



## Aquatic invasions: new record and first data on the biology of *Gambusia holbrooki* (Girard, 1859) (Pisces, Poeciliidae) in Argentina

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**Abstract:** Fish of the genus *Gambusia* have a wide distribution and are part of the 100 most harmful invasive species in the world. In Argentina, there are records of *Gambusia affinis* Baird y Girard, 1854 and *Gambusia holbrooki* Girard, 1859. The presence of *G. holbrooki* has been reported in some regions of the country. However, there is no comprehensive information available regarding the biology and habitat of this fish. The aim of this study is to report a new locality for *G. holbrooki* in Argentina and to investigate various aspects of its biology and of the ecosystem where it was captured. The research was conducted at La Helvecia shallow lake (95 ha, 33°25'23"S, 62°54'02"W), representing the first record for the province of Córdoba. Four seasonal samplings were performed. The water exhibited elevated values of conductivity (9,685  $\mu\text{S cm}^{-1}$ ) and pH (8.9), coupled with low transparency (36 cm). The maximum temperature reached 25.1°C. The total length (TL) range of *G. holbrooki* was 20 to 50 mm, with a female-to-male ratio of 2.48:1. Pregnancy stage was observed from 25 mm TL and was most prevalent in spring. The standard length (StL)-TL ratio of the population was: TL (mm) = 3.47 + 1.06 StL (n=228;  $r^2=0.96$ ). Differences in body condition indices were observed between seasons of the year ( $p<0.001$ ). *Gambusia holbrooki* exhibits a higher catch per unit effort as temperatures increase and submerged hydrophyte coverage becomes more extensive. The results obtained indicate the urgent need to reinforce management measures aimed at controlling the spread of *Gambusia* in the central region of Argentina.

**Key words:** fish, exotic species, mosquitofish, ecosystem, limnology.

**Invasiones acuáticas: nuevo registro y primeros datos sobre la biología de *Gambusia holbrooki* (Girard, 1859) (Pisces, Poeciliidae) en Argentina. Resumen:** Los peces del género *Gambusia* tienen una amplia distribución y forman parte de las 100 especies invasoras más dañinas del mundo. En Argentina existen registros de *Gambusia affinis* Baird y Girard, 1854 y *Gambusia holbrooki* Girard, 1859. La presencia de *G. holbrooki* ha sido reportada en algunas regiones del país, sin embargo, no se dispone de información conjunta sobre la biología y el hábitat de este pez. El objetivo del trabajo es reportar una nueva localidad para *G. holbrooki* en Argentina y estudiar diferentes aspectos de su biología y del ecosistema donde se capturó. La

investigación se realizó en la laguna La Helvecia (95 ha, 33°25'23"S, 62°54'02"W), siendo este el primer registro para la provincia de Córdoba. Se realizaron cuatro muestreos estacionales. El agua exhibió valores elevados de conductividad ( $9.685 \mu\text{S cm}^{-1}$ ) y pH (8,9), junto con una baja transparencia (36 cm). La temperatura máxima alcanzó los 25,1°C. El rango de longitud total (LT) de *G. holbrooki* fue de 20 a 50 mm, con una proporción hembra:macho de 2,48:1. La etapa de preñez se observó a partir de los 25 mm LT y fue más importante en primavera. La relación entre la longitud estándar (LSt) - LT de la población fue:  $\text{LT (mm)} = 3,47 + 1,06 \text{ LSt}$  ( $n=228$ ;  $r^2=0,96$ ). Se observaron diferencias en los índices de condición corporal entre estaciones del año ( $p<0,001$ ). *Gambusia holbrooki* exhibe una mayor captura por unidad de esfuerzo a medida que aumenta la temperatura y la cobertura de hidrófitos sumergidos. Los resultados obtenidos indican la urgente necesidad de reforzar las medidas de manejo enfocadas a controlar la propagación de *Gambusia* en la región central de Argentina.

**Palabras clave:** peces, especies exóticas, pez mosquito, ecosistema, limnología.

## Introduction

Freshwater fish have been widely introduced worldwide, and aquatic ecosystems are among the most affected by biological invasions, hence they are well-documented among animal taxa. While the majority of publications address specific aspects of aquatic invasions, there still exists a significant lack of integral information (Bernery *et al.* 2022).

