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Scientific Note

# Ultrasonography in the reproductive management of the Brazilian sardine *Sardinella brasiliensis*

Sabrina Lara da Luz[1]*, Ingrith Aparecida Mazuhy Santarosa[2], Maria Alcina Martins de Castro[1], Carolina Perenguez Riofrio[1], Letícia Cordeiro Koppe de França[1], Everton Danilo dos Santos[1], Bernardo Viero de Oliveira[3], Aline Brum[1], Caio Magnotti[1]

[1]Universidade Federal de Santa Catarina – Centro de Ciências Agrárias, Departamento de Aquicultura, Laboratório de Piscicultura Marinha – Florianópolis (SC), Brazil.
[2]Independent Research – Florianópolis (SC), Brazil.
[3]Universidade Federal de Santa Catarina – Centro de Ciências Biológicas, Departamento de Biologia Celular, Embriologia e Genética – Florianópolis (SC), Brazil.
*Corresponding author: luzsabrina328@gmail.com

## ABSTRACT

Ultrasonography is a fast and non-invasive method that offers a promising alternative for sex identification and gonadal maturation assessment in fish. This technique is especially relevant to Brazilian sardine (*Sardinella brasiliensis*), a species of high commercial value whose small body makes traditional sexing methods unfeasible. Ultrasonography was applied to 55 individuals undergoing their first gonadal maturation. All fish were anesthetized for examination and subsequently euthanized for macroscopic evaluation to confirm sex and determine the maturation phase. These findings were supported by histological analysis, which confirmed the accuracy of the results. Ultrasonography using a multifrequency transducer (8–13 MHz) achieved 98% accuracy in sex identification, and 46 males and nine females were identified. Histological examination revealed 32 reproductively capable males (phase C) and 14 immature individuals (phase A), while all females were classified as phase C (ready for reproduction and spawning). Although ultrasonography proved effective for sex differentiation, it showed limitations in determining gonadal maturation phases. Further studies and the development of a specific protocol are required for accurate phase classification. Therefore, ultrasonography demonstrates great potential as a practical and effective tool in reproductive monitoring. This study is the first report of its application to the Brazilian sardine.

**Keywords:** Clupeidae; Diagnostic imaging; Marine fish; Sex identification; Ultrasonography.

## Ultrassonografia na gestão reprodutiva da sardinha-verdadeira *Sardinella brasiliensis*

## RESUMO

A sardinha-verdadeira é uma espécie de grande importância econômica e ecológica, amplamente explorada pela pesca comercial. Sua reprodução em cativeiro apresenta desafios, especialmente pelo pequeno porte da espécie, que inviabiliza o uso de técnicas invasivas, como a canulação, para determinação sexual e avaliação gonadal. A ultrassonografia, cada vez mais utilizada em programas de reprodução na aquicultura, destaca-se por ser uma ferramenta não invasiva, rápida e eficiente para identificação do sexo e análise reprodutiva. Neste estudo, 55 sardinhas foram anestesiadas para avaliação ultrassonográfica, utilizando um transdutor linear multifrequencial com frequências entre 8 e 13 MHz. As imagens capturadas evidenciaram diferenças de ecogenicidade entre as gônadas. Nas fêmeas, os ovários apresentaram aspecto hiperecogênico e heterogêneo, enquanto nos machos os testículos exibiram padrão hipoecogênico e homogêneo, em comparação às estruturas corporais adjacentes. Para a confirmação dos resultados, foram realizadas análises macroscópicas, que exigiram a eutanásia de todos os peixes, e análises histológicas. Essas análises permitiram não apenas validar a sexagem com uma acurácia de 98,18%, identificando 46 machos e nove fêmeas, mas também determinar as fases de maturação gonadal de cada indivíduo. Tanto na avaliação macroscópica quanto na microscópica foi possível observar características específicas das gônadas que indicaram as fases de desenvolvimento reprodutivo, contribuindo para uma compreensão mais detalhada do ciclo reprodutivo da espécie.

