


Age and growth patterns of the ten spotted live-bearing fish (*Cnesterodon decemmaculatus*) along a polluted freshwater system

Micaela J. Zambrano^{1,2} | Alejandro D. Bolzán^{3,4} | Alejo F. Bonifacio^{1,2} |
Juan M. Brito^{1,2} | María V. Amé⁵ | Andrea C. Hued^{1,2} 

¹Instituto de Diversidad y Ecología Animal (IDEA), CONICET (Consejo Nacional de Investigaciones Científicas Y Técnicas), Córdoba, Argentina

²Facultad de Ciencias Exactas, Físicas y Naturales, Cátedra de Diversidad Biológica IV, Universidad Nacional de Córdoba, Córdoba, Argentina

³Laboratorio de Citogenética y Mutagénesis, Instituto Multidisciplinario de Biología Celular (IMBICE, CICPBA-UNLP-CONICET La Plata), La Plata, Argentina

⁴Facultad de Ciencias Naturales y Museo, UNLP, La Plata, Argentina

⁵Facultad Ciencias Químicas, Dto. Bioquímica Clínica-CIBICI, Universidad Nacional de Córdoba-CONICET, Haya de La Torre Esq. Medina Allende, Ciudad Universitaria, Córdoba, Argentina

Correspondence

Andrea C. Hued, Instituto de Diversidad y Ecología Animal (IDEA), CONICET-UNC, Facultad de Ciencias Exactas, Físicas y Naturales, Universidad Nacional de Córdoba, Córdoba, CP 5000, Argentina.
Email: andrea.hued@unc.edu.ar

Funding information

Consejo Nacional de Investigaciones Científicas y Tecnológicas, Grant/Award Number: PIP 112- 201101-01084; Fondo para la Investigación Científica y Tecnológica, Grant/Award Number: PICT 2019-3161; Secretaría de Ciencia y Tecnología - Universidad Nacional de Córdoba, Grant/Award Number: 2018-2021. Res No: 411/ 18

Abstract

Age and growth patterns of fish provide important information about the effects of environmental disturbances, which can be used as comparative tools in subsequent studies that attempt to assess freshwater quality. The main goal of our study was to provide information on relevant biological aspects of a native fish species used as a bioindicator in an extensive area of South America. In particular, we evaluated the age and growth patterns of *Cnesterodon decemmaculatus* (Poeciliidae) to establish reference species values and to compare them in different sites along an environmental quality gradient in a South American freshwater system. Water quality assessments indicated increasing environmental degradation downstream, reflecting anthropogenic impacts. The estimated ages of *C. decemmaculatus* along this quality gradient varied across sampling sites. The longest-lived individuals were those from the reference site, also presenting the highest average age value (almost 2 years). The best model to describe the growth in length of the individuals was the Logistic model. According to the parameters estimated from the growth curves, individuals from the most disturbed site showed slower growth although they reached greater maximum lengths than fish from the other sites. These results suggest that fish would tolerate the adverse conditions of the most altered sites, allocating their energy differentially. Our study provides valuable information on the age and growth patterns of *C. decemmaculatus*, a species endemic to the Neotropical region and a useful bioindicator in ecotoxicological studies.

KEYWORDS

age determination, *Cnesterodon decemmaculatus*, fish growth, otolith, water quality

1 | INTRODUCTION

Population growth and associated human activities are causing aquatic ecosystems to become increasingly threatened worldwide. Growth and survival of aquatic biota are severely affected by chronic exposure to

contaminated freshwater environments (Burbank et al., 2021). Like other biological aspects of the affected organisms, both age and growth patterns of the species can provide information about the effects of environmental alterations, which can be used as comparative tools in subsequent studies that attempt to assess freshwater quality.

Widely distributed in three South American countries, Brazil, Uruguay, and Argentina (Lucinda, 2005), the fish species *Cnesterodon decemmaculatus* has been classified as a tolerant fish species to a wide degree of environmental quality conditions occurring in both quasi-pristine and highly contaminated freshwater systems (Hued & de los Bistoni, 2005; Masson et al., 2024). Some attributes, such as its small size, easy acclimation and handling under laboratory conditions, and its wide range of tolerance to environmental variations, have made *C. decemmaculatus* a valuable bioindicator for both field studies and laboratory bioassays (Bernal-Rey et al., 2020; Bonifacio et al., 2020; Rautenberg et al., 2022; Vidal et al., 2018; Zambrano et al., 2018).

Although *C. decemmaculatus* has been used as a fish model in different studies, crucial biological information that remains unknown is related to its age and growth patterns. These aspects are relevant to understanding species biology, population dynamics, and also community structure and ecosystem functioning (Volpedo & Vaz-dos-Santos, 2015). To determine the age of teleost fish, the most widely used method is based on the microstructural analysis of otoliths (Campana & Thorrold, 2001; Spich & Fey, 2022). These calcified structures involved in sound detection and responsible for maintaining the balance and orientation of fish contain microstructural and chemical information related to fish growth and the environment where they lived. The otolith structure changes based on the daily or sub-daily deposition of calcium carbonate, organic matter, and other constituents that form the otolith (Campana, 1999; Morales-Nin, 2000). The analysis of these depositions allows us to know the age and growth rate of fish and also gives information about the environmental conditions where they live (Elfman et al., 2000; Ofelio et al., 2023).

The study of age determination is closely related to growth patterns. In fish, the growth of most species tends to occur indeterminately throughout their lifetime (Mommsen, 2001). In studies of fish population dynamics, somatic growth is a necessary process that determines biomass production, fecundity, and mortality (Lorenzen, 2000; Röpke et al., 2021), and its characterization provides comprehensive information about the effects of environmental characteristics on fish (Zapfe & Rakocinski, 2008). In this sense, growth parameters have been used to compare habitat quality, to assess fish response to pollution and climate change, and to perform fish population management (Burbank et al., 2021; Huang et al., 2021; Pasingi, 2021; Searcy et al., 2007). When age information is available, it is possible to use mathematical functions to describe how body length increases over time and estimate the absolute growth through parameters that can be compared among different populations (Wilson et al., 2019). On the contrary, different studies have demonstrated that both the otolith growth rate and the growth patterns of individuals can vary between populations, depending significantly on the environmental conditions in which these individuals develop (Araújo & Monteiro, 2013; Campana & Thorrold, 2001; Reznick et al., 1989).

In Argentina, where *C. decemmaculatus* has a wide distribution, the Suquía River basin, located in Córdoba Province, is considered a vital element of the landscape; however, it receives a complex mixture of contaminants from different sources (Bertrand et al., 2023; Merlo

et al., 2011; Zambrano et al., 2018). Through the studies conducted over the past 10 years, the presence of several pesticides, pharmaceuticals, and heavy metals has been recorded in concentrations that exceed the limits established by the National Secretariat of Water Resources (Bonansea et al., 2013; Rautenberg et al., 2015, 2022; Reyna et al., 2019; Valdés et al., 2014). Given that the basin presents river sections with high water quality, it becomes an excellent study area to establish reference values on the age and growth of the ten spotted live-bearer fish to be compared with the age and growth of individuals from sites with moderate-to-severe environmental pollution. We hypothesize that fish from pristine sites present different average ages and growth patterns than those from more contaminated sites. Therefore, our main goal was to determine the age and growth patterns of *C. decemmaculatus* in sites with high water quality and compare these findings with the results obtained in populations from polluted river sections.

2 | MATERIALS AND METHODS

2.1 | Study area

The Suquía River basin is located in the semiarid region of the province of Córdoba, Argentina, with a drainage area covering approximately 7700 km², of which 900 km² corresponds to the drainage area of the city of Córdoba. The San Antonio and Cosquín rivers are its main tributaries, which feed into the San Roque Dam. The Suquía River originates from the dam and passes through small towns before entering the city of Córdoba (1.57 million inhabitants, INDEC, 2022), flowing through a concrete channel for approximately 6 km and then alternating with open banks for about 40 km (Amé & Pesce, 2015). Along most of its course through the city, the river is accompanied by two coastal roads, one on each bank. Over the past 20 years, the city's population has nearly doubled, and increasing industrialization has increased the risk of toxic effluents being discharged into the river. La Cañada stream, located in the city center of Córdoba, contributes to the river's flow. Further downstream, near the city's eastern edge, the Suquía River is affected by municipal wastewater discharge from the wastewater treatment plant. It then continues its course through the plains, pouring its waters into the Mar Chiquita lagoon, 450 km from its source (Pasquini et al., 2012).

The flow regime of the rivers and streams that form the Suquía River drainage network is exclusively rainfall-driven, with marked flow seasonality due to the irregular distribution of precipitation throughout the year (Pasquini et al., 2012). The system experiences a high-flow period (greater than 15 m³ · s⁻¹) from December to April, whereas during the dry season from May to November, the estimated flow ranges between 5 and 10 m³ · s⁻¹ (Dasso, 1998).

