Embryonic development and effect of temperature on larval growth of the Peruvian Anchovy *Engraulis ringens*

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ABSTRACT

Understanding aspects of the biology of early life stages of marine fish is critical if one hopes to reveal the factors and processes that impact the survival and recruitment (year class) strength. The Peruvian anchovy (*Engraulis ringens*) is a key species in the Humboldt Current System and the present study provides the first description of the embryonic and larval development of this species reared in captivity. Embryonic and early exogenous feeding stages of larvae were illustrated in detail at 18.5°C. Hatching was completed within 42 and 48 hours post-fertilization at 18.5°C and 14.5°C, respectively. Mean ± 95% CI standard length (L_S) at hatch (3.40 ± 0.10 mm at 18.5°C and 2.76 ± 0.34 mm at 14.5°C) was significantly different between the two temperatures. Larval behaviour was assessed at 18.5°C; at the onset of exogenous feeding (3 days post-hatch [dph]), larvae were fed small, motile dinoflagellates, *Akashiwo sanguinea*. At 7 dph, larvae started to feed almost exclusively on zooplankton (rotifers and *Artemia* nauplii). Larval activity increased with age and the first sign of schooling was noted at 31 dph (18.56 mm L_S) at 18.5°C. Temperature had a significant effect on size-at-age, but not on body shape (depth to L_S ratio). The size-at-age data for larvae (this study) was used to parameterize a temperature-corrected von Bertalanffy growth function for Peruvian anchovy, the accuracy of which was assessed for juveniles and adults (literature values).

KEYWORDS: embryo, fish larvae, fishery resources, Humboldt Current, ontogeny, Peru

1. Introduction

Small pelagic fish play an important ecological role in marine ecosystems due to their significant biomass and intermediate position in the food web (Cury *et al*., 2000; Essington *et al*., 2015; Palomera *et al*., 2007). The Peruvian anchovy, *Engraulis ringens* Jenyns 1842, is a key species of the Humboldt Current System (HCS), one of the four major Eastern Boundary Upwelling Ecosystems (EBUE's) of the world (Chavez and Messié, 2009). This small pelagic fish forages on plankton and supports diverse predators such as marine mammals, seabirds, and fish, connecting lower and upper trophic levels (Espinoza and Bertrand, 2008). The Peruvian anchovy fishery is considered one of the world's largest single-species fisheries (Aranda, 2009; Bakun and Weeks, 2008), with > 6 million tons landed in Peru in 2018 (PRODUCE - Peru, 2019). Even though the fishery on Peruvian anchovy is highly monitored (*e.g.* Boerema and Gulland, 1973; Schreiber and Halliday, 2013; Tveteras *et al*., 2011), and numerous surveys have examined the dynamics of this anchovy stock (*e.g.* Bakun and Broad, 2003; Barrett *et al*., 1985; Gutiérrez *et al*., 2007; Walsh *et al*., 1980), studies on the biology and physiology of this species are scarce. Early life stages, particularly the embryonic, first-feeding, and pelagic larval phases, are still poorly studied despite the importance of the survival of these stages for recruitment success in marine pelagic (and other) fishes (Garrido *et al*., 2016; Houde, 2001; Peck *et al*., 2013). Due to the inherent difficulties in working with small pelagic species in the laboratory (*e.g.* inability to maintain broodstock fish in good condition, or successful rearing of eggs or larvae, see Peck *et al*. (2021)), little information exists on embryonic and larval development in many species including *E. ringens*.

The first studies on early life stages of *E. ringens* were carried out at the end of the 1950s and beginning of the 1960s, focusing on morphological descriptions of eggs and larvae (Einarsson and Rojas de Mendiola, 1963; Fischer, 1958). During the 1970s and 1980s, studies on larval

diet (De Mendiola, 1974) and behaviour of first-feeding larvae (Walsh *et al*., 1980; Ware *et al*., 1981) were published and Pauly (1987) pointed out the correlation between the abundance of anchovy larvae and high phytoplankton concentrations in the HCS, referring to it as a "recruitment window". Since the 2000s, the number of studies on anchovy eggs and larvae collected in Chilean coastal waters has increased (Contreras *et al*., 2017; Hernandez and Castro, 2000; Llanos-Rivera and Castro, 2004; Yañez-Rubio *et al*., 2011) and, among them, Moreno *et al*. (2011) reported the successful rearing of *E. ringens* larvae and juveniles until an age of 163 days post-hatch (dph). However, very few laboratory studies have focused on the ontogeny and behaviour of this species (Llanos-Rivera and Castro, 2006; Tarifeño *et al*., 2008). Nevertheless, in a particular ecosystem such as the HCS, strongly influenced by El Niño-La Niña cycles, understanding how these early life stages can be affected by environmental factors, such as temperature, is of fundamental importance to predict future biomass resource and for sustainable fisheries management (Hinrichsen *et al*., 2011).

Working on eggs and larvae of *E. ringens* reared in the laboratory, this study has four goals. First, we provide a detailed description of the embryonic and larval development of the Peruvian anchovy using high-quality pictures. Second, we determine the embryonic and larval stage duration of the Peruvian anchovy at two temperatures representing those encountered by anchovy off central Peru and along the Chilean coast. Third, we validate the developmental suitability of larvae reared in captivity, an essential step before carrying out laboratory experiments whose results may be confidently extrapolated to the natural environment. Finally, we parameterize a mechanistic growth model for *E. ringens* from larval size-at-age data, which takes into account the effect of temperature, and we assess its accuracy for juveniles and adults.

2. Materials and methods

2.1. Broodstock

Approximately 1600 wild adults of Peruvian anchovies (*E. ringens*) were captured on 18 July 2019 using a commercial purse seine off Santa Rosa (11°48'06.9"S, 77°14'54.7"W). The adults were placed in four 300-L tanks with constant aeration and transferred the same day to the Laboratory of Aquatic Ecophysiology at Instituto del Mar del Perú (IMARPE, 12°4′1.21″S, 77°9′28.39″W). In the laboratory, the adults were maintained in three cylindrical, dark blue, 2000-L fiberglass tanks connected to a recirculating aquaculture system (a total recirculation flow rate of 4000 L h^{-1} allowed exchange of the tank volume every 30 minutes). The tanks were filled with 1-µm filtered sea water and a biofilter (filled with plastic bio balls) ensured water quality. The water inflow established a circular current with a velocity of 0.030 ± 0.007 m s^{-1}. A light regime of 13 L:11 D and constant aeration were provided. After an acclimation period of 30 days, fish mortality was very low and two tanks were maintained with approximately 250 adults each and a sex ratio of about 1:1. A chiller unit was used to stabilize the water temperature, with a maximum variation of 0.5°C. The temperature was set at 18°C for 4 months and then a weekly water temperature cycle of 17-19°C was established with cooling and heating times of 5 and 20h, respectively, that aimed to synchronize spawning. Anchovies were fed by hand until apparent satiation 3 times a day with 2 mm commercial pellets (slow-sinking type, Otohime EP2, 48% protein, and 14% lipids). The pellets were enriched by soaking them in fish oil and using a multivitamin supplement (vitamins A, E, and C, Hematec® TQC, 0.05% of food weight). This enrichment appeared to be critical to obtain viable eggs.

2.2. Spawning and eggs collection

Spawning of buoyant eggs of *E. ringens* occurred naturally after five weeks in captivity. To collect the eggs, a PVC overflow was used, with a pipe cut lengthwise set at the water surface on half the diameter of the tank (Figure 1). Eggs were transported from the surface of the tank and gently trapped onto a 100-µm mesh collector located in a small container outside the tank. The outgoing water was reintroduced into the recirculating system. Spawning occurred at night at an average frequency of 5 times per week, and collectors were set up from 16:00 to 08:00h every day. The collected eggs were always kept in 1 to 2 L of water while being transferred to rectangular 4.5-L transparent plastic rearing tanks. Two experimental temperatures, for different egg batches, were set: 18.5 ± 0.02°C (warm condition), that corresponds to the preferentially inhabited water masses of *E. ringens* off Peru (Swartzman *et al*., 2008), and 14.5 ± 0.1°C (cold condition), which represents the mean sea surface temperature along the Chilean coast, between 20°S and 33°S (Hormazabal *et al*., 2001). To avoid any thermal stress, the cold condition was established by decreasing the temperature from 18.5 to 14.5°C by 0.5°C h^{-1}. A light regime of 24 L:0 D was provided during the incubation period. Non-viable or unfertilized eggs sunk to the bottom of the incubation tanks and were removed every day by siphoning.

2.3. Larval rearing

The recently hatched larvae were transferred into two 100-L cylindrical black fiberglass tanks, filled with 1-µm filtered (cartridge filter) and UV-sterilized sea water. The two tanks were kept at the same temperatures that were set for the warm and cold incubation conditions, using a chiller. Anchovy larvae were reared using a 24-hr photoperiod from hatching to 23 dph, and with a light regime of 13 L:11 D from 23 dph until the end of the experiment (33 dph), using neon lamps (approx. 1000 lux at water surface) and constant gentle aeration. Our first experiences, prior to this study, showed that non-motile prey were neglected. Larvae were thus

fed, at 18.5°C, with motile dinoflagellate *Akashiwo sanguinea* (100 cells mL^{-1}) from 1 until 7 dph. From 3 to 13 dph, S strain of the rotifer *Brachionus plicatilis* (20 individuals mL^{-1}) was added, which was replaced with the M-L strain (same concentration) from 12 to 19 dph. From 18 dph, larvae were fed with brine shrimp *Artemia sp.* nauplii (5 to 10 individuals mL^{-1}) until 29 dph, and with 24-hr old *Artemia* metanauplii from 28 to 33 dph (same concentration). In addition, phytoplankton *Tetraselmis sp.* (10^5 cells mL^{-1}) was added from 1 until 26 dph (Figure 2). The concentration of food in the tanks was checked daily and adjusted as needed after water exchange.