**Palavras-chave:** Aquicultura; Clupeidae; Diagnóstico por imagem; Peixes marinhos; Sexagem.

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## INTRODUCTION

The genus *Sardinella*, belonging to the family Clupeidae, is one of the world's most widely distributed groups of pelagic fish, with occurrences recorded in tropical and subtropical waters (Cergole & Dias Neto, 2011). More than 65 valid species are currently recognized within the genus (FishBase, 2024). Among them, *Sardinella aurita* stands out as one of the most commercially exploited species globally, accounting for 37% of worldwide fish landings (FAO, 2024). In Brazil, the Brazilian sardine, *Sardinella brasiliensis* (Steindachner, 1879), is the main species targeted by commercial fisheries, particularly in the Southeast and South regions of the country (Cergole & Dias Neto, 2011). Its catch supports both the pole-and-line fishery for skipjack tuna, *Katsuwonus pelamis*, in which it is used as live bait, and the canned fish industry (Cerqueira et al., 2020; Figueiredo et al., 2010; IBAMA, 2007).

Sardines exhibit a high capacity to rapidly adapt to controlled laboratory conditions, which makes them promising candidates for aquaculture. They are also classified as a low-trophic level species, whose production may contribute to the sustainability of aquaculture and fisheries systems (Owatari et al., 2024). One of the main challenges for developing captive reproduction protocols is the difficulty in sex identification due to the lack of clear external sexual dimorphism (Cergole & Dias Neto, 2011; Doi et al., 2024; Silva, 2013).

Regarding its reproductive biology, the Brazilian sardine, *S. brasiliensis*, reaches first sexual maturity at 160–170 mm total length, at approximately 1.5 year old, with all individuals considered mature at 210–220 mm (Cergole & Dias Neto, 2011). Reproductive processes occur mainly during spring, summer, and early autumn, with a spawning peak in December and January, under temperatures ranging from 22 to 28°C (Gigliotti et al., 2010; Matsuura, 1983; 1998).

Based on this, the selection of individuals in their first gonadal maturation cycle enables the evaluation of early reproductive development stages, which is essential for improving non-invasive sex identification techniques.

Sex identification in fish can be performed using several techniques, including radioimmunoassays for the analysis of sex steroid hormones (Chang et al., 1999; Sangalang et al., 1978) and the immunoagglutination technique (Le Bail & Breton, 1981). However, commonly used approaches for sex identification and gonadal assessment include abdominal massage to induce milt extrusion in males at full gonadal maturation and urogenital cannulation, which allow the collection of ovarian samples for oocyte measurement in females (Crepaldi et al., 2006; Crepaldi & Rotta, 2007). Despite their effectiveness, most techniques used to assess sexual maturation are invasive and may negatively affect fish health and reproductive performance (Oliveira et al., 2024). This limitation is particularly evident in small species, such as the Brazilian sardine, which has a narrow genital pore, making safe insertion of the cannula difficult or even unfeasible (Cerqueira et al., 2020).

Ultrasonography is a well-established and safe imaging tool for routine examinations, with no reported adverse biological effects on either the subject or the operator. As a non-invasive method, it is generally well tolerated by animals (Preston & Shaw, 2001). It is effective for visualizing soft tissues at various depths within the scanned region, based on the reflection of sound waves by tissues with different acoustic impedances, which are then converted into images (Griffin & Ginther, 1992; Goddard, 1995).

In aquaculture, ultrasonography can be considered a viable alternative, enabling faster and more effective broodstock management, reducing potential sources of stress for the fish, and allowing continuous monitoring of gonadal development (Novelo & Tiersch, 2012; Rotta & Marques, 2007).

The first use of ultrasonography in fish was reported in Coho salmon (*Oncorhynchus kisutch*), marking a pioneering advancement in sex identification (Martin et al., 1983). Since then, its application has been widely explored. Santarosa et al. (2025) compiled 15 studies documenting the use of ultrasonography in marine and diadromous fish species, including pacific halibut (*Hippoglossus stenolepis*) (Loher & Stephens, 2011), European sea bass (*Dicentrarchus labrax*) (Macrí et al., 2013), sturgeon (*Acipenser gueldenstaedtii*) (Memis et al., 2016), flathead grey mullet (*Mugil cephalus*) (Masoudifard et al., 2023), pufferfish (*Arothron manilensis*) (Doi et al., 2024), among others.