The study of ichthyofauna in the Argentine Republic has become increasingly relevant in recent decades. In this regard, research efforts have not only prioritized species of high sporting, recreational, or environmental value but also extended to exotic or invasive species (Espínola *et al.* 2022). One of the objectives is to minimize the threats posed to natural ecosystems and the native species that inhabit them.

The vulnerability of aquatic systems and their native fauna to biological invasions is related to climatic and limnological characteristics of the region. It is well known that Argentina boasts a diverse range of aquatic environments, both natural and artificial, with varying altitudes and water characteristics. In particular, in the province of Córdoba located in the center of the country, the salinity spectrum encompasses freshwater environments such as rivers and reservoirs in the mountainous region, extending to shallow lakes with salinity levels several times higher than that of the sea (Bucher, 2006). In this context, recent studies have confirmed the presence of 42 native and 10 introduced species in Córdoba (Bistoni *et al.* 2022).

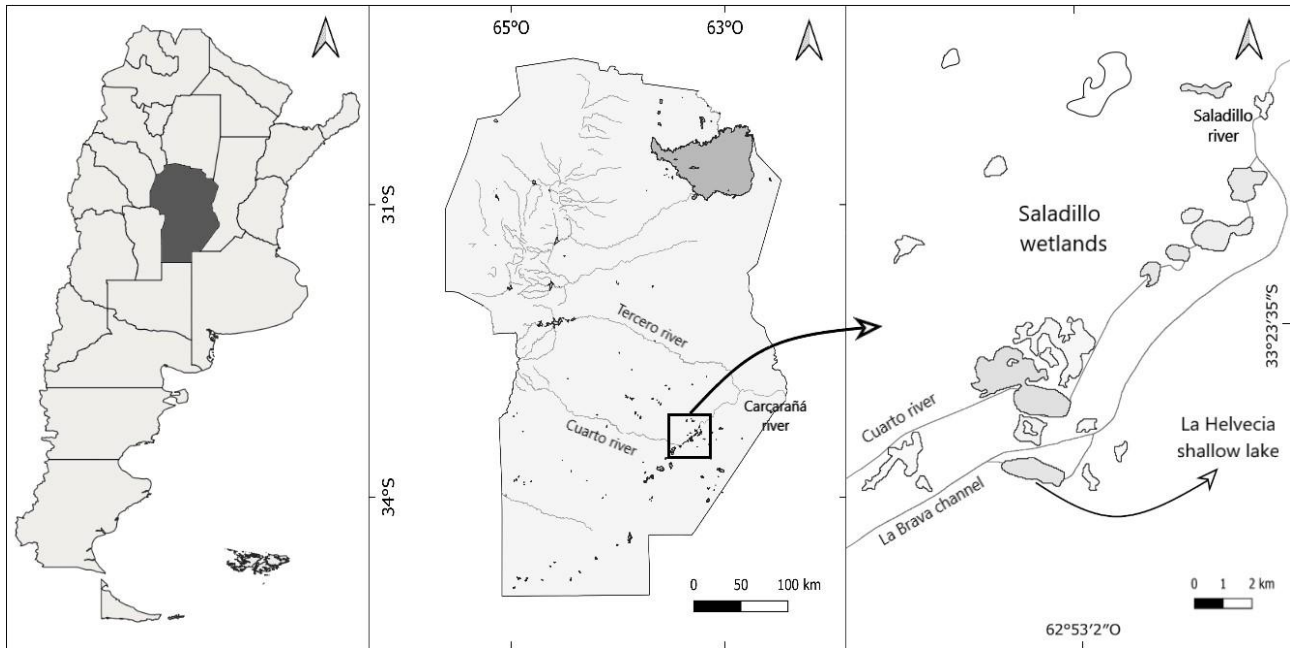
Fish of the genus *Gambusia*, commonly known as mosquitofish, have a wide global distribution. Two exotic species inhabit Argentina: *Gambusia affinis* Baird y Girard, 1854 and *Gambusia holbrooki* Girard, 1859 (Cardoso *et al.* 2015; Cabrera *et al.* 2017; Espínola *et al.* 2022),

both introduced decades ago for malaria control (Ringuelet *et al.* 1967; Menni, 2004). Despite their seemingly innocuous appearance, they are considered among the 100 most harmful invasive species worldwide. The IUCN lists them as invasive species due to their introduction as a biological control agent for mosquitoes. *Gambusia* exhibit typical characteristics of invasive species, including rapid sexual maturity, high population abundance, a varied trophic spectrum, swift colonization and wide environmental tolerance (Moreno Varcárcel, 2006).

