ORIGINAL RESEARCH

# Baseline physiological data and enzymatic valorization of fishery waste for female *Odontesthes argentinensis* nutrition

Yamila E. Rodriguez[1, 2, *], Juana C. del Valle[1], Ivana S. Friedman[1], María Victoria Laitano[1], Clara Liébana[3], Aldo N. Zanazzi[2] and Analia V. Fernández-Gimenez[1, *]

[1]Instituto de Investigaciones Marinas y Costeras (IIMyC), Facultad de Ciencias Exactas y Naturales (FCEyN),Universidad Nacional de Mar del Plata (UNMdP), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Juan B. Justo 2550, B7608FBY - Mar del Plata, Argentina. [2]Universidad Tecnológica Nacional (UTN), Facultad Regional Mar del Plata, Av. Dorrego 281, B7600CLF - Mar del Plata, Argentina. [3]Instituto Nacional de Investigación y Desarrollo Pesquero (INIDEP), Paseo Victoria Ocampo Nº 1, Escollera Norte, B7602HSA - Mar del Plata, Argentina. ORCID *Yamila E. Rodriguez* https://orcid.org/0000-0002-4596-3586, *Juana C. del Valle* https://orcid.org/0000-0003-1095-8098, *Ivana S. Friedman* https://orcid.org/0000-0002-9075-0539, *María Victoria Laitano* https://orcid.org/0000-0003-2436-0839, *Clara Liébana* https://orcid.org/0000-0002-7062-8675, *Aldo N. Zanazzi* https://orcid.org/0009-0008-7736-2567, *Analia V. Fernández Gimenez* https://orcid.org/0000-0001-9232-4560

**ABSTRACT.** This study investigated the energy storage patterns in wild female *Odontesthes argentinensis* collected during autumn in the coastal waters of Mar del Plata (Argentina) to assess their suitability as breeders and explored innovative enzyme-based feeding strategies to improve food quality, nutrient utilization, and nutritional management of breeders in this species. Biochemical analyses showed that the liver was the primary storage organ for lipids and proteins, while the fatty acid profiles were consistent with a capital breeding strategy associated with seasonal reproduction. However, because this study was based on a single seasonal sampling and lacked ovarian stage classification, gonadosomatic or hepatosomatic indices, and a temporal series, these findings should be interpreted as preliminary evidence and should not be taken as sufficient to definitively classify the reproductive strategy. To improve the nutritional value of soybean meal, enzymatic treatment with peptidases from *Urophycis brasiliensis* under acidic conditions (pH 2) reduced antinutritional compounds while preserving bioactive molecules, thereby enhancing protein quality. In addition, *in vitro* combination of exogenous peptidase extracts from fisheries waste with digestive enzymes of *O. argentinensis* increased proteolytic activity, suggesting synergistic interactions that warrant testing in future feeding trials. Together, these findings provide physiological and nutritional insights that can contribute to the development of feeding strategies for *O. argentinensis* females. Furthermore, the use of fishery waste as sources of digestive enzymes represents a promising biotechnological approach to improve feed digestibility while promoting more sustainable aquaculture practices. Nevertheless, further studies assessing digestibility, fish performance, extraction yields, safety, economic feasibility, and environmental impacts are needed to substantiate these potential benefits.

**Key words:** Peptidases, lipases, antinutritional factors, fish, soybean meal, fish wastes.

**Datos fisiológicos basales y valorización enzimática de residuos pesqueros para la nutrición de hembras de *Odontesthes argentinensis***

**RESUMEN.** Este estudio investigó los patrones de almacenamiento de energía en hembras silvestres de *Odontesthes argentinensis* capturadas durante el otoño en aguas costeras de Mar del Plata (Argentina) para evaluar su idoneidad como reproductoras, y exploró estrategias de alimentación innovadoras basadas en enzimas para mejorar la calidad del alimento, la utilización de nutrientes y el manejo nutricional de los reproductores de esta especie. Los análisis bioquímicos mostraron que

Marine and Fishery Sciences MAFIS

OPEN ACCESS

*Correspondence:
fgimenez@mdp.edu.ar
yrodriguez@mdp.edu.ar

Received: 9 June 2026
Accepted: 17 September 2026

ISSN 2683-7595 (print)
ISSN 2683-7951 (online)

https://ojs.inidep.edu.ar

Journal of the Instituto Nacional de Investigación y Desarrollo Pesquero (INIDEP)

el hígado fue el principal órgano de almacenamiento de lípidos y proteínas, mientras que los perfiles de ácidos grasos fueron coherentes con una estrategia reproductiva basada en reservas corporales (*capital breeding*) asociada a la reproducción estacional. Sin embargo, dado que este estudio se basó en un único muestreo estacional y careció de clasificación de estadios ováricos, índices gonadosomáticos o hepatosomáticos y series temporales, estos hallazgos deben interpretarse como evidencia preliminar y no considerarse suficientes para clasificar definitivamente la estrategia reproductiva. Para mejorar el valor nutricional de la harina de soja, el tratamiento enzimático con peptidasas de *Urophycis brasiliensis* en condiciones ácidas (pH 2) redujo los compuestos antinutricionales y preservó las moléculas bioactivas, mejorando así la calidad proteica. Además, la combinación *in vitro* de extractos de peptidasas exógenas provenientes de residuos pesqueros con enzimas digestivas de *O. argentinensis* aumentó la actividad proteolítica, lo que sugiere interacciones sinérgicas que justifican su evaluación en futuros ensayos de alimentación. En conjunto, estos hallazgos aportan información fisiológica y nutricional que puede contribuir al desarrollo de estrategias de alimentación para hembras de *O. argentinensis*. Asimismo, el uso de residuos pesqueros como fuente de enzimas digestivas representa un enfoque biotecnológico prometedor para mejorar la digestibilidad del alimento y promover prácticas acuícolas más sostenibles. No obstante, se requieren estudios adicionales que evalúen la digestibilidad, el desempeño de los peces, los rendimientos de extracción, la seguridad, la viabilidad económica y los impactos ambientales para corroborar estos beneficios potenciales.

**Palabras clave:** Peptidasas, lipasas, factores antinutricionales, peces, harina de soja, residuos pesqueros.

## INTRODUCTION

*Odontesthes argentinensis* (Valenciennes, 1835) (Atherinopsidae, Atheriniformes), commonly known as the Southwest American marine silverside, inhabits temperate and cold-temperate coastal waters of the southern Atlantic Ocean, ranging from southern Brazil to northeastern Argentine Patagonia (Llompart et al. 2013). Owing to its commercial value, desirable flesh quality, and promising aquaculture potential, this species has attracted increasing interest as a candidate for marine aquaculture (Guidi et al. 2021). However, important aspects of its reproductive biology, energy allocation, digestive physiology, and nutritional requirements remain poorly understood, limiting the development of species-specific culture technologies.

Reproduction is one of the most energetically demanding phases of the fish life cycle, requiring the mobilization of stored energy to support gonadal development, spawning, and embryo production. In *O. argentinensis* the reproductive cycle is characterized by marked changes in the hepatosomatic index and body condition, suggesting shifts in energy allocation throughout the reproductive season (Llompart et al. 2013). Consequently, characterizing the quantity and distribution of energy reserves in storage tissues is essential for understanding the reproductive physiology of the species and for designing appropriate nutritional strategies for broodstock management (Rahman et al. 2020).

Recent physiological studies of the population of *O. argentinensis* from Mar Chiquita Coastal Lagoon (Argentina) have revealed marked seasonal and ontogenetic plasticity in digestive and metabolic traits associated with reproduction. Méndez et al. (2024) showed that females maintain body condition throughout ovarian development by adjusting digestive capacity and energy metabolism; in particular, during the spawning-capable phase, increased intestinal aminopeptidase-N activity was accompanied by the selective mobilization of hepatic and muscle glycogen, triglycerides, and proteins to support gonadal maturation. In addition, Méndez et al. (2026) demonstrated marked seasonal changes in digestive capacity, reflected by changes in the intestinal alkaline phosphatase activity, as well as in the mobilization of energy reserves. Collectively, these findings indicate that *O. argentinensis* relies on endogenous energy reserves that are mobilized in a coordinated and tissue-specific manner to meet the energetic demands of reproduction. However, these studies were conducted on an estuarine population and did not include fatty acid profiling or a direct comparison of the biochemical composition of liver and ovary. The present study therefore extends this knowledge by providing the first fatty acid profile and a comprehensive biochemical characterization of liver and ovary in wild females from the marine population off Mar del Plata, together with baseline digestive peptidase and lipase activities that have not previously been reported for this species.

Digestive physiology is another key aspect influencing feed utilization and reproductive performance in aquaculture. Unlike many teleosts, *O. argentinensis* lacks both a stomach and pyloric caeca. As in other agastric fish species, the intestine is relatively long, increasing the surface area available for nutrient digestion and absorption (Le et al. 2019). Under these conditions, alkaline digestive enzymes, particularly peptidases and lipases, play a central role in nutrient hydrolysis. Nevertheless, the digestive enzyme profile of *O. argentinensis* remains poorly characterized, representing an important knowledge gap for the development of nutritionally adequate formulated diets.

