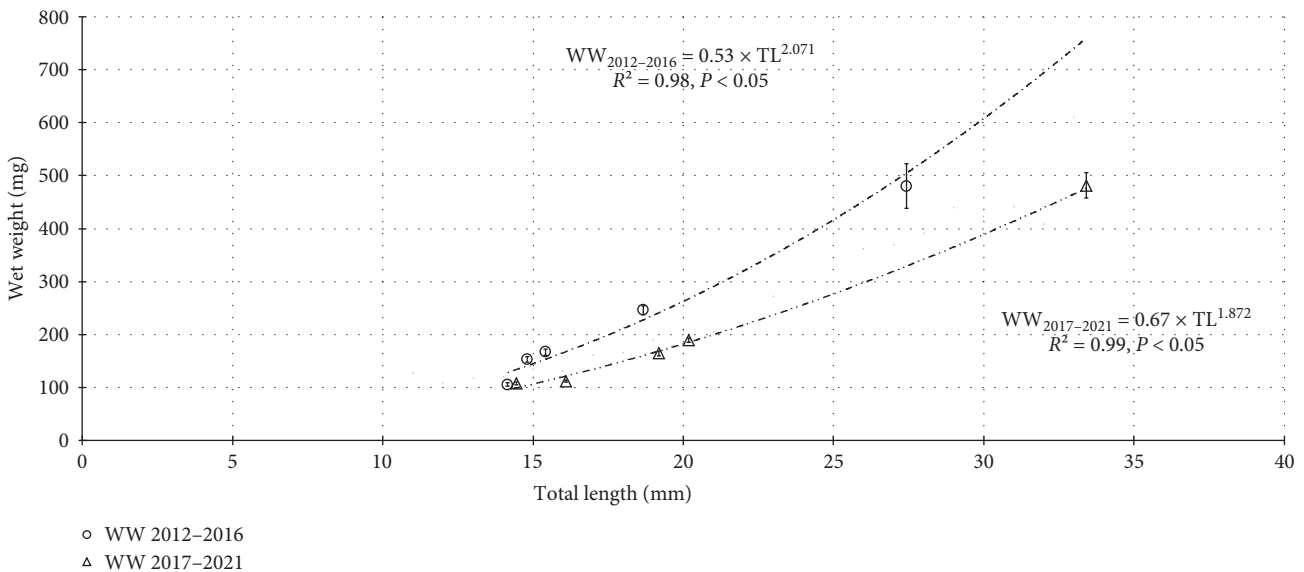


(b)

FIGURE 2: Continued.



(c)



(d)

FIGURE 2: Growth of *E. megalocyathus* paralarvae over 90 days (from hatching to settlement) during two different production periods. (a) Wet weight (WW) in mg versus paralarvae age. The curve and equation show the exponential regression fit to all data. The slope is the growth rate in $\ln(\text{mg}) \text{ day}^{-1}$ after hatching, the slope $\times 100$ is the specific growth rate (SGR) in $\% \text{ day}^{-1}$ after hatching. (b) Total length (TL) in mm versus paralarvae age. The curve and equation show the exponential regression fitted to all data. The slope is the TL-growth rate in $\ln(\text{mm}) \text{ day}^{-1}$ after hatching. (c) Morphometric ratio, arm length (AL)/mantle length (ML) at different paralarvae ages. (d) Allometric equation between TL and WW. Bars show the standard error of average values per age in each period. Different superscript values show significant differences between periods ($P < 0.05$).

at 135 DPS the 2017 juveniles were double the weight of the 2012 samples at the same age ($F_{1,125} = 30.15, P < 0.00001$) (Figure 3(a)). The SGR were 1.88% and $2.52\% \text{ day}^{-1}$ postsettlement for 2012 and 2017 batches, respectively (Figure 3(a)) with the highest value in 2017 ($F_{1,193} = 42.16, P < 0.05$).

In initial total length, the juveniles produced in 2017 presented higher values than in 2012 ($F_{1,41} = 7.50, P = 0.009$), and the same trend was observed at 135 DPS ($H(1, N = 127) = 17.21, P < 0.0001$) (Figure 3(b)). The exponential TL-growth rates

postsettlement were 0.0089 and 0.0103 day^{-1} for 2012 and 2017 batches, respectively, without differences between them (Figure 3(b)). In the AL/ML relationship, there were no differences between groups at 1 DPS, while at 30 DPS, the 2012 group showed higher values ($F_{1,25} = 7.11; P = 0.01$) and at 135 DPS the 2017 group of juveniles showed higher values ($H(1, N = 127) = 4.71, P = 0.03$). The linear regression adjustment of AL/ML to the age of the juveniles of both productions had similar slopes of 0.009 and 0.0115 day^{-1} postsettlement, respectively (Figure 3(c)).

TABLE 3: Performance parameters and fatty acid composition of Patagonian red octopus paralarvae fed three food supplies or fasted for 15 days from hatching.