Following different previous studies that confirm the water quality gradient of the Suquía River basin (Hued & de los Bistoni, 2005; Maggioni et al., 2012; Rautenberg et al., 2022; Zambrano et al., 2018), we selected three sampling sites (Figure 1): (1) Puente Zuviría (PZ), located on the Yuspe River, a tributary of the Cosquín River,

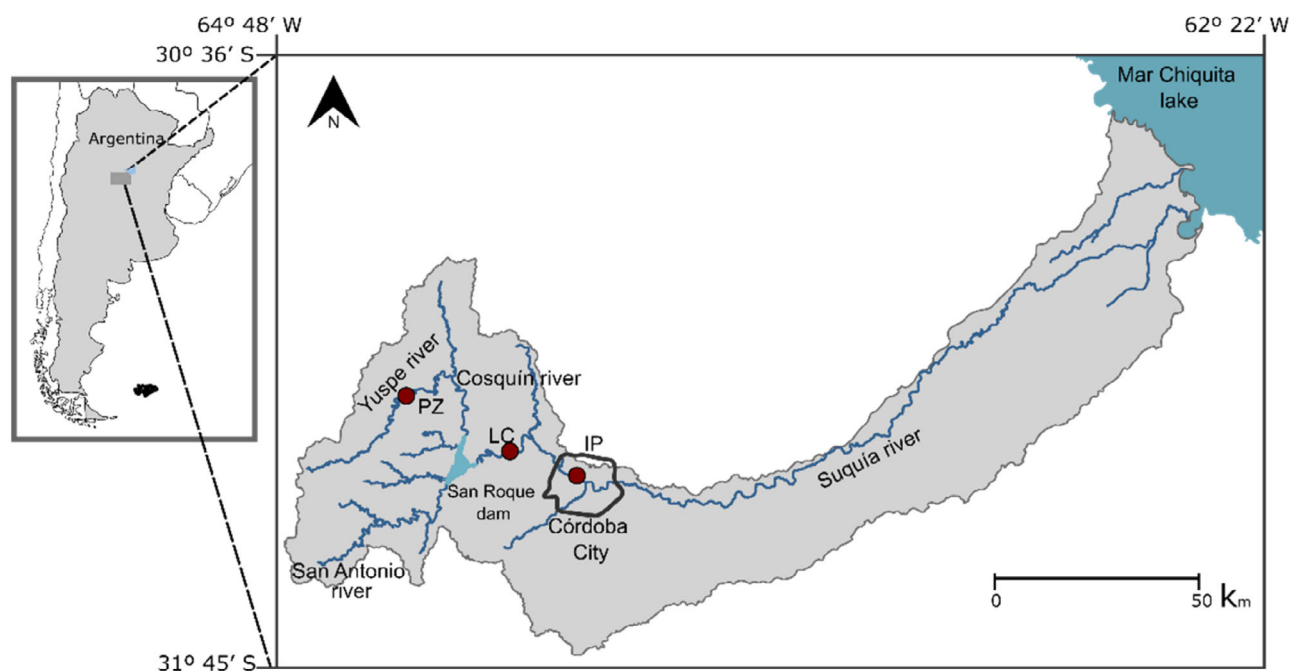


FIGURE 1 Map of the Suquia River basin and the *Cnesterodon decemmaculatus* sampling site's location. IP, Isla de los Patos; LC, La Calera; PZ, Puente Zuviria. Modified from Reyna et al. (2019).

considered *quasi*-pristine site; (2) La Calera (LC), located on the Suquia River, 15 km downstream from San Roque Dam; (3) Isla de los Patos (IP), situated in the center of Córdoba city and therefore subject to the effects of urban activities (Merlo et al., 2011; Pasquini et al., 2012; Rautenberg et al., 2022; Zambrano et al., 2018).

2.2 | Fish collection and water quality assessment

Individuals of *C. decemmaculatus* were collected using hand fishing nets with a 2-mm mesh at each site, twice per site (April 2019 and September 2021), and transferred alive to the laboratory in water tanks, where their total length (TL) was measured. Afterward they were euthanized by decapitation. All procedures were complied with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health, 2011). To characterize the water quality condition at each sampling site, a water sample was simultaneously collected with fish capture. The following physical, chemical, and biological parameters were measured: conductivity (mS/cm), pH, temperature (°C), dissolved oxygen (DO, mg/L), 5-day biological oxygen demand (BOD-5, mg/L), nitrites (mg/L), nitrates (mg/L), ammonia (mg/L), sulfates (mg/L), phosphorus (mg/L), dissolved and total solids (mg/L), total hardness (mg/L), Ca^{+2} (mg/L), Mg^{+2} (mg/L), chlorides (mg/L), and total coliforms (colony-forming units [CFU]/100 mL). The measurements were conducted following standard techniques (APHA, 2005). Based on these parameters, the water quality index (WQI) was calculated according to Pesce and Wunderlin (Pesce & Wunderline, 2000). This index requires normalizing each parameter on a scale of 0–100 and assigning different weighting factors based on the parameter's importance as an indicator of water quality. The index is calculated

using the following formula: $\text{WQI} = \Sigma (C_i \times P_i) / \Sigma P_i$, where C_i is the normalized value assigned to each parameter, and the value of P_i ranges from 1 to 4, with 4 representing a parameter of highest relevance for the conservation of aquatic life (e.g., dissolved oxygen), whereas a value of 1 means the parameter has a lesser impact on biota (e.g., chloride). The WQI provides a dimensionless number associated with a percentage of quality, ranging from 0% to 100%, representing the lowest-to-highest water quality.

In addition, another water sample was collected at each site to measure pesticides, following Bonansea et al. (2013): 4,4'-DDD, 4,4'-DDE, 4,4'-DDT, aldrin, dieldrin, endrin, endrin aldehyde, alpha-chlordane, gamma-chlordane, alpha-hexachlorocyclohexane, delta-hexachlorocyclohexane, gamma-hexachlorocyclohexane, chlorobenzilate, chloroneb, chlorothalonil, chlorpyrifos, *cis*-permethrin, *trans*-permethrin, alfa-endosulfan, beta-endosulfan, endosulfan sulfate, hexachlorobenzene (HCB), heptachlor, heptachlor epoxide, methoxychlor, propachlor, and trifluralin. The limits of detection (LOD) obtained for pesticide analysis were calculated as three times the SD of the detector response in repetitive injections of blank samples, according to de los González Sagrario et al. (2002), ranging between 0.5 and 20 ng/L. To complete these measurements, a compilation of the pesticides detected along the Suquia basin was added from the research published in Rautenberg et al. (2022). These authors perform data collection at the same sites that were studied in the present work.

2.3 | Age and growth estimation

The periodicity of otolith growth increments in *C. decemmaculatus* was determined on laboratory-reared individuals of known age. Adult

fish collected at Puente Zuviría (reference site) were kept in a 120-L aquarium, where they were allowed to reproduce naturally. The newly hatched fry was separated and kept in 15-L tanks under a light-to-dark cycle of 12 h:12 h and fed with commercial fish food (Shulet) twice daily. Ten individuals were collected every 10 days during the first 30 days, starting from the hatching day. The pair of sagitta otoliths were extracted using a stereoscopic microscope for each age group (10, 20, and 30 days old). *Sagitta* otoliths were chosen because they are the largest in the studied fish species. The same procedure was repeated for the remaining individuals at 30 days and subsequently every 60 days for 725 days. Before the otolith extraction, the sex of each individual (males and females) was determined. Newborn individuals whose gonads could not be differentiated due to their small size and development were classified as “indeterminate.”

After extraction, each otolith was mounted on a glass slide using cyanoacrylate adhesive and then polished with P2500 water sandpaper to enhance the visualization of increments. After that, they were photographed again at 100× magnification to be used for the daily increment count. For this purpose, three radii were selected in each otolith from the nucleus to the margin. The number of increments was counted on each radio, resulting in three readings per otolith. The following criteria were considered when selecting the radii: first, according to the length of the radius, short axes usually do not contain the complete sequence of increments, whereas longer axes frequently exhibit sub-daily rings; thus, the shorter axes that presented the complete sequence of increments were chosen; and the second criterion was related to the clarity of the rings, selecting axes with the best visibility (Stevenson & Campana, 1992). The count was performed using the ImageJ software (version 1.51).

CV was calculated using the Chang (1982) formula to determine the precision of the readings:

$$CV_j = \frac{\sqrt{\sum_{i=1}^R \frac{(X_{ij} - X_j)^2}{R-1}}}{X_j} \times 100\%$$

where X_{ij} is the i th age determination of the j th fish, X_j is the mean age estimate of the j th fish, and R is the number of times each fish is aged.

