Neotrop. Helminthol., 7(1), 2013

ISSN: 2218-6425 impreso / ISSN: 1995-1043 on line

aphia
ASOCIACIÓN PERUANA DE HELMINTOLOGÍA E INVERTEBRADOS AFINES

# ORIGINAL ARTICLE / ARTÍCULO ORIGINAL

## MICROHABITAT OF MONOGENEA AND COPEPODIDS OF *LERNAEA CYPRINACEA* ON THE GILLS OF FOUR BRAZILIAN FRESHWATER FISH

## MICRO HÁBITAT BRANQUIAL DE MONOGENEA Y COPEPODITOS DE *LERNAEA CYPRINACEA* EN LAS BRANQUIAS DE CUATRO PECES BRASILEÑOS DE AGUA DULCE

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Suggested citation: Jerônimo, GT, Gonçalves, ELT, Bampi, D, Paseto, A, Pádua, SB, Ishikawa, MM & Martins, ML. 2013. Gill microhabitat of monogenea and copepodids of *Lernaea cyprinacea* on the gills of four Brazilian freshwater fish. Neotropical Helminthology, vol. 7, N°1, jan-jun, pp. 65 - 74.

## Abstract

This study evaluated the ectoparasite distribution on the gill archs of pond-reared pacu (*Piaractus mesopotamicus* Holmberg, 1887), hybrid patinga (*P. mesopotamicus* female x *P. brachypomus* (Cuvier, 1817) male), hybrid tambacu (*Colossoma macropomum* (Cuvier, 1816) female x *P. mesopotamicus* male) and hybrid surubim (*Pseudoplatystoma reticulatum* female x *P. corruscans* Spix & Agassiz, 1829 male). A total of 300 fish were examined from fish farms in West-Central Brazil for parasitological assessment of the gill arches I, II, III and IV, from the outer to inner arch, respectively. Only the monogenean *Mymarothecium boegeri* Cohen & Kohn, 2005 and *Anacanthorus penilabiatus* Boeger, Husak & Martins, 1995 from the hybrid patinga showed the greatest ($p<0.05$) mean intensities on the gill arch I when compared to gill arch IV. Microhabitat preference was not observed in the other fish examined. Copepodids of *Lernaea cyprinacea* Linnaeus 1758 showed no microhabitat preference. This study highlighted the fact that under culture conditions, homogeneous distribution of parasites on the gills may occur.

**Keywords:** *Anacanthorus* - Copepoda - Characidae - *Mymarothecium* - parasitologia - Siluriformes.

## Resumen

Este estudio evaluó la distribución de ectoparásitos en los arcos branquiales de pacu (*Piaractus mesopotamicus*), hibrido patinga (*P. mesopotamicus* hembra x *P. brachypomus* macho), híbrido tambacu (*Colossoma macropomum* hembra x *P. mesopotamicus* macho) e híbrido surubim (*Pseudoplastystoma reticulatum* hembra x *P. corruscans* macho) cultivados en viveros. Un total de 300 peces fueran examinados en piscifactorías del Centro Oeste de Brasil para un levantamiento parasitológico en los arcos branquiales denominados I, II, III y IV, del arco más externo al más interno, respectivamente. Solamente los monogeneos *Mymarothecium viatorum* y *Anacanthorus penilabiatus*, parásitos del híbrido patinga, presentaran mayores ($p<0,05$) intensidades promedios en el I arco cuando es comparado con el IV arco branquial. Aun así, ninguna preferencia por micro-hábitat por los parásitos en los otros peces fuera observado. Copepoditos de *Lernaea cyprinacea* no presentan preferencia por micro-hábitat. Este estudio muestra que en condiciones de cautiverio puede ocurrir una distribución homogénea de los parásitos en las branquias.

**Palabras clave**: *Anacanthorus* - Copepoda - Characidae - *Mymarothecium* - parasitologia - Siluriformes.

## INTRODUCTION

The Central-Western region of Brazil presents propitiated characteristics for freshwater fish culture development. Nowadays, it has been observed significant increase in the production of characid and silurid fish and also their hybrids. According to MPA (2012), in 2010, the production of those fishes in the region was responsible for about 70 tons, in which we could notice the improvement of 35% in relation to 2008.

