

Short-term infection dynamics of the monogenean parasite *Ligictaluridus floridanus* (Monogenea: Dactylogyridea: Ancyrocephalidae) on channel catfish *Ictalurus punctatus* (Actinopterygii: Siluriformes: Ictaluridae)

Jesús Genaro SÁNCHEZ-MARTÍNEZ¹, Isidro Otoniel MONTELONGO-ALFARO^{1,2},
Jaime Luis RÁBAGO-CASTRO³, Roberto PÉREZ-CASTAÑEDA¹,
María de la Luz VÁZQUEZ-SAUCEDA¹, Flaviano BENAVIDES-GONZÁLEZ¹,
Jorge LOREDO-OSTI¹

¹ Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma de Tamaulipas, Ciudad Victoria, TAM, Mexico

² Universidad Tecnológica del Mar de Tamaulipas Bicentenario, Poblado La Pesca, Soto la Marina, TAM, Mexico

³ Secretaría de Investigación y Posgrado, Universidad Autónoma de Tamaulipas, Ciudad Victoria, TAM, Mexico

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Corresponding author: Roberto Pérez-Castañeda (roperez@docentes.uat.edu.mx)

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Abstract

Ligictaluridus floridanus (Mueller, 1936) is a monogenean parasite that affects farmed channel catfish, *Ictalurus punctatus* (Rafinesque, 1818). No studies, however, have been conducted on this monogenean's infection dynamics. The aim of this study was to determine the prevalence and mean abundance of *L. floridanus* on channel catfish fry in a cohabitation experiment. The experiment was performed in triplicate and involved the introduction of five infected fish to a 40-L aquarium that contained 30 naive fish. The trial lasted 29 days and was conducted in a system with a continuous water flow. The percentage of naive fish that were infected increased sharply, from 0% to 100%, between the second and third days after cohabitation. On the subsequent days of the experiment, 100% prevalence was observed, except for days 23 and 26, when it was 66%. The dynamics of infection with *L. floridanus* showed its main peak five days after cohabitation, exhibiting a dome-shaped function. The left side of the fitted function represented the abrupt increase in parasite abundance in a few days, while the right side depicted its gradual decrease. One would expect that when *L. floridanus* enters the catfish culture system, it will be able to infect all fish (100% prevalence) within a few days. This would occur primarily in floating cage culture systems, where fish density is usually high. The parasite can disperse rapidly throughout all the gill arches when its abundance exceeds a certain threshold. Further studies on the reproductive patterns of this parasite and the factors affecting its variability are necessary to have a better understanding of its host dynamics and life cycle parameters.

Keywords

aquaculture, cohabitation, experimental infection, farmed fish, parasite

Introduction

Aquaculture in Tamaulipas, Mexico, plays a significant role in channel catfish production using controlled systems such as hatcheries and floating cages (Lara-Rivera et al. 2015). The state ranks fourth nationally, with historical production volumes ranging from 124 to 913 tons per year (CONAPESCA 1998, 2022). Parasites, such as monogeneans, can impair productivity in intensive aquaculture by causing lesions on fish skin, gills, and other tissues (Rubio-Godoy 2007).

Ligictaluridus floridanus (Mueller, 1936) Beverley-Burton, 1984, a prevalent monogenean in Mexican catfish cages (Rábago-Castro et al. 2011), negatively affects weight gain and growth rates (Rábago-Castro et al. 2014), leading to costly treatments due to high infection rates in densely stocked fish (50–120 ind. m⁻³) (Lara-Rivera et al. 2015). Understanding the infection dynamics of *L. floridanus* is crucial for effective management strategies.

While studies exist on infection dynamics for other monogenean parasites (Scott and Anderson 1984; Buchmann and Bresciani 1998; Bakke et al. 2002), none have been conducted for *L. floridanus*. Therefore, this study aims to explore the prevalence and infection dynamics of *L. floridanus* in channel catfish under controlled conditions to mirror the infection process in a culture system.