2.4. Eggs and larvae development

The embryonic development study was performed following Fischer (1958). Eggs from the warm condition (18.5°C) were sampled every 2 hours from 08:00 to 16:00h and were divided into five developmental stages. The time to reach each stage (hours post-fertilization, hpf) was determined by assuming that the spawning peak (and fertilization) occurred around 22:00 h (Alheit *et al*., 1984). Eggs (n 22) were sampled by pipetting from the incubation tanks and pictures were taken under the microscope (Leica DM1000 LED at 40x magnification, Leica Application Suite 4.0 software). The longest (a) and shortest (b) axes were measured in µm (± 1 µm) and mean egg volume (± 95% confidence interval [CI]) was calculated using the following equation for ellipsoids:

$$V = (4/3)\,\pi\,(½a)\,(½b)^2 \qquad (1)$$

Subsequently, between 3 and 17 anchovy larvae were sampled weekly from each tank, using a

500-mL beaker, and anesthetized with Tricaine (MS-222). Standard length (L_S) was measured from the tip of the snout to the end of the notochord, as well as body depth at the anus (D_B), in mm (± 0.01 mm), using a stereo microscope (Leica S8 APO at a 10-25x magnification, Leica Application Suite 4.0 software). Pictures of larvae longer than 1.3 mm L_S were taken with Nikon Coolpix AW130 camera and analyzed to the nearest 0.01 mm with ImageJ program (National Institutes of Health), using graph paper as a scale. Pictures of body details were taken under the microscope (Leica DM1000 LED at 40x magnification) and stereo microscope (Zeiss SteREO Discovery.V12, ZEN 2.6 blue edition software) to assess morphological traits and determine the developmental stage at each experimental temperature. Additionally, early life traits (*e.g.* age at first-feeding, feeding behaviour, schooling) were recorded. In total, 132 larvae were measured and mean L_S (± 95% CI) at age was calculated. For the determination of length at hatch for each temperature, measurements data from complementary batches were used (n 13 and n 51 for 14.5 and 18.5°C, respectively). The relationship between body depth at the anus (D_B, mm) and standard length (L_S, mm) for larvae reared at each of the two temperatures was described using a linear regression model:

$$D_B = a + b * L_S \quad (2)$$

where a is the intercept, and b the slope of the regression line. Additionally, an analysis of covariance (ANCOVA) was performed to test for a significant effect of the temperature on body shape (D_B to L_S ratio) and size-at-age using L_S and age as covariates, respectively.

2.5. Growth model

For both temperatures (18.5 and 14.5°C), the relationship between length and age of the larvae was described using the von Bertalanffy growth function (VBGF, von Bertalanffy, 1938), a mechanistic model based on a differential equation describing the change in weight as a difference between anabolic and catabolic processes. The form of the VBGF proposed by Kooijman (2010) was used:

$$L_t = L_\infty - (L_\infty - L_0)\,(\exp(-T_{corr} * K_d * t)) \quad (3)$$

where L_t is the length (mm) at age t, L_∞ is the asymptotic length (mm), L_0 is the mean length (mm) when $t = 0$ (corresponding to hatching), T_{corr} is the temperature correction, K_d is the von Bertalanffy growth rate (d^{-1}) and t is time since hatching (d). The parameters L_0 and L_∞ were fixed: L_0 was fixed using the mean standard length at hatch for each temperature, and L_∞ was fixed at 17.7 cm (L_S = total length (L_T) /1.14, with L_∞ = 20.2 cm L_T from Fishbase, Froese and Pauly, 2019) for both temperature conditions, since this parameter is not affected by temperature (Kooijman, 2010). According to Kooijman (2010), T_{corr} depends on the Arrhenius temperature (T_A) and can be estimated as:

$$T_{corr} = \exp((T_A / T_{ref}) - (T_A / T)) \quad (4)$$

where T_{corr} is the temperature correction (K), T_A is the species-specific Arrhenius temperature (K), T_{ref} is the reference temperature (K) and T is the absolute temperature (K) at which the growth is monitored. The temperature of 18.5°C (291.7 K) was taken as the reference temperature (where T_{corr} = 1). The goodness of model fit was assessed by examining the

distribution of the residuals.

The accuracy of the VBGF for larvae was further assessed by applying it across a broader range in ages and sizes based on measurements made in other studies on Peruvian anchovy. Size-at-age data for *E. ringens* at 18 and 15°C (close to our rearing temperatures of 18.5 and 14.5°C) were extracted from FishBase (Froese and Pauly, 2019) and the 95% CI of the dataset for each temperature was calculated. For this model (complete life cycle), the units were length = cm, time = years (y), and $K = y^{-1}$ which was renamed K_y. T_{corr} was calculated from Eq. (4) for 18 and 15°C, using T_A obtained for the larval stages.

2.6. Ethical statement

All animal handling procedures complied with the Peruvian animal welfare law (Ley N° 30407 de Protección y Bienestar Animal).

3. Results

3.1. Embryonic development

Despite experiencing temperature and light cycles, spawning was not completely synchronized and occurred almost daily (average frequency of 5 times per week) at highly variable magnitude (50-3000 eggs per spawning). Eggs of *E. ringens* were ellipsoidal, transparent, had a smooth texture, segmented yolk, and lacked an oil globule. The mean (± 95% CI) of the longest and shortest axis was 1343 ± 37 and 687 ± 13 µm, respectively, and the mean (± 95% CI) egg volume was 0.33 ± 0.018 mm^3. In the incubation tanks, eggs were buoyant and distributed at the water surface (the shortest axis of the ellipsoid directed upwards). Eggs tended to aggregate and form groups of up to 2000 eggs. In these patches, the eggs were positioned

vertically (longest axis upwards) at the water surface.

Since spawning occurred during the night and eggs were collected in the morning, embryogenesis could only be described from the end of stage I to stage V. At 18.5°C, the end of stage I was reached at 10 hpf and the germ ring was formed at the animal pole by the thickening of the blastoderm marginal region (Figure 3a). Stage II was reached at 12 hpf, when the epiboly, the process of migration of the germ ring, started. The blastoderm started to spread and embrace the yolk-sac, and the germ ring approached an equatorial position (½ epiboly) (Figure 3b). Stage III was reached at 14 hpf. The blastoderm expanded and the germ ring passed the equatorial position. A local accumulation of cells in the germ ring formed the embryonic shield (Figure 3c,d). At the end of stage III, epiboly reached 80% and the notochord started to develop (Figure 3e). Stage IV was reached at 18 hpf, with the closure of the blastopore (end of epiboly). At the end of stage IV, the embryo surrounded almost half of the yolk and somites started to form. The tail bud became visible and the optic capsules started to develop in the cephalic region (Figure 3f). Stage V was reached at 34 hpf, when the embryo surrounded the two-thirds of the yolk. At the same time, the body thickened, the notochord lengthened and the number of somites increased. At this stage the optic capsules were well defined as well as the otic vesicles containing the otoliths, and the heart started to beat (Figure 3g,h). Just before hatching, larvae showed increasing signs of activity. Hatching started at 36 hpf (Figure 3i,j) at 18.5°C and was completed within 42 hpf. At 14.5°C, hatching was completed within 48 hpf.

3.2. Larval development

Larval development was divided into four main stages (Table 1). Stage 1, which started at hatching, ranged from 2.82 to 4.03 mm *Ls* and from 2.04 to 3.88 mm *Ls* at 18.5 and 14.5°C,

respectively (Figure 4). At this stage, yolk-sac larvae were transparent, the mouth was closed and the eyes and body were not pigmented (Figure 5a-c). A primordial fin extended along the dorsal and ventral side of the body (Figure 5a), and the notochord was straight in its entire length (preflexion stage, Figure 5b). At 1 dph, the pectoral fin began to develop and was clearly visible at 2 dph, when the mouth was open, the eyes pigmented and some yolk-sac was still present (Figure 5d,e,f).

The complete absorption of the yolk-sac defined the beginning of stage 2 (Figure 5g) and occurred at 3 dph, at 18.5°C. This stage included three substages. In the substage 2a, the dorsal fin was not yet posteriorly separated from the primordial fin, forming a marginal finfold which surrounded the larva. This stage included larvae between 4 and 11 dph and ranged from 4.35 to 9.53 mm L_S at 18.5°C, while it included larvae between 8 and 17 dph and ranged from 4.05 to 8.13 mm L_S at 14.5°C (Figure 4). At 3 dph, microalgae (*Tetraselmis sp.*) was observed in the digestive tract of first-feeding larvae. From 4 dph (4.55 ± 0.32 mm L_S at 18.5°C) onward, larvae started to feed on the dinoflagellate *A. sanguinea*. At 7 dph and 18.5°C, the diet of *E. ringens* switched from phyto- to almost exclusive zooplankton. As size increased, larvae were able to feed on larger prey items (rotifers from 6 dph and *Artemia* from 18 dph). The substage 2b was defined when the division between the posterior edge of the dorsal fin and the posterior part of the primordial fin was completed, but the lobe of the dorsal fin did not markedly protrude beside or above the primordial fin (Figure 5h). This stage included larvae between 11 and 18 dph at 18.5°C and 24-dph larvae at 14.5°C, and ranged from 7.00 to 14.04 mm L_S (18.5°C) and from 6.06 to 11.71 mm L_S (14.5°C) (Figure 4). In substage 2c, the dorsal fin was completely separated from the primordial fin and the edge of the dorsal fin protruding markedly, but the tip of the notochord was still straight at its posterior end (Figure 5i). This stage included larvae between 12 and 19 dph at 18.5°C and 31-dph larvae at 14.5°C, and larval size ranged from 8.15

to 12.75 mm *Ls* at 18.5°C and from 9.51 to 12.97 mm *Ls* at 14.5°C (Figure 4).

Stage 3 was characterized by the flexion of the posterior tip of the notochord, the complete separation of the caudal fin from the primordial fin, and the beginning of the caudal fin rays formation (Figure 5j). At this stage, the anal fin was not yet completely separated posteriorly from the ventral primordial fin. Only individuals from the warm condition attained this stage, which included larvae between 19 and 26 dph, and ranged from 10.89 to 15.03 mm *Ls* (Figure 4).

At the end of the laboratory rearing (33 dph), larvae from 18.5°C attained stage 4 (17.42 to 23.65 mm *Ls*) (Figures 5k). At this stage, melanophores developed on the side (Figure 6a) and the top (Figure 6b) of the head, and the gills were visible (Figure 6c). The dorsal and anal fins were completely formed (Figure 6d) and melanophores along the body increased in size and became large stellates (Figure 6e). The caudal fin was completely formed and melanophores were present (Figure 6f). The structure of the jaw had not yet acquired the typical structure of adult filter-feeding anchovies and the fish were performing raptorial hunting for their prey.

3.3 Larval behaviour

Activity of *E. ringens* larvae can be divided into different patterns: recently hatched larvae stayed motionless with the head downwards. After some hours, this pattern changed and intermittent swimming began with some bursts of continuous swimming. When the larvae started feeding on rotifers, the larva formed an S-shaped posture and extended in a striking motion towards the prey. The time spent in active swimming increased with increasing larval age and the first sign of schooling (continuous swimming as a group, Hunter and Coyne, 1982) occurred at 31 dph at 18.5°C.

3.4. Larval size-at-age

The mean ± 95% CI size at hatch of *E. ringens* larvae was 2.76 ± 0.34 and 3.40 ± 0.10 mm L_S at 14.5 and 18.5°C, respectively, and larvae at the colder temperature were significantly smaller (*t*-test = 3.9; df = 15; $P < 0.05$). Larvae reared at 18.5°C reached a mean ± 95% CI size of 20.49 ± 0.86 mm L_S at 33 dph, while larvae reared at 14.5°C reached a size of 11.53 ± 0.65 mm L_S at 30 dph (Figure 7), and temperature had a positive and significant effect on growth (ANCOVA, $P < 0.001$), within the temperature range evaluated. There was no change in shape during the larval period and the relationship between body depth at anus (D_B) and L_S was linear for the two rearing temperatures (Figure 8, Table 2). This morphometric characteristic was not significantly affected by temperature (ANCOVA, $P > 0.05$).

