# Analyses of body condition and digestive/metabolic parameters of *Odontesthes argentinensis* (Atherinopsidae) from Mar Chiquita Coastal Lagoon (Argentina) during different phases of ovarian development

**Correspondence:**
Mariano González-Castro
gocastro@mdp.edu.ar

Eugenia Mendez, Camila Albanesi, María Soledad Michiels,
Alejandra López-Mañanes and Mariano González-Castro

This study analyses body condition and digestive/metabolic parameters of *Odontesthes argentinensis* from Mar Chiquita Coastal Lagoon (MChCL) during different ovary maturity phases (II: developing; III: spawning capable; IIIas: actively spawning subphase). Silverside individuals in phases II and III maintained their body condition, while it improved in actively spawning subphase. Total weight and Fulton's condition factor increase in 70% and 50%, respectively. No changes were observed during the ovary maturation phases in hepatosomatic index and intestinal coefficient. Maltase activity in the intestine remained unchanged, while Aminopeptidase-N was higher, about 300%, in the spawning capable phase (III). Alkaline phosphatase in the intestine was unchanged pointing out the maintenance of intestinal homeostasis. Liver glycogen concentration decreased, about 40%, in the actively spawning subphase (IIIas), while muscle glycogen abruptly decreased in phase III and recovered in subphase IIIas. In intestinal fat, triglycerides concentration was lower 70% in subphase IIIas, while no changes occurred in liver and muscle. Muscle protein concentration decreased about 50% in actively spawning subphase. The findings shed light on the remarkable adaptability of this population of *O. argentinensis* from MChCL to support its gonadal maturation and active spawning maintaining and even improving its physical condition.

Keywords: Digestive enzymes, Energy reserves, Euryhaline fishes, Reproductive ecology, Silverside.

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1 Instituto de Investigaciones Marinas y Costeras (IIMyC), Universidad Nacional de Mar del Plata CONICET - FCEyN, Funes 3250 (7600) Mar del Plata, Argentina. (EM) mendezeugenia@hotmail.com, (CA) camila.albanesi2@gmail.com, (MSM) michiels@mdp.edu.ar, (ALM) mananes@mdp.edu.ar, (MGC) gocastro@mdp.edu.ar (corresponding author).

Este trabajo analiza los parámetros digestivo/metabólicos y de condición corporal de *Odontesthes argentinensis* de la Laguna Costera Mar Chiquita (LCMCh) en diferentes fases de madurez ovárica (II: maduración; III: capacidad de desove; IIIas: subfase activo desovante). Pejerreyes en fases II y III mantuvieron su condición corporal, mientras que la misma mejoró en la subfase activo desovante. El peso total y el factor de condición de Fulton aumentaron en un 50% y 70% respectivamente. No se observaron cambios en el índice hepatosomático ni en el coeficiente intestinal en las diferentes fases de maduración ovárica. La actividad de maltasa en el intestino no varió, mientras que la actividad de N-aminopeptidasa fue 300% mayor en la fase con capacidad de desove (III). La actividad de fosfatasa alcalina en intestino no cambió, indicando el mantenimiento de la homeostasis intestinal. La concentración de glucógeno en hígado disminuyó un 40% en la subfase activo desovante (IIIas), mientras que el glucógeno en músculo disminuyó abruptamente en la fase III y se recuperó en la subfase IIIas. En grasa intestinal, la concentración de triglicéridos fue 70% menor en la subfase IIIas mientras que no hubo cambios en hígado y músculo. La concentración de proteínas en músculo disminuyó un 50% en la subfase activo desovante. Estos resultados destacan la capacidad de adaptación de esta población de *O. argentinensis* de la LCMCh para sostener la maduración gonadal y el desove manteniendo e incluso mejorando la condición corporal.

Palabras clave: Ecología reproductiva, Enzimas digestivas, Peces eurihalinos, Pejerrey, Reservas de energía

## INTRODUCTION

The New World silversides (Atherinopsidae) are widespread freshwater and marine fish commonly occurring in schools in shallow waters (Helfman *et al*., 2009). In Argentina, this family is represented by one genus (*Odontesthes* Evermann & Kendall, 1906) which comprises ten species, commonly known as "pejerreyes". *Odontesthes argentinensis* (Valenciennes, 1835) inhabits both estuaries and coastal waters (Cousseau, Perrotta, 2013) possess a wide distribution along the southwestern Atlantic Ocean between Rio de Janeiro, Brazil (22°S), and Rawson, Argentina (43°S) (Dyer, 2000; Llompart *et al*., 2013; Di Dario *et al*., 2014; González-Castro *et al*., 2016, 2019, 2022). This species has singular economic importance due to is target by recreational and artisanal fisheries in Buenos Aires Province (Dyer, 2006; Llompart, 2011; Llompart *et al*., 2013; Cousseau, Perrotta, 2013; González-Castro *et al*., 2016, 2019).

Mar Chiquita Coastal Lagoon (MChCL) is located in the Buenos Aires Province (Argentina) (37°32' – 37°45'S, 57°19' – 57°26'W) and was declared since 1996 as a World Biosphere Reserve by UNESCO. It constitutes a heterogeneous and challenged habitat since it is subjected to highly dynamic environmental conditions that modulate biological communities (Reta *et al*., 2001; González-Castro *et al*., 2009; Marquez *et al*., 2022). Inside this lagoon, a population of *O. argentinensis* performs its entire life cycle (González-Castro *et al*., 2009, 2016, 2019, 2022), representing a "hot spot" in the topics of evolutionary biology. Reports strongly suggest that it represents an evolutionary significant unit (ESU) (*sensu* Waples, 2004) or even an incipient species (González-Castro *et al*., 2019, 2022; Hughes *et al*., 2020; Levy *et al*., 2021).

*Odontesthes argentinensis* from MChCL present a multiple-batch spawning strategy (González-Castro *et al*., 2009), *e.g*., their ovaries exhibit oocytes in different growth phases. However, it is not yet clear whether breeding females feed throughout the spawning season (winter-spring), or if they do so intermittently during the period between batch spawning (González-Castro *et al*., 2009). Therefore, along the gonadal maturation and spawning period, the maintenance of body condition could be compromised. In fishes, body condition refers to a physiological or nutritional state, which can be assessed by a variety of parameters such as biological indices and weight-length relationships. Body condition is a particularly important attribute of fish success because it has a large influence on growth, reproduction and survival (Lloret *et al*., 2002; Kanno *et al*., 2023; Vagnon *et al*., 2024; Wolf *et al*., 2024). Hepatosomatic Index serves as an indicator of a fish's general health condition, considering that some fishes do not always rely on muscle somatic tissue as their primary energy storage reserve, but on specific organs such as the liver (do Carmo Silva *et al*., 2019).

