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Key aspects of egg incubation in Patagonian red octopus (*Enteroctopus megalocyathus*) for cultivation purposes

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ABSTRACT

Patagonian red octopus, *Enteroctopus megalocyathus*, is a valuable resource of the artisanal fishery in southern Chile, however, due to overfishing the *E. megalocyathus* fishery was banned for 3 years in Chile, therefore its cultivation became a target for the Chilean aquaculture. The rearing of octopus paralarvae is currently the biggest bottleneck for the aquaculture of merobenthic octopus species. Besides, the embryos of Patagonian red octopus require 5 months for embryonic development, a very long period of incubation that involves high risks of contamination and detachment, therefore, little advance has been achieved in the larviculture of this species. This study represents the first investigation to evaluate the embryo viability, embryo morphometrics, embryo growth and the biochemical composition and fatty acid dynamics during early development of Patagonian red octopus under captive reproduction. The eggs obtained from broodstock conditioning were incubated under maternal care and the embryos were studied until hatching in their main morphometric and biochemical features during development. Most females showed a tending behavior of the eggs along the incubation period, losing between 40 and 100% of the eggs in the first 3 months of incubation. The results of incubation at 11 °C were successful observing the complete gastrulation, onset of organogenesis and first inversion about 48 days after spawning, the complete organogenesis and second reversion were observed about 152 days after spawning, hatching was observed 168 days after spawning without any external yolk-sac on the hatched paralarvae. During their development, embryos showed an exponential growth rate in length and weight, fueled by the protein and lipids of external yolk-sac. Metabolism of lipids showed over a 70% depletion of the saturated fatty acids (SFA), and the fatty acids 16:1, 18:2n-6, 18:3n-3 and 22:5n-3 during development. The DHA/EPA ratio remained constant throughout the incubation period. The early embryos showed a fatty acid profile dominated by both SFA and highly unsaturated fatty acids (HUFA), while the newly hatched paralarvae showed a profile dominated by HUFA.

1. Introduction

Over the last few years, aquaculture research in Chile has been strongly focused towards the diversification of species that have problems in fisheries and are demanded in the global markets. Two Chilean octopus species have been investigated as serious candidates for aquaculture in terms of their biological and market potential, one of them is the Changos octopus (*Octopus mimus*), found from Tumbes, Northern Perú (3° 34′ 00″S) to San Vicente Bay in Chile (33° 37′ 60″N), and the other is found in Southern Chile (from 34° 20´S to 72° 00´W to the Beagle Channel in Chile, up to Southern Argentina), known as Patagonian red octopus, *Enteroctopus megalocyathus* (Cardoso et al., 2004; Ibáñez and Chong, 2008; Ortíz et al., 2006; Rocha and Vega, 2003). In November 2008, the *E. megalocyathus* fishery was banned for 3 years in Chile, a reason that has made their aquaculture an important issue (Uriarte et al. 2011).

According to Uriarte et al. (2011), the embryo incubation is a bottleneck for the aquaculture of this particular species, and this difficulty adds up to the problem of cultivating paralarvae of developing octopuses with merobenthic development (Berger, 2010; Iglesias et al., 2007).

It is highly likely that *E. megalocyathus* might be a species that stems from a predecessor of direct development belonging to clade 5 as proposed by Ibáñez et al. (2013) that re-evolved into a species with pelagic paralarva, therefore, according to these authors it should be a species with a low to moderate fertility, large eggs and a paralarva that should be very large, with ambiguous swimming behavior in between pelagic and benthic. The information available from the literature shows that *E. megalocyathus* fits well with the predictions of Ibáñez et al. (2013) because under culture conditions this species has shown moderate spawning that does not exceed 5000 eggs sized approximately 10 mm

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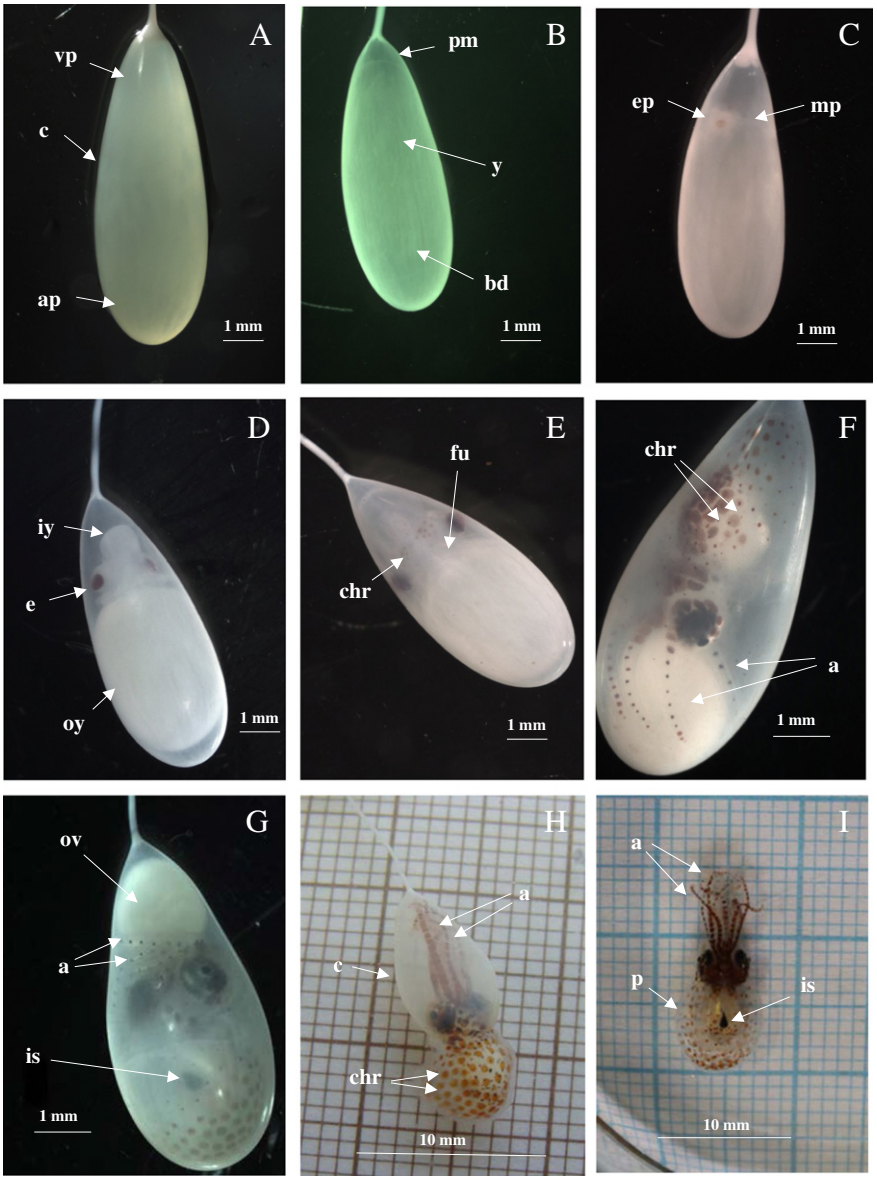