Arrhenius temperature for *E. ringens*, calculated from larval stages, was T_A = 11680.90 K (Table 3) and the fitting of the VBGF was highly significant ($P < 0.001$) for both larval stages and the complete life cycle (Table 3). However, the fitting of the model was not totally accurate for the earliest larval stages. Length at age 4 and 5 dph from the warm condition, and 8 and 10 dph from the cold condition were predicted by the model to be longer than sizes observed at those ages and temperatures (Figure 7). In accordance with the model fitting, distribution of residuals showed that at length between 5 and 6 mm L_S for warm and cold conditions, model predictions were overestimated in comparison to the observations (Figure 9). Additionally, although the VBGF prediction curves for the complete life cycle of *E. ringens* were found within the 95% confidence interval for each temperature, length at 3 years seemed underestimated, particularly at 18°C (Figure 10).

4. Discussion

It is challenging to maintain and spawn adults of small pelagic (Clupeid) fish in laboratory conditions due to their sensitivity to manual handling. Furthermore, larval growth can be hampered by the inability to provide suitable (high quality) prey (Peck *et al*., 2021) and, thus, information from the controlled rearing for early life stages is often scarce. This study documents the captive breeding of adult Peruvian anchovy and the successful rearing and development of its eggs and larvae. This study represents the first detailed description of the early development of this species in the laboratory. The high-quality images of various stages can be compared with knowledge gained on field-caught eggs and larvae to corroborate the successful development of larvae in the laboratory, a fundamental step that helps ensure that measurements made in the laboratory can be generalized to the wild. We also provide the first model describing the temperature-specific size-at-age of Peruvian anchovy that includes data on larvae, juveniles, and adults.

4.1. Peruvian anchovy rearing

Successful rearing of *E. ringens* required that larvae were provided small mobile prey in high abundance during the first days of feeding; larvae did not feed on immobile prey. The presence of dinoflagellates such as *Akashiwo sanguinea* (former name *Gymnodinium splendens*), that are small (40-80 µm) motile cells is, thus, critical for the proper development of Peruvian anchovy larvae. Similar results were obtained in rearing experiments with the larvae of Northern anchovy (*Engraulis mordax* Girard 1858) (Hunter, 1976; Lasker *et al*., 1970; Scura and Jerde, 1977), with some authors emphasizing the importance of the abundance of small motile prey in concentrations ranging from 20 to 40 cells mL^{-1} for the survival and growth of

anchovy larvae (Lasker and Smith, 1977; Lasker and Zweifel, 1978). Moreno *et al*. (2011) reported successful rearing of *E. ringens* until 163 dph in the absence of dinoflagellates but in the presence of three species of nanoflagellates (*Isochrysis galbana*, $3x10^4$ cells mL^{-1}; *Dunaliella tertiolecta*, $2x10^4$ cells mL^{-1}; and *Tetraselmis suecica*, $1x10^4$ cells mL^{-1}). On the contrary, the Japanese anchovy (*Engraulis japonicus* Temminck & Schlegel 1846) was successfully reared in absence of phytoplankton, replaced by concentrations of the rotifer *B. plicatilis* of ≥ 20 individuals mL^{-1} (Fukuhara, 1983). Nevertheless, results from the present study are consistent with the finding that gut contents of field-caught Peruvian anchovy larvae almost exclusively contained flagellates and dinoflagellates (Muck *et al*., 1989). Field surveys along the Peruvian coast also reported that spawning activity is associated with areas of high phytoplankton concentrations (Walsh *et al*., 1980). Of the two annual spawning peaks of *E. ringens*, the smallest one (occurring in February-March) contributes most to the recruitment of this population (Pauly, 1987), and during the austral summer and autumn, phytoplankton biovolume is dominated by dinoflagellates along the Humboldt Current System off Peru (Ochoa *et al*., 2010; Rojas de Mendiola, 1989). Walsh *et al*. (1980) and Pauly (1987) suggested that the co-occurrence of spawning and high levels of dinoflagellates allows successful first feeding and survival of anchovy larvae.

4.2. Embryonic development

In the present study, size and embryonic development (time from spawning to hatching) of eggs of *E. ringens* spawned in captivity were very similar to those of eggs caught in the wild off central Peru and central and southern Chile, although smaller sizes were reported in eggs of the Peruvian anchovy caught off northern Chile (Table 4). Maternal influence and adaptations to specific environmental conditions can result in a differentiation of egg traits

among populations of the same species (Castro *et al*., 2009). Egg size could, therefore, be an indicator of contrasting environmental conditions, such as the coastal areas of central Peru and northern Chile, both within the Humboldt Current system: the coast of central Peru is formed by a broad continental shelf with high primary production, while in the northern area of Chile the continental shelf is narrower, the upwelling strength and the productivity are weaker (Karstensen and Ulloa, 2019; Montecino and Lange, 2009).

Egg size is fairly similar between various *Engraulis* species, such as the Northern anchovy *E. mordax*, the Argentine anchovy *Engraulis anchoita* Hubbs & Marini 1935 and the European anchovy *Engraulis encrasicolus* (Linnaeus 1758), although eggs of the Japanese anchovy *E. japonicus* are relatively small (Table 4). However, the time required for embryonic development reported in most of the studies on *E. ringens* was shorter than that reported in studies on the other *Engraulis* species (Table 4). Most of the eggs of *E. ringens* were incubated at relatively higher temperature, which can increase the rate of embryonic development as demonstrated in European anchovy (*E. encrasicolus*) by Bernal *et al*. (2012), thereby, explaining part of the discrepancy in development times among these various studies.

4.3. Larval development

In the laboratory study, the newly hatched larvae at 18.5°C were significantly longer than those hatched at 14.5°C. Although the lack of data on the size of the eggs incubated at 14.5°C made no comparison possible with the eggs incubated at 18.5°C, and thus did not allow to determine if bigger eggs leaded to a longer size-at-hatch, it could be that temperature, by affecting embryonic metabolism rates, influenced positively larval size-at-hatch, as for the Atlantic cod (*Gadus morhua* L.) (Pepin *et al*., 1997). The size-at-hatch of larvae from eggs caught in the

wild in central Peru and along the Chilean coast was similar to that observed at 14.5°C in the present study (Table 4). Some of the measurements of these studies were made on preserved larvae (Table 4) and the shrinkage due to preservation, which is more noticeable for small larvae (Theilacker, 1980), could account for some of the smallest values. In addition to temperature, maternal effects and local adaptations of adults can influence larval size at hatch (Llanos-Rivera and Castro, 2006). As a consequence of maternal effects on both eggs and larvae, smaller eggs from northern Chile produced smaller larvae, while anchovy population from southern Chile produced relatively larger eggs and larger larvae at hatch. Those authors suggested that the larger size in southern Chile was an adaptation by this species providing the larvae a better chance to survive the particularly adverse environmental conditions in that area (Llanos-Rivera and Castro, 2006). Furthermore, adult anchovies from the present study were maintained in captivity and were fed until satiation every day. They may have assimilated more energy, accumulated more reserves and consequently allocated more energy for reproduction. Larger size at hatch reported here could, thus, reflect better feeding conditions of broodstock fish as it was demonstrated for various species, such as Icelandic cod *Gadus morhua* L. (Marteinsdottir and Steinarsson, 1998), Atlantic herring *Clupea harengus* L. (Bang *et al*., 2006), European sea bass *Dicentrarchus labrax* (L.) (Saillant *et al*., 2001) and common sole *Solea solea* (L.) (Ofelio *et al*., 2020). Nevertheless, biochemical analysis (*e.g.* lipids and proteins) of laboratory-reared eggs and larvae are needed to add support to this hypothesis.

Similarly, *E. ringens* larvae reared in the laboratory at 18.5°C had a greater length-at-hatch than the larvae of congeners (Table 4). The timing of developmental events observed here (*e.g.* age at mouth opening, yolk-sac absorption and first-feeding), however, was similar to that observed in previous studies on this and other engraulids such as *E. mordax*, *E. japonicus* and *E. anchoita*. Body pigmentation of laboratory-reared *E. ringens* larvae from the present study

followed the pattern described by other authors working on this species (Einarsson and Rojas de Mendiola, 1963; Fischer, 1958) and others working on *E. japonicus* (Fukuhara, 1983). Furthermore, timing of eye pigmentation agrees with observations made for *Engraulis* genus. Similar to most marine teleosts, Muck *et al*. (1989) showed that mouth and eye functionality of *E. ringens* were closely related, which is not surprising since anchovy larvae are visual feeders (De Mendiola, 1974). Moreover, as demonstrated for *E. mordax* (O'Connell, 1981) and *E. anchoita* (Miranda *et al*., 2020), early-feeding anchovy larvae have effective, binocular vision, although the retina and the lens retractor muscle develop at later stages.

The staging system used here was based on the external morphology of *E. ringens* specimens, accordingly to previous classifications for pelagic larvae (see Table 1). Similar to the early morphology and characteristics of *E. japonicus* reported by Takahashi and Watanabe (2004), larvae of *E. ringens* were initially translucent. They went through a series of morphological changes within days of hatching but the developmental landmarks at later larval stages are more gradual in engraulids than in other species, such as pleuronectids (Takahashi and Watanabe, 2004). This gradual development makes it challenging to determine a simple morphological criteria separating larvae from juveniles. Takahashi and Watanabe (2004) adopted a different staging system to describe the morphological development in *E. japonicus*, using the degree of guanine deposition as the criteria to identify the larval-juvenile metamorphosis. These authors also reported that changes in relative body depth (body depth to body length ratio) were completed towards the end of metamorphosis, just prior to the juvenile stage. On the other hand, Moreno *et al*. (2011) indicated that the start and end of metamorphosis (called "transition period") were better defined by changes in body morphometry as a function of age, suggesting the first inflection point to occur at an average age of 46 days. In the present study, the degree of guanine deposition was not taken into consideration for morphological

description, and a difference in body shape was not consistently registered as larvae aged. In the present study, the rearing experiment had to stop when the quarantine due to the covid-19 pandemic started in Peru, and data on larvae older than 33 dph could not be collected. However, based on our final morphological assessment, the oldest individuals reared in this study had not yet entered into the metamorphic period but had reached the final stages of the larval period. Our results agree with those of Plaza *et al*. (2018) on Peruvian anchovies caught in northern Chile. That study reported that the pre-recruit stage (*i.e.* juvenile period after transition) started from 2.7 cm. Peck *et al*. (2005) demonstrated the prolonged changes in body shape that can occur as Clupeid larvae metamorphose into juveniles. Future rearing of larvae through metamorphosis will allow us to better understand the ontogenetic changes occurring throughout early development of the Peruvian anchovy.

