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**Embryonic development, hatching time and newborn juveniles of *Octopus tehuelchus* under two culture temperatures**

Ramiro Braga<sup>a,b</sup>, Silvina Van der Molen<sup>a</sup>, Julián Pontones<sup>a,b</sup>, Nicolás Ortiz<sup>a</sup>

<sup>a</sup>Laboratory of Cephalopods, Instituto de Biología de Organismos Marinos, IBIOMAR-CONICET. Boulevard Brown 2915, (9120), Puerto Madryn, Chubut, Argentina.

<sup>b</sup>Universidad Nacional de la Patagonia San Juan Bosco. Ciudad Universitaria, 25 de Mayo km 4- (9000), Comodoro Rivadavia, Chubut, Argentina.

Corresponding author:

Nicolás Ortiz

nicortiz@cenpat-conicet.gob.ar

Phone: +54 280 4883184

**Abstract**

The development of cephalopods early life stages is strongly influenced by environmental variables, especially temperature. *Octopus tehuelchus* (d'Orbigny, 1834) is an Atlantic Patagonian fishery resource currently being studied as a new species for cultivation; however it is not known how temperature modulates its early life stages. In this work, egg masses were artificially incubated at 13 and 16°C under controlled aquarium conditions. In each thermal treatment, the stages of embryonic development, embryo morphometry and survival throughout embryogenesis, as well as embryogenesis duration were recorded. After hatching, the morphological description of the juveniles was achieved and survival time in starvation was calculated for both temperatures. At 16°C the mean embryonic duration was 85 days shorter than at 13°C. For both thermal treatments, the highest mortalities occurred up to the beginning of organogenesis, and no significant differences in hatching success were observed. The temperature also showed the potential to increase or decrease the juvenile performance at the early post hatching period. This resulted in a significant reduction in size and weight of new born juveniles at 13°C but also in an average increment of 7 days in their survival in starvation when compared to octopus reared at 16°C. The chromatophore pattern was similar for both thermal treatments and was characteristic of juveniles of this species. The observed differences seem to show adaptive mechanisms that optimize embryos and juveniles viability under the different environmental temperatures that can be found in the northern Atlantic Patagonian coast. From a practical point of view, our findings are important to define the biological parameters and associated procedures for the cultivation of the early life stages of *O. tehuelchus*.

**Keywords:** cephalopod, incubation temperature, octopus culture, hatchlings performance, octopus early life stages.

## 1. Introduction

The knowledge of the biological aspects of cephalopod's early life stages and a precise understanding of the species responses to environmental factors is required to achieve significant survival values under culture (Boletzky and Villanueva, 2014; Oosthuizen et al., 2002; Sakurai et al., 1996; Uriarte et al., 2018; Vidal et al., 2002a). From an ecological perspective, this information is relevant to understand the influence of environmental factors on the population structure and dynamics of octopuses in wild populations (Boletzky, 2003, 1989; Juárez et al., 2015; Villanueva et al., 2016).

Octopodidae species may exhibit a holobenthic or merobenthic mode of life, which is determinant both for culture experiences and for a better understanding of species distribution. In holobenthic species the full life cycle is associated with benthos (Iglesias et al., 2014; Vidal et al., 2014; Villanueva et al., 2016): the hatched juveniles are large and benthic, which favors their feeding and survival under controlled culture conditions (Rosas et al., 2007; Solorzano et al., 2009; Vidal et al., 2014). In contrast, in merobenthic octopuses, hatchlings are planktonic before they reach the benthic mode of life (i.e. paralarvae) and the lack of appropriate food under culture conditions for the planktonic stage gives rise to a high mortality rate that has so far hampered their scaling production (Espinoza et al., 2017; Farías et al., 2016; Uriarte and Farías, 2014; Valverde et al., 2019; Zúñiga et al., 2013).

Whether they are holobenthic or merobenthic species, temperature directly influences the cephalopods' life cycle (Forsythe et al., 2001; Iglesias et al., 2014; Schwarz et al., 2018; Villanueva et al., 2016). It is the main factor modulating the duration of embryogenesis. Also, the thermal condition affects especially the embryo development and growth, the yolk absorption, and the hatching rate (Boletzky, 1987; Grigoriou and Richardson, 2008; Uriarte et al., 2016; Vidal et al., 2014). In several

cephalopod species, the duration of embryogenesis can be shortened when the incubation of the eggs is carried out at high temperatures within the species optimum temperature range (Caamal-Monsreal et al., 2016; Uriarte et al., 2016, 2012). Temperatures at the extremes of the thermal range generate morphological anomalies and death of the embryos, low hatching success, and a deficiency in the use of inner yolk in newborn hatchlings, causing low growth rates and short survival times (Rosa et al., 2012; Vidal and Boletzky, 2014; Vidal et al., 2002b; Villanueva et al., 2003; Zheng et al., 2014).

Of the few species of coastal octopods found in the Argentine Sea, the “Pulpito” *Octopus tehuelchus* is the most studied, particularly its ecological, biological and fisheries aspects (Fassiano et al., 2017; Iribarne et al., 1991; Ré, 1998; Storero and Narvarte, 2013). This species endemic to South America is found in the Atlantic Ocean between the south of Brazil (16°S) and the northern part of Argentine Patagonia (44°S), and from the intertidal up to 100m deep (Narvarte et al., 2006; Ré, 1998; Ré and Ortiz, 2011; Storero et al., 2010). In North Patagonia coastal areas, in the southern limit of its distribution, *O. tehuelchus* artisanal fishery has been carried out since the 1950s (Bocco et al., 2019; Narvarte et al., 2006; Ré, 1998). Currently, this fishery is not only the support of the fishermen community but also benefits the local gastronomy by positively influencing the growth of the economy of the area (Narvarte et al., 2006; Ré, 1998). *O. tehuelchus* is a holobenthic species, with a small adult size (up to 150 g) and a seasonal reproductive cycle (Ré, 1998; Storero et al., 2010). In North Patagonia, the laying and brooding season runs from autumn to spring, frequently in shallow subtidal areas (<20m) (Iribarne, 1990; Ré, 1998; Storero et al., 2010). The species is categorized as simultaneous terminal spawner and its fecundity is low, with a maximum of 220 eggs per laying. The egg clutches are generally attached to rocks or mollusks' shells; they are

conformed by large eggs (9-12 mm long x 3-5 mm width and a stalk 4-6 mm long), arranged individually or in groups of two or three eggs, giving raise to large benthic juveniles (Iribarne, 1990; Pujals, 1986; Ré, 1998; Storero et al., 2012).

Experiences carried out under controlled aquarium conditions indicate that *O. tehuelchus* can adapt well to captivity (Berrueta et al. 2020; Iribarne et al., 1991; Klaich et al., 2008, 2006). For example, Klaich et al. (2006) showed that at 15°C the rates of instantaneous growth and daily feeding in adults and subadults were higher than at 10°C. Little has been documented on the early life stages of this species. Previous attempts performed in aquariums by Ré (1989) suggested that the embryogenesis could demand 120 days at 15°C, and that juveniles can survive in starvation up to 10 days. However, there is a lack of studies regarding the embryonic development, juvenile morphology and performance, and their relation to temperature.

The existing knowledge about the biological attributes of *O. tehuelchus*, its good tolerance to captivity, the absence of a paralarvae stage, and its great acceptance in the regional market, in addition to a global context with high demand for this type of products (FAO, 2018; Vidal et al., 2014) generate great interest in the cultivation of this species. The comprehension of how temperature modulates embryogenesis and newly born juveniles is crucial to evaluate the biological culture feasibility and to define and plan the conditions for the *Octopus tehuelchus* early life stages culture. In turn, determining how temperature affects populations would help to understand the distribution and abundance of this species in nature. Thereby, the aim of this work is to determine and evaluate the effect of two thermal regimes on embryos development time, and on the morphology and survival of embryos and newly hatched juveniles.

## 2. Materials and methods

### 2.1. Capture and eggs conditioning

*Octopus tehuelchus* spawning females along with their egg clutches were collected during the 2016 spawning period. Specimens and clutches were gathered at a depth of 10 m using traps in the northwest coast of Nuevo Gulf (Argentina) (42° 42'S 65° 36' W), on the north Atlantic Patagonian coast. These traps were hoisted by diving and checked on the vessel to verify the presence of eggs and spawning females. Traps with spawning females and eggs were carried in aerated seawater to the CCT CONICET-CENPAT (Centro Científico Tecnológico, Consejo Nacional de Investigaciones Científicas y Técnicas - Centro Nacional Patagónico) Experimental Aquarium where the specimens were acclimated for 24 hours.

### 2.2. Artificial incubation

Egg clutches were separated from females and the embryo development stages were determined under a magnifying glass following Naef (1928). The 20 stages of Naef's embryonic scale encompass the blastulation (stages I-II) and gastrulation (stages III-VI) processes, stage VII, which is considered a transitional phase, and the organogenesis (stages VIII-XVI) and post-organogenesis (stages XVII-XX) processes. Five complete egg clutches of 68, 44, 40, 64 and 40 eggs between stages I-II were selected to perform the incubation experiments. The eggs in each clutch were randomly separated into two groups with the same number of eggs and were artificially incubated in controlled temperature chambers at two experimental temperatures:  $13\pm 1^{\circ}\text{C}$  and  $16\pm 1^{\circ}\text{C}$  (i.e. the seawater temperatures found during the spawning and brooding period in the sampling area) (Ortiz et al., 2011; Williams et al., 2018). For these experiments, five glass aquariums with five liters of filtered (10, 5, and 1  $\mu\text{m}$ ), UV-sterilized, and aerated seawater were used for each temperature. Water in the aquariums was partially replaced

every two days and its quality was monitored daily. Dissolved oxygen was  $>5$  mg/l, pH range 7.5-8.4, total ammonia concentration lower than  $1 \text{ mg L}^{-1}$  and salinity of 33-35 ppt. The photoperiod was 12 h light: 12 h dark.

