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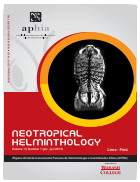

ORIGINAL ARTICLE / ARTÍCULO ORIGINAL

SPATIAL DISTRIBUTION OF TWO DIGENEA METACERCARIAE IN GILLS OF THE SILVERSIDE *ODONTESTHES ARGENTINENSIS* (ATHERINIFORMES) FROM *DE LA PLATA* RIVER (URUGUAY, SOUTHWESTERN ATLANTIC OCEAN)

DISTRIBUCIÓN ESPACIAL DE DOS METACERCARIAS DE DIGENEOS EN BRANQUIAS DEL PEJERREY *ODONTHESTES ARGENTINENSIS* (ATHERINIFORMES) DEL RIO DE LA PLATA (URUGUAY)

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ABSTRACT

The silverside (*Odonthestes argentinensis* Valenciennes, 1835) is an important aquatic resource in coasts of *de la Plata* River both in Argentina and Uruguay. This fish species acts as intermediate host of several parasites, included trematode metacercariae. It was found in gills of silversides collected in Uruguayan waters, metacercariae of *Ascocotyle* sp. Looss, 1899 (Heterophyidae) and *Stephanoprora uruguayensis* Holcman-Spector & Olagüe, 1989 (Echinostomatidae). The aim of this study was determinate the prevalence and mean intensity of digenean metacercariae in the gills of *O. argentinensis* from the Uruguayan coast of de la Plata River, its relationship with fish size and sampling season, and to describe the spatial distribution of that metacercariae in gills. A total of 279 fish were examined. The prevalence and mean intensity of the parasites was 53.04% and 26.38 respectively for *S. uruguayensis* and 34.05% and 11.38 for *Ascocotyle* sp. We found a different distribution in the metacercariae between fore and rear gill arches. While *S. uruguayensis* is distributed mainly in the gills archs 1 and 2; *Ascocotyle* sp. are mainly in the gills archs 3 and 4. Although *S. uruguayensis* metacercariae were mainly found in the proximal region of the gill filament, (K-S = 4.80; $p<0.01$) and *Ascocotyle* sp., which was mainly present in the distal region (K-S =4.89; $p<0.01$). This restriction of transverse and lateral niche may correspond to different penetration route and the different size of the cercariae.

Keywords: *Ascocotyle* – metacercariae – *Stephanoprora* – *Odonthestes argentinensis*

RESUMEN

El pejerrey (*Odonthestes argentinensis* Valenciennes, 1835) es un importante recurso acuático en las costas del Río de la Plata. Este pez actúa como huesped intermediario de varios parásitos incluyendo metacercárias de trematodes. En las branquias de estos peces colectados en la costa uruguaya del Rio de la Plata encontramos metacercárias de *Ascocotyle* sp. Looss, 1899 (Heterophiydae) y *Stephanoprora uruguayensis* Holcman-Spector & Olagüe, 1989 (Echinostomatidae). El objeto de este trabajo fue determinar la prevalencia e intensidad media de infestación, así como describir la distribución especial de estas metacercarias en las branquias. Se examinaron 279 peces. La prevalencia e intensidad media de la parasitosis fue de 34,05 % y 11,38 para *Ascocotyle* sp. y de 53,04% y 26,38 para *S. uruguayensis*. Observamos una distribución desigual de las metacercarias entre los arcos branquiales internos y externos. Mientras que *S. uruguayensis* se distribuye principalmente en los arcos branquiales 1 y 2; *Ascocotyle* sp. es más abundante en los arcos branquiales 3 y 4. Además las metacercárias de *S. uruguayensis* se encontraron principalmente en la región proximal del filamento branquial (KS = 4.80; p <0.01) mientras que las de *Ascocotyle* sp. están principalmente en la región distal (KS = 4,89; p <0,01). Los autores postulan que esta restricción del nicho transversal y lateral puede corresponder a diferentes vías de penetración y al diferente tamaño de las cercárias.

