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MICROHABITAT PREFERENCE AND SEASONALITY OF GILL MONOGENEANS IN NILE TILAPIA REARED IN SOUTHERN BRAZIL

PREFERENCIA DE MICROHÁBITAT Y ESTACIONALIDAD DE MONOGENEOS BRANQUIALES DE LA TILAPIA DEL NILO CULTIVADA EN EL SURESTE DE BRASIL

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Abstract

Few studies have been done to evaluate the monogenean microhabitat preference. This study evaluated the monogenean distribution among different gill arches of Nile tilapia Oreochromis niloticus and seasonality. A total of 89 fish were captured in a pond located in Chapecó, Santa Catarina, for parasitological analysis. After fish euthanasia, the gill arches were removed, separated from the most external to the most internal and named as I, II, III and IV. Transparency, pH, oxygen, temperature, alkalinity, conductivity, ammonia, iron, orthophosphate, sulfide, nitrite and nitrate of water pond were measured. In spring 2008 the mean abundances of the monogeneans (Scutogyrus longicornis, Cychlidogyrus sclerosus, C. thurstonae and C. tilapiae) in the arches I, II, III and IV were, respectively, 11.45, 14.45, 13.8, 13.15, in summer 2008 they were 13.12, 12.25, 14.94, 21.53, in summer 2009 they were 2.75, 3.25, 2.45, 2.45, in autumn 2009 they were 13.44, 18.78, 11.78, 9.22 and in winter 2009 they were 0.35, 0.5, 0.3, 0.5. Monogenean preference for microhabitat in the gills of farmed Nile Tilapia was not observed. A significant increase of parasites in spring and summer 2008 and autumn 2009 was observed. The highest number of parasites coincided with seasons of high water temperature. The lack of microhabitat preference among the gill arches suggest homogenous distribution of parasites combined with water quality parameters.

Keywords: Oreochromis – monogenean parasites - gills - season.
Resumen

Pocos estudios han sido hechos para evaluar la preferencia por el microhabitat de monogenea. Este estudio evaluó la distribución y estacionalidad de monogenea entre los diferentes arcos branquiales de tilapia del Nilo. Un total de 89 peces fueron capturados en una laguna en la ciudad de Chapecó, Santa Catarina, para el análisis parasitológico. Después de la eutanasia de los peces, fueron extraídos los arcos branquiales separadamente a partir de la región externa hasta la interna y numerados como I, II, III e IV. Fueron evaluadas la transparencia del agua, el pH, el oxígeno disuelto, la temperatura, la alcalinidad, la conductividad, el amonio, el hierro, el ortofosfato, el sulfato, el nitrato y el nitrato. En la primavera de 2008 las abundancias promedio de monogenea (*Scutogyrus longicornis*, *Cychlidogyrus sclerosus*, *C. thurstonae* y *C. tilapiae*) en los arcos I, II, III y IV fueron, respectivamente, 11,45, 14,45; 13,8; 13,15; en el verano de 2008 de 13,12; 12,25; 14,94; 21,53; en el verano de 2009 de 2,75; 3,25; 2,45; 2,45; en el otoño de 2009 de 13,44; 18,78; 11,78; 9,22 y en el invierno de 2009 de 0,35; 0,5; 0,3; 0,5. No fue observada preferencia por monogenea por el microhabitat en las branquias de la tilapia del Nilo en cultivo. Fue observado un aumento significativo de los helmintos en la primavera y verano de 2008 y otoño de 2009. El mayor número de parásitos coincidió con las estaciones de mayor temperatura del agua. La falta de preferencia por microhabitat entre los arcos branquiales sugiere una distribución homogénea de los parásitos combinados con los parámetros de calidad de agua.

Palabras clave: branquias - estacion *Oreochromis* - parásitos.

INTRODUCTION

Monogenean helminthes are among the major parasites on Nile tilapia, even though in Brazil there are few cases of mass mortalities caused by high parasite number. They are responsible for significant economical losses, especially when present with both bacterial and parasitic concurrent infections (Xu *et al.*, 2007). Its reproduction is favored by water quality, stocking density and temperature (Moraes & Martins, 2004). The parasite may inhabit and live well adapted to the host with no mortality outbreaks. On the other hand, if fish are exposed to stressful conditions, disease can occur (Buchmann & Bresciani, 1997).


