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RESEARCH NOTE

A first experience of Patagonian sprat *Sprattus fuegensis* spawning in captivity: Adult acclimation, egg and larval measurements

Una primera experiencia de desove de sardina austral *Sprattus fuegensis* en cautiverio:
Aclimatación de adultos, mediciones de huevos y larvas

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Abstract. Relevant traits of eggs and larvae of Patagonian sprat (*Sprattus fuegensis*), obtained from adult reproduction in captivity conditions, were studied. Temperature for adult acclimation and for incubation of eggs and larvae was not controlled; however it ranged with a mean of 13 ± 2 °C. Adult sprat maintained in cylindrical ponds of 2000-L spawned spontaneously at the first night after catch, probably due to catch stress. The eggs were spherical with a diameter ranging between 0.89 and 1.06 mm (mean 1.00 ± 0.05 mm, $n=54$). The larvae hatched between the fourth and fifth day after spawning and their size varied between 2.83 and 4.11 mm long (mean 3.56 ± 0.34 mm, $n=39$). The yolk sac reached a volume of between 0.11 and 0.22 mm³ (mean 0.18 ± 0.033 mm³, $n=32$) and was exhausted by the third day post hatching (dph). Larval growth only occurred during the endogenous feeding period (efp) and survival did not exceed 6 dph, probably due to inappropriate conditions for feeding and growth.

Key words: *Sprattus fuegensis*, captivity, egg, larvae

INTRODUCTION

In the small pelagic fish dynamic, egg and larval survival is a key factor to annual individual recruitment for the population. Its variability is modulated by environmental (Houde 2010) and biological factors such as the broodstock condition, initial egg size, yolk sac absorption (first exogenous feeding), or larval growing rate (Chambers *et al.* 1989, Pepin 1991, Houde 1994, Castro *et al.* 2009, Teletchea & Fontaine 2010).

Patagonian sprat *Sprattus fuegensis* (Jenyns, 1842) is an important commercial and ecological species of small pelagic fish in the fjord ecosystem of southern Chile. This sprat appears to play an important trophodynamics role since it provides a base for upper levels in the food marine web (Landaeta *et al.* 2011).

Studies of *S. fuegensis* and its biological features have focused on adult specimens. Aranís *et al.* (2007) confirm the presence of *S. fuegensis* in the inner sea of Chiloé, Chile. In this area, Leal *et al.* (2011) described its reproduction, reporting higher reproductive activity in spring and female maturation with an average fork length of 13.5 cm. Cerna *et al.* (2013) studied growth and mortality of *S. fuegensis* larvae based on otolith age analysis. On egg and larval traits, De Ciechomski (1971) used plankton samples taken from the Argentinian coast to describe embryonic development in Patagonian sprat. Studies carried out in Chile have only considered the distribution and

egg and larva size in the fjords of the southern Chilean region (41.5°-53°S) (Bustos *et al.* 2008, Landaeta *et al.* 2011, Contreras *et al.* 2014). Thus, little is known about the basic features of early developmental stages. The few available studies have been done using samples taken from natural environment. Therefore, data regarding certain relevant traits, remain unclear.

To date, published studies have neither dealt with the maintenance of this species in captivity nor have they dealt with egg and larval collection in this condition. Thus, the objective of these first experiments was to describe the procedure for catching and maintaining adult specimens as well as to present our findings on relevant traits of the first developmental stages of *S. fuegensis*. This study examined the egg size, endogenous feeding period (efp) and larvae growing rate, among others aspects, under laboratory conditions.

MATERIALS AND METHODS

ADULT CATCHES AND ACCLIMATION

In two catch events (October 13 and 26, 2016), adult living specimens of Patagonian sprat were collected from the inner sea of Chiloé, (Fig. 1) using purse seine boats. Specimens were collected with a 25-L bucket and kept in two 250-L tanks with recirculating sea water during transport to the hatchery.

