

## Preference for microhabitat by Monogenea on the gills of the south american catfish *Rhamdia quelen* at different stocking densities under laboratory conditions

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### Summary

This study aimed to verify preferences for microhabitat by the monogeneans on the gills of the south american catfish, *Rhamdia quelen* (Quoy & Gaimard, 1824), at different stocking densities under controlled laboratory conditions. Three stocking densities were used: 14, 28 and 42 fish per tank (50 L) and the fish were sampled initially, at day 5 and 10 of the experiment. *Aphanoblastella mastigatus* (Suriano, 1986) was noted as the most abundant species at all stocking densities, except for the initial collection day. The gill arches I and II were the most parasitized, showing the highest mean abundance in the dorsal region, at the highest density. The pattern of microhabitat preference of *A. mastigatus* for outer regions of the gills of the host was independent of the stocking density and collection day. Higher prevalence and abundance of *Scleroductus* sp. at the initial collection reflected the parasitic infection of the fish at the farm from where the fish were taken. The abundance of *Scleroductus* sp. decreased along the experiment, and no preference for gill arches was recorded for the species, probably due to the low abundance of this parasite on the gills.

**Keywords:** fish; parasitology; monogenean; ectoparasites; ecology

### Introduction

In Southern Brazil, the south american catfish locally known as “jundiá” (*Rhamdia quelen*) is one of the most cultivated fish species, along with the Nile tilapia *Oreochromis niloticus* (Linnaeus, 1758); common carp *Cyprinus carpio* Linnaeus, 1758; grass carp *Ctenopharyngodon idella* (Valenciennes, 1844); silver carp *Hypophthalmichthys molitrix* (Valenciennes, 1844); bighead carp *Hypophthalmichthys nobilis* (Richardson, 1845) and rainbow trout *Oncorhynchus mykiss* (Walbaum, 1792). South american catfish production represented 1.8 % of Brazilian fish production in 2010 and it has increased to 2.4 % in 2011, responsible for 215 tons more than the anterior year (Silveira & Silva, 2011). In order to obtain maximum production at low costs, frequently the highest quantity of fish per tank as possible is used, and fish frequently cul-

tivated under high densities that culminate in disease outbreaks. Monogeneans are responsible for major diseases outbreaks in aquaculture, causing great mortality in intensive fish farming (Pappas *et al.*, 1984; Thoney & Hargis, 1991; Moraes & Martins, 2004; Tu *et al.*, 2015).

Monogeneans are generally host specific; one species of parasite occurs in a single species or closely related species of hosts (Thatcher *et al.*, 2006). In Brazil, a considerable number of studies on monogeneans parasites of fish were performed (Kohn & Cohen, 1998; Takemoto *et al.*, 2009; Jerônimo *et al.*, 2011a; Kritsky *et al.*, 2013; Marchiori *et al.*, 2013), but few studies focused on their possible microhabitat preference (Oliva & Luque, 1998; Jerônimo *et al.*, 2013).

Some hypothesis have been thought and tested to explain the choice of the parasite for a microhabitat. The suggested explana-

tions include the heterogeneous water flow in the gills (El Hafidi *et al.*, 1998; Rubio-Godoy & Tinsley, 2002; Rubio-Godoy, 2008; Jeannette *et al.*, 2010; Soler-Jiménez & Fajer-Ávila, 2012), parasite migration (Arme & Halton, 1972; Buchmann & Bresciani, 1997; Rubio-Godoy & Tinsley, 2002), intraspecific interaction of parasites with aggregation to facilitate the reproduction (Rohde, 1979; Kadlec *et al.*, 2003; Raymond *et al.*, 2006; Jeannette *et al.*, 2010), inhibition of a possible hybridization between species (Rubio-Godoy, 2008; Soler-Jiménez & Fajer-Ávila, 2012) and differences in the area or size of the gills (Buchmann, 1989; Rubio-Godoy, 2008; Soler-Jiménez & Fajer-Ávila, 2012).

