



## Ecology and implications of parasitism by *Lernaea cyprinacea* (Crustacea: Copepoda) on Argentinian silverside *Odontesthes bonariensis* (Teleostei: Atherinopsidae)

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**Abstract.** *Lernaea cyprinacea* is a cosmopolitan copepod that affects a wide variety of fish. Parasite infestations severity is influenced by environmental factors and varies among fish species. The Argentinian silverside (*Odontesthes bonariensis*) is one of the most important fish in Argentina's fisheries. The main characteristics of the water of a Pampean shallow lake in central Argentina (34°38'S, 63°44'W) and different parameters of *L. cyprinacea* in *O. bonariensis* were evaluated. The prevalence of *L. cyprinacea* was of 44.9%, with an average abundance and intensity of 2.2 and 4.0 parasites per fish respectively, with no preference found for the fixation site. A positive correlation was observed between the number of parasites vs. length and fish age. The relative weight (*Wr*) of the parasitized vs. non-parasitized fish did not show significant differences. The growth parameters of the *O. bonariensis* evaluated were framed within characteristic values. It is concluded that *L. cyprinacea* does not alter the body condition of the *O. bonariensis* with the observed parasitic intensity and water characteristics (2.98 ±0.16 g.L<sup>-1</sup> of salinity and temperature of 17.1 ±1.5 °C). However, esthetic deterioration is observed in fish with high parasitic load.

**Key words:** Anchor worm, Ectoparasite, Argentinian silverside, Pampean shallow lake.

**Resumen:** Ecología e implicaciones del parasitismo por *Lernaea cyprinacea* (Crustacea: Copepoda) en el pejerrey argentino *Odontesthes bonariensis* (Teleostei: Atherinopsidae).

*Lernaea cyprinacea* es un copépodo cosmopolita que afecta a una gran variedad de peces. La gravedad de las infestaciones del parásito está influenciada por factores ambientales y varía entre especies de peces. El pejerrey (*Odontesthes bonariensis*) es uno de los peces más importantes de las pesquerías de Argentina. Se evaluaron las principales características del agua de una laguna pampeana del centro de Argentina (34°38'S, 63°44'W) y diferentes parámetros de *L. cyprinacea* en *O. bonariensis*. La prevalencia de *L. cyprinacea* fue de 44,9%, con una abundancia e intensidad promedio de 2,2 y 4,0 parásitos por pez respectivamente, sin hallarse preferencia por el sitio de fijación. Se observó una correlación positiva entre el número de parásitos vs. longitud y edad de los peces. El peso relativo (*Wr*) de los peces parasitados vs. no parasitados no arrojó diferencias significativas. Los parámetros de crecimiento de *O. bonariensis* evaluados se encuadraron dentro de los valores característicos. Se concluye que *L. cyprinacea* no altera la condición corporal de *O. bonariensis* en la intensidad parasitaria y

características del agua observadas ( $2,98 \pm 0,16$  g.L<sup>-1</sup> de salinidad y temperatura de  $17,1 \pm 1,5$  °C), sin embargo, se observa un deterioro estético en peces con alta carga parasitaria.

**Palabras clave:** Ectoparásito, Gusano ancla, Laguna pampeana, Pejerrey argentino.

## Introduction

The cyclopoid copepods include a wide variety of freshwater species and some of them are fish parasites. In Argentina, one of the most frequent is *Lernaea cyprinacea* (Linnaeus, 1758), which was identified by its morphology and by molecular techniques (Mancini *et al.* 2008a, Soares *et al.* 2018). This copepod affects fish for human consumption, as well as aquarium, farmed and wild fish from a wide geographical area, with a tendency towards an increase in cases, of the number of affected species (Mancini *et al.* 2008a) and their geographical distribution (Waicheim *et al.* 2019).