Palabras clave: *Ascocotyle* – metacercarias – *Stephanoprora* – *Odonthestes argentinensis*

INTRODUCTION

The silverside *Odontesthes argentinensis* Valenciennes, 1835; is distributed from the state of Santa Catarina in Brazil, to Bahía Blanca in Argentina, and is one of the species caught by commercial and recreational fishermen in "de la Plata" River. This fish acts as intermediate host of several trematodes (Alarcos *et al*., 2010). In Uruguay were identified two metacercariae in gills of *O. argentinensis*: *Stephanoprora uruguayensis* (Echinostomatidae) and *Ascocotyle* sp. (Heterophyidae) (Letamendia *et al*., 2010; Carnevia *et al*., 2012; Castro *et al*., 2012; Maidana *et al*., 2012).

The niche separation facilitates the coexistence of several species by reducing competition for resources (Slagsvold & Wiebe, 2007). There is an interaction between parasites that inhabit the same host and constant competitive interaction leads to niche diversification by segregation, eventually causing site specificity. The mechanisms responsible for niche restriction in parasites are competition, predation, and reinforcement of reproductive barriers (Rohde, 1979). The gills are a microhabitat within the host, and several niches can be recognized within it. The selection of the implantation site of parasites in the gills may respond to several factors: water flows into the gill chamber, availability of gill area for clamping sheets, differences in blood flow in different areas of gill arches, immunity level, etc. (Gutiérrez & Martorelli, 1999a). There are few studies on spatial distribution and interaction of parasites in the gills of fish. Most of this works concerns the ecology of parasitic monogeneans (Gutiérrez & Martorelli, 1999b, 1999c; Simková *et al*., 2000; Gutiérrez, 2001; Turgut *et al*., 2006; Soylu *et al*., 2010) and copepods (Benz & Dupre, 1987; Bashirullah, 2000; Timi, 2003; Failla, 2012) and also exists few researchs aimed in the spatial distribution of other parasites as myxosporidia or Digenea (Nie, 1996; Molnar, 2002; Tombi *et al*., 2010).

The objective of this study was determinate the prevalence and mean intensity of digenean metacercariae in the gills of *O. argentinensis* from the Uruguayan coast of the Río de la Plata, its relationship with fish size and sampling season, and to describe the spatial distribution of that metacercariae in gills.

MATERIAL AND METHODS

Monthly samplings were made during a year in coasts of de la Plata River in Montevideo city (56º07`W, 34º54`S), in each sampling a minimum of 20 fishes were collected. The fishes were obtained from an artisanal fisherman, already dead and were transported refrigerated to the laboratory. Each fish was measured and weighed, then dissected and gill arches were removed. The arches were numbered 1 to 4 in an anteroposterior direction (considering the arches 1 and 2 as fore and 3 and 4 as rear) and the blades of each gill arch were divided into proximal and distal region (fig. 1). Each gill arch was observed under a light microscope in fresh, and the number and location of the parasites were recorded.

With analytical purposes was adopted the criteria of two thermal seasons in de la Plata River estuary proposed by Guerrero *et al.* (1997), this implies the existence of a warm season (December to March) with a mean surface temperature of 12°C in our sampling site and a cold season (June to September) with 22°C of mean surface temperature in the same site. Prevalence and mean intensity of parasitism were calculated according to Bush *et al.* (1997). The relationship between the mean intensity and prevalence with the fish size was analyzed by a correlation test (fish were divided into 7 classes respect to the total length). To compare the prevalence between seasons the null hypothesis of equal proportions by Z test was tested. For the analysis of differences in infrapopulation of parasites between the gills arches or arch sectors a nonparametric Kolmogorov-Smirnov test was performed because the data were not normally distributed (Zar, 2010). We used the statistical software Statgraphic Plus 5.1 and significance level was established at $p<0.05$.

Ethic aspects: The authors declare that all the ethical aspects of the country and international ones were fulfilled.