The selection of microhabitats on the sites of infection of monogeneans does not show evident pattern. Therefore, the preference for microhabitat is a result of a complex process where several possibilities can be considered (Gutiérrez & Martorelli, 1999). This assay investigated the influence of the stocking density of fish on the monogenean distribution on the gills of the south american catfish under laboratory conditions.

## Material and Methods

Fish used in the assay were albino south american of the same spawn, obtained from a disease-free stock of the fish farming Khiran, in Pomerode, Santa Catarina State, Brazil. They were

naturally parasitized by monogeneans. Fish presented  $21.24 \pm 6.91$  g weight and  $12.8 \pm 61.44$  cm of total length. They were distributed into 18 aquaria of 50 L capacity with individual biological filters and aeration. Fish were fed twice a day "*ad libitum*" with commercial diet of 42 % crude protein, and divided at the following stocking densities (treatments): 14, 28 fish and 42 fish per tank (50 L), with six replicates of each treatment. The tested stocking densities were similar to Pouey *et al.* (2011). During the assay, the following water variables were monitored: pH  $8.2 \pm 0.5$  (measured with Quimis® equipment), dissolved oxygen  $7.6 \pm 0.5$  mg.L<sup>-1</sup> (measured at every 3 days with a multiparameter HANNA®), and water temperature  $28.0 \pm 1.0$  °C (measured with a multiparameter HANNA®) controlled with thermostates and daily monitored.

At the beginning of the assay, 18 fish were examined for parasites. In addition, parasitological evaluation of fish occurred at day five and at day ten of the assay (six fish of three replicates of each treatment were collected, a total of 18). These experimental units were then discharged.

Fish were killed by a lethal dose of eugenol dissolved in water (approved by the Ethic Committee CEUA/UFSC-PP00756). Their gills were cutted out from the fish (Eiras *et al.*, 2006), the gill arches were separated according to the right and left side, and numbered from the outer to inner region of the operculum as arches I, II, III and IV. The arches were separated in three sectors: ventral, me-

Table 1. Prevalence (%), mean and standard deviation of intensity and abundance of *Scleroductus* sp. and *A. mastigatus* in *R. quelen* at different collection days and stocking density. Mean intensities and mean abundances with the same letters did not differ significantly; mean intensities and mean abundances with different letters differ significantly ( $p < 0.05$ )

<i>Scleroductus</i> sp.				
Density	Collection	Prevalence	Mean intensity	Mean abundance
14 fish per tank	Initial	66.7	$4.33 \pm 6.34^a$	$2.89 \pm 5.52^a$
	Day 5	33.3	$2.17 \pm 1.17^a$	$0.72 \pm 1.23^b$
28 fish per tank	Initial	66.7	$4.33 \pm 6.34^a$	$2.89 \pm 5.52^a$
	Day 5	44.4	$2.63 \pm 1.77^a$	$1.17 \pm 1.76^a$
	Day 10	60.0	$11.33 \pm 17.56^a$	$6.80 \pm 14.34^a$
42 fish per tank	Initial	66.7	$4.33 \pm 6.34^a$	$2.89 \pm 5.52^a$
	Day 5	38.9	$2.14 \pm 2.19^a$	$0.83 \pm 1.69^b$
	Day 10	33.3	$2.17 \pm 1.17^a$	$0.72 \pm 1.23^b$
<i>Aphanoblastella mastigatus</i>				
14 fish per tank	Initial	61.1	$4.18 \pm 5.23^{Aa}$	$2.56 \pm 4.53^{Aa}$
	Day 5	72.2	$3.62 \pm 1.85^{Aa}$	$2.61 \pm 2.28^{Aa}$
28 fish per tank	Initial	61.1	$4.18 \pm 5.23^{Aa}$	$2.56 \pm 4.53^{Aa}$
	Day 5	83.3	$3.07 \pm 2.25^{Aa}$	$2.56 \pm 2.36^{Ab}$
	Day 10	100.0	$9.50 \pm 3.57^{Bb}$	$9.50 \pm 3.57^{Bc}$
42 fish per tank	Initial	61.1	$4.18 \pm 5.23^{Aa}$	$2.56 \pm 4.53^{Aa}$
	Day 5	83.3	$3.13 \pm 2.17^{Aa}$	$2.61 \pm 2.30^{Aa}$
	Day 10	100.00	$4.72 \pm 7.31^{Ca}$	$4.72 \pm 7.31^{Ca}$