4.4 Larval behaviour

Swimming and feeding behaviour observed during early larval development of *E. ringens* reared in the laboratory matched those described for *E. mordax* (Hunter, 1972), *E. ringens* (Ware *et al.,* 1981) and *E. japonicus* (Fukuhara, 1983; Fukuhara and Takao, 1988). Similar observations of "continuous swimming" and "intermittent swimming" were described for Northern anchovy, as well as the "S-shape striking posture" when feeding (Hunter, 1972). Compared to other species, such as reef fish families, anchovy larvae are poor swimmers. Nevertheless, as for many other fish species, with increases in swimming activity, foraging success improves (Houde and Schekter, 1980; Yúfera and Darias, 2007) as well as escape responses (Folkvord and Hunter, 1986; Fuiman, 1994; Gibb *et al*., 2006), with a positive effect on survival. Additionally, in the present study, no schooling was observed in larvae < 31 dph (18.5 mm L_S, calculated from the VBGF model fitting) at 18.5°C. This is longer than the values

of 13 to 15 mm L_S observed in *E. mordax*, for the onset of schooling (Hunter and Coyne, 1982). However, the rearing at a colder temperature (15.6-16.4°C) of *E. mordax* larvae might account for this variation. At larger body sizes, *E. ringens* larvae are potentially able to swim strongly enough to actively participate in their dispersal.

4.5. Modelling temperature-specific size-at-age

The body size at specific developmental stages was similar at both temperatures and the mean size-at-age increased more rapidly at 18.5 compared to 14.5°C. The VBGF, being a mechanistic and bioenergetics-based model (Sousa *et al*., 2010), provides a metabolic interpretation of the model parameters directly linked to the physiology of the species. Furthermore, the VBGF presented here, parameterized from the larval stages, gives a simple and unique equation to describe larval growth at different temperatures, when it includes the temperature correction function T_{corr} (Eq. 4), within the optimum temperature range of the species. Moreover, the fitted VBGF proved to capture well juvenile and adult growth, suggesting that temperature effect on Peruvian anchovy larvae can be extrapolated to the entire life cycle. This highlights the major role playing by temperature in the bioenergetics of this species, and the potential of the model presented here for fisheries management and environmental variability scenarios. Consequently, this VBGF seems to be suitable to describe growth in Peruvian anchovy, if one aims to reduce the number of functions and parameters to describe *E. ringens* growth throughout its life cycle. Nevertheless, although the VBGF provides an accurate model to describe *E. ringens* growth, an adjustment is needed to improve model predictions for the earliest stages, until day 10 post hatch. The overestimation of size-at-age of the earliest larvae is likely due to the poor foraging ability and feeding success of these life stages. Feeding success has been reported to increase from 11% at first-feeding (3 dph) to 50% at age 8 dph in

E. mordax (Hunter, 1972). Furthermore, the maturation of the eyes and digestive system, that show extensive development after yolk absorption (O'Connell, 1981), probably causes a delay in the growth. Finally, ingestion capacity and digestive efficiency might not be optimum at first-feeding (Kooijman *et al*., 2011; Ofelio *et al*., 2019). Such developmental changes generate growth stanzas, that are identified as metabolic accelerations and are now well documented in the context of the Dynamic Energy Budget (DEB) theory (Kooijman *et al*., 2011; Kooijman, 2013; Lika *et al*., 2014). If taken into proper consideration it could provide a better adjustment for fish growth models at their early larval stages (Kooijman, 2014). Longer laboratory rearing experiments at different prey concentrations and across more temperatures are needed to fully explore the dynamics and environmental constraints affecting the somatic growth of early life stages in the Peruvian anchovy. Also, as part of further work on the Peruvian anchovy life cycle, an adjustment should be performed both on larval and adult data simultaneously, to improve the VBGF model fitting.

Finally, although temperature had a direct effect on the rates of growth and development in this laboratory study, it is important to note that temperature in the field is linked with a broader range of abiotic and biotic parameters, such as water stratification (Ladd and Stabeno, 2012), or distribution and productivity of phytoplankton communities (Polovina *et al*., 2008). These additional factors linked to in situ temperature may indirectly influence larval and juvenile growth, and they have to be taken into consideration for a better understanding of Peruvian anchovy early life history.

The present study suggests that embryos and larvae of the Peruvian anchovy reared in the laboratory compare well to that reported for field-collected individuals. The morphological staging system described here allowed a better understanding of the ontogeny of this species.

Furthermore, the VBGF proved to be appropriate to describe Peruvian anchovy growth, including the direct effect of temperature. The present work also gives a reliable and repeatable methodology to obtain and rear Peruvian anchovy larvae in the laboratory, providing opportunities for further experiments and measurements on the ecophysiology of the larvae of this species.

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Author contributions

AAV participated in funding acquisition for the "PpR de Acuicultura" of IMARPE. AAV, CO, FR and MRS conceived and designed the study. JDA collected and ensured the maintenance of the broodstock. FR and MRS sampled the eggs and FR and CO sampled the larvae. FR, CO and AAV analyzed the data. FR, CO and AAV wrote the manuscript and MAP contributed to its review and edition. All authors read and approved the final manuscript.

Significance Statement

The Peruvian anchovy supports the largest single-species fishery on the planet. However, information about its early life stages remains scarce. This study represents an updated description of its embryonic and early larval development, illustrated by high quality pictures. A predictive model for larval growth is provided, which includes the effect of temperature. This model can be extended to the complete life cycle, which is essential to improve fishery management, in the actual context of climate change.

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FIGURE 1 Schematic representation of the rearing tank showing the broodstock, eggs and egg collector. The egg collection system consisted of: water intake (a); rearing tank containing the broodstock (b); overflow for bottom water, without eggs (c); fertilized anchovy eggs (d); half-pipe at the water surface (e); PVC pipes (f); valve (g); 100-µm mesh collector and container (h); water outlet to recirculating system (i). Blue arrows indicate direction of water flow.

FIGURE 2 Feeding schedule for Peruvian anchovy *Engraulis ringens* larvae rearing at 18.5°C, from 1 to 33 days post-hatch (dph).

FIGURE 3 Embryonic development of Peruvian anchovy *Engraulis ringens* at 18.5°C: end of phase I, 10 hours post-fertilization (hpf) (a); phase II, 12 hpf (b); phase III front view, 14 hpf (c); phase III side view, 14 hpf (d); end of phase III, 17 hpf (e); phase IV side view, 18 hpf (f); phase V, 34 hpf (g); phase V side view, 34 hpf (h); larva breaking the chorion during hatching 36 hpf (i); newly hatched larva, 36 hpf (j). an, anus; ch, chorion; es, embryonic shield; gr, germ ring; ht, heart; n, notochord; oc, optic capsule; ov, otic vesicle including the otoliths; s, somites; t, tail; tb, tailbud; ys, yolk-sac. Scale bar: 0.5 mm.

FIGURE 4 Standard length (mm) at each developmental stage for Peruvian anchovy *Engraulis ringens* larvae and juvenile reared at 18.5°C (red dots) and at 14.5°C (blue dots).

FIGURE 5 Larval development of Peruvian anchovy *Engraulis ringens* from yolk-sac to juvenile stage at 18.5°C: stage 1, 0 days post-hatch (dph), lateral anterior (a), lateral posterior (b) and ventral (c) view; stage 1, 1 dph, lateral (d) and dorsal (e) view; stage 1, 2 dph, dorsal view (f); stage 2a, 3 dph, lateral view (g); stage 2b, 11 dph (h); stage 2c, 12 dph (i); stage 3, 18 dph (j); stage 4, 28 dph, juvenile stage (k). a, *Artemia*; af, anal fin; cf, caudal fin; df, dorsal fin;

e, eye; fe, fin edge; n, notochord; pf, pectoral fin; pr, primordial fin; ys, yolk-sac.

FIGURE 6 Details and lateral view of 33 days post-hatch (dph) Peruvian anchovy *Engraulis ringens* juvenile, reared at 18.5°C: details of the head, melanophores and gills. Right side view (a); top view (b); left side bottom view (c); details of dorsal fin and anal fin (d), stellate melanophores near the dorsal fin (e); caudal fin (f); Scale bar: 1 mm.

FIGURE 7 Standard length (mm) at age in days (0 = hatching) for Peruvian anchovy *Engraulis ringens* larvae reared at 18.5°C (filled circles) and at 14.5°C (open circles). Solid lines represent the VBGF fitting for 18.5°C (red) and 14.5°C (blue). See Table 3 for estimated model parameters.

FIGURE 8 Relationship between body depth at the anus (D_B), and standard length (L_S) for Peruvian anchovy *Engraulis ringens* larvae reared at 18.5°C (filles circles) and 14.5°C (open circles). Solid lines represent linear regression fitting for 18.5°C (red) and 14.5°C (blue). Estimated parameters are given in Table 2.

FIGURE 9 Distribution of residuals of the VBGF fitting for 18.5°C (a) and 14.5°C (b). Arrows indicate overestimated values.

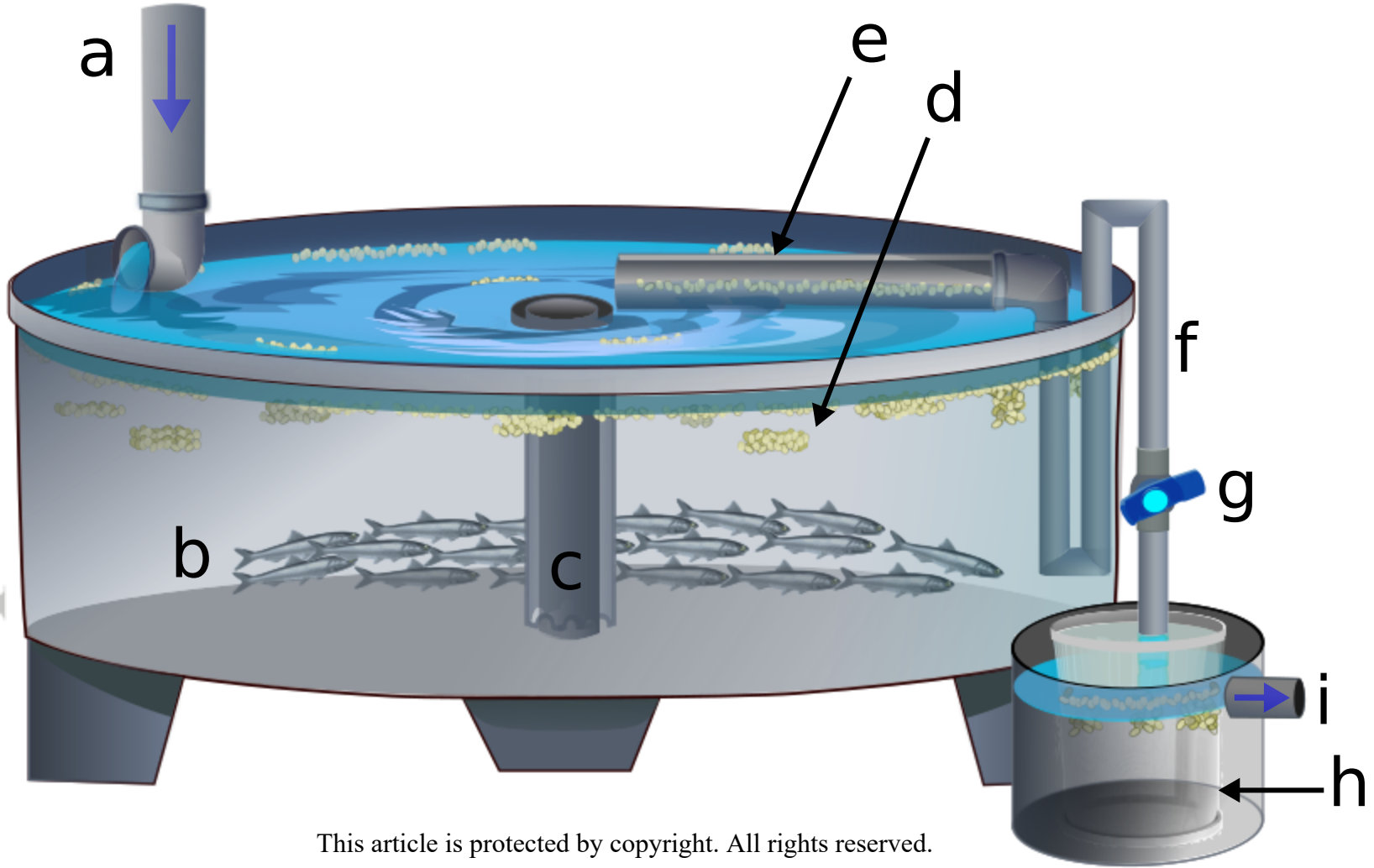
FIGURE 10 Standard length at age and VBGF fitted curves for the full life cycle of Peruvian anchovy *Engraulis ringens*, at two different temperatures. Data from FishBase is represented with red dots (18°C) and blue dots (15°C) and 95% confidence interval is represented with red shaded area (18°C) and blue shaded area (15°C); VBGF fitted curves are represented with red line (18°C) and blue line (15°C).