The CV was calculated for each otolith, and then a CV average was calculated per individual. Readings with a CV value equal to or less than seven were considered accurate, whereas otoliths with CV values greater than seven were re-read. They were discarded if they continued to be inaccurate (Campana, 2001).

To validate the daily increments in the otolith formation in individuals with known age, a linear regression was performed between the number of daily growth increments and the actual age in days. Before the statistical analyses, the distribution of model residuals was checked to ensure that the model's assumptions were met. All analyses were conducted using R version 4.2.1 (R Core Team, 2022).

The age of *C. decemmaculatus* individuals collected at each sampling site was estimated following the methodology explained previously. On the contrary, fish growth patterns were evaluated in fish raised in the laboratory and those collected from the field separately.

Three growth models were explored: the von Bertalanffy, the Gompertz, and the Logistic models.

The von Bertalanffy model has three parameters in its equation:

$$L_t = L_\infty \left(1 - e^{-k(X-X_0)} \right)$$

where L_t is the size at a given time (X), L_∞ is the asymptotic maximum length (it has the same biological meaning in the Gompertz and Logistic models), K is the growth constant, and X_0 is the age of the fish when they hypothetically have zero length.

The Gompertz model was calculated based on the following equation:

$$L_t = L_\infty e^{[-e^{-G(X-X_0)}]}$$

where L_t is the size at a given time (X), L_∞ is the asymptotic maximum length, G is the instantaneous growth rate at age X_0 , and X_0 is the point of inflection of the curve corresponding to the age at which the absolute growth rate begins to decline.

The Logistic model has three parameters in its equation:

$$L_t = L_\infty \left(1 + e^{-G(X-X_0)} \right)^{-1}$$

where L_t is the size at a specific time (X), L_∞ is the asymptotic maximum length, G is the instantaneous growth rate at the origin of the curve, and X_0 is the age at the inflection point of the curve and the age of maximum absolute growth rate.

The parameters of each model were estimated following the methodology proposed by Ogle (2018).

The corrected AIC (AICc) was used to determine the model that best fits the data. If more than one model had $\Delta AICc < 2$, they were validated by taking the known age data and comparing it with the model estimates. Then, each model's mean squared error (MSE) was calculated, and the model with the lowest value was selected.

The effects of WQI, site, age, and sampling years on TL in *C. decemmaculatus* were assessed using a general linear model. To address potential multicollinearity, variance inflation factors (VIF) and correlation matrices were computed. Further model selection was performed using the “stepAIC” function from the “MASS” package, which facilitated automatic selection based on the AIC. Post hoc analysis of least-squares means was conducted using the “lsmeans” and “emmeans” packages. All analyses were performed using R, version 4.2.1 (R Core Team, 2022).

3 | RESULTS

3.1 | Water quality assessment

The physical, chemical, and biological parameters measured in water varied along the Suquia River (Table 1), showing a downstream decrease in water quality conditions. The highest values recorded for all the parameters, except pH and dissolved oxygen, correspond to IP. At this site, nutrient concentrations (ammonium, nitrates, nitrites,

TABLE 1 Physical, chemical, and biological parameters measured in water samples along the Suquia River basin.

Parameters	Sites		
	PZ	LC	IP
WQI	87.97 ± 4.20	80.16 ± 1.99	70.16 ± 6.41
Conductivity (mS/cm)	0.109 ± 0.038	0.211 ± 0.042	0.499 ± 0.010
pH	8.025 ± 0.177	8.405 ± 0.148	7.315 ± 0.191
Temperature (°C)	16.675 ± 0.459	20.500 ± 1.952	20.900 ± 0.849
Dissolve oxygen (mg/L)	8.550 ± 0.212	9.550 ± 0.495	8.150 ± 0.071
BOD-5 (mg/L)	0.550 ± 0.212	1.025 ± 1.025	2.100 ± 0.566
N-Nitrites (mg/L)	0.001 ± 0.001	0.004 ± 0.002	0.018 ± 0.020
N-Nitrates (mg/L)	1.620 ± 1.160	2.820 ± 0.000	5.340 ± 1.344
N-Ammonia (mg/L)	1.360 ± 1.527	1.835 ± 1.393	4.145 ± 3.033
Sulfates (mg/L)	13.400 ± 3.960	23.200 ± 2.404	75.100 ± 38.184
Phosphorus (mg/L)	0.004 ± 0.000	0.017 ± 0.018	0.105 ± 0.092
Total solids (mg/L)	77.700 ± 17.961	171.200 ± 86.550	628.600 ± 57.417
Dissolve solids (mg/L)	31.500 ± 9.192	53.500 ± 37.477	358.000 ± 250.316
Total hardness (mg/L)	36.000 ± 4.243	62.650 ± 5.728	168.850 ± 118.016
Ca ²⁺ (mg/L)	29.850 ± 2.616	39.150 ± 3.323	109.800 ± 62.084
Mg ²⁺ (mg/L)	1.650 ± 0.212	3.600 ± 0.849	4.800 ± 0.141
Chlorides (mg/L)	8.700 ± 1.697	11.000 ± 4.101	35.200 ± 18.950
Total coliforms (CFU/100 mL)	350.000 ± 212.132	3023.000 ± 2792.900	5933.000 ± 3016.518
Pesticides			
Atrazine (ng/L)	0.01 ± 0.01	<LOD	<LOD
Pirimiphos-methyl (ng/L)	0.08 ± 0.04	0.03 ± 0.00	0.05 ± 0.02
Aldrin (ng/L)*	<LOD	<LOD	0.30 ± 0.42
Dieldrin (ng/L)*	0.15 ± 0.30	0.76 ± 0.77	0.60 ± 0.00
α-Chlordane (ng/L)*	<LOD	0.15 ± 0.30	0.30 ± 0.42
α-Hexachlorocyclohexane (ng/L)*	0.45 ± 0.30	0.15 ± 0.30	0.00 ± 0.00
d-Hexachlorocyclohexane (ng/L)*	0.45 ± 0.30	0.60 ± 0.00	0.60 ± 0.00
g-Hexachlorocyclohexane (ng/L)*	0.30 ± 0.35	<LOD	<LOD
Chlorobenzilate (ng/L)*	<LOD	0.15 ± 0.30	<LOD
Chloroneb (ng/L)*	0.15 ± 0.30	2.70 ± 5.33	16.03 ± 22.67
Chlorpyrifos (ng/L)*	0.75 ± 1.50	0.79 ± 1.57	<LOD
cis-Permethrin (ng/L)*	<LOD	5.28 ± 9.17	<LOD
trans-Permethrin (ng/L)*	4.00 ± 4.63	8.17 ± 9.61	9.14 ± 0.85
Hexachlorobenzene (ng/L)*	0.30 ± 0.35	3.15 ± 3.57	0.60 ± 0.00
Heptachlor (ng/L)*	0.30 ± 0.35	0.45 ± 0.30	0.60 ± 0.00
Propachlor (ng/L)*	<LOD	<LOD	4.86 ± 6.87
Trifluralin (ng/L)*	3.51 ± 7.01	33.50 ± 67.00	2.17 ± 3.07

Note: Values are expressed as mean ± SD. Asterisk indicates pesticides measured by Rautenberg et al. (2022).

Abbreviations: BOD-5, biological oxygen demand; CFUs, colony-forming unit; IP, Isla de los Patos; LC, La Calera; LOD, lower than detection limit; PZ, Puente Zuviría; WQI, water quality index.

phosphates) and dissolved and total solids were 4–25 times higher than those recorded at the reference site (PZ). Regarding the WQI values, a marked decrease was observed along the basin, ranging from the highest average value registered at PZ (88%) followed by 80% at LC and 70% at IP. Table 1 shows the concentrations of the 17 pesticides measured in the present work and those taken from Rautenberg et al. (2022).

3.2 | Age and growth estimation

In the present study, the number of daily growth increments (y) as a function of the actual age in days (x) of laboratory-raised *C. decemmaculatus* individuals (between 0 and 725 days of age) was fitted using a linear model. The equation obtained was the following: $y = 43 + 0.91 \times x$ ($n = 68$, $R^2 = 0.99$).

