

## SPONTANEOUS SPAWNING OF BRAZILIAN SARDINE IN CAPTIVITY

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### ABSTRACT

In one year, 76 first generation (F1) Brazilian sardine *Sardinella brasiliensis* breeders spawned 84 times spontaneously in captivity. This is the first report for the genus *Sardinella*. Following the pattern found in the natural environment, the number of spawns, as well as fertilization and hatching rates were higher in spring, summer and fall than in winter. Until now, winter spawning was only presumed to occur in a natural environment. Water temperature, and not photoperiod, was positively correlated with reproductive performance.

**Keywords:** aquaculture; Clupeidae; photoperiod; reproduction; temperature

### DESOVAS ESPONTÂNEAS DA SARDINHA-VERDADEIRA EM CATIVEIRO

### RESUMO

Durante um ano, 76 reprodutores de primeira geração (F1) de sardinha-verdadeira *Sardinella brasiliensis* desovaram espontaneamente 84 vezes em cativeiro. Esse é o primeiro relato dentro do gênero *Sardinella*. Seguindo o padrão do ambiente natural, o número de desovas e as taxas de fertilização e eclosão foram maiores na primavera, verão e outono que no inverno. Até o momento, a desova no inverno foi presumida apenas no ambiente natural. A temperatura da água, e não o fotoperíodo, apresentou correlação positiva com o desempenho reprodutivo.

**Palavras-chave:** aquicultura; Clupeidae; fotoperíodo; reprodução; temperatura

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## INTRODUCTION

Species of the genus *Sardinella* are widely distributed around the world, inhabiting the Atlantic, Indian and Pacific Oceans. Although species of great importance to commercial fishing are included in this genus, abrupt variations in capture occur annually (Jablonski, 2007), so captive production is an alternative to supply existing demand. On the Brazilian coast, *Sardinella brasiliensis* Steindachner, 1879 is found from Rio de Janeiro (São Tomé Cape) (22 °S) to Santa Catarina (south of Cape Santa Marta) (29 °S), at depths of 30 to 100 m (Cergole, 1995; Jablonski, 2007).

The species begins sexual maturation with 160-170 mm of total length at 1.5 years, and all individuals mature at 210-220 mm (Cergole and Dias-Neto, 2011). They have a bath-spawning strategy, as females spawn several batches of oocytes in a single reproductive season with average fecundity varying from 20,000 to 35,000 oocytes per female or approximately 356 to 431 g<sup>-1</sup> spawning eggs (Vazzoler and Rossi-Wongtschowski, 1976; Matsuura, 1977; Isaac-Nahum et al., 1988).

In the wild, maturation, spawning and gonadal recovery occur mainly in spring, summer and early fall. Spawning peaks occur in December and January, when the maximum frequency of spawning individuals was identified, with a spawning interval of 4 to 11 days (Cergole and Dias-Neto, 2011). However, Katsuragawa et al. (2006) report the presence of eggs and larvae in all seasons, indicating that *S. brasiliensis* spawn throughout the year. Eggs are planktonic, with spherical shape and 1.20 mm mean diameter, varying from 1.00 to 1.40 mm (Saccardo and Rossi-Wongtschowski, 1991).

At the Laboratório de Piscicultura Marinha (LAPMAR-UFSC) juvenile fish collected from the wild were reared until they reached adult size and responded well to hormonal induction using an analog of LHRH (des-Gly<sup>10</sup>, D-Ala<sup>6</sup> LH-RH ethylamide acetate salt hydrate, L4513, Sigma-Aldrich, St. Louis, USA). Due to lack of sexual dimorphism in *S. brasiliensis*, the same hormone dosage (75 µg kg<sup>-1</sup>) was used for both sexes (Reis da Silva, 2013; Cerqueira et al., 2017), generating viable eggs from the year 2012 until 2017. In 2017, a first generation of captive bred fish (eggs hatched in May 2015) began to spawn spontaneously. In this context, the present study aimed to describe spontaneous spawning and egg quality of Brazilian sardine breeders.

## MATERIAL AND METHODS

Spawns and eggs from this brood stock were monitored for one year (from October 1, 2017 to September 30, 2018), and fertilization and hatching rates were estimated. Data were correlated with environmental parameters of water temperature and photoperiod to evaluate influence of annual seasons on spawning frequency and quality. All procedures with the fish were approved by the Ethical Committee on Animal Use of UFSC (CEUA 9027020718).

The brood stock was kept in an outdoor 8,000 L black circular tank with constant aeration and water renewal of 350% per day (19 to 20 L min<sup>-1</sup>). The water was continuously supplied by direct pumping from the ocean, collected at Mozambique beach,

Florianópolis, Brazil (27°34'02"S, 48°25'44"W). Water quality was monitored daily: oxygen and temperature (YSI pro 20, Yellow Springs, Ohio, USA), as well as pH (YSI pH10A). Photoperiod was determined from official data available on the internet (Dateandtime.info, 2018). The tank was equipped with an anti-bird net (2 cm mesh) and a shade cover (50% light reduction) to reduce light intensity and protect fish from direct sun exposure. Water temperature, salinity, pH and photoperiod varied naturally throughout the period evaluated.