Index	Diet			
	T1: copepods	T2: <i>Artemia</i>	T3: fasting	T4: crabs
Performance parameters				
Final wet weight (mg)	104.2 ± 12.9	107.3 ± 2.3	73.5 ± 1.2*	68.7 ± 12.0*
Survival (%)	80.0 ± 0.0	33.3 ± 17.6*	60.0 ± 11.5	80.0 ± 11.5
SGR (% day ⁻¹)	2.4 ± 1.0	2.7 ± 0.2	-0.2 ± 0.1	-0.9 ± 1.4
Protein retention (mg paralarvae ⁻¹)	12.45 ± 6.90	-3.65 ± 19.71	-1.29 ± 0.67	-3.39 ± 6.94
Lipid retention (mg paralarvae ⁻¹)	4.40 ± 1.90	0.05 ± 5.46	1.01 ± 0.19	0.50 ± 1.99
Energy retention (kJ paralarvae ⁻¹)	0.47 ± 0.24	-0.09 ± 0.68	0.01 ± 0.02	-0.06 ± 0.25
Fatty acid composition (% of total fatty acids)				
16:0	23.18 ± 2.26	21.97 ± 0.38	16.93 ± 2.64	21.14 ± 2.61
18:0	8.28 ± 1.21	9.84 ± 0.32	4.74 ± 4.74	8.53 ± 0.23
18:1n-7	0.48 ± 0.48	3.33 ± 1.1	0.76 ± 0.76	0.58 ± 0.58
18:2n-6	0.81 ± 0.81	3.78 ± 1.55	0.75 ± 0.75	0.0
18:3n-3	7.27 ± 0.21	6.13 ± 0.06	3.51 ± 3.51	7.30 ± 1.70
20:4n-6	5.74 ± 0.87	5.34 ± 0.63	2.60 ± 2.60	4.01 ± 0.04
20:5n-3	20.34 ± 0.39	17.45 ± 1.15	16.48 ± 2.79	18.48 ± 4.11
22:6n-3	27.73 ± 1.21	22.59 ± 0.73	22.02 ± 3.35	23.66 ± 0.85
SFA	33.11 ± 0.50	32.69 ± 0.81	46.84 ± 15.65	36.54 ± 8.36
MUFA	2.45 ± 1.44	9.02 ± 3.56	3.65 ± 0.01	5.37 ± 0.43
PUFA	55.39 ± 0.89	46.13 ± 2.00	42.94 ± 10.58	47.41 ± 6.25
n-3	56.93 ± 0.24	46.93 ± 2.70	43.85 ± 11.49	50.700 ± 4.52
n-6	6.55 ± 1.68	9.61 ± 1.68	3.35 ± 1.85	4.59 ± 0.61
DHA/EPA	1.36 ± 0.03	1.29 ± 0.04	1.34 ± 0.02	1.34 ± 0.25
EPA/ARA	3.62 ± 0.48	3.34 ± 0.61	3.71	4.59 ± 0.98

Values with different superindexes are different with $P < 0.05$. Values with * show trends with $0.05 < P < 0.095$.

The allometry between weight and total length of juveniles showed exponents 2.018 and 2.398 for 2012 and 2017 batches (Figure 3(d)), respectively, with the highest value for 2017 batch ($F_{1,193} = 51.00$, $P < 0.05$). Therefore, the weight condition index for juveniles of 2012 and 2017 were $Wr = WW/0.056 \times TL^{2.018}$ and $Wr = WW/0.028 \times TL^{2.398}$, respectively.

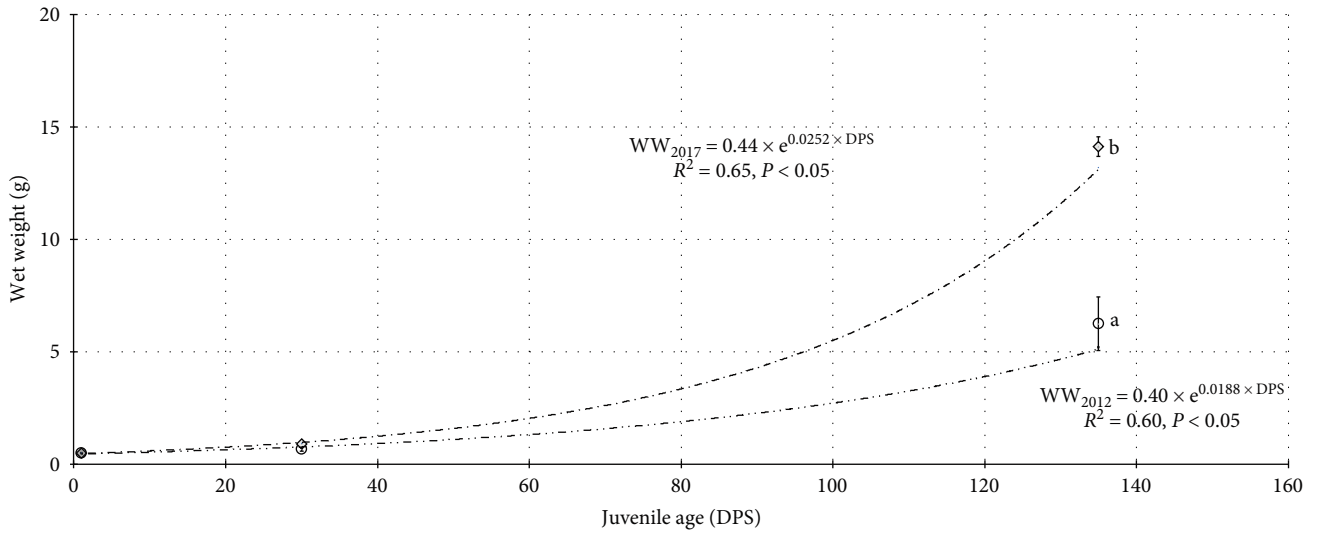
In the thermal experiment of juveniles, no differences were observed by temperature effect in the AL/ML ratio, SGR, survival, standardized food intake (percentage body weight per day), or condition index (Wr) (Table 4). The culture temperature also did not significantly affect protein, lipid, or energy efficiency ratios (Table 4), but there was a negative correlation between temperature and survival ($r = -0.55$, $P = 0.026$). Besides, a trend to negative correlation was obtained between temperature and FCR ($r = -0.48$, $P = 0.098$). The AM/ML relationship showed a positive correlation with SGR ($r = 0.76$, $P = 0.002$), and negative one with FCR ($r = -0.85$, $P = 0.0003$).