Beyond its reproductive physiology, *O. argentinensis* is a euryhaline species that inhabits both marine and estuarine environments, and its digestive and metabolic traits reflect this ecological plasticity. In this sense, Albanesi et al. (2025) showed that estuarine individuals from Mar Chiquita Coastal Lagoon display higher intestinal carbohydrase activities (amylase, maltase, sucrase) and greater lipid and glycogen reserves than marine conspecifics, with no differences in protein content or trypsin activity, highlighting the species' capacity for habitat-related digestive/metabolic adjustment. At the interspecific level, Albanesi et al. (2026) compared juveniles of *O. argentinensis* with the detritivorous *Mugil liza* and the carnivorous *Oligosarcus jenynsii* within the same lagoon system and found that *O. argentinensis* exhibits an intermediate enzymatic profile (combining carbohydrase and peptidase activities) together with a marked reliance on hepatic triglycerides as its principal energy reserve, consistent with its omnivorous feeding habits.

Adequate broodstock nutrition is essential to ensure successful ovarian development, spawning performance, and offspring quality. Fishmeal remains the primary protein source in aquafeeds, whereas soybean meal is widely used as sustainable alternative because of its availability and favorable nutritional composition (Soltan et al. 2023). However, soybean meal contains several antinutritional factors, including trypsin inhibitors, which can impair digestive enzyme activity, reduce nutrient utilization, and negatively affect fish performance (Weng et al. 2023). Enzymatic treatment has emerged as an effective strategy to reduce these antinutritional compounds while improving protein digestibility and generating bioactive peptides that may benefit intestinal microbiota (Deng et al. 2023; Soltan et al. 2023). Marine enzymes recovered from fishery by-products represent an alternative to commercial enzymes for feed processing. Previous studies have demonstrated the biotechnological potential of digestive enzyme extracts obtained from discarded marine organisms, including *Percophis brasiliensis* (Quoy and Gaimard, 1824), *Urophycis brasiliensis* (Kaup, 1858), and the crab *Ovalipes trimaculatus* (de Haan, 1833). In addition, dietary supplementation with exogenous digestive enzymes has been shown to improve digestive efficiency and nutrient utilization in several cultured fish species (Bonadero et al. 2022; Friedman et al. 2022). Nevertheless, the effectiveness of this approach depends on species-specific digestive physiology, and the interactions between endogenous and exogenous enzymes remain insufficiently understood (Liang et al. 2022). *In vitro* studies have demonstrated that these interactions may be synergistic or inhibitory, highlighting the importance of evaluating their compatibility before application in formulated diets (Rodriguez et al. 2017, 2021; del Valle et al. 2022).

Considering the limited knowledge of the reproductive physiology and digestive biology of *O. argentinensis*, this study aimed to generate information supporting the development of nutritional strategies for broodstock culture. Specifically, the objectives were: (1) to characterize the energy reserves in the muscle, liver, and ovaries of wild female collected during autumn as a partial indicator of nutritional and physiological status during the reproductive period, and (2) to assess the effects of two functional ingredients for diet by evaluating their effects *in vitro* (i) soybean meal treated with marine enzymes as an improved protein source, and (ii) exogenous marine enzymes as dietary supplements.

## MATERIALS AND METHODS

### Fish sampling

Twenty wild adult females *Odontesthes argentinensis* (body weight: 186.70 g ± 7.17; total length: 30.02 cm ± 0.42) were collected during autumn (April-May) by artisanal fishers off the coast of Mar del Plata, Argentina (38° 04′ S, 57° 30′ W) (supplementary material, Table S1). Fish were transported on ice to the laboratory and immediately dissected. The digestive tract, liver, ovaries, and muscle were individually excised for subsequent analyses. Only females with ovaries at a resting stage and no food contents in the digestive tract were included in the study; individuals that did not meet either of these criteria were excluded.

*Energy reserve characterization*

Crude protein contents in muscle, liver, and ovary tissues (n = 20) were determined using the Kjeldahl method (AOAC Method No. 2001.11) with a nitrogen-to-protein conversion factor of 6.25 (Thiex et al. 2002). Crude lipid content was determined using the Soxhlet extraction method (AOAC Method 920.39C) with petroleum ether as solvent (AOAC 1995). Glycogen concentration was determined according to the method of Dubois et al. (1956). Fatty acid profiles were analyzed by gas chromatography with flame ionization detection (GC-FID) following COI-T.20; DOC. 24-2001. All biochemical analyses were performed by CEDEAC Group Laboratories (Mar del Plata, Argentina).

### Digestive enzyme extraction and characterization

Digestive tracts were individually homogenized in ice-cold 50 mM Tris-HCl buffer (pH 7.5) at a ratio of 1:2 (w/v) using a Teflon-glass homogenizer. Homogenates were centrifuged at 10,000 × g for 30 min at 4 °C (KIMADI 3H12RI, China), and the resulting supernatants were stored at -20 °C as the *O. argentinensis* digestive enzyme extract (Oa).

Alkaline peptidase activity was determined according to García-Carreño (1992) using 0.5% (w/v) azocasein (Sigma A2765) prepared in 50 mM Tris-HCl buffer (pH 7.5) as substrate. Absorbance was measured at 366 nm using a SPECTROstar Nano microplate spectrophotometer (BMG LABTECH, Germany) following González-Zamorano et al. (2013). Lipase activity was determined according to the micro-method described by Nolasco-Soria et al. (2018) using 20 mM β-naphthyl caprylate (Goldbio N-100) as substrate. β-Naphthol release was measured at 540 nm.

One unit (U) of enzyme activity was defined as the change in absorbance per minute. Total activity (TA) was expressed as U $ml^{-1}$. Protein concentration was determined following Bradford (1976), and specific activity (SA) was calculated as U mg $protein^{-1}$. All enzymatic assays were performed in triplicate.

### Marine enzymes for functional feed applications

Digestive enzyme extracts were obtained from the viscera of *Percophis brasiliensis* (Pb), *Urophycis brasiliensis* (Ub), and *Ovalipes trimaculatus* (Ot). Fish specimens were collected by commercial fishing vessels operating off the coast of Mar del Plata, Argentina. Fish stomachs and intestine-pyloric caeca, as well as crab midgut glands, were excised immediately after capture and transported on ice until processing. Cephalothorax by-products of the Argentine red shrimp *Pleoticus muelleri* (Pm) were obtained from a local seafood processing plant (Moscuzza SACI, Mar del Plata, Argentina). Enzyme extracts were prepared as previously described. Ot extracts were used both for soybean meal treatment and as exogenous enzyme supplements, whereas Pb and Ub extracts were used exclusively for soybean meal treatment and Pm extracts only as dietary enzyme supplements.

**Soybean meal treatment with marine enzymes**

Acid peptidase activity from fish stomach extracts (Pb and Ub) was determined at pH 2 using 100 mM glycine-HCl buffer containing 0.5% hemoglobin (Sigma H2625) as substrate (Anson 1938). Absorbance was measured at 280 nm, and total activity was calculated as described above. Alkaline peptidase activity from intestine-pyloric caeca (Pb and Ub) and crab midgut gland (Ot) extracts was measured at pH 8 following the method described above.

Soybean meal (5% w/v) was hydrolyzed according to Wu and Muir (2010), with modifications. Each enzyme preparation was added at a concentration of 5 U $g^{-1}$ soybean meal, and reactions were carried out under the optimal pH conditions previously established for each extract (Bonadero et al. 2022; Friedman et al. 2022). Hydrolysis was performed at 30 °C for 150 min under gentle stirring using stomach extracts at pH 2 (Pb2 and Ub2) and intestinal or crab extracts at pH 8 (Pb8, Ub8, and Ot8). Reaction pH was maintained by continuous adjustment with 0.5 M NaOH or HCl. Following incubation, samples were centrifuged (6,000 *g*, 20 min), washed with distilled water, resuspended in 10 mL distilled water, centrifuged again, dried at 40 °C, and stored at room temperature until analysis. All treatments were performed in triplicate.