Fishes require different sources of energy to maintain their physical condition and fundamental processes, such as growth, metabolism, and reproduction (Karasov, Douglas, 2013; Steinberg, 2018; Small, 2022). Digestive enzyme activity and energy reserves provide insights into nutrient utilization under different physiological conditions (Karasov, Douglas, 2013; Yang *et al*., 2019; Lallès, 2020; Albanesi *et al*., 2022). Membrane-bound enzymes like maltase and aminopeptidase-N indicate intestinal maturity and digestion progress (Holt, 2011; del Valle *et al*., 2016; Albanesi *et al*., 2021). Alkaline phosphatases play multiple roles in digestion and absorption of nutrients (Estaki *et al*., 2014; Lallès, 2014, 2020; Bilski *et al*., 2017; Ducatelle *et al*., 2018). The determination of the concentration of energy reserves such as glycogen, triglycerides and protein in storage organs is a common tool used to evaluate the metabolic characteristics and adjustments at the biochemical level of an individual (Karasov, Douglas, 2013; Albanesi *et al*., 2023; 2024). Digestive/metabolic adjustments such as differential modulation of key digestive enzyme activity in the intestine and a selective use of energy reserves in different storage organs could occur in order to make successfully the individuals adaptation to variations in external and/or internal factors (Karasov, Douglas, 2013). Changes in tissue-biochemical composition of reserve organs have been related to gonadal maturity phases in fishes (Tyler, Sumpter, 1996) and reproductive displacements (Šmejkal *et al*., 2017). The gonads undergo developmental changes with the onset of maturation, which are closely accompanied by conspicuous cellular, biochemical, molecular and endocrinological adjustments (Nagahama, 1983; Guraya, 2000). As the gonads increase in size, somatic growth slows down and eventually stops and energy reserves are mobilized from the somatic tissues and transferred to the gonads (Aksnes *et al*., 1986).

For the reasons exposed above, the aim of this work was to analyse several body condition and digestive/metabolic parameters in different ovary development phases (II: developing; III: spawning capable; IIIas: actively spawning subphase) of the resident population of *O. argentinensis* from MChCL, including: a) GSI and HSI, intestinal coefficient (IC) and condition factor K; b) maltase, aminopeptidase-N (APN) and alkaline phosphatase (AP) activities in the intestine and c) glycogen, free glucose, triglycerides and protein concentration in energy storage sites. We hypothesize that the individuals of the resident population of *O. argentinensis* from MChCL exhibit changes in digestive/metabolic parameters, that could allow them to sustain their body condition throughout their development and ovary maturity phases.

## MATERIAL AND METHODS

**Study area and fish sampling.** Specimens (n = 75 for Fulton condition factor K analysis; n = 41 for morphological and biochemical analysis) of the resident population of *O. argentinensis* from MChCL were captured in Zone III (mixo-oligohaline waters) and its freshwater tributaries (Fig. 1) according to González-Castro *et al*. (2009). Zone III is located in the extreme north of the lagoon, where spawning events of *O. argentinensis* have been recorded (González-Castro *et al*., 2009). Voucher specimens of *O. argentinensis* (UNMDP-T 1450, UNMDP-T 1451, UNMDP-T 1452, UNMDP-T 1453) were deposited at the ichthyological collection of the Instituto de Investigaciones Marinas y Costeras, Universidad Nacional de Mar del Plata, Argentina. Field samples were monthly performed, employing beach seine-nets, during spring, summer and early autumn with an average water temperature of 17 °C. Adult females were transported on ice to the laboratory and stored at -20 °C. Fish were taxonomically identified according to Cousseau, Perrotta (2013) and González-Castro *et al*. (2016, 2022). Total length (TL), standard length (SL), total weight (TW), gonad (GW), liver (LW) and intestinal (IW)

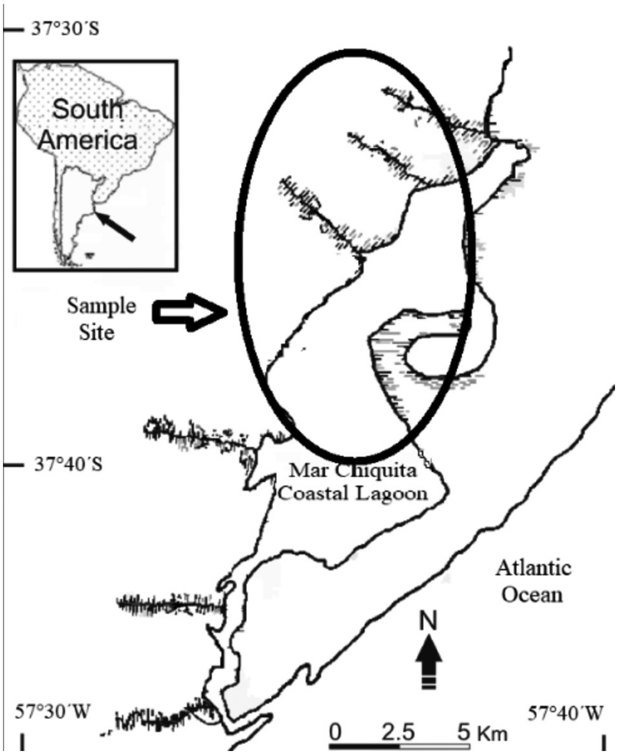

**FIGURE 1** | Study area, Mar Chiquita Coastal Lagoon, Buenos Aires Province, Argentina.

weights, intestinal length (IL) and intestinal-content weight of each individual were recorded, employing a digital caliper to the nearest millimeter and an electronic balance (0.1 g). Gonadosomatic index (GSI %): GW/ (TW-GW) x 100, hepatosomatic index (HSI %): LW/TW-LW) × 100), intestinal coefficient (IC) (IL/SL) and condition factor of Fulton (K) K = (TW / TLb) x 100, were estimated. Length-weight relationships for individuals of different ovary development phases were estimated using a linear regression based on the power equation, $Y = a \times TL^b$ with the b parameter (slope) related to the rate of weight gain as a function of length.