## MATERIALS AND METHODS

### Study site and experimental animals

The experiment was conducted at the Laboratory of Marine Fish Farming (27°35'8.960"S; 48°26'24.235"W), affiliated with the Universidade Federal de Santa Catarina (UFSC). Prior to execution, the project was submitted for evaluation and approval by the Animal Ethics and Use Committee (CEUA/UFSC - protocol number 7367220424), ensuring compliance with ethical standards for animal research. The experimental animals were sixth-generation broodstock (F6) in their first reproductive cycle, with no previous spawning history and presenting early stages of gonadal development, maintained in a circular tank with a total volume of 8,000 L. Stocking density was limited to a maximum biomass of 1 kg·m$^{-3}$, corresponding

to approximately 170 individuals in the tank. Water temperature varied according to ambient conditions (17–30°C), with a salinity of 35‰, natural photoperiod (latitude 27° S, with maximum daylight of 13 h 53 min and minimum of 10 h 23 min), and continuous water flow (Magnotti et al., 2020). The experiment was conducted in September, corresponding to the spring season in the Southern Hemisphere. Reproductive processes in *S. brasiliensis* occur mainly during spring, summer, and early autumn, with a spawning peak in December and January (Gigliotti et al., 2010; Matsuura, 1983; 1998).

Fish were fed a commercial diet (Bernaqua Wean Prime micro-extruded feed) with a particle size of 0.8–1.7 mm, containing 450 g/kg crude protein, 120 g/kg moisture, and 40 g/kg crude fiber. The diet was provided twice daily, fed to apparent satiation.

## Sex identification of sardine

A triple-approach methodology was adopted in this study, encompassing macroscopic, ultrasonographic, and histological analyses of the gonads. Macroscopic and ultrasonographic evaluations were performed on all individuals, whereas histological analysis was conducted on a subset of samples (n = 19).

For gonadal identification, 55 sardines were anesthetized with benzocaine at a concentration of 50 $mg \cdot L^{-1}$ (Takeuchi, 2012). A multifrequency linear transducer (8–13 MHz) was positioned on the right and left lateral regions of the coelomic cavity, with the fish placed in dorsal and lateral recumbency (Fig. 1a). Sonographic images were obtained in the right and left sagittal planes, as well as in the transverse plane. Lateral sweeps were performed to allow complete visualization of the gonads in all planes, and bilateral measurements of gonadal length and width were recorded. In the sagittal plane, images were obtained with the cranial end of the transducer oriented toward the head of the fish, corresponding to the left side of the ultrasound screen (Fig. 1b). Transverse scans were performed with the cranial end of the transducer oriented toward the right or left lateral side of the fish.

Subsequently, biometric and morphometric measurements were recorded, including body weight (g), standard and total length (cm), body width at the level of the first dorsal fin ray (BW), and body height at the same level (BH) (Szpilman, 2000).

The animals were then dissected by ventral aorta sectioning for macroscopic evaluation of the gonads, which were photographed for later analysis and sex identification, allowing validation of ultrasonography accuracy (Crepaldi & Rotta, 2007). Macroscopic evaluation followed the criteria established by Vazzoler (1997), classifying gonads into the following phases: immature (phase A), maturing (phase B), mature (phase C), and spent (phase D).

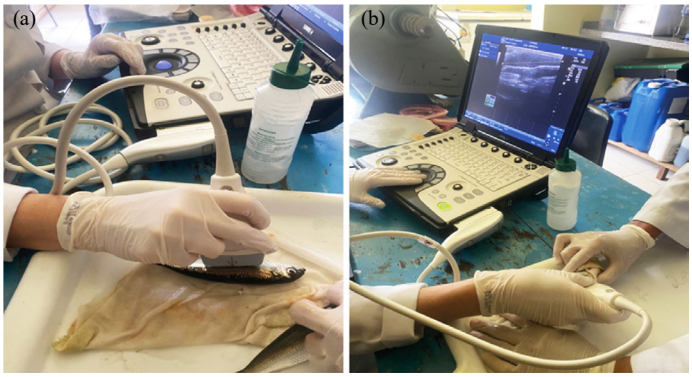



**Figure 1.** Ultrasonographic procedure for sex identification in *Sardinella brasiliensis*. (a) Positioning of the multifrequency linear transducer on the lateral regions of the coelomic cavity, with the fish in lateral recumbency. (b) Acquisition of sonographic images in the sagittal plane, with the fish in dorsal recumbency.

Histological analyses of the gonads were conducted at the Laboratory of Health of Aquatic Organisms at UFSC, following the terminology proposed by Brown-Peterson et al. (2011), allowing a more detailed characterization of gonadal development stages. A total of 19 samples were collected, comprising six females and 13 males.

Gonads were dehydrated in a graded ethanol series, cleared in xylene, embedded in paraffin at 60°C, sectioned at 4 µm using a rotary microtome (MRP09, Lupetec, Brazil), and stained with hematoxylin and eosin (H&E) according to a protocol adapted from Martins et al. (2018). The slides were subsequently mounted with Entellan and examined under a light microscope (DM750, Leica, Germany), equipped with an image acquisition and processing system (Leica LAS EX V2.1.0). To assess differences in germ cell development, a morphological analysis of the various cell types present in the gonads was performed. The description of gonadal maturation phases was adapted according to the terminology proposed by Brown-Peterson et al. (2011).