**Evaluation of antinutritional and antioxidant properties**

Antinutritional activity was evaluated by peptidase inhibition assays adapted from Alarcón et al. (1999), with modifications. Aqueous extracts of untreated soybean meal (SM) and enzyme-treated soybean meals (Pb2, Pb8, Ub2, Ub8, and Ot8) were prepared at 100 mg $ml^{-1}$ by shaking for 60 min. Following centrifugation (6,000 *g*, 10 min), 10 µl of supernatant was mixed with 10 µl of Oa digestive extract and incubated at 25 °C for 60 min. Control reactions contained digestive extract and Tris-HCl buffer only. Residual peptidase activity was determined as described above and expressed as U $ml^{-1}$. Antioxidant activity was evaluated by electron paramagnetic resonance (EPR) spectroscopy using the DPPH radical scavenging assay according to Díaz et al. (2014). Soybean meal samples (20 mg) were suspended in 900 µL absolute ethanol, sonicated for 30 min, mixed with 100 µL DPPH solution (0.8 mmol $l^{-1}$), and incubated in darkness for 1 h. Spectra were recorded at 293 K using a Bruker ELEXSYS E500T spectrometer equipped with an ER 4102ST rectangular resonator operating in the TE102 mode at X-band frequency (9.8 GHz). Total radical scavenging activity was quantified from the area under the first integral of each EPR spectrum, which is proportional to the remaining DPPH concentration.

***In vitro* compatibility of exogenous enzymes with digestive enzymes of *O. argentinensis***

Potential synergistic interactions between endogenous digestive enzymes of *O. argentinensis* (Oa) and exogenous crustacean enzyme extracts from *Pleoticus muelleri* (Pm) and *Ovalipes trimaculatus* (Ot) were evaluated *in vitro*. Equal volumes (5 µl) of Oa extract, and each exogenous enzyme preparation were mixed prior to determining alkaline peptidase and lipase activities. Individual enzyme extracts were assayed separately under identical conditions and served as controls.

**Statistical analysis**

Differences among soybean meal treatments were evaluated by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. Comparisons between endogenous enzyme activity and endogenous + exogenous enzyme activity were performed separately for proteases and lipases and for Pm and Ot, using Student's t-tests with individual fish considered as the experimental unit (n = 10). Data were tested for normality and homogeneity of variance before statistical analyses. All analyses were performed using R software (R Core Team 2023).

## RESULTS AND DISCUSSION

Although recent studies on *O. argentinensis* aquaculture have addressed larval nutrition and feeding ecology (Führ et al. 2016; Thompson and Volpedo 2018), broodstock reproductive physiology and digestive enzyme characterization remain poorly understood, representing key knowledge gaps for the development of culture technologies. Nutrition is essential during reproduction because it directly influences maturation, fecundity, and larval survival. Successful reproduction requires sufficient energy reserves to meet the high energetic demands of gamete production and reproductive behavior. Females invest substantial amounts of energy in maintaining egg reserves (Volkoff and London 2018; Hernandez de Dios et al. 2022). Therefore, maternal nutrition strongly influences egg quality and larval development during endogenous feeding (Izquierdo et al. 2001). In fish, the liver and ovary are the main organs involved in nutrient storage and provisioning for reproduction (Volkoff and London 2018). In the present study, the liver and ovary of wild adult females exhibited similar glycogen concentrations. In contrast, the liver contained higher concentrations of fatty acids and proteins than the ovary (Table 1). The predominance of the liver as a major storage site for lipids and proteins is consistent with previous findings for this species. In *O. argentinensis* from Mar Chiquita Coastal Lagoon, hepatic glycogen, triglycerides, and proteins are mobilized in relation to reproductive stage and season, whereas muscle appears to play a comparatively minor role in energy storage (Méndez et al. 2024, 2026; Albanesi et al. 2025). Notably, hepatic triglycerides have been identified as the main lipid reserve in this species, in contrast to the muscle glycogen or protein-based energy storage strategies reported for co-occurring species (Albanesi et al. 2026). These findings further support the importance of hepatic lipid reserves in meeting the energetic demands of gonadal development in *O. argentinensis*.

In fish, glucose serves as a primary energy source for gonadal maturation. Additionally, glycogen may serve as the main energy source for oocytes during the first days after ovulation (Soengas et al. 1995). Before vitellogenesis, proteins accumulate in the female liver to support vitellogenin synthesis (Yilmaz et al. 2024). Protein mobilization from the liver to the ovary is regulated by estrogens. The higher protein content observed in the liver of *O. argentinensis* compared with the ovary suggests that this organ constitutes an important source of amino acids for the synthesis of the yolk precursor protein vitellogenin (Table 1). Adequate protein reserves in females facilitate the synthesis of lipoproteins, hormones, and enzymes

Table 1. Biochemical composition of liver and ovary in female *Odontesthes argentinensis*. Values are means ± SE (n = 20).

| | Liver | Ovary | Muscle |
|---|---|---|---|
| Glycogen (mg glucose/g tissue) | 5.7 ± 0.02 | 5.8 ± 0.07 | 2.0 ± 0.02 |
| Protein (%) | 15.4 ± 0.20 | 15.0 ± 0.17 | 22.3 ± 0.25 |
| Total fatty acids (%) | 18.4 ± 0.24 | 4.4 ± 0.03 | 1.43 ± 0.01 |

required for oocyte development, thereby influencing reproductive performance in aquaculture. Proteins are the major nutrient in fish eggs and play a pivotal role throughout the reproductive cycle, affecting spawning success, fertilization, embryonic development, hatching rate, and larval survival and growth (Hernandez de Dios et al. 2022).

Fish obtain lipids primarily from their diet, particularly essential fatty acids that cannot be synthesized *de novo*. Seasonal capital breeders, such as the marine silverside, rely on energy reserves accumulated before the reproductive period (Fuiman and Perez 2015). In these species, lipids are mainly stored in the liver and muscle and are subsequently mobilized to the ovary during gametogenesis for incorporation into the egg yolk (Volkoff and London 2018). Accordingly, the liver of female *O. argentinensis* contained the highest lipid content (18.4%), followed by the ovary (4.4%), whereas muscle did not represent a major lipid storage tissue (Table 1). Previous studies in red drum have shown that maternal nutrition can induce long-term metabolic effects in the offspring (Fuiman and Perez 2015). Maternal provisioning of essential fatty acids enhances offspring fitness. In this context, palmitic acid and unsaturated fatty acids stored in the liver of *O. argentinensis* females may serve as energy reserves that are transferred to the ovaries during the reproductive season to support follicular development and egg provisioning (Table 2). Palmitic acid (16:0) was the predominant saturated fatty acid in both the liver and ovary (Table 2), and may provide an important substrate for ATP production during vitellogenesis (Zhao et al. 2023). Likewise, the presence of the polyunsaturated fatty acids linoleic acid (18:2 n-6), alpha linolenic acid (18:3 n-3), and arachidonic acid (20:4 n-6) (Table 2) may contribute to eicosanoid (e.g. prostaglandin) synthesis, which is required for steroid hormone production (Izquierdo et al. 2001), whereas vitellogenin synthesized in the liver serves as the primary precursor of egg yolk (Hara et al. 2016).

The finding of arachidonic acid (20:4n-6) as the only HUFA in the present work, together with the absence of detectable EPA and DHA, may reflect differences in fatty acid metabolism and tissue-specific lipid allocation rather than an analytical inconsistency. Marine fish have a limited capacity to synthesize long-chain polyunsaturated fatty acids from C18 precursors and therefore rely largely on dietary sources to meet their requirements for these fatty acids (Xu et al. 2022). Under conditions of low dietary n-3-PUFA availability, changes in tissue fatty acid composition may support the incorporation of n-6 fatty acids, including 20:4n-6 and 20:3n-6, into membrane phospholipids, helping to preserve membrane structure and function (Bou et al. 2017). This reciprocal relationship between n-6 and n-3 fatty acids has been reported in marine fish, including Atlantic salmon, in which increased dietary 18:2n-6 resulted in higher tissue ARA (20:4n-6) levels accompanied by a reduction in EPA (Katan et al. 2020; Sissener et al. 2020).

Tissue fatty acid profiles are also influenced by lipid class composition and by the physiological role of each tissue. Dietary effects, for example, can be particularly evident in liver polar lipids, whereas tissues such as the brain and retina tend to maintain relatively conserved fatty acid profiles (Sissener et al. 2020). Reproductive tissues may show an additional degree of regulation, as fatty acids can be selectively retained or mobilized according to reproductive demands rather than simply reflecting dietary intake (Rodríguez-Barreto et al. 2012). In this context, the presence of 20:4n-6 as the only detectable HUFA in the present samples could be related to preferential retention of ARA, reduced availability of dietary n-3-PUFA, or selective utilization of EPA and DHA. Nevertheless, these mechanisms cannot be distinguished from the present data alone, and the absence of detectable EPA and DHA should therefore be interpreted with caution.