Sex and ovary development phases of adult specimens were determined employing the macro and microscopy terminology of five phases proposed by Brown-Peterson *et al*. (2011). Then, individuals from the following phases were analysed: II) developing (early gonadal growth, including the presence of cortical alveoli, primary [Vtg1], secondary [Vtg2], and tertiary [Vtg3] vitellogenesis oocytes according to Brown-Peterson *et al*., 2011); III) spawning capable (Vtg3 oocytes, with fish being capable of spawning within the current reproductive cycle); IIIas) actively spawning subphase (defined by the presence of oocytes in late Germinal Vesicle Migration, germinal vesicle breakdown, hydration, ovulation, or newly collapsed post-ovulatory follicles [POFs]) (Brown-Peterson *et al*., 2011).

**Biochemical assays.** With the purpose of preparing the different tissues for conducting the determinations of enzymatic activities and energy reserves, intestine, liver, muscle and intestinal fat were extracted, weighed and placed in individual containers on ice for subsequent processing. The intestine and liver were separately homogenized in 50 mM Tris/HCl, pH 7.4, 4 ml of tissue per gram, using a bench mixer (homogeniser model X-9120 - Schaft T10F -Ingenieurbüro CAT, M. Zipperer GmbH), on ice until complete tissue processing (del Valle *et al*., 2016; Albanesi *et al*., 2022). The same procedure was followed for the body muscle although 8 ml of tissue per gram was used (del Valle *et al*., 2016; Albanesi *et al*., 2022). Subsequently, aliquots of each homogenate were separated into Eppendorf tubes and stored at -20 °C until their use.

Maltase activity was determined by measuring the glucose released from the specific substrate (Albanesi *et al*., 2022). The sample was incubated during 10 min at 37 °C with 42 mM of maltose or sucrose in 0.1 M malate buffer (pH 6, 4) at 37 °C (Albanesi *et al*., 2022). The reaction was stopped with 1.5 mL of a glycemia kit (Wiener Lab Glicemia AA) and further incubated during 5 min at 37 °C. Glucose amount was quantified reading absorbance at 505 nm of the coloured quinone complex.

Aminopeptidase-N activity was determined by using L-alanine-p-nitroanilide (L-Ala-pNA) as substrate (Roncari, Zuber, 1969) with modifications (Michiels *et al*., 2015, 2017; del Valle *et al*., 2016; Albanesi *et al*., 2022). The reaction was initiated by the addition of substrate (final concentration 0.33mM) to a reaction mixture containing the sample in 50 mM Tris-HCl buffer at pH 7.4. After incubation for 15 min at 45 °C (Michiels *et al*., 2015, 2017; del Valle *et al*., 2016; Albanesi *et al*., 2022), the reaction was stopped with 0.2 ml of cold 2 M acetic acid and absorbance was determined at 384 nm.

Alkaline phosphatase (AP) activity was determined as previously described (Méndez *et al*., 2021). In the standard assay, AP activity was determined by measuring p-nitrophenyl phosphate (pNPP) hydrolysis in a reaction medium containing 4 mM MgSO4 in 0.1 M Tris-HCl buffer at pH 9.0. After pre-incubation of the sample in the assay mixture for 5 min at 37 °C, pNPP, final concentration of 10 mM, was added and incubation proceeded for 10 min. The reaction was stopped by adding 0.1 M KOH.

Glycogen was determined by hydrolysis of α-amyloglucosidase (Sigma Chemicals) (del Valle *et al*., 2016; Albanesi *et al*., 2022). The corresponding sample was boiled for 4 min and then incubated in acetate buffer at pH 4.8 in the presence and absence of 0.2 mg ml-1 of α-amyloglucosidase for 2.5 h at 55 °C (del Valle *et al*., 2016; Albanesi *et al*., 2022). After incubation, samples were centrifuged at 6,000 rpm for 15 min. Glucose was quantified in the supernatant using the commercial kit for enzyme glycemia (Wiener Lab AA). Released glucose from glycogen was determined as the difference between the tests with and without enzyme. Results were presented as mg glucose x g of tissue$^{-1}$. Free glucose content was determined from assay performed in the absence of α-amyloglucosidase.

Triglycerides (TG) were measured by the colorimetric method of glycerol phosphate oxidase (TG Wiener-Lab AA code 861110001). An aliquot of the corresponding sample was incubated with this reactant for 5 min at 37 °C (Albanesi *et al*., 2022). The amount of released glycerol was determined by reading the absorbance at 505 nm of the colored quinone complex. Proteins were assayed according to Bradford (1976). Bovine serum albumin (0.96 mg x ml$^{-1}$) was used as standard.

**Statistical analysis.** Both, a Student T and a Fisher's tests were employed in order to test isometric/allometric growth of the different ovary development phases. The obtained coefficients b for each group were employed to estimate factor K. A One-way ANOVA was employed to test comparisons among K values of the different development phases studied.

To study the differences of the biological indexes (HSI, GSI, IC) and biochemical data among the different ovary development phases, statistical analyses were performed using the Sigma-Stat statistical package 3.0 for Windows. This software automatically pre-tests for equality of variances and normality. One-Way ANOVA was performed to estimate statistical differences, with significance set at $p < 0.05$. Post-hoc tests were conducted to identify differences.

## RESULTS

**Body condition parameters.** Morphological determinations showed that TL, BW, GW, LW were greater in phase IIIas compared to phase II and phase III. The IW did not vary between the three phases. Neither was different the intestinal content weight found in the fish intestine between the three phases. Individuals in the phases II, III and subphase IIIas presented a similar IC (mean 1.1) and HSI (II: 1.92 ± 0.71; III: 2.31 ± 1.84; IIIas: 2.17 ± 0.79) (Tab. 1).

The lowest GSI value was found in phase II, with a mean of 0.78 ± 0.33. In phase III, the mean GSI calculated was 2.11 ± 0.62. The highest GSI mean 8.9 ± 3.35 was estimated in subphase IIIas (Tab. 1).

Fish analysed presented an isometric growth in the three phases of ovary development. However, Fisher's test revealed significant differences between coefficients 'b' of the three phases. A significant variation of the factor condition was observed between the different phases of ovary development with a maximum in phase IIIas (mean II = 0.49 ± 0.05; III = 0.59 ± 0.06; IIIas = 0.75 ± 0.07) (Fig. 2).