Materials and methods

Source and fish maintenance. A group of clinically healthy fry of channel catfish, *Ictalurus punctatus* (Rafinesque, 1818), 2.18 ± 0.06 cm fork length, were purchased from a fish hatchery in Abasolo, Tamaulipas, Mexico, where fish are grown in concrete tanks and are generally free of most external parasites. The absence of infection with *L. floridanus* was confirmed by gill examination of a sample of 5 fish at the time of collection in the hatchery. The fish were then transported to the aquaculture facilities at the Facultad de Medicina Veterinaria y Zootecnia (Universidad Autónoma de Tamaulipas) in Ciudad Victoria, Tamaulipas, Mexico. The fish were transported in a fiberglass tank with aerated well water at a temperature of approximately 20°C.

Another group of juvenile fish, 17.5 ± 0.50 cm fork length, were simultaneously collected from a floating cage at a different fish farm (also located in Tamaulipas) with reports of *L. floridanus* chronic presence. We confirmed the presence of the parasite in the farm cages by taking a sample of 5 fish from a cage with infected fish. Both the hatchery and the fish farm are supplied with water from the Soto la Marina River. These infected fish were transported in another tank to the same facilities mentioned above, where they were kept completely separated from naive fish. Both groups of fish (infected and non-infected) were acclimatized over two weeks in two separate and independent tanks. During the acclimatization and experimental period, fish were fed a commercial feed based on their size, twice a day, ad libitum, with no waste.

This resulted in a feed rate of approximately 15% of the body weight day⁻¹ (the individual fish weight was 0.26 ± 0.01 g). The well water supply to the aquaria was constant (36 L h⁻¹), and a blower (0.74 kW) provided the aeration, utilizing air stones. Dissolved oxygen (mg L⁻¹), temperature (°C), un-ionized ammonia (mg L⁻¹), pH, alkalinity (mg L⁻¹ as CaCO₃), and hardness (mg L⁻¹ as CaCO₃) were all measured using an oximeter (YSI®, USA) and a portable kit (LaMotte®, USA). The fish were subjected to natural light and dark periods during the experiment.

We assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional guides on the care and use of laboratory animals.

Experimental design. The absence of infection in naive fish was confirmed after the acclimatization period, just before beginning the experimental trial, by analyzing a sample of 10 fish from this group. The mean intensity (±SD) of *L. floridanus* in the group of infected fish utilized in the experiment was of 228 ± 86 parasites per fish ($n = 5$), which was determined prior to the start of cohabitation.

For the cohabitation experiment, 30 naive catfish (non-infected) were placed in a 40-L aquarium and then five infected catfish were added using the Del Rio-Zaragoza et al. (2011) methodology with some modifications. Both groups of fish (infected and non-infected) were kept physically separated in the same aquarium. The separation was done with a plastic mesh screen, allowing for water flow and parasite infection while preventing direct contact between the two groups of fish (Fig. 1). No data are available regarding the dimensions of the eggs or oncomiracidia. However, we utilized a sufficiently large mesh size to guarantee their passage to the opposite side of the aquarium, given that the mesh size (2000 µm) exceeded the body length of the adult parasites (307–600 µm) (Beverley-Burton 1984). This experimental trial was performed in triplicate to calculate the average values of the response variables that were evaluated in this study. The trial lasted 29 days and was conducted in a system with a continuous flow of water. Well water was used to supply the experimental aquaria. No filtration system was used on the incoming water supply. The water from the well was pumped to an elevated tank at an approximate height of 10 m. It was then gravity-fed down to enter the laboratory with the aquaria. Water quality parameters (mean ± SD) were within the range suitable for channel catfish: dissolved oxygen at 6.0 ± 0.50 mg L⁻¹, temperature at 25.06 ± 1.60°C, un-ionized ammonia at 0.0018 mg L⁻¹, pH at 7.50 ± 0.12, alkalinity at 324.0 ± 3.22 mg L⁻¹, and hardness at 297.60 ± 3.80 mg L⁻¹. There were no fish mortalities during the trial.