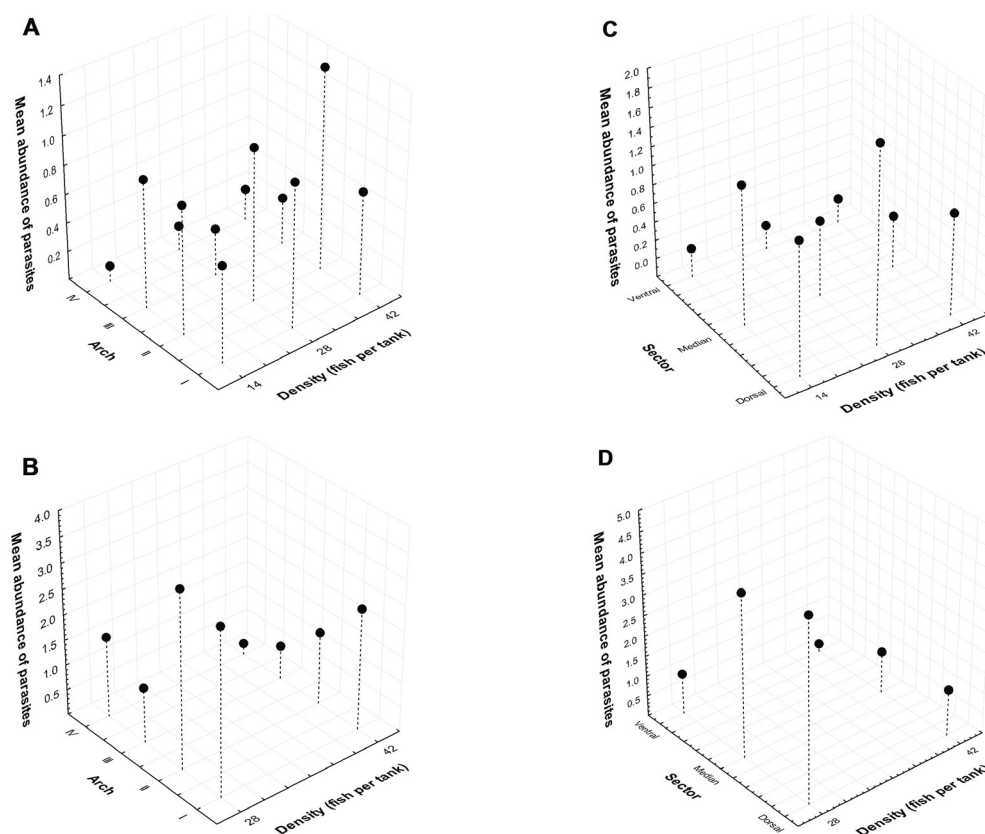


Fig. 1. Mean abundance of the parasite *Aphanoblastella mastigatus* on *Rhamdia quelen*, comparing the gill arches and stocking densities at day 5 (A) and day 10 (B) of the experiment, and comparing the gill sectors and stocking densities at day 5 (C) and day 10 (D) of the experiment

dium and dorsal according to Dzika (1999). After the collection, the gills were bathed in hot water (55 °C) for parasite release and fixed in alcohol 70 % (Jerônimo *et al.*, 2011b).

The parasites were mounted in Hoyer's medium and identified according to Kritsky *et al.* (1995) and Suriano (1986). Prevalence, mean intensity and mean abundance were calculated according to Bush *et al.* (1997).