The presence of *G. holbrooki* has been documented in some regions of Argentina (Cabrera *et al.* 2017; Andreoli Bize & Fernandez, 2019; Liotta, 2024). However, there is no comprehensive information available regarding the biology and habitat of this species. The objective of this study is to report a new locality for the *G. holbrooki* in Argentina and to investigate various aspects of its biology and the ecosystem where it was captured.

## Materials and methods

**Study area:** The research was conducted in La Helvecia shallow lake (33°25'23"S, 62°54'02"W, 95 ha), situated near Canals in the southeast region of the province of Córdoba, Argentina (Fig. 1). In the wetland areas of the Argentine Republic, La Helvecia shallow lake is situated within the Pampa region, specifically within the sub-region of brackish lakes of the Interior Pampa (Benzaquén *et al.* 2017). In terms of ichthyogeographic provinces, it is situated within the Pampean Province associated with the locality encompassed by the Tercero, Cuarto, and Carcarañá rivers (López *et al.* 2008). The area encompasses both permanent and temporary aquatic environments, constituting Saladillo wetlands. This region receives water from the Cuarto river, the Tegua-Chazón, Carnerillo and



**Figure 1.** Geographic location of La Helvecia shallow lake in the province of Córdoba (Argentina).

Chucul streams as well as contributions from the Tigre Muerto wetland and La Salada-La Brava system via the La Brava channel. Upon confluence with the Tercero river, these waters amalgamate to create the Carcarañá river (Santinelli *et al.* 2020). La Helvecia is one of the few remaining shallow lakes in the Saladillo wetlands, and it holds significant value as an emblematic environment of the region due to the ecosystem benefits it provides.

In periods of abundant precipitation, different lotic environments and shallow lakes in this region naturally connect with La Helvecia and there are also canalizations for this purpose (Santinelli *et al.* 2020). This situation allows for the movement of large quantities of different fish species, some of which are used for recreative fishing (Mancini *et al.* 2014). Limnological studies indicate that La Helvecia is an eutrophic lake with typical nutrient concentrations, algal biomass, and phytoplankton composition found in enriched systems (Izaguirre *et al.* 2015). The region has an annual mean temperature of 16.4 °C with average records of 24.0 °C and 9.2 °C for January and July, respectively. The mean annual precipitation is 871 mm, with most of it falling between the months of November to March.

**Environmental characteristics:** The average depth of the lake was assessed in March 2022 using a sounding lead along predetermined transects. In each sampling, conducted in March, May, August and October 2022, pH and conductivity were measured *in situ* using a Milwaukee MI 806 digital equipment.

Water and air temperatures, dissolved oxygen (measured with a Hanna HI 98103 oximeter), water transparency (Secchi disk) and wind speed (measured with a digital anemometer) were also recorded. In addition, a water sample was collected for its subsequent laboratory analysis, which included dissolved solids, cations, anions, hardness and alkalinity. The sample was collected, transported and analyzed in accordance with the recommendations outlined in APHA (2001).

To classify La Helvecia as clear or turbid, we used the quotient between the mean depth ( $Z_m$ ) and the depth of the photic zone ( $Z_f$ ) following the method described by Quirós *et al.* (2002). Additionally, the percentage of oxygen saturation was estimated based on the atmospheric pressure at the location and the salinity of the water. The hydrophyte species were identified and their abundance was quantified using the Braun-Blanquet coverage scale as outlined by Mazzeo (1999).

**Fish capture and analysis:** Fish were captured in the littoral zone using rectangular hand nets (70 × 45 cm, 1 mm mesh). Subsequently, they were transported alive to laboratory and then narcotized with a benzocaine solution at a concentration of 300 mg l<sup>-1</sup>. *Gambusia* species identification followed the methods of Walters & Freeman (2000) and Cabrera *et al.* (2017), which considered the morphology of the gonopodium (Rosen & Bailey, 1963) and the number of rays of the dorsal and anal fins according to Rivas (1963). This process involved the use of a

stereo microscope and microscope. Standard length (StL) and total length (TL) were measured with a precision of 0.1 mm and the weight was recorded with a digital scale accurate to 0.001g. Using the length and weight data, the StL-TL and StL-Weight relationships were calculated, along with the Fulton and LeCren index (Froese, 2006; Erguden, 2013; Eagderi & Radkhah, 2015; Perez Bote & López, 2005). An aliquot of the captured specimens was deposited in the Ichthyological Collection of the Faculty of Agronomy and Veterinary (National University of Río Cuarto). The Kruskal - Wallis test was employed to determine whether there were significant differences in length, weight and condition indices between seasons. The catch per unit effort (CPUE) was calculated as the average number of fish caught per sampling and per fishing effort. Principal components analysis (PCA) was conducted to identify general trends and relationships between environmental variables and CPUE.