The analysis of the 669 individuals (paralarvae + juveniles) belonging to the periods 2012–2016 and 2017–2021 revealed that paralarvae weighed 0.10 g at 1 DAH and 90 days later they reached 0.48 g. The ML/TL ratio decreased from 0.48 to 0.43 in the same period and AL/TL increased from 0.44 to 0.50 (Figure 4). In the juvenile period of 1–135 DPS, weight varied between 0.48 and 14.1 g, while ML/TL decreased to 0.23 and AL/TL increased to 0.70 (Figure 4).

4. Discussion

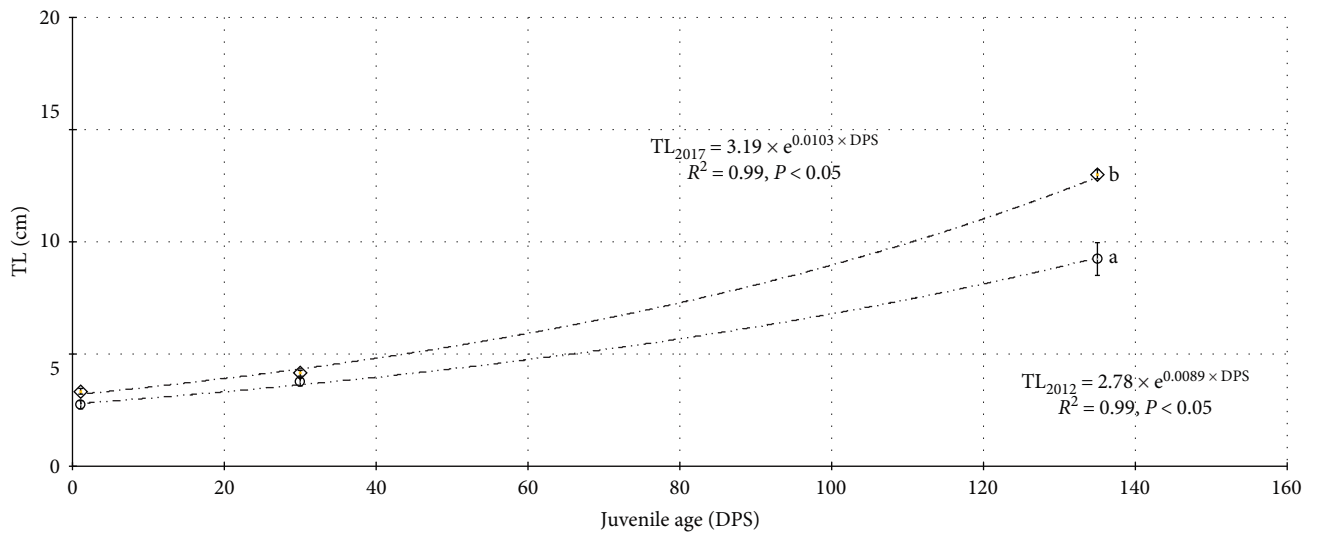
Patagonian red octopus reproduction was successfully achieved with different fresh diets. Although a higher feed intake was observed with the crab diet, it was not associated with female growth rate during the period, subsequent fecundity, or the hatching percentage. The egg composition also did not vary due to the female diet. According to Farías et al. [5], *E. megalocyathus* females tend to have high-quality eggs, although fecundity is reduced when food is deficient. This implies that eggs with appropriate biochemical content should result from all diets tested, explaining why the relative amounts of eggs of the females were similar, regardless of whether females were fertilized by males. These results are very similar to those documented by Gutiérrez et al. [24], who also found no differences in the fecundity of females or in the proximal composition of their spawn when feeding on various prey, including fish.