In Brazil, few studies regarding the gill microhabitat preference of parasites in farmed fish are found. Jerônimo *et al.* (2013) have reported higher mean intensity of the monogeneans *Mymarothecium viatorum* Boeger, Piasecki & Sobecka, 2002 and *Anacanthorus penilabiatus* Boeger, Husak & Martins, 1995 in the gill arch I than in the arch IV of pacu (*Piaractus mesopotamicus* Holmberg, 1887) native farmed fish.

On this view, the aim of this work was to evaluate the monogenean preference for gill microhabitat in Nile tilapia, *Oreochromis niloticus* Linnaeus 1758 (Cichlidae) reared in ponds in Southern Brazil and its occurrence in each season of the year.
**MATERIAL AND METHODS**

From spring 2008 to winter 2009, a total of 89 Nile tilapia were captured with nets in a fish pond located in Chapecó in the State of Santa Catarina, Southern Brazil (27° 5' 48" S and 52° 37' 7" W) for parasitological diagnosis. Fish were kept in 2000 m² earthen ponds at a stocking density of 2 to 3 fish/m² and fed twice a day with commercial diet. In each season 20 fish were examined, except in autumn 2009 when only 9 animals were captured. Parasitological analysis was made in situ and fixed material was stored and transported to the laboratory. In the sampling day pH (Alfakit phmeter), transparency (Secchi disc), dissolved oxygen (Alfakit oxymeter at-150), water temperature, electrical conductivity (PHTEK CD203), alkalinity, ammonia, iron, orthophosphate, sulfide, nitrite and nitrate (measured with colorimetric kit Alfakit) of pond water were measured in the morning and afternoon.

After anesthetizes in a benzocaine solution (75 mg L⁻¹) and euthanasia (Ethic Committee number 23080.019034/2008-98/CEUA/UFSC), smears of the gill filaments were examined under microscope between a glass slide and a cover slip (Jerônimo et al., 2011). Gill arches were carefully separated from the most external to the most internal, named as I, II, III and IV, embedded into 60°C hot water to relax parasites and fixed in 5% formalin solution for further quantification in the laboratory using marked Petri dishes. The parasites were mounted using the Hoyer's method for identification through the esclerotized parts of the parasites (Kritsky et al., 1995; Eiras, 1994) and identified according to Paperna & Thurston (1969), Ergens (1981), Douêllou (1993), Pariselle & Euzet (1995) and Pariselle et al. (2003). The monogeneans are deposited in the Laboratory AQUOS, Santa Catarina, Brazil collection under the numbers ARS 1-5. The data of prevalence, mean intensity and mean abundance for all monogenean joined were calculated as suggested by Bush et al. (1997).

In order to evaluate microhabitat preference among gill arches and differences in parasitism between seasons, monogenean counting data were transformed to and submitted to factorial analysis of variance (ANOVA). Principal components analysis was also used as an exploratory technique to assess relation between seasons and every measured parameter. All analysis were performed using StatSoft's Statistica 7.0 software, using Bartlett's test to verify homogeneity of variances and Tukey's test used for mean comparison.

**RESULTS**

The means and standard deviation of weight (g) and total length (cm) were as follows: spring 2008 (357.5±97.4 and 27.2±2.3), summer 2008 (520.7±72.7 and 30.3±1.6), summer 2009 (110.1±32 and 20.6±1.8), autumn 2009 (152.2±95.3 and 22.1±3.5) and winter 2009 (251.6±51.2 and 23.8±1.8).

Almost all water quality parameters were constant during the whole collection period (Figure 1). As expected, the highest water temperature was found in summer and depletion of dissolved oxygen occurred in autumn. Water transparency was between 20 and 30 cm as measured with Secchi disc.


Prevalence rate of parasitism of all monogenean was high in all seasons, except in winter 2009 (Table 1). ANOVA data comparison showed no difference (P>0.05) in monogenean intensity between gill arches (Figure 2), despite of season, indicating there was no microhabitat preference in the current study. When gill arches were compared among seasons (Figure 3), significantly higher numbers of helminthes were found in spring 2008, summer 2008 and autumn 2009. Factorial ANOVA showed no significant interaction between seasons and gill arches preference (Figure 4).
Both prevalence and number of parasites were lower (P<0.05) in winter than that observed in the other seasons (Figure 3). Despite the high prevalence rate in summer 2009 (89%) mean intensity and mean abundance showed low values (3.1 and 2.7, respectively).