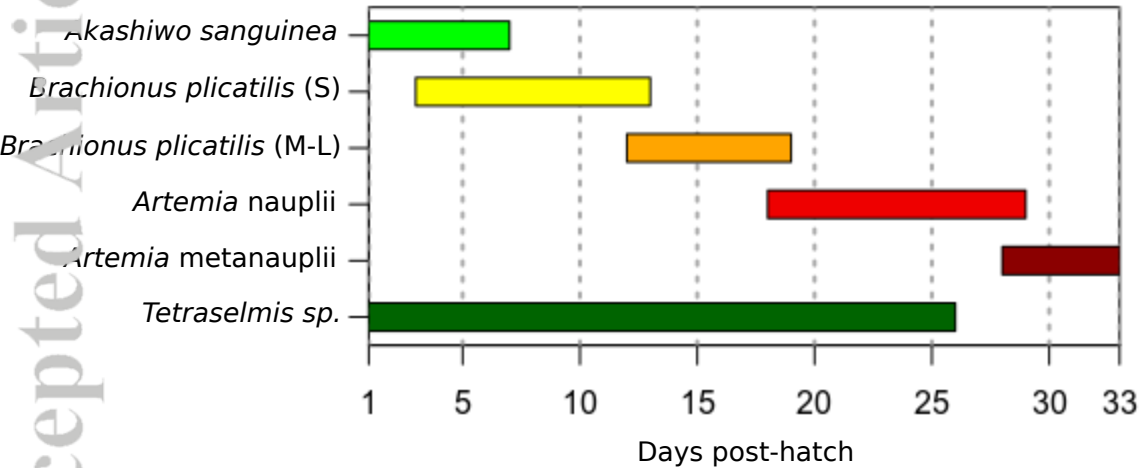
TABLE 1 Morphological staging of Peruvian anchovy *Engraulis ringens* reared in captivity, compared to other species.

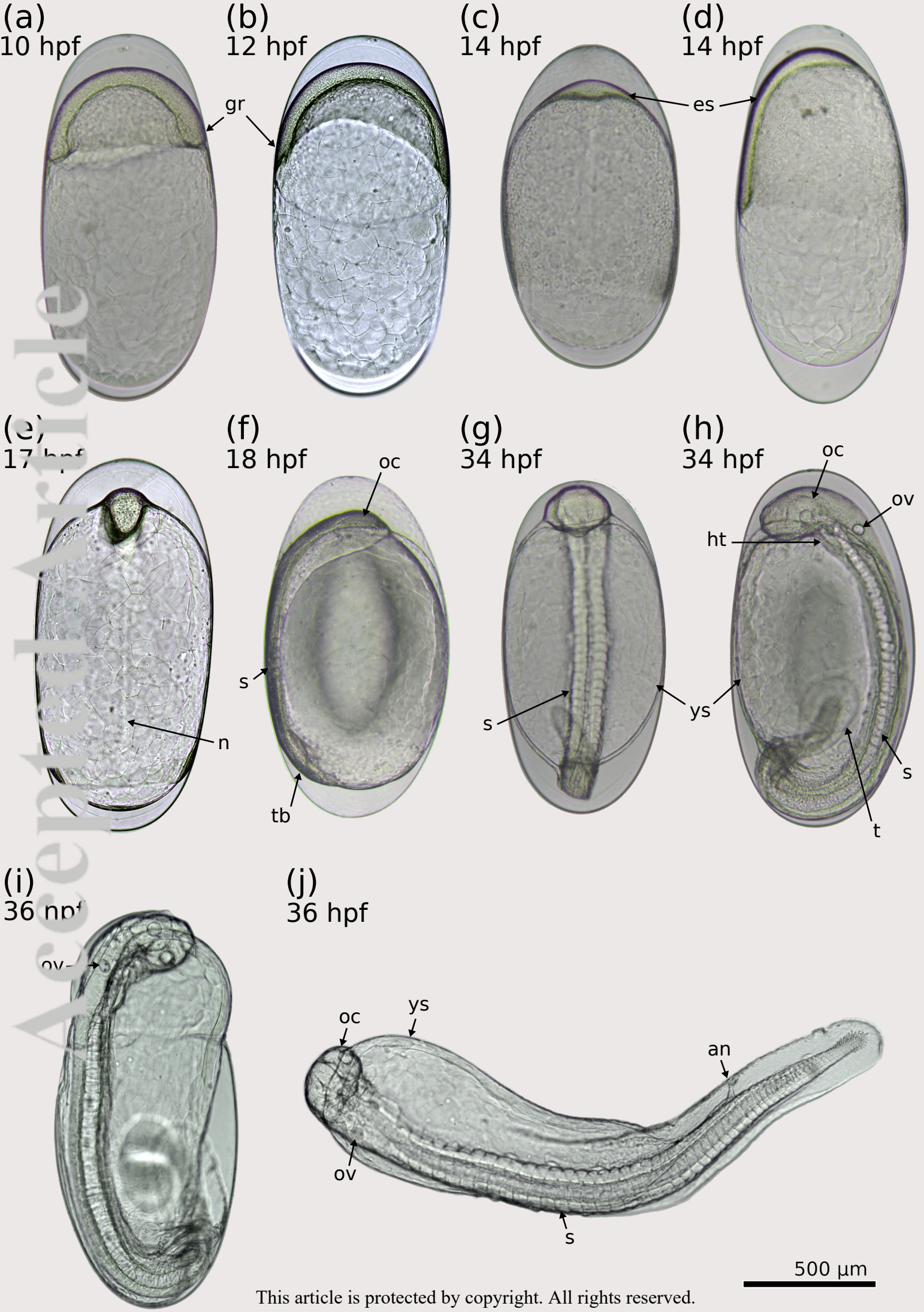
TABLE 2 Estimates of linear regression parameters for fitted relationship between body depth at the anus (D_B) and standard length (L_S) for Peruvian anchovy *Engraulis ringens* larvae reared at two temperatures (18.5°C and 14°C). All model estimates are significant ($P < 0.001$).

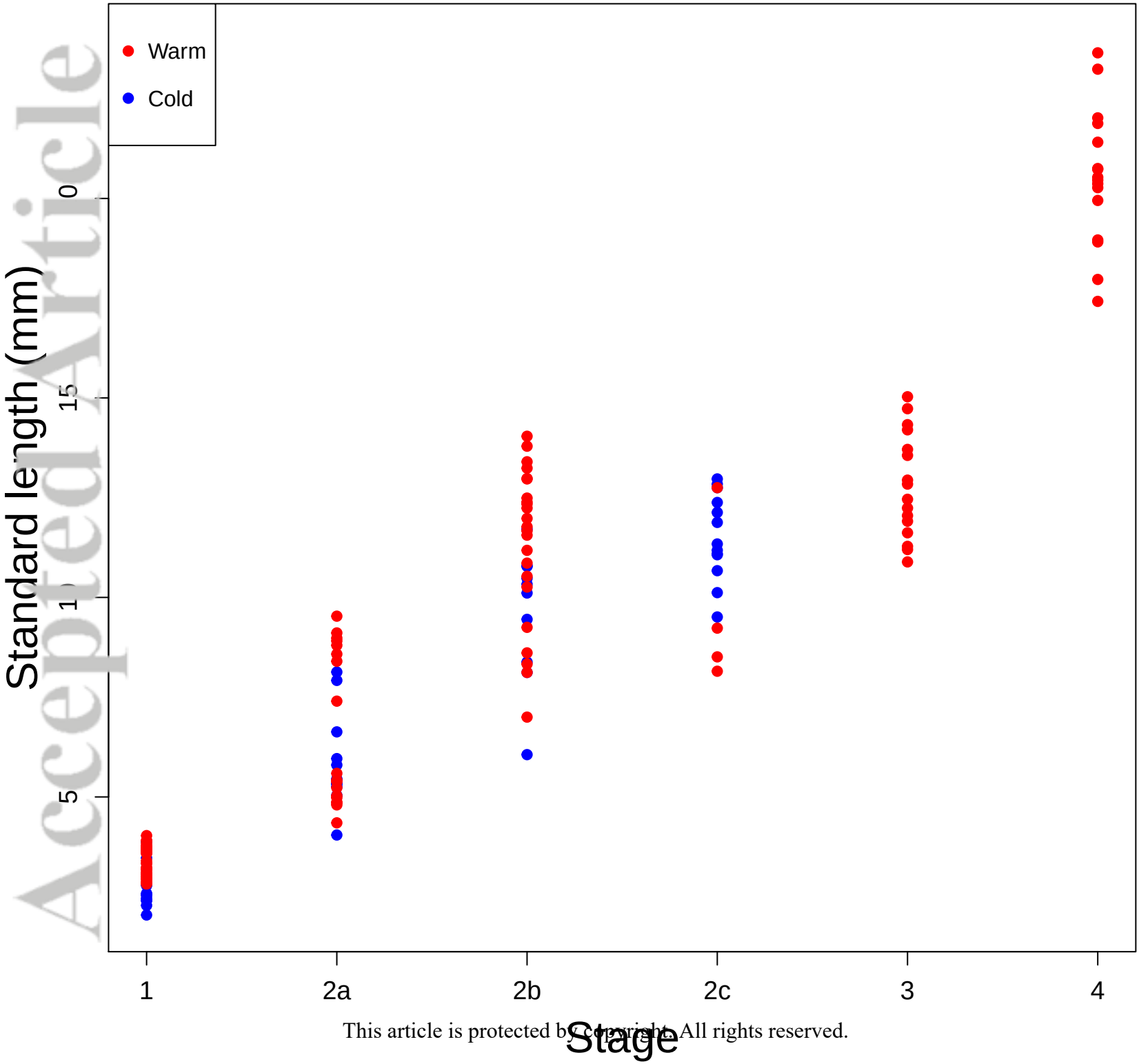
TABLE 3 Estimates of VBGF parameters for fitted growth in standard length of Peruvian anchovy *Engraulis ringens* larvae at 18.5 and 14.5°C, and for *E. ringens* complete life cycle at 18 and 15°C. All model estimates are significant ($P < 0.001$).