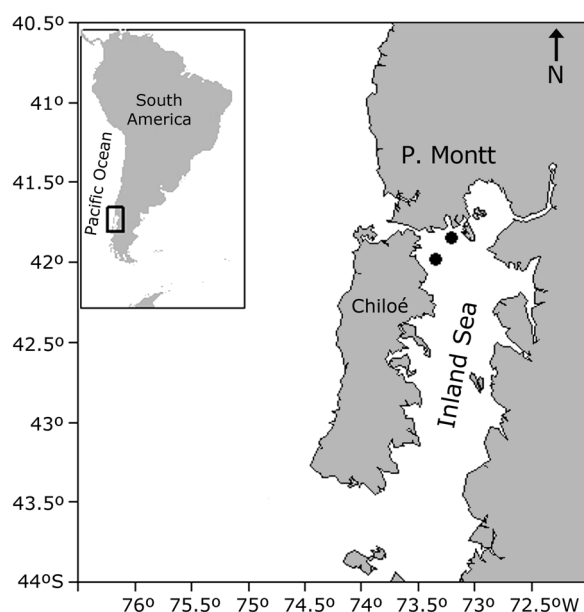


Figure 1. Approximate position of catch event (black dots) of patagonian sprat, collected from the inner sea of Chiloé / Posición aproximada de los eventos de captura (círculos negros) de sardina austral colectada en la zona del mar interior de Chiloé

Approximately 550 and 1,100 specimens from the first and second catch, respectively began the acclimation process in 2 cylindrical ponds (2000-L each), with a density of 225 and 550 ind m⁻³ for each case. In both experiments, the fish were kept in filtered sea water (37 µm), with open flow and no additional oxygen. This was monitored 3 times a day and ranged mostly between 8 and 12 mg l⁻¹. Water temperature was not regulated, but it ranged between 11 and 15 °C depending on the time of day. From the third day on, adults were fed *ad libitum* with a diet of pellets (Golden Rc 5220, Biomar Chile S.A.). Daily mortality counts were done and the dead fish were removed from the ponds. Only in the second experiment, a circular current (vortex) was fixed inside the ponds to stimulate swimming in the specimens

EXPERIMENT DESIGN FOR EGG AND LARVAL REARING

After spawning, the eggs were collected immediately using a 200-µm sieve placed outside the spawning tanks and then incubated in an 80-L container with filtered sea water, open flow, and temperatures of 13 ± 2 °C. Living zooplankton food was not added to the setting which was enriched with microalgae (*Chaetoceros*, *Isochrysis*, *Nannochloropsis*) and a protein complex (Mcrovit Hi Protein) before the larvae completely absorbed the yolk sacs.

Among 12 and 15 eggs and larvae were measured daily *in vivo* and preserved in 90% ethanol and 5% formalin, respectively. Eggs, larvae, and yolk sacs were all photographed with a built-in camera of a stereo microscope (10X magnification). Measurements were done with Image-Pro Plus software version 5.1 (©Media Cybernetics, Inc.). Yolk sac volume (mm³) was calculated according to the equation of a spheroid (De Ciechowski 1971), $\frac{4}{3} \pi (a \cdot b^2)$, where *a* is half the maximum yolk sac length and *b* is half the maximum width of the yolk sac. The daily larval growth rate was estimated through a linear model: length (mm) = *a* + *b* * age (days).

RESULTS AND DISCUSSION

During transport to the hatchery, adult mortality was low, less than 2%. Nevertheless, it was high during the first 5 days of acclimation, reaching 90% and 77% in the first and second experiments, respectively. Mortality stabilized after day 6. In experiment one, all the fish had died by day 18. In experiment two, mortality stopped on day 28 of acclimation, and some 210 fully acclimated specimens (19%) remained in the ponds by day 40, until they were sacrificed for other analyses. In both experiments, the first adult fish began to accept food after day 5, while all individuals in the ponds were eating around day 15.

The 2 experiments differed in terms of adult acclimation. Experiment 2 procedures were more successful. These included recirculating seawater (without additional oxygen), circular currents in the ponds, and an initial number of around 1,000 specimens (500 per ponds).

Reproduction occurred only the first night after the catch in both experiments close to 23:30 h. Specimens spawned spontaneously, probably due to the catch stress. Previous experiences with other species indicate that, given suitable reproductive conditions, the stress of being caught may trigger spontaneous spawning in adults within 24 h of capture (Cleary *et al.* 2002, Plaza & Cerna 2015). Because the catches occurred during the reproductive period peak for Patagonian sprat (Leal *et al.* 2011), it was expected that the specimens would have spawned spontaneously within the first day in captivity.