RESULTS

A total of 279 fish were examined, 60.2% of them were collected in cold season and 39.8% in warm season. The prevalence and mean intensity of the parasites was 53.04% and 26.38 respectively for *S. uruguayensis* and 34.05% and 11.38 for *Ascocotyle* sp. Prevalence and mean intensity of *S. uruguayensis* were no correlated with fish size ($p=0.08$ and $p=0.16$, respectively). However the prevalence and the mean intensity of *Ascocotyle* sp. were correlated with fish size ($p=0.01$ and 0.03, respectively). The prevalence for *S. uruguayensis* and *Ascocotyle* sp. in warm season was 27.02% and 13.5% respectively, while in cold season was 70.65% and 48.5% respectively. There was a difference between the prevalence in the warm and cold season for both *S. uruguayensis* ($Z=-7.15$, $p<0.01$) and *Ascocotyle* sp. ($Z=-6.02$, $p<0.01$). We

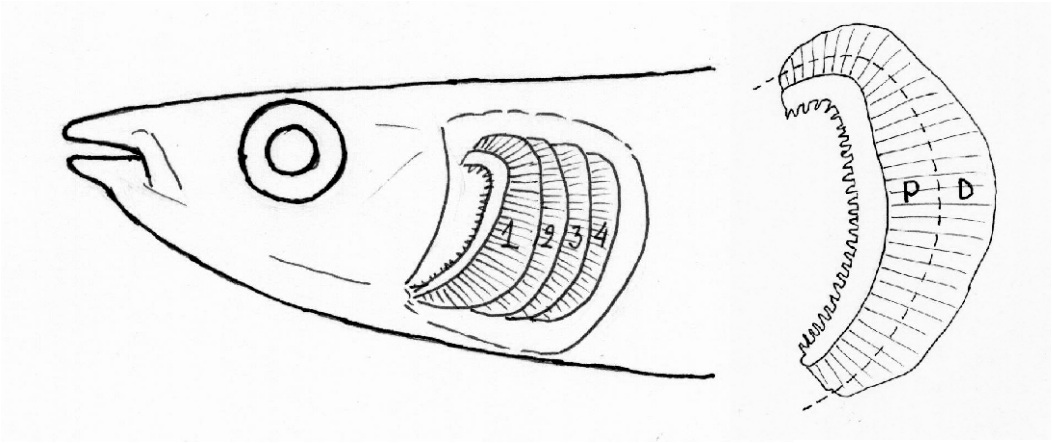

Figure 1. Diagram showing gill arches position in *Odontesthes argentinensis* (operculum removed) and division of gill arch in proximal and distal region (P and D).

found a different distribution in the metacercariae between fore and rear gill arches. While *S. uruguayensis* is distributed mainly in the gills archs 1 and 2; *Ascocotyle* sp. are mainly in the gills archs 3 and 4. Differences were found between the total number of metacercariae of *S. uruguayensis* (K-S = 3.03; $p<0.01$) and *Ascocotyle* sp. (K-S = 1.82; $p<0.01$) present in the gills 1 and 2 with respect to 3 and 4. Differences were found between the number of metacercariae of *S. uruguayensis* (K-S = 4.80; $p<0.01$), which were mainly found in the proximal region of the gill filament, and *Ascocotyle* sp. (K-S =4.89; $p<0.01$), which was mainly present in the distal region (Table 1).

DISCUSSION

The prevalence of *S. uruguayensis* found in the silverside (53.04%) was higher than that found in other fish species in Patagonia (*Galaxias maculatus* Jenyns, 1842, 29.54%, *Brachygalaxias bulbcki* Regan, 1908. 26.9% , *Aplochiton zebra* Jenyns, 1842, 3%) (Viozzi *et al.,* 2008, 2009; Fernández *et al.,* 2012), but lesser than the prevalence of *S. aylacostoma* Ostrowski & Quintana, 2007; found in fishes from the Parana River (88-100%) (Ostrowski & Quintana, 2007). Instead the mean intensity of infection (26.38) was much higher than that cited by these researchers (1 to 3.8).