Interestingly, a difference in protein was only observed in the perivitelline fluid of the eggs, with the highest values found in the eggs of crab-fed females, which could be important for the metabolism of embryos with this diet. Gutiérrez et al. [24] found no differences in the proximal composition of perivitelline liquid but did find differences in the C/N ratio of this compartment, where the C/N ratio of the perivitelline liquid adjusts linearly with the protein/energy ratio of the



○ WW (g) 2012
◇ WW (g) 2017

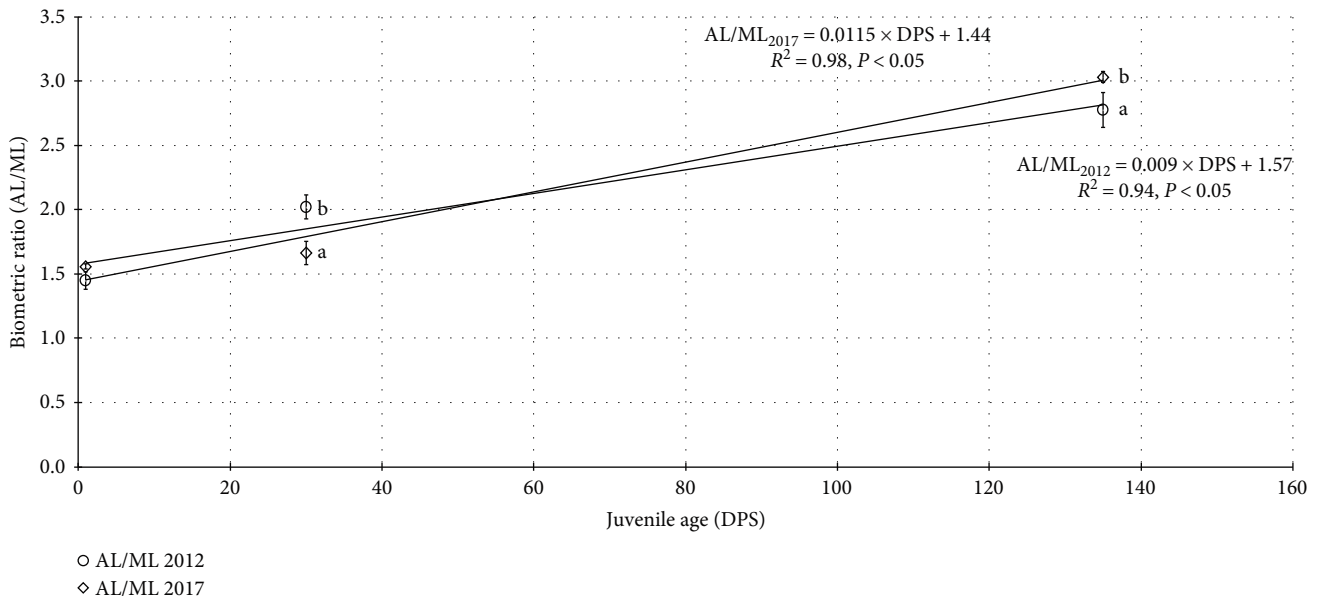
(a)



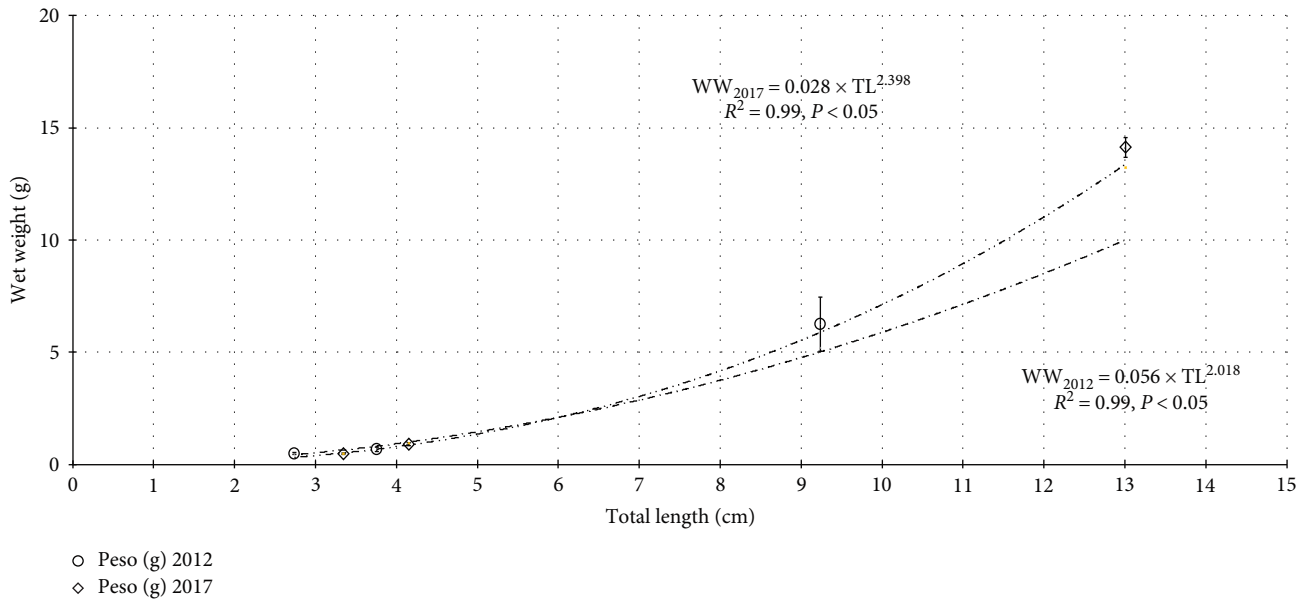
○ TL (cm) 2012
◇ TL (cm) 2017

(b)

FIGURE 3: Continued.



