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# Behavioral responses of *Poecilia vivipara* (Osteichthyies: Cyprinodontiformes) to experimental infections of *Acanthocollaritrema umbilicatum* (Digenea: Cryptogonimidae)

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ABSTRACT

The swimming behavior of *Poecilia vivipara* was evaluated using an image analysis system comparing laboratory-reared uninfected fish before and after experimental infection with different intensities of cercariae of the trematode *Acanthocollaritrema umbilicatum*. Two experiments were performed, each with 30 fish which were individually exposed to 30 and 50 cercariae, respectively, shed from experimentally infected molluscs, *Heleobia australis*. Before and after (17–27 days) infection, the behavior of each fish was monitored in terms of Distance travelled, Ambulatory time, Stereotypic time, Resting time and Average speed. At the end of the experiments, the fish were dissected to count the number of metacercariae recovered. In the experiment with 30 cercariae, fish with 2–10 metacercariae did not exhibit any significant differences in their swimming activity, but those with 11–22 metacercariae had a significantly enhanced Stereotypic time and a reduced Time Resting. In the experiment with 50 cercariae, fish with 5–22 metacercariae had an enhanced Distance travelled and a reduced Average speed; highly significant differences occurred with regard to all behavioral parameters when considering the subgroup 23–36 metacercariae: Distance travelled, Stereotypic time, Resting time, Ambulatory time and Average speed. The swimming behavior of *P. vivipara* changed influenced by an intensity-dependence on metacercariae of *A. umbilicatum*, supporting the prediction that parasites are able to alter the behavior of their hosts.

## 1. Introduction

Fish have been considered good sentinels for behavioral experiments, as their swimming behavior can be readily observed and quantified in controlled conditions (Scott et al., 2003). Their swimming activity has been also used as a biosensor in ecotoxicological assays using a real time monitoring system together with an image analysis system (Gerhardt, 1998; Scott et al., 2003; Magalhães et al., 2007). Nevertheless, these studies did not take in account the phenomenon of parasitism, which is known to cause behavioral changes in hosts (Barber, 2007).

Parasites can induce different biochemical or physiological host responses that may reflect on their behavior (Shariff et al., 1980; Seppälä et al., 2004; Poulin, 2002), which in some cases may facilitate their predation by definitive hosts (Seppälä et al., 2004, 2005; Poulin, 2010). According to Barber et al. (2000), such behavioral changes associated with parasitic infections may affect the host's foraging efficiency, time budget, habitat selection, competitive ability, predator–prey relationship, swimming performance, and sexual behavior. Some of these changes may appear to enhance the parasite's chances of completing its life cycle (Holmes and Bethel, 1972; Lafferty and Morris, 1996; Moore and Gotelli, 1990; Poulin, 1995, 1998). In relation to the guppy *Poecilia reticulata* (Peters, 1859), Brassard et al. (1982) reported that fish exposed to small numbers of trematode cercariae exhibited reduced activity, apparently increasing their susceptibility to predation by brook trout *Salvelinus fontinalis* (Mitchill, 1814).

*Poecilia vivipara* Bloch and Schneider, 1801 is one of the most common fish species in small ponds, rivers, and coastal lagoon ecosystems of Brazil. This fish has found to be naturally parasitized by metacercariae of *Pygidiopsis macrostomum* Travassos, 1928, *Ascocotyle (Phagicola) pindoramensis* Travassos, 1928, *Ascocotyle (Phagicola) diminuta* (Stunkard and Haviland, 1924) and *Acanthocollaritrema umbilicatum* Travassos, Freitas and Bührnheim, 1965 in the Rodrigo de Freitas lagoon at Rio de Janeiro. The life cycles of these parasites have been described and are maintained in our laboratory (Simões et al., 2005, 2006, 2008, 2009).

*A. umbilicatum*, for instance, has a complex life cycle, including the snail *Heleobia australis* (d'Orbigny, 1835) as the first intermediate host, *P. vivipara* as the second intermediate host and the common snook *Centropomus undecimalis* (Bloch, 1792) as the definitive host. The common snook is a *lie-in-wait* predator (sensu Greenway, 1965; Moyle and Cech, 2000), adapted for capturing prey from ambush, with a sudden strong and quick dash after a prey. Given that,

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the swimming activity of the fish intermediate host may be decisive to influence their risk of predation.

Considering the availability of *A. umbilicatum* larval stages under experimental conditions, the aim of this paper is to evaluate whether this parasite can alter the swimming behavior of its fish intermediate host, *P. vivipara*, comparing individuals before and after infection.

## 2. Materials and methods

### 2.1. Animals

Sixty adult *P. vivipara* born and reared in the laboratory, free of parasites, weighing 0.05–0.36 g, with a standard body length varying from 14 to 24 mm, more than 90 days old were used in the experiments. Fish were individually isolated, unsexed, in boxes and identified to be used in the experiments. Fishes were not fed for 12 h prior to the experiments. Each of the 60 fish was analyzed using an image monitoring system prior to the infection, thus forming their own ‘control group’.

Uninfected stocks of the hydrobiid snail *H. australis* born and reared in the laboratory, were exposed to infection by placing in their aquaria eggs of *A. umbilicatum*, obtained from naturally infected *C. undecimalis* from Rio de Janeiro, Brazil (22°57’2”S, 43°11’9”W) (Simões et al., 2008). Forty-two days post-infection, the shedding of the cercariae started. Thirty laboratory-reared *P. vivipara* were individually exposed to 30 cercariae (first experiment) and another 30 with 50 cercariae (second experiment). The fish were exposed to cercariae for 2 h or until the latter had disappeared from the beaker glass.