To allow a constant circulation of water over the eggs, proper visual inspection and correct handling, each egg group was incubated in plastic mesh baskets. In the absence of the females' parental care, the eggs were cleaned by the same operator with a fine brush (Ortiz and Ré, 2011; Ortiz et al., 2006) every three days, thus avoiding fouling accumulation on the eggs' chorion, which acts as a barrier between the embryo and the external environment (Parra et al., 2000; Uriarte et al., 2012). During handling, the eggs were held by the remaining cement of the egg stalk to avoid any kind of embryo disturbance, either by breaking the stalk or the embryo chamber. Manual cleaning was interrupted at stage XIX to prevent any premature hatching (Ortiz and Ré, 2011) that may reduce survival chances (Boletzky and Villanueva, 2014; Vidal et al., 2014).

### *2.3. Embryos: morphological analysis, survival and development time*

The eggs were monitored daily and potentially non-viable eggs (i.e. eggs with abnormal development) were counted, assigned to an embryonic stage and separated in an individual plastic mesh to verify the non-viability with time. Random samples of 5 eggs from each aquarium were observed every 7 days under a stereo microscope to check the embryo development stage. From these data, the relative embryonic survival (RES%) was calculated as  $\text{RES}\% = n^\circ \text{ viable eggs in stage } i \times 100 / n^\circ \text{ viable eggs in stage } i-1$ , where  $i$  is the embryonic stage in which the non-viability of the egg was registered. In addition, total embryo survival was evaluated, and the embryonic development duration at each temperature treatment was recorded. Likewise, the hatching period was defined as the difference in time between the first and the last



hatching in the same laying (Vidal et al., 2014). In addition, every 10 days, eggs were photographed under a stereomicroscope, and embryos' morphological parameters (mm) such as dorsal mantle length (DML), arm length (AL), eye diameter (ED) and outer yolk sac volume (OYSV) were obtained as development indicators, with the ImageJ software (Schneider et al., 2012). The OYSV ( $\text{mm}^3$ ) was calculated as  $\text{OYSV} = \frac{4\pi AB^2}{3}$ , where  $A$  is the half of the outer yolk sac length, and  $B$  is the half of the outer yolk sac width (Uriarte et al., 2014; Vidal et al., 2002a).

#### *2.4. Juveniles: morphological description and starvation survival time*

For the morphological descriptions, a total of 33 one day post-hatching juveniles from the 13°C treatment and 34 from the 16°C treatment were randomly sampled from all aquariums. The juveniles were anesthetized using 96% ethanol, thus minimizing stress (Butler-Struben et al., 2018). Ethanol was gradually added to the seawater, starting at 0.5% v/v and increasing to 1 and 1.5% at 2 minute intervals until sedation. Afterward, using the same methodology as with the embryos, the following morphometric measurements (mm) were obtained: eye diameter (ED), mantle width (MW), head width (HW), ventral mantle length (VML), total length (TL), dorsal mantle length (DML), arm length (AL), and funnel length (FL). In addition, the total wet weight (TW) (in grams), the chromatophore pattern, and the number of suckers were recorded. The morphometric measurements and the description of juveniles were made following Hochberg et al., (1992), Sweeney et al., (1992) and Young et al., (1989). For observations with scanning electron microscopy (SEM), samples were fixed in 2.5% glutaraldehyde in phosphate buffer for 3 hours and then dehydrated with the critical point method for subsequent gold coating (Ortiz and Ré, 2011).

To calculate survival time in starvation, juveniles hatched in each thermal treatment were separated by hatching date in three-liter plastic aquariums. A total of 62 juveniles

distributed in 36 aquariums at 13°C, and 44 juveniles distributed in 21 aquariums at 16°C, were studied (density: 1 specimen/liter). Juveniles were kept under the same temperature, photoperiod, and water quality conditions that they were incubated in. In the rearing tanks, two pebbles per octopus were provided as refuge.

### 2.5. Data analysis

Throughout the embryonic development, each morphological parameter was compared between and within temperature treatments. For this, a linear mixed effects model was constructed (Pinheiro et al., 2016), where the morphometric parameters were considered as the response variable, the temperature and embryogenesis time as fixed explanatory variables and the aquarium as a random explanatory variable. A generalized least squares model was used to evaluate the effect of the temperature on the morphological parameters on the same day of embryogenesis. In turn, a Tukey test was used to compare among embryogenesis days within each thermal treatment. To analyze the effect of temperature and embryo development stages on RES%, a generalized linear model of mixed effects (GLMM) (Zuur et al., 2009) with a binomial distribution (link=logit) was performed, considering the aquarium as a random variable. To analyze the effect of temperature on total embryonic survival, a generalized linear model (GLM) with a quasi-binomial distribution (link=logit) was used. To evaluate the effect of temperature on the survival time in starvation of juveniles, a GLM with a Gamma distribution (link= inverse) was constructed. In this case, the aquarium was not used as a random variable since it explained only 0.06 % of the total variance. For model selection, the Akaike information criterion was used for each analysis. The effect of temperature on morphological parameters of newborn juveniles was evaluated with a one-way Anova. In addition, to assess the relationship between starvation survival time

and hatching time, Pearson's correlation coefficient was calculated (Zar, 1996). The aquariums were considered as replicates, except when comparing morphological parameters of the newly born juveniles in which the specimens were considered as replicates. Statistical analyzes were performed with the R statistical package, version 4.0.0, the level of significance used was  $p < 0.05$ .

### 3. Results

#### 3.1. Embryo development time and morphological description

Temperature strongly affects the embryogenesis of *O. tehuelchus*. Embryonic development (mean  $\pm$  SD) took  $186 \pm 8.68$  days until the first hatching at  $13^{\circ}\text{C}$  and  $101 \pm 5.68$  days at  $16^{\circ}\text{C}$ . The average ( $\pm$  SD) hatching period was  $38 \pm 12.5$  days at  $13^{\circ}\text{C}$  and  $21 \pm 12.58$  days at  $16^{\circ}\text{C}$ . The time elapsed between the first and the last hatching was 68 and 37 days at  $13^{\circ}\text{C}$  and  $16^{\circ}\text{C}$  respectively (Fig. 1). For the same incubation time, embryos treated at  $16^{\circ}\text{C}$  presented a more advanced development stage than those treated at  $13^{\circ}\text{C}$ . At  $16^{\circ}\text{C}$ , the progress of embryonic development seemed to be nearly constant until hatching. However, at  $13^{\circ}\text{C}$  two phases could be distinguished: the first one running from stages I-II up to the end of organogenesis at stages XV-XVI, and the second, slower than the first, going from stages XV-XVI up to hatching (Fig. 1).

For both temperatures, during the first 30 days of embryonic development, most of the eggs were in blastulation, with the formation of the germinal disc at the animal pole (stage I-II) (Fig. 2A), or with the blastoderm cells migrating over the surface of the yolk (gastrulation process) and covering the egg partially (Fig. 2B) or totally, a process that occurs from stage III to stage VI. From day 45, at both temperatures most of the embryos were at the vegetal pole (i.e. end of the first inversion). At this time the perivitelline membrane encloses the embryo, the egg chamber becomes internally separated from the stalk and the organ rudiments of the embryo (seen as an increase of

distinct tissue concentration) begins to appear (i.e. beginning of organogenesis) (stage VIII) (Fig. 2C, D). On day 50, all embryos under the higher temperature treatment were between stages IX-X. In these stages, the retina pigment in the eyes is evident, the mantle complex in the cephalic region is more developed, and more elongated arms with the first recognizable suckers were observed (Fig. 2E). However, none of the embryos subjected to 13°C exceeded stages VII-VIII (Fig. 1). On day 65, at 16°C some of the embryos reached stage XIII-XIV, with the mantle and the arms more elongated, the suckers more developed (Fig. 2F) and the optical complex in the form of a cup; at 13°C no embryo exceeded stages IX-X (Fig. 1). By day 75, most embryos subjected to higher temperatures were between stages XV-XVI, where the first chromatophores appear on the dorsal head, the eye becomes full pigmented and the systemic and branchial heart is pulsating. Between days 90 and 95, most embryos at 16°C reached stages XVII-XVIII. At stage XVIII, chromatophores begin to appear in mantle and arms and the inner yolk sac become larger (Fig. 2G), while at 13°C most embryos are still at stages IX-X. At 16°C most embryos reached stages XIX-XX between days 100 and 105, while at 13°C those stages were attained between days 180 and 200 (Fig. 1). At stage XIX, the embryos return to the animal pole (end of the second inversion), and the outer yolk sac is almost completely absorbed (Fig. 2H). After the complete absorption of the external yolk, specimens would be ready to hatch. Not all embryos made the first inversion, and complete their normal development directly at the animal pole side (Fig. 2F).