*Lernaea cyprinacea*, known commonly as anchor worm, is a cosmopolitan copepod that affects a wide variety of fish (Sanchez-Hernández 2017, Hossain *et al.* 2018, Waicheim *et al.* 2019). The “anchoring” site of this ectoparasite is variable, some authors suggest that fins constitute the most frequent fixation zones, but it can also be found in gills, mouth, and nostrils (Gutiérrez-Galindo & Lacasa-Millán 2005, Acosta *et al.* 2013, Guagliardo & Tanzola 2016, Idoumou *et al.* 2019). Its degree of pathogenicity is variable, it produces injuries in the skin that lead to secondary bacterial and fungal infections. In small fish species it can have serious and even lethal effects with high impact at a population level (Guagliardo & Tanzola 2016). The highest parasitic intensity is not always associated to the size and age of the host fish and sometimes affects the body condition (Gutiérrez-Galindo & Lacasa-Millán 2005, Barson *et al.* 2008, Sánchez-Hernández 2011).

The gravity of the infestations of *L. cyprinacea*, as well as the prevalence, intensity, and abundance are influenced by environmental factors among which pH, temperature, and the concentration of dissolved oxygen in the water stand out (Dalu *et al.* 2012, Sánchez Hernández 2017). Other authors also highlight the role of salinity in the survival of *L. cyprinacea* (Idris & Amba 2011, Maceda-Veiga *et al.* 2019). In this sense, the limnologic characteristics where the host fish inhabit play an important role in the presence/absence of the parasite (Welicky *et al.* 2017).

The Argentinian silverside *Odontesthes bonariensis* (Valenciennes, 1835) locally known as pejerrey (view Fig. 3a), is the fish with greatest

importance in the centre-northern region of Argentina due to the intense social and economic movement that is generated by recreational fishing and the capture of hundreds of tons of protein for consumption that are extracted annually through that modality (Mancini *et al.* 2016a). This fish inhabits a wide variety of environments with different limnologic characteristics, especially freshwater reservoirs and Pampean shallow lakes. Even though the latter constitute the habitat par excellence of the silverside, they possess a wide range of salinity, which is why the largest number of cases of lerneosis refer to reservoirs (Mancini *et al.* 2008a,b, Bethular *et al.* 2014, Mancini *et al.* 2019).

Although studies exist on the presence of *L. cyprinacea* in *O. bonariensis*, these do not address different aspects of ecology, nor were epidemiologic records of this parasite found at the population level. The objectives of this work were to evaluate: a) the main characteristics of water of the study environment; b) different parameters of *L. cyprinacea* in the population of Argentinian silverside and, c) the clinical signs that the parasite produces and if it affects the body condition of the fish.

## Materials and Methods

**Study area and water characteristics:** The studied environment is a representative Pampean shallow lake, located in Córdoba province, in the central region of Argentina (34°38'S, 63°44'W). The following water characteristics were evaluated *in situ* and in laboratory (n = 5): temperature, pH (digital pH meter Milwaukee MI 806, Romania), dissolved oxygen (digital oximeter Hanna HI 98193, Romania), water transparency (Secchi disk), salinity, alkalinity, and hardness (APHA, 1992). To classify the lake as “clear” or “turbid”, the quotient between the average depth of the lake ( $Z_m$ ) and the depth of the photic zone ( $Z_p$ ) was used (Quirós *et al.* 2002).

**Fish collection and analysis of population biological parameters:** Fish were caught with trawl and gillnets during March, 2018. In fish of different size intervals, the standard (StL) and total (TL) length was registered with an ichthiometre graduated in mm and the weight with a digital scale with 0.1 g precision. Moreover, the standard length-weight relation was calculated,  $W = a \cdot \text{StL}^b$ , where W is the

weight,  $a$  is the intercept and  $b$  the slope obtained by means of the regression analysis, previous logarithmic transformation according to the  $\log P = \log a + b \log \text{StL}$  model (Froese 2006). The age of the fish was defined by means of scale reading and the back-calculation method was applied to estimate mean lengths at different past ages. Growth was estimated by means of the von Bertalanffy equation:  $\text{StL} = L_{\infty} * (1 - e^{-k*(t-t_0)})$  where  $L_{\infty}$  is the asymptotic length,  $k$  is the growth rate and  $t_0$  is the theoretical time in which the length of the fish is zero (Moreau 1987). The relative weight ( $Wr$ ) for individuals greater than a 120 mm StL was used to describe body condition (Colautti *et al.* 2006), who sustain that this index does not produce biases based on the length of the fish.