**TABLE 1** | Measurements of morphological/morphometrical and body condition parameters of *Odontesthes argentinensis* from Mar Chiquita Coastal Lagoon, at different phases of ovarian development. N = Number of samples; SD = Standard deviation; GSI = Gonadosomatic index; HIS = Hepatosomatic index; IC = Intestinal coefficient. Different letters indicate significant differences (one-way ANOVA; $p < 0.05$).

| | **Phase II** N = 18 | | | **Phase III** N = 10 | | | **Phase IIIas** N = 13 | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Physical Traits | Mean | Range | SD | Mean | Range | SD | Mean | Range | SD | P value |
| Total length (mm) | 317 **a/b** | 255–378 | 36.92 | 300 **a** | 294–385 | 48.5 | 349 **b** | 331–400 | 39.7 | 0.01 |
| Body weight (g) | 255 **a** | 112–423 | 91 | 222.1 **a** | 214–414 | 99 | 382 **b** | 256–635 | 149 | 0.003 |
| Gonad weight (g) | 2.00 **a** | 0.2–3.5 | 1.1 | 4.1 **a** | 1.2–7 | 1.6 | 31.56**b** | 10.3–73.5 | 18.4 | <0.0001 |
| Liver weight (g) | 5.1 **a** | 1.3–11.5 | 3.20 | 4.5 **a** | 1.8–9 | 2.37 | 8.8 **b** | 0.7–19.2 | 5.6 | 0.02 |
| Intestinal length (mm) | 279 **a/b** | 175–370 | 44 | 266 **a** | 147–320 | 56 | 324 **b** | 205–414 | 66 | 0.03 |
| Intestinal weight (g) | 7.6 | 2.7–17 | 3.6 | 5.5 | 1–10.3 | 3.1 | 11.2 | 1.9–38.1 | 10.9 | 0.1 |
| Intestinal content weight (g) | 4.6 | 0.7–9.2 | 2.5 | 3.0 | 1.0–6.8 | 1.9 | 6.6 | 0.5–31.9 | 3.3 | 0.2 |
| GSI (%) | 0.8 **a** | 0.1–1.8 | 0.3 | 2.1 **b** | 1.0–5.3 | 0.6 | 8.9 **c** | 3.6–10.7 | 3.3 | <0.0001 |
| HSI (%) | 1.9 | 1.2–3.4 | 0.7 | 2.3 | 1.4–3.0 | 1.8 | 2.2 | 1.5–3.1 | 0.8 | 0.09 |
| IC (%) | 1.1 | 0.7–1.3 | 0.1 | 1.1 | 0.9–1.4 | 0.2 | 1.1 | 0.9–1.2 | 0.1 | 0.4 |

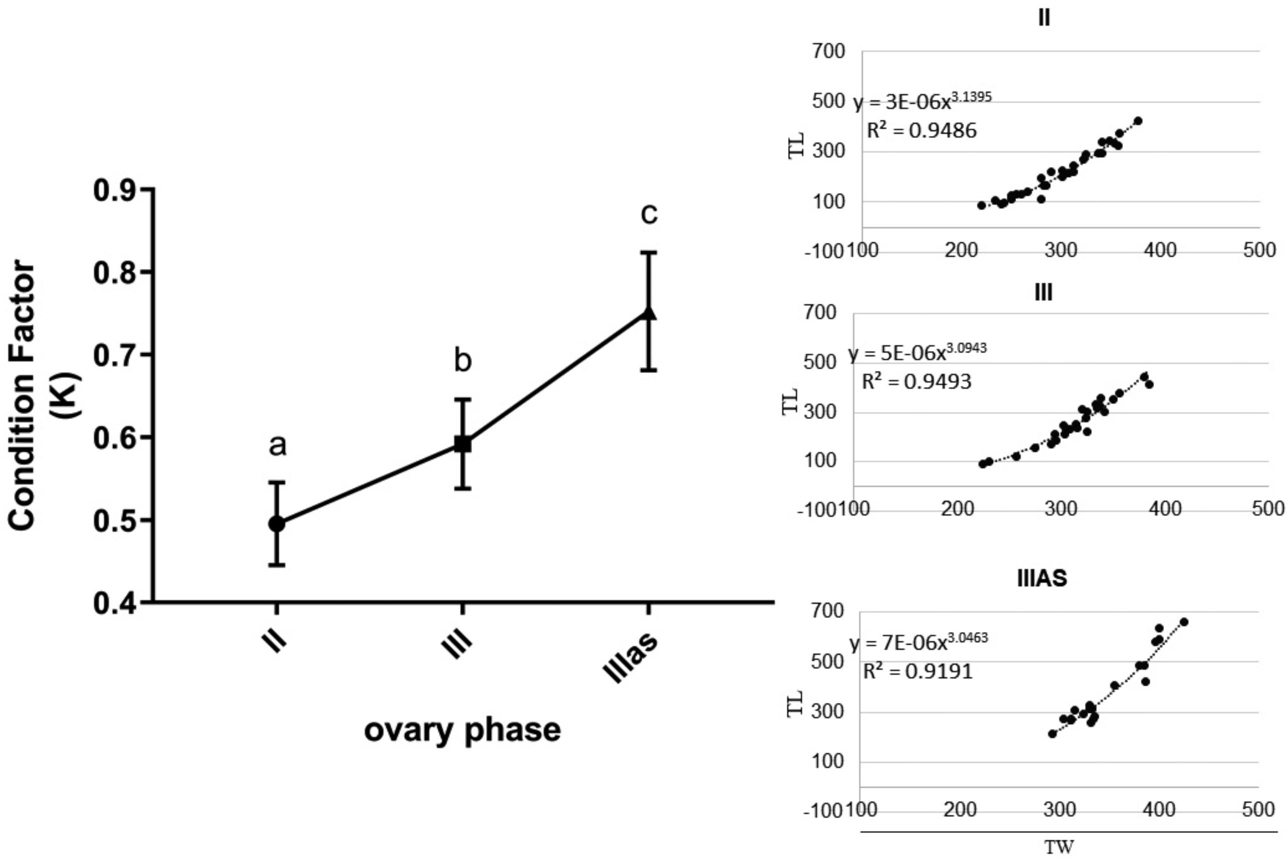

**FIGURE 2** | Allometric coefficient and condition factor for *Odontesthes argentinensis* at different phases of ovary development. TL = Total length; TW = Total weight. Different letters indicate significant differences (one-way ANOVA; $p < 0.05$).