Besides the intensification and aquaculture development, fish farms are predisposed to epizootics being the ectoparasites the most important agents responsible for economic losses (Shoemaker *et al*., 2012). Contrarily to endoparasites, ectoparasites can be directly affected by the water quality favoring or not their reproduction which can culminate to fish mortality (Moraes & Martins, 2004). According to Jerônimo *et al*. (2011a) the relationship host/parasite consists in an important biotic factor in fish, both intra and interspecific being directly related to water temperature, light intensity, photoperiod, chemical water composition and stocking density. Moreover, parasitic fauna of fish depends on the geographical location of the ecosystem and seasonality (Jerônimo *et al*., 2011a), on the water characteristics and the environmental fauna (Dogiel, 1970).

The study of parasitic communities is important for understanding the ecological relationship between the hosts and parasites once it depends mostly of populational dynamics, which allow observing the relation among the different parasite taxa competing or not by specific sites of attachment (Luque & Cezar, 2004). The fish gills represent one of the most exploited microhabitat by the ectoparasites (Fernando & Hanek, 1976) revealing the specific site of preference in the hosts (Jeannette *et al*., 2010).

There may be different outcomes to explain the microhabitat selection of ectoparasites on fish gills. Geets *et al*. (1997) suggested that the parasite attachment to different sites in the gills is a result of food availability. Another hypothesis is that the distribution on the gill arches may be a result of variation in the water flow and such mechanism would restrict the adaptation of parasites to the attachment sites (Arme & Halton, 1972; Davey, 1980; Etchegoin & Sardella, 1990; Poulin *et al.*, 1991). The third hypothesis was due to parasite reproduction in different sites and that the preference for microhabitat could be related to the age of parasite (Rohde, 1994). On this view, Timi (2003) suggested that parasite selection for specific site might occur in order to enhance the parasite reproduction chances or by interspecific competition.

Some investigators have focused the microhabitat studies in fish from natural (Geets *et al*., 1997; Oliva & Luque, 1998; Lo & Morand, 2001; Baker *et al*., 2005; Raymond *et al.*, 2006; Jeannette *et al.*, 2010; Hermida *et al.* 2012, Iannacone & Alvariño, 2012) and culture environment (Buchmann, 1989; Heinecke *et al.*, 2007; Rubio-Godoy, 2008), but no studies were performed in Brazilian cultured fish until this moment.

By supporting the above statements, the hypothesis of this study was that ectoparasites may present microhabitat preference depending on the host and/or parasite taxon in culture conditions. This study aimed to evaluate the microhabitat distribution of ectoparasites on the gills of four fishes of economic importance in Brazil.

## MATERIAL AND METHODS

In the period of July 2009 through July 2011, a total of 300 fishes were captured from ponds located at Grande Dourados, Mato Grosso do Sul (2212'54.45"S 5448'26.35"W), Central-Western region of Brazil. From these, 60 pacu (*Piaractus mesopotamicus* Holmberg, 1887), 60 hibrids patinga (*P. mesopotamicus* fêmea x *P. brachypomus* Cuvier 1818 macho), 60 hibrids tambacu (*Colossoma macropomum* Cuvier 1818 female x *P. mesopotamicus* male) and 120 hibrids surubim (*Pseudoplatystoma reticulatum* (Eigenmann & Eigenmnn, 1889) female x *P. corruscans* (Spix and Agassiz, 1829) male) were examined.

The fishes were measured and weighed and the water quality monitored during the culture of each fish. Water temperature, dissolved oxygen, electrical conductivity and pH were measured with a HANNA multiparameter. The alkalinity was measured by titulation method, transparency was measured with a Secchi disc and total ammonia, nitrate, nitrite, iron and orthophosphate was measured with a colorimetric method ALFAKIT.

The fishes were captured with net and transported to the fish farm Laboratory of Embrapa Western Agriculture and deeply anesthetized in clove oil (75 $mg.L^{-1}$) (Ethic Comittee 23080.0 29979/2009-05/CEUA/UFSC) for parasitological analysis.