In the principal components analysis (Figure 5 a-b), the score and loading plots summarizes data influence over two factors, also called principal components (PC), which represent 71.81% of variance among samples. It shows a strong relation between sulfide and nitrite, opposed to water temperature and monogenean as well as relation between transparency and dissolved oxygen, opposed to conductivity. All toxic components, ammonia, nitrite, nitrate and sulfide, grouped in the same region of axis. Regarding seasons, summer 2008 and spring 2008 were more related with monogenean and water temperature, while summer 2009 was related to toxic components and autumn 2009 to transparency and dissolved oxygen.

Table 1. Parasitological indexes of Oreochromis niloticus cultured in Chapecó. Santa Catarina State. Southern Brazil, along the seasons and in each gill arch. IG: infected gills, EG: examined gills, IF: infected fish, EF: examined fish.

<table>
<thead>
<tr>
<th>Seasons</th>
<th>IG/EG</th>
<th>P (%)</th>
<th>Mean Intensity</th>
<th>Mean Abundance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spring 2008</td>
<td>80/80</td>
<td>100</td>
<td>13.2±11.2</td>
<td>13.2±11.2</td>
</tr>
<tr>
<td>Summer 2008</td>
<td>79/80</td>
<td>98</td>
<td>15.5±10.0</td>
<td>15.7±9.9</td>
</tr>
<tr>
<td>Summer 2009</td>
<td>71/80</td>
<td>89</td>
<td>3.1±1.8</td>
<td>2.7±1.9</td>
</tr>
<tr>
<td>Autumn 2009</td>
<td>35/36</td>
<td>97</td>
<td>13.7±9.6</td>
<td>13.3±9.7</td>
</tr>
<tr>
<td>Winter 2009</td>
<td>24/80</td>
<td>30</td>
<td>1.4±0.6</td>
<td>0.4±0.7</td>
</tr>
<tr>
<td>Gill arches</td>
<td>IF/EF</td>
<td>P (%)</td>
<td>Mean Intensity</td>
<td>Mean Abundance</td>
</tr>
<tr>
<td>Arch 1</td>
<td>71/89</td>
<td>80</td>
<td>9.8±8.3</td>
<td>7.8±8.4</td>
</tr>
<tr>
<td>Arch 2</td>
<td>74/89</td>
<td>83</td>
<td>10.5±10.3</td>
<td>8.7±10.1</td>
</tr>
<tr>
<td>Arch 3</td>
<td>69/89</td>
<td>77</td>
<td>10.7±8.9</td>
<td>8.2±9.0</td>
</tr>
<tr>
<td>Arch 4</td>
<td>75/89</td>
<td>84</td>
<td>10.9±12.8</td>
<td>9.2±12.4</td>
</tr>
</tbody>
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<tbody>
<tr>
<td>Water Temperature (°C)</td>
<td>20.15</td>
<td>26.9</td>
<td>19.05</td>
<td>20.45</td>
<td>12.6</td>
</tr>
<tr>
<td>pH</td>
<td>6.63</td>
<td>7.375</td>
<td>8.45</td>
<td>7.2</td>
<td>6.965</td>
</tr>
<tr>
<td>Transparency (cm)</td>
<td>20</td>
<td>20</td>
<td>22.5</td>
<td>27.5</td>
<td>22.5</td>
</tr>
<tr>
<td>Ammonia (mg/L)</td>
<td>0.1</td>
<td>0.1</td>
<td>1.025</td>
<td>0.45</td>
<td>0.1</td>
</tr>
<tr>
<td>Nitrite (mg/L)</td>
<td>0</td>
<td>0</td>
<td>0.03</td>
<td>0.03</td>
<td>0.005</td>
</tr>
<tr>
<td>Nitrate (mg/L)</td>
<td>0.05</td>
<td>0</td>
<td>0.85</td>
<td>0.4</td>
<td>1.035</td>
</tr>
<tr>
<td>Dissolved oxygen (mg/L)</td>
<td>3.985</td>
<td>8.665</td>
<td>0</td>
<td>20</td>
<td>10.655</td>
</tr>
<tr>
<td>Sulfide (mg/L)</td>
<td>0</td>
<td>0</td>
<td>0.025</td>
<td>0.025</td>
<td>0.0125</td>
</tr>
<tr>
<td>Conductivity (µS/cm)</td>
<td>78</td>
<td>74</td>
<td>79</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>Orthophosphate (mg/L)</td>
<td>0.75</td>
<td>0</td>
<td>0.75</td>
<td>0.75</td>
<td>1.25</td>
</tr>
<tr>
<td>Iron (mg/L)</td>
<td>0.75</td>
<td>0</td>
<td>0.25</td>
<td>0.9</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Figure 1. Water quality parameters from the pond in Chapecó, Santa Catarina State, Southern Brazil.
Figure 2. ANOVA comparison of transformed (\( \sqrt{x} \)) Monogenean count data between gill arches of Oreochromis niloticus cultured in Chapecó, Santa Catarina State, Southern Brazil.