### 3.2. *Embryos morphological analysis*

The ED and AL were significantly affected by temperature and embryogenesis time, while DML and OYSV were significantly affected by the interaction between both

factors (Table 1). Significant differences were firstly observed on day 45 of embryonic development when DML, ED, and AL were larger in the embryos subjected to higher temperatures (Table 2, Fig. 3 A, B, C). For OYSV statistical differences were registered at day 65, with lower volumes in embryos maintained at 16 than those at 13°C (Fig. 3D). These trends continued until the end of embryonic development (Table 2, Fig. 3). Regarding comparisons within temperature treatments, embryos at 13°C showed a significant increase in DML ( $F=51.91$ ,  $p<0.0001$ ), ED ( $F=18.01$ ,  $p<0.0001$ ) and AL ( $F=73.91$ ,  $p<0.0001$ ) from day 75 on, while at 16°C this pattern was observed from day 45 onwards (DML:  $F=35.10$ ,  $p<0.0001$ ; ED:  $F=28.58$ ,  $p<0.0001$ ; AL:  $F=23.70$ ,  $p<0.0001$ ). However, at 16°C, the OYSV decreased significantly ( $F=35.32$ ,  $p<0.0001$ ) from day 65 onwards, while at the lower temperature this was evident twenty days before ( $F=38.10$ ,  $p<0.0001$ ).

### 3.3. Embryonic survival

Relative embryonic survival (RES%) was significantly affected only by the embryo development stages ( $\text{Chisq} = 6.653$ ,  $p = 0.0098$ ). For both temperature treatments, the highest mortality was recorded in the early stages of embryogenesis (Fig. 4). The lowest RES% occurred between stages III and VI (88% and 86% for 13°C and 16°C, respectively), and it increased between stages IX and XII until reaching its maximum levels (100%). Towards the end of embryonic development (stages XIX-XX), the RES% decreased slightly, reaching percentages of 96% at 16°C and 95% at 13°C (Fig. 4). Regarding total embryo survival, no significant differences were observed between temperatures ( $\text{Chisq}=0.0257$ ,  $p=0.8724$ ) (Fig. 5), the average hatching success was 64%.

#### 3.4. Morphological features of juveniles

At hatching, the large *O. tehuelchus* juveniles completely adopt the benthic mode of life, lying on the floor or hiding in shelters. No significant differences were found in the DML, AL and FL. However, the ED, MW, HW, VML, TL and TW were significantly higher in juveniles from the higher temperature treatment (Table 3).

In both thermal treatments, specimens presented their head, arms, interbrachial membrane (arm webs), eyes, dorsal mantle, and posterior cap of the mantle densely covered in small brown-reddish and black tegumental chromatophores. In the funnel, the anterior margin of the ventral mantle and the perivisceral epithelium there was no pigmentation. The mantle has a flask shape (Fig. 6). For each arm, a total of 50 suckers along long stalks were counted. The two suckers closest to the mouth were arranged linearly, whereas the rest were disposed in a zigzag row and decreased in size towards the tip, which lacked terminal suckers. There were emerged fascicles of the Kölliker's organs spread over the skin of the arms, mantle and head (Fig. 7). These bristle-like structures could also be seen as iridescent dots in living animals under incident light (Fig. 6).

#### 3.5. Juveniles' survival time in starvation

Even when juveniles' maximum survival time was similar between treatments (54 and 59 days at 13 and 16°C, respectively), the survival time in starvation was significantly higher (Chisq = 5.6482,  $p = 0.0174$ ) in juveniles subjected to the lower temperature treatment compared to those under a higher temperature (24 and 17 days on average at 13 and 16°C, respectively). For both temperatures, the Pearson correlation coefficient between day of hatching and survival time in starvation was significant and

negative ( $p < 0.0001$ ,  $r = -0.51$  and  $p = 0.0109$ ,  $r = -0.38$  for 13 and 16 °C), this means that the firsts to hatch survived longer (Fig. 8). No cannibalism was observed among juveniles.

#### 4. Discussion

Cephalopods' embryo development time is characterized by the yolkiness of the eggs and it is modulated by temperature (Boletzky, 2003). In general terms, small eggs present a shorter embryonic development than larger ones, and species adapted to cold water environments show longer embryogenesis duration than tropical ones (Boletzky, 1987, 1984; Clarke, 1982; Vidal et al., 2014). Furthermore, within the range of temperature adaptation of cephalopod species, higher temperatures promote shorter embryonic development time without affecting the hatching success (Boletzky, 2003; Caamal-Monsreal et al., 2016; Uriarte et al., 2016). Beyond the thermal threshold, embryos experience irreversible physiological damages (Pimentel et al., 2012; Repolho et al., 2014; Sánchez-García et al., 2017). In *Octopus tehuelchus*, a temperate species which lays large eggs, the results of the present study showed a long embryological period, of around six months at 13°C, which can be reduced by half with a 3°C increase, without showing significantly thermal-related effects on the relative nor the total embryonic survival. Moreover, the average hatching success of *O. tehuelchus* (64%), was similar to that found in artificial incubation experiences under optimal thermal conditions for *Enteroctopus megalocyathus* (39.6%-65.8%), a species adapted to cold water (Uriarte et al., 2016) and for *O. maya* (60-76%), a tropical species (Caamal-Monsreal et al., 2016).

Under culture conditions, the period that goes from hatching up to stage VIII, when organogenesis begins, seems to be critical for the survival of *O. tehuelchus* embryos,

regardless culture temperature. During that period the blastulation and gastrulation processes take place, and according to Boletzky (2003), the growth of the blastula is a phase susceptible to malformations. Particularly, nonviable eggs at these stages exhibited loss of turgidity, swelling and abnormal yellow coloring, as it was observed in *E. megalocyathus* (Uriarte et al., 2011) and *Loligo vulgaris reynaudii* (Oosthuizen et al., 2002). *O. tehuelchus* nonviable eggs also showed alterations such as a collapsed or deformed (i.e. granulated or partially obliterated) yolk mass, which result in abnormal morphogenesis and unviable embryos. In addition, during these development stages there is no separation between the egg chamber and the egg stalk; this connection is interrupted when the previtelline membrane is fully shaped at stage VIII. The connection between the egg chamber and the stalk has been only described for *O. bimaculoides* (Monsalvo-Spencer et al., 2013), a species that also produces large eggs and benthic hatchlings. This particular embryological feature should be considered in order to avoid *O. tehuelchus* embryonic mortality due to stalk break by wrong egg handling before the beginning of organogenesis.

Temperature has a conditioning effect on the embryonic metabolic processes, such as the rate and efficiency of yolk absorption, and growth of the embryos (Boletzky, 1987; Caamal-Monsreal et al., 2016; Uriarte et al., 2012; Vidal et al., 2002b; Villanueva et al., 2007). In this sense, *O. tehuelchus* embryonic development showed different patterns between incubation temperatures. While at 16°C embryo development rate seems to be nearly constant throughout embryogenesis, at 13°C it slows down from stages XV-XVI until hatching (i.e. post-organogenesis). Two similar phases were observed in the embryo development pattern of *L. vulgaris reynaudii* (Oosthuizen et al., 2002). From day 45 onwards, *O. tehuelchus* embryos at higher temperatures were larger than those maintained at lower temperatures. On the other hand, at both temperatures, as



a concomitantly effect of organogenesis processes, an increase in yolk consumption became relevant at stages IX-X, indicating that embryos require greater amounts of yolk to satisfy the high energy demands for organ formation and growth. These observations agree with those for *O. maya* in which, regardless the incubation temperature, the amount of outer yolk remained constant up to embryonic stage X, but the yolk consumption rate increased afterwards (Camaal-Monsreal et al., 2016).

During the post-organogenesis period there are steep morphophysiological changes during which embryos grow and go through the pre-hatching stages, acquiring the competence to hatch (Boletzky, 2003; Naef, 1928; Olivares et al., 2019). Throughout this period, the remaining outer yolk sac is transferred to the inner one and transfer rate is influenced by temperature at which the embryo develops (Boletzky, 2003, 1994; Bouchaud and Daguzan, 1990). The inner yolk sac acts as an energetic reserve during the first days after hatching (Boletzky et al., 2002; Camaal-Monsreal et al., 2016; Naef, 1928; Sanchez-García et al., 2017). Since the rate of yolk absorption in *O. tehuelchus* embryos subject to low temperatures slows down during the post-organogenesis stages, almost duplicating the total development time, the speed of this process would determine the individual hatching time and, consequently, the hatching period. Moreover, the inverse relationship observed between hatching time and survival time could be indicating an energetic tradeoff between the pre-hatching stages and hatchlings performance. With a longer pre-hatching phase, the inner yolk would be consumed inside the egg restricting the available energy during the first days of free life. In other words, the processes occurring during the pre-hatching period would have a pivotal role on the individual hatching time and the newborn juveniles of *O. tehuelchus*' performance.