The gills arches were carefully separated from the outer to the inner arch and named as I, II, III and IV, respectively. After that, the gills were bathed in hot water 60°C, for parasite relax and fixed in 5% formalin solution for posterior quantification according to Jerônimo *et al.* (2011b). The parasites were identified by the method of Hoyer's to observe the sclerotic structures (Kritsky *et al.*, 1995) and identified according to Boeger *et al*. (1995, 2002), Cohen & Kohn (2005) and Thacther (2006). The prevalence, mean intensity and mean abundance were calculated as suggested by Bush *et al*. (1997), wich for monogeneans were not separated by species. The monogeneans are deposited in the Laboratory AQUOS, Santa Catarina, Brazil collection under the numbers GTJ 1-20.

For statistical analysis, the hypothesis tests were used to compare the values parasitic values among the arches (I, II, III and IV), being the null hypothesis the absence of microhabitat preference. The behavior of the data was verified by analyzing the residual and predictions. The homocedasticity was analyzed by the Bartlett test with transformations. The data were submitted to a one way or factorial ANOVA, and the averages compared by the Tukey test.

Monogenean values from pacu was transformed in root (y), once these values presented proportional variation to the means and posteriorly submitted to variance analysis. To other hosts the monogenean values were transformed to root (y) and the *Lernaea cyprinacea* copepodids values were transformed in root (y+0.5), once these data presented elevated numbers below 10 and mostly equal zero.

## RESULTS

The means and standard deviation of weight and total length were as follows, respectively: pacu 689.2 ± 288.7 g and 28.8 ± 3.7 cm, hybrid patinga 724.3 ± 173.0 g and 32.5 ± 2.3 cm, hybrid tambacu 1,141.0 ± 295.6 g and 37.6 3.4 cm, and hybrid surubim 784.2 ± 371.3 g and 44.9 ± 6.2 cm.

The ectoparasites of the Monogenea class were identified as *Anacanthorus penilabiatus* Boeger, Husak & Martins, 1995 in pacu, hybrid patinga and hybrid tambacu; *Mymarothecium boegeri* Cohen & Kohn 2005 and *M. viatorum* Boeger, Piasecki & Sobecka, 2002 in the hybrids tambacu and patinga, respectively; *Amphocleithrium paraguayensis* Price & Romero 1969, *Vancleaveus fungulus* Kritsky, Thatcher & Boeger, 1986, *V. ciccinus* Kritsky, Thatcher & Boeger, 1986, *V. janacauensis* Kritsky, Thatcher & Boeger, 1986 and *Ameloblastella* sp. Kritsky, Mendonza-Franco & Scholz, 2000 in the hybrid surubim. Crustacea was identified as *Lernaea cyprinacea* Linnaeus, 1758 copepodids in the hybrids patinga, tambacu and surubim.

The water quality was kept within the recommended levels for freshwater fish (Table 1), except for ammonia concentration that was slightly higher in pacu culture.

More than 95% prevalence was found in all monogenean-parasitized fishes (Table 2). The highest mean intensity of monogenean in the gills was observed in pacu followed by the hybrids surubim, patinga and tambacu. On the other hand, the hybrids patinga and tambacu showed 40% and 36% prevalence of copepodids, respectively, contrarily to that found in the hybrid surubim that presented low prevalence (16%) and pacu that was not found to be parasitized by copepodids (Table 3).

For monogeneans from pacu neither the variance analysis nor the Tukey test means comparison showed significant difference (Fig. 1).

The Fig. 2 presents the parasitic values of monogeneans transformed in log (y+1) in order to reduce the intervals between the highest and the lowest values of the data. Only the hybrid patinga showed higher mean intensity and mean abundance of infection in the gill arch I that that observed in the arch IV (Table 2).

According to the variance analysis and Tukey test means comparison no significant difference ($p>0.05$) was found in the parasitism by copepodids among the gills arches and fishes examined.