By comparison, the proximate composition of *O. argentinensis* muscle showed a protein content of 22.3%, glycogen content of 2.0%, and a low lipid content (1.43%). The low glycogen concentration suggests limited carbohydrate storage in skeletal

Table 2. Fatty acid profile (% total fatty acid) of liver and ovary of *Odontesthes argentinensis* female (n = 20) collected from the coastal waters of Mar del Plata during autumn.

| Fatty acid | Liver | Ovary |
|---|---|---|
| 14:0 | 5.2 | 8.2 |
| 16:0 | 31.1 | 37.1 |
| 17:0 | 1.2 | 1.3 |
| 18:0 | 8.3 | 9.1 |
| 22:0 | 0.2 | 1.5 |
| 24:0 | 0.1 | 1.1 |
| ∑SFA | 46.1 | 58.3 |
| 16:1 n-7 | 11.7 | 7.8 |
| 17: 1: n-7 | 0.9 | 0.7 |
| 18:1 n-9 | 33.5 | 27.2 |
| 20:1 n-11 | 1.0 | 0.6 |
| ∑MUFA | 47.1 | 36.3 |
| 18:2 n-6 | 4.3 | 4.0 |
| 18:3 n-3 | 2.0 | 1.2 |
| ∑PUFA | 6.3 | 5.2 |
| 20:4 n-6 | 0.5 | 0.3 |
| ∑HUFA | 0.5 | 0.3 |
| Ratio ∑ω6/∑ω3 | 2.4 | 3.6 |

∑SFA: sum of saturated fatty acids. ∑MUFA: sum of monounsaturated fatty acids. ∑PUFA: sum of polyunsaturated fatty acids (18:2n-6, 18:3n-3). ∑HUFA: sum of highly unsaturated fatty acids (≥ 20 carbons, ≥ 3 double bonds); in this study, represented only by arachidonic acid (20:4n-6), as eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) were below the quantification limit of the GC-FID method used and are therefore not reported. Values represent percentage of total fatty acids identified.

muscle, consistent with the metabolic characteristics of marine teleost, which rely primarily on hepatic glycogen reserves. Furthermore, the low lipid content (< 2%) indicates that *O. argentinensis* can be classified as a lean fish with high nutritional value due to its elevated protein content.

The biochemical composition observed in female *O. argentinensis* collected during autumn suggests that the liver represents the principal energy reserve, particularly for proteins and lipids, which are likely to support vitellogenesis. This pattern is consistent with the central role of the liver in nutrient storage and mobilization described for many marine teleosts. However, because the present study was based on wild adult females' samples at a single seasonal point, these findings should be interpreted as a physiological snapshot rather than definitive evidence of the species' overall reproductive energy allocation strategy. This limitation is consistent with previous evidence from the same species in Mar Chiquita Coastal Lagoon, where digestive and metabolic traits vary substantially across both seasons and stages of ovarian maturation (Méndez et al. 2024, 2026). In this population, hepatic glycogen content and intestinal alkaline phosphatase activity decline during winter-spring, coinciding with increased investment in gonadal development. Conversely, lipid reserves accumulated during summer-autumn are subsequently mobilized to support spawning, while muscle protein reserves decrease during active reproduction. These patterns highlight the extent of physiological remodeling that occurs throughout the reproductive cycle and underscore the need for future studies covering multiple seasons to fully characterize the energy allocation strategy of *O. argentinensis* from Mar del Plata. While our results provide a detailed biochemical baseline for females in autumn, the absence of a temporal series prevents a direct quantification of energy mobilization or a definitive classification of the reproductive strategy. These findings represent a physiological state prior to the peak of spawning, which aligns with, but does not independently prove, the seasonal trajectories described for other populations of the species.

Comparisons with other marine fish indicate that *O. argentinensis* shares some characteristics with species that rely, at least partially, on stored endogenous reserves for reproduction. For example, the Patagonian grouper (*Acanthistius patachonicus*) exhibits mobilization of hepatic lipid reserves to the developing gonads, a pattern consistent with a capital breeding strategy, although concurrent feeding during the reproductive period suggests a certain degree of energy income (López et al. 2025). Likewise, the Patagonian redfish (*Sebastes oculatus*) accumulates proteins and lipids in muscle and gonadal tissues before reproduction, also displaying characteristics associated with capital breeding (Rodrigues et al. 2025). In contrast, reproductive energy allocation in the European sardine (*Sardina pilchardus*) varies geographically, behaving as a typical capital breeder in the northern Mediterranean through seasonal lipid accumulation prior to spawning, whereas populations from the northern Adriatic show greater dependence on recently acquired resources (Schismenou et al. 2024). Similarly, the snapper (*Chrysophrys auratus*) exhibits a mixed strategy in which hepatic fatty acid reserves coexist with ovarian enrichment in polyunsaturated fatty acids, reflecting contributions from both stored and recently assimilated nutrients (Sabetian et al. 2025).

In the present study, the relatively high protein content and low lipid concentration in muscle indicate that *O. argentinensis* is a lean species, while the low muscle glycogen content is consistent with the limited carbohydrate storage generally reported for marine teleosts. Together with the high protein and lipid contents observed in the liver, these findings suggest that hepatic reserves may play an important role in supporting ovarian development. Nevertheless, because the present work was restricted to autumn-collected females, further studies covering the complete reproductive cycle, together with assessments of seasonal changes in tissue biochemical composition and feeding activity, will be necessary to determine whether *O. argentinensis* follows a predominantly capital, income, or mixed breeding strategy.

The digestive physiology of *O. argentinensis* has received limited attention despite its importance for understanding the species' biology and feeding ecology. In the present study, the characterization of digestive enzymes revealed measurable alkaline peptidase and lipase activities in wild adult females (Table 3). These baseline enzyme activities provide fundamental insights into the digestive capacity of the species. Silversides are agastric fish; therefore, they lack gastric digestion and rely on an elongated intestine with an increased microvillar surface

Table 3. Peptidase and lipase activities in the digestive tract of female *Odontesthes argentinensis*. Values are means ± SE (n = 20).

| | Total activity (U $ml^{-1}$) | Specific activity (U mg $protein^{-1}$) |
|---|---|---|
| Peptidase | 0.86 ± 0.10 | 0.33 ± 0.04 |
| Lipase | 33.50 ± 6.91 | 12.67 ± 2.36 |

area to maximize nutrient digestion and absorption (Horn et al. 2006; Toledo-Cuevas et al. 2024). Early development in related *Odontesthes* species (*O. bonariensis* and *O. hatcheri*) is characterized by the rapid onset of high cytosolic peptidase activity, indicating early functional maturation of the digestive tract (Toledo-Cuevas et al. 2024). This adaptation compensates for the absence of a stomach and supports efficient nutrient digestion despite the simplified gastrointestinal anatomy.

It has been reported that other silverside species possess a broad range of digestive enzymes, including amylase, maltase, sucrase, lipase, trypsin, and aminopeptidase-N, enabling the efficient hydrolysis of carbohydrates, proteins, and lipids (Horn et al. 2006; Logothetis et al. 2001). Digestive enzyme profiles in atheriniform fishes are associated with ecological niche and feeding strategy, with estuarine herbivores exhibiting longer intestines and higher carbohydrase activity, whereas carnivorous species have shorter intestines and higher peptidase activity (Logothetis et al. 2001). Building on the intermediate, omnivorous enzyme profile established for *Odontesthes argentinensis* (Albanesi et al. 2026), comparisons between estuarine and marine populations revealed stable trypsin activity alongside variable carbohydrase levels and energy reserves. This suggests that proteolytic capacity remains conservative across habitats, whereas carbohydrate digestion and lipid storage respond dynamically to environmental and dietary variations (Albanesi et al. 2025). In this work, the observed alkaline peptidase and lipase activities (Table 3) align with prior findings, supporting an omnivorous digestive profile in *O. argentinensis*.

Diet quality has received increasing attention in aquaculture because broodstock nutrition directly influences egg quality, larval survival, and reproductive performance. Protein source selection is particularly important for maintaining energy reserves and ensuring offspring fitness. In recent decades, soybean meal has become one of the main protein sources used to partially replace fishmeal in aquafeeds owing to its consistent availability and favorable nutritional properties. Soybean meal provides a high protein content, a balanced amino acid profile, and bioactive compounds with antioxidant properties, making it a valuable ingredient in aquafeed formulations (Alarcón et al. 2001; Coscueta et al. 2016). However, its use is often limited by the presence of antinutritional factors (ANFs), including protease inhibitors, which negatively affect digestive processes (Alarcón et al. 2001).

Trypsin is an endogenous digestive enzyme that plays a key role in protein hydrolysis. Protease inhibitors interfere with trypsin activity, thereby reducing protein digestibility and exerting antinutritional effects. Soybean meal contains two major classes of protease inhibitors: Kunitz trypsin inhibitors, which primarily inhibit trypsin, and Bowman-Birk inhibitors, which inhibit both trypsin and chymotrypsin (Erdaw and Beyene 2018). Therefore, selecting an appropriate processing method is essential when incorporating soybean meal into aquafeeds (Vagadia et al. 2017). Enzymatic treatment has been extensively investigated as an effective approach to improve the functional properties and nutritional quality of soybean meal (Wu and Muir 2010), thereby enhancing its value as a functional feed ingredient.