The plasticity in the hatching time of cephalopods could represent an advantage for their survival under heterogeneous environmental conditions (Boletzky, 2003). According to Boletzky (1994, 1987), hatching could be delayed if embryonic developmental temperature is close to the lower temperature limit to which the species is adapted. A longer pre-hatching period might allow juveniles to hatch when food is available, and thus it might have a regulatory function in places where food availability shows seasonal fluctuations (Boletzky, 1994; Clarke, 1982). This would be the case in temperate environments of the northern Atlantic Patagonia, at the southern end of the *O. tehuelchus* distribution. In this area, water temperature ranges from 11 to 19°C and primary production is strongly seasonal with two peaks, one in autumn and one in spring (Rivas et al., 2006; Williams et al., 2018). Furthermore, the progressive and discontinuous recruitment of juveniles observed between spring and early summer in North Patagonia populations (Iribarne, 1991), could be explained by their temperature-related hatching period that can spread from weeks to months, coupled with a laying period that extends from early autumn to early winter (Iribarne, 1991; Ré, 1998; Storero et al., 2010). On the contrary, a shorter embryonic development and hatching period due to an increase in temperature would allow the octopuses to hatch earlier, grow faster and survive to reproductive age, increasing population fitness (Nande et al., 2018; Uriarte et al., 2012). This would be the case in the inner shelf of the subtropical waters of southern Brazil, where water temperature ranges between 12 and 24°C and productivity is relatively high (Ciotti et al., 1995), allowing the year-round availability of food for hatchlings (Alves and Haimovici, 2011; Ciotti et al., 1995).

In this work, temperature showed the potential to increase or decrease the performance of juveniles at the early post hatching period. Studies on *Sepia officinalis*, *L. opalescens* and *O. vulgaris* recorded that hatchlings obtained at low temperatures

were larger and heavier than those incubated at warmer temperatures (Bouchaud, 1991; Repolho et al., 2014; Vidal et al., 2002a). In turn, Caamal-Monsreal et al. (2016) established that in *O. maya* the best hatchling performance occurred in embryos incubated at intermediate temperatures (22-26°C) and not at thermal extremes (18-30°C). In *O. tehuelchus*, a higher incubation and juveniles' maintenance temperature seems to optimize the efficiency of yolk conversion, giving rise to larger juveniles, as is reflected by cephalic measurements, TL, VML and in the wet weight. In contrast, at lower temperatures the energy obtained through yolk absorption during embryonic development would be used to guarantee basic hatchling metabolic processes and for physiological maintenance, not for growth. Furthermore, at higher temperatures, metabolic rates would be greater, so yolk absorption would be faster with the subsequent reduction of survival time in starvation.

Since morphometric features can change with temperature and undergo changes during the ontogeny of hatchlings, they would not be enough to perform the correct identification of newly hatched juveniles from wild populations (Hochberg et al., 1992; Kubodera and Okutani, 1981; Messenger, 2001; Young et al., 1989). In contrast, chromatophore patterns are species-specific and remain unchanged throughout early growth (Sweeney et al., 1992; Villanueva and Norman, 2008). Moreover, in *O. tehuelchus* juveniles, chromatophore patterns seem showed to be independent of temperature. In this sense, the presence of chromatophores densely covering the mantle, heads and arms, and the absence of pigmentation in the funnel, might serve to distinguish the newborn *O. tehuelchus* from other coastal sympatric species, such as *Enteroctopus megalocyathus* and *Robsonella fontaniana*, already described in North Patagonia (Ortiz and Ré, 2011; Ortiz et al., 2006).

The differences observed between both experimental temperatures seem to show adaptive mechanisms that optimize the viability of embryos and juveniles under the different environmental temperatures that can be found in the northern Patagonian coast, where *O. tehuelchus* has evolved. However, these compensatory mechanisms are expected to trigger metabolic costs and behavioral changes on juveniles (Higgins et al., 2012; Noyola et al., 2013a, 2013b) and hence, both consequences should be investigated in order to set culture conditions for further rearing trials. From a practical point of view, knowing the proper handling conditions of egg masses and the temperature-related effects on embryogenesis and offspring survival would help organize laboratory procedures and plan the availability of newborn juveniles of this potential aquaculture resource at different times of the year.

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### **References**

Alves, J., Haimovici, M., 2011. Reproductive biology of *Octopus tehuelchus* d'Orbigny, 1834 (Cephalopoda: Octopodidae) in southern Brazil. *Nautilus* (Philadelphia). 125, 150–158.

- Berrueta, M., Desiderio, J.A. Agliano, F., López, A.V, AristizabalAbud, E., Ortiz, N., 2020. Mating behavior of Patagonian octopus (*Octopus tehuelchus*) under laboratory conditions. *Marine and Fishery Science*. 33(1), 115-120.
- Bocco, G., Cinti, A., Vezub, J., Sánchez-Carnero, N., Chávez, M., 2019. Lugar y sentido de lugar en un camino de la costa atlántica patagónica, 1950-1970. *Región Y Soc.* 31, e1127. doi:10.22198/rys2019/31/1127
- Boletzky, S.V., 1984. Of The Octopus *Scaevurgus unicirrhus* (Mollusca, Cephalopoda). *Vie Milieu*. 34, 87-93.
- Boletzky, S.V., 1987. Embryonic phase, in: Boyle PR (Ed.), *Cephalopod life cycles*, vol 2. Acad Press., London, pp. 5-31.
- Boletzky, S.V., 1989. Recent studies on spawning, embryonic development, and hatching in the Cephalopoda. In *Advances in Marine Biology*, vol. 25. Acad Press., London, pp. 85-115.
- Boletzky, S.V., 1994. Embryonic development of cephalopods at low temperatures. *Antarct. Sci.* 6, 139–142. doi:10.1017/S0954102094000210
- Boletzky, S.V., Fuentès, M., Offner, N., 2002. Developmental features of *Octopus macropus* Risso, 1826 (Mollusca, Cephalopoda). *Vie Milieu* 52, 209–215.
- Boletzky, S.V., 2003. Biology of early life stages in cephalopod molluscs. *Adv. Mar. Biol.* 44, 143–203. doi:10.1016/S0065-2881(03)44003-0
- Boletzky, S.V., Villanueva, R., 2014. Cephalopod Biology, in: Iglesias J., Fuentes, L. Villanueva, R. (Eds.), *Cephalopod Culture*. Springer, Dordrecht, pp. 3-16.
- Bouchaud, O., Daguzan, J., 1990. Etude expérimentale de l'influence de la température sur le déroulement embryonnaire de la seiche *Sepia officinalis* L. (Céphalopode, Sepioidae). *Cah. Biol. Mar.* 31, 131–145.

- Bouchaud, O., 1991. Energy consumption of the cuttlefish *Sepia officinalis* L. (Mollusca: Cephalopoda) during embryonic development, preliminary results. *Bulletin of Marine Science*, 49(1-2), 333-340.
- Butler-Struben, H.M., Brophy, S.M., Johnson, N. A., Crook, R. J., 2018. In vivo recording of neural and behavioral correlates of anesthesia induction, reversal, and euthanasia in cephalopod molluscs. *Front. Physiol.* 9, 1–18. doi:10.3389/fphys.2018.00109
- Caamal-Monsreal, C., Uriarte, I., Farias, A., Díaz, F., Sánchez, A., Re, D., Rosas, C., 2016. Effects of temperature on embryo development and metabolism of *O. maya*. *Aquaculture* 451, 156–162. doi:10.1016/j.aquaculture.2015.09.011
- Ciotti, Á.M., Odebrecht, C., Fillmann, G., Moller, O.O., 1995. Freshwater outflow and Subtropical Convergence influence on phytoplankton biomass on the southern Brazilian continental shelf. *Cont. Shelf Res.* 15, 1737–1756. doi:10.1016/0278-4343(94)00091-Z
- Clarke, A., 1982. Temperature and embryonic development in polar marine invertebrates. *Int. J. Invertebr. Repr.* 5(2), 71-82. doi:10.1080/01651269.1982.10553456
- Espinoza, V., Viana, M.T., Rosas, C., Uriarte, I., Farías, A., 2017. Effect of starvation on the performance of baby octopus (*Robsonella fontaniana*) paralarvae. *Aquac. Res.* 48, 5650–5658. doi:10.1111/are.13387
- FAO. 2018. The State of World Fisheries and Aquaculture 2018 - Meeting the sustainable development goals. Rome. Licence: CC BY-NC-SA 3.0 IGO.
- Farías, A., Martínez-Montañó, E., Espinoza, V., Hernández, J., Viana, M.T., Uriarte, I., 2016. Effect of zooplankton as diet for the early paralarvae of Patagonian red

- octopus, *Enteroctopus megalocyathus*, grown under controlled environment. *Aquac. Nutr.* 22, 1328–1339. doi:10.1111/anu.12334
- Fassiano, A.V., Ortiz, N., Ríos de Molina, M. del C., 2017. Reproductive status, antioxidant defences and lipid peroxidation in *Octopus tehuelchus* (Cephalopoda: Octopodidae) females. *J. Nat. Hist.* 51, 2645–2660.  
doi:10.1080/00222933.2017.1329460
- Forsythe, J.W., Walsh, L.S., Turk, P.E., Lee, P.G., 2001. Impact of temperature on juvenile growth age at first egg-laying of the Pacific reef squid *Sepioteuthis lessoniana* reared in captivity. *Mar. Biol.* 138, 103–112. doi:10.1007/s002270000450
- Grigoriou, P., Richardson, C.A., 2008. The effect of ration size, temperature and body weight on specific dynamic action of the common cuttlefish *Sepia officinalis*. *Mar. Biol.* 154, 1085–1095. doi:10.1007/s00227-008-1002-3
- Higgins, F.A., Bates, A.E., Lamare, M.D., 2012. Heat tolerance, behavioural temperature selection and temperature-dependent respiration in larval *Octopus huttoni*. *J. Therm. Biol.* 37, 83–88. doi:10.1016/j.jtherbio.2011.11.004
- Hochberg, F.G., Nixon, M., Toll, R.B., 1992. Order OCTOPODA Leach, 1818. *Smithson. Contrib. to Zool.* 513, 213-280.
- Iglesias, J., Villanueva, R., Fuentes, L., 2014. *Cephalopod culture*. Springer, Dordrecht. doi:10.1007/978-94-017-8648-5
- Iribarne, O.O., 1990. Use of shelter by the small Patagonian octopus *Octopus tehuelchus*: availability, selection and effects on fecundity. *Mar. Ecol. Prog. Ser.* 66, 251–258.
- Iribarne, O.O., 1991. Life history and distribution of the small south-western Atlantic octopus, *Octopus tehuelchus*. *J. Zool.* 223, 549–565.  
doi:10.1111/j.1469-7998.1991.tb04387.x