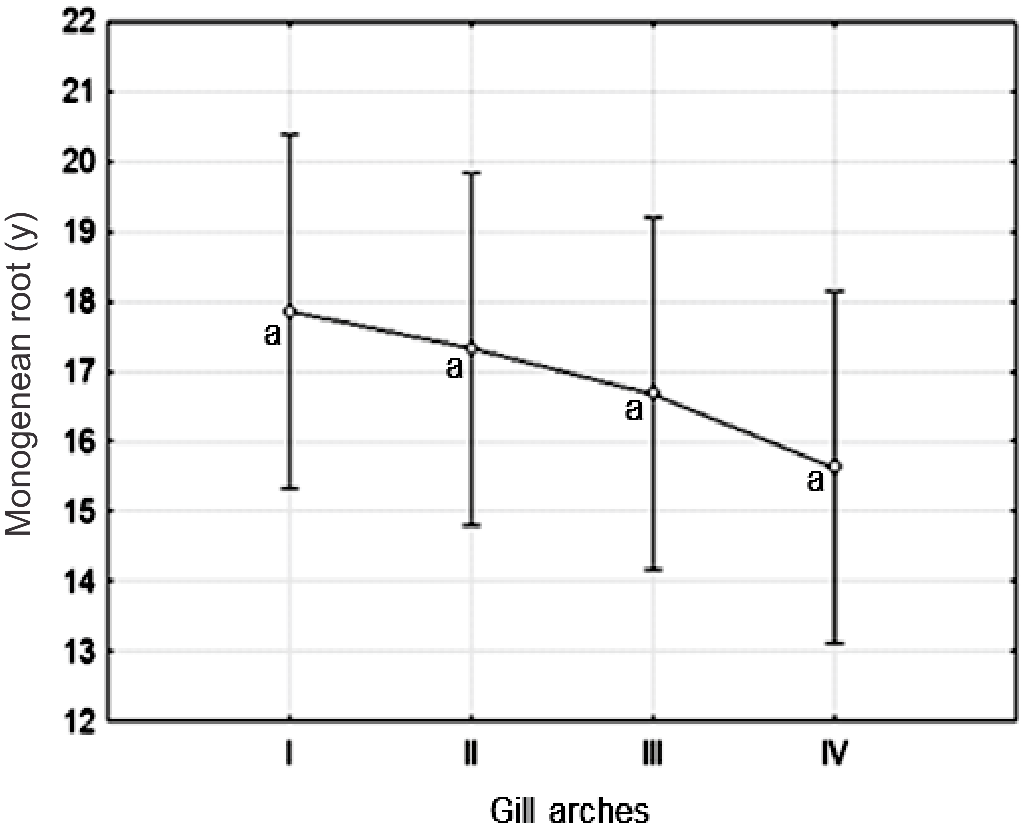


**Figure 1.** Variance analysis comparing transformed data of monogeneans among the gill arches of pacu, *Piaractus mesopotamicus*. Different letters indicate significant difference among the arches in confidence intervals of 95%.