During the 12-month period, 76 fish were kept as breeders and there was no mortality. The size of the fish in October 2018 was 82.9 ± 11.6 g in weight and 19.1 ± 1.3 cm in total length (biomass = 0.8 kg m<sup>-3</sup>). They were fed with a commercial diet (0.8 - 1.0 mm, 45% crude protein, 13% moisture, 9% lipid, 3.6% fiber and 4500 cal kg<sup>-1</sup>) three times daily until apparent satiation.

Spontaneous spawning occurred in the brood stock tank. The floating eggs were automatically collected through the overflow water and passed into a 35 L cylindrical conical tank. This collector has lateral openings screened by a 500 µm size mesh net. Every day at 8:00 AM the presence of eggs was monitored. Once spawns occurred, eggs were removed from the collector only after blastopore closure (embryonic stage).

Eggs were quantified by counting three 5.5 mL samples and the fertilization rate was determined with stereomicroscopic observation (Leica EZ4HD). Subsequently, samples of 100 eggs L<sup>-1</sup> were transferred into 2 L Beckers, in triplicate, to obtain the hatch rate. Eggs were incubated at the same temperature as the breeding tank, maintained by a thermostatic bath controlled in a room cooled by air conditioning, a 500W submerged heater with thermostat, at a photoperiod of 24 h light (600 Lux), and smooth, constant aeration using a porous stone.

Data were analyzed according to month, year and season: spring (September 22 to December 21), summer (December 22 to March 19), fall (March 20 to June 21) and winter (22 from June to September 21). Before statistical analysis, fertilization and hatching rates, expressed as a percentage, were transformed to arcsen (x)<sup>0.5</sup>. Data for spawning rates were submitted to simple Anova and Tukey tests (P<0.05) were performed with Statistica 7.0 software (Hill and Lewicki, 2007). To verify the influence of water temperature and photoperiod on the number of spawns, linear regression (y = a + bx) was applied to analyze water temperature, polynomial regression (y = ax<sup>2</sup> + bx + c) for photoperiod and Pearson correlation for both.

## RESULTS AND DISCUSSION

Photoperiod and water temperature varied daily during the period evaluated. The minimum photoperiod was on June 22 (10h and 24min or 624min of light) and the maximum on December 22 (13h and 54min or 834min), at (latitude 27 °S) (Dateandtime.info, 2018). In the brood stock tank water, temperature varied from 17.4 °C in August to 27.6 °C in March, dissolved oxygen was 5.7 ± 0.9 mg L<sup>-1</sup>, pH 8.2 ± 0.1 and salinity 34.5 ± 1.0.

During the 12-month period (October 1, 2017 to September 30, 2018) the F1 brood stock of *S. brasiliensis* spawned 84 times

totaling about 2.3 million eggs collected. Unlike in a natural environment, it was possible to obtain spawns in captivity in all months of the year. A positive relation was observed between the number of spawns per month and water temperature ( $R^2 = 0.74$ ;  $P < 0.05$ ;  $n = 12$ ;  $R = 0.86$ ): the higher the water temperature, the more spawns obtained. Photoperiod did not influence the number of spawns ( $R^2 = 0.41$ ;  $P > 0.05$ ;  $n = 12$ ;  $R = 0.51$ ) (Figure 1).

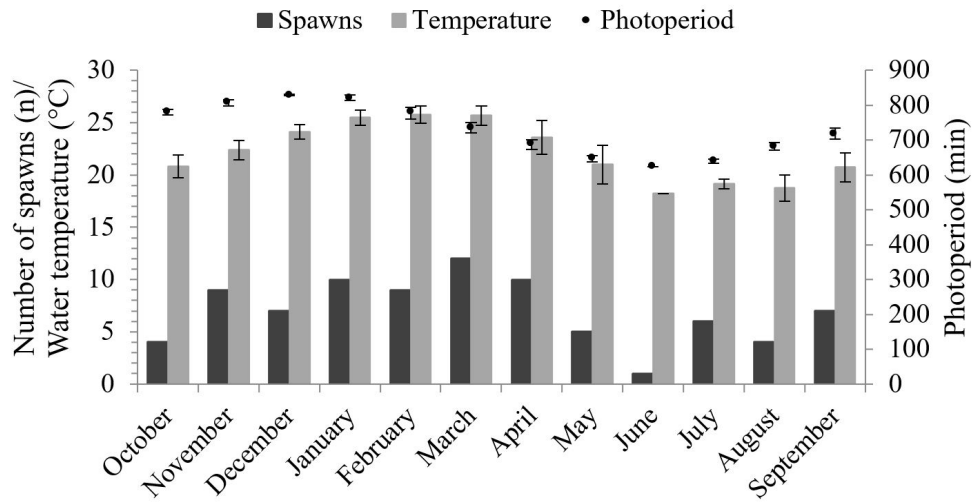
All variables evaluated were influenced by the season and water temperature. In winter, when water temperature was the lowest, a lower number of spawns, and reduced fertilization and hatching rates ( $P < 0.05$ ) were observed. In summer, when water temperature was the highest ( $P < 0.05$ ), a high number of spawns and eggs were found. There were no differences in fertilization and hatching rates during spring and fall ( $P > 0.05$ ) (Table 1).