**Enzyme activities in the intestine.** Maltase, APN and AP activities in the intestine of *O. argentinensis* at different phases of ovary development are shown in Figs. 3A–C. Maltase activity was not significantly different among the three phases (Fig. 3A). Aminopeptidase-N revealed significantly higher activity in phase III compared to phases II and IIIas (Fig. 3B). No differences in the AP activity were detected in the intestine of *O. argentinensis* among the three phases (Fig. 3C).

**Energy reserves in reserve organs.** Glycogen and free glucose concentration in liver are presented in Fig. 4, showing similar values in phase II and phase III, but it was 60% lower in phase IIIas (Fig. 4A). No significant differences in free glucose concentration were detected among the three phases (Fig. 4B). Glycogen concentration in muscle markedly decreased (99%) in phase III compared with phase II (Fig. 4C). Phase IIIas exhibited similar values of muscle glycogen concentration to those of phase II (Fig. 4C). Free glucose concentration was significantly higher (600%) in phase III than in phase II and IIIas.

Triglyceride concentration (TG) in liver, muscle and intestinal fat are shown in Figs. 5A–C, no difference in the TG concentration were detected in liver (Fig. 5A) and muscle (Fig. 5B) of *O. argentinensis* among the three phases studied. Meanwhile, TG concentration in intestinal fat was similar in phase II and phase III, but with lower values (50%) in phase IIIas (Fig. 5C).

Protein concentration in liver and muscle are shown in Figs. 6A–B. In liver, no differences were recorded (Fig. 6A). Meanwhile, in muscle, protein concentration was similar in phase II and III, while in phase IIIas was lower (50%) compared to stage III (Fig. 6B).

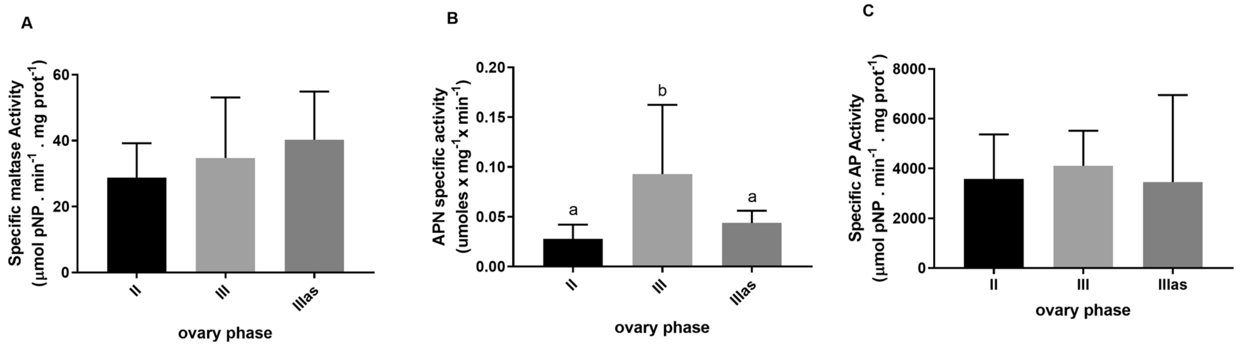

**FIGURE 3** | **A.** Maltase; **B.** Aminopeptidase-N (APN); **C.** Alkaline phosphates (AP) specific activity in *Odontesthes argentinensis* at different phases of ovary development (II: developing; III: spawning capable; IIIas: actively spawning subphase). Different letters indicate significant differences (one-way ANOVA; $p < 0.05$). Data are the mean ± S.E. for six individuals.