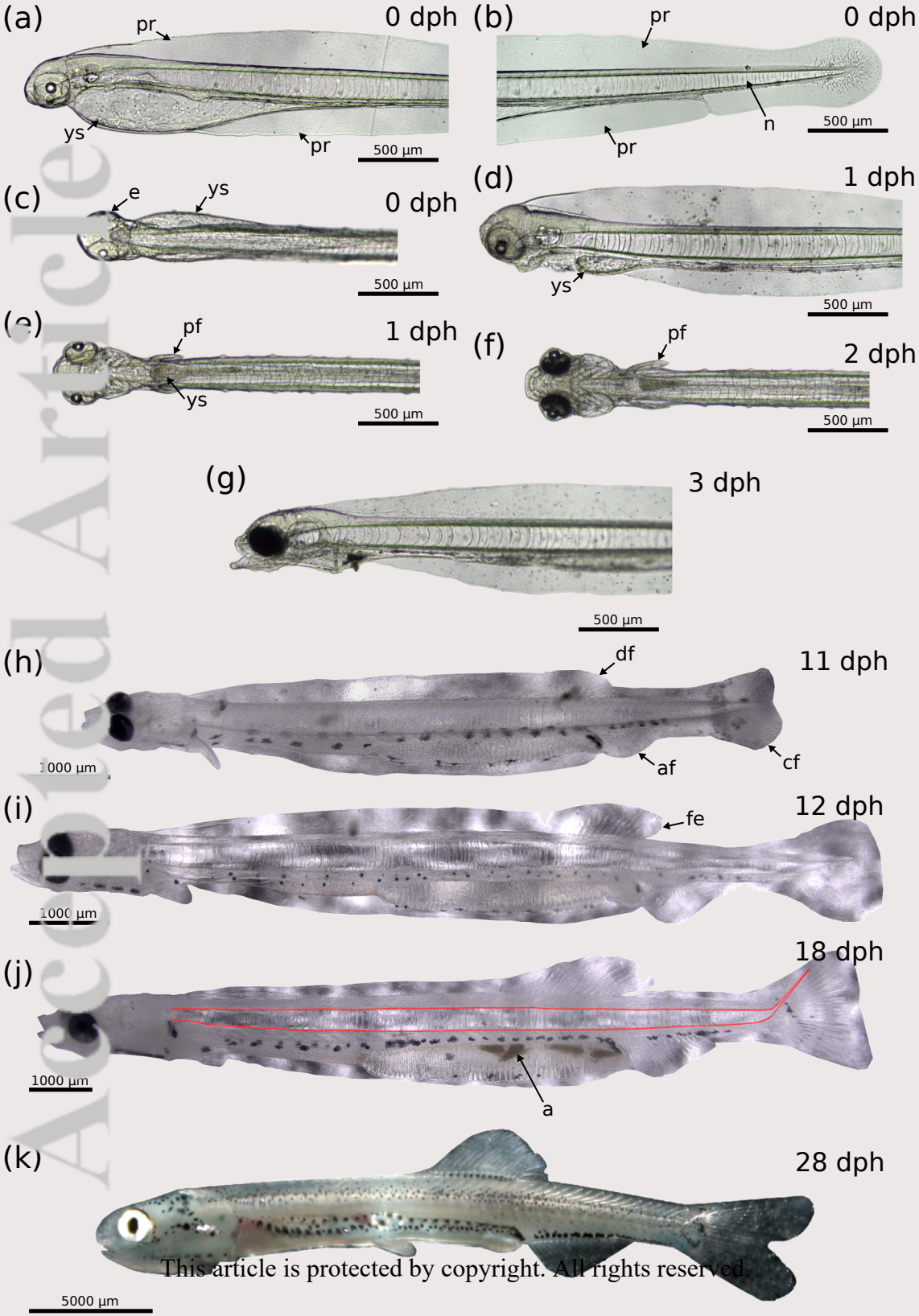
TABLE 4 Early life traits in different species from *Engraulis* genus.