- Iribarne, O.O., Fernandez, M.E., Zucchini, H., 1991. Prey selection by the small Patagonian octopus *Octopus tehuelchus* d'Orbigny. J. Exp. Mar. Bio. Ecol. 148(2), 271–281. doi:10.1016/0165-7836(91)90020-G
- Juárez, O.E., Galindo-Sánchez, C.E., Díaz, F., Re, D., Sánchez-García, A.M., Camaal-Monsreal, C., Rosas, C., 2015. Is temperature conditioning *Octopus maya* fitness? J. Exp. Mar. Bio. Ecol. 467, 71–76. doi:10.1016/j.jembe.2015.02.020
- Klaich, M.J., Re, M.E., Pedraza, S.N., 2006. Effect of temperature, sexual maturity and sex on growth, food intake and gross growth efficiency in the “Pulpito” *Octopus tehuelchus* (D'orbigny, 1834). J. Shellfish Res. 25, 979–986. doi:10.2983/0730-8000(2006)25[979:EOTSMA]2.0.CO;2
- Klaich, M.J., Ré, M.E., Pedraza, S.N., 2008. Gross growth efficiency as a function of food intake level in the “Pulpito” *Octopus tehuelchus*: A multimodel inference application. Aquaculture 284, 272–276. doi:10.1016/j.aquaculture.2008.07.054
- Kubodera, T., Okutani, T., 1981. The systematics and identification of larval Cephalopods from the northern north Pacific. Res. Inst. N. Pac. Fish. spe, 131–159.
- McCullagh, P., Nelder, J.A., 1989. Generalized Linear Models. Monographs on Statistics and Applied Probability Vol. 37. Taylor & Francis Group., New York.
- Messenger, J.B., 2001. Cephalopod chromatophores: Neurobiology and natural history. Biol. Rev. Camb. Philos. Soc. 76, 473–528. doi:10.1017/S1464793101005772
- Monsalvo-Spencer, P., Salinas-Zavala, C.A., Reynoso-Granados, T., 2013. Morfología de la membrana coriónica de los huevos de *Octopus bimaculoides* y *Octopus hubbsorum* (Cephalopoda: Octopodidae). Hidrobiologica 23, 124–129.
- Naef, A., 1928. Die Cephalopoden. Embryologie. Die Fauna Flora Golf Neapel, 35(2),



1-357.

- Nande, A.M., Domingues, P., Rosas, C., 2018. Effects of Temperature on the Embryonic Development of *Octopus vulgaris* 37, 1013–1019. doi:10.2983/035.037.0512
- Narvarte, M., González, R., Fernández, M., 2006. Comparison of Tehuelche octopus (*Octopus tehuelchus*) abundance between an open-access fishing ground and a marine protected area: Evidence from a direct development species. Fish. Res. 79, 112–119. doi:10.1016/j.fishres.2006.02.013
- Noyola, J., Caamal-Monsreal, C., Díaz, F., Re, D., Sánchez, A., Rosas, C., 2013a. Thermopreference, tolerance and metabolic rate of early stages juvenile *Octopus maya* acclimated to different temperatures. J. Therm. Biol. 38, 14–19. doi:10.1016/j.jtherbio.2012.09.001
- Noyola, J., Mascaró, M., Caamal-Monsreal, C., Noreña-Barroso, E., Díaz, F., Re, D., Sánchez, A., Rosas, C., 2013b. Effect of temperature on energetic balance and fatty acid composition of early juveniles of *Octopus maya*. J. Exp. Mar. Bio. Ecol. 445, 156–165. doi:10.1016/j.jembe.2013.04.008
- Olivares, A., Rodríguez-Fuentes, G., Mascaró, M., Arteaga, A.S., Ortega, K., Monsreal, C.C., Rosas, C., 2019. Maturation trade-offs in octopus females and their progeny: energy, digestion and defence indicators. PeerJ, 7, e6618.
- Oosthuizen, A., Roberts, M.J., Sauer, W.H.H., 2002. Temperature effects on the embryonic development and hatching success of the squid *Loligo vulgaris reynaudii*. Bull. Mar. Sci. 71, 619–632.
- Ortiz, N., Ré, M.E., Márquez, F., 2006. First description of eggs, hatchlings and hatchling behaviour of *Enteroctopus megalocyathus* (Cephalopoda: Octopodidae). J. Plankton Res. 28, 881–890. doi:10.1093/plankt/fbl023

- Ortiz, N., Ré, M.E., 2011. The eggs and hatchlings of the octopus *Robsonella fontaniana* (Cephalopoda : Octopodidae) 91, 705–713.  
doi:10.1017/S0025315410001232
- Ortiz, N., Ré, M.E., Márquez, F., Glebocki, N.G., 2011. The reproductive cycle of the red octopus *Enteroctopus megalocyathus* in fishing areas of Northern Patagonian coast. Fish. Res. 110, 217–223. doi:10.1016/j.fishres.2011.03.016
- Parra, G., Villanueva, R., Yúfera, M., 2000. Respiration rates in late eggs and early hatchlings of the common octopus, *Octopus vulgaris*. J. Mar. Biol. Assoc. U.K., 80(3), 557-558. doi:10.1017/S0025315400002319
- Pimentel, M.S., Trübenbach, K., Faleiro, F., Boavida-Portugal, J., Repolho, T., Rosa, R., 2012. Impact of ocean warming on the early ontogeny of cephalopods: A metabolic approach. Mar. Biol. 159, 2051–2059. doi:10.1007/s00227-012-1991-9
- Pinheiro, J.C., Bates, D.J., DebRoy, S., Sakar, D., R Core Team, 2016. Linear and Nonlinear Mixed Effects Models. R package 3.1-128. URL: <http://CRAN.R-project.org/package=nlme>.
- Pujals, M. A., 1986. Contribución al conocimiento de la biología de *Octopus tehuelchus* d'Orbigny (Mollusca: Cephalopoda). Anales de la Sociedad Científica Argentina, Serie I, 214, 29-71.
- Ré, M.E., 1989. Estudios ecológicos sobre el crecimiento y la alimentación de *Octopus tehuelchus* d'Orbigny en Puerto Lobos, Golfo San Matías. Universidad Nacional de La Plata
- Ré, M.E., 1998. Pulpos octopódidos (Cephalopoda, Octopodidae), in: Boschi E.E. (Ed.), El Mar Argentino y sus Recursos Pesqueros, Tomo 2. Instituto Nacional de Investigación y Desarrollo Pesquero, Mar del Plata, pp69-98.

- Ré, M.E., Ortiz, N., 2011. Cefalópodos capturados en la Campaña “Concacen noviembre 2009”, B/O Puerto Deseado. VIII Congreso Latinoamericano de Malacología. Libro de resúmenes. 249.
- Repolho, T., Baptista, M., Pimentel, M.S., Dionísio, G., Trübenbach, K., Lopes, V.M., Lopes, A.R., Calado, R., Diniz, M., Rosa, R., 2014. Developmental and physiological challenges of octopus (*Octopus vulgaris*) early life stages under ocean warming. J. Comp. Physiol. B Biochem. Syst. Environ. Physiol. 184, 55–64. doi:10.1007/s00360-013-0783-y
- Rivas, A.L., Dogliotti, A.I., Gagliardini, D.A., 2006. Seasonal variability in satellite-measured surface chlorophyll in the Patagonian Shelf. Cont. Shelf Res. 26, 703–720. doi:10.1016/j.csr.2006.01.013
- Rosa, R., Pimentel, M.S., Boavida-Portugal, J., Teixeira, T., Trübenbach, K., Diniz, M., 2012. Ocean warming enhances malformations, premature hatching, metabolic suppression and oxidative stress in the early life stages of a keystone squid. PLoS One 7. doi:10.1371/journal.pone.0038282
- Rosas, C., Cuzon, G., Pascual, C., Gaxiola, G., Chay, D., López, N., Maldonado, T., Domingues, P.M., 2007. Energy balance of *Octopus maya* fed crab or an artificial diet. Mar. Biol. 152, 371–381. doi:10.1007/s00227-007-0692-2
- Sakurai, Y., Bower, J.R., Nakamura, Y., Yamamoto, S., Watanabe, K., 1996. Effect of temperature on development and survival of *Todarodes pacificus* embryos and paralarvae. Am. Malacol. Bull. 13, 89–95.
- Sanchez-García, A., Rodríguez-Fuentes, G., Díaz, F., Galindo-Sánchez, C.E., Ortega, K., Mascaró, M., López, E., Caamal-Monsreal, C., Juárez, O., Noreña-Barroso, E., Re, D., Rosas, C., 2017. Thermal sensitivity of *O. maya* embryos as a tool for monitoring the effects of environmental warming in the Southern of Gulf of Mexico.