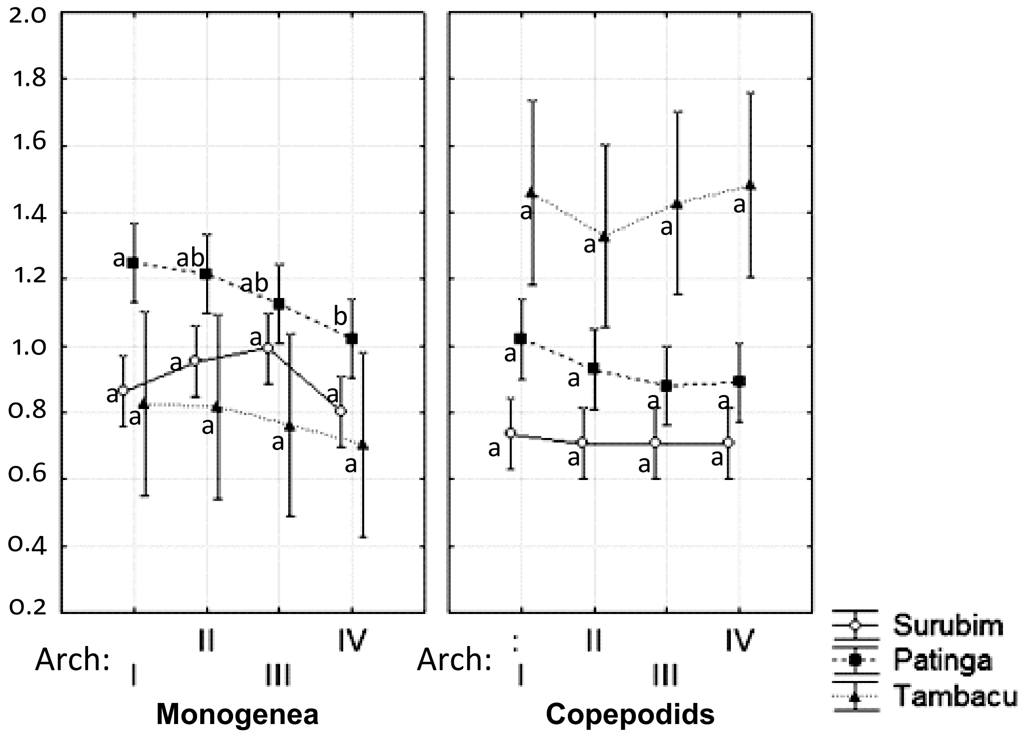


**Figure 2.** Variance analysis comparing transformed data of monogeneans and copepodids of *Lernaea cyprinacea* among the gill arches of the hybrids surubim, patinga e tambacu. Different letters indicate significant difference among the arches in confidence intervals of 95%.

**Table 1.** Mean values of water quality during the culture of Brazilian freshwater fishes.

| Parameter | Fish | | | | Recommended values by Boyd & Tucker (1998) |
|---|---|---|---|---|---|
| | Pacu | Hybrid patinga | Hybrid tambacu | Hybrid surubim | |
| Alkalinity (mg.L$^{-1}$) | 57.98 8.01 | 40.72 5.83 | 49.82 9.35 | 39.03 6.43 | 20 a 300 |
| pH | 7.02 0.36 | 7.41 0.98 | 7.19 0.45 | 7.14 0.49 | 6 a 8 |
| Conductivity ( S.cm$^{-1}$) | 55.11 7.34 | 34.04 8.11 | 62.99 9.94 | 26.00 4.93 | < 1.000 |
| Dissolved oxygen (mg.L$^{-1}$) | 4.21 1.99 | 7.38 2.23 | 6.61 1.29 | 6.30 1.47 | 5 a 15 |
| Temperature (°C) | 22.8 2.23 | 22.17 3.90 | 21.99 4.09 | 23.53 4.18 | 20 a 30 |
| Transparency (cm) | - | 24.55 7.89 | 17.62 4.68 | 23.78 8.36 | 20 a 50 |
| Ammonia (mg.L$^{-1}$) | 0.45 0.21 | 0.18 0.17 | 0.15 0.09 | 0.25 0.15 | < 0,3 |
| Iron (mg.L$^{-1}$ Fe) | 0.46 028 | 0.26 0.37 | 0.33 0.53 | 0.12 0.19 | 0.05 a 0.5 |
| Nitrate (mg.L$^{-1}$ $NO_3$) | - | 0.00 0.00 | 0.00 0.00 | 0.00 0.00 | 0.2 a 10 |
| Nitrite (mg.L$^{-1}$ $NO_2$) | - | 0.10 0.32 | 0.03 0.06 | 0.02 0.07 | < 0.03 |
| Ortophosphate (mg.L$^{-1}$ $PO_4$) | - | 0.08 0.24 | 0.45 0.39 | 0.17 0.32 | 0.005 a 2 |

**Table 2.** Prevalence (P), mean intensity (MI), mean abundance (MA) and minimum and maximum values (Min – Max) of monogeneans on the gill arches of the examined hybrids. Different letters indicate significant difference among the arches (p<0.05).