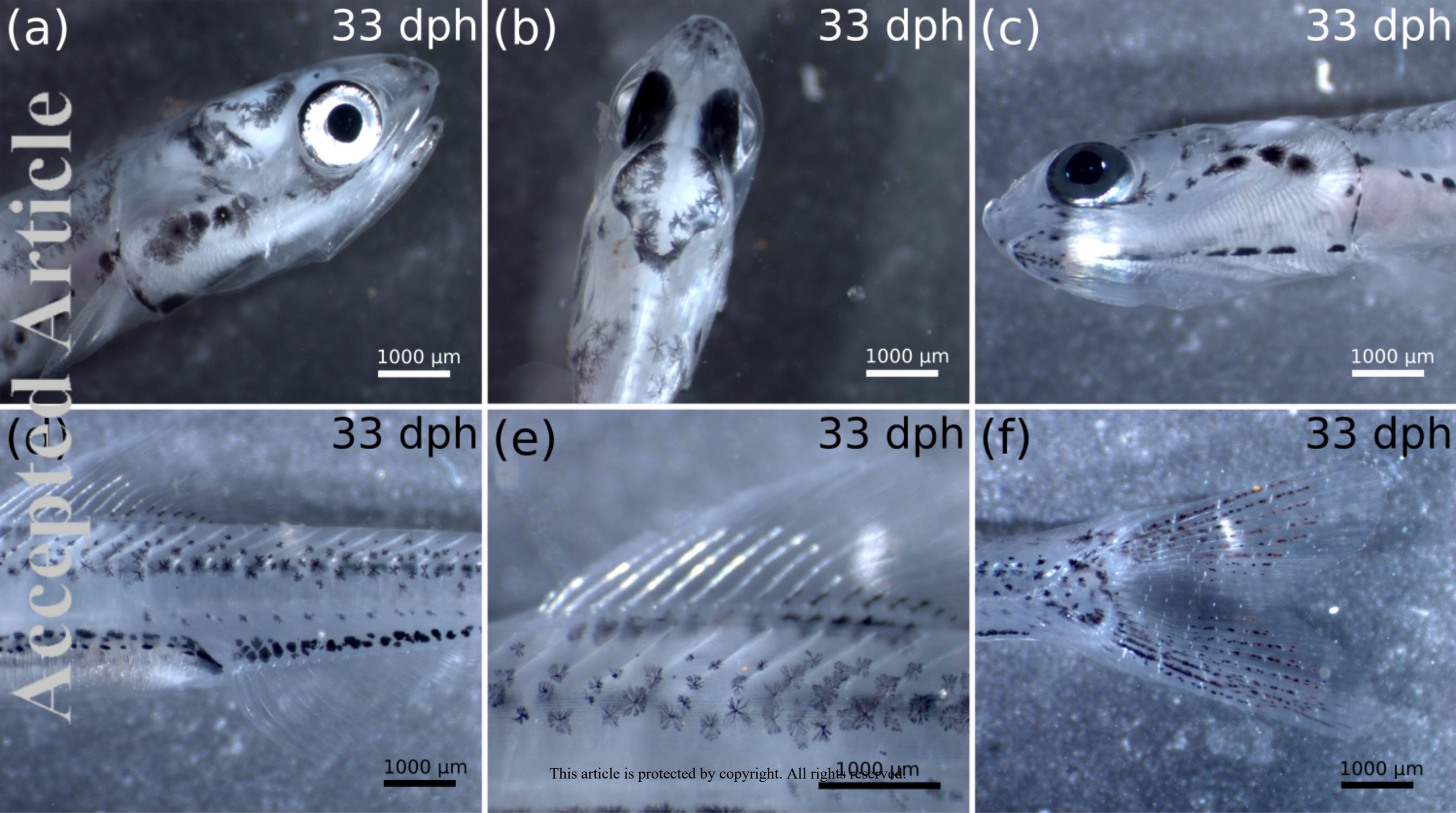


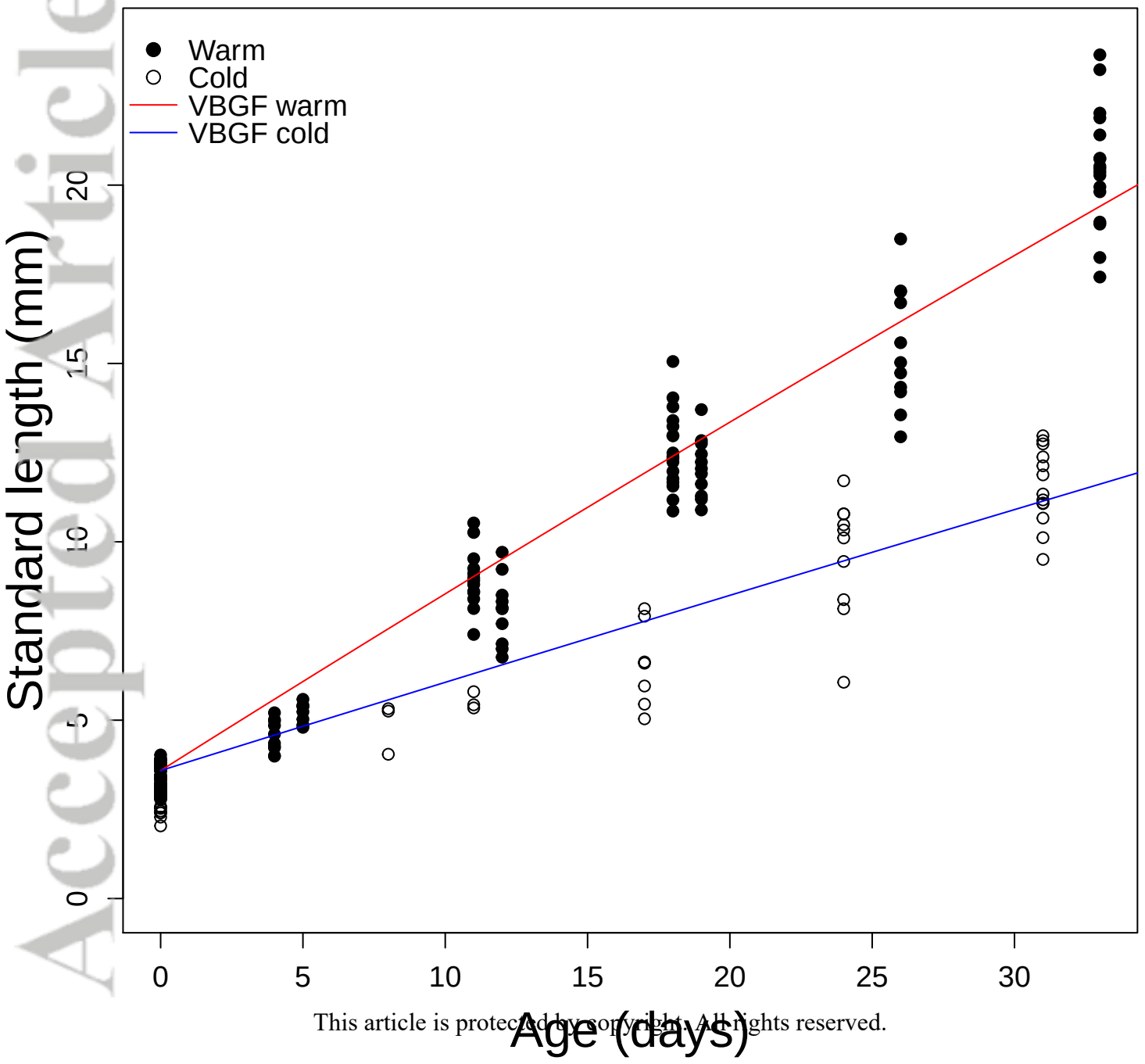


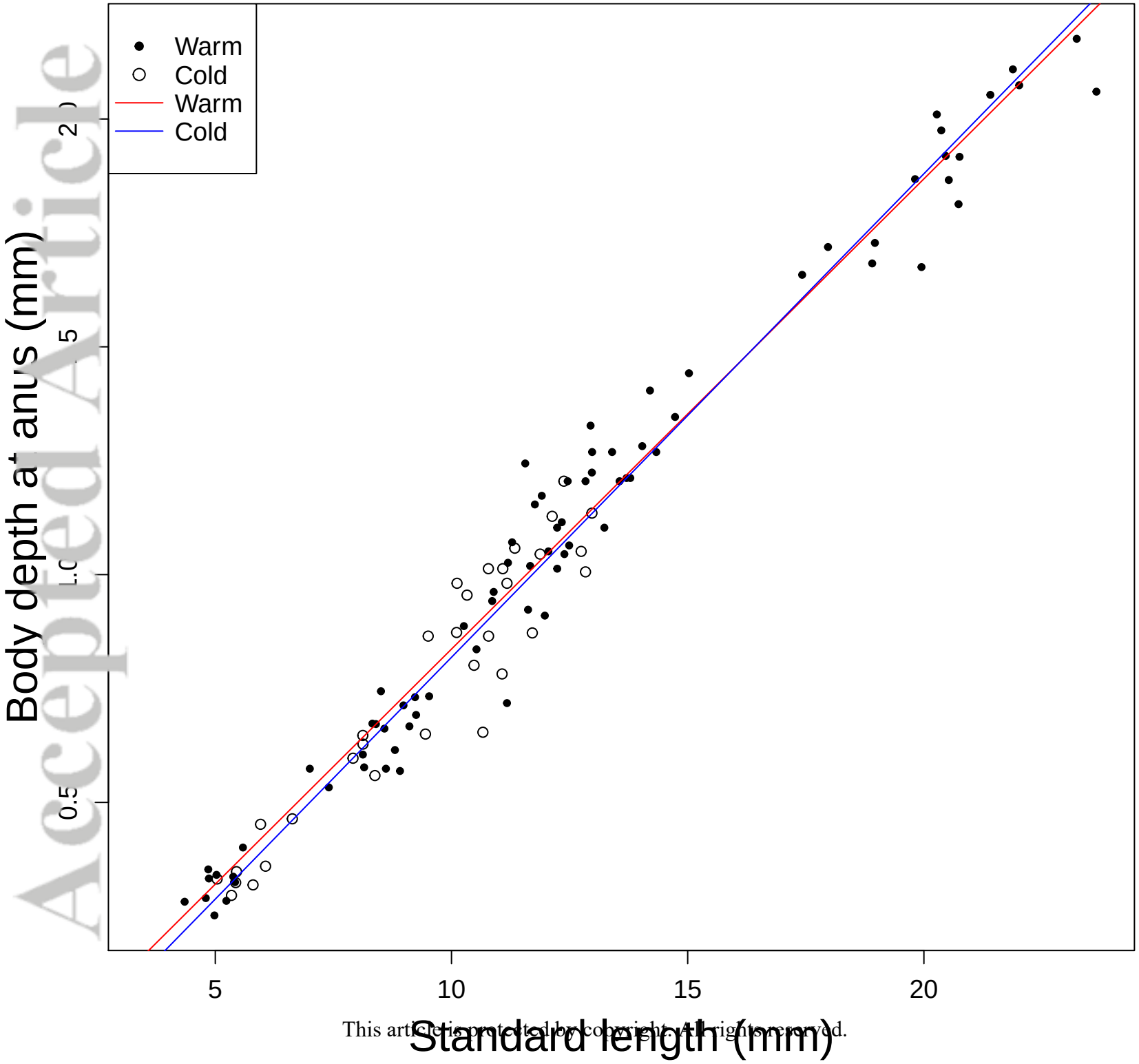


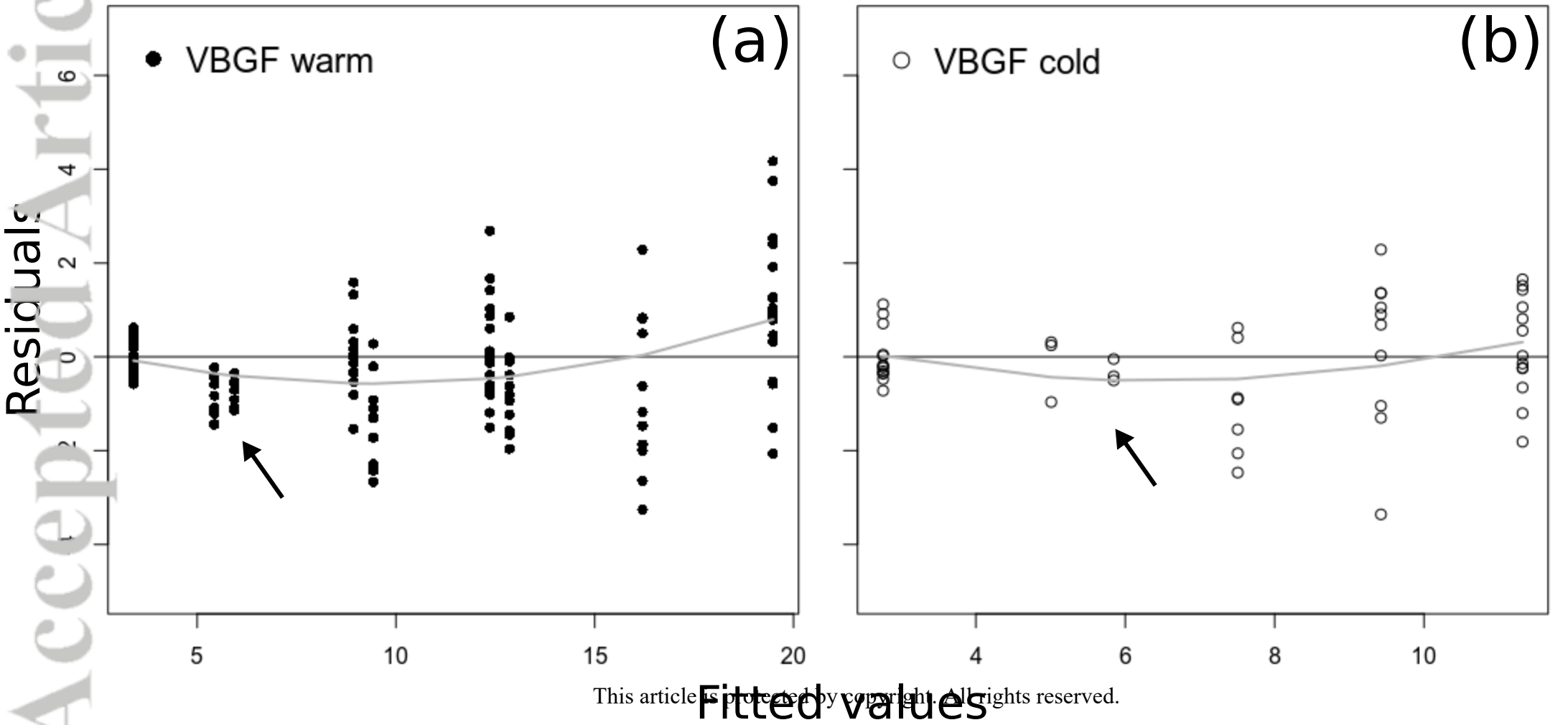


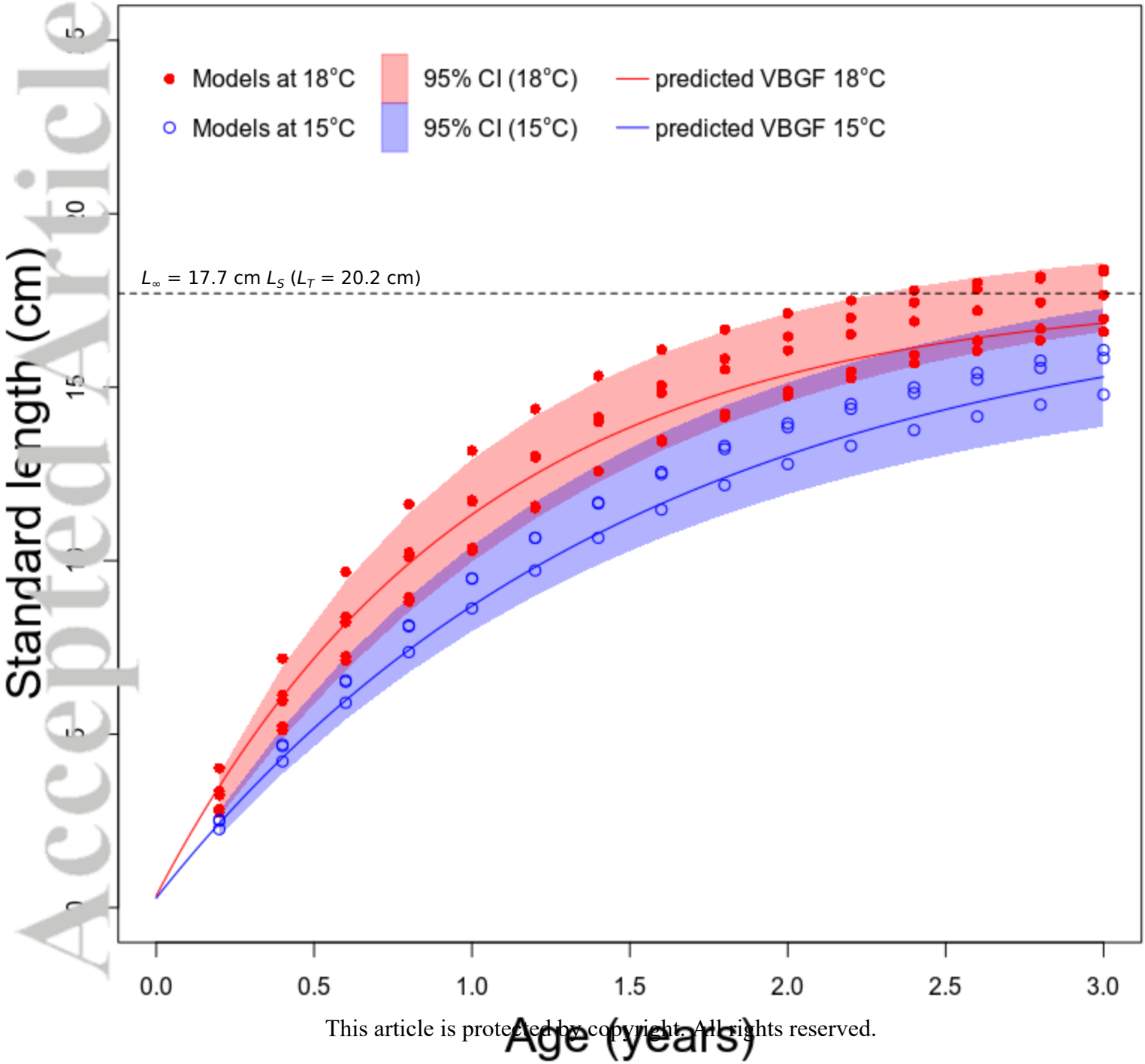












Significance Statement

The Peruvian anchovy supports the largest single-species fishery on the planet. However, information about its early life stages remains scarce. This study represents an updated description of its embryonic and early larval development, illustrated by high quality pictures. A predictive model for larval growth is provided, which includes the effect of temperature. This model can be extended to the complete life cycle, which is essential to improve fishery management, in the actual context of climate change.