Fig. 1. Embryo development stages for *E. megalocyathus*. A) Embryo of 4 days after spawning (stages I–II of Naef), *c*, chorion; *vp*, vegetal pole; *ap*, animal pole. B) Embryo of 29 days after spawning (stage V of Naef), blastoderm covers 30% of egg surface (*bd*) in the animal pole, perivitelline membrane (pm) spreads over the yolk (*y*) towards the vegetal pole. C) Post-first inversion. Embryo of 67 days after spawning (stage IX of Naef), *mp*, mantle primordium; *ep*, eye primordia. D) Embryo of 83 days after spawning (stage XIII of Naef), *e*, eye; *iy*, inner yolk-sac; *oy*, outer yolk-sac. E) Embryo of 102 days after spawning (stage XV of Naef), *fu*, funnel; *chr*, chromatophores. F) Embryo of 134 days after spawning (stage XVIII of Naef), *chr*, chromatophore; *a*, arms. G) Post-second inversion. Embryo of 152 days after spawning (stage XIX of Naef), oy, outer yolk-sac; a, arms; is, ink sac. H) Hatching. Embryo of 172 days after spawning (stage XX of Naef), *c*, chorion; chr, chromatophore. I) Paralarva of 173 days after spawning, P, paralarva; a, arms; is, ink sac.

(Uriarte et al., 2013), the hatched paralarvae exceed 10 mm and they present a swimming behaviour that ranges in between pelagic and benthic which has been described as suprabenthic hatchlings by Ortíz et al. (2006) and planktonic hatchlings *Enteroctopus*-type by Villanueva and Norman (2008).

The embryonic development and maturation of the embryo of *E. megalocyathus* to generate a large hatching paralarva have not been achieved easily; there are descriptions of 1) hatchlings from embryonated eggs captured on ground and reared for 88 days in laboratory at 11.7 °C (Ortíz et al., 2006), 2) critical bacterial infection during the embryonic period under controlled conditions (Uriarte et al., 2011) and 3) absence of embryonic development in 12 clutches obtained from broodstock conditioning under controlled conditions (Farías et al., 2011). Embryo development depends on the nutritional reserves of the egg, making relevant the studies on feeding females during the reproductive conditioning period that showed a strategy to maintain the quality of eggs under unfavorable environmental conditions (Farías et al., 2011). Sargent et al. (1999) have shown that in cold and hard environments fish larvae hatch with a high lipid content to ensure the survival, which could be the case for *E. megalocyathus* paralarvae hatching in the southern waters of South America. In cold marine waters, fish larvae show essential requirements of highly unsaturated fatty acids (HUFA i.e.,fatty acids with 20 or more carbon atoms and 3 or more double bonds) such as arachidonic acid (AA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), to maintain high DHA for neural development and to encourage a best species specific ratio EPA:AA affecting eicosanoid actions (Sargent et al., 1999), this would also be expected from newly hatched paralarvae of *E. megalocyathus* in Patagonic waters of South America.

This study aimed to characterize the morphometric variations and nutritional reserves of *E. megalocyathus* embryos from spawning to paralarvae hatching, obtained under controlled conditions of