## Data analysis

Normality and homogeneity of variance were assessed using the Shapiro–Wilk and Levene's tests, respectively. When assumptions were not met, data were arcsine square root transformed ($\sqrt{x}$). Comparisons between biometric variables and gonadal maturation phases were performed using Student's t-test, considering a significance level of $p < 0.05$. Pearson's correlation analyses, as well as linear and polynomial regressions, were used to investigate the relationships between biometric variables, maturation phases, and sex identification methods.

## RESULTS

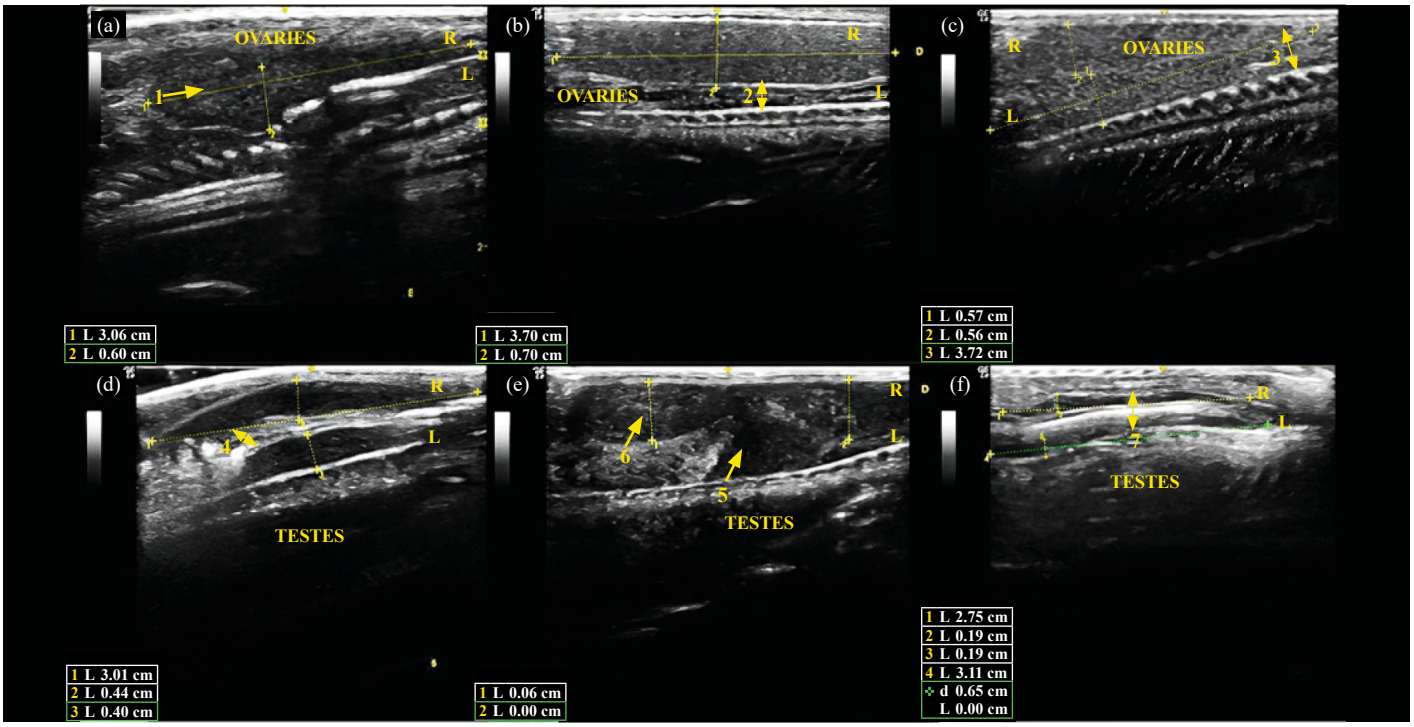
During ultrasonography evaluation, 46 fish were identified as males and nine as females. Macroscopic confirmation revealed only one misidentification: one male was mistakenly identified as a female using the ultrasonography technique.

The anatomical boundaries of the gonads were defined by the cranial region, in contact with the stomach, and the caudal region, near the urogenital pore. Between the gonads, the intestine was consistently identified in all fish as a tubular and linear structure, extending from the stomach to the urogenital pore, predominantly located in the middle region of the dorsal cavity.