(c)



(d)

FIGURE 3: Growth of juvenile *E. megalocyathus* over 135 days after settlement for two different production periods. (a) Wet weight (WW) in g versus juvenile age. The curve and equation show the exponential regression fit to all data. The slope is the growth rate in $\ln(g) \text{ day}^{-1}$ postsettlement, the slope $\times 100$ is the specific growth rate (SGR) in $\% \text{ day}^{-1}$ postsettlement. (b) Total length (TL) in cm versus juvenile age. The curve and equation show the exponential regression fitted to all data. The slope is the TL-growth rate in $\ln(\text{cm}) \text{ day}^{-1}$ postsettlement. (c) Ratio AL/ML at different juvenile ages. The curve and equation show the linear regression fit to all data. DPS is days postsettlement. (d) Allometric equation between TL and WW. Bars show the standard error of average values for age in each period. Different superscript values show significant differences between periods ($P < 0.05$).

breeder diet. Similarly, current results showed a linear fit of the perivitelline protein with the protein/energy ratio of the broodstock diet ($R^2 = 0.69$), indicating that the female diet specifically affects this component of the egg and not others. If we add the positive correlation of the perivitelline protein with the lipid of broodstock diet, it is likely that diets with more nonprotein energy delivered to the broodstock allow a greater availability of protein in the perivitelline compartment. According to Uriarte

et al. [27], the perivitelline protein in *Robsonella fontaniana* eggs was relevant for energy and morphogenesis of the embryo. During the embryonic development of *E. megalocyathus*, the perivitelline protein is reduced by 74% and the vitelline protein by 19% with respect to the first day of incubation, indicating that the perivitelline protein is an energy substrate for the embryos [6]. Therefore, the crab-based diet leads to a higher protein content in the perivitelline

TABLE 4: Performance parameters and nutrient efficiency ratio of *E. megalocyathus* early juveniles reared under different temperatures.

Index	Rearing temperature (°C)		
	11°C	13°C	15°C
Performance indicators			
Final wet weight (g)	0.88 ± 0.27	1.25 ± 0.35	1.79 ± 0.67
AL/ML	1.98 ± 0.15	2.23 ± 0.17	2.48 ± 0.19
SGR (% day ⁻¹)	0.86 ± 0.34	1.33 ± 0.29	1.77 ± 0.71
Survival (%)	86.33 ± 16.66	83.33 ± 16.66	50.00 ± 16.67
Feed intake (% BW day ⁻¹)	2.30 ± 0.19	2.26 ± 0.28	2.33 ± 0.43
Wr	0.98 ± 0.09	0.88 ± 0.07	1.00 ± 0.10
Nutrient efficiencies			
Protein efficiency ratio (mg ww gain/mg dw protein intake)	0.60 ± 0.16	1.29 ± 0.32	1.19 ± 0.47
Lipid efficiency ratio (mg ww gain/mg dw lipid intake)	2.26 ± 0.86	4.65 ± 1.24	5.04 ± 1.96
Energy efficiency ratio (mg ww gain/kJ energy intake)	17.83 ± 4.81	36.87 ± 8.92	35.42 ± 13.85
Feed conversion ratio (mg dry weight feed intake/mg wet gain)	4.94 ± 1.66	4.31 ± 3.02	2.53 ± 1.38

Values with different superindexes are different with $P < 0.05$. Values with * show trends with $0.05 < P < 0.095$.