In the present study, digestive enzymes recovered from marine fishery by-products were characterized and evaluated for their potential to improve soybean meal as a protein source. Digestive extracts obtained from *P. brasiliensis* (Pb), *U. brasiliensis* (Ub), and *O. trimaculatus* (Ot) were selected because these by-products contain enzymes with favorable technological properties, including stability over a broad pH range and at moderate temperatures, while being readily inactivated at relatively low temperatures (Bonadero et al. 2022; Friedman et al. 2022). Their enzymatic activity was confirmed prior to soybean meal treatment (supplementary material, Table S2).

Incubation of *O. argentinensis* digestive extracts with untreated commercial soybean meal significantly reduced alkaline peptidase activity, indicating the presence of residual antinutritional factors despite industrial heat processing (Figure 1). This inhibition is likely associated with the presence of thermostable Bowman-Birk inhibitors, which can persist after conventional heat treatment (Chen et al. 2014). In contrast, incubation with soybean meal treated using marine enzymes (Pb2, Ub2, and Ub8) resulted in significantly higher peptidase activity than that observed with untreated soybean meal (supplementary material, Table S3; Figure 1). These findings indicate that enzymatic treatment with marine enzymes effectively reduces the antinutritional effects of soybean meal, most likely through the hydrolysis of protease inhibitors such as Bowman-Birk inhibitors. Nevertheless, additional *in vivo* studies are required to confirm the nutritional benefits of this approach.

Proteins from soybean meal have been shown to provide health benefits, either as intact proteins or in partially digested forms, known as bioactive peptides. Previous studies have demonstrated that enzymatic treatment of soybean meal can increase the production of soy-derived peptides with antioxidant properties (Sila and Bougatef 2016). In the present study, DPPH radical scavenging activity was used to evaluate the antioxidant capacity of untreated and enzyme-treated soybean meal. Both commercial soybean meal and soybean meal treated with marine enzymes recovered from fishery waste exhibited DPPH radical scavenging activity, although significant differences were observed among treatments (supplementary material, Table S3; Figure 2). Commercial soybean meal (control) showed the highest antioxidant activity. Similarly, soybean meal treated with *U. brasiliensis* enzymes under acidic conditions (Ub2) and with *O. trimaculatus* enzymes under alkaline conditions (Ot8)

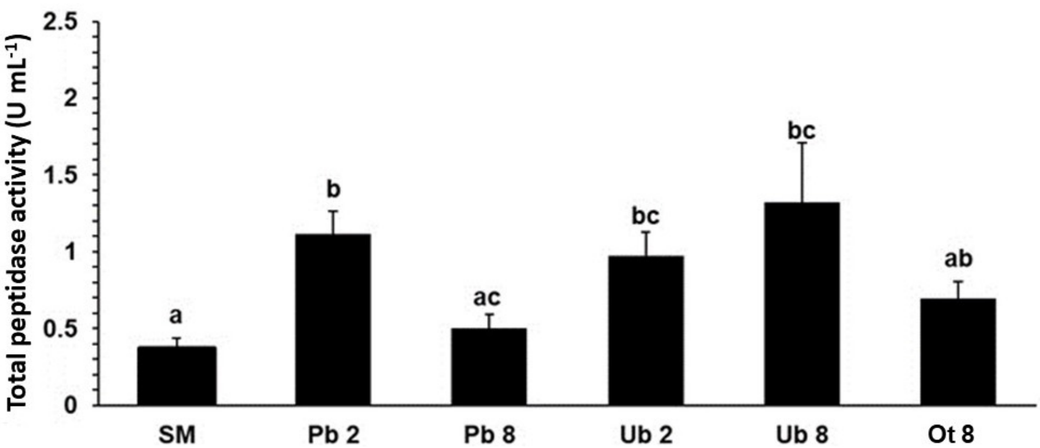


Figure 1. Peptidase activity of *Odontesthes argentinensis* with commercial (SM) and enzyme-treated soybean meal. *P. brasiliensis* at pH 2 and 8 (Pb2, Pb8), *U. brasiliensis* at pH 2 and 8 (Ub2, Ub8), and *O. trimaculatus* at pH 8 (Ot8). Bars represent means ± SE. Different letters indicate significant differences.

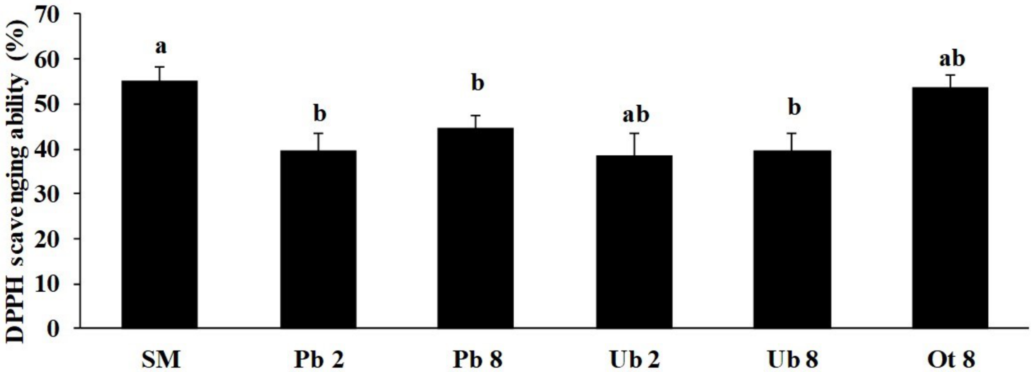


Figure 2. Antioxidant activity of commercial (SM) and enzyme-treated soybean meal: *P. brasiliensis* at pH 2 and 8 (Pb2, Pb8), *U. brasiliensis* at pH 2 and 8 (Ub2, Ub8), and *O. trimaculatus* at pH 8 (Ot8). Bars represent means ± SE. Different letters indicate significant differences.

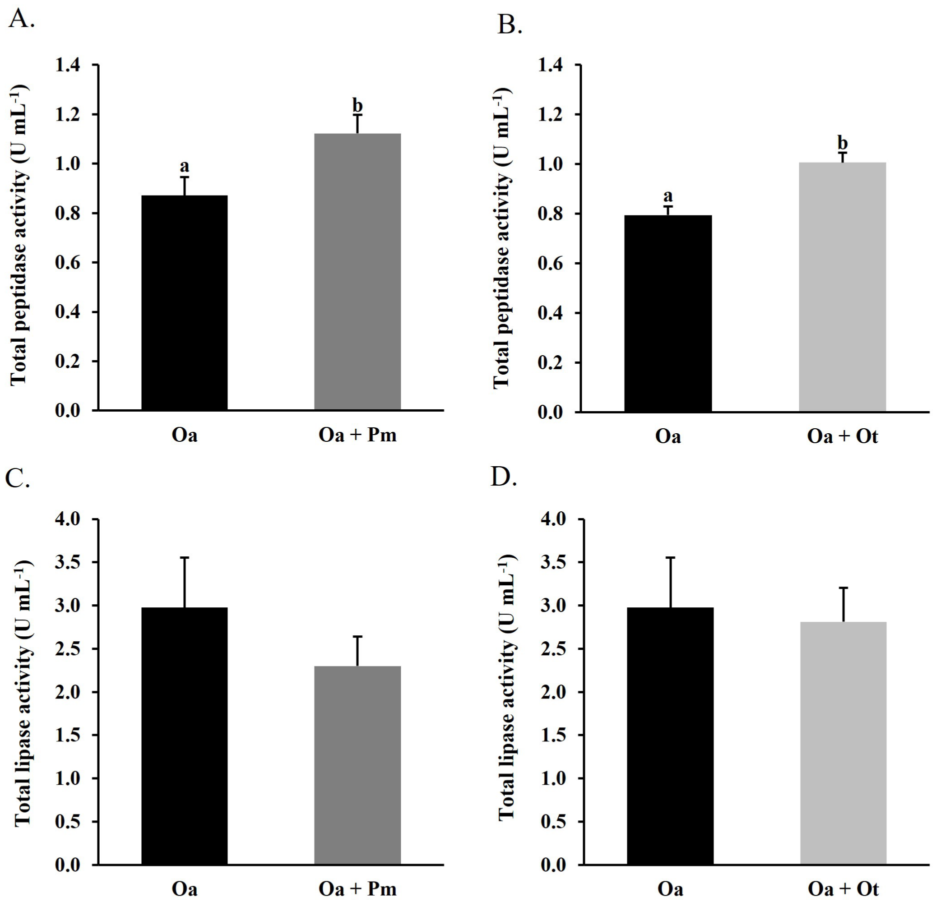


Figure 3. Total activity of peptidase (A and B) and lipase activity (C and D) of *Odontesthes argentinensis* (Oa) mixed with exogenous enzymes of fisheries waste (*P. muelleri* (Pm) or *O. trimaculatus* (Ot)). Vertical bars represent the mean and standard error. Different letters indicate significant differences.

exhibited antioxidant activities comparable to the control (Figure 2). In contrast, antioxidant activity decreased in soybean meal treated with *P. brasiliensis* enzymes under acidic conditions (Pb2) and with *P. brasiliensis* and *U. brasiliensis* enzymes under alkaline conditions (Pb8 and Ub8) compared with the control (Figure 2).