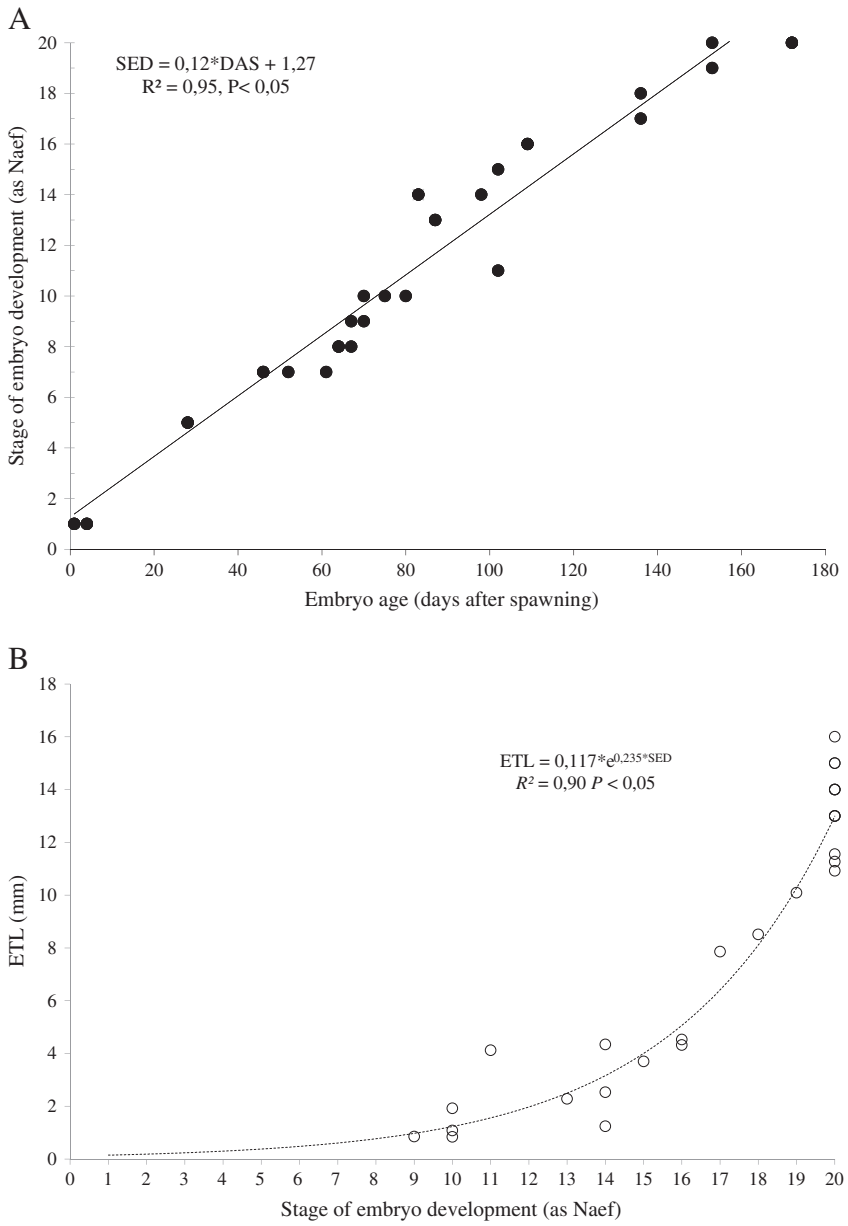


Fig. 2. *E. megalocyathus*. A) Stages of embryo development (*SED*) in relation to the age of the embryo, B) Embryo total length (*ETL*) depending on the stage of embryo development. *DAS* is the age registered as days after spawning. R^2 is the coefficient of determination and *P* indicates the significance of the regression.

reproduction, to improve both egg incubation protocols obtaining development indicators over the five month period of embryonic life, and the future formulation of broodstock diets for more efficient captive reproduction of Patagonian red octopus *E. megalocyathus*.

2. Material and methods

2.1. Conditioning of females

16 females weighing 0.79 kg (± 0.079) and 12 males weighing 0.60 kg (± 0.031) of *E. megalocyathus* were captured in their natural environment at Hueihue, Xth region of Chile (42° lat.S), and transferred to conditioning tanks in the Marine Invertebrate Hatchery at Universidad Austral de Chile (HIM-UACH). The octopuses were kept in individual 100 L tanks using a circulation system with 5 μm-filtered and UV-sterilized sea water, and a 5 to 10% daily water exchange. Salinity was kept at 30‰ and the temperature at 11 ± 1 °C using a temperature control system and a chiller. A fresh diet based on crabs, squids and fish was dosed at a feed ratio of 5% body weight day^{-1}. During the reproductive conditioning period, which lasted between 4 and 6 months, the females increased their weights 2.8 times, when they began to reduce their food intake and were paired with 2 or more males according to the size ratio recommended by Gutiérrez et al. (2012). When the spawnings begun, the egg clutch was left to the care of females, during this period the females were fed a maintenance diet based on silverside fish bits (*Odontestes* sp), until they died at different times during or after the incubation period.

2.2. Morphometric analyses and yolk quantification on the embryonic stages

For this study was selected the best clutch showing the highest percentage of complete embryonic development, and the lowest percentages of infection, and detached eggs from the walls of the den. Twice every week 2 eggs were randomly sampled from the clutch to monitor the developmental stages. The eggs and their corresponding embryos were measured and photographed using a light microscope

(Stemi 2000-C) coupled to an Axio Cam (ICc3; Zeiss) camera. The stages of embryonic development were defined according to Naef (1928) and the photographs were used to determine morphometric relationships: egg length (EL), egg weight (EW), embryo total length (ETL), mantle length (ML), arm length (AL), and eye diameter (ED) during the development period until hatching.

The yolk sac volume was estimated by superimposing standard geometric forms onto the shape of the yolk sac (Uriarte et al., 2009; Vidal et al., 2002). The anterior yolk sac, also called the outer yolk sac, was measured by superimposing cylindrical, or spherical forms during embryonic development. The formulas used to determine the anterior yolk sac volume were:

i. Cylindrical volume (CV) $= \pi^{*}r^{2*}H$
ii. Spherical volume (SV) $= 4/3^{*}\pi^{*}r^{3}$
iii. Ellipsoid $= (4^{*}\pi^{*}AB^{2})/3$

where r was the ratio of the cylinder base or the ratio of the sphere, π was 3.1416, H was the cylinder length, A and B were ratio of half of the length and half of the width of the yolk sac, respectively. Volumes of anterior and posterior yolk sac were then added together to form the total yolk. Yolk volumes were multiplied by a density of 1.036 mg mm^{-3} to convert them to wet weights (Vidal et al., 2002). The criteria of Naef (1928) represented with Roman numerals were used to discriminate the developmental stages until hatching.