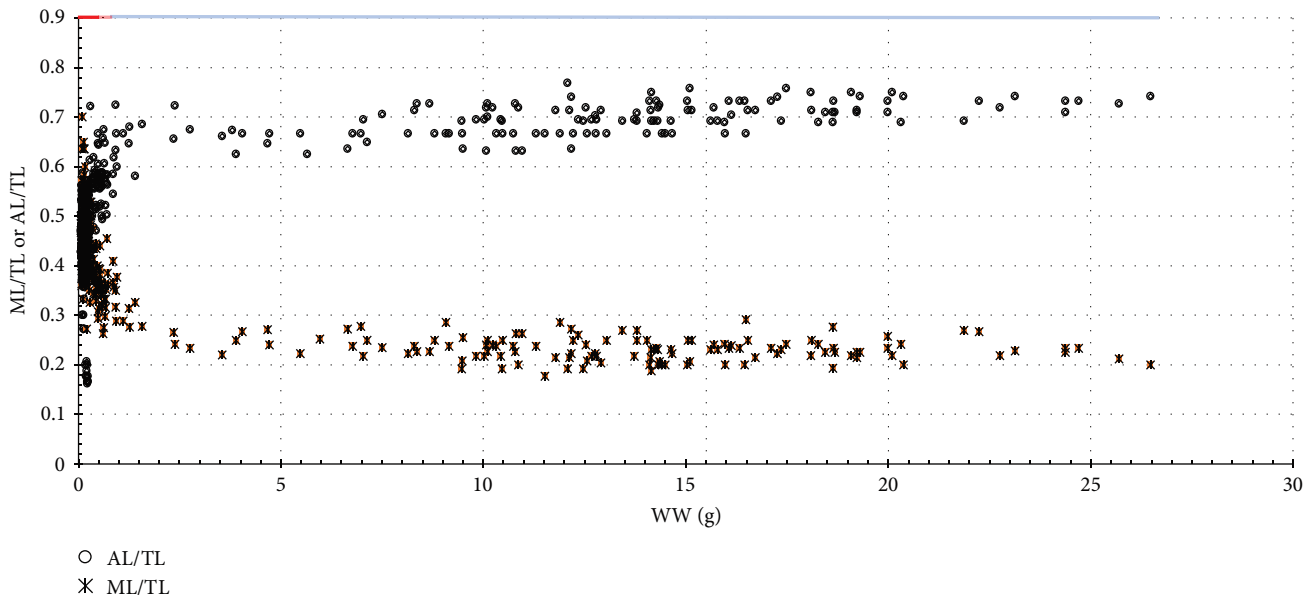


FIGURE 4: Changes in body proportions ML/TL and AL/TL of *E. megalocyathus* paralarvae and early juveniles as weight increases. The red line shows the weight range for paralarvae stages, while the blue line shows weight range for juvenile stages.

liquid that could improve the supply of energy and amino acids for embryonic development.

The ineffective crossing or lack of fertilization of the conditioned females with fertile males generated clutches without embryonic development, which was independent of the diets. Future studies should focus on nondisruptive indicators of conditioned females and males to determine the optimal moment to carry out the crosses and avoid failed fertilization.

It is interesting to note that females' loss weight during egg-brooding was independent of the fertilization success and it was related only with the size of the eggs through a negative exponential slope ($R^2 = 0.69$). As a result, we suggest two hypotheses that should be tested in the future: (1) females spend their body reserves incubating eggs whether

fertilized or not, so it is likely they do not distinguish between the two situations and (2) females having greater weight loss with smaller eggs than with those larger, probably due to higher energy expenditure when there are more units to clean during maternal care.

Regarding embryos, this is the first study that reports the results of incubating embryos at 8°C and shows that development can be completed at this temperature. This result indicates that under natural conditions of 8°C, females would require 9 months without feeding to care for their offspring. According to Robinson et al. [29], *Graneledone boreopacifica* octopus females incubate eggs for 53 months without feeding in the Arctic Sea at depths greater than 1,000 m. In this sense, a 9-month incubation period for *E. megalocyathus* could be common in the Magallanes Region, which constitutes the

southern limit of the species where 4°C were registered [30]. The low survival of embryos at 8°C (16%) and a biological zero at 1.5°C could also imply a lower abundance that would partly explain the scarce fishery of this species in its sub-Antarctic limit.

Temperature is a key factor for the speed of embryonic development of cephalopods and for the different stages, such as preorganogenesis, organogenesis, and growth until hatching [31]. Under controlled incubation, Uriarte et al. [7] documented the hatching efficiency of Patagonian red octopus eggs with the best results at 14°C (65.8%) and the worst results at 16°C (28.6%). They also observed that temperatures of 15 and 16°C did not shorten the development time with respect to 14°C, nor did these temperatures cause deformations of the embryos. In the current study, at 18°C, the incubation time was not reduced compared to 14°C, but there were deformed embryos and the incubation efficiency was reduced to a negligible level of 0.4%, indicating that at 18°C reproduction is not possible.

Reduction of incubation time has been tested in other octopus species with similar results. In *O. maya*, the embryo development time was reduced by 50% when embryos were incubated at 30°C compared with embryos maintained at 22 or 26°C [32]. Even though 30°C accelerated the rate of embryo development, the high temperature had negative effects on development, causing high metabolic rates, lower growth rates, and poor use of yolk reserves, leading to deformations and the death of a high proportion of embryos compared with those produced at lower temperatures. It was also observed that at temperatures above 27°C, embryos experienced changes into the antioxidant defense system and were not able to recover, suggesting that there is an equilibrium between the embryo development and the time that animals require to successfully complete the development processes. The effects of high temperature were also observed in *O. vulgaris* embryos, where temperatures higher than 18°C provoked an excess of radical oxygen species [33]. That result suggests that, as was observed in *E. megalocyathus* megalocyathus embryos, octopus species have evolved to develop in specific thermal ranges where growth, survival, and maximum performance is expressed both in the wild and in aquaculture conditions [34]. At the three temperatures tested for *E. megalocyathus* embryos, they showed similar ATU and DD. So, degree-days are not decreased with increasing temperature as proposed by Márquez et al. [31]. The reason may be the loss of linearity at 18°C for *E. megalocyathus* embryos.

The biochemical composition of the Patagonian red octopus embryos was not affected by temperature, except for the protein content of the chorion. This could be indirectly related to temperature because the increase in temperature has been observed to cause an exponential increase in respiratory rate in *Octopus mimus* [35] and *E. megalocyathus* embryos [7]. Therefore, the reduction in chorionic protein associated with higher temperature could be linked with a need to reduce the thickness of the chorion layer that wraps around the egg to increase oxygen exchange as temperature rises.