## Results

The mean depth of the lake was 1.62 m. Throughout the annual cycle, the Secchi disk reading consistently remained below 42 cm, water temperature exhibited significant variations and the pH remained alkaline. Afternoon oxygen concentration readings consistently showed supersaturation and the conductivity reached a maximum value of 11,310  $\mu\text{S cm}^{-1}$  (Table I), coinciding with the highest salinity value (7.75  $\text{g l}^{-1}$ ). Water hardness reached its maximum during autumn (Table II). Two aquatic macrophyte species, *Schoenoplectus californicus* (C.A. Mey.) Soják and *Stuckenia striata* (Ruiz & Pav.) Holub were identified. The area occupied by *S. californicus* remained unchanged across all four seasons. However, *S. striata*, characterized by its "spots", experienced a substantial reduction in these features from summer to winter.

**Table I.** Environmental characteristics analyzed *in situ* in La Helvecia shallow lake.

Variable		Summer	Autumn	Winter	Spring
Transparency	cm	42	23	37	42
pH	pH	8.34	8.75	9.12	9.40
Oxygen	$\text{mg l}^{-1}$	8.32	11.90	11.65	8.95
Saturation	%	101	128	107	112
Water Temperature	$^{\circ}\text{C}$	23.8	16.8	9.3	25.1
Wind speed	$\text{km/h}$	5.6	27.0	19.4	4.5
Air Temperature	$^{\circ}\text{C}$	21.6	14.5	8.9	28.9
Conductivity	$\mu\text{S cm}^{-1}$	7,320	10,970	9,140	11,310
Macrophytes coverage	Cat.	IV	II	I	III

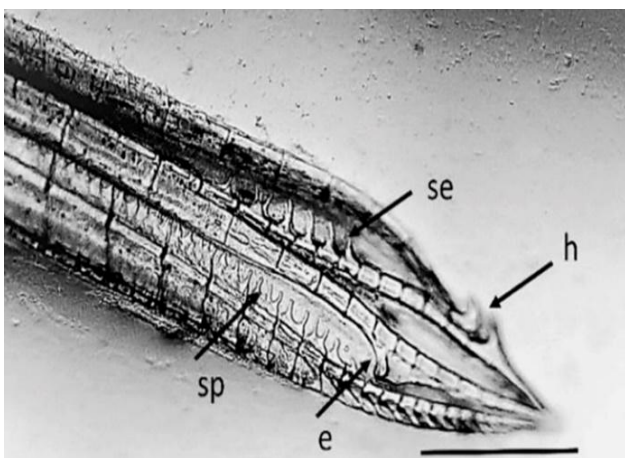
**Table II.** Chemical characteristics of the water of La Helvecia shallow lake.

Determination	Unit	Summer	Autumn	Winter	Spring
Total Salts	$\text{g l}^{-1}$	5.23	7.25	7.53	7.75
Carbonates	$\text{mg l}^{-1}$	3	51	51	150
Bicarbonates	$\text{mg l}^{-1}$	415	598	550	382
Sulfates	$\text{mg l}^{-1}$	1,826	2,173	2,428	1,812
Chlorides	$\text{mg l}^{-1}$	1,178	1,500	1,785	2,300
Sodium	$\text{mg l}^{-1}$	1,638	2,654	2,548	2,952
Potassium	$\text{mg l}^{-1}$	62	76	27	81
Calcium	$\text{mg l}^{-1}$	46	55	23	15
Magnesium	$\text{mg l}^{-1}$	31	60	42	47
Nitrate	$\text{mg l}^{-1}$	24	75	69	s/d
Fluoride	$\text{mg l}^{-1}$	1.1	4.1	1.9	1.1
Total Hardness	$\text{ppm CO}_3\text{Ca}$	244	384	228	228
Alkalinity	$\text{ppm CO}_3\text{Ca}$	336	563	524	553