2.3. Gravimetric and biochemical analyses of the eggs

On days 1, 55, 84, 102 and 118 after spawning two samples of 10 eggs were randomly collected from the clutch under maternal care, totaling 100 sampled eggs. After 118 days, the eggs were not manipulated again to avoid premature hatching, resulting in paralarvae still having their outer yolk-sac. Newly hatched paralarvae without outer yolk-sac were sampled as representatives of the last stage of development. The eggs were individually measured for length with a caliper (± 0.1 mm) and weighed on a Sartorius analytical scale (± 0.0001 g), their contents were then emptied into a previously weighed Eppendorf tube, centrifuged at 1000 rpm at 4 °C for 10 min, separating the supernatant corresponding to the perivitelline liquid in a new tube from the precipitate corresponding to the yolk plus the embryo, both samples were freeze-dried in a SAVANT freeze-dryer and weighed to determine their dry weight, then they were ground to make them available for biochemical analysis.

The carbon, hydrogen, and nitrogen content were analyzed with a LECO autoanalyzer CHN over 1 mg freeze-dried samples weighted in a Mettler Toledo micro scale using a precision of 0.1 μg. Crude protein was obtained after multiplying 6.25 by nitrogen content. The total lipid content was gravimetrically obtained after extracting the freeze-dried yolk samples through the Bligh and Dyer method (1959). Energetic value of protein was 23.7 KJ g^{-1} and lipid was 39.5 KJ g^{-1}. Methylation and quantification of fatty acids were carried out according to the methodology of Bell et al. (1993). Fatty acid methyl ester (FAME) from the total lipids was analyzed in a THERMO gas chromatographer, equipped with an auto sampler and a split-splitless injection system. The separation was done using hydrogen as the transporting gas in a RESTEC RT-2560 capillary column of 100 m with 0.25 mm internal diameter and 0.2 μm film, at an initial temperature of 140 °C during 5 min and a temperature of 140 °C to 240 °C during 20 min at a 4 °C/min rate and blocking it at 240 °C the last 20 min, with the detector at 260 °C. Nonadecenoic acid (19:0) was used as the internal standard, and the FAMEs were identified by comparison with the retention timed observed in the Mix FAME Supelco 37 and the Sigma docosapentaenoic acid (DPA; 22:5n-3).