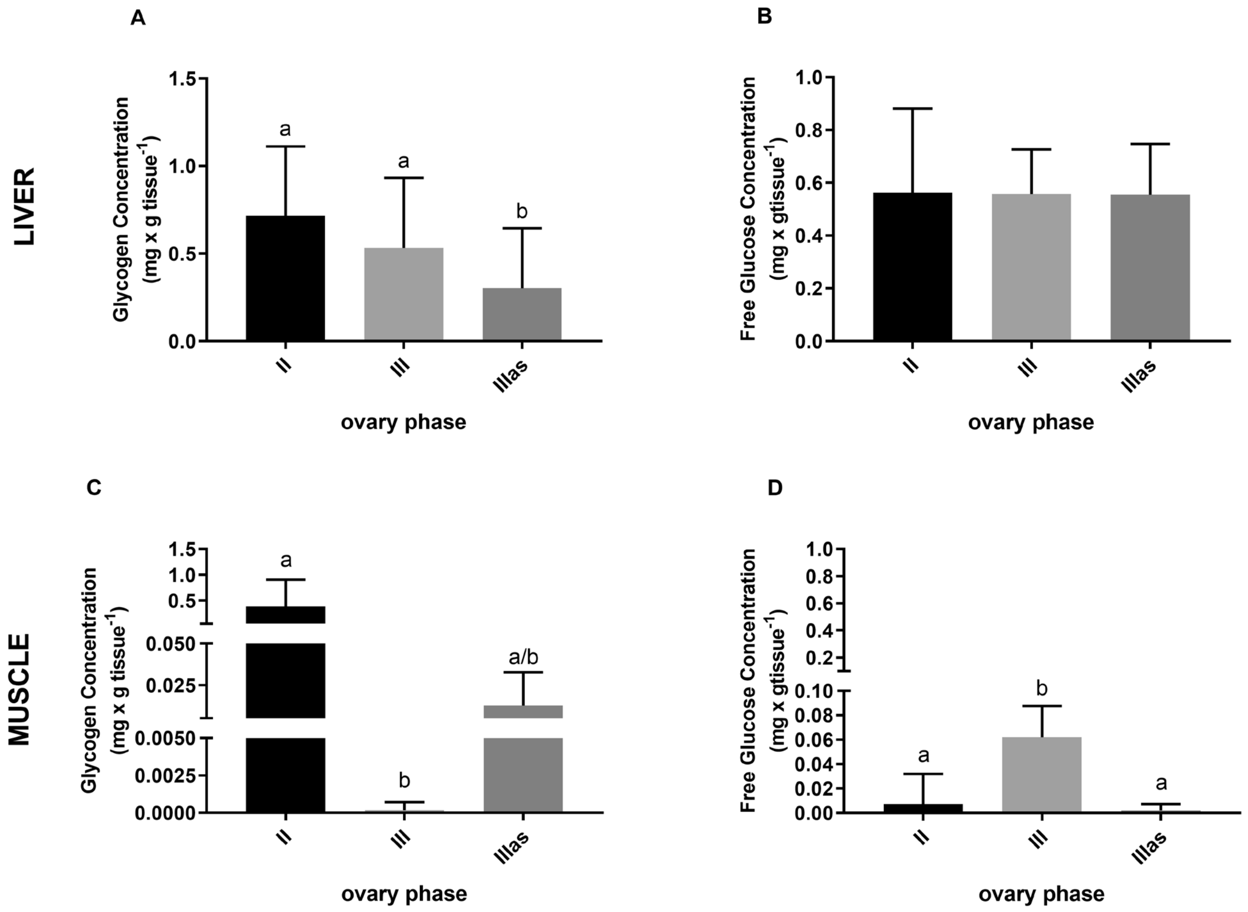

**FIGURE 4** | Glycogen concentration in liver (**A**) and muscle (**C**) and free glucose concentration in liver (**B**) and muscle (**D**) of *Odontesthes argentinensis* at different phases of ovary development (II: developing; III: spawning capable; IIIas: actively spawning subphase). Different letters indicate significant differences (oneway ANOVA; $p < 0.05$). Data are the mean ± S.E. for 6 to 16 individuals.

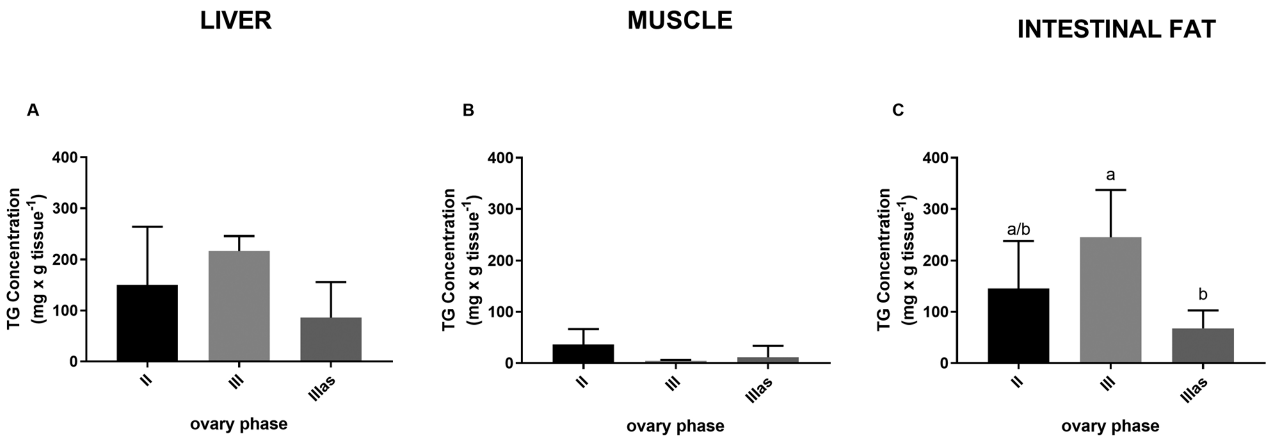

**FIGURE 5** | Triglycerides (TG) concentration in liver (**A**), muscle (**B**) and intestinal fat (**C**) of *Odontesthes argentinensis* at different phases of ovary development (II: developing; III: spawning capable; IIIas: actively spawning subphase. Different letters indicate significant differences (oneway ANOVA; $p < 0.05$). Data are the mean ± S.E. for 6 to 16 individuals.

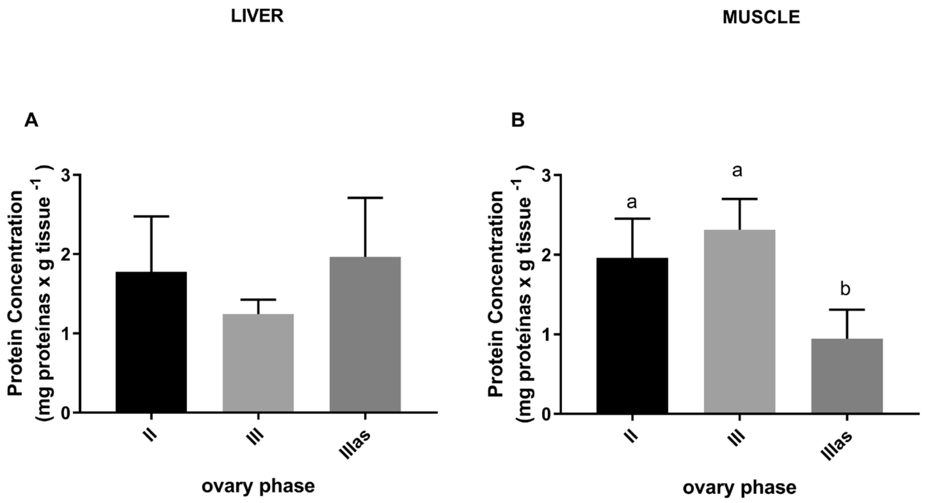

**FIGURE 6 |** Protein concentration in liver (**A**) and muscle (**B**) of *Odontesthes argentinensis* at different phases of ovary development (II: developing; III: spawning capable; IIIas: actively spawning subphase). Different letters indicate significant differences (one-way ANOVA; $p < 0.05$). Data are the mean ± S.E. for 6 to 16 individuals.

## DISCUSSION

The resident population of *O. argentinensis* in MChCL has been intensively studied in the last two decades, due to its remarkable morphological, meristic, genetic and ecological features (González-Castro *et al*., 2009, 2016, 2019, 2022; Levy *et al*., 2021). González-Castro *et al*. (2009) suggested that a population of the marine silverside (*O. argentinensis*) would perform its whole life cycle inside MChCL, at water salinities ranging freshwater values, pointing out the necessity of deeper studies on this taxon. After that, González-Castro *et al*. (2016) demonstrated significant-meristic and body shape differences between the MChCL and marine individuals of *O. argentinensis*, suggesting they behave as well differentiated populations, or even incipient ecological species. They also reported a new group of silverside morphotypes. The suitability of DNA Barcoding to analyse the diversity and distribution of haplotypes in some putative populations of *O. argentinensis* was assessed by González-Castro *et al*. (2019). The Fst (genetic variation among individuals within populations) pairwise comparisons within *O. argentinensis* localities supported the existence of three population-groups: one composed by MChCL specimens, and the others by Mar del Plata/Mar Chiquita coast and San Blas Bay coastal specimens, respectively. These authors suggest that specimens from MChCL should be considered as a marine to freshwater incipient speciation event. Recently, Levy *et al*. (2021), combining evidence from fish meristic analysis and parasite ecology, demonstrated that the structure and composition of parasite assemblages are indicators of isolation between the populations of *O. argentinensis* from MChCL and their neighbours of MCh coastal marine waters, again suggesting an incipient speciation processes. At last, González-Castro *et al*. (2022) based on environmental, genomic and geometric-morphometric data demonstrated a clear signature of population genetic structure, distinguishing *O. argentinensis* residents of MChCL from the marine populations of this species, that also was supported by distinctive morphometric and ecological features among these groups. All these previous results indicate that the resident population of *O. argentinensis* from MChCL can be considered an evolutionary significant unit (ESU), *sensu* Waples (2004), or even an incipient species, constituting it as a hot spot in evolutionary biology. Phenotypic differences can result in local adaptations when, among other necessary conditions, populations live in unstable environments, exchange few or no migrants, and they are subject to differential selective pressures (Ghalambor *et al*., 2007). In fact, several studies have documented the persistent tendency of *O. argentinensis* to establish estuarine (Beheregaray, Levy, 2000; Moresco, Bemvenuti, 2006), lagoon (González-Castro *et al*., 2009, 2016) or even landlocked (Colautti *et al*., 2020) populations.