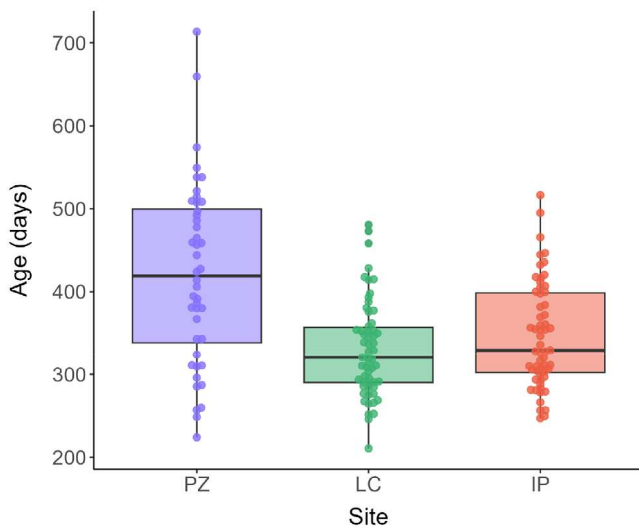


FIGURE 2 Estimated age (days) in *sagitta* otoliths of *Cnesterodon decemmaculatus* collected from different sites in the Suquía River. PZ, Puente Zuviría ($n = 44$); LC, La Calera ($n = 58$); IP, Isla de los Patos ($n = 59$).

The analysis of *sagitta* otolith microstructure from *C. decemmaculatus* females revealed that the average age of fish living in PZ was 421 days, 336 days for LC individuals, and 357 days for IP individuals (Figure 2). Furthermore, when comparing the mean ages at each site, we observed that the reference site (PZ) had the oldest individuals ($F_{2,29} = 3.79$, $p = 0.03$), with fish of 700 days of age being observed.

From the three candidate models we used to model the growth of laboratory-reared *C. decemmaculatus* individuals ($n = 96$), the Logistic model provided the best fit for describing the length growth of the individuals, as evidenced by the lowest AICc and Δ AICc values (Table 2). In contrast, the von Bertalanffy model exhibited the poorest fit.

According to the Logistic model, in the studied population of *C. decemmaculatus*, the point of inflection (X_0) and the age at which the growth rate is maximum occur at 87 days, and the asymptotic length (L_∞) corresponds to 25 mm. When fitting the Logistic growth model separately based on sex, we obtained the following parameters: $L_\infty = 23.72$ mm, $G = 0.01$, and $X_0 = 73.1$ days for males, and $L_\infty = 27.47$ mm, $G = 0.008$, and $X_0 = 117.71$ days for females.

The three models applied on fish individuals collected along the Suquía River showed a good fit, with Δ AICc values < 2 (Table 2). However, the Logistic model presented the lowest MSE value and was chosen as the best model.

According to the Logistic model, females collected at IP would reach the highest maximum length ($L_\infty = 49$ mm; Figure 3) followed by fish from the reference site ($L_\infty = 42$ mm). In contrast, the lowest value for this parameter was estimated in individuals from LC ($L_\infty = 40$ mm). On the contrary, in females inhabiting IP, the growth inflection point (X_0), which is the age at which the growth rate is at its maximum, occurs about 60 days later than in fish from the other sites. Fish from IP reach this inflection point at 255 days of age, whereas in

PZ and LC, the maximum growth rate is achieved at 202 and 194 days of age, respectively. Finally, the growth rate at the curve's origin (G) is slightly lower in IP females than the others.

Finally, the best linear model explaining the variation in LT includes the independent variables WQI, age, and year of sampling. The LT variation was significantly related to WQI and age ($F_1 = 28.13$, $p = 3.7 \times 10^{-7}$; $F_1 = 644.39$, $p = 2.2 \times 10^{-16}$), whereas the relation with sampling year was not significant ($F_1 = 3.22$, $p = 0.07$).

4 | DISCUSSION

Our results provide new information about the fish *C. decemmaculatus*, a species endemic to the Neotropical region. The determination of age and growth patterns in both reference and polluted freshwater environments gives new insights about this useful bioindicator. Our study is significant for two important factors: first, the species considered, because it is a useful and widely used bioindicator, but there is still a lack of information on different aspects of its biology; and second, it inhabits a basin with a confirmed history of contamination, which makes it a model study area for this type of approach.

4.1 | Water quality assessment

Fishes from the Suquía River are living under different levels of pollution. Our study showed a variation pattern of water quality conditions along the Suquía River basin and demonstrated the negative impact of human activities from Córdoba city. Relevant parameters for the aquatic biota, such as biochemical oxygen demand (BOD5), nitrogen compounds, and total coliform bacteria, showed the highest values at the site in Córdoba city (IP). Additionally, the WQI values decreased (18%) at IP compared to the reference site (PZ). The variation pattern of WQI coincides with the results of studies from many years ago, which demonstrated that the river has experienced high pollution levels for almost 25 years, leading to a critical situation in the last decade (de los Bistoni et al., 1999; Hued & de los Bistoni, 2005; Maggioni et al., 2012; Merlo et al., 2011; Nimptsch et al., 2005; Pesce & Wunderline, 2000; Rautenberg et al., 2015, 2022; Wunderlin et al., 2001; Zambrano et al., 2018).

Urbanization has been considered one of the leading causes of river habitat degradation through channelization, increased erosion and sedimentation rates, increased runoff, nutrient enrichment, alteration of flood regimes, and the entry of numerous toxic substances from human activities (Andrade-Muñoz et al., 2023; Miserendino et al., 2011; Spanjer et al., 2018). Another consequence of urbanization is the removal of riparian vegetation, which is crucial for maintaining stream temperature and mitigating the entry of nutrients and sediments (Fornells et al., 1998). In particular, the low flow of the Suquía River basin, its seasonal variation, and its lower self-purification capacity, because of the short distance to its mouth into the Mar Chiquita Lagoon, make the river highly vulnerable to alterations from anthropogenic activities (Contardo-Jara et al., 2009;

Models	K	AICc	Δ AICc	W AICc	Cum W	LL	MSE
Laboratory-reared fish							
Logistic	4	418.16	0	0.81	0.81	-204.86	-
Gompertz	4	421.22	3.07	0.17	0.98	-206.39	-
Von Bertalanffy	4	425.51	7.35	0.02	1	-208.54	-
Fish from the Suquía River							
Logistic	4	651.35	0.00	0.41	0.41	-321.55	50.77
Gompertz	4	651.66	0.30	0.35	0.76	-321.70	51.05
Von Bertalanffy	4	652.46	0.11	0.24	1.00	-322.10	50.96

TABLE 2 Selection criteria for estimated growth models in *Cnesterodon decemmaculatus* from laboratory-reared individuals and those collected from the Suquía River.

Note: Parameters of the best fitting models are shown in bold.

Abbreviations: AICc, corrected AIC; Cum Wt, cumulative weight; K, number of parameters of each model; LL, log likelihood; MSE, mean square error; W AICc, Akaike weight; Δ AICc, criterion difference to the best candidate model.

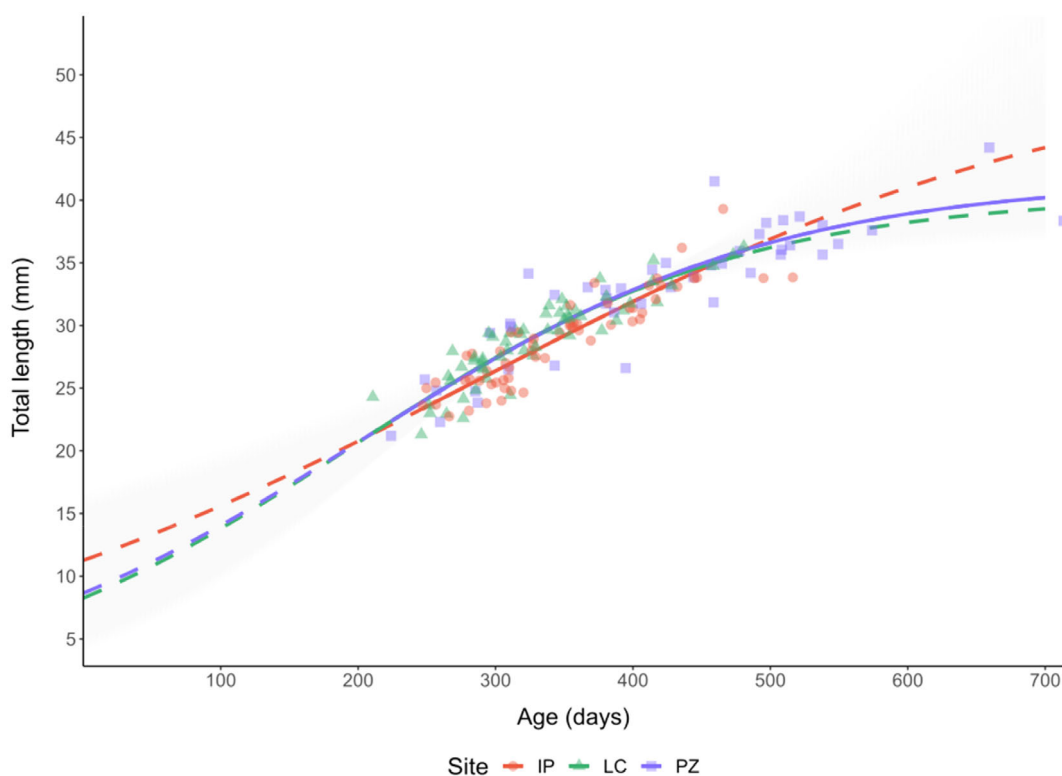


FIGURE 3 Logistic growth model adjusted to females of *Cnesterodon decemmaculatus* collected from different sites of the Suquía River. The solid line represents the fit of the observed data, and the dotted line represents the estimated data. IP, Isla de los Patos; LC, La Calera; PZ: Puente Zuviria.