The ovaries were identified as elongated structures, with slightly tapered or rounded cranial and caudal extremities, occupying a large portion of the coelomic cavity. They were bordered by a thin hyperechoic line, which was thicker in the mid-region. Sonographically, the ovaries exhibited a hyperechoic, heterogeneous pattern with a coarse echotexture (Figs. 2a–2c).

Due to increased volume and contact between the ovaries, the separation plane between the right and left gonads was poorly defined in five females (55%) (Fig. 2a). Among these, three individuals (33%) exhibited unilateral development, with the contralateral gonad highly reduced, being difficult to be identified, and with undefined boundaries. When visible, an underdeveloped gonad displayed irregular contours, a homogeneous hypoechoic appearance, and a markedly reduced size (Fig. 2b). One female (11%) showed bilateral clearing of the gonads (Fig. 2c).

In males, the gonads appeared thinner than the ovaries. In 28 individuals (61%), the sonographic pattern was hypoechoic, homogeneous, and characterized by a fine echotexture, with some specimens showing even thinner gonads (Figs. 2d and 2f). Three fish (6%) presented enlarged gonads with mixed echogenicity, displaying hyperechoic and heterogeneous areas interspersed with hypoechoic and homogeneous regions. One of these was initially identified as a female via ultrasonography, but we confirmed later to be male through macroscopic evaluation (Fig. 2e).




R: right; L: left.

**Figure 2.** Gonads of Brazilian sardine (*Sardinella brasiliensis*) in longitudinal view. (a–c) Ovaries: (a) poorly defined separation between ovaries; (b) unilateral gonadal reduction; (c) bilateral clearing of ovaries. (d–f) Testes: (d) homogeneous with fine echotexture; (e) mixed echogenicity; (f) thin, homogeneous, and hypoechoic testes. Arrows indicate: (1) poorly defined separation between ovaries; (2) unilateral gonadal reduction; (3) bilateral clearing of ovaries; (4) bilateral clearing of the testes; (5) hypoechoic areas; (6) hyperechoic areas.

The analysis of biometric, morphometric, and ultrasonographic measurements revealed significant differences between males and females. Females exhibited higher values for body weight, standard length, total length, BW, and BH ($p < 0.05$). The length and width of the right gonad, assessed by ultrasonography, were also greater in females ($p < 0.05$). In contrast, gonad weight and the gonadosomatic index did not differ between sexes ($p > 0.05$) (Table 1).

**Table 1.** Biometric, morphometric, and ultrasonographic measurements of gonads in Brazilian sardine (*Sardinella brasiliensis*)*.

| Measurement | Males (n = 46) Mean ± SD | Females (n = 9) Mean ± SD |
|---|---|---|
| Weight (g) | 38.62 ± 7.79 A | 46.81 ± 7.38 B |
| Standard length (cm) | 11.83 ± 3.49 A | 13.21 ± 1.07 B |
| Total length (cm) | 14.93 ± 1.29 A | 16.17 ± 1.32 B |
| BW (cm) | 1.55 ± 0.18 A | 1.71 ± 0.21 B |
| BH (cm) | 2.82 ± 0.28 A | 3.14 ± 0.28 B |
| Gonad weight (g) | 3.14 ± 1.54 A | 3.89 ± 1.34 A |
| Gonadosomatic index (%) | 7.98 ± 3.33 A | 8.30 ± 2.25 A |
| Ultrasonography – Right gonad length (cm) | 3.05 ± 0.73 A | 3.81 ± 0.06 B |
| Ultrasonography – Right gonad width (cm) | 0.42 ± 0.19 A | 0.61 ± 0.14 B |

BW: body width at the level of the first dorsal fin ray; BH: body height at the level of the first dorsal fin ray; *Different uppercase letters indicate statistically significant differences between males and females ($p < 0.05$).

Macroscopic analysis of the gonads enabled the confirmation of sex and identification of gonadal maturation phases (Vazzoler, 1997). All the females were classified as mature (Figs. 3a–3c), with one exhibiting symmetrical gonads (Fig. 3a) and two showing asymmetrical gonads (Figs. 3b and 3c). Among the males, 14 were classified as immature (Fig. 3d) and 32 as mature (Figs. 3e and 3g). Among them, 19 had symmetrical gonads (Fig. 3e), and 12 had asymmetrical gonads (Figs. 3f and 3g), while symmetry could not be determined in the immature individuals (Fig. 3d).