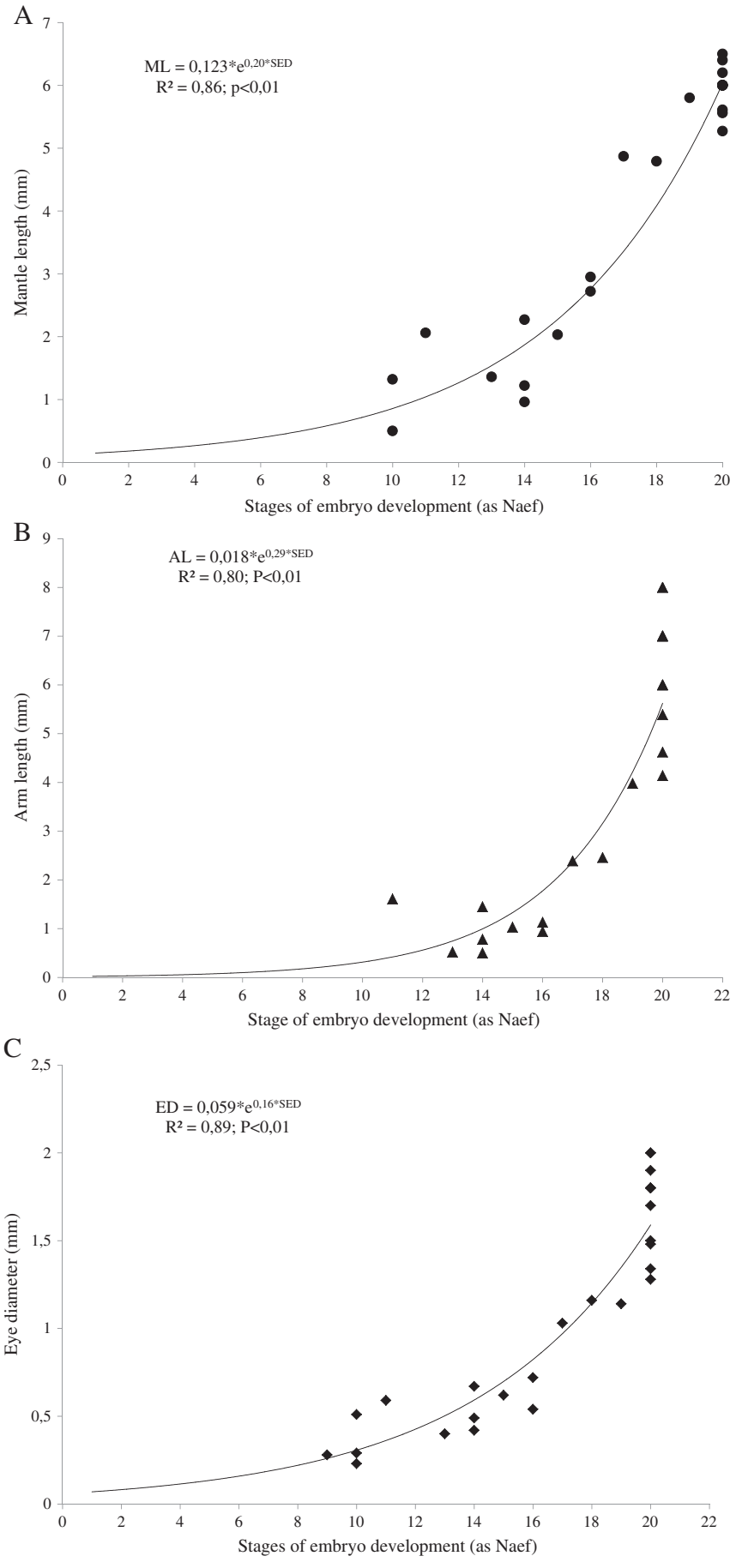
2.4. Data analysis

Exponential equation was used to relate EL, ETL, AL, ML, or ED with octopus age (t): morphological characteristic (mm) $= a * e^{bt}$, where e was the base of the natural logarithm, the constants a and b were the intercept and slope, and t was the age in days during embryonic development. Other models were also adjusted to the morphological data as required (linear: $Y = bX + a$, or logarithmic: $Y = a + b^{*}\ln(X)$, or allometric: $Y = a^{*}X^{b}$; where Y was the dependent variable and X was the independent variable). Data were analyzed statistically in order to compare morphometric differences among embryos of different ages. Fatty acids were compared between the stage VII embryo (early organogenesis) and the newly hatched paralarva (in absolute and relative values). All fatty acids were analyzed giving emphasis to the essential fatty acids AA, EPA and DHA. The fatty acid consumption during the embryonic period was estimated by comparing the stage VII embryo and the newly hatched paralarva. One way analysis of variance (ANOVA) was used when data were normal. No normal data were analyzed using a Kruskal–Wallis test (Sokal and Rohlf, 1981).

3. Results

Out of 16 females that conditioned, 11 had laid eggs on the walls of their dens (69%) and four clutches showed complete embryonic development (36.4%). The average absolute fecundity was 2095 eggs (±425) and average relative fecundity was 924 eggs kg^{-1} (±198). The clutches consisted of bunch sets of 34 eggs (±3, $n = 110$ bunch sets). Two clutches of eggs were totally swept by the females (18.2% of egg clutches). Five clutches did not show any organogenesis signs (45.4%). From 4 egg clutches with embryonic development, females swept up and detached to 40% of the eggs, and it was observed a reduced paralarvae hatching due to bacterial contamination and non-developed eggs. Hatching rate was 15.3% (±8.2, $n = 4$ clutches), and the selecting criteria for the study were choosing the clutch that had been less sweeping by the female and showed the lowest egg contamination and highest egg hatching.

E. megalocyathus eggs are centrolecithal, consisting mainly of yolk with an outer shell or corion that forms the stalk in the vegetal pole (Fig. 1A). Eggs post-fecundation had an active segmentation during the first days that was evident with the formation of a blastodisc in the animal pole, while the yolk remained undivided. During the gastrulation process, the blastodermic cells migrate over the yolk surface and at day 29 in stage V it covers 30% of the egg surface (Fig. 1B), whereas at day 48 in stage VII it already covers 60% of the egg, reaching 100% in stage VIII at 64 days forming the yolk sac. During gastrulation between stages IV and VII, the embryo starts the first reversion and it is placed on the base of the egg in the stage VIII. At stage VII was conspicuous that the eggs were actually fertilized since it was then possible to observe the organ primordia. From stage VII, due to organogenesis, the pattern of the body of the embryo starts to develop and gradually differentiates from the yolk, with the retinal pigment appearing in the stage IX (Fig. 1C), and being completely separated from the yolk in stage XIII (Fig. 1D). From stage VIII, each one of the germinal units originating the arms can be recognized as rudiments, being the 8 arms clearly distinguished in stage XIII (Fig. 1D), when they begin to extend. Among the organogenesis processes associated to gastrulation, the formation of the optical complex stands out, especially pigmentation of the retina which is already observed in stage IX (Fig. 1C), reaching the cup form at stage XIV and its maximum coloration intensity in stage XV (Fig. 1E). The complex pallial-mantle can be outlined early in stage IX, in the form of a protuberance in the embryo's cephalic portion and gains characteristic morphology in stage XV (Fig. 1E). The chromatophores appear on the dorsal and ventral areas of the head in stage XV (Fig. 1E), and are totally visible on the arms in stage XVIII at 134 days, when arms cover approximately 50% of the external yolk sac (Fig. 1F). The complete organogenesis and second reversion were observed about 152 days after spawning at the end of stage XIX, when hatching may occur by external stimulus (Fig. 1G). At 172 days the embryo has absorbed all of the external yolk, reaching the stage XX (Fig. 1H) and hatching as planktonic paralarva (Fig. 1I).


A
ML = 0,123*e0,20*SED
R² = 0,86; p<0,01
Mantle length (mm)
Stages of embryo development (as Naef)
B
AL = 0,018*e0,29*SED
R² = 0,80; P<0,01
Arm length (mm)
Stage of embryo development (as Naef)
C
ED = 0,059*e0,16*SED
R² = 0,89; P<0,01
Eye diameter (mm)
Stages of embryo development (as Naef)

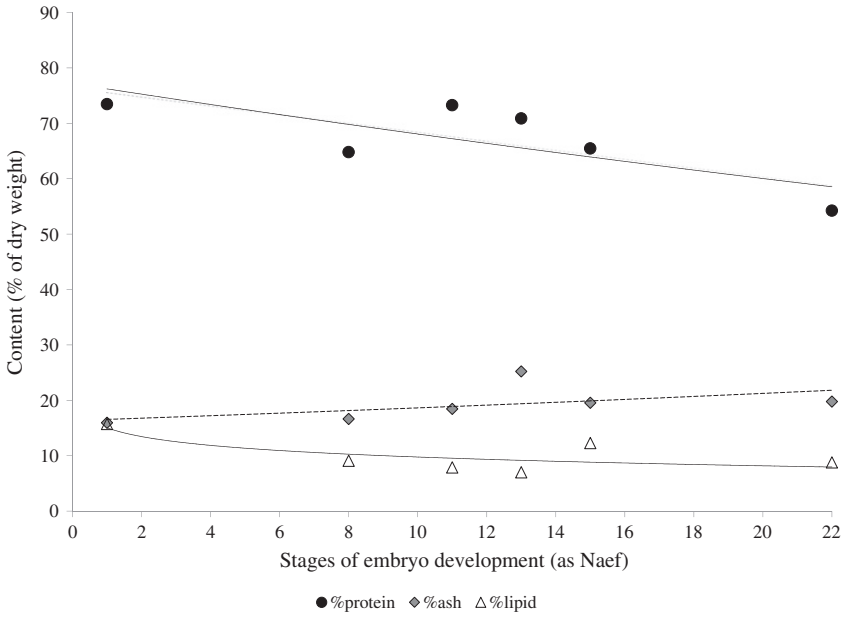


Fig. 4. *E. megalocyathus* protein, lipid and ash content of embryo-yolk set with regard to the stages of embryo development.