The fact that the best results were obtained during the hottest period of the year (summer) corroborates studies that describe the species in nature, where they spawn from spring to early fall, at temperatures from 22 °C to 28 °C (Matsuura, 1998; Gigliotti et al., 2010). Spontaneous spawning in captivity during winter (with mean temperature of  $19.2 \pm 0.8$ ) is an important result, even though fertilization and hatching rates were lower than other periods. These spawning events confirm that *S. brasiliensis* can spawn in all seasons of the year, as suggested by Katsuragawa et al. (2006). Moreover, spawning during winter in captivity is also related to

good management practices, fish domestication (F1 individuals) and adequate feed (energy, nutrients quality and quantity) (Valdebenito et al., 2015).

Between late autumn and early winter there was a significant reduction in the number of monthly spawns and egg collection, probably due to the reduction in the average water temperature ( $23.6 \pm 1.6$  °C in April to  $18.2 \pm 1.2$  °C in June). During these months, 339,766 eggs were obtained from 10 spawnings; 27,950 eggs from 5 spawnings; and 20,100 eggs from only 1 spawning in April, May and June, respectively. Based on the results expressed in Table 1, the winter season had the second highest number of eggs collected and the highest average eggs per spawning, even with fewer spawnings. However, since rates of egg fertilization and eclosion were lower than in other seasons ( $P < 0.05$ ), winter was not considered the best period for sardine reproduction.

Regarding other species such as Sea Bass (*Dicentrarchus labrax*) (Mylonas et al., 2003) and Tilapia (*Oreochromis spilurus*) (Ridha et al., 1998), the number of eggs per spawn decreased after consecutive spawning, probably because of energy spent on the reproductive process, leading to breeder exhaustion (Ridha et al., 1998). Temperature reduction caused greater intervals among spawning, which allowed reestablishment of body energy reserves, gonad recovery and gamete development (McBride et al., 2015). In this study, this recovery period may have favored more significant



**Figure 1.** Number of spawns (n), water temperature (°C) and photoperiod (min.) during 12 months of spontaneous spawning of *Sardinella brasiliensis* in captivity.

**Table 1.** Productive indexes obtained from spontaneous spawning of *Sardinella brasiliensis* per season (means ± SD).

	Spring	Summer	Fall	Winter
Number of spawns	22	29	20	14
Total collected eggs	400,587	869,419	451,581	571,322
Eggs per spawn	20,029 ± 17,557	29,980 ± 32,063	22,579 ± 17,543	40,809 ± 47,146
Egg fertilization (%)	95.1 ± 5.8 <sup>a</sup>	95.4 ± 5.0 <sup>a</sup>	93.8 ± 9.9 <sup>a</sup>	76.5 ± 18.8 <sup>b</sup>
Egg eclosion (%)	91.9 ± 6.1 <sup>a</sup>	91.7 ± 9.2 <sup>a</sup>	90.0 ± 12.6 <sup>a</sup>	73.7 ± 23.1 <sup>b</sup>
Temperature (°C)	22.5 ± 1.3 <sup>b</sup>	25.6 ± 0.8 <sup>a</sup>	22.2 ± 2.4 <sup>b</sup>	19.2 ± 0.8 <sup>c</sup>

Superscript letters demonstrate significant difference by simple Anova and Tukey tests ( $P < 0.05$ ).

spawning events during winter: 40.809 average eggs spawned<sup>-1</sup>, compared to 29.980, 22.579 and 20.029 in summer, autumn and spring, respectively (Table 1).

During the period evaluated, from 2,000 to 156,000 eggs were spawned by the brood stock per day. Due to this large variation, there was no seasonal difference. Variation in the number of eggs released may follow a pattern found in nature related to reproductive strategy, in which only a few individuals in each batch spawn at a time, instead of all together (Vazzoler and Rossi-Wongtschowski, 1976).

Photoperiod and water temperature are responsible for activating or inhibiting reproductive processes. These parameters trigger processes on the hypothalamo-pituitary-gonadal (HPG) axis, through synthesis and release of hormones essential for reproduction such as peptide gonadotropin-releasing hormones (GnRH), follicle-stimulating hormone (FSH) and luteinizing hormone (LH), influencing sexual cycle, development and functioning of gametes (Luks'iene and Svedang, 1997; Pankhurst and Munday, 2011; Valdebenito et al., 2015). During the period analyzed, there was no correlation between photoperiod and the number of spawns. However, further specific experiments are needed to better elucidate the influence of light on biochemical and physiological processes of gonad maturation and spawn induction.

This unprecedented study demonstrates the control of the complete life cycle of *Sardinella brasiliensis* in captivity, which is very important in marine fish farming. The occurrence of spontaneous spawning during all months of the year by the F1 breeders is useful for providing a constant supply of Brazilian sardine larvae, which can stimulate the development of marine fish farming and improve scientific knowledge in coming years.

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