Differences on parasitism between the right and left sides of the gills were evaluated using the Mann-Whitney's U test. To compare the number of parasites between different gill arches, gill sectors and collection days the Kruskal-Wallis test was used.

## Results

Two species of monogeneans were identified parasitizing the gills: *Scleroductus* sp. (Gyrodactylidae) and *Aphanoblastella mastigatus* Suriano, 1986 (Dactylogyridae).

Fish maintained at the lowest density presented high number of the protozoan *Ichthyophthirius multifiliis* (Fouquet, 1876) and necrosis foci on the fins and tail caused by bacteria. At day 10, two experimental units were lost at the density 14 fish per tank and one at the density fish per tank.

*Scleroductus* sp.

In total, 101 specimens of *Scleroductus* sp. were collected at day 5, and 133 at day 10. In relation to left and right side of the gills, there was no significant difference on the abundance of parasites found. Higher abundance and prevalence of *Scleroductus* sp. were observed at the initial sampling and in fish maintained at 28 fish per tank at day 10 (Table 1). Fish maintained at 14 fish per tank showed the highest mean abundance of monogeneans at the initial sampling when compared to day 5. Considering all collection days, neither mean intensity, mean abundance nor prevalence of *Scleroductus* sp. showed differences between gill arches, gill sectors, stocking densities and collection days (Fig. 2).

*Aphanoblastella mastigatus*

In total, 186 specimens of *A. mastigatus* were collected at day 5, and 228 at day 10. No significant difference was observed in the mean intensity and abundance among the stocking densities at day 5 (Table 1). The highest mean intensity and abundance were observed at day 10 in fish maintained at 28 fish per tank, followed by those at 42 fish per tank and initial collection. In fish maintained at 28 fish per tank and 42 fish per tank, the prevalence increased along the experimental period reaching 100 % at day 10.

On the initial collection, no preference for gill arches and sec-

Table 2. Results of Mann-Whitney's U test between the abundance of *Scleroductus* sp. and *A. mastigatus* on the gills of *R. quelen*, at three stocking densities, at different collection day. \*Significant values ( $P \leq 0.05$ )

<i>Scleroductus</i> sp. x <i>A. mastigatus</i>		Mann-Whitney	
Density	Collection	U	P
14 fish per tank	Initial	152.00	0.734
	Day 5	81.00	0.007*
28 fish per tank	Day 5	95.00	0.029*
	Day 10	20.50	0.025*
42 fish per tank	Day 5	73.50	0.004*
	Day 10	38.00	0.001*

tors were observed. At day 5, fish maintained at 14 fish per tank showed preference for the gill arches II and III ( $p=0.035$ ). *Aphanoblastella mastigatus* showed preference for the first gill arches in fish maintained at 28 fish per tank ( $p=0.012$ ). The mean abundance was higher in the gill arches I and II in fish maintained at 42 fish per tank ( $p=0.001$ ).

*Aphanoblastella mastigatus* showed preference for the dorsal and medium sectors (50.00 %) when compared to the ventral sector (11.11 %) in fish maintained at 14 fish per tank. In fish maintained at 28 fish per tank and 42 fish per tank the dorsal and medium

sectors presented the highest abundance, respectively, at days 5 and 10 (Fig. 1).

#### *Scleroductus* sp. versus *Aphanoblastella mastigatus*

There was significant difference between the abundance of *Scleroductus* sp. and *A. mastigatus* in all treatments and collection days, except for the initial collection. Higher abundance of *A. mastigatus* was observed in all treatments. After 10 days, there was a high mortality of fish maintained at the density of 14 fish per tank, and due to significant losses these tanks were excluded from the analyzes (Table 2).