The eggs of the selected clutch measured 8.35 ± 0.42 mm and weighed 57.3 ± 0.6 mg wet weight immediately after they were laid (Fig. 1A) and by the end of the embryonic period reached 10.05 ± 0.03 mm and a wet weight of 171 ± 22.5 mg. The eggs grew exponentially in size at a rate of 0.1% day^{-1} and in weight at a rate of 0. 9% day^{-1}. Egg length (EL) and egg weight (EW) showed an allometric relationship along embryonic development as $EW = 0.001EL^{5.21}$ ($R^2 = 0.81$ $P < 0.00001$). The embryonic developmental stages increased linearly in relation to the age of the embryo (Fig. 2A) at incubation temperature of 12 °C with maternal care, while the total length of the embryo increased exponentially depending on the stage of development (Fig. 2B).

The total length of the mantle, the arms, and the eye diameter increased exponentially along embryonic development at rates of 0.20, 0.29, and 0.16 $stage^{-1}$, respectively (Fig. 3A, B, C).

At the time of hatching, paralarvae showed a length of 14.1 mm (±0.35) and a wet weight of 148.7 mg (±10.6). Paralarvae at hatching time showed a planktonic behaviour with swimming by jet propulsion in a column water of 50 cm with intermittent short periods close to the tank bottom. The ratio between arm length and the mantle length (AL/ML) in new-born paralarvae was 1.13 (±0.05).

It was observed that the yolk weight (EY) decreases exponentially with the age of the embryo (DAS = days after spawning), reaching its lowest value after 150 days, right before they hatch ($EY = 1.75{*}e^{-0.019*DAS}$; $R^2 = 0.75$; $P << 0.001$). It was observed a lineal decrease in perivitelline protein with reduction of 79.4 after 118 days of embryonic development ($F = 5.93$; $df = 4, 7$; $P = 0.02$). The biochemical composition of embryo-yolk set, from spawning until the day of hatching of the paralarva, showed an exponential decrease of the protein, from 73 to 54% dry weight and a log reduction of lipids from 16 to 8% dry weight (Fig. 4), while ash remained relatively constant at around 19.3% dry weight throughout embryonic development. The decrease in the weight of external yolk converted into energy of protein and lipid of the vitellus (Fig. 5) showed that the major source of energy for the development of the embryo came from protein (80–90%), followed by 10–20% of the energy provided by lipid.

The comparison of fatty acid profiles between stage VII embryos and newly hatched paralarvae showed a decrease of 57.92% of the total fatty acids during development (Table 1). It was observed that the newly hatched paralarvae had less of nearly all fatty acids than embryos, including essential fatty acids AA, EPA and DHA, which resulted in the smaller amounts of SFA, MUFA, PUFA, HUFA, PUFA n-3 and PUFA n-6 at the time of hatching. The highest percentages of consumption during development were observed in fatty acids 4:0, 8:0, 11:0, 15:0, 16:0, 16:1, 17:0, 18:2n-6, 18:3n-3, 22:5n-3 between stage VII embryos and newly hatched paralarvae. Saturated fatty acids were the most consumed during the period of embryonic development (69.77%). Therefore, the embryos showed a profile of fatty acids dominated by SFA and HUFA while paralarvae showed a profile dominated by HUFA. In both, stage VII embryos and newly hatched paralarvae, n-3 PUFA had much higher values than n-6 PUFA, whereas MUFA were much lower than PUFA (Table 1). In relative amounts the n-3 PUFA consumption was similar to n-6 PUFA, but in absolute amounts the consumption of n-3 PUFA reached 10.98 μg mg^{-1} DW while in n-6 PUFA it was only 2.15 μg mg^{-1} DW. The fatty acid 16:0 was the main fatty acid that carried out energy functions with a contribution of 15.98 μg mg^{-1} DW during the embryonic period. The fatty acids AA, EPA and DHA decreased during embryonic development although the DHA/EPA ratio was constant between the beginning of embryonic development and paralarva, and the EPA/AA ratio decreased slightly.

4. Discussion

This study represents the first investigation to evaluate the viability, morphometrics, growth and the biochemical composition and fatty acids dynamics during embryo development of Patagonian red octopus under captive reproduction.

The tending and loosening of eggs in most observed spawnings in this study could be a normal maternal behavior to remove contaminated eggs. The fact that some females lost all the laid eggs could be a response to stress caused on them by the sampling of eggs.

The stages observed during the embryonic development of *E. megalocyathus* were similar to those reported in several octopus

Fig. 3. *E. megalocyathus* clutch selected for morphometric analyses: relationship between A) mantle length (*ML*), B) arm length (*AL*) and C) eye diameter (*ED*) along of stages of embryo development (*SED*). R^2 is the coefficient of determination and *P* indicates the significance of the regression.

