

***Clinostomum complanatum* (Digenea, Clinostomidae) Density in *Rhamdia quelen* (Siluriformes, Pimelodidae) from South Brazil**

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ABSTRACT

The density of *Clinostomum complanatum* metacercariae by the body regions and among the size classes of *Rhamdia quelen* was compared. The host size classes were defined by cluster analysis, using weight and total length as attributes. These values were related with environmental characteristics and host biological data. In all the host size classes H and PeF, CF and PF, and P and AF regions pairs, showed similar density (tested by X^2 test). The hosts with 30-36 cm showed largest parasite density in all the regions, except in CAV. Fishes smaller than 11 cm did not show significant differences of parasite density in all body regions. In the other classes, H was the most infected region, followed by PeF. The results suggested that the environmental conditions had little importance on the host colonization by metacercariae.

Key words: *Clinostomum complanatum*, *Rhamdia quelen*, metacercariae density, Jundiá, South Brazil, fish disease, aquaculture

INTRODUCTION

The South American Catfish, *Rhamdia quelen* (Quoy and Gaimard, 1824) (Siluriformes, Pimelodidae) has importance for the pisciculture (Kossowski, 1996) and is found in South and Central America (Silfvergrip, 1996). This fish prefers the muddy bottom covered by leaves and decaying wood that provide protection during the day, and eat on benthic crustacean and zooplankton (Le Bail et al., 2000). *R. quelen* has a benthic behaviour (Guedes, 1980; Grosser et al., 1994; Bizerril, 1998; Bizerril and Lima, 2000) which facilitate the Digenea recruitment (Eiras et

al., 1999, Guzmán-Cornejo and García-Prieto, 1999). *Clinostomum complanatum* (Rudolphi, 1814) Braum, 1899 (Clinostomidae) metacercariae infect the skin, muscle, fins, head and viscera, causing pathologies and changes in the host behavior, with consequent economic losses in fish farms (Kagei et al., 1984; Eiras, 1994; Mitchell, 1995). The *Clinostomum* infection increases with the host size, suggesting a cumulative process (Kalantan et al., 1987).

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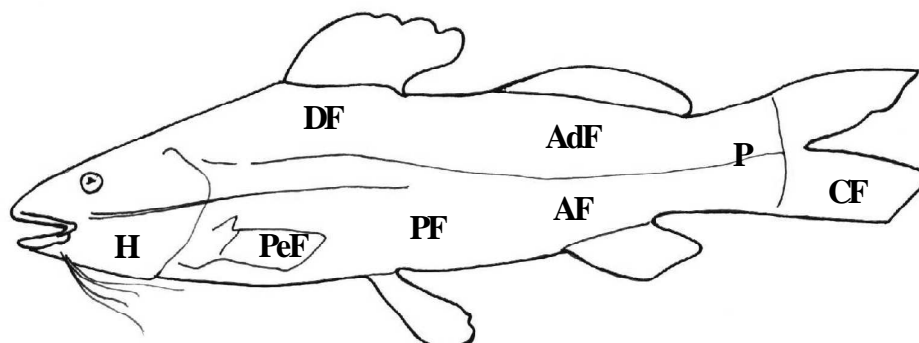


Figure 1 - Body regions of *Rhamdia quelen* used for the density calculation of *Clinostomum complanatum* metacercárias. AdF - Adipose fin; AF - Anal fin; CF - Caudal fin; DF - Dorsal fin; H - Head; P - Peduncle; PeF - Pectoral fin; PF - Pelvic fin.

The site of infection is the place in the host body that provides the requirements of the parasite. The occupied site by the parasite in each life cycle stages depends of the type of immediately anterior stage, and the location of the metacercariae will depend on the behavior pattern showed by cercariae (Erasmus, 1972).

In this study the distribution of *C. complanatum* metacercariae infrapopulations in the body regions of *R. quelen* of Southern Brazil was defined in distinct host size classes. These results were confronted with the host ontogenetic changes and its environment distribution in order to establish if environmental conditions influenced the host colonization by the metacercariae.

MATERIALS AND METHODS

One hundred and twenty specimens of *Rhamdia quelen*, caught in the Sarandí stream (32°00'-32°10'S and 32°20'-32°30'W) in Rio Grande, Rio Grande do Sul State, Brazil, between September 1999 and August 2000 were examined in order to determine parasitological index of *Clinostomum complanatum* metacercariae. For necropsy, each host body was divided in nine regions: Head (H), Pectoral fin (PeF), Dorsal fin (DF), Adipose fin (AdF), Caudal Peduncle (P), Caudal fin (CF), Anal fin (AF), Pelvic fin (PF), Abdominal cavity (CAV) (Fig. 1). The density values (*sensu* Bush et al., 1997) of *C. complanatum* by host body regions

were compared among and inside each size class using the chi-square test.