#### Discussion

Parasitological indexes of *Scleroductus* sp. and *A. mastigatus* were compared to evaluate whether one of them presented relation to the stocking density, collection day and distribution on the gills. The monogenean *A. mastigatus* was found to be the most abundant in all stocking densities with exception of the initial collection day. It is suggested that the environmental conditions were responsible for the high number of this parasite species, once farmed fish from the initial collection day showed differences when compared to laboratory facilities. According to Moraes and

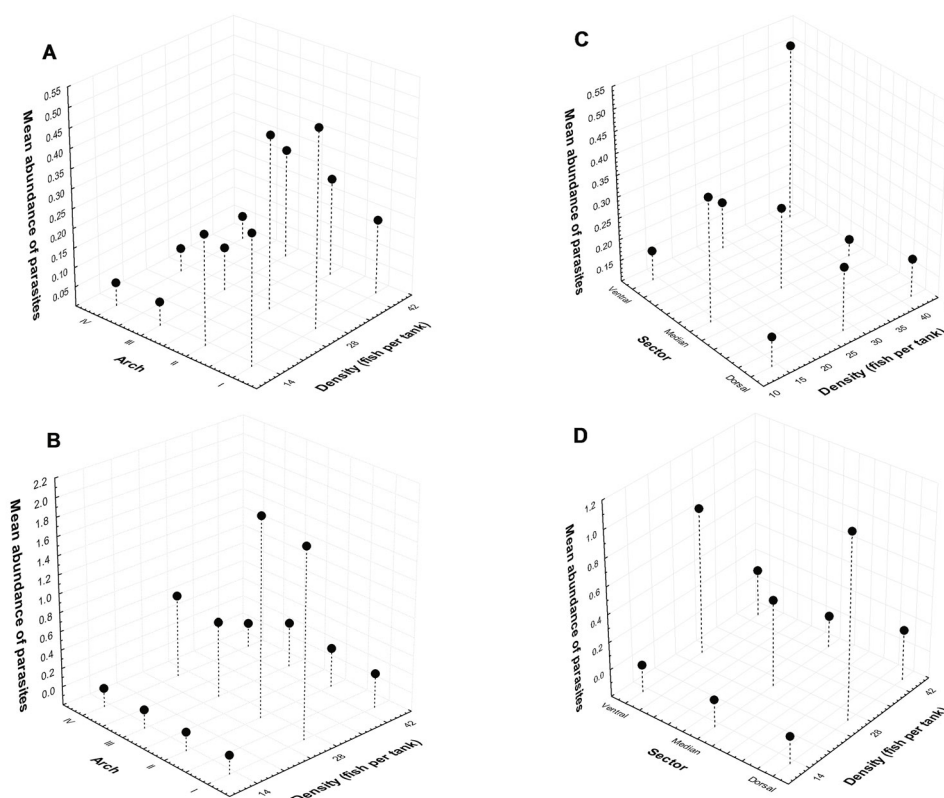


Fig. 2. Mean abundance of the parasite *Scleroductus* sp. on *Rhamdia quelen*, comparing the gill arches and stocking densities at day 5 (A) and day 10 (B) of the experiment, and comparing the gill sectors and stocking densities at day 5 (C) and day 10 (D) of the experiment

Martins (2004) the water quality and farming conditions may favor proliferation. This study showed that the south american catfish maintained at high density were apparently more resistant when compared to the ones maintained at low density.

Ichthyophthiriasis and bacteriosis found in fish maintained at low density indicate that this environment was not favorable for the south american catfish under laboratory conditions, considering that all other variables were controlled except for the stocking density. *Rhamdia quelen* inhabits lakes and the bottom of rivers and prefers lentic waters (Baldiasserotto & Gomes, 2010). The fish maintained in laboratory are more exposed to stress, what favours spreading of the disease pathogens; in fish maintained at 42 fish per tank more protection could be observed with fish agglomeration, possibly favoring their welfare.

Venancio *et al.* (2010) recognized *Aphanoblastella* sp. as a dominant parasite on the gills of the south american catfish in the Paraíba do Sul river, State of Rio de Janeiro. Regarding the preference for the left and right side of the gills, no significant differences were observed by other authors (Iannacone & Alvarino, 2012; Jeannette *et al.*, 2010; Raymond *et al.*, 2006; Rubio-Godoy, 2008; Backer *et al.*, 2005), similar to the present assay, suggesting that the host is symmetric and water flow must be the same through both sides.