On the first five days of cohabitation, one fish from the non-infected section was removed from each aquarium and euthanized by cervical transection, followed by pithing (AVMA 2020). Each euthanized fish was analyzed to quantify the number of parasites. Then, from the eighth day to the end of the trial this procedure was performed

every three days. During the trial the examined fish were not replaced, and the original infected fish remained in the aquarium. The total number of fish examined in the present study was 42 (one fish from each aquarium \times three aquaria \times 14 collection dates).

The gill arches were removed from each euthanized fish and examined under a stereoscope to determine the number of *L. floridanus* per gill arch, as well as the total number per fish. The parasite was identified according to Hoffman (1999). The collected data were used to calculate the proportion of infected fish and the mean abundance of parasites at each collection time, according to the experimental design (see Fig. 1).

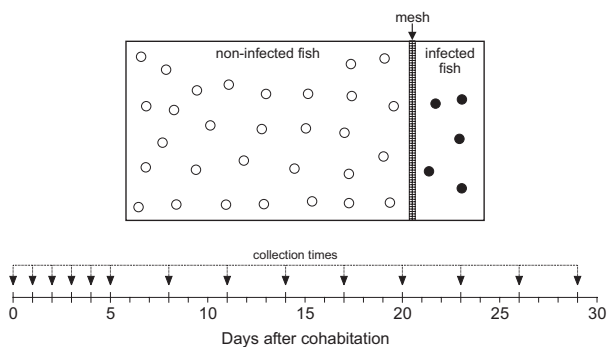


Figure 1. Design of the 29-day cohobitation experiment in a 40-L aquarium with water circulation (top view). Five catfish (*Ictalurus punctatus*) infected with the monogenean *Ligictalurus floridanus* were kept in cohobitation with 30 non-infected catfish. A mesh screen separating the two groups of fish allowed water to pass through, but not fish. The black triangles on the timeline indicate fish collection times, from the left section of the aquarium (non-infected fish), to determine the number of parasites in their gills.

The proportion of infected fish (dependent variable 1) and mean parasite abundance (dependent variable 2) were expressed separately as a function of the number of days after cohobitation (independent variable) using nonlinear models. Likewise, the percentage of infected gill arches (dependent variable) was nonlinearly related to the mean abundance of parasites (independent variable). The corresponding functional relationships were fitted using non-linear least squares and the quasi-Newton algorithm.

Results

No parasites were found on fish gills in the first two days after cohobitation, but they were found in all the collected fish (100% prevalence) in the subsequent collection times, except for days 23 and 26 of the trial, where the proportion of infected fish was 0.66. The proportion of infected fish increased sharply from 0% to 100% within 24 h, from day 2 to day 3 of the trial. The changes in the proportion of infected fish over the entire period of the experiment were best described by a sigmoid function ($r^2 = 0.90$) (Fig. 2).

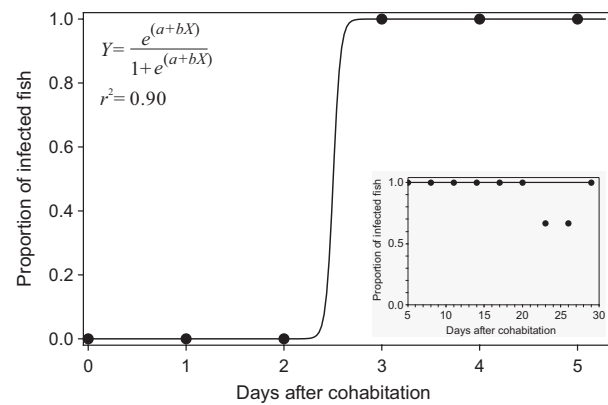


Figure 2. Proportion of infected channel catfish (*Ictalurus punctatus*) with *Ligictalurus floridanus* during the cohobitation experiment. A sigmoid model was fitted to describe the fish infection over time. Results are shown for the first 5 days of the experiment. From the third day of cohobitation, the proportion of infected fish was 1, excluding days 23 and 26, when it was 0.67 (see graph in the shaded area). The estimated parameters for the fitted equation were as follows: $a = -67.06$; $b = 26.79$.