In the present study, results showed a differential modulation of key components of carbohydrates, lipid and protein metabolism that would allow to maintain the body condition of the resident population of *O. argentinensis* from MChCL. The condition factor can be influenced by reproductive mode, food availability, habitat and environmental condition (Morato *et al*., 2001; Palazón-Fernandez *et al*., 2001; Eastwood, Counture, 2002). Fulton's condition factor (K) of *O. argentinensis* from MChCL increased, thus notably showing an improvement during phase IIIas. This result can be explained by the fact that *O. argentinensis* individuals continued feeding during both, gonadal maturation and actively spawning phases, as it was recorded by the permanent presence of food in their digestive tract. The fact that HSI did not change among the three phases of ovary development studied could mean that ovary development did not limit reserve store in liver (Dambo *et al*., 2021). The maintenance of HSI values recorded in the present work could be attributed to the continued intake of food. An increased K with no change in HSI is common in fishes that continue to feed during maturation and spawning seasons (Militelli, Macchi, 2006; Domínguez-Petit, Saborido-Rey, 2010). Then, the energy demand through the ovarian cycle of *O. argentinensis* (*e.g*., GSI increases as the ovaries develop), could be supported by metabolic reorganization (*e.g*., activity of key enzymes in the digestive tract, differential use of reserves) (Hany *et al*., 2018). Llompart *et al*. (2013) assessed the relationship between annual cycles of different biological indices with growth patterns of *O. argentinensis* in San Blas Bay, North Patagonia. The reproductive cycle was also coupled with metabolic processes related to energy allocation as shown by changes in the HSI and body condition. Similarly, the results obtained in the present work evidenced an increase in Fulton's condition factor, which accompanies the actively spawning subphase (IIIas) of *O. argentinensis* from MChCL.

The comparison of digestive enzyme activities allows to evaluate the possible adjustments in digestive capacity for the corresponding substrate (Pradhan *et al*., 2013; Small, 2022). The fact that maltase activity in the intestine of *O. argentinensis* did not vary between the phases of ovary development, suggests the maintenance of the potential capacity for final steps of carbohydrate digestion. On the other hand, the higher APN activity in intestine of individuals in phase III, suggests the occurrence of a specific modulation of the potential capacity for the final steps of protein digestion. In fishes, the digestion of proteins by proteases and the absorption processes in the intestine play a crucial role in providing amino acids necessary for the construction of tissues and the maintenance of key functions (Karasov, Douglas, 2013; Cassidy *et al*., 2016; Steinberg, 2018). Phase III involves Vtg3 oocytes, a critical stage in yolk accumulation, necessary to progress to subphase IIIas. In this context, the potentially increased digestive capacity for final steps of protein digestion during this phase could lead to a major availability of metabolites to support this process.

In fishes, intestinal AP is involved in several functions and is an index of the maintenance of intestinal homeostasis (Lallès, 2020; Pérez-Sirkin *et al*., 2020). The absence of changes in AP activity suggests that *O. argentinensis* could maintain intestinal functionality consistently throughout the different phases of ovary development. Changes in the length of the gut and therefore variations in surface area and total volume can affect both digestion and absorption and impact body condition in various fish (Krogdahl *et al*., 2005; Vidal *et al*., 2019; Small, 2022). The absence of changes in the IC among the three ovary development phases pointed out that this would not be the case for the resident population of *O. argentinensis* from MChCL. Furthermore, the unchanged IC throughout the ovarian development suggested that the variations in APN activity could be due to the modulation of the preexisting enzymes by chemical messengers, or by the synthesis and degradation of the enzymes, rather than changes in the intestinal structure or morphology (Albanesi *et al*., 2023).

Levels and types of energy reserves are an expression of the metabolic characteristics of an animal (del Valle, López Mañanes, 2012). The amount of glycogen stored and/or mobilized in the liver depends on different factors faced by the fishes (Coban, Sen, 2011). Similarly to other fishes (Tolussi *et al*., 2018), the lower concentration in actively spawning subphase (IIIas) indicates a mobilization (*e.g*., degradation) of glycogen reserves in liver upon active spawning in *O. argentinensis.* This decrease in liver glycogen storage could support energy demands for oocyte growth and development. The fact that the decreased glycogen concentration did not result in an enhancement of free glucose suggests that liver of *O. argentinensis* can act as a source of glucose for other tissues. In several fish the liver has a central role in maintaining glucose homeostasis (Polakof *et al*., 2012; Albanesi *et al*., 2024). In some fishes, muscle glycogen content is lowest during the breeding period (Coban, Sen, 2011). This appears to be the case for *O. argentinensis* since the diminished glycogen concentration in muscle point out the mobilization of this reserve during the spawning capable phase (III). The concomitant enhancement in free glucose concentration would lead to an increased availability of this metabolite to be used locally in muscle. This could sustain the glucose requirements for movements inside Zone III/ freshwater tributaries of specimens in phase III while searching for proper conditions for spawning. The glycogen concentration in muscle, which is the main source of energy that fishes use to swim, can vary depending on the needs of the fish such as for rapid movement (Hardy, Kaushik, 2021). On the other hand, the increase of glycogen concentration in muscle of *O. argentinensis* along with the decrease in free glucose concentration suggests the re-building of the reserve in actively spawning subphase (IIIas). In fish, skeletal muscle is the main glucose entry site via a specific glucose transporter subjected to regulation (Yang *et al*., 2021).