- Ecol. Indic. 72, 574–585. doi:10.1016/j.ecolind.2016.08.043
- Schneider, C.A., Rasband, W.S., Eliceiri, K.W., 2012. NIH Image to ImageJ: 25 years of image analysis. Nat. Methods 9, 671–675. doi:10.1038/nmeth.2089
- Schwarz, R., Piatkowski, U., Hoving, H.J.T., 2018. Impact of environmental temperature on the lifespan of octopods. Mar. Ecol. Prog. Ser. 605, 151–164. doi:10.3354/meps12749
- Solorzano, Y., Viana, M.T., López, L.M., Correa, J.G., True, C.C., Rosas, C., 2009. Response of newly hatched *Octopus bimaculoides* fed enriched *Artemia salina*: Growth performance, ontogeny of the digestive enzyme and tissue amino acid content. Aquaculture 289, 84–90. doi:10.1016/j.aquaculture.2008.12.036
- Storero, L.P., Narvarte, M.A., González, R.A., 2012. Reproductive traits of the small Patagonian octopus *Octopustehuelchus*. Helgol. Mar. Res. 66, 651–659. doi:10.1007/s10152-012-0298-z
- Storero, L.P., Ocampo-Reinaldo, M., González, R.A., Narvarte, M.A., 2010. Growth and life span of the small octopus *Octopus tehuelchus* in San Matías Gulf (Patagonia): Three decades of study. Mar. Biol. 157, 555–564. doi:10.1007/s00227-009-1341-8
- Storero, L.P., Narvarte, M.A., 2013. Coccidian infection may explain the differences in the life history of octopus host populations. J. Invertebr. Pathol. 114, 222–225. doi:10.1016/j.jip.2013.08.006
- Sweeney, M.J., Roper, C.F., Mangold, K.M., Clark, M.R., Boletzky, S.V., 1992. "Larval" and juvenile cephalopods: a manual for their identification. Smithsonian Contribution to Zoology 513. Smithsonian Institution Press, Washington, DC:
- Uriarte, I., Iglesias, J., Domingues, P., Rosas, C., Viana, M.T., Navarro, J.C., Seixas, P., Vidal, E., Ausburger, A., Pereda, S., Godoy, F., Paschke, K., Farías, A., Olivares, A.,

- Zuñiga, O., 2011. Current Status and Bottle Neck of Octopod Aquaculture: The Case of American Species. *J. World Aquac. Soc.* 42, 735–752. doi:10.1111/j.1749-7345.2011.00524.x
- Uriarte, I., Espinoza, V., Herrera, M., Zúñiga, O., Olivares, A., Carbonell, P., Pino, S., Farías, A., Rosas, C., 2012. Effect of temperature on embryonic development of *Octopus mimus* under controlled conditions. *J. Exp. Mar. Bio. Ecol.* 416–417, 168–175. doi:10.1016/j.jembe.2012.03.003
- Uriarte, I., Espinoza, V., Gutiérrez, R., Zúñiga, O., Olivares, A., Rosas, C., Pino, S., Farías, A., 2014. Key aspects of egg incubation in Patagonian red octopus (*Enteroctopus megalocyathus*) for cultivation purposes. *Aquaculture* 424–425, 158–166. doi:10.1016/j.aquaculture.2013.12.039
- Uriarte, I., Farías, A., 2014. *Enteroctopus megalocyathus*, in: Iglesias J., Fuentes, L., Villanueva, R. (Eds.), *Cephalopod Culture*. Springer., Dordrecht, pp. 365-382.
- Uriarte, I., Martínez-Montañó, E., Espinoza, V., Rosas, C., Hernández, J., Farías, A., 2016. Effect of temperature increase on the embryonic development of Patagonian red octopus *Enteroctopus megalocyathus* in controlled culture. *Aquac. Res.* 47, 2582–2593. doi:10.1111/are.12707
- Uriarte, I., Rosas, C., Espinoza, V., Hernández, J., Farías, A., 2018. Thermal tolerance of paralarvae of Patagonian red octopus *Enteroctopus megalocyathus*. *Aquac. Res.* 49, 2119–2127. doi:10.1111/are.13666
- Valverde, J.C., Granero, M.D., Giménez, F.A., García, B.G., 2019. Successful rearing of common octopus (*Octopus vulgaris*) fed a formulated feed in an offshore cage 968–972. doi:10.1111/are.13955

- Vidal, E.A.G., DiMarco, F.P., Wormuth, J.H., Lee, P.G., 2002a. Influence of temperature and food availability on survival, growth and yolk utilization in hatchling squid. *Bulletin of Marine Science*, 71(2), 915-931.
- Vidal, E.A.G., DiMarco, F.P., Wormuth, J.H., Lee, P.G., 2002b. Optimizing rearing conditions of hatchling loliginid squid. *Mar. Biol.* 140, 117–127. doi:10.1007/s002270100683
- Vidal, E.A.G., Boletzky, S.V., 2014. *Loligo vulgaris* and *Doryteuthis opalescens*. In cephalopod culture. Springer., Dordrecht. pp. 271-313.
- Vidal, E.A.G., Villanueva, R., Andrade, J.P., Gleadall, I.G., Iglesias, J., Koueta, N., Rosas, C., Segawa, S., Grasse, B., Franco-Santos, R.M., Albertin, C.B., Caamal-Monsreal, C., Chimal, M.E., Edsinger-Gonzales, E., Gallardo, P., Le Pabic, C., Pascual, C., Roubledakis, K., Wood, J., 2014. Cephalopod culture: Current status of main biological models and research priorities, *Advances in Marine Biology*. doi:10.1016/B978-0-12-800287-2.00001-9
- Villanueva, R., Arkhipkin, A., Jereb, P., Lefkaditou, E., Lipinski, M.R., Perales-Raya, C., Riba, J., Rocha, F., 2003. Embryonic life of the loliginid squid *Loligo vulgaris*: Comparison between statoliths of Atlantic and Mediterranean populations. *Mar. Ecol. Prog. Ser.* 253, 197–208. doi:10.3354/meps253197
- Villanueva, R., Moltschanivskyj, N.A., Bozzano, A., 2007. Abiotic influences on embryo growth: Statoliths as experimental tools in the squid early life history. *Rev. Fish Biol. Fish.* 17, 101–110. doi:10.1007/s11160-006-9022-x
- Villanueva, R., Norman, M.D., 2008. Biology of the planktonic stages of benthic octopuses. *Oceanogr. Mar. Biol.* 46, 105–208. doi:10.1201/9781420065756.ch4
- Villanueva, R., Vidal, E.A.G., Fernando, A., 2016. Early Mode of Life and Hatchling

- Size in Cephalopod Molluscs: Influence on the Species Distributional Ranges. PLoS ONE 11(11): e0165334. doi:10.1371/journal.pone.0165334
- Williams, G.N., Solís, M.E., Esteves, J.L., 2018. Satellite-measured phytoplankton and environmental factors in north Patagonian Gulfs. *Plankt. Ecol. Southwest. Atl. From Subtrop. to Subantarctic Realm* 307–325.  
doi:10.1007/978-3-319-77869-3\_15
- Young, R.E., Harman, R.F., Hochberg, F.G., 1989. Octopodid paralarvae from Hawaiian waters. *The Veliger*, 32(2), 152-165.
- Zar, J.H., 1996. *Biostatistical analysis*. Prentice-Hall, Upper Saddle River, New Jersey
- Zheng, X.D., Qian, Y.S., Liu, C., Li, Q., 2014. *Octopus minor*, in: Iglesias J., Fuentes, L. Villanueva, R. (Eds.), *Cephalopod Culture*. Springer, Dordrecht, pp. 415-426.
- Zúñiga, O., Olivares, A., Rojo, M., Chimal, M.E., Díaz, F., Uriarte, I., Rosas, C., 2013. Thermoregulatory behavior and oxygen consumption of *Octopus mimus* paralarvae: The effect of age. *J. Therm. Biol.* 38, 86–91.  
doi:10.1016/j.jtherbio.2012.11.003
- Zuur, A., Ieno, E.N., Walker, N., Saveliev, A.A., Smith, G.M., 2009. *Mixed effects models and extensions in ecology with R*. Springer Sci. Bus. Media. doi:10.1007/978-0-387-87458-6\_1

## Figure captions

**Fig.1.** *Octopus tehuelchus* embryo development stages versus time at 13°C (gray squares) and 16°C (black squares). Each square represents the mode of 25 observations, vertical lines show the maximum and minimum embryonic stages observed. Horizontal lines indicate the hatching period at 13°C (gray line) and 16°C (black line).

**Fig.2.** *Octopus tehuelchus* embryo development stages. A) Stages I-II, B) Stages V-VI: blastoderm cells occupying nearly 30-50% of the egg, C-D) Stage VIII: end of the first inversion and beginning of organogenesis, E) Stages IX-X, F) Stage XIII-XIV, G) Stage XVIII, H) Stage XIX: end of the second inversion. (a) arms, (ap) animal pole, (apr) arm primordium, (bd) blastoderm, (c) chorion, (chr) chromatophores, (dh) dorsal side of head, (dm) dorsal side of mantle, (e) eye, (gd) germinal disk, (iys) inner yolk sac, (m) mantle complex, (mp) mantle primordium, (op) optic vesicle, (oys) outer yolk sac, (pm) perivitelline membrane, (su) suckers, (y) yolk, (vp) vegetal pole. Note that the embryo in image F did not perform the 1st inversion.