The dynamics of the mean abundance of *L. floridanus* in the naive fish showed its main peak 5 days after cohobitation, whereas in the early phase of the trial (days 0 to 2), the mean abundance of this parasite was zero. During the ten days following the peak infection of *L. floridanus* (127 parasites fish⁻¹), its abundance fell to 8 parasites fish⁻¹. Later, in the second half of the experiment, the mean abundance fluctuated between 11 and 35 parasites per fish (Fig. 3). The dynamics of infection with *L. floridanus* in terms of the mean abundance of parasites per fish over time exhibited a dome-shaped response ($r^2 = 0.76$). The left side of the curve represented the abrupt increase in parasite abundance in a few days, while the right side of the curve depicted its gradual decrease (Fig. 3).

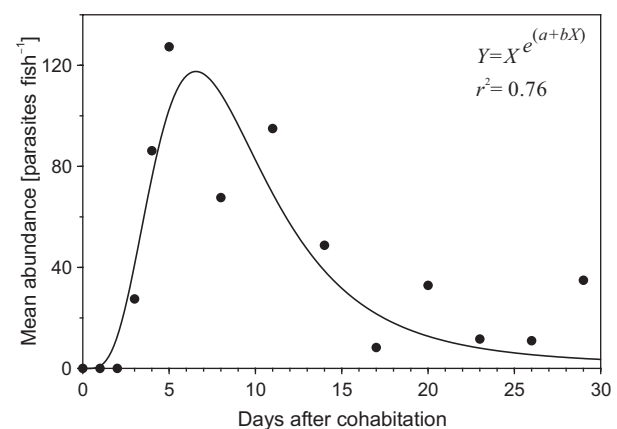


Figure 3. Mean abundance of *Ligictalurus floridanus* on channel catfish (*Ictalurus punctatus*) throughout the cohobitation experiment. A dome-shaped curve was fitted to describe parasite abundance over time. Three aquaria were used to calculate each mean abundance value. The estimated parameters and their significance levels for the fitted equation were as follows: $a = 1.46$ ($P < 0.01$); $b = -0.08$ ($P < 0.01$).

An asymptotic function was fitted ($r^2 = 0.98$) between % of infected gill arches and the mean abundance of *L. floridanus*, indicating an increase in gill arch infection as parasite abundance increases until reaching 100% of infected gill arches. The existence of a threshold value of parasitic abundance above which all gill arches are infected was also observed (Fig. 4).

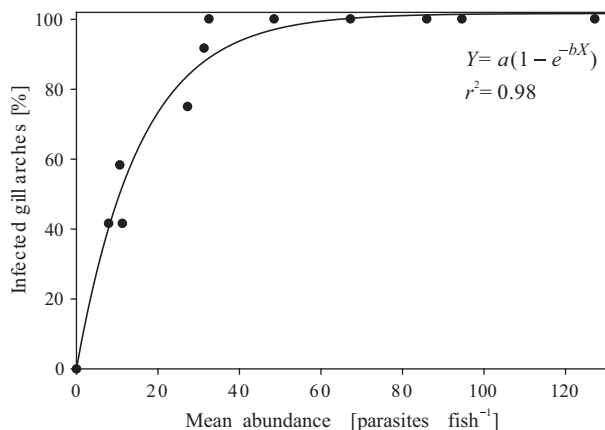


Figure 4. Percentage of infected gill arches per fish (*Ictalurus punctatus*) as a function of the mean abundance of parasites (*Ligictaluridus floridanus*). This relationship is described by an asymptotic function, which is included in the graph. Three aquaria were used to calculate the mean abundance of parasites and the percentage of infected gill arches. The estimated parameters and their significance levels for the fitted equation were as follows: $a = 101.70$ ($P < 0.01$); $b = 0.06$ ($P < 0.01$).

Discussion

The variation in prevalence and mean abundance of *L. floridanus* in channel catfish (*I. punctatus*) as a function of days after cohabitation may be directly related to the parasite's reproductive characteristics. In this sense, the peaks in prevalence (from day 3) and mean abundance (at day 5) observed in our study for *L. floridanus* could be attributed to its egg hatching rate dynamics.