To assess the degree of parasitism, we proceeded to the detailed inspection and observation of fins, skin, gills, operculums, nostrils, and mouth following the methodology described by Noga (1996) and Eiras *et al.* (2003). The prevalence, mean abundance and mean intensity were calculated according to Bush *et al.* (1997), grouping fish by age class. A non-parametric correlation analysis was performed (Spearman's rho,  $r_s$ ) to evaluate the existent relationship between the number of parasites in silversides with their standard length, age, and  $Wr$ . The Kruskal-Wallis statistic test was also applied (Sokal & Rohlf 2002) to check if differences existed in the number of parasites according to the age of the fish. Finally, the Mann-Whitney U test (Siegel & Castellan 1995) was applied to verify the existence of significant differences between the  $Wr$  of the parasitized and non-parasitized individuals.

## Results

The water characteristics during the present study are presented in Table I.

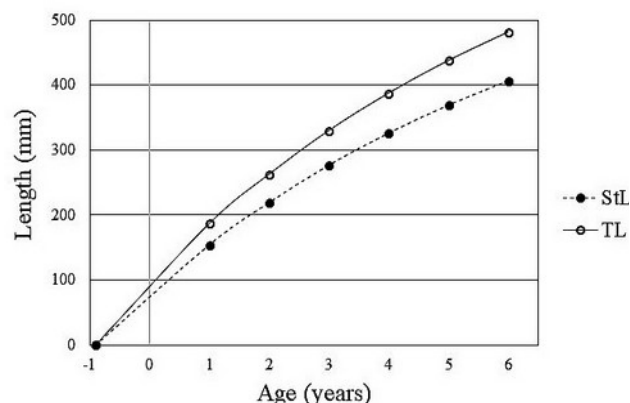
A total of 89 Argentinian silversides were analyzed and a summary (mean and range) of the measured StL, TL, and weight can be observed in Table II. The length-weight relationship of the evaluated population was,  $P = 0.00000964 * \text{StL}^{3.118}$  ( $R^2 = 0.99$ ; CI of  $b = 3.07-3.15$ ). The presence of silversides of up to 6 years old was found. The estimated growth parameters were  $L_{\infty} = 677.5$  mm,  $k = 0.13$ , and  $t_0 = -0.95$ . The growth curve as a function of the age of the fish is presented in Figure 1. Excluding extreme values (outliers), the average  $Wr$  of *O. bonariensis* was  $101.6 (\pm 5.0)$ .

**Table I.** Water characteristics analyzed (mean values and standard deviation).

Variable	Unit	Mean ( $\pm$ S.D.)
Transparency	cm	23.7 ( $\pm 5.6$ )
pH	pH	9.05 ( $\pm 0.21$ )
Oxygen	mg.L <sup>-1</sup>	8.77 ( $\pm 1.26$ )
Water Temperature	°C	17.1 ( $\pm 1.5$ )
Salinity	g.L <sup>-1</sup>	2.98 ( $\pm 0.16$ )
Total hardness	ppm CO <sub>3</sub> Ca	390 ( $\pm 19$ )
Alkalinity	ppm CO <sub>3</sub> Ca	205 ( $\pm 22$ )