All analyzed specimens displayed dorsal and caudal fins with one to three rows of small rounded spots. In some cases, a subocular spot was present, and all mature females exhibited anal spot, although it was not clearly visible in specimens in 70% alcohol (Fig. 2). The counting of fin rays and lateral line scales is detailed in Table III. The gonopodium morphology in all analyzed specimens revealed the presence of small spines in the third fin-ray, where the number of spines per segment (one in the first) appearing to increase toward the cranial end. It also has an elbow and serrae in the fourth ray, as well as terminal hooks in the rays 4 and 5 (Fig. 3).



**Figure 2.** Specimens of *Gambusia holbrooki* from La Helvecia shallow lake (Argentina): a) gravid female with its typical anal spot; b) female and c) male specimens in 70% alcohol. Scale: 10 mm.



**Figure 3.** Gonopodium of *Gambusia holbrooki* observed under microscope (se: serrae; h: hooks; e: elbow; sp: spines). Scale: 1 mm.

**Table III.** Fin rays and lateral line scales number of *Gambusia holbrooki* from La Helvecia shallow lake.

Specimen /Sex	Dorsal	Anal	Caudal	Scales
<b>Male</b>				
n	8	11	23	31
10	(7-8)	(10-11)	(21-25)	(31-33)
<b>Female</b>				
n	8	11	23	31
10	(7-8)		(22-24)	(30-33)

*Gambusia holbrooki* represented the 28.6 % of the total captures (Table IV). The StL, TL and weight ranges are detailed in Table V, with a TL range of 20 to 32 mm for males and 20 to 50 mm for females. The standard length (StL)-TL ratio of the population was: TL (mm)= 3.47 + 1.06 StL (n=228; r<sup>2</sup>=0.96). The lengths and weights of the analyzed fish did not show significant differences between samplings (p>0.05), but they did between sexes (p<0.01). Of the total fish caught, there were more females than males (ratio 2.48:1). Pregnancy was at its maximum during spring and absent during winter and it was observed in fish with a length above 25 mm TL.

The relationship between length and weight is detailed in Table VI, with females consistently exhibiting greater growth. The Fulton index for the entire population revealed significant differences between males and females (p<0.001). The Le Cren index also displayed significant differences between samplings (p<0.001) with the highest value observed in spring (Fig. 4).

Principal Components Analysis revealed that CPUE during autumn and winter exhibited different behavior compared to that during spring and summer. The latter was found to be associated with temperature, water transparency (Secchi disk) and macrophyte coverage (Fig. 5).

**Discussion**

The central region of Argentina exhibits a considerable diversity of aquatic environments. The average depth of La Helvecia falls within the range observed for shallow lakes in the Pampean region as described in Quirós *et al.* (2002). In terms of water transparency, this environment is classified as turbid, which aligns with previous studies conducted at this site (Mancini *et al.* 2014). The high pH values and other chemical variables analyzed are also

**Table IV.** CPUE of different species captured in the littoral zone of La Helvecia shallow lake (A= absence; Su=summer; Au=autumn; Wi=winter; Sp=spring).

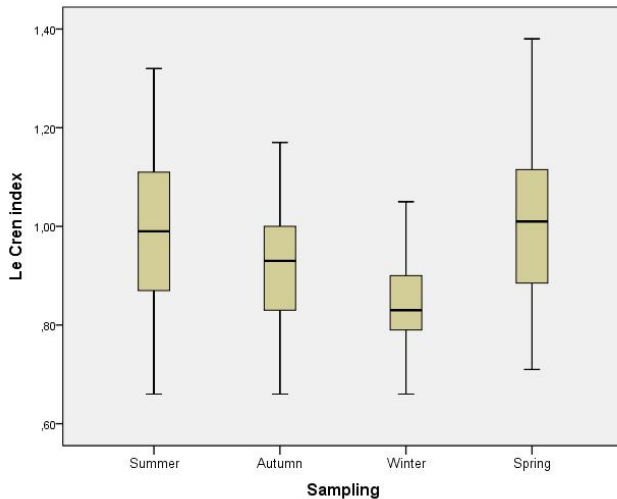
Species	Summer	Autumn	Winter	Spring
<i>Cnesterodon decemmaculatus</i> Jenyns,1842	5.2	7.1	12.1	49.0
<i>Jenynsia lineata</i> Jenyns, 1842	4.0	4.8	0.8	1.0
<i>Australoheros facetus</i> Jenyns, 1842	4.7	A	A	A
<i>Gambusia holbrooki</i> Girard, 1859	11.2	7.5	6.3	10.6

**Table V.** Standard length (StL), total length (TL) and weight records of *Gambusia holbrooki* from La Helvecia shallow lake.