The prevalence of *Ascocotyle* sp. found in this study (34.05%) was lower than that found in other fish species (*A. longa* Ransom,1920, 35%, *A. tenuicollis* Price, 1935, 58%, *A. ampullacea* Miller & Harkima, 1962, 80% and *A. mcintoshi* Price, 1936, 87%). The mean intensity of infection (11.38) was similar to that reported for *A. ampullacea* and *A. mcintoshi* (9.1 to 10.8) but lower than *A. longa* (93.06) (Perretta *et al*., 2005; Brock & Font, 2009; Galvan-Borja *et al*., 2010; Shoaibi *et al*., 2010; Martorelli *et al*., 2012).

If well the existence of a positive correlation between the prevalence and mean intensity of metacercariae with the fish size has been reported previously, it is still discussed; several authors argue this existence by means a cumulative effects and the increment of parasite penetration with the increase of the body surface of the fishes (Coleman & Travis, 1998; Yamada *et al*., 2007; Namba *et al*., 2012), but on the other hand, Portes *et al*. (2013) for example, do not found correlations between the number of metacercariae of *A. longa* and the fish size.

The high prevalence and intensity levels found in the cold season in this study contrast the findings made by others. Coleman & Travis (1998) found an increased prevalence and intensity of infection to *Ascocotyle pachycystis* Schroeder & Leigh, 1965; metacercariae in the warm season, which was explained by the lower survival of highly parasitized fish (*Cyprinodon variegatus* Lacépède, 1803) during the winter, and by the low production of cercariae by lowering the temperature (Steinauer & Font, 2003). A possible explanation of our findings could be that *O. argentinensis* breeding in the cold season, many juveniles being captured in the warm season, so the cumulative effect is more pronounced during the cold season.

There is little information that allows us to interpret the preferential location of *Ascocotyle* sp. and *S. uruguayensis* in the gills of *O.argentinensis.* The morphological and physiological factors that determine the selection of a specific parasite are still unknown for most species. In the case of the gill parasites, differences in the flow of water between gills, as well as differences in blood flow

Table 1. Metacercariae in the gills of *Odontesthes argentinensis* from Rio de la Plata, Uruguay (average, SD, minimum-maximun) (n = 279).

site	*Stephanoprora uruguayensis*	*Ascocotyle* sp.
Gills arches 1 and 2	98.91 ± 71.32 (1 – 308)	1.64 ± 2.14 (0 – 8)
Gills arches 3 and 4	19.65 ± 16.19 (1 – 62)	2.17 ± 2.23 (0 – 8)
Proximal region of gills	56.32 ± 62.31 (1 – 308)	0.32 ± 1.08 (0 – 8)
Distal region of gills	2. 94 ± 13.92 (0 – 113)	1.58 ± 2.02 (0 – 7)

to areas of the gills, have been postulated as the main factors that determine the preferential localization (Rohde, 1979). We postulate a preferential location of *Ascocotyle* and *Stephanoprora* metacercariae in gills of *O. argentinensis* based on the different route of arrival to the gill and different cercariae sizes (*Ascocotyle* 140-265 µm, *Stephanoprora* 85-141 µm). According to Stein (1968) and Leigh (1974) cercariae of the genus *Ascocotyle* penetrate through the gills of fish, then migrate to the target organs. By contrast, cercariae of Echinostomatidae have a great tail and takes intense movements near the surface to attract fish that ingest, afterwards penetrate the wall gut and would travel through the bloodstream to the target organs. Gill would be reached via the branchial artery afferent to settle at the secondary lamellae (Koie, 1986; Paller & Uga, 2008).

Odontesthes argentinensis could be parasitized with trematode metacercariae of the family Heterophyidae (*Ascocotyle* sp.) and Echinostomatidae (*Stephanoprora uruguayensis*) in gills, with higher prevalence and intensity of infection in the cold season. Metacercariae are differentially located in the gills: *S. uruguayensis* dominates arches 1 and 2 in the proximal area, while *Ascocotyle* sp. predominates in arches 3 and 4 in the distal zone. This restriction of transverse and lateral niche may correspond to different penetration route and the different size of the cercariae.

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