Oxidative stress contributes to the development of several diseases and accelerates the aging process; therefore, antioxidants play an important role in maintaining health. In aquaculture, the use of antioxidant-enriched diets has been associated with several beneficial effects. Chen et al. (1995) hydrolyzed soybean meal using different peptidases and observed a significant increase in antioxidant activity due to the production of bioactive peptides. Ma et al. (2023) proposed that the bioactivity of soybean peptides depends mainly on peptide chain length, amino acid composition, and amino acid sequence. Amino acids such as tryptophan, tyrosine, glycine, histidine, alanine, phenylalanine, methionine, leucine, and valine have been identified as key constituents of antioxidant peptides (Sun et al. 2022). Recent studies on marine fish have shown that fermentation or enzymatic hydrolysis of soybean meal can enhance *in vitro* antioxidant capacity (Zhang et al. 2023). In the present study, however, the DPPH results did not consistently follow this pattern. The control (SM) exhibited the highest antioxidant activity, with only the Ot8 and Ub2 treatments reaching values that were statistically comparable to those of the control. In contrast, the remaining enzyme-treated meals exhibited significantly lower antioxidant activity.

In recent years, the application of exogenous enzymes in aquaculture has increased because of their ability to promote growth, enhance immune function, and improve the overall health of cultured fish. Furthermore, the use of exogenous enzymes as feed additives has been proposed as a strategy for enhancing nutrient digestibility in aquaculture. Previous studies have demonstrated the effectiveness of exogenous enzymes in improving the *in vitro* digestibility of dietary proteins (Rodriguez et al. 2017, 2021; del Valle et al. 2022). Consequently, these enzymes have become valuable feed additives, attracting considerable interest from both the aquafeed industry and the research community (Zheng et al. 2020).

In the present study, the compatibility between the endogenous digestive enzymes of *O. argentinensis* and exogenous enzymes derived from the crustaceans *P. muelleri* (Pm) and *O. trimaculatus* (Ot) was evaluated to assess potential synergistic effects. When *O. argentinensis* digestive extracts (Oa) were combined with either Pm or Ot enzyme extracts, alkaline peptidase activity increased by more than 25% compared with Oa alone (supplementary material, Table S3; Figure 3 A and 3 B). This result provides the first experimental evidence that digestive enzymes recovered from crustacean processing by-products are functionally compatible with the endogenous proteases of *O. argentinensis*, under *in vitro* conditions tested, providing support for further evaluation of their potential as exogenous feed enzymes *in vivo* trials with this species. In contrast, lipase activity did not differ significantly after combining Oa with either crustacean enzyme extract (supplementary material, Table S3; Figure 3 C and 3 D). Collectively, these *in vitro* results demonstrate that crustacean-derived enzyme extracts are compatible with the digestive physiology of *O. argentinensis* and can increase proteolytic activity. This represents a promising strategy for improving dietary protein utilization while simultaneously promoting the valorization of crustacean processing by-products within a circular bioeconomy framework. However, testing is required in future feeding trials.

## CONCLUSIONS

This study provides the biological characterization of wild female *O. argentinensis*, contributing fundamental knowledge to the biology of atheriniform fishes. Energy reserve analysis revealed distinct allocation patterns, suggesting that the liver is a key site of lipid concentration in autumn, whereas muscle showed the highest protein concentration among the tissues analyzed. The characterization of digestive enzymes provides baseline values for alkaline peptidase and lipase activities that may help fill an important gap in the understanding of the digestive physiology of this species and could provide comparative data for other members of the Atherinopsidae family. These findings may also contribute to a better understanding of the digestive adaptations of agastric fishes.

Additionally, this study explores potential strategies for the future development of highly digestible feeds through a circular economy approach based on the valorization of fishery waste. Enzymatic treatment of soybean meal with *U. brasiliensis* enzymes at pH 2 reduced antinutritional factors, and the treated meal retained DPPH antioxidant activity comparable to that of the untreated control. Furthermore, cross-species enzyme compatibility assays showed that mixtures of exogenous marine enzymes with the endogenous digestive extract of *O. argentinensis* maintained or increased proteolytic activity relative to the endogenous extract alone, indicating the biotechnological potential of fishery by-products as feed additives. Likewise, exogenous enzymes recovered from crustacean processing waste resulted in greater activity in mixtures than with the endogenous extract alone.

Overall, these findings provide a valuable physiological and biochemical baseline for *O. argentinensis* and establish a foundation for future studies aimed at developing nutritional strategies for broodstock management, and could support the diversification of marine aquaculture in South America through the culture of a native fish species. Moreover, this study provides in vitro evidence that enzymatic treatment of soybean meal and cross-species enzyme mixtures can reduce antinutritional factors and increase proteolytic activity relative to the endogenous digestive extract of *O. argentinensis*, indicating the biotechnological potential of fishery by-products as candidate feed additives. However, these conclusions are based exclusively on *in vitro* assays; before these enzymatic strategies could be considered for incorporation into feeding programs for this species, their stability under feed processing and storage conditions, safety, digestibility and efficacy *in vivo*, production costs, and environmental impact would need to be specifically evaluated.

## ACKNOWLEDGEMENTS

The authors thank Dr. Luis I. Granone for assistance with EPR spectra determinations and Lic. Cecilia Bonadero for assistance with laboratory assays. This work was supported by the Technological Development and Innovation Promotion National Agency from Argentina [PICT 2020, 01851; AVFG], CONICET Multiyear Research Projects [PIP 2021/2023, 353 11220200101093CO; AVFG] and Mar del Plata National University [EXA 1194/24; AVFG]. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. This is INIDEP contribution no 2544.

### Conflict of interest

The authors declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

### Author contributions

Yamila E. Rodriguez: conceptualization; methodology; formal analysis and investigation; writing-original draft preparation. Juana C. del Valle: conceptualization; methodology; writing-original draft preparation; supervision. Ivana S. Friedman: methodology; formal analysis and investigation; writing, review and editing. María Victoria Laitano:

methodology; formal analysis and investigation; writing, review and editing. Clara Liébana: methodology; formal analysis and investigation; writing, review and editing. Aldo N. Zanazzi: methodology. Analia V. Fernández-Gimenez: conceptualization; methodology; writing-original draft preparation; writing, review and editing; funding acquisition; resources; supervision.

## REFERENCES


Alarcón F, García-Carreño F, Navarrete del Toro MA. 2001. Effect of plant protease inhibitors on digestive proteases in two fish species, *Lutjanus argentiventris* and *L. novemfasciatus*. Fish Physiol Biochem. 24: 179-189. DOI: https://doi.org/10.1023/A:1014079919461

Alarcón FJ, Moyano FJ, Díaz M. 1999. Effect of inhibitors present in protein sources on digestive proteases of juvenile sea bream (*Sparus aurata*). Aquat Living Resour. 12 (4): 233-238. DOI: https://doi.org/10.1016/S0990-7440(00)86633-4

Albanesi C, Méndez E, González-Castro M, Michiels MS. 2025. Digestive, metabolic, and morphological adaptations of *Odontesthes argentinensis* to estuarine and marine environments: insights into phenotypic plasticity and ecological differentiation. Estuar Coast Shelf Sci. 324: 109446. DOI: https://doi.org/10.1016/j.ecss.2025.109446

Albanesi C, Méndez E, Toraño JC, Michiels MS. 2026. Integrated digestive and metabolic traits underlie trophic differentiation in juvenile fish assemblages. J Fish Biol. DOI: https://doi.org/10.1111/jfb.70594

Anson ML. 1938. The estimation of pepsin, trypsin, papain, and cathepsin with hemoglobin. J Gen Physiol. 22 (1): 79. DOI: https://doi.org/10.1085/jgp.22.1.79

[AOAC] Association of Official Agricultural Chemists. 1995. Official methods of analysis. 16th ed. Washington: Association of official analytical chemists.