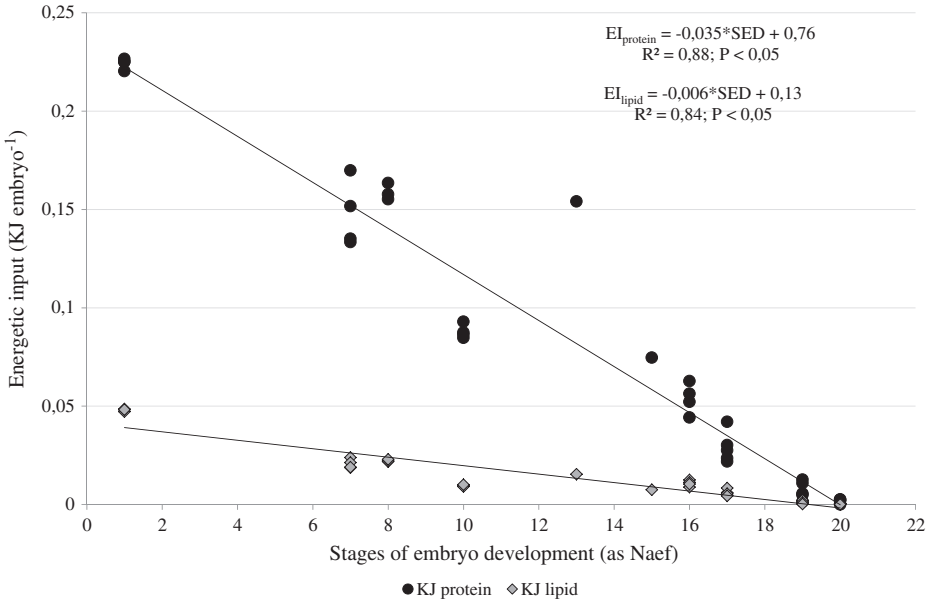


Fig. 5. *E. megalocyathus*. The protein and lipid of external yolk-sac used as energy source along the stages of embryo development (*SED*). R^2 is the coefficient of determination and *P* indicates the significance of the regression.

species (Boletzky, 2003). Taking into account the morphologic features from the moment the eggs were laid until the hatching of paralarvae (planktonic hatchling), the following phases were observed: post-fecundation, segmentation, gastrulation, first reversion, second reversion and hatching (Boletzky, 2003; Castro et al., 2002), what required 8.15 days for each stage of development (Fig. 2A) during which the embryo grew exponentially (Fig. 2B).

Both mantle and arms grew exponentially throughout the development, with highest rate for arm length, which determined that the newborn paralarvas had arms 13% longer than the mantle. These long arms were expected for large planktonic hatchlings (*Enteroctopus*-type) as has been described by Villanueva and Norman (2008).

E. megalocyathus embryos showed lower growth rates (0.9% day^{-1}) than those described for *R. fontaniana* (1.1% day^{-1}) (Uriarte et al., 2009), suggesting that subpolar waters of Patagonian environments

Table 1
Enteroctopus megalocyathus. Absolute (μg mg^{-1} DW) and relative (%TFA) fatty acid methyl esters (FAME) in total lipids in embryos plus yolk (55 DAS) and new born hatchlings (173 DAS). Mean values ± standard errors of as content of dry weight and as % of total fatty acids. DAS is days after spawning, ESVII is embryo at stage VII, NB is new born hatchling.

	Absolute FAME (μg mg^{-1} DW)		Relative FAME (%TFA)		Embryonic consumption	
	ESVII 55 DAS	NB 173 DAS	ESVII 55 DAS	NB 173 DAS	μg mg^{-1} DW	%
4:0	2.13 ± 1.60	0.34 ± 0.23	2.89 ± 0.56	1.03 ± 0.39	1.79	83.98
6:0	0.06 ± 0.06	0.35 ± 0.24	0.05 ± 0.05	1.03 ± 0.44	−0.29	
8:0	0.32 ± 0.28	0.08 ± 0.08	0.37 ± 0.18	0.45 ± 0.45	0.24	75.16
11:0	1.12 ± 0.77	0.22 ± 0.19	1.64 ± 0.11	0.61 ± 0.40	0.90	80.24
15:0	0.30 ± 0.25	0.03 ± 0.03	0.35 ± 0.16	0.94 ± 0.62	0.27	90.43
16:0	22.13 ± 15.18	6.15 ± 1.17	32.33 ± 2.14	23.18 ± 5.01	15.98	72.2
16:1	1.13 ± 0.66	0.27 ± 0.13	1.83 ± 0.18	0.89 ± 0.11	0.86	76.03
17:0	0.89 ± 0.58	0.20 ± 0.03	1.36 ± 0.01	0.87 ± 0.46	0.69	77.6
18:0	4.57 ± 2.96	2.02 ± 0.68	6.97 ± 0.02	7.16 ± 0.44	2.55	55.73
18:1n-9	1.73 ± 1.24	1.49 ± 0.59	2.42 ± 0.32	5.11 ± 0.05	0.24	13.89
c18:2 n-6	0.21 ± 0.14	0.00 ± 0.00	0.30 ± 0.03	0.39 ± 0.39	0.21	100
20:1n-9	3.84 ± 2.41	1.80 ± 0.24	5.99 ± 0.22	6.95 ± 1.89	2.04	53.15
18:3n-3	0.48 ± 0.24	0.09 ± 0.09	0.84 ± 0.18	0.52 ± 0.52	0.39	80.78
22:1n-9	0.43 ± 0.13	0.65 ± 0.12	0.91 ± 0.39	2.47 ± 0.55	−0.22	
20:3n-3	0.16 ± 0.05	0.41 ± 0.14	0.09 ± 0.09	1.45 ± 0.10	−0.24	
20:4n-6	4.22 ± 2.81	2.40 ± 0.69	6.30 ± 0.19	8.67 ± 1.00	1.82	43.1
20:5n-3	9.83 ± 6.26	4.70 ± 2.53	15.18 ± 0.31	15.13 ± 2.82	5.12	52.15
22:5n-3	0.22 ± 0.17	0.00 ± 0.00	0.22 ± 0.00	0.00 ± 0.00	0.22	100
22:6n-3	11.56 ± 6.62	6.06 ± 3.38	19.11 ± 2.31	19.30 ± 4.13	5.50	47.57
TFA	65.85 ± 42.74	27.71 ± 10.19			38.14	57.92
SFA	31.53 ± 21.68	9.53 ± 2.45	45.97 ± 3.20	35.70 ± 4.42	22.00	69.77
MUFA	7.12 ± 4.45	4.40 ± 1.21	11.16 ± 0.47	15.97 ± 2.05	2.73	38.31
PUFA	27.19 ± 16.61	13.78 ± 6.53	42.88 ± 2.73	48.33 ± 6.47	13.41	49.32
HUFA	26.05 ± 15.98	13.57 ± 6.74	40.95 ± 2.45	44.54 ± 5.85	12.48	47.9
n-3	22.24 ± 13.35	11.26 ± 5.96	35.43 ± 2.88	36.39 ± 6.33	10.98	49.36
n-6	4.55 ± 3.08	2.40 ± 0.69	6.72 ± 0.34	9.06 ± 1.40	2.15	47.3
n-3/n-6	5.36 ± 0.70	4.34 ± 1.23	5.36 ± 0.70	4.34 ± 1.23		
MUFA/PUFA	0.26 ± 0.01	0.33 ± 0.08	0.26 ± 0.01	0.33 ± 0.08		
DHA/EPA	1.26 ± 0.13	1.27 ± 0.04	1.26 ± 0.13	1.27 ± 0.04		
EPA/ARA	2.41 ± 0.12	1.81 ± 0.53	2.41 ± 0.12	1.81 ± 0.53		