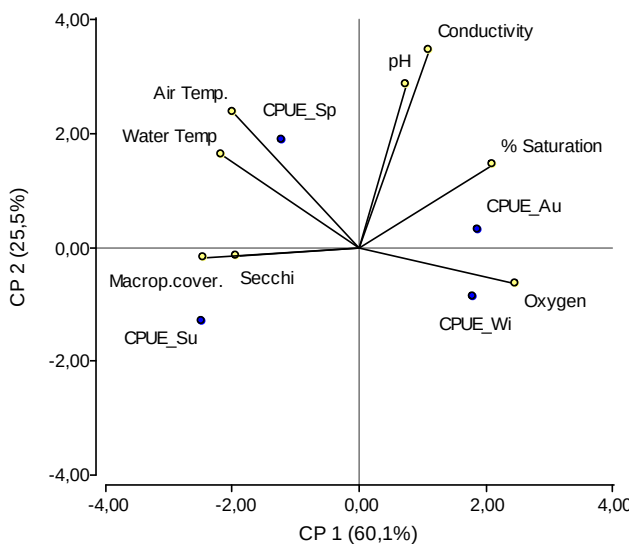
Variable	Summer	Autumn	Winter	Spring
n	45	90	93	52
<b>StL (mm)</b>				
Mean ± SD	27 ± 7.7	25.6 ± 5.5	26.4 ± 3	26.6 ± 6.1
Minimum	16	17	18	17
Maximum	43	42	32	39
<b>TL (mm)</b>				
Mean ± SD	32.2 ± 8.5	30.2 ± 5.9	30.5 ± 3.5	30.7 ± 6.6
Minimum	20	22	23	20
Maximum	50	49	39	45
<b>Weight (g)</b>				
Mean ± SD	0.43 ± 0.35	0.29 ± 0.19	0.25 ± 0.09	0.36 ± 0.25
Minimum	0.08	0.08	0.09	0.09
Maximum	1.45	1.07	0.55	0.93
<b>Sex</b>				
Ratio f/m	10.2:1	2.7:1	3.4:1	0.7:1
Pregnancy (%)	39.0	4.5	0	90.4

**Table VI.** Total length-weight and standard length-total length relationship, and Fulton index of *Gambusia holbrooki* from La Helvecia shallow lake.

Variables	Summer	Autumn	Winter	Spring
<b>TL-Weight</b>				
<b>Males</b>				
a		4.348*10 <sup>-6</sup>	3.158*10 <sup>-5</sup>	2.064*10 <sup>-5</sup>
b		2.54	2.60	2.76
r <sup>2</sup>		0.82	0.90	0.89
<b>Females</b>				
a	3.946*10 <sup>-6</sup>	6.958*10 <sup>-6</sup>	7.889*10 <sup>-6</sup>	4.338*10 <sup>-6</sup>
b	3.26	3.08	3.02	3.25
r <sup>2</sup>	0.98	0.95	0.94	0.93
<b>Both Sexes</b>				
a	3.786*10 <sup>-6</sup>	8.854*10 <sup>-6</sup>	8.917*10 <sup>-6</sup>	3.40*10 <sup>-6</sup>
b	3.27	3.02	2.98	3.31
r <sup>2</sup>	0.98	0.95	0.93	0.94
<b>StL - TL</b>				
a	2.83	3.02	2.92	2.22
b	1.08	1.06	1.12	1.07
r <sup>2</sup>	0.98	0.96	0.95	0.98
Fulton index	0.99 ± 0.13	0.92 ± 0.11	0.84 ± 0.08	1.01 ± 0.15



**Figure 4.** Le Cren index of *Gambusia holbrooki* at different seasons from La Helvecia shallow lake.



**Figure 5.** Analysis of principal components (biplots) of the environmental variables and CPUE from La Helvecia shallow lake.