**Table II.** Standard length, total length, and weight of the studied silversides (n= 89).

Variable	Unit	Mean ( $\pm$ S.D.)	Minimum - Maximum
StL	mm	207 ( $\pm 133$ )	63 – 419
TL	mm	239 ( $\pm 158$ )	78 – 504
Weight	g	283.8 ( $\pm 363.0$ )	2.8 – 994.0



**Figure 1.** Length of *Odontesthes bonariensis* at different ages.

The prevalence of *L. cyprinacea* in the population of *O. bonariensis* was 44.9% with an average abundance and intensity of 2.2 and 4.0 parasites per fish respectively. The separation of these indicators by fish age is shown in Table III. The parasite was observed from the size of 149 mm of StL (180 mm of TL) onwards. The maximum intensity (16), was registered in a specimen of 5+ years of life. Considering all examined individuals, a significant positive correlation was found between the number of parasites vs. StL ( $r_s = 0.72$ ;  $p = 0.000$ ) and the fish age ( $r_s = 0.76$ ,  $p = 0.000$ ). On the other hand, when analyzing the average abundance of *L.*

**Table III.** Population indicators of *Lernaea cyprinacea* in Argentinian silversides of different ages.

Reference/Age	Age (years)							Total
	0+	1+	2+	3+	4+	5+	6+	
Prevalence	3.1	25.0	66.6	100.0	91.3	93.7	88.8	44.9
Mean abundane	0.03	0.25	1.00	1.50	3.30	4.75	4.56	2.2
Mean intensity	1.00	1.00	1.50	1.50	3.62	5.07	5.13	4.0

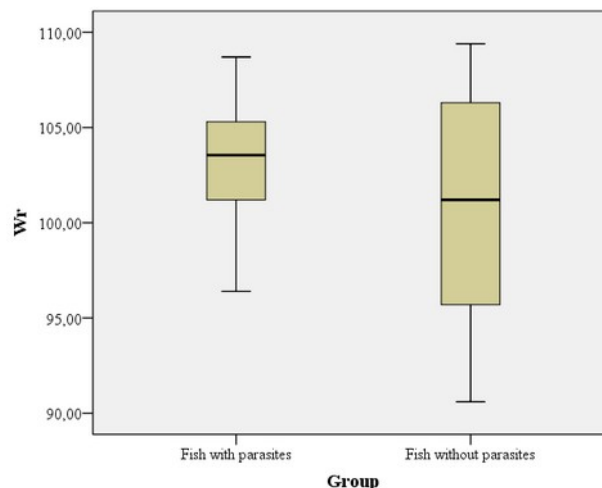
*cyprinacea* with the age of *O. bonariensis*, a high correlation was obtained ( $r_s = 0.96$ ;  $p = 0.000$ ). The number of parasites presented significant difference according to the age of the silversides ( $p < 0.001$ ). The parasitic intensity showed no correlation with the body condition ( $r_s = -0.054$ ,  $p > 0.05$ ). Lastly, significant differences were not found ( $p > 0.05$ ) between the relative weight of the parasitized ( $Wr = 100.9$ ) vs non-parasitized fish ( $Wr = 103.1$ ) (Fig. 2).

There was no preference found for the fixation site of the parasite. *Lernae acyprinacea* was present in the base of the anal fin (Fig. 3b), at the level of the stole below the dorsal fin and close to the pectoral fin (Figs. 3c and 3d), even contiguous to the eye (Fig. 3e) and inside of the operculum, but not in the gills. In the insertion area, small ulcers with hyperemia, fibrosis, and petechial and diffuse hemorrhages were observed in the skin (Fig. 3f). The latter was present in 31% of the parasitized silversides coinciding with those with the highest age and parasitic intensity.

## Discussion

The studied environment is framed within the turbid shallow lake, with water of hypohaline characteristics and very hard (Quirós *et al.* 2002, Conzonno 2009). The pH, oxygen, temperature and salinity values are framed within the expected values for the Pampean shallow lakes, which constitute the par excellence habitat of *O. bonariensis* (Mancini *et al.* 2016b).