Histological sections of the ovaries revealed that all females were in the spawning-capable phase, according to the classification of Brown-Peterson et al. (2011). This phase is characterized by the presence of tertiary vitellogenic oocytes (Vtg3), post-ovulatory follicles, and occasionally, atretic oocytes. At this phase, oocytes can undergo final maturation and spawning. Macroscopically, the ovaries appeared turgid, occupying a large portion of the coelomic cavity, and contained large, yellowish, and opaque oocytes.

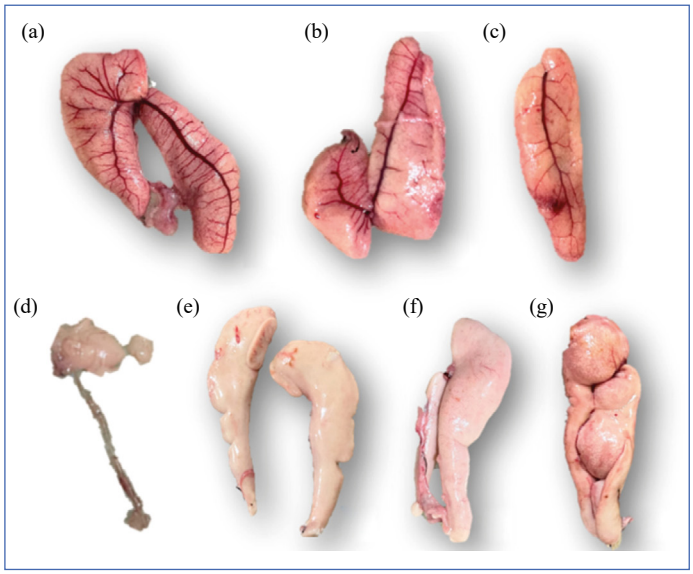
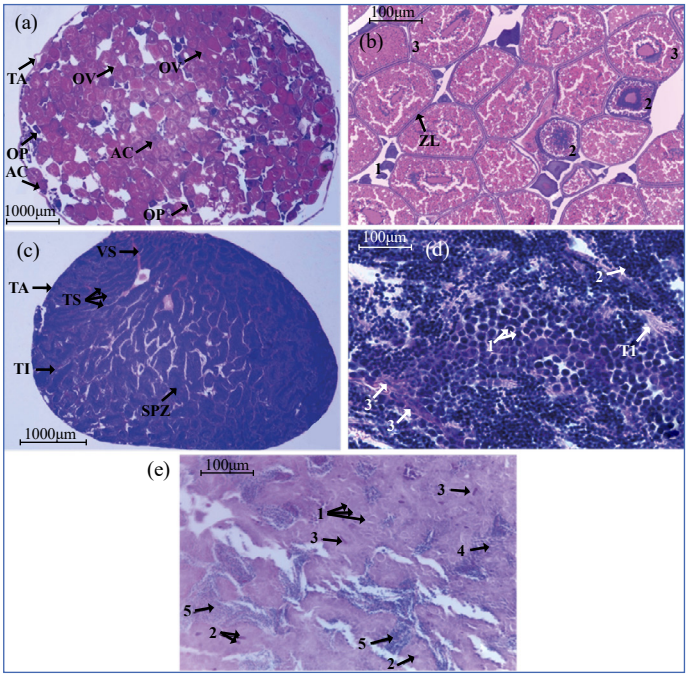



**Figure 3.** Morphological aspect of the macroscopic analysis of gonads in the Brazilian sardine (*Sardinella brasiliensis*). (a, b, and c) Female gonads at the mature phases: (a) symmetrical gonads; (b and c) asymmetrical gonads. (d, e, f, and g) male gonads: (d) at the immature phases, (e) mature (symmetrical), (f and g) mature (asymmetrical). Images are illustrative only and do not represent proportional size among specimens.

The observation revealed the presence of the tunica albuginea (TA) surrounding the ovary, with germ cells at various developmental stages (Fig. 4a), including perinucleolar oocytes (1), cortical alveoli oocytes (2), vitellogenic oocytes (3), and the zona radiata (arrow), consistent with ovaries at the final phase of development (Fig. 4b). Females exhibited an abundance of vitellogenic oocytes, indicating advanced maturation and proximity to spawning. This supports the pattern of batch spawning and the high reproductive adaptability of the species, as previously reported even under controlled conditions (Cergole & Dias Neto, 2011; Magnotti et al., 2020).