Wunderlin et al., 2001). In addition to this, the presence of a cocktail of chemicals, including heavy metals, pesticides, pharmaceuticals, and personal care products, has been documented along the basin, posing a risk to the entire aquatic biota (Bertrand et al., 2023; Rautenberg et al., 2022; Santiago et al., 2015; Valdés et al., 2014). In parallel to our measurements, another data collection was carried out for a research project published by our working group in Rautenberg et al. (2022). In this study, numerous compounds were recorded along the Suquía River basin, demonstrating the diversity of substances that characterize the environments of our sampling sites.

Compounds such as aldrin, dieldrin, chlordane, hexachlorocyclohexane (lindane), propachlor, trifluralin, heptachlor, and permethrin have been registered at IP in the past decade (Bonansea et al., 2013; Rautenberg et al., 2022; Zambrano et al., 2018). Particularly, Rautenberg et al. (2022) reported the presence of the pesticide permethrin (*cis* and *trans* isomers) in concentrations exceeding the limit of 4 ng/L established for aquatic biota preservation according to the Argentinian Environmental Water Quality Guidelines (Subsecretaría de Recursos Hídricos de la Nación [SRHN], 2017). Permethrin is a synthetic pyrethroid insecticide, widely used to protect crops or kill parasites in

livestock (Kaneko et al., 2000), and it is also used as a domestic pesticide for mosquito control and as a pediculicide for veterinary and human use. It has been indicated that this insecticide can cause oxidative stress and immunosuppressive effects in fish (Parent et al., 2011). An intermediate condition was observed in LC. Although this site presented a high WQI value, Rautenberg et al. (2015) registered the presence of heavy metals such as copper and mercury and a high concentration of cypermethrin, possibly from sewage and urban runoff from small towns along the river.

Recently, Bertrand et al. (2023) conducted an analysis of the incidence and ecological risk assessment of currently used pesticides (CUPs) and pharmaceutical and personal care products (PPCPs) measured in the Suquia River basin. The most frequently registered herbicides were acetochlor and atrazine, whereas among insecticides, chlorpyrifos and cypermethrin were the most frequently detected. Regarding PPCPs, those showing the highest detection frequency were analgesic/anti-inflammatory compounds (paracetamol, ibuprofen, naproxen, and diclofenac), H1 H2 receptor antagonists (ranitidine), psychiatric drugs (fluoxetine and carbamazepine), and sweeteners (saccharin). As for the environmental risk assessment, the authors found a very high risk in LC and Córdoba city. In LC, the major contributors to this risk were cypermethrin, glyphosate, and chlorpyrifos, whereas in the city of Córdoba, the calculated risk was associated with PPCPs, with paracetamol, ibuprofen, and the antibiotic clarithromycin standing out as the main contributors.

All the aforementioned studies have highlighted the severe pollution along the Suquia River basin, reflected not only in the lowest WQI values but also in the large quantity of xenobiotics present in the aquatic system whose effects on the biota are unknown.

4.2 | Age and growth estimation

The study of fish growth through age estimation using otoliths requires, first, a validation of the daily deposition of otoliths in the species (Campana, 1983). The value of $b = 0.913$ obtained in the present work indicates that each ring would take slightly less than 1 day to form. Furthermore, the intersection of the line presents a value of 43, demonstrating that at the time of birth, the fish otoliths already have 43 daily rings. In this sense, Pisam et al. (2002) observed that *Danio rerio* (zebrafish) otoliths are formed at 50-h postfertilization. Considering that *C. decemmaculatus* pregnancy lasts between 30 and 40 days (Lorier & Beoris, 1995), the intercept value we found from the regression analysis is a good estimator of the fry age (in days) at birth.

According to the estimated age for *C. decemmaculatus* females collected throughout the Suquia River basin, the longest-lived individuals reached approximately 2 years (713 days). As was mentioned earlier, these specimens were collected at the reference site (PZ), where the average age was 434 days. On the contrary, at the remaining river sections, individuals rarely exceeded 500 days of age, with the average age in LC and IP being 336 and 357 days, respectively. These differences could be attributed to the presence of top predators such as

Hoplias malabaricus in LC and IP, and the high abundance of the invasive species *Gambusia affinis*. We suggest that this invasive fish could compete for the resources used by *C. decemmaculatus*. The negative effect of *G. affinis* on native fish fauna has been confirmed in previous studies, highlighting not only the competition but also the species' resistance to pollution and aggression in the newly invaded areas (Pyke, 2008; Raghavan et al., 2008; Raja & Ravikanth, 2020).

Furthermore, both *H. malabaricus* and *G. affinis* are absent in PZ; therefore, *C. decemmaculatus* individuals would reach greater longevity as they are not under the pressure of this invasive fish. The maximum age we registered in the present work for *C. decemmaculatus* is similar to the results from Erguden (2013), who reported a maximum age of 2 years for *Gambusia holbrooki* in Lake Seyhan (Turkey), whereas other authors have estimated ages up to 1 year for *G. affinis* and *G. holbrooki* (Nguyen et al., 2021; Öztürk & İKİZ, 2004; Patimar et al., 2011). Krumholz (1948) also postulated that *Gambusia* sp. can live up to 5 years in aquariums as many other species. However, they rarely survive more than 2 years in the wild, consistent with our results.

Regarding the study of fish growth through mathematical modeling of age, the most common practice has been to preselect the von Bertalanffy model and fit it to the data. However, applying this model could result in biased point estimates and a false assessment of the accuracy of growth parameters (Katsanevakis & Maravelias, 2008). Therefore, we decided to apply three candidate models to model the *C. decemmaculatus* growth: the von Bertalanffy model, the Gompertz model, and the Logistic model. In laboratory-reared fish, the Logistic model showed the best fit to describe the length growth of individuals, whereas the von Bertalanffy model exhibited the poorest fit. Consistent with our results, the Logistic model has been identified as the best model to describe the growth of phylogenetically close species to *C. decemmaculatus*, such as *Poecilia vivipara*, *Poecilia velifera*, and *Anableps anableps* (Araújo & Monteiro, 2013; de Albuquerque Oliveira et al., 2011; Sa-nguansil, 2009).

The Logistic model is non-linear and sigmoidal, where the growth rate initially increases until an inflection point and then decreases toward the asymptotic length. The inflection point separates growth into two phases: acceleration and deceleration (Araújo & Monteiro, 2013). According to the Logistic model applied to laboratory-reared *C. decemmaculatus* individuals, the inflection point (X_0) and the age at which the maximum growth rate would occur at 87 days, and the asymptotic length (L_∞) would be 25 mm.

Due to the marked sexual dimorphism in this species, the Logistic growth model was fitted separately for males and females. This model shows that females reach a greater maximum length ($L_\infty = 27.47$ mm). Like most species, female poeciliids exhibit indeterminate growth, which occurs practically throughout their life span. Contrary to the female growth pattern, male growth ($L_\infty = 23.72$ mm) is limited by the development of the gonopodium (through the elongation of the rays that make it up) during the maturation stages. The development of morphological sexual characteristics, such as differentiation of the anal fin into a copulatory organ, is under strict androgenic control, beginning at the onset of sexual maturity and ceasing once individuals

are sexually mature (Angus et al., 2001; Turner, 1942a, 1942b). Bisazza (1993) observed that in species with marked sexual dimorphism, males tend to mature earlier than females. In contrast, de Albuquerque Oliveira et al. (2011) indicated that males need to attain a minimal length rapidly to compete for female access. In our study, this phenomenon was reflected in the lower value of the estimated X_0 parameter in males, where the inflection point in male growth occurs ~45 days earlier than in females.

Females reared under laboratory conditions reach smaller sizes than those collected at the field, with a maximum length approximately 14 mm smaller than fish collected at the reference site on Suquia River. These results are consistent with those reported by Araújo and Monteiro (2013), who found a difference of 10–20 mm in length between aquarium-reared and wild-caught *P. vivipara* individuals. The authors attributed these results to differences in the quality and quantity of food accessed by fish depending on their rearing environment and the higher population density they experienced during their growth under laboratory conditions.