The Figure 2 shows Patagonian sprat eggs and larvae in different developmental stages. In experiment 2, high incidence of unviable eggs and malformed larvae was observed. Thus, results for egg and larval measurements were referred to only in experiment one.

Eggs were spherical, between 0.89 and 1.06 mm in diameter (mean 1.00 ± 0.05 mm, *n* = 54). Despite some overlap, eggs in this study were smaller than those reported for Patagonian sprat

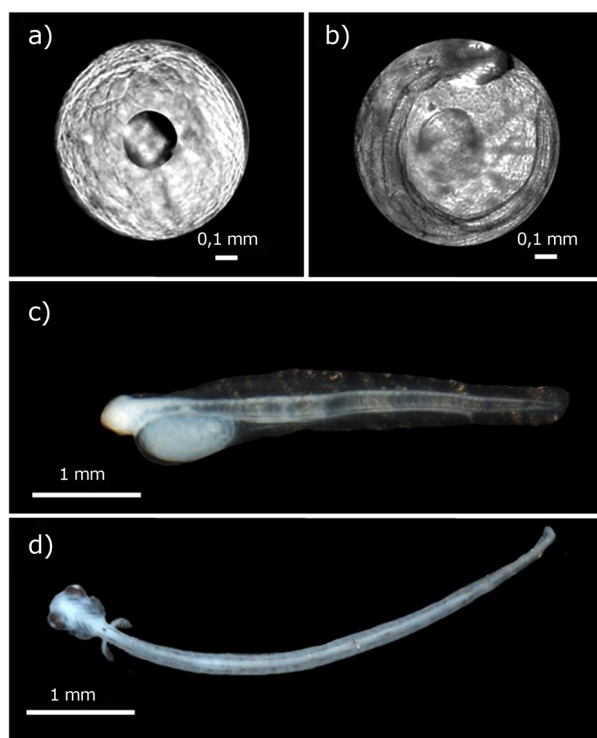


Figure 2. Patagonian sprat eggs and larvae in different stages of development. a) Newly fertilized egg (day 0); b) advanced embryo shortly before hatching (day 4); c) larva with yolk sac, 3 dph (lateral view); d) larva with completely absorbed yolk sac, 6 dph (dorsal view) / Huevos y larvas de sardina austral en diferentes estados de desarrollo. a) Huevo recién fecundado (día 0); b) embrión avanzado poco antes de la eclosión (día 4); c) larva con saco vitelino, 3 dpe (vista lateral); d) larva con saco vitelino completamente reabsorbido, 6 dpe (vista dorsal)

by Landaeta *et al.* (2011) (0.922-1.361 mm), which included samples taken from the natural environment. These authors reported larger eggs in the colder waters (9 °C average) of southern Chile (50°-53°S). Our results differ even more regarding the range (1.035-1.175 mm) reported by De Ciechomsky (1971) for *S. fuegensis* eggs captured off the coast of southern Argentina (50°43'S). Those eggs were also collected from colder waters (6°C average) than the temperature registered in our experiment (13 ± 2°C). Several reports have indicated this inverse relationship between egg size and water temperature for fish populations (Funamoto & Aoki 2002, Castro *et al.* 2009). For *S. sprattus* in the Baltic Sea, Nissling (2004) reported egg size ranging from 1.21 to 1.51 mm in different times during the spawning season in temperatures reaching from 3.9 to 6.3 °C. In addition to environmental factors (*e.g.*, temperature), maternal effect is also known to determine egg and larva size and/or quality (Chambers & Laggett 1996, Castro *et al.* 2009, Mark *et al.* 2014). Accordingly, possible differences in the physiological condition of the broodstock

between the two experiments could have influenced the low hatching percentage, malformed larvae, and high mortality in experiment two.

Larval hatching occurred between days 4 and 5 after spawning measuring between 2.83 and 4.11 mm long (mean 3.56 ± 0.34 mm, $n=39$). These results fully correspond with Garrido *et al.* (2015) for *Sardina pilchardus*. The authors used laboratory studies to show that the larvae averaged 3.6 ± 0.34 mm on hatching, which occurred 72 h after spawning at constant temperature of 15 °C.