Higher prevalence and abundance of *Scleroductus* sp. at the initial collection reflected the parasitic infection of the fish at the farm from where the fish were taken. The decrease in the abundance of *Scleroductus* sp. was observed along the experiment, while for *A. mastigatus* an increase in prevalence, mean intensity and mean abundance was observed along the assay. No preference of *Scleroductus* sp. for the gill arches was recorded, probably due to the low abundance of parasites on the gills. However, it seems probable that *Scleroductus* sp. may not exhibit the preference for the microhabitat, as already observed by Soylu *et al.* (2010) for *Dactylogyrus crucifer* Wagener, 1857 in *Rutilus rutilus* (Linnaeus, 1758) and Iannacone and Alvarino (2012) for *Mexicana* sp. in *Anisotremus scapularis* (Tschudi, 1846).

Monogenean parasites exhibited preference for the first gill arches. Oliva and Luque (1998) found high abundance of the monogenean *Microcotyle nemadactylus* Dillon & Hargis 1965 on the arch I and dorsal region of the wild fish *Cheilodactylus variegatus* Valenciennes, 1833, suggesting the enhancement of couple formation for reproduction. The preference for the gill arch I of *Diclidophora merlangi* (Kuhn In Nordmann, 1832) Krøyer, 1851 in whiting *Gadus merlangus* Linnaeus, 1758 proposed some host/parasite or parasite/parasite relationship (Arme & Halton, 1972). Buchmann (1989) found arches I and II mostly parasitized by *Pseudodactylogyrus bini* (Kikuchi 1929) whereas *P. anguillae* (Yin & Sproston, 1948) preferred the arches II and IV. The author suggested that the reason of this site selection was the size and development of the host *Anguilla anguilla* and not an interspecific factor. In Brazil, Jerônimo *et al.* (2013) have studied the microhabitat preference of two monogeneans, *Mymarothecium boegeri* Cohen & Khon, 2005 and *Anacanthorus penilabiatus* Boeger, Husak & Martins, 1995, on

different hybrid fish. In the hybrid patinga (*Piaractus mesopotamicus* female x *P. brachypomus* male) preference for gill arch I were noted but no other preferences were found in other studied fish, the hybrids tambacu (*Colossoma macropomum* female x *P. mesopotamicus* male) and surubim (*Pseudoplatystoma corruscans*). The authors suggested that the results could be related to the size of the arches and culture conditions, and the differences were not enough to support the hypothesis of microhabitat preference.

*Aphanoblastella mastigatus* showed preference for the arches I and II in the south american catfish, and no preference for gill sectors.. Rubio-Godoy and Tinsley (2002) and Rubio-Godoy (2008) have observed high number of the adult parasite *Discocotyle sagittata* in the first gill arches, and high number of larval stages on the arches III and IV of rainbow trout, suggesting that the larval stages reached the gill using the water flow in this region and then they would migrate to arches I and II to get space for new larvae. According to Buchmann (1989), the real reason for the preference for microhabitats, or niche restriction, is unknown, but chances for mating may be increased when microhabitats are narrow.

Based on the results of the present study we conclude that the abundance of parasites in the south american catfish is correlated to environmental conditions, supported by two evidences during the experiment. First, there was a turnover in the dominance of the species in relation to the environment of fish. *Scleroductus* sp. was dominant in the fish farm while *A. mastigatus* was dominant in the experiment tanks, showing the opportunistic feature of the species. Then, independently of the stocking density, *A. mastigatus* showed preference for regions more exposed to the external environment, and this could be related to a more favorable microhabitat for attachment, feeding and/or reproduction of the monogenean parasites. Future studies should investigate which factors are more important to determine this distribution pattern, and if it extends to other species of parasites and host.

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