**Fig.3.** Relationship between embryo structures and age of *Octopus tehuelchus* reared at 13°C (grey squares) and 16°C (black squares). a) Dorsal mantle length, b) eye diameter, c) arm length, and d) outer yolk sac volume. The vertical bars indicate the standard error.

**Fig.4.** *Octopus tehuelchus* mean relative embryo survival (RES%) as a function of embryo development stages (grey bars: 13°C, black bars: 16°C).

**Fig.5.** Mean ( $\pm$  SE) total embryo survival as a function of temperature (grey bars: 13 °C, black bars: 16 °C).

**Fig.6.** Lateral view of a newly hatched juvenile of *Octopus tehuelchus*, showing the dorsal and ventral mantle, head, arms and eyes densely covered with chromatophores,



and the funnel and anterior margin of the ventral mantle without a chromatophore cover.

Note the Kölliker's organs seen as iridescent dots in the ventral mantle.

**Fig.7.** Kölliker's organ detail from scanning electron micrograph on the mantle of *Octopus tehuelchus* juvenile.

**Fig.8.** *Octopus tehuelchus* juveniles' survival time in starvation, depending on the hatching time at 13 °C (white circles) and 16 °C (black triangles).

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**Ramiro Braga:** Conceptualization, Methodology, Investigation, Formal analysis, Writing - Original Draft, Visualisation, Writing - Review & Editing.

**Silvina Van der Molen:** Conceptualization, Investigation, Resources, Writing - Review & Editing, Project administration, Funding acquisition.

**Julián Pontones:** Investigation, Formal analysis.

**Nicolás Ortiz:** Conceptualization, Methodology, Validation, Investigation, Resources, Supervision, Formal analysis, Writing - Review & Editing, Project administration, Funding acquisition.

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**Declaration of interests**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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**Table 1.** Results of the Linear mixed-effects model. Effect of temperature, embryogenesis time, their interaction, and the aquarium as a random effect on embryos morphometric parameters of *Octopus tehuelchus*.

<b>Fixed effects</b>	DML		ED		AL		OYSV	
	F-value	p-value	F-value	p-value	F-value	p-value	F-value	p-value
Temperature	11.710	0.0091	7.613	0.0247	3.909	0.0534	1.066	0.3319
Embryogenesis time	504.606	<.0001	176.627	<.0001	413.480	<.0001	205.219	<.0001
Temp*Embryo. time	8.853	0.0034	2.132	0.1463	0.700	0.4046	28.091	<.0001
<b>Random effects</b>	L.Ratio	p-value	L.Ratio	p-value	L.Ratio	p-value	L.Ratio	p-value
Aquarium	47.266	<.0001	27.751	<.0001	35.393	<.0001	0.299	0.584

**Table 2.** Results of the Generalized least squares model. Effect of temperature on the embryos morphometric parameters of *Octopus tehuelchus* throughout the embryogenesis time.

Embryogenesis time (days)	DML		ED		AL		OYSV	
	F-value	p-value	F-value	p-value	F-value	p-value	F-value	p-value
<b>25</b>	1.179	0.2988	1.019	0.3325	2.306	0.1547	0.447	0.5163
<b>45</b>	7.755	0.0127	12.015	0.003	7.581	0.0136	0.088	0.7703
<b>55</b>	24.521	1e <sup>-04</sup>	12.667	0.0022	15.089	0.0015	0.001	0.9662
<b>65</b>	72.288	<.0001	18.084	1e <sup>-04</sup>	55.163	<.0001	11.641	0.0015
<b>75</b>	126.148	<.0001	61.941	<.0001	67.341	<.0001	133.962	<.0001
<b>95</b>	12.800	0.0117	25.937	0.0022	19.045	0.0073	12.222	0.0129

**Table 3.** Average (mm or g, +/- SE) of the different morphological variables of anesthetized *Octopus tehuelchus* at hatching, and comparison of the morphological variables between temperatures.

Morphological measures	Incubation temperatures		Statistical parameters	
	16°C	13°C	F-value	p-value
Number of juveniles	34	33		
Eye diameter (ED)	1.48 ± 0.31	1.23 ± 0.18	4.79	0.0413
Mantle width (MW)	4.62 ± 0.31	4.27 ± 0.35	6.08	0.0234
Head width (HW)	4.95 ± 0.28	4.26 ± 0.45	18.22	0.0004
Ventral mantle length (VML)	5.05 ± 0.96	3.93 ± 0.49	11.11	0.0035
Total length (TL)	17.35 ± 1.43	15.78 ± 1.80	4.92	0.0389
Total weight (TW)	0.18 ± 0.03	0.16 ± 0.03	4.58	0.0368
Arm length (AL)	8.46 ± 1.44	8.15 ± 1.41	0.25	0.6249
Funnel length (FL)	1.87 ± 0.45	1.73 ± 0.42	0.54	0.4720
Dorsal mantle length (DML)	6.15 ± 0.59	5.87 ± 0.64	1.12	0.3039

**Highlights**

- 1) Embryonic survival increases when the gastrulation process comes to an end.
- 2) Temperature influences the embryonic development pattern and embryos morphometrics.
- 3) Temperature determines the individual hatching time and the hatching period.
- 4) The first octopuses to hatch survive longer.
- 5) Temperature could increase or decrease *Octopus tehuelchus* hatchlings performance.

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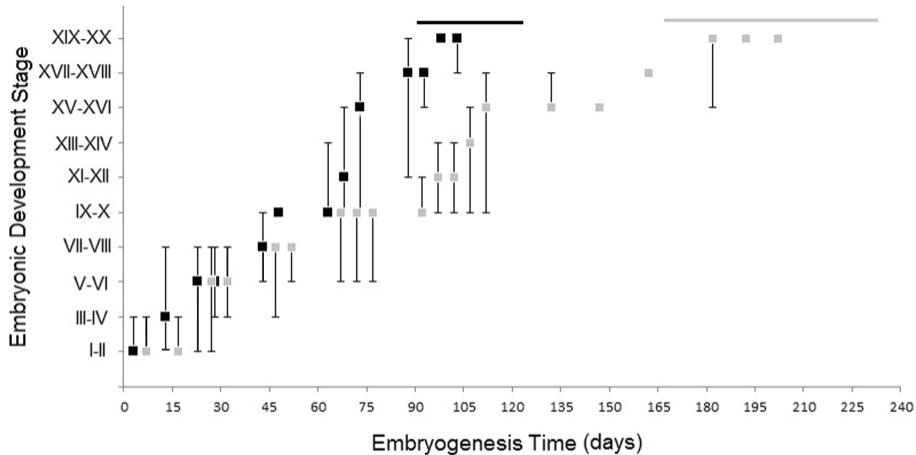