The definition of the host size classes limits was assayed in two ways: Initially by using the Sturgers equations ($K = 1 + 3.3 \times \log n$) and total width ($h = \text{higher length value} - \text{smaller length value} / K$) (Arango, 2001), which resulted eight host size classes. In the second assay, weight (g) and total length (cm) mean values of the eight classes obtained with Sturgers equation were used as attributes in the non-parametric Cluster analysis (Ward Method - Euclidian distance) (Arango, 2001). With this, dendrograms with 5, 6 and 7 size classes were created (Table 3). To determine the best dendrogram, relationships were established with the host biology (*R. quelen* or similar cogenetic species when necessary) and with space distribution using available dates on the environmental (Table 1 and Fig. 2). The importance and discriminatory capacity of each attribute for cluster definition was obtained by "K - means" algorithym, that was used to eliminate tendentious definition in its composition (Digby and Kempton, 1991).

RESULTS

Among 120 examined fishes, 77 were infected with a total of 17777 *C. complanatum* metacercariae. The 7 size classes dendrogram was adopted because showed the best relationship with

host biological and environmental host distribution features (Fig 2). The first sexual maturation length and largest frequencies sizes (Table 1) were determinants for the separation of 3-7 size classes hosts that occurred in the area III (Table 2 and line c from Fig. 2). The general density of *C. complanatum* showed significant differences both between and within the different *R. quelen* size classes. The density values of *C. complanatum* showed no significant differences in all host size classes at the following pairs of body regions: H and PeF; CF and VF; P and AF. The size class 5 shows higher density in all regions, except in CAV (Table 4) and AF. The size class 5 shows higher density in all regions, except in CAV (Table 4). The density into each size class did not show significant difference in class 1 in all host body regions. In the others classes, there were differences and H showed higher densities values, followed by PeF (Table 4).

DISCUSSION

Mardini et al. (1981) observations showed that host class 1 was observed only in area I (Table 2

and Fig. 2) corroborating these results. Although shallow and with little current places, the area I showed low transparency. These features could favor fish larvae survival, as reported by Behr et al. (1999) and also favored the infection by *C. complanatum*, since its cercariae swimmer actively (Eiras et al., 1999).

However, the smallest infection values (absolute and relative) were observed in the size class 1.

The class 2 hosts were collected in the area II and the differences of environmental features were related with changes in alimentary habit (Guedes, 1980) and alterations of host reproductive behavior (Narahara et al., 1985) (Table 1). The influence of the first sexual maturation length and the largest frequencies sizes on the distribution of *R. quelen* in the Sarandí stream was similar to observations move by Arratia (1983) who showed Siluriformes environmental distribution changing with their growth. In the present study, *C. complanatum* density in *R. quelen* was largest in the head region, especially in the oro-branchial cavity. In *Aphanius dispar* (Cyprinodontidae) the largest density of these metacercariae was found in the trunk, followed by the head and tail (Kalantan et al., 1987).

Table 1 - Relationships among frequency space distribution and others host biology available data (especially reproduction) of *Rhamdia quelen* or similar cogenetic species when necessary (ND = numbers in line D of Fig. 2).

ND	Features	Reference
1	<i>R. quelen</i> spawns and reproduces at places with low transparency and little current. Fingerlings and juveniles stay at these places	Mardini et al., (1981); Behr et al., (1999)
2	Males of <i>R. hilarii</i> with 11 - 13 cm more frequent (Jaguari river, SP, 1973-1975).	Narahara et al., (1985)
3	First maturation (L50) of males (14 cm) and females (13 cm) in <i>R. hilarii</i> .	Narahara et al., (1985)
4	Total maturation (L 100) in males (16 cm) and females (17 cm) in <i>R. hilarii</i> .	Narahara et al., (1985)
5	Males of <i>R. hilarii</i> with 17 - 23 cm. (1973 -1974).	Narahara et al., (1985)
6	Male predominance of <i>R. hilarii</i> with length between 25 and 27 cm.	Narahara et al., (1985)
7	Larger weight in males of <i>R. quelen</i> , Santa Catarina swamp, RS.	Weis (1983)
8	Higher frequencies of males (34cm) and females (32 cm).	Weis (1983)
9	Larger total length of males of <i>R. quelen</i> (33 cm) and females (56 cm).	Garro and Fialho (1997)

Table 2 - Characterization of collection areas in the Sarandí stream, Rio Grande, RS, Brazil, station occurrence and the size classes of *Rhamdia quelen* (line E of Fig. 2).

Area	Features	Collected classes (annual occurrence)
I	Flooded area between November/1999 and March/2000 (30-50 cm depth) on both banks. Creeping submerged vegetation (especially Graminae). Low transparency and current, 26 - 28°C (Dec.; Jan.; Feb.- summer); 23 - 27 °C (Mar.; Ap.; May.- autumn) water temperature.	1 (summer; autumn)
II	Burrow areas. (40-80 cm depth). Submerged and/or emerged vegetation on the banks. Strong stream, little