In fishes, TG stored in the liver, muscle and/or adipose tissue constitute a main source of energy for physiological processes such as growth, reproduction and movement, including migration (Steinberg, 2018; Small, 2022). The unchanged TG concentration in liver and muscle in phases II, III and subphase IIIas, suggests that adjustments in key components of the lipid metabolism in these organs are not involved throughout the gonadal development and spawning. The decrease in TG concentration in subphase IIIas point to that intestinal fat could be a source of lipid metabolites to meet the energy demands of the gonadal maturation. The fact that TG concentration was only varied in the intestinal fat suggests the occurrence of tissue-specific modulation of lipids reserves during active spawning subphase. In other fishes, TG in intestinal fat is an important fuel reserve for facing the energy cost of reproduction (Huynh *et al*., 2007; Weil *et al*., 2013).

Protein content in fish may vary in response to certain conditions such as the season, effect of spawning and migration, and food availability (Abdullahi, 2001). Liver proteins content is not expected to change, due to its structural role (Medford, Mackay, 1978; Hardy, Kaushik, 2021). This appears to be the case for *O. argentinensis* in different gonadal maturity phases, since no differences in liver protein reserve were found. On the opposite, muscle protein concentration decreased in subphase IIIas, suggesting that protein reserves in muscle would be mobilized to face the energy demand for spawning.

In conclusion, this study confirms the differential modulation of key components of carbohydrate, lipid, and protein metabolism (Fig. 7), suggesting that *O. argentinensis* from MChCL undergoes a metabolic reorganisation to maintain and even improve the physiological condition during their gonadal maturation and active spawning. The findings shed light on the remarkable adaptability of this population of *O. argentinensis* to fluctuating environmental conditions. Further research, comparing different months throughout the whole spawning season, may provide deeper insights into the energy dynamics of this remarkable evolutionarily significant unit of *O. argentinensis* from MChCL during the reproductive season.

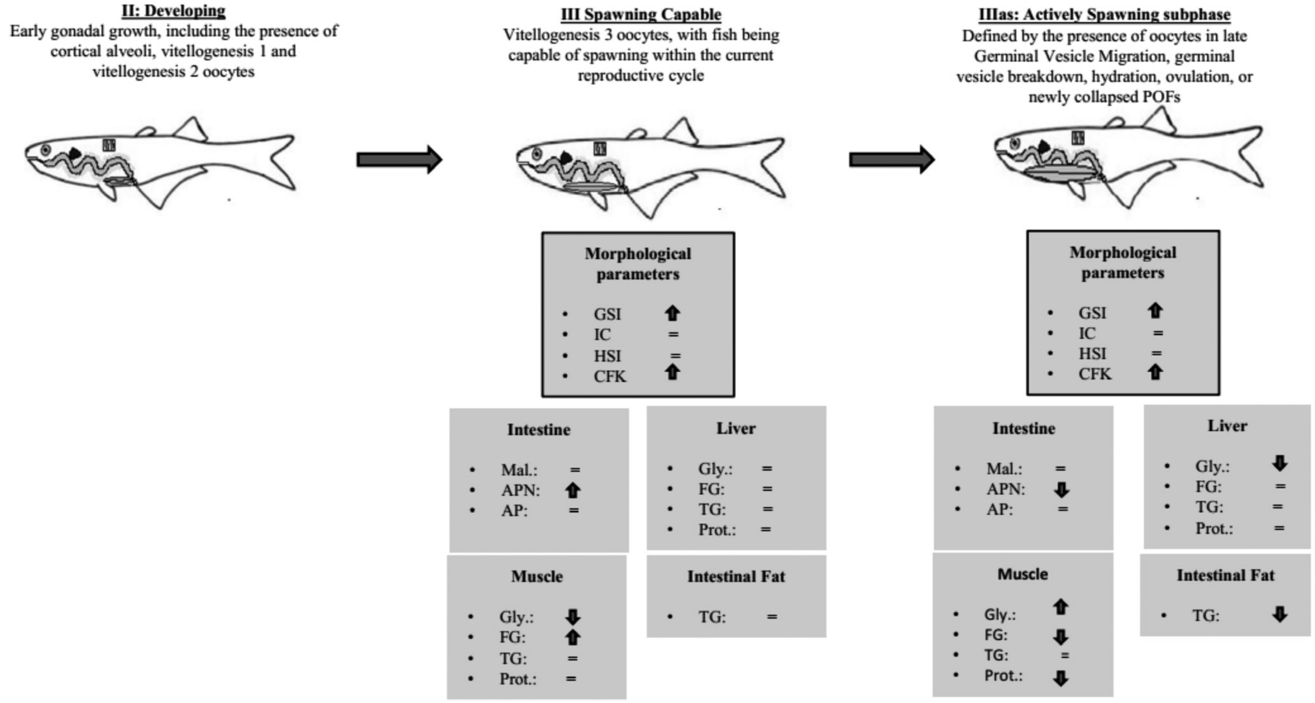

**FIGURE 7** | Summary of morphological, digestive and metabolic parameters at different phases of ovary development of *Odontesthes argentinensis*. GSI: gonadosomatic index; HIS: hepatosomatic index; IC: intestinal coefficient; CFK: condition factor of Fulton; Mal: maltase; APN: aminopeptidase-N; AP: alkaline phosphatase; Gly: glycogen; FG: free glucose; TG: triglycerides; Prot: protein. Comparisons were made between phases as is described in Results. ↑, ↓, = indicate increase, decrease and unchanged, respectively.

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## AUTHORS' CONTRIBUTION

**Eugenia Mendez:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Visualization, Writing-original draft, Writing-review and editing.

**Camila Albanesi:** Data curation, Formal analysis, Investigation, Methodology, Software.

**María Soledad Michiels:** Data curation, Formal analysis, Investigation, Methodology, Software.

**Alejandra López-Mañanes:** Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing-original draft, Writing-review and editing.

**Mariano González-Castro:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Visualization, Writing-original draft, Writing-review and editing.

## ETHICAL STATEMENT

This study was approved (RD 2022–105, FCEyN) and conducted following the regulations and statements of Ethics Committee CICUAL (OCA 1499/12; FCEyN, UNMdP, Argentina).

## COMPETING INTERESTS

The author declares no competing interests.

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