characteristic of these particular aquatic environments of Argentina (Izaguirre *et al.* 2014). Oxygen saturation percentages consistently exceeded the reference value, considering the time of measurement, prevailing winds, and the water turbidity of La Helvecia with high chlorophyll *a* concentration (Izaguirre *et al.* 2014). In terms of salinity and hardness, the water was classified as mesohaline and hard (Conzono, 2009). In this regard, the salinity values were much higher than the average of 35 shallow lakes in the central region of Argentina (Mancini *et al.* 2016a), but they are characteristic of the Saladillo wetlands region, where maximum values of  $7.22 \text{ g l}^{-1}$  (Rodríguez *et al.*

2000) and  $10,400 \mu\text{S cm}^{-1}$  (Mancini *et al.* 2016b) have been reported.

According to the geographical distribution of Argentina, divided into provinces, *G. holbrooki* was not listed among the recently confirmed exotic species in the province of Córdoba (Bistoni *et al.* 2022; Liotta, 2024). In this context, previous reports of *G. affinis* might have been misidentifications of *G. holbrooki* due to the high similarity between the two species (Odagiu *et al.* 2020). According to Rauchenberger (1989) and Walters & Freeman (2000), the count of the caudal and anal rays in females and the morphology of the gonopodium of the specimens studied here are characteristics of *G. holbrooki* and is consistent with reports from nearby localities corresponding to the provinces of San Luis and Santa Fe. These reports have been further confirmed through molecular studies (Cabrera *et al.* 2017). Conversely, the count of caudal rays and lateral line scales overlaps with *G. affinis* and it is in line with what has been indicated in Qoraqulovich *et al.* (2022), therefore, these characteristics were not considered sufficient to distinguish between the species.

The ratio of females in the population of *G. holbrooki* from La Helvecia is consistent with findings from numerous studies. However, it is important to note that this aspect may not always be observed in all populations (Perez Bote & López, 2015; Kurtul & Sari, 2020).

*Gambusia holbrooki* exhibits a wide range of the *b* value corresponding to the length-weight relationship, with females generally showing isometric or positive allometric growth (Kurtul & Sari, 2020), as observed in this study. Variations in body condition over the year are likely related to environmental factors, such as temperature, which can increase metabolism associated with greater prey consumption and reproductive phenomena (Perez Bote & López, 2015). This was observed in La Helvecia, where the highest body condition was recorded in the two seasons where the temperature and the pregnancy percentage were higher. Differences in Fulton index, favoring females, were also noted by Erguden (2013).

The introduction of *G. holbrooki* to this environment may have occurred during the great floods in recent years that impacted the Bañados del Saladillo region (Santinelli *et al.* 2020). The physical-chemical characteristics of the water in this region do not seem to limit the invasive success of the species, given its remarkable ability to adapt to environmental variables (Pike, 2005; Alcaraz &

García Berthou, 2007; Ruiz-Navarro *et al.* 2011). The threat of its expansion is further exacerbated by the existing natural and anthropic connections that allow the movement of fish between numerous lotic and lentic environments (Marzuoli *et al.* 2023). In this sense, furthermore, the percentage cover of *S. striata* varied between the seasons of the year and was related to mosquitofish CPUE, which increased at higher temperatures, in accordance with the observations of Pike (2005).

In conclusion, our findings confirm a new record of *G. holbrooki* in Argentina, and concurrently, this represents the first official report of the species in the province of Córdoba. The population exhibits a wide range of sizes (20 to 50 mm TL), with a female-to-male ratio of 2.48, varying body conditions and pregnancy rates throughout the year. *Gambusia holbrooki* reproduces for much of the year after females reach a size of 25 mm LT. All the environmental variables analyzed, even with maximum levels of water conductivity exceeding 11,000  $\mu\text{S cm}^{-1}$ , appear to be well-tolerated by this exotic species, which is recognized as one of the most invasive in the world. It shares its microhabitat in the littoral zone with native species *Cnesterodon decemmaculatus*, *Jenynsia lineata* and *Australoheros facetus*. The results obtained indicate the urgent need to reinforce management measures aimed at controlling the spread of *Gambusia* in the central region of Argentina, taking extreme precautions, especially with biological controls of mosquitoes and the use of bait fish in the recreational fisheries.

### Ethics statement

Collection of fish and other biological samples were conducted following all applicable ethical regulations regarding the use and experimentation with animal.

### Acknowledgments

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