Histological sections of the testes revealed two distinct phases: 32 males were in the reproductively active phase, with all stages of spermatogenesis present (spermatocytes, spermatids, and spermatozoa) and a filled lumen (Figs. 4c and 4d). The remaining 14 males were in the developing phase, exhibiting spermatogonia differentiation and lumen formation, but no mature spermatozoa (Fig. 4e). Macroscopically, mature testes appeared turgid, firm, and whitish, occupying a large portion of the coelomic cavity. In contrast, immature testes were filamentous, translucent, and occupied less than one-third of the cavity.




TA: tunica albuginea; OV: vitellogenic oocytes; OP: perinucleolar oocytes; AC: cortical alveoli; ZL: zona radiata layer; TI: interstitial tissue; VS: blood vessels; TS: seminiferous tubules; SPZ: spermatozoa.

**Figure 4.** Transverse histological sections of (a, b) mature ovaries, (c, d) mature testes, and (e) developing testes of Brazilian sardine (*Sardinella brasiliensis*).

In teleosts, testes can be classified as either lobular or tubular, depending on the species (Grier et al., 1981; Koulish et al., 2002). In *S. brasiliensis*, histological analysis revealed linearly arranged seminiferous tubules, with germ cell cysts and free spermatozoa in the lumen, confirming the tubular pattern.

## DISCUSSION

The ultrasonographic method used in this study demonstrated an accuracy of 98% and can be considered a viable technique for sex identification of *S. brasiliensis* during the reproductive period. The predominance of males in this study, with 46 identified compared to only nine females, contrasts with findings in the European sardine (*Sardina pilchardus*)—females represented 71% of the sampled individuals (Zorica et al., 2019), while Znari and Mounir (2021) reported a balanced sex ratio. This variation in the number of male and female fish may be influenced by multiple factors, including environmental and physiological conditions. Although temperature has been reported as a factor affecting sex determination in some fish species, this aspect was not evaluated in the present study (Baroiller et al., 2009; Devlin & Nagahama, 2002; Mank et al., 2006).

The occurrence of gonadal asymmetry in 18 individuals (13 males and five females) is unprecedented for the Brazilian sardine (*S. brasiliensis*). Its irregular formation may suggest the influence of multiple factors, but the underlying causes were not investigated in the present study. Similar cases have been reported in other species, such as Baltic herring (*Clupea harengus membras*), in which environmental changes have been associated with impaired gonadal development (Rajasilta et al., 2016). Such anomalies have also been linked to chronic exposure to contaminants and endocrine-disrupting chemicals in other contexts (Coady et al., 2005).

Biometric analyses revealed that females of *S. brasiliensis* exhibited significantly greater length and weight than males, a pattern previously reported in other Clupeiform species (Lopes et al., 2017). The larger body size observed in females may be associated with delayed onset of first sexual maturation, which would allow for prior accumulation of energy reserves and, consequently, greater success in fractional spawning (Jonsson & Jonsson, 2015; Roff, 2002). In marine fish with partial fecundity, such as the Brazilian sardine, there is a positive correlation between female size and oocyte production, highlighting the importance of somatic growth prior to reproduction (Cergole & Dias Neto, 2011).

Despite the observed differences in body size between sexes, no significant differences were found in gonad weight or gonadosomatic index. This result may be associated with variability in gonadal development among individuals. Although females were classified as mature, not all individuals may have been at peak vitellogenic stage at the time of sampling. In addition, males also exhibited advanced gonadal development, which may have contributed to similar gonadosomatic index values between sexes (Brown-Peterson et al., 2011; Vazzoler, 1997).

Using ultrasonography, ovaries exhibited a hyperechoic appearance, whereas testes showed a predominantly hypoechoic pattern. This observation is consistent with reports in species such as Atlantic cod (*Gadus morhua*), flathead grey mullet (*M. cephalus*), and Lebranche mullet (*Mugil liza*), in which ovaries at advanced maturation phase reflect ultrasound waves more strongly due to oocyte development (Karlsen & Holm, 1994; Masoudifard et al., 2023; Santarosa et al., 2025).

The 8 to 13 MHz transducer proved effective for sex identification but was insufficient for visualizing oocytes or accurately determining gonadal maturation phase. A similar result was reported in *M. liza*, whose oocyte measurement was not possible at this frequency range either (Santarosa et al., 2025). Considering these limitations, higher-frequency probes ranging from 18 to 22 MHz are a viable alternative, as they provide

greater resolution for small-scale structures (Barcaui et al., 2015). Devices operating at 20 MHz, for example, are successfully used in human dermatological examinations, allowing the distinction of fine tissue layers with high definition (Jasaitiene et al., 2011).