Figure 3. ANOVA comparison of transformed (\( \sqrt{x} \)) Monogenoidea count data between seasons in Chapecó, Santa Catarina State, Southern Brazil.
DISCUSSION

Water temperature, which presents an important role in fish development by influencing directly its metabolic rates and also, several times, influencing parasite reproduction (Moraes & Martins, 2004), was a strong component related to monogenean, especially for 2008’s summer and spring.

Water quality parameters were kept within tolerance levels for fish maintenance (Sipaúba-Tavares, 1998). According to Pereira & Mercante (2005) ammonia may cause damage in fish production system and its levels depend on the amount fish, feeding rate, diet quality and temperature. In summer 2008 and especially in summer 2009 higher levels of ammonia were observed due to enhanced fish metabolism and increase of feeding related to higher water temperatures. Despite the low levels of oxygen in autumn 2009 and high ammonia concentration in summer 2008 and 2009 no abnormal fish behavior was observed. It could be explained due to lower water temperature in Southern Brazil when compared to Southeastern region (Tavares-Dias et al., 2008).

Parasitological analysis showed highest prevalence rates in spring and summer and the lowest in winter, results that confirmed the observations of Koskivaara et al. (1992) who found greater abundance of Dactylogyrus Diesing, 1850 in the gills of roach in spring and summer. As demonstrated in this study and compared to other Brazilian regions, water temperature has a strong influence on the parasite settlement (Buchmann & Bresciani, 1997). Garcia et al. (2003) related negative correlation between temperature and Urocleidoides sp. (Mizelle & Price, 1964) Kritsky, Thatcher & Boeger, 1986 in sword tail

Figure 4. Factorial ANOVA comparison of transformed (\(\sqrt{x}\)) Monogenean count data between seasons and gill arches of Oreochromis niloticus cultured in Chapecó, Santa Catarina State, Southern Brazil.
**Figure 5.** Principal component analysis (PCA) of water quality parameters and Monogenean count data obtained from a fish pond located in Chapecó, Santa Catarina State, Southern Brazil. DO = dissolved oxygen; TR = transparency; WT = water temperature; CT = electrical conductivity; AK = alkalinity; AM = ammonia; IR = iron; OP = orthophosphate; SF = sulphide; NI = nitrite; NA = nitrate; MO = Monogenoidea.
and platy fish (\textit{Xiphophorus hellerii} Heckel, 1848 and \textit{X. maculatus} Gunther, 1866). It must be added that not only the water temperature, but also other factors like oxygen concentration (Molnar, 1994) and handling management (Jerônimo \textit{et al.}, 2011) are responsible for modulating the parasite life cycle.

Seasonal occurrence of monogenean helminthes was mostly studied in reservoirs (Rawson & Rogers, 1973; Ranzani-Paiva \textit{et al.}, 2005) and lakes (Ozturk & Altunel, 2006). The population of \textit{Gyrodactylus macrochiri} Hoffman et Putz, 1964 on largemouth bass (\textit{Micropterus salmoides} Lacepède, 1802) increased as the water temperature reached 28°C (Rawson & Rogers, 1973). Except for autumn 2009 our results are in agreement with Koskivaara \textit{et al.} (1992). The authors also observed high mean abundance of \textit{Dactylogyrus} species in spring and summer.

The present results are supported by these authors where the highest mean intensity and abundance were found in spring and summer. Similarly to Ozturk & Altunel (2006) in autumn 2009 the majority of fish (97%) were infected and in consequence the mean intensity and abundance were statistically similar to spring and summer.

The water temperature has an important influence on the parasite abundance and egg production in monogenean life cycle (Tinsley & Jackson, 2002). The low infection observed in summer 2009 might be related not only to the water temperature (similar to autumn) but also other environmental factors, like toxic components as ammonia.

The seasonal variation of monogenean infections in intensive fish farming is poorly known, especially in Brazil. Flores-Crespo \textit{et al.} (1992) observed high infection by \textit{Dactylogyrus} sp. in reared tilapia. Later, Ranzani-Paiva \textit{et al.} (2005) confirmed high mean intensity of monogenean in spring, peaking in summer.