Another monitoring system analysis was performed 17–27 days post-infection (d.p.i.). At the end of the experiments, the fish were dissected, and any metacercariae recovered were isolated and counted. For statistical analysis, both fish groups were subdivided in two subgroups according with the median number of metacercariae recovered.

### 2.2. Image analysis biomonitoring system (IABS)

The image analysis biomonitoring system used followed Magalhães et al. (2007). During the experiments, fish were kept in filtered dechlorinated tap-water with oxygen (6 ± 0.6 mg/dL), temperature (23.7 ± 0.9 °C) and pH (6.1 ± 0.4) being controlled. Fish were placed individually in eight holding boxes ($4 \times 4 \times 2$ cm each) made of opaque acrylate with 3 mm holes, which were kept inside an opaque glass aquarium of 30 L capacity ($35 \times 35 \times 25$ cm). Submerged water pumps maintained the water circulation. An illuminating cabin provided shadowless, diffused soft lighting. A recording cabin made of acrylate held the analogical video camera. Images were sent to a Videomex V® (Columbus Instruments, Ohio, USA) and analyzed using the software Travelled Distance of Multiple Objects (TDMO). Data were sent to a computer and recorded in an Excel spreadsheet for subsequent analyses.

Each experiment was performed during 5 h, with 1 h of acclimation period and 4 h of biomonitoring analysis. The monitoring period was recorded in 48 intervals of 5 min and all values of each interval of Distance travelled, Ambulatory time, Stereotypic time, Resting time and Average speed were used for the statistical analyses. ‘Distance travelled’ is the total distance (mm) travelled by the animal during the session. ‘Ambulatory time’ is the total number of seconds during the session which were spent in travelling movement. ‘Stereotypic time’ is the total number of seconds during the session in which fish performed some activity other than travelling. In contrast, ‘Resting time’ is the total number of seconds during the session, which was spent resting. The ‘Average speed’ of animal movement was calculated as the distance travelled divided by the ambulatory time.

### 2.3. Statistical analysis

All the statistical tests were performed using software Statistica v8.0 (StatSoft Inc.). In order to assess the normality of the parameters, statistical analyses of all data were carried out using the W Shapiro–Wilk test. Normality could not be achieved by transforming the data using log $(x + 1)$, square root, exponential and arc-sine. Therefore, a Wilcoxon paired-sample test was used to determine significant differences between all values of each behavioral parameter before and after infection. The data used for the Pearson correlation test were transformed as log $(x + 1)$ in order to meet the requirement of the parametric assumption for assessing any association between the length and weight of the fishes and (i) the number of the metacercariae, and (ii) the mean values of each parameter of the locomotory activity. The same test was used to evaluate if the swimming activity of fish was influenced by the variation on days post infection.

## 3. Results

All experimentally infected fish ($N = 60$) were dissected at the end of the experiments and harbored metacercariae encysted under the scales, fins, musculature, and buccal cavity. The mean intensity of the metacercariae recovered from the 30 fish of the first experiment (exposed to 30 cercariae) was 10 (range 2–22) which was divided into two subgroups for statistical analysis, i.e. those with 2–10 and 11–22 metacercariae. The second experiment with the remaining 30 fish (exposed to 50 cercariae) presented a mean intensity of 22 (5–36) metacercariae, which were also divided into two subgroups, i.e. with 5–22 and 23–36 metacercariae, for statistical analysis.

In the first experiment, the fish measured 20.1 ± 1.9 (15–24) mm in standard body length and weighed 0.20 ± 0.06 (0.08–0.36) g. In the subgroup with 2–10 metacercariae per fish, the Wilcoxon test did not indicate any significant differences between the data before and after infection for any of the analyzed parameters: Distance travelled ($Z = 1.21$; $P = 0.22$), Average speed ($Z = 0.61$; $P = 0.53$), Ambulatory time ($Z = 1.71$; $P = 0.08$), Stereotypic time ($Z = 1.53$; $P = 0.12$) and Resting time ($Z = 1.49$; $P = 0.13$) (Fig. 1A–E). Nevertheless, significant differences were found when considering the parameters of Stereotypic time ($Z = 3.87$; $P < 0.01$) and Resting time ($Z = 2.08$; $P = 0.03$) in the subgroup with 11–22 metacercariae (Fig. 1 C and D). No significant differences occurred when considering the Distance travelled ($Z = 1.00$; $P = 0.31$), Ambulatory time ($Z = 1.67$; $P = 0.09$) and Average speed ($Z = 0.05$; $P = 0.95$) (Fig. 1A, B and E). Although experimental infections were performed in different days, the Pearson correlation test showed no influence in the swimming activity of fish (Distance Travelled $r = 0.009$ $P = 0.960$; Stereotypic time $r = 0.04$ $P = 0.79$; Ambulatory time $r = -0.07$ $P = 0.707$; Resting time $r = -0.02$ $P = 0.884$ and Speed Average $r = 0.20$ $P = 0.268$).

In the second experiment, fish exposed to 50 cercariae measured 17.4 ± 2.2 (14–24) mm in standard body length and weighed 0.12 ± 0.05 (0.05–0.33) g. In the subgroup with 5–22 metacercariae per fish, the Wilcoxon test showed significant differences only for Distance travelled ($Z = 2.32$; $P = 0.02$) and Average speed ($Z = 2.22$; $P = 0.02$) before and after infection (Fig. 2A, E). However, significant differences occurred for all behavioral parameters when considering the subgroup with 23–36 metacercariae: Distance travelled ($Z = 2.87$; $P < 0.01$), Average speed ($Z = 2.00$; $P = 0.04$), Ambulatory time ($Z = 4.20$; $P < 0.01$), Stereotypic time ($Z = 3.96$; $P < 0.01$) and Resting time ($Z = 5.52$; $P < 0.01$) (Fig. 2A–E). Once again, differences