The efp or yolk sac absorption occurred during 3 days post-hatch (dph) (Fig. 3A). The yolk sac reached a volume of between 0.11 and 0.22 mm³ (mean 0.18 ± 0.033 mm³, $n=32$). Larvae with the fully absorbed yolk sac measured 5.91 ± 0.47 mm long on average.

During the first 5 days, the larvae grew according to the linear model (Fig. 3B), following the equation ($n=66$): Longitude (mm) = $3.85 + 0.42 \times \text{age (days)}$ ($R^2=0.64$, $P<0.001$). Larvae of day 6 was not considered in the lineal model, since some of the measurements were made on dead larvae.

Previous reports on *S. fuegensis* larval growth rates are unknown. Nevertheless, reports about the *S. sprattus phalericus* in the Adriatic Sea (Dulcic 1998) estimated larvae growth rates between 0.40 and 0.42 mm·day⁻¹ with an average temperature of 10.8 °C. Also, Valenzuela & Vargas (2003), using otolith analyses, estimated growth rates for *S. sprattus* larvae, which ranged from 0.36 to 0.40 mm·day⁻¹ at temperatures ranging between 11 and 13.5 °C in the North Sea. For *S. pilchardus* under laboratory conditions, Garrido *et al.* (2015) indicated larval rate growth of 0.25 mm·day⁻¹ but for a larger incubation period (75 dph). The same authors claimed that in the lack of optimal feeding conditions, larval growth of *S. pilchardus* only occurred during the efp and survival did not exceed 11 dph. In our experiment, larvae survived only 6 dph (3 days after efp) In this case, starvation effected growth and massive larval mortality occurred due to the fact that the water was enriched only with microalgae and a protein complex. Living food supply (zooplankton) has been described as an essential requirement for larval fish survival in laboratory studies by several authors (Garrido *et al.* 2012, Peck *et al.* 2013, Caldeira *et al.* 2014).

Although we obtained relevant egg and larval measurements, we failed to achieve satisfactory larval survival rates over time. Thus, future experiments will require a better acclimation setting through greater control of temperature, along with sterilization and trials with different living food types (zooplankton). This will allow improving the larva growth rate estimation, including the exogenous feeding times.

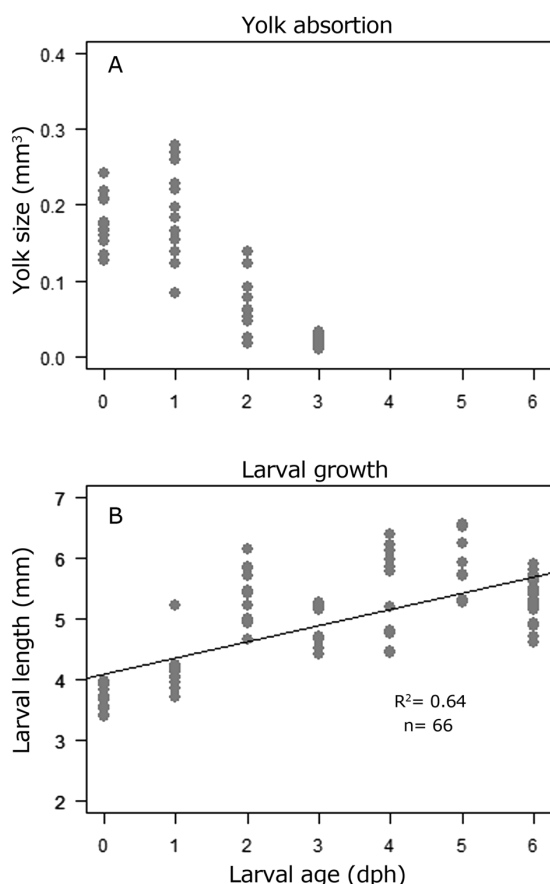


Figure 3. Patagonian sprat yolk sac absorption (A) and larval growth (B), under laboratory conditions / Absorción del saco vitelino (A) y crecimiento de las larvas (B) de sardina austral bajo condiciones de laboratorio

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