| | Arch I | Arch II | Arch III | Arch IV | Total |
|---|---|---|---|---|---|
| | | | **Pacu (n=60)** | | |
| **P (%)** | 100 | 100 | 100 | 100 | 100 |
| **MI** | 416.5 353.2[a] | 395.1 348.9[a] | 373.0 328.1[a] | 344.7 327.8[a] | 1529 1312 |
| **MA** | 416.5 353.2[a] | 395.1 348.9[a] | 373.0 328.1[a] | 344.7 327.8[a] | 1529 1312 |
| **Min – Max** | 3 – 1245 | 2 – 1315 | 8 – 970 | 2 – 1008 | 24 - 4269 |
| | | | **Hybrid patinga (n=58)** | | |
| **P (%)** | 100 | 98 | 97 | 93 | 100 |
| **MI** | 25.7 22.9[a] | 21.9 17.9[ab] | 20.1 23.7[ab] | 15.7 13.4[b] | 81 58 |
| **MA** | 25.7 22.9[a] | 21.6 18.0[ab] | 19.5 23.6[ab] | 14.6 13.5[b] | 81 58 |
| **Min – Max** | 1 – 87 | 1 – 81 | 1 – 171 | 1 – 71 | 3 - 314 |
| | | | **Hybrid tambacu (n=60)** | | |
| **P (%)** | 86 | 78 | 76 | 78 | 97 |
| **MI** | 18 24.8[a] | 18.6 21.4[a] | 16.6 18.4[a] | 12.8 16.6[a] | 60.9 81 |
| **MA** | 15.5 23.9[a] | 14.4 20.3[a] | 12.6 17.5[a] | 9.9 15.5[a] | 52.5 77 |
| **Min – Max** | 1 – 105 | 1 – 83 | 1 – 75 | 1 – 88 | 1 - 285 |
| | | | **Hybrid surubim (n=120)** | | |
| **P (%)** | 91 | 92 | 94 | 92 | 95 |
| **MI** | 28.0 27.8[a] | 35.0 39.8[a] | 36.9 38.7[a] | 33.9 43.5[a] | 130 138 |
| **MA** | 25.4 27.7[a] | 32.1 39.2[a] | 34.8 38.2[a] | 31.0 42.5[a] | 123 158 |
| **Min – Max** | 1 – 170 | 1 – 311 | 1 – 179 | 1 – 274 | 2 - 934 |

**Table 3**. Prevalence (P), mean intensity (MI), mean abundance (MA) and minimum and maximum values (Min – Max) of copepodids of *Lernaea cyprinacea* on the gill arches of the examined hybrids. Different letters indicate significant difference among the fishes (p<0.05).

| | Arch I | Arch II | Arch III | Arch IV | Total |
|---|---|---|---|---|---|
| | | | **Hybrid patinga (n=60)** | | |
| **P (%)** | 28.3[a] | 18.3[a] | 16.7[a] | 23.3[a] | 40[a] |
| **MI** | 3.0 2.9[a] | 3.5 3.1[a] | 2.6 1.7[a] | 1.8 1.3[a] | 6 7 |
| **MA** | 0.9 2.0[a] | 0.6 1.9[a] | 0.4 1.2[a] | 0.4 1.0[a] | 2 5 |
| **Min – Max** | 1 – 10 | 1 – 12 | 1 – 6 | 1 – 6 | 1 - 30 |
| | | | **Hybrid tambacu (n=58)** | | |
| **P (%)** | 28.3[a] | 26.7[a] | 25[a] | 30[a] | 36[a] |
| **MI** | 12.6 12.5[a] | 9.6 7.8[a] | 14.1 15.1[a] | 12.1 11.7[a] | 38 41 |
| **MA** | 3.6 8.8[a] | 2.6 5.9[a] | 3.5 9.8[a] | 3.6 8.5[a] | 14 30 |
| **Min – Max** | 1 – 51 | 1 – 34 | 1 – 64 | 1 – 42 | 1 - 180 |
| | | | **Hybrid surubim (n=120)** | | |
| **P (%)** | 5.8[b] | 10[b] | 5[b] | 10.8[b] | 16[b] |
| **MI** | 2.0 0.6[a] | 1.8 0.6[a] | 2.7 0.7[a] | 3.0 1.2[a] | 5 4 |
| **MA** | 0.1 0.5[a] | 0.2 0.6[a] | 0.1 0.6[a] | 0.3 1.2[a] | 1 3 |
| **Min – Max** | 1 – 4 | 1 – 4 | 1 – 5 | 1 – 7 | 1 - 11 |