Another interesting aspect of the embryos is that at 12°C, the development time was constant under both maternal incubation and in artificial incubators [2, 7], respectively). However, the hatchability or survival of the embryos until hatching was affected, reaching only an average of $15.3\% \pm 8.2\%$ under maternal care [2] and almost tripling to $42.8\% \pm 7.6\%$ in an artificial incubator [7]. This difference could be related to the risk that they may be detached and/or swept away in the presence of the female octopus [2]. Maternal care involves cleaning the eggs with their arms and directing jets of seawater from the funnel to the eggs, leading to the gentle rubbing of eggs against one another [36]. During this time, brooding females usually decrease their food intake or reject food completely, which frequently leads to their death around the end of the egg-brooding period [37]. In captivity, using an artificial incubator will depend on the water quality obtained in each facility. If the water quality of the incubator is not enough to maintain healthy embryos, it is better to maintain the eggs in maternal care, mainly in merobenthic octopus species that spawn 100,000 to 700,000 eggs per female [38]. In species such as *O. tetricus* lower survival of embryos was attributed to an accumulation of invertebrate fauna in the incubator that was presumed to have killed the eggs [38]. On the other hand, if the water quality of the incubator is high and free of fauna and pathogens, then it is preferable the use an incubator that will reduce the maintenance labor of the production of the embryo and allow sold the female before the loss of their quality due to maternal care. In *O. vulgaris*, on the contrary, no differences were found in development time when comparing both types of incubation [34].

One of the most critical aspects in the culture of octopus paralarvae is feeding, which explains a large part of the mortality in this stage of development for several species [10, 39]. In *O. sinensis* [8], feeding paralarvae with marine zooplankton increased the growth rate of paralarvae by 4.9 times and survival in more than nine times compared to *Artemia*. In the feeding experiment of *E. megalocyathus*, survival rate increased by four times when Patagonian red octopus paralarvae were fed copepods, but growth rate did not improve compared to *Artemia*. The positive correlation of the DHA/EPA ratio with paralarvae survival showed that the higher quality of fatty acids in copepods can explain the improved survival, while the negative correlation of survival with $18:1n-7$ explains the higher mortality of paralarvae fed with *Artemia*. The fact that the growth rate did not improve with copepods could be related to the copepod size, which was 2 mm in length, the lower limit to feed newly hatched *E. megalocyathus* paralarvae according to Espinoza et al. [12]. Therefore, while the diet was probably of high nutritional quality, the ration was suboptimal for growth. The SGR was favored mainly by the ingestion of prey; therefore, the paralarvae fed copepods or *Artemia* showed similar growth. However, in the case of juvenile crabs, the paralarvae had difficulty preying on them, and when there was ingestion, it was through the joint action of several paralarvae attacking a crab (in situ observation). Until now, cofeeding on juvenile crabs had been documented from 60 DAH [2];

however, these current results show that some paralarvae manifest predation behavior of benthic juvenile crabs from 1 to 15 DAH. The foregoing can be related to the difference in the AL/ML ratio of the paralarvae 15 DAH observed between batches, corroborating that some paralarvae attain a ratio $AL/ML > 1$ earlier than others.

Regarding the comparison between groups of *E. megalocyathus* paralarvae, it is again evident that arm lengthening is the main characteristic of the transition to benthic life, as described by Dan et al. [40] for *O. sinensis*. According to the morphometric relationships studied for *E. megalocyathus*, AL/ML remains under one (<1) almost until 15 DAH, then rises to 1.1 at 25 DAH and remains almost constant until 50 DAH where it reaches 1.2 and then progressively increases until 90 DAH. According to the characterization of Dan et al. [40], the AL/ML ratio < 1.0 is typical of planktonic life, while $AL/ML > 1.0$ indicates the transition to benthic life. On the other hand, the paralarvae produced between 2012 and 2016 had less arm-length development than the paralarvae of the 2017–2021 period, which could be associated with both genetic variability and the progressive standardization of the *E. megalocyathus* paralarvae culture [11, 12, 22].

Regarding the effect of temperature in accelerating growth of Patagonian red octopus juveniles, this is the first study on newly settled juveniles. Although there were no significant effects due to temperatures of 11, 13, and 15°C on SGR and survival, a trend toward lower FCR and higher PER were observed at 15°C, which would mean that at this temperature the juveniles better utilize the food for growth. Another aspect of this study showed that the highest SGR and lowest FCR were observed in the juveniles with the highest AL/ML ratio showing that this index reflects a relevant capacity to improve their feeding efficiency for this phase of development. The negative correlation between survival and temperature shows that there is a possible decrease in the homeostasis of juveniles by increasing the temperature up to 15°C. Future studies should determine whether such high temperatures can be used throughout the on growing until harvest size while maintaining Patagonian red octopus welfare.