Although the highest prevalence rates observed in this study, the abundance of \textit{Cichlidogyrus} species was similar to that found by Lizama \textit{et al.} (2007). Kadlec \textit{et al.} (2003) found \textit{Dactylogyrus carpathicus} Zachvatkin, 1951 as the most abundant parasite in the gills of barbel (\textit{Barbus barbus} Linnaeus, 1758) in August. Contrarily, \textit{Dactylogyrus malleus} Linstow, 1877 showed significant higher abundance in April. It's necessary to remember that Kadlec \textit{et al.} (2003) obtained their results from the Danube River. In a fish pond system, where stocking density and feeding conditions are relatively stable, the parasite abundance may not be affected, as here demonstrated.

In terms of microhabitat, 68 to 73% of \textit{Diclidophora merlangi} Nordmann, 1832 found on whiting (\textit{Gadus merlangus} Linnaeus, 1758) from the Irish Sea were in the first gill arch (Arme & Halton, 1972). These authors explained that in a single infection the majority of worms could be located on the arch I, but in multiple infections the parasites would be found on the other arches. In part, this comment could be applied to our research in which the community of parasites was composed by infrapopulations. Buchmann (1989) has demonstrated preference of \textit{P. bini} for arches I and II and \textit{P. anguillae} preference for arches III and IV in European eel (\textit{Anguilla anguilla} Linnaeus, 1758). Although, in this study, parasite species were not analyzed separately, the results suggest that monogenean infrapopulation in tilapia reared in ponds may not vary among gill arches.

In the studies of Ramasamy \textit{et al.} (1985) parasites \textit{Scomberoides} species from the Bay of Bengal, India. Polyopisthocotyleans \textit{Vallisia indica} Unnithan, 1962 showed preference for arch I, but, when low parasite intensities were observed, they were also found on arches II and III. In the present work, parasite intensity can be considered lower than those normally observed in cultured fish (Tavares-Dias \textit{et al.}, 2001), supporting the hypothesis of Ramasamy \textit{et al.} (1985).

El Hafidi \textit{et al.} (1998) reported the preference of microhabitat in two species of Monogenea, \textit{Metamicrotecta cephalus} Azim 1939 and
Microcotyle mugilis Vogt, 1878 in the gills of Mugil cephalus Linnaeus, 1758. They found that when two species coexist, the intensity of infection is higher than that in monospecific infection. Both species showed preference for gill arch I. It's normally reported that two species coexisting may cause more damage on the host than monospecific infections (Koskivaara et al., 1992, Moraes & Martins, 2004). On the other hand, the second arch was the most preferred location by Pseudodactylogyrus in eel (Matejusová et al., 2003). According to Kadlec et al. (2003) the preference for microhabitat within gills is modulated by the parasite abundance. They found that the overlap between species increased in cases of greatest abundances. This could also be explaining the lack of difference among the gill arches in the present study.

The preference for the middle arches may be related, among other factors, to greater surface area (Ramasamy et al., 1985). According to Geets et al. (1997) Pseudo haliotrema Yamaguti, 1952 was more abundant in the first two arches of rabbit fish (Siganus sutor Valenciennes, 1835) while Microcotyle mouwoi Ishii et Sawada, 1938 did not show preference for gill arch. Even though, other factors are involved in the parasite distribution on the gills such as health status, parasite density and diversity. Another point that must be emphasized is the distinct environment where fish were captured.

The studies of Rubio-Godoy & Tinsley (2002) showed that the parasites migrate from the site of initial attachment to the most favorable place on the gill arches. In contrast, this study did not show any difference among the gill arches. Possibly this could be related to low parasite abundance when compared to those observed by Rubio-Godoy (2008) and even to parasite species.

This study suggests that parasitic fauna in cultured tilapia may not vary considerably in low water temperature. On the other hand, the pond management such as feeding rate, water flow, fish stocking density and the presence or not of swine manure has a strong influence on the parasitic life cycle. Further studies must be carried out in other Brazilian fish farming facilities to verify the parasite population in each gill arch, which varies in morphology according to fish species. Moreover, not only studies with the Brazilian wild fishes must be encouraged to improve the knowledge of the host parasite environment system but also experimental trials with different parasite density in controlled conditions.

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BIBLIOGRAPHIC REFERENCES


from cichlid fishes of Lake Kariba (Zimbabwe) with descriptions of five new species. Systematic Parasitology, vol. 25, pp. 159-186.


Molnár, K. 1994. Effect of decreased water oxygen content on common carp fry with Dactylogyrus vastator (Monogenea)