Stage	Doyle, 1977	Balon, 1999	Takahashi and Watanabe, 2004	Garrido *et al*., 2016	Present study
	Clupea harengus		***Engraulis japonicus***	***Sardina pilchardus***	***Engraulis ringens***
1	Post-hatching. Yolk-sac morphology (substage a, b, c)	Embryo	Larval stage	Hatching day, no pigmentation	Finfolded larva, yolk-sac, no pigmentation
				Pigmentation, yolk-sac, mouth opened.	
2	Yolk-sac absent. Dorsal fin differentiating from the dorsal primordial fin (substage a, b, c)	Finfolded Larva	-	Pectoral fins and beginning of caudal fin formation	Finfolded larva, eyes pigmented, mouth opened, pectoral fins and beginning of caudal fin formation, dorsal fin not yet separated posteriorly from primordial fin, yolk-sac absent.
2.1	Dorsal fin not yet separated posteriorly from primordial fin. Yolk-sac absent.				
2b	Division between the posterior edge of the dorsal fin and the posterior part of the primordial fin complete. Lobe of dorsal fin not protruding markedly beyond the posterior primordial fin nor growing along beside it.	Finformed Larva	-	-	Finformed larva, division between the posterior edge of the dorsal fin and the posterior part of the primordial fin completed. Lobe of dorsal fin not protruding markedly. Beginning of anal fin formation.

2c	Dorsal fin protruding markedly beyond or beside the primordial fin and extending posteriorly above it. The dorsal fin is completely separate from the primordial fin posteriorly. The posterior tip of the notochord is still more or less straight.	-	-	-	Finformed larva, dorsal fin protruding markedly and completely separated from the primordial fin. The posterior tip of the notochord is still more or less straight.
3	Notochord turns dorsally at its posterior tip. The dorsal fin is clearly differentiated. The pelvic fins are not yet visibly protruding ventrally below the gut when the larva is viewed from the side (substage a, b, c)	-	Early metamorphosing	Beginning of notochord flexion Beginning of dorsal fin development	Finformed larvae, the dorsal fin is clearly differentiated, beginning of notochord flexion, caudal fin completely separated from the primordial fin, beginning of caudal fin rays formation, the anal fin is not yet completely separated posteriorly from the ventral primordial fin.
4	Pelvic fins are visible, protruding ventrally below the gut, when the larva is viewed from the side. The gut is shortening relative to the body length (substage a, b, c, d)	-	Late metamorphosing	Notochord flexion completed Dorsal fin completed Caudal fin completed	Dorsal fin completed, caudal fin completed, dorsal and ventral stellate melanophores

4a	At least 24 myomeres are present between the posterior point of insertion of the pelvic fin and the anus	-	-	-	-
4b	At least 21 such myomeres but less than 24	-	-	-	-
4c	At least 18 such myomeres but less than 21	-	-	-	-
4d	Adult-like appearance. At least 15 such myomeres but less than 18	Juvenile	Juvenile	-	-

Model	Temperature	Estimates	Std. Error	P-value	r^2
$D_B = a + b * L_S$	18.5	a = -0.20	0.026	5.93e-11	0.97
		b = 0.10	0.0019	< 2e-16	
	14.5	a = -0.24	0.063	0.00052	0.90
		b = 0.11	0.0064	< 2e-16	

	Model	Temperature	Estimates	Std. Error	*P*-value
von Bertalanffy (larval stages)	$L_t = L_\infty - (L_\infty - L_0)\,(\exp(-T_{corr} * K_d * t))$	18.5	$K_d = 392.46$	0.013	< 2e-16
			$T_{corr} = 1$		
		14.5	$K_d = 392.46$	0.013	< 2e-16
			$T_{corr} = 0.57$		
von Bertalanffy (complete life cycle)	$L_t = L_\infty - (L_\infty - L_0)\,(\exp(-T_{corr} * K_y * t))$	18	$K_y = 1.075$	0.013	< 2e-16
			$T_{corr} = 0.93$		
		15	$K_y = 1.075$	0.013	< 2e-16

T_{corr} = 0.61

Arrhenius temperature T_A = 11680.90 K

Author	Species	Egg origin	Temperature (°C)	Egg size (volume in mm³)	Embryonic development	Sample state	Size at hatch (mm)	Mouth opening	Eye pigmentation	Yolk-sac absorption	First-feeding
Present study	*E. ringens*	Laboratory	18.5	0.33 ± 0.018	42 hpf	Fresh	3.40 ± 0.10	2 dph	2 dph	3 dph	3 dph
		Laboratory	14.5	-	48 hpf	Fresh	2.76 ± 0.34	-	-	-	-
Fischer, 1958	*E. ringens*	Valparaiso, central Chile	10.5-12.5	0.266	4 days	Fresh	2.9-3.1	-	2 dph	-	-
Einarsson and Rojas de Mendiola, 1963	*E. ringens*	Callao, central Peru	-	0.372	-	Preserved	1.71-2.25	-	3.2-3.5 mm	> 4 mm	-
Santander and Sandoval de Castillo, 1973	*E. ringens*	Northern Peru	14.9-16.9	-	48 -52h	-	-	-	-	-	-
Ivare *et al*., 1981	*E. ringens*	Samanco Bay (Peru)	17-19	-	-	Fresh	2.8	64h	64h	64h	3.5-6.8 dph (average 4.4 dph)
Llanos-Rivera and Castro, 2004	*E. ringens*	Iquique, northern Chile	-	0.201 ± 0.031	-	-	-	-	-	-	-
		Antofagasta, northern Chile	-	0.243 ± 0.034	-	-	-	-	-	-	-
		Valparaiso, central Chile	-	0.298 ± 0.026	-	-	-	-	-	-	-

		Talcahuano, southern Chile	-	0.312 ± 0.030	-	-	-	-	-	-	-
Rivera and Castro, 2006	*E. ringens*	Antofagasta, northern Chile	15	-	-	Fresh	2.50 ± 0.14	-	-	4.16 ± 0.14	-
			18	-	-	Fresh	2.29 ± 0.24	-	-	4.14 ± 0.14	-
		Talcahuano, southern Chile	15	-	-	Fresh	2.60 ± 0.08	-	-	4.54 ± 0.15	-
			18	-	-	Fresh	2.66 ± 0.20	-	-	4.52 ± 0.15	-
Cubillos *et al*., 2007	*E. ringens*	southern Chile	18	-	<60h	-	-	-	-	-	-
			15	-	>60h	-	-	-	-	-	-
Tarifeño *et al*., 2008	*E. ringens*	Antofagasta, northern Chile	18	-	37h (50% hatched)	-	-	-	-	-	-
			15	-	45h (50% hatched)	-	-	-	-	-	-
		Talcahuano, Southern Chile	18	-	39h (50% hatched)	-	-	-	-	-	-
			15	-	46h (50% hatched)	-	-	-	-	-	-
Castro *et al*., 2009	*E. ringens*	Iquique, northern Chile	-	0.279 ± 0.03	-	-	-	-	-	-	-

		Talcahuano, Southern Chile	-	0.339 ± 0.03	-	-	-	-	-	-	-
Kramer and Zweifel, 1970	*E. mordax*	California	17	-	-	Fresh	3.2 ± 0.17	-	-	-	-
Lasker *et al*., 1970	*E. mordax*	California	17.5 ± 1	-	1-2 days after collection	Fresh	3.4	-	-	-	3.9 mm (2 dph)
[illegible], 1977	*E. mordax*	Southern California	13-16	0.306	59.8h	-	2.86 ± 0.028	3.8-4.4 mm	3.8-4.4 mm	3.8-4.4 mm	3.8-4.4 mm
Fukuhara, 1983	*E. japonicus*	Laboratory	17.5	0.251	52h	Preserved	2.76 ± 0.19	2 dph	2 dph	2 dph	2-3 dph
Fukuhara and [illegible], 1988	*E. japonicus*	Laboratory	21 ± 0.5	-	-	Preserved	2.62-2.92	2 dph	3 dph	3 dph	3 dph
[illegible]da, 2011	*E. japonicus*	Laboratory	23.2 ± 1.0	0.268	-	-	2.80 ± 0.06	-	2 dph	-	-
Wan and [illegible],	*E. japonicus*	Yellow Sea	-	0.278 ± 0.023	-	-	-	-	-	-	-
		East China Sea	-	0.264 ± 0.028	-	-	-	-	-	-	-
[illegible]era *et al*., 1988	*E. encrasicolus*	East Mediterranean Sea	18.49-26.35	-	-	Preserved	-	3.2 mm	-	3.5 mm	2 dph
[illegible]os *et al*., 2004	*E. encrasicolus*	Bay of Biscay	14.0-17.3	0.37	-	-	-	-	-	-	-

Goarant *et al*., 2007	*E. encrasicolus*	Bay of Biscay	14.84-17.63	0.26	-	-	-	-	-	-	-
Aldanondo *et al*., 2008	*E. encrasicolus*	Laboratory	17.6-22.3	-	within 72h	Preserved	2.97 ± 0.24	-	-	-	-
Bernal *et al*., [illegible]	*E. encrasicolus*	Laboratory	18.55	-	54.88h	-	-	-	-	-	
De Ciechomski, 1966	*E. anchoita*	Mar del Plata (Argentina)	16-18	0.372	60h	Fresh	2.85-3.60	70-80h	70-80h	70-90 h	-
De Ciechomski, 1973	*E. anchoita*	Northern Argentina	13.9-14.5	0.287	-	-	-	-	-	-	-
		Southern Argentina	12.3-14.3	0.291	-	-	-	-	-	-	-