According to Robin et al. [41], the most delicate changes and the highest mortality in cephalopods correspond to the transition from embryo to paralarvae and from paralarvae to subadult. It is interesting to note that juveniles from 2017 batch reached a 10 g individual much faster than those from 2012. This could be due to differences in the transition from paralarvae, since at the time of settlement, paralarvae from the 2017–2021 batches presented a higher TL than the paralarvae of the 2012–2016 batches, which could have favored greater prey intake and accelerated growth. During the juvenile period, the main morphometric characteristic was the elongation of the arms that began at 1.5 times ML at the time of settlement (1 DPS) and reached values higher than 2.5 times ML at 135 DPS. Dan et al. [40] defined the fourth phase of truly benthic *O. sinensis* juvenile as that in which the development of the octopus has almost been completed, acquiring the proportions required for the benthic phase. This phase, according to the same authors, starts when ML

is 32% of TL and AL is 50% of TL, with AL continuing to increase with respect to TL. Given this definition, the Patagonian red octopus would be fully benthic at 35 DPS when the mantle and arms represent around 30% and 60% of TL, respectively. Therefore, in the first juvenile period of this study, between 1 and 35 DPS, *E. megalocyathus* would be in the third phase of development, the provisionally benthic stage, which begins when the mantle represents 37% of TL.

It is interesting that under controlled culture conditions, paralarvae have a lower growth rate than early juveniles and that both stages demonstrate positive allometry between weight and total length, although it is 56% higher in juveniles than in paralarvae. This is consistent with the delicate stage of the paralarvae and with the robustness that the juveniles acquire when they start benthic life.

Therefore, it is very important to have basic indicators that allow conditions to be determined during rearing without sacrificing the organisms, as is the case of Wr, PER, and FCR. In the case of paralarvae, these indicators are expected to improve when a more suitable diet to be found for this stage of development.

5. Conclusions

In conclusion, this study shows as follows:

- (1) The female protects and attends to the eggs throughout the period of development of the embryo, regardless of whether the eggs are fertilized.
- (2) The biological zero for Patagonian red octopus embryos is 1.5°C.
- (3) The egg-brooding period to reach stage XX of hatching is linearly reduced with the increase in temperature up to 14°C when the minimum incubation time is reached.
- (4) The survival of paralarvae-fed copepods and juvenile crabs quadrupled compared to those fed *Artemia*.
- (5) The best relative weight indicator for paralarvae is $Wr = WW/0.53 \times TL^{2.07}$, WW in mg and TL in mm.
- (6) The on-growing of juveniles at 15°C leads to a lower FCR and higher PER without significantly affecting the survival.
- (7) The best relative weight indicator for juveniles is $Wr = WW/0.028 \times TL^{2.398}$, WW in g and TL in cm.

Data Availability

Data available upon reasonable request.

Disclosure

The funders had no role in the design of study; in the analysis, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

Conflicts of Interest

The authors declare no conflicts of interest.

Authors' Contributions

Iker Uriarte and Carlos Rosas contributed to conceptualization. María Hernández, Fernanda Peñailillo, Nicole Montero, and Ranferi Gutiérrez contributed to data curation. María Hernández, Fernanda Peñailillo, Nicole Montero, Ranferi Gutiérrez, and Ana Farías contributed to formal analysis. Iker Uriarte, Carlos Rosas, and Ana Farías contributed to funding acquisition. Iker Uriarte, Jorge Hernández, and Viviana Espinoza contributed to investigation. Iker Uriarte, Jorge Hernández, and Viviana Espinoza contributed to methodology. Iker Uriarte contributed to supervision. Ana Farías contributed to validation. Ana Farías, Carlos Rosas, and Iker Uriarte contributed to writing—review and editing.

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Supplementary Materials

Figure S1: *E. megalocyathus*. The lost weight by females during incubation in relation of hatching percentage of the spawned eggs from experiment 1 with females conditioned with three different fresh diets 100% crab (black circle), 100% squid (grey circle), and 50% crab + 50% squid (empty circle). Each value is one female and its clutch. Eggs with 0% hatching were not fecundated by males before spawning, so these eggs not shown embryo development nor hatching. Figure S2: *E. megalocyathus*. The lost weight by females during incubation in relation of egg size as percentage of female weight from experiment 1 with females conditioned with three different fresh diets: 100% crab (black circle), 100% squid (grey circle), and 50% crab + 50% squid (empty circle). Each value is one female and its clutch. Figure S3: *E. megalocyathus*. Protein and lipid content of just spawned eggs after reproductive conditioning from experiment 1 with three different fresh diets. The values are mean of three different females and its clutches. Figure S4: *E. megalocyathus*. The perivitelline protein of egg at the beginning of incubation in relation of female diet expressed as protein/energy ratio (P/E) from experiment 1 with females conditioned with three different fresh diets: 100% crab (35 mg kJ^{-1}), 100% squid (51 mg kJ^{-1}), and 50% crab + 50% squid (42.8 mg kJ^{-1}). Each value is a sample of 10 eggs per clutch. Figure S5: *E. megalocyathus*. Time development in degree-days was calculated as ATU and DD, and hatching in percentage of incubated eggs for embryos reared at three temperatures. Each point is the mean of the three independent clutches belonging to different females. Values with different superindexes are different with $P < 0.05$. (Supplementary Materials)

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