The infestation by *L. cyprinacea* can show significant differences in the different species that live in the same environment (Raissy *et al.* 2013). Even though it is recognized that the increase in the water temperature in summer season is reflected in the intensity increase of *L. cyprinacea* in Argentinian silverside (Mancini *et al.* 2008b, Bethular *et al.* 2014), some authors suggest that in certain species it is greater during the cold and dry season with a range from 19 to 24 °C (Dalu *et al.* 2012). It is observed that to values proximate to 17 °C, lerneosis is manifested in *O. bonariensis*, even

**Figure 2.** Relative weight ( $Wr$ ) boxplot of the Argentinian silversides with and without *Lernaea cyprinacea*.

though that variable is significantly less than the optimum reported for the propagation of the parasite according to Innal *et al.* (2017). On the other hand, the oxygen and pH values present in the water could promote the proliferation of *L. cyprinacea* according to Dalu *et al.* (2012).

Concerning salinity and according to the results obtained, the concentration of 3 g.L<sup>-1</sup> does not affect the survival of *L. cyprinacea*, which coincides with Hossain *et al.* (2018), who reported that salinity greater than 7 g.L<sup>-1</sup> would be necessary to prevent at least reproduction of the copepod. In this sense, it is important to highlight that although salinity increase affects the survival of *L. cyprinacea*, it can also affect the body condition of stenohaline fish (Idris & Amba 2011), which is an advantage for the silverside, since it is a species of marked euryhalinity (Mancini *et al.* 2016a).

Many authors have reflected the existing relationship between the infestation of *L. cyprinacea* with the age, weight, and size of the fish. The studied length-weight relation and the growth of the population of *O. bonariensis* is framed within the characteristic parameters of the species (Mancini *et*



**Figure 3.** Image of a specimen of *Odontesthes bonariensis* (a); *Lernaea cyprinacea* in the base of the anal fin (b); at the level of the stole (c); next to the pectoral fin (d) and the eye (e); superficial hemorrhages in an individual with high parasitic intensity (f).

*al.* 2016a). In this study, the presence of the copepod increases with the size and age of the silverside, unlike what happens in several cyprinid species where no significant relationship was found between these variables (Raissy *et al.* 2013), as well as in the brown trout (*Salmo trutta*, Linnaeus 1758) according to what was expressed by Sánchez-Hernandez (2011). Innal *et al.* (2017), also found no significant differences in the prevalence and intensity of *L. cyprinacea* among different ages in various fish species in the same environment, perhaps due to the size of the samples of each species analyzed.

Parasitic copepods feed on blood and body fluids of the hosts, high infestations by *Lernaea* can produce fatal cases in some fish (Hossain *et al.* 2013). The body condition expressed through the relative weight (*Wr*) of *O. bonariensis* did not present significant differences between parasitized

and non-parasitized fish, results that agree with other populations of the same species (Mancini *et al.* 2008a), but not with observations of Sánchez-Hernandez (2011), who reported differences in the Fulton condition factor among wild groups of brown trout (*S. trutta*).

Regarding the fixation site of *L. cyprinacea*, no preference was observed in the present study, being reported in different parts of the body. The location next to the eye agrees with Smith (2019), who maintains that occasionally it can parasitize the cornea or the surrounding tissues of the eye and also cause the presence of fibrosis and hemorrhages, a situation observed in the most parasitized fish. It is important to remember that in silversides and others fish with lerneosis, these hemorrhages have been associated with infections by opportunistic bacteria

such as *Aeromonas hydrophila* (Stainer 1943, Mancini *et al.* 2006, Hossain *et al.* 2013).

It is concluded that, although the intensity of *L. cyprinacea* increases positively with the age and size of the *O. bonariensis*, and that it also aesthetically affects the most parasitized individuals, its presence does not alter the body condition of this species in the parasitic intensity observed.

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