influence differently both sympatric species probably because the size of the eggs and the adults is very different [*E. megalocyathus* with eggs of 8–10 mm and adults weighing about 3 kg, (Farías et al., 2011; Ortíz et al., 2011), *R. fontaniana* with 2 mm eggs and adults weighing around 0.07 kg (Ortíz and Ré, 2011, Uriarte et al., 2009)]. The difference on the egg sizes could explain that the eggs of *R. fontaniana* take only half the time to develop (70 days) compared to the eggs of *E. megalocyathus*. However, both species also show differences in paralarval behavior, being both planktonic. A purely planktonic paralarva has been described for *R. fontaniana* (Uriarte et al., 2010), while for the Patagonian red octopus an ambiguous planktonic-benthic behavior has been described, which is called suprabenthic behavior (Ortíz et al., 2006).

The low speed at which Patagonian red octopus embryos use the nutrients in the yolk is consistent with a big egg, in which the female makes efficient accumulation of the nutrients during spring and summer. Thus, females of Patagonian red octopus prefer spawning eggs from summer to the fall (Chong et al., 1999), and the eggs will spend part of the fall and winter (5 months) under embryonic development until they hatch just in spring, when the environmental conditions for planktonic prey availability may be high in the Pacific and Atlantic tip of South America.

Furthermore, apart from the above mentioned strategy it could be added that there is a compromise between female fecundity and quality of the eggs, which has been observed in the female of Patagonian red octopus under laboratory conditions of food limitation, with a reduction of fertility without losing the quality of the eggs (Farías et al., 2011). There was a net decrease in yolk-sac protein content until hatching, suggesting that most proteins incorporated in the vitellum must have been either consumed as an energy source or converted into other components. As protein was determined by CHN it means that protein and free aminoacids (FAA) were measured as protein, future studies will have to discriminate if the FAA in the *E. megalocyathus* embryos are as relevant as in other embryos (Krautz et al., 2010, Martínez et al., 2008).

The high reproductive investment of octopuses in a single spawning makes relevant the feeding of the females, because the yolk is the only source of nutrients during the long period of embryonic development of this species. Furthermore, according to Tocher et al. (2008) and Tocher (2010) the cold water marine carnivores are organisms with increased demand for highly unsaturated fatty acids, used to form membrane phospholipids which regulate membrane fluidity, and with a high demand for DHA to form a well-developed nervous system that ensures predatory skills of newly hatched individuals, therefore it is expected that fatty acids of embryos and paralarvae and *E. megalocyathus* are dominated by n-3 HUFA, especially DHA and EPA. On the other hand, AA levels were relatively high, which is typical in species thriving in stressful environments (Sargent et al., 1999). During the embryonic development of *E. megalocyathus* it could be observed a significant reduction of SFA and low tendency to use HUFA, keeping the DHA:EPA ratio, this is similar to what is observed in species from cold waters with large eggs (Faleiro and Narciso, 2010). On the other hand, according to Lahdes et al. (2010) the combination MUFA/PUFA in membrane phospholipids is essential regarding the adaptation of biological membranes to temperature, and since the MUFA/PUFA value observed in the total lipids of embryos and paralarvae varied between 26% and 33% (Table 1) it would suggest that *E. megalocyathus* is a cold-adapted stenothermic species, which might be very affected by the temperature modification to improve the embryonic development period.

We can conclude that from the incubation of Patagonian red octopus eggs generated from broodstock conditioning, it is possible to obtain 15% of hatching with females themselves being highly responsible for the loss of eggs. Further studies are needed to shorten the period of embryonic development, since the currently reported difference of 150 to 172 days indicates that it is possible to achieve changes in this period by the temperature incubation. Given the strong influence that temperature can have on changing the speed of development in this species, it is interesting to study that the developing stages were consistently associated with growth without detrimental effects of elevated temperature in the percentages of hatchability or survival of early hatchlings.

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