According to the parameters estimated from the growth curves, it is observed that individuals in the IP population grow more slowly than those in the PZ and LC populations. In fact, individuals from IP reach the inflection point in their growth ~60 days later than individuals from the other sites. However, the size that the organisms can reach at an older age is greater in IP, being able to reach maximum lengths ~8 mm greater than PZ and LC fish. These observed differences in growth patterns between populations may be because individuals in the more contaminated site (IP) grow more slowly because they allocate energy toward detoxification processes to cope with the contamination, rather than to biomass growth, resulting in slower growth over time (Wendelaar Bonga, 1997). These results, together with the persistence and development of the species' populations in IP, are supported by the opportunistic feeding habits of this non-migratory species (Quintans et al., 2009) and its high tolerance to severe polluted environments (Hued & de los Bistoni, 2005; Rautenberg et al., 2022; Masson et al., 2024).

5 | CONCLUSIONS

Based on the findings presented in this study, it is important to highlight the significance of estimating age through otolith daily increments. The short life span of *C. decemmaculatus* emphasizes the importance of age estimation through daily increments, as it allows for more precise inferences regarding population dynamics and individual growth, compared to the information obtained from classic estimations based on annual growth analysis in scales or seasonal rings in otoliths (Nguyen et al., 2021). In addition, it is necessary to emphasize the importance of fitting more than one model when studying fish growth because, as our study shows, it may happen that the von Bertalanffy model is not the most appropriate for modeling individual growth.

Our results provide new information on *C. decemmaculatus*, which is endemic to the Neotropical region and a highly relevant species in

ecotoxicological studies. At the same time, its usefulness as a bioindicator is revalued. The use of this species as a biological model, together with the use of a basin with high levels of pollution as a case of study, allows addressing the use of *C. decemmaculatus* as a bioindicator in the evaluation of environmental quality not only for this main aquatic system but also for an extensive area of South America where this species inhabits.

AUTHOR CONTRIBUTIONS

All authors contributed to the study's conception and design. Micaela J. Zambrano, Juan M. Brito, and Alejo D. Bonifacio performed material preparation, data collection, and analysis. Maria V. Amé was in charge of the measurements in the water samples. The first draft of the manuscript was written by Micaela J. Zambrano, Andrea C. Hued, and Alejandro D. Bolzán, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

ACKNOWLEDGEMENTS

We thank German Gonzalez for his helpful collaboration in the statistical analysis.

FUNDING INFORMATION

This work was supported by grants from Consejo Nacional de Investigaciones Científicas y Tecnológicas, Argentina (CONICET, PIP 112-201, 101-01084), Fondo para la Investigación Científica y Tecnológica (FONCYT, PICT 2019-3161), and Secretaría de Ciencia y Tecnología de la Universidad Nacional de Córdoba (SECyT, 2018-2021. Res number: 411/18).

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

ORCID

Andrea C. Hued  <https://orcid.org/0000-0003-4866-3367>

REFERENCES

- Amé, M. V., & Pesce, S. F. (2015). Spatial and Temporal Changes in Water Quality Along the Basin. In *The Handbook of Environmental Chemistry*. Springer.
- American Public Health Association (APHA), American Water Works Association (AWWA), & Water Environment Federation (WEF). (2005). In L. S. Clesceri, E. W. Rice, & A. H. Greenberg (Eds.), *Standard methods for the examination of water and wastewater* (21st ed.). Eaton, A. D.
- Andrade-Muñoz, A. S., di, C., Assef, Y. A., Kutschker, A. M., Alday, G., Dromaz, W. M., Quinteros, P., & Miserendino, M. L. (2023). Implications of wastewater discharges on environmental features and fish communities in an urban river. *Urban Ecosystems*, 26, 779–791. <https://doi.org/10.1007/s11252-023-01331-1>
- Angus, R. A., McNatt, H. B., Howell, W. M., & Peoples, S. D. (2001). Gonopodium development in Normal male and 11-Ketotestosterone-treated female mosquitofish (*Gambusia affinis*): A quantitative study using computer image analysis. *General and Comparative Endocrinology*, 123, 222–234. <https://doi.org/10.1006/gcen.2001.7669>
- Araújo, L. G. B. R., & Monteiro, L. R. (2013). Growth pattern and survival in populations of *Poecilia vivipara* (Teleostei; Poeciliidae) inhabiting an environmental gradient: A common garden study. *Environmental*