The egg hatching time of monogenean parasites can vary according to the species (Gannicott and Tinsley 1998; Oliveira Maciel et al. 2017; Wan Sajiri et al. 2023). However, it also varies depending on the incubation temperature, with higher temperatures resulting in shorter hatching times. The latter has been observed in the monogeneans *Thaparocleidus vistulensis* (Siwak, 1932) (see Wan Sajiri et al. 2023) and *Dactylogyrus extensus* Mueller et Van Cleave, 1932 (see Turgut 2012), which parasitize the gills of European catfish (*S. glanis*) and carp (*Cyprinus carpio*), respectively. When the eggs of both monogeneans were kept at a temperature similar to that of the presently reported study (around 23°C), they hatched after approximately 3 days. This period coincides with the time it took for *L. floridanus* to be detected in the catfish's gills during the current cohabitation experiment. The above suggests that *L. floridanus* eggs' hatching time could be like that of the other monogeneans mentioned above.

The effectiveness of the fish's immune response significantly impacts the parasite outbreak peaks (Harris 1988), which could have influenced the dynamics of the mean parasite abundance during the cohabitation experiment. The decrease in parasite abundance following the outbreak peak (i.e., after day 5; Fig. 3) could be attributed primarily to an immune response by the naive fish. The presence of *L. floridanus* in the gills of naive fish after the third day of cohabitation may have triggered their immune response, contributing to the decrease in parasite abundance during the remaining time of the trial. Gills secrete mucus containing different innate immunity compounds. These secretions act as a barrier against pathogens, such as monogenean parasites (Salinas et al. 2021), which may have occurred in this experiment.

Serum epithelial lysozymes, complement system molecules, antibacterial peptides, and lectins, which can degrade and neutralize pathogens, can be found in the mucus of teleost fish (Watts et al. 2001). In fact, it has been indicated that mucus may affect the survival of invading monogeneans and could be responsible for the antiparasitic response in the later stages of infection, leading to decrease of the ectoparasite population (Buchmann 2013).

During the first days after cohabitation, not only did the prevalence and mean parasite abundance increase, but so did the percentage of infected gill arches (i.e., the occupancy percentage of the available habitat). *Ligictaluridus floridanus* was found in all gill arches when the parasite count exceeded 40 per fish (Fig. 4). This abundance threshold occurred in the middle of the cohabitation period, specifically on days 4–14 (Fig. 3). The fact that *L. floridanus* spread over all gill arches as abundance increased would be a mechanism to reduce intraspecific competition for space. It has been noted, however, that monogeneans may show intraspecific interactions because of their proclivity to exhibit aggregated distributions for reproductive intentions (Karvonen et al. 2007).

On the other hand, some authors have documented that when the abundance of monogenean parasites increases, their distribution becomes more widely dispersed on the gills (Bagge et al. 2005). Similarly, we found that as the abundance of *L. floridanus* increased, the percentage of infected gill arches also increased. This could be due, on the one hand, to a response in the parasites' dispersal to enhance their mating opportunity (Karvonen et al. 2007). But it could also be related to changes in susceptibility to infection of the different microhabitats of the host (Buchmann and Uldal 1997).

Based on the results of the present experiment, one would expect that when *L. floridanus* enters the catfish culture system, it will be able to infect all fish (100% prevalence) within a few days. This would occur primarily in floating cage culture systems, where fish density is usually higher than in ponds. Furthermore, *L. floridanus* can disperse rapidly in the fish's available habitat, being present in all gill arches as long as its abundance exceeds a certain threshold.

Conclusion

This study demonstrated the rapid dispersal and infection dynamics of *Ligictaluridus floridanus* in channel catfish fry, highlighting a significant peak in parasite abundance shortly after infection and a subsequent gradual decline. The parasite spread rapidly in the available habitat of the fish, increasing the percentage of infected gill arches as its abundance increased. Further studies

on the reproductive patterns of this parasite and the factors affecting its variability are necessary.

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