## DISCUSSION

This study evaluated whether ectoparasites in Brazilian cultured fishes present microhabitat preference on the gills under culture conditions. The greatest monogenean values ($p<0.05$) in the arch I of the hybrid patinga were similar to that found by Rohde & Watson (1985), who reported high mean intensity of *Kuhnia scombri* Kuhn, 1829 e *K. sprostonae* Price, 1961 (Monogenea: Polyopisthocotylea) in the arches I and II. In fact, these authors suggested microhabitat difference on the gills of *Scomber australasicus* Cuvier, 1832, *S. scombrus* Linnaeus, 1758 and *S. japonicas* Houttuyn, 1782 captured in Pacific and Atlantic Ocean. Similarly to the present results, Ramasamy *et al.* (1985) also observed high mean intensity of infection by *Vallisia indica* Unnithan, 1962 and *Allodiscocotyla chorinemi* Yamaguti, 1953 (Monogenea: Polyopisthocotylea) in the arch I of *Scomberoides commersonnianus* Lacepède, 1801. In European eel, *Anguilla Anguilla* Linnaeus, 1758, Buchmann (1989) also stated high mean intensity of *Pseudodactylogyrus bini* Kiknuchi, 1929 in the arches I and II. On the other hand, Raymond *et al.* (2006) evaluated the distribution of *Afrodiplozoon polycotyleus* Paperna, 1973 on the gills of *Barbus neumayeri* Fischer, 1884 from the river Mpanga, Uganda and concluded that the parasites have been concentrated on the gill arches II and IV, differently of our results. The other fish examined in this study no difference was found among the gill arches corroborating the results of Soylu *et al.* (2010) on the spatial distribution of *Dactylogyrus crucifer* Wagener, 1857 on the gills of *Rutilus rutilus* Linnaeus, 1758 in Lake Spanca, Turkey.

Similarly to that observed by Ramasamy *et al.* (1985) in *S. commersonnianus* Lacepède, 1801 parasitized by copepod *Caligus* sp., our results did not show significant difference neither among the gill arches nor among the fishes parasitized by the copepodids of *L. cyprinacea*. This could be explained by the larval stage of parasite. According to Timi (2003) adult parasites of *Lernanthropus cynoscicola* Timi and Etchegoin, 1996 in *Cynoscion guatucupa* Cuvier, 1830 occupied different sites of attachment and greater prevalence in the gill arch IV followed by the arches III, II and I. On the other hand, females showed high prevalence and mean abundance in the arch II (Timi, 2003). Ho & Do (1985) studying the specific niche of *Lernanthropus* concluded that the body shape of these copepods has been developed to minimize the resistance against the income water stream in the respiratory process. On this view, copepods of this genus could be used as model to study the microhabitat. It can be inferred that the body shape of *Lernaea* copepodids also present resistance to water flow on the gills being unneeded the specific microhabitat on this stage in the life cycle.

The analyses found in this study corroborate the null hypothesis of the non-existence of microhabitat preference on the gills at least on these fishes examined.

Unpublished data (Martins *et al.*) also showed no preference by gill microhabitat in Nile tilapia parasitized by the monogeneans *Scutogyrus longicornis* Paperna & Thurston, 1969, *Cychlidogyrus sclerosus* Paperna & Thurston, 1969, *Cychlidogyrus thurstonae* Ergens, 1981 and *Cychlidogyrus tilapiae* Paperna, 1960 in fish farm in Southern Brazil.

We consider that the differences found between arch I and arch IV in the hybrid patinga were not enough to justify our hypothesis once they can also be related to difference in size between the arches. Another reason to this support could be related to culture conditions. Differently from fish examined in natural environment, high stocking density in culture conditions could provoke the homogeneous distribution on the gills. Further studies must be carried out on other cultured fish considering the competition of parasites and specific surface gill tissue.

## ACKNOWLEDGEMENTS

The authors thank National Council for Scientific and Technological Development (CNPq) for financial support (CNPq

577657/2008-9) and grant to M.L. Martins (CNPq 302493/2012-7); Ministry of Fisheries and Aquaculture; Embrapa Western Agriculture, Dourados, MS, Brazil; Macroprogama 1 "Bases Tecnológicas para o Densenvolvimento Sustentável da Aquicultura - AQUABRASIL" and Coordination for the Improvement of Higher Education Personnel (CAPES) for doctoral schollarship to G.T. Jerônimo (CAPES/BEX 9655-11-5).

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Received January 8, 2013.
Accepted March 11, 2013.

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