The choice of scanning plane directly influences the quality of information obtained through ultrasonography. While longitudinal scans provide a panoramic view of gonadal morphology, transverse scans are more suitable for detailed internal analysis, especially when the aims are to assess echogenicity and estimate the diameter of internal structures (Brown-Peterson et al., 2011). This distinction is particularly relevant for identifying echotextural patterns, which have been consistently described across various species. Studies of Atlantic halibut (*Hippoglossus hippoglossus*) indicate that mature ovaries exhibit a granular, hyperechoic appearance, whereas testes appear more homogeneous and hypoechoic (Karlsen & Holm, 1994; Martin-Robichaud & Rommens, 2001). Similar patterns have also been reported in striped bass (*Morone saxatilis*) (Blythe et al., 1994) and European eel (*Anguilla anguilla*) (Jéhannet et al., 2017), suggesting that echogenic characteristics of gonads remain stable across species.

The application and comparison of ultrasonographic findings with histological analysis is essential for accurately determining the maturation phase of gonads, allowing identification of phases such as imminent gamete release, regression, and regeneration distinctions that are particularly relevant in batch-spawning species like *S. brasiliensis* (Brown-Peterson et al., 2011; Vazzoler, 1997). The comparison between macroscopic and microscopic analyses revealed that macroscopic assessment alone may not accurately reflect gonadal development in male Brazilian sardines, as some individuals with immature appearance exhibited clear signs of progressing spermatogenesis. This underscores the importance of histology as a complementary tool for reproductive assessment, especially in early developmental stages (Brown-Peterson et al., 2011).

Consistency was observed in the results obtained with all three approaches. Both mature females and males identified by ultrasonography were confirmed through macroscopic and microscopic analyses, which placed these individuals at an advanced reproductive phase. In contrast, males classified as immature via ultrasonography exhibited smaller and thinner gonads, an observation that was validated by both macroscopic and microscopic evaluations, indicating a developmental phase. Unlike previous studies that reported difficulties in visualizing immature gonads and restricted their analyses to individuals in the mature phase (Loher & Stephens, 2011; Moghim et al., 2002; Newman et al., 2008), the present study successfully identified the developmental phase of immature gonads. These findings highlight the value of combining ultrasonography with macroscopic and microscopic analyses, to overcome limitations reported in earlier research and provide a comprehensive and accurate overview of gonadal maturation phases.

To further enhance the accuracy of maturation phase identification, future studies should aim to establish standardized gonadal measurements using ultrasonography, creating a reference framework for each developmental phase. Such research could focus on defining average values considered characteristic for immature, developing, and mature gonads. Establishing this standard would allow a more objective classification of maturation phases and support more consistent and reproducible reproductive assessments in captive environments, while also facilitating comparisons across studies and species.

## CONCLUSION

Ultrasonography proved to be an effective, rapid, and non-invasive tool for sex identification of the Brazilian sardine (*S. brasiliensis*), including in individuals with immature gonads. This technique represents a valuable alternative to traditional invasive methods, particularly for small pelagic species, in which conventional approaches are limited or unfeasible. Its application may improve broodstock management, reduce handling stress, and support the advancement of captive reproduction protocols for this species and other similar taxa.

## CONFLICT OF INTEREST

Nothing to declare.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

## AUTHORS' CONTRIBUTIONS

**Conceptualization:** Luz, L.S.; Data curation: Luz, L.S.; **Formal Analysis:** Luz, L.S.; **Investigation:** Luz, L.S., Santarosa, I.A.M., Castro, M.A.M., França, L.C.K., Santos, E.D.; **Writing – original draft:** Luz, L.S.; **Methodology:** Santarosa, I.A.M., França, L.C.K., Santos, E.D.; **Writing – review & editing:** Riofrio, C.P.; Oliveira, B.V.; **Funding acquisition:** Brum, A., Magnotti, C.; **Project administration:** Brum, A., Magnotti, C.; Resources: Brum, A., Magnotti, C.; **Supervision:** Magnotti, C.; **Final approval:** Luz, L.S.

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## DECLARATION OF USE OF ARTIFICIAL INTELLIGENCE TOOLS

Artificial intelligence tools, specifically ChatGPT by OpenAI, were used to assist with language editing, translation, and reference formatting. All scientific content and interpretations were reviewed and validated by the authors, who take full responsibility for the final manuscript.

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