Bou M, Berge GM, Baeverfjord G, Sigholt T, Østbye TK, Romarheim OH, Ruyter B. 2017. Requirements of n-3 very long-chain PUFA in Atlantic salmon (*Salmo salar* L): effects of different dietary levels of EPA and DHA on fish performance and tissue composition and integrity. Br J Nutr. 117 (1): 30-47. DOI: https://doi:10.1017/S0007114516004396

Bradford MM. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. Anal Biochem. 72 (1-2): 248-254. DOI: https://doi.org/10.1016/0003-2697(76)90527-3

Bonadero MC, Laitano MV, del Valle JC, Fernández-Gimenez AV. 2022. Marine sources for biotechnology: preliminary digestive enzymes assessment of seven decapod species from the southwest Atlantic. Reg Stud Mar Sci. 55: 102515. DOI: https://doi.org/10.1016/j.rsma.2022.102515

Chen H, Muramoto K, Yamauchi F. 1995. Structural analysis of antioxidative peptides from soybean β-conglycinin. J Agric Food Chem. 43: 574-578. DOI: https://doi.org/10.1021/jf00051a004

Chen Y, Xu Z, Zhang C, Kong X, Hua Y. 2014. Heat-induced inactivation mechanisms of Kunitz trypsin inhibitor and Bowman-Birk inhibitor in soymilk processing. Food Chemistry. 154: 108-116. DOI: https://doi.org/10.1016/j.foodchem.2013.12.092

Chen Z, Fei S, Liu C, Duan Y, Liu H, Han D, Jin J, Yang Y, Zhu X, Xie S. 2023. Compared to fishmeal, dietary soybean meal improves the reproductive performance of female yellow catfish (*Pelteobagrus fulvidraco*) broodstock. Aquac Nutr. 2023: 6240803. DOI: https://doi.org/10.1155/2023/6240803

Coscueta ER, Amorim MM, Voss GB, Nerli BB, Picó GA, Pintado ME. 2016. Bioactive properties of peptides obtained from Argentinian defatted soy flour protein by Corolase PP hydrolysis. Food Chem. 198: 36-44. DOI: https://doi.org/10.1016/j.foodchem.2015.11.068

del Valle JC, Zanazzi AN, Rodriguez YE, Haran NS, Laitano MV, Mallo JC, Fernández-Gimenez AV. 2022. Morphological changes, peptidase activity, and effects of exogenous enzymes in the early ontogeny of Nile tilapia, *Oreochromis niloticus*. Aquac Int. 30 (4): 1645-1658. DOI: https://doi.org/10.1007/s10499-022-00932-5

Deng Z, Duarte ME, Kim SY, Hwang Y, Kim SW. 2023. Comparative effects of soy protein concentrate, enzyme-treated soybean meal, and fermented soybean meal replacing animal protein supplements in feeds on growth performance and intestinal health of nursery pigs. J Anim Sci Biotechnol. 14 (1): 89. DOI: https://doi.org/10.1186/s40104-023-00888-3

Díaz AC, Velurtas SM, Espino ML, Fenucci JL. 2014. Effect of dietary astaxanthin on free radical scavenging capacity and nitrite stress tolerance of postlarvae shrimp, *Pleoticus muelleri*. J Agric Food Chem. 62 (51): 12326-12331. DOI: https://doi.org/10.1021/jf503754q

Dubois M, Gilles KA, Hamilton JK, Rebers PT, Smith F. 1956. Colorimetric method for determination of sugars and related substances. Anal Chem. 28 (3): 350-356. DOI: https://doi.org/10.1021/ac60111a017

Erdaw MM, Beyene WT. 2018. Anti-nutrients reduce poultry productivity: influence of trypsin inhibitors on pancreas. Asian J Poult Sci. 12 (1): 14-24. DOI: https://doi.org/10.3923/ajpsaj.2018.14.24

Friedman IS, Behrens LA, Pereira NA, Contreras E, Fernández-Gimenez AV. 2022. Digestive proteases from fish processing wastes: their partial characterization and comparison. J Fish Biol. 100: 150-160. DOI: https://doi.org/10.1111/jfb.14929

Führ F, Tesser MB, Rodrigues RV, Pedron J, Romano LA. 2016. *Artemia* enriched with hydrolyzed yeast improves growth and stress resistance of marine pejerrey *Odontesthes argentinensis* larvae. Aquaculture. 450: 173-181. DOI: https://doi.org/10.1016/j.aquaculture.2015.07.018

Fuiman LA, Perez KO. 2015. Metabolic programming mediated by an essential fatty acid alters body composition and survival skills of a marine fish. Proceedings of the Royal Society B: Biological Sciences. 282 (1819): 20151414. DOI: https://doi.org/10.1098/rspb.2015.1414

García-Carreño FL. 1992. The digestive proteases of langostilla (*Pleuroncodes planipes*, Decapoda): their partial characterization, and the effect of feed on their composition. Comp Biochem Physiol B: Biochem Mol Biol. 103: 575-578. DOI: https://doi.org/10.1016/0305-0491(92)90373-Y

González-Castro M, Rosso JJ, Delpiani SM. 2019. Inferring boundaries among fish species of the new world silversides (Atherinopsidae; genus *Odontesthes*): new evidences of incipient speciation between marine and brackish populations of *Odontesthes argentinensis*. Genetica. 147: 217-229. DOI: https://doi.org/10.1007/s10709-019-00066-2

González-Zamorano M, Navarrete del Toro MA, García-Carreño FL. 2013. Exogenous proteinases as feed supplement for shrimp: in vitro evaluation. Aquac Nutr. 19 (5): 731-740. DOI: https://doi.org/10.1111/anu.12020

Guidi C, Baigún CRM, Ginter LG, Soricetti M, Rivas FG, Morawicki S, Quezada F, Bazzani, JL, Solimano PJ. 2021. Characteristics, preferences and perceptions of recreational fishers in northern Patagonia, Argentina. Reg Stud Mar Sci. 45: 101828. DOI: https://doi.org/10.1016/j.rsma.2021.101828

Hara A, Hiramatsu N, Fujita T. 2016. Vitellogenesis and choriogenesis in fishes. Fish Sci. 82: 187-202. DOI: https://doi.org/10.1007/s12562-015-0957-5

Hernandez de Dios MA, Tovar-Ramírez D, Maldonado-García D, Galaviz-Espinoza MA, Spanopoulos Zarco M, Maldonado-García MC. 2022. Functional additives as a boost to reproductive performance in marine fish: A review. Fishes. 7 (5): 262. DOI: https://doi.org/10.3390/fishes7050262

Horn MH, Gawlicka A, German DP, Logothetis E, Cavanagh J, Boyle K. 2006. Structure and function of the stomachless digestive system in three related species of New World silverside fishes (Atherinopsidae) representing herbivory, omnivory, and carnivory. Mar Biol. 149: 1237-1245. DOI: https://doi.org/10.1007/S00227-006-0281-9

Izquierdo MS, Fernandez-Palacios H, Tacon AGJ. 2001. Effect of broodstock nutrition on reproductive performance of fish. Aquaculture. 197 (1-4): 25-42. DOI: https://doi.org/10.1016/S0044-8486(01)00581-6

Katan T, Xue X, Caballero-Solares A, Taylor RG, Rise ML, Parrish CC. 2020. Influence of dietary long-chain polyunsaturated fatty acids and ω6 to ω3 ratios on head kidney lipid composition and expression of fatty acid and eicosanoid metabolism genes in Atlantic salmon (*Salmo salar*). Front Mol Biosci. 7: 602587. DOI: https://doi.org/10.3389/fmolb.2020.602587

Le HT, Shao X, Krogdahl Å, Kortner TM, Lein I, Kousoulaki K, Sæle Ø. 2019. Intestinal function of the stomachless fish, ballan wrasse (*Labrus bergylta*). Front Mar Sci. 6: 140. DOI: https://doi.org/10.3389/fmars.2019.00140

Liang Q, Yuan M, Xu L, Lio E, Zhang F, Mou H, Secundo. 2022. Application of enzymes as a feed additive in aquaculture. Mar Life Sci Technol. 4 (2): 208-221. DOI: https://doi.org/10.1007/s42995-022-00128-z

Llompart FM, Colautti DC, Maiztegui T, Cruz-Jiménez AM, Baigún CRM. 2013. Biological traits and growth patterns of pejerrey *Odontesthes argentinensis*. J Fish Biol. 82 (2): 458-474. DOI: https://doi.org/10.1111/j.1095-8649.2012.03494.x

Logothetis E, Horn M, Dickson K. 2001. Gut morphology and function in *Atherinops affinis* (Teleostei: Atherinopsidae), a stomachless omnivore feeding on macroalgae. J Fish Biol. 59: 1298-1312. DOI: https://doi.org/10.1111/J.1095-8649.2001.TB00193.X

López S, Militelli MI, Riestra CM, Macchi GJ. 2025. Energy allocation strategy during the reproductive cycle of Patagonian grouper (*Acanthistius patachonicus*) from the southwestern Atlantic Ocean. Mar Fish Sci. 38 (3): 375-388. DOI: https://doi.org/10.47193/mafis.3832025010704

Ma J, Su K, Chen M, Wang S. 2023. Study on the antioxidant activity of peptides from soybean meal by fermentation based on the chemical method and AAPH-induced oxidative stress. Food Sci Nutr. 11: 6634-6647. DOI: https://doi.org/10.1002/fsn3.3612

Méndez E, Albanesi C, Michiels MS, López-Mañanes A, González-Castro M. 2024. Analyses of body condition and digestive/metabolic parameters of *Odontesthes argentinensis* (Atherinopsidae) from Mar Chiquita Coastal Lagoon (Argentina) during different phases of ovarian development. Neotrop Ichthyol. 22 (3): e230139. DOI: https://doi.org/10.1590/1982-0224-2023-0139

Méndez E, Albanesi C, Michiels MS, López Mañanes A, González-Castro M. 2026. Metabolic flexibility and seasonal physiological responses of the resident silverside *Odontesthes argentinensis* in a southwestern Atlantic coastal lagoon. J Fish Biol. 1-10. DOI: https://doi.org/10.1111/jfb.70482

Nolasco-Soria H, Moyano-López F, Vega-Villasante F, Del Monte A, Espinoza-Chaurand D, Gisbert E. 2018. Lipases and phospholipases: methods and protocols. In: Sandoval G, editor. Methods in molecular biology. Totowa: Springer, Humana Press. p. 139-167.