- Biology of Fishes*, 96, 941–951. <https://doi.org/10.1007/s10641-012-0089-z>
- Bernal-Rey, D. L., Cantera, C. G., dos Santos Afonso, M., & Menéndez-Helman, R. J. (2020). Seasonal variations in the dose-response relationship of acetylcholinesterase activity in freshwater fish exposed to chlorpyrifos and glyphosate. *Ecotoxicology and Environmental Safety*, 187, 109673. <https://doi.org/10.1016/j.ecoenv.2019.109673>
- Bertrand, L., Iturburu, F. G., Valdés, M. E., Menone, M. L., & Amé, M. V. (2023). Risk evaluation and prioritization of contaminants of emerging concern and other organic micropollutants in two river basins of central Argentina. *Science of the Total Environment*, 878, 163029. <https://doi.org/10.1016/j.scitotenv.2023.163029>
- Bisazza, A. (1993). Male competition, female mate choice and sexual size dimorphism in poeciliid fishes. *Marine and Freshwater Behaviour and Physiology*, 23, 257–286. <https://doi.org/10.1080/10236249309378869>
- Bonanse, R. I., Amé, V., & Wunderlin, D. A. (2013). Determination of priority pesticides in water samples combining SPE and SPME coupled to GC-MS. A case study: Suquia River basin (Argentina). *Chemosphere*, 90, 1860–1869. <https://doi.org/10.1016/j.chemosphere.2012.10.007>
- Bonifacio, A. F., Zambrano, M. J., & Hued, A. C. (2020). Integrated ecotoxicological assessment of the complex interactions between chlorpyrifos and glyphosate on a non-target species *Cnesterodon decemmaculatus* (Jenyns, 1842). *Chemosphere*, 261, 127782. <https://doi.org/10.1016/j.chemosphere.2020.127782>
- Burbank, J., Drake, D. A. R., & Power, M. (2021). Urbanization correlates with altered growth and reduced survival of a small-bodied, imperilled freshwater fish. *Ecology of Freshwater Fish*, 30, 478–489. <https://doi.org/10.1111/eff.12598>
- Campana, S. E. (1983). Calcium deposition and otolith check formation during periods of stress in coho salmon, *Oncorhynchus kisutch*. *Comparative Biochemistry and Physiology Part A: Physiology*, 75, 215–220. [https://doi.org/10.1016/0300-9629\(83\)90073-7](https://doi.org/10.1016/0300-9629(83)90073-7)
- Campana, S. E. (1999). Chemistry and composition of fish otoliths: Pathways, mechanisms and applications. *Marine Ecology Progress Series*, 188, 263–297. <https://doi.org/10.3354/meps188263>
- Campana, S. E. (2001). Accuracy, precision and quality control in age determination, including a review of the use and abuse of age validation methods. *Journal of Fish Biology*, 59, 197–242.
- Campana, S. E., & Thorrold, S. R. (2001). Otoliths, increments, and elements: Keys to a comprehensive understanding of fish populations? *Canadian Journal of Fisheries and Aquatic Sciences*, 58, 30–38. <https://doi.org/10.1139/f00-177>
- Chang, W. Y. B. (1982). A statistical method for evaluating the reproducibility of age determination. *Canadian Journal of Fisheries and Aquatic Sciences*, 39, 1208–1210.
- Contardo-Jara, V., Galanti, L. N., Amé, M. V., Monferrán, M. V., Wunderlin, D. A., & Wiegand, C. (2009). Biotransformation and antioxidant enzymes of *Limnoperna fortunei* detect site impact in watercourses of Córdoba, Argentina. *Ecotoxicology and Environmental Safety*, 72, 1871–1880. <https://doi.org/10.1016/j.ecoenv.2009.07.001>
- Dasso, C. (1998). CIRSA (Centro de la Región Semiárida). INA, Instituto Nacional del Agua y del Ambiente.
- de Albuquerque Oliveira, V., Ferreira Fontoura, N., & Fogaça de Assis Montag, L. (2011). Reproductive characteristics and the weight-length relationship in *Anableps anableps* (Linnaeus, 1758) (Cyprinodontiformes: Anablepidae) from the Amazon estuary. *Neotropical Ichthyology*, 9, 57–766.
- de los Bistoni, M. A., Hued, A. C., Videla, M. M., & Sagretti, L. (1999). Efectos de la calidad del agua sobre las comunidades ícticas de la región central de Argentina. *Revista Chilena de Historia Natural*, 72, 325–335.
- de los González Sagrario, M. A., Miglioranza, K. S. B., Aizpún de Moreno, J. E., Moreno, V. J., & Escalante, A. H. (2002). Polychlorinated biphenyls in different trophic levels from a shallow lake in Argentina. *Chemosphere*, 48, 1113–1122. [https://doi.org/10.1016/S0045-6535\(02\)00149-2](https://doi.org/10.1016/S0045-6535(02)00149-2)
- Elfman, M., Limburg, K. E., Kristiansson, P., Svedäng, H., Westin, L., Wickström, H., Malmqvist, K., & Pallon, J. (2000). Complex life histories of fishes revealed through natural information storage devices: Case studies of diadromous events as recorded by otoliths. *Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms*, 161–163, 877–881. [https://doi.org/10.1016/S0168-583X\(99\)00923-4](https://doi.org/10.1016/S0168-583X(99)00923-4)
- Erguden, S. A. (2013). Age, growth, sex ratio and diet of eastern mosquitofish *Gambusia holbrooki* Girard, 1859 in Seyhan dam Lake (Adana/Turkey). *Iranian Journal of Fisheries Sciences*, 12, 204–218.
- Fornells, N. P., Solá Ortega, C., & Munné, A. (1998). QBR: un índice rápido para la evaluación de la calidad de los ecosistemas de ribera. *Tecnología del Agua*, 177, 20–39.
- Huang, M., Ding, L., Wang, J., Ding, C., & Tao, J. (2021). The impacts of climate change on fish growth: A summary of conducted studies and current knowledge. *Ecological Indicators*, 121, 106976. <https://doi.org/10.1016/j.ecolind.2020.106976>
- Hued, A. C., & de los Bistoni, M. A. (2005). Development and validation of a biotic index for evaluation of environmental quality in the central region of Argentina. *Hydrobiologia*, 543, 279–298. <https://doi.org/10.1007/s10750-004-7893-1>
- Instituto Nacional de Estadística y Censos (INDEC). (2022). República Argentina. https://censo.gob.ar/index.php/mapa_poblacion/2/
- Kaneko, A., Taleo, G., Kalkoa, M., Yamar, S., Kobayakawa, T., & Björkman, A. (2000). Malaria eradication on islands. *The Lancet*, 356, 1560–1564. [https://doi.org/10.1016/S0140-6736\(00\)03127-5](https://doi.org/10.1016/S0140-6736(00)03127-5)
- Katsanevakis, S., & Maravelias, C. D. (2008). Modelling fish growth: Multi-model inference as a better alternative to a priori using von Bertalanffy equation. *Fish and Fisheries*, 9, 178–187. <https://doi.org/10.1111/j.1467-2979.2008.00279.x>
- Krumholz, L. A. (1948). Reproduction in the Western mosquitofish, *Gambusia affinis affinis* (Baird & Girard), and its use in mosquito control. *Ecological Monographs*, 18, 1–43.
- Lorenzen, K. (2000). Allometry of natural mortality as a basis for assessing optimal release size in fish-stocking programmes. *Canadian Journal of Fisheries and Aquatic Sciences*, 57, 2374–2381. <https://doi.org/10.1111/j.1095-8649.1996.tb00060.x>
- Lorier, E., & Beoris, N. (1995). Reproducción Y Nutrición Embrionaria En *Cnesterodon decemmaculatus*. *Revista Brasileira de Biologia*, 55, 27–44.
- Lucinda, P. H. F. (2005). Systematics of the genus *Cnesterodon* Garman, 1895 (Cyprinodontiformes: Poeciliidae: Poeciliinae). *Neotropical Ichthyology*, 3, 259–270. <https://doi.org/10.1590/S1679-62252005000200003>
- Maggioni, T., Hued, A. C., Monferrán, M. V., Bonanse, R. I., Galanti, L. N., & Amé, M. V. (2012). Bioindicators and biomarkers of environmental pollution in the middle-lower basin of the Suquia River (Córdoba, Argentina). *Archives of Environmental Contamination and Toxicology*, 63, 337–353. <https://doi.org/10.1007/s00244-012-9785-0>
- Masson, I., Bertora, A., Rosso, J. J., Gonzalez Castelain, J., Dubny, S. A., Othax, N. E., & Peluso, F. O. (2024). Longitudinal patterns in water quality and fish assemblage structure in a low-gradient, temperate neotropical stream disrupted by a point-source urban effluent. *Limnetica*, 43(1), 1. <https://doi.org/10.23818/limn.43.08>
- Merlo, C., Abril, A., Amé, M. V., Argüello, G. A., Carreras, H. A., Chiappero, M. S., Hued, A. C., Wannaz, E., Galanti, L. N., Monferrán, M. V., González, C. M., & Solís, V. M. (2011). Integral assessment of pollution in the Suquia River (Córdoba, Argentina) as a contribution to lotic ecosystem restoration programs. *Science of the Total Environment*, 409, 5034–5045. <https://doi.org/10.1016/j.scitotenv.2011.08.037>
- Miserendino, M. L., Casaux, R., Archangelsky, M., Di Prinzi, C. Y., Brand, C., & Kutschker, A. M. (2011). Assessing land-use effects on water quality, in-stream habitat, riparian ecosystems and biodiversity in Patagonian northwest streams. *Science of the Total Environment*, 409, 612–624. <https://doi.org/10.1016/j.scitotenv.2010.10.034>