Figure 1



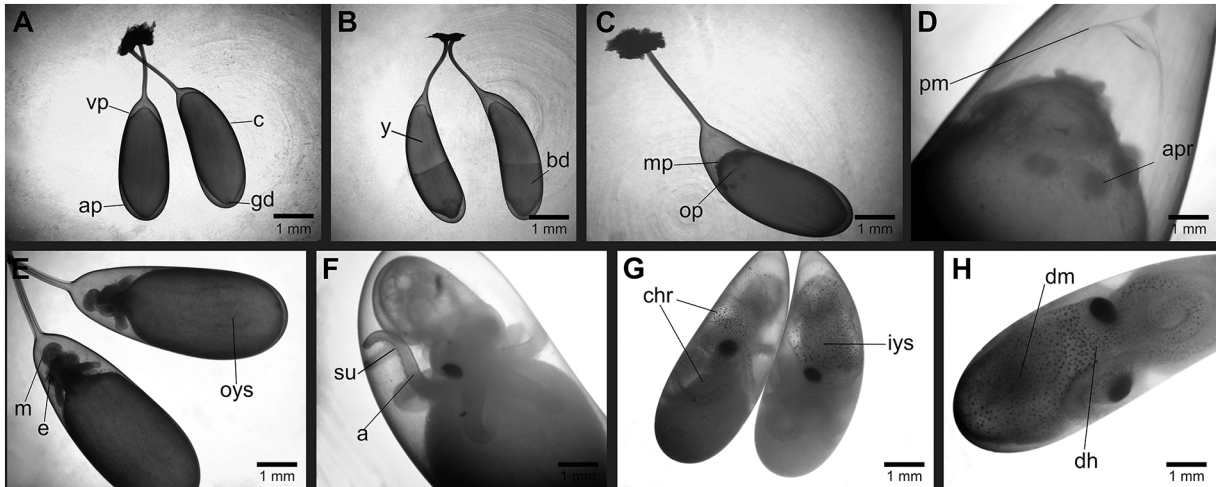


Figure 2

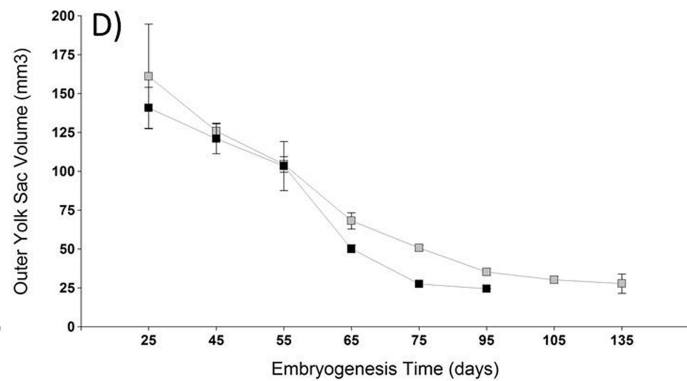
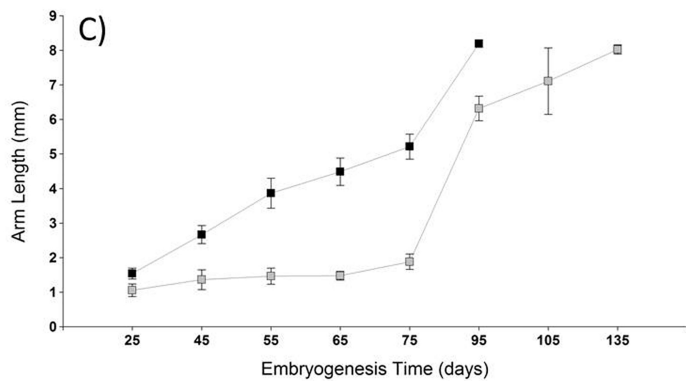
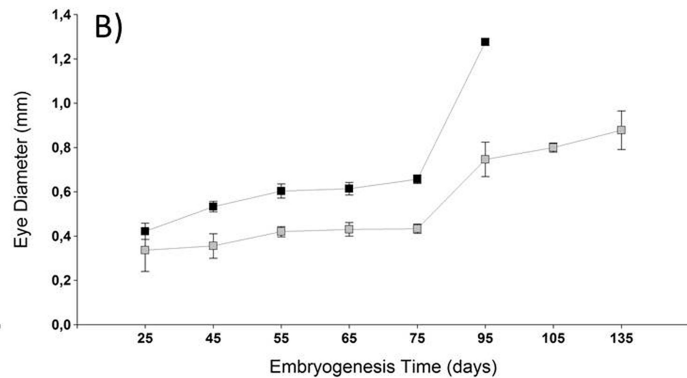
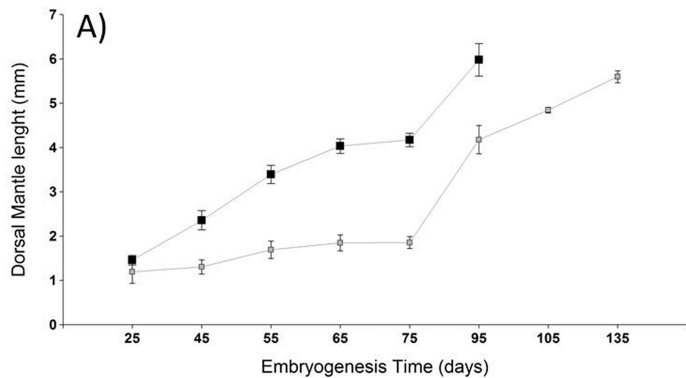


Figure 3

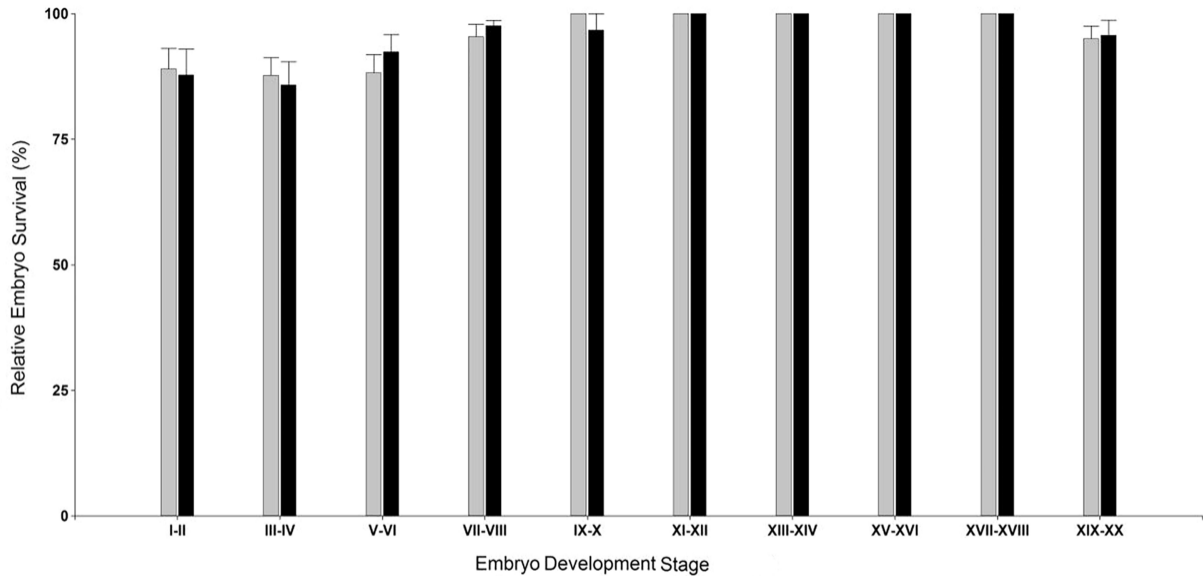


Figure 4

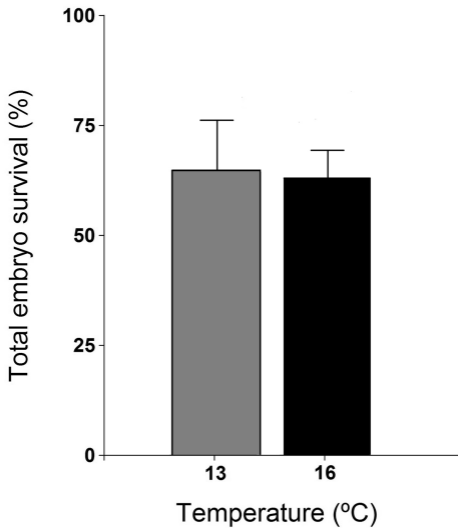


Figure 5

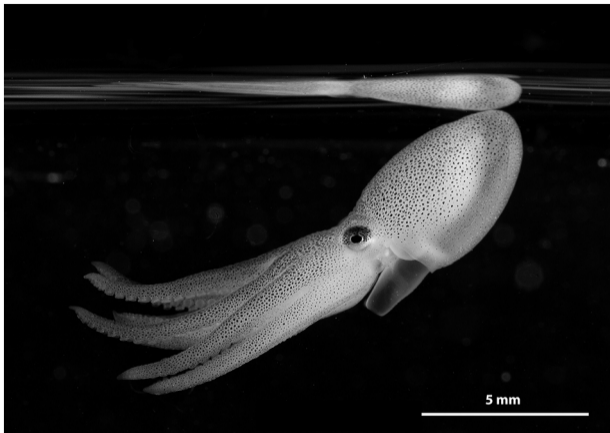


Figure 6

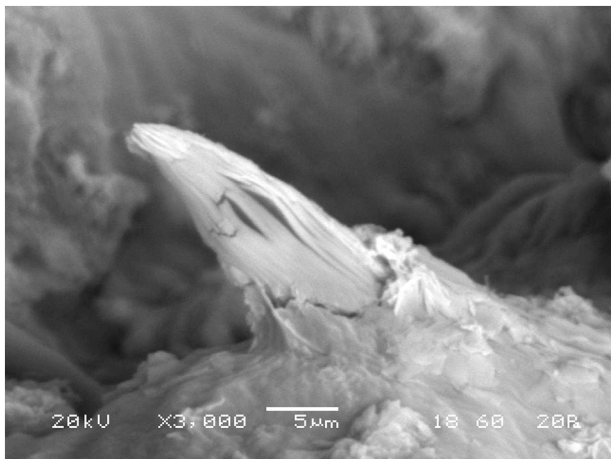


Figure 7

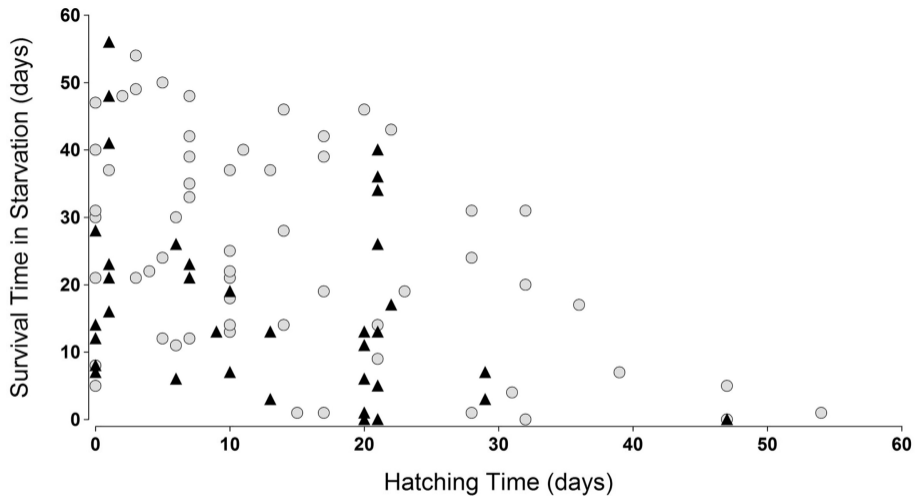


Figure 8