Rahman MS, Riad HM, Mahmud MS, Fatema MK, Shahjahan M. 2020. Farmers' perception on quality of fish feed, brood stock and fingerling produced in commercial fish farms of Bangladesh. Bangladesh J Fish. 32 (1): 141-147. DOI: https://doi.org/10.52168/bjf.2020.32.16

R Core Team. 2023. R: A language and environment for statistical computing. R Foundation for Statistical Computing. https://www.r-project.org/.

RODRIGUES KA, LEONARDUZZI E, MILITELLI M., Macchi GJ. 2025. Condition and energy allocation during the reproductive cycle of the Patagonian redfish *Sebastes oculatus* from the Argentine continental shelf. Mar Fish Sci. 38 (4): 575-589. DOI: https://doi.org/10.47193/mafis.3842025011002

RODRIGUEZ YE, PEREIRA NA, HARAN NS, MALLO JC, FERNÁNDEZ-GIMENEZ AV. 2017. A new approach to fishery waste revalorization to enhance Nile tilapia (*Oreochromis niloticus*) digestion process. Aquac Nutr. 23: 1351-1361. DOI: https://doi.org/10.1111/anu.12510

RODRIGUEZ YE, PEREIRA NA, LAITANO MV, MORENO P, FERNÁNDEZ-GIMENEZ AV. 2021. Exogenous proteases from seafood processing waste as functional additives in rainbow trout aquaculture. Aquac Res. 52 (9): 4350-4361. DOI: https://doi.org/10.1111/are.15272

RODRÍGUEZ-BARRETO D, JEREZ S, CEJAS JR, MARTÍN MV, ACOSTA NG, BOLAÑOS A, LORENZO A. 2012. Comparative study of lipid and fatty acid composition in different tissues of wild and cultured female broodstock of greater amberjack (*Seriola dumerili*). Aquaculture. 360: 1-9. DOI: https://doi.org/10.1016/j.aquaculture.2012.07.013

SABETIAN A, HOANG LH, ZHANG J, WHITE WL, CHEN T, WANG H, LILKENDEY J. 2025. Fatty acid biomarkers reveal maternal energy allocation strategies in spawning Snapper (*Chrysophrys auratus*). New Zealand J Mar Freshw Res. 59 (5): 1117-1130. DOI: https://doi.org/10.1080/00288330.2025.2464048

SCHISMENOU E, CHATZIFOTIS S, TSIARAS K, SOMARAKIS S. 2024. Anchovy and sardine condition and energy content in the North Aegean Sea (eastern Mediterranean) in relation to their contrasting reproductive strategies. J Fish Biol. 105 (4): 1178-1188. DOI: https://doi.org/10.1111/jfb.15872

SILA A, BOUGATEF A. 2016. Antioxidant peptides from marine by-products: Isolation, identification and application in food systems. A review. J Funct Foods. 21: 10-26. DOI: https://doi.org/10.1016/j.jff.2015.11.007

SISSENER NH, ARAUJO P, SÆLE Ø, ROSENLUND G, STUBHAUG I, SANDEN M. 2020. Dietary 18: 2n-6 affects EPA (20: 5n-3) and ARA (20: 4n-6) content in cell membranes and eicosanoid production in Atlantic salmon (*Salmo salar* L.). Aquaculture 522: 735098. DOI: https://doi.org/10.1016/j.aquaculture.2020.735098

SOENGAS JL, BARCIELA P, ALDEGUNDE M. 1995. Variations in carbohydrate metabolism during gonad maturation in female turbot (*Scophthalmus maximus*). Mar Biol. 123: 11-18. DOI: https://doi.org/10.1007/BF00350318

SOLTAN NM, SOAUDY MR, ABDELLA MM, HASSAAN MS. 2023. Partial dietary fishmeal replacement with mixture of plant protein sources supplemented with exogenous enzymes modify growth performance, digestibility, intestinal morphology, haemato-biochemical and immune responses for Nile tilapia, *Oreochromis niloticus*. Anim Feed Sci Technol. 299: 115642. DOI: https://doi.org/10.1016/j.anifeedsci.2023.115642

SUN K, GAO M, WANG Y, LI, XR, WANG P, WANG B. 2022. Antioxidant peptides from protein hydrolysate of marine red algae *Eucheuma cottonii*: Preparation, identification and cytoprotective mechanisms on H2O2 oxidative damaged HUVECs. Front Microbiol. 13: 791248. DOI: https://doi.org/10.3389/fmicb.2022.791248

THIEX NJ, MANSON H, ANDERSON S, PERSSON JÅ. 2002. Determination of crude protein in animal feed, forage, grain, and oilseeds by using block digestion with a copper catalyst and steam distillation into boric acid: collaborative study. J AOAC Int. 85 (2): 309-317. DOI: https://doi.org/10.1093/jaoac/85.2.309

THOMPSON GA, VOLPEDO AV. 2018. Diet composition and feeding strategy of the New World silverside *Odontesthes argentinensis* in a temperate coastal area (South America). Mar

Coast Fish. 10 (1): 80-88. DOI: https://doi.org/10.1002/mcf2.10005

Toledo-Cuevas E, Chávez-Sánchez MC, Herrera-Vargas MA, Abad Rosales SM, Cortés Ortiz E, Strüssmann CA, Raggi L. 2024. Early anatomical development of the digestive system and high intestinal digestive enzyme activity in two silverside species, *Odontesthes bonariensis* and *O. hatcheri* (Atherinomorpha: Atherinopsidae). Lat Am J Aquat Res. 52 (3): 368-382. DOI: https://doi.org/10.3856/vol52-issue3-fulltext-3155

Vagadia BH, Vanga SK, Raghavan V. 2017. Inactivation methods of soybean trypsin inhibitor - a review. Trends Food Sci Technol. 64: 115-125. DOI: https://doi.org/10.1016/j.tifs.2017.02.003

Volkoff H, London S. 2018. Nutrition and reproduction in fish. In: Skinner MA, editor. Encyclopedia of reproduction. New York: Academic Press. p. 743-748.

Weng L, Wang Z, Zhuang W, Yang T, Xu X, Liu J, Liu J, Xu Z, Chen R, Wang Q, et al. 2023. Effect of replacing fish meal using fermented soybean meal on growth performance, intestine bacterial diversity, and key gene expression of largemouth bass (*Micropterus salmoides*). Fermentation. 9 (6): 520. DOI: https://doi.org/10.3390/fermentation9060520

Wu J, Muir AD. 2010. Isoflavone during protease hydrolysis of defatted soybean meal. Food Chem. 118 (2): 328-332. DOI: https://doi.org/10.1016/j.foodchem.2009.04.129

Xu H, Meng X, Wei Y, Ma Q, Liang M, Turchini GM. 2022. Arachidonic acid matters. Reviews in Aquaculture. 14 (4): 1912-1944. DOI: https://doi.org/10.1111/raq.12679

Yilmaz O, Sullivan CV, Bobe J, Norberg B. 2024. The role of multiple vitellogenins in early development of fishes. Gen Comp Endocrinol. 351: 114479. DOI: https://doi.org/1016/j.ygcen.2024.114479

Zhang Q, Li F, Guo M, Qin M, Wang J, Yu H, Xu J, Liu Y, Tong T. 2023. Growth performance, antioxidant and immunity capacity were significantly affected by feeding fermented soybean meal in juvenile coho salmon (*Oncorhynchus kisutch*). Animals. 13 (5): 945. DOI: https://doi.org/10.3390/ani13050945

Zhao Z, Zhao Q, Wan H, Wei L, Wang S, Li S, Wang Z. 2023. Integrated transcriptomic and metabolomic analyses identify key factors in the vitellogenesis of juvenile Sichuan bream (*Sinibrama taeniatus*). Front Mar Sci. 10: 1243767. DOI: https://doi.org/10.3389/fmars.2023.1243767

Zheng CC, Wu JW, Jin ZH, Ye ZF, Yang S, Sun YQ, Fei H. 2020. Exogenous enzymes as functional additives in finfish aquaculture. Aquac Nut. 26 (2): 213-224. DOI: https://doi.org/10.1111/anu.12995