- Mommsen, T. P. (2001). Paradigms of growth in fish. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, 129, 207–219. [https://doi.org/10.1016/S1096-4959\(01\)00312-8](https://doi.org/10.1016/S1096-4959(01)00312-8)
- Morales-Nin, B. (2000). Review of the growth regulation processes of otolith daily increment formation. *Fisheries Research*, 46, 53–67. [https://doi.org/10.1016/S0165-7836\(00\)00133-8](https://doi.org/10.1016/S0165-7836(00)00133-8)
- National Institutes of Health. (2011). *Guide for the care and use of laboratory animals*. The National Academies Press.
- Nguyen, H., Bell, J. D., & Patil, J. G. (2021). Daily ageing to delineate population dynamics of the invasive fish *Gambusia holbrooki*: Implications for management and control. *Biological Invasions*, 23, 2261–2270. <https://doi.org/10.1007/s10530-021-02504-9>
- Nimtsch, J., Wunderlin, D. A., Dollan, A., & Pflugmacher, S. (2005). Antioxidant and biotransformation enzymes in *Myriophyllum quitense* as biomarkers of heavy metal exposure and eutrophication in Suquia River basin (Córdoba, Argentina). *Chemosphere*, 61, 147–157. <https://doi.org/10.1016/j.chemosphere.2005.02.079>
- Ofelio, C., Moyano, M., Sswat, M., Rioual, F., Moullec, F., Aguirre-Velarde, A., & Peck, M. A. (2023). Temperature and prey density drive growth and otolith formation of the world's most valuable fish stock. *Scientific Reports*, 13, 16001. <https://doi.org/10.1038/s41598-023-43168-w>
- Ogle, D. H. (2018). *Introductory fisheries analyses with R*. Taylor & Fran.
- Öztürk, Ş., & İlkiz, R. (2004). Some biological properties of mosquitofish populations (*Gambusia affinis*) living in inland waters of the Western Mediterranean region of Turkey. *Turkish Journal of Veterinary and Animal Sciences*, 28, 355–361.
- Parent, L. M., DeLorenzo, M. E., & Fulton, M. H. (2011). Effects of the synthetic pyrethroid insecticide, permethrin, on two estuarine fish species. *Journal of Environmental Science and Health, Part B*, 46, 615–622. <https://doi.org/10.1080/03601234.2011.589316>
- Pasingi, N. (2021). Growth and mortality rate of Scad (*Decapterus macrerosoma*, Bleeker 1851) landed at Inengo fish Landing Base, bone Bolango, Gorontalo. *Biota: Biologi Dan Pendidikan Biologi*, 14, 74–86.
- Pasquini, A. I., Formica, S. M., & Sacchi, G. A. (2012). Hydrochemistry and nutrients dynamic in the Suquia River urban catchment's, Córdoba, Argentina. *Environmental Earth Sciences*, 65, 453–467. <https://doi.org/10.1007/s12665-011-0978-z>
- Patimar, R., Ghorbani, M., Gol-Mohammadi, A., & Azimi-Glugahi, H. (2011). Life history pattern of mosquitofish *Gambusia holbrooki* (Girard, 1859) in the Tajan River (southern Caspian Sea to Iran). *Chinese Journal of Oceanology and Limnology*, 29, 167–173. <https://doi.org/10.1007/s00343-011-0110-y>
- Pesce, S. F., & Wunderline, D. A. (2000). Use of water quality indices to verify the impact of Córdoba City (Argentina) on Suquia River. *Water Research*, 34, 2915–2926. [https://doi.org/10.1016/S0043-1354\(00\)00036-1](https://doi.org/10.1016/S0043-1354(00)00036-1)
- Pisam, M., Jammet, C., & Laurent, D. (2002). First steps of otolith formation of the zebrafish: Role of glycogen? *Cell and Tissue Research*, 310, 163–168. <https://doi.org/10.1007/s00441-002-0622-z>
- Pyke, G. H. (2008). Plague minnow or mosquito fish? A review of the biology and impacts of introduced *Gambusia* species. *The Annual Review of Ecology, Evolution, and Systematics*, 39, 171–191.
- Quintans, F., Scasso, F., Loureiro, M., & Yafe, A. (2009). Diet of *Cnesterodon decemmaculatus* (Poeciliidae) and *Jenynsia multidentata* (Anablepidae) in a hypertrophic shallow lake of Uruguay. *Iheringia*, 99, 99–115.
- R Core Team. (2022). *R: A language and environment for statistical computing*. R Foundation for statistical computing. <https://www.R-project.org/>
- Raghavan, R., Prasad, G., Anvar-Ali, P. H., & Pereira, B. (2008). Exotic fish species in a global biodiversity hotspot: Observations from river Chalakudy, part of Western Ghats, Kerala, India. *Biological Invasions*, 10, 37–40. <https://doi.org/10.1007/s10530-007-9104-2>
- Raja, M. N., & Ravikanth, G. (2020). The enemy of my enemy is still my enemy: The biological invasion and management of *Gambusia* in peninsular India. *Current Science*, 119, 1752–1759. <https://doi.org/10.18520/cs/v119/i11/1752-1759>
- Rautenberg, G. E., Amé, V., Monferrán, M. V., Bonansea, R. I., & Hued, A. C. (2015). A multi-level approach using *Gambusia affinis* as a bioindicator of environmental pollution in the middle-lower basin of Suquia River. *Ecological Indicators*, 48, 706–720. <https://doi.org/10.1016/j.ecolind.2014.09.025>
- Rautenberg, G. E., Bonifacio, A. F., Chiappero, M. B., Amé, M. V., & Hued, A. C. (2022). Genetic structure of a native Neotropical fish species: New insights about a south American bioindicator. *Archives of Environmental Contamination and Toxicology*, 83, 168–179. <https://doi.org/10.1007/s00244-022-00952-5>
- Reyna, P. B., Ballesteros, M. L., Albá, M. L., Bertrand, L., González, M., Miglioranza, K. S. B., Tatián, M., & Hued, A. C. (2019). A multilevel response approach reveals the Asian clam *Corbicula largillierti* as a mirror of aquatic pollution. *Science of the Total Environment*, 692, 175–187. <https://doi.org/10.1016/j.scitotenv.2019.07.194>
- Reznick, D., Lindbeck, E., & Bryga, H. (1989). Slower growth results in larger stoliths: An experimental test with guppies (*Poecilia reticulata*). *Canadian Journal of Fisheries and Aquatic Sciences*, 46, 108–112.
- Röpke, C., Pires, T. H. S., Zuanon, J., Freitas, C. E. C., Hernandez, M. C., Souza, F., & Amadio, S. (2021). Growth–reproduction trade-off and fecundity regulate population stability in Amazon floodplain fishes. *Freshwater Biology*, 66, 1101–1109. <https://doi.org/10.1111/fwb.13702>
- Sa-nguansil, S. (2009). *Population characteristics and diet of sailfin Molly Poecilia velifera (Regan, 1914) (Poeciliidae: Cyprinodontiformes): An alien fish invading Songkhla Lake Basin, South Thailand Suebpong*. Prince of Sonkla University.
- Santiago, A. N., Bonansea, R. I., & Valdés, M. E. (2015). Organic pollutants in the Suquia River basin. In D. A. Wunderlin (Ed.), *The Suquia River basin (Córdoba, Argentina). The handbook of environmental chemistry* (Vol. 62). Springer. https://doi.org/10.1007/698_2015_431
- Searcy, S. P., Eggleston, D. B., & Hare, J. A. (2007). Is growth a reliable indicator of habitat quality and essential fish habitat for a juvenile estuarine fish? *Canadian Journal of Fisheries and Aquatic Sciences*, 64, 681–691.
- Spanjer, A. R., Moran, P. W., Larsen, K. A., Wetzel, L. A., Hansen, A. G., & Beauchamp, D. A. (2018). Juvenile coho salmon growth and health in streams across an urbanization gradient. *Science of the Total Environment*, 625, 1003–1012. <https://doi.org/10.1016/j.scitotenv.2017.12.327>
- Spich, K., & Fey, D. P. (2022). Using otolith microstructure analysis in studies on the ecology of the early life stages of cod, *Gadus morhua* L.: A review. *Fisheries Research*, 250, 106265. <https://doi.org/10.1016/j.fishres.2022.106265>
- Subsecretaría de Recursos Hídricos de la Nación (SRHN). (2017). *Niveles Guía Nacionales de Calidad de Agua Ambiente*. Subsecretaría de Recursos Hídricos de la Nación.
- Stevenson, D. K., & Campana, S. E. (1992). Otolith microstructure examination and analysis. In *Canadian special publication of fisheries and aquatic sciences*. Canada Communication Group-Publishin.
- Turner, C. L. (1942a). A quantitative study of the effects of different concentrations of ethynyl testosterone and methyl testosterone in the production of gonopodia in females of *Gambusia affinis*. *Physiological Zoology*, 15, 263–280.
- Turner, C. L. (1942b). Morphogenesis of the gonopodial suspensorium in *Gambusia affinis* and the induction of male suspensorial characters in the female by androgenic hormones. *Journal of Experimental Zoology*, 9, 167–193.
- Valdés, M. E., Amé, M. V., Bistoni, M. D. L. A., & Wunderlin, D. A. (2014). Occurrence and bioaccumulation of pharmaceuticals in a fish species inhabiting the Suquia River basin (Córdoba, Argentina). *Science of the*

- Total Environment*, 472, 389–396. <https://doi.org/10.1016/j.scitotenv.2013.10.124>
- Vidal, N., Loureiro, M., Hued, A. C., Eguren, G., & De Mello, F. T. (2018). Female masculinization and reproductive success in *Cnesterodon decemmaculatus* (Jenyns, 1842) (Cyprinodontiforme: Poeciliidae) under anthropogenic impact. *Ecotoxicology*, 27, 1331–1340.
- Volpedo, A. V., & Vaz-dos-Santos, A. M. (2015). *Métodos de estudios con otolitos: principios y aplicaciones*. CAFP-BA-PIESCI.
- Wendelaar Bonga, S. E. (1997). The stress response in fish. *Physiological Reviews*, 77, 591–625.
- Wilson, K. L., De Gisi, J., Cahill, C. L., Barker, O. E., & Post, J. R. (2019). Life-history variation along environmental and harvest clines of a northern freshwater fish: Plasticity and adaptation. *Journal of Animal Ecology*, 88, 717–733. <https://doi.org/10.1111/1365-2656.12965>
- Wunderlin, D. A., del Díaz, M. P., Amé, M. V., Fabiana, P. S., Cecilia, H. A., & de Los Angeles, B. M. (2001). Pattern recognition techniques for the evaluation of spatial and temporal variations in water quality. A case study: Suquía River basin (Córdoba-Argentina). *Water Research*, 35, 2881–2894. [https://doi.org/10.1016/S0043-1354\(00\)00592-3](https://doi.org/10.1016/S0043-1354(00)00592-3)
- Zambrano, M. J., Rautenberg, G. E., Bonifacio, A. F., Filippi, I., Amé, M. V., Bonansea, R. I., & Hued, A. C. (2018). Effects of water quality on aspects of reproductive biology of *Cnesterodon decemmaculatus*. *Science of the Total Environment*, 645, 10–21. <https://doi.org/10.1016/j.scitotenv.2018.07.084>
- Zapfe, G. A., & Rakocinski, C. F. (2008). Coherent growth and diet patterns in juvenile spot (*Leiostomus xanthurus* Lacepède) reflect effects of hydrology on access to shoreline habitat. *Fisheries Research*, 91, 107–111. <https://doi.org/10.1016/j.fishres.2007.12.014>

How to cite this article: Zambrano, M. J., Bolzán, A. D., Bonifacio, A. F., Brito, J. M., Amé, M. V., & Hued, A. C. (2025). Age and growth patterns of the ten spotted live-bearing fish (*Cnesterodon decemmaculatus*) along a polluted freshwater system. *Journal of Fish Biology*, 106(4), 1148–1159. <https://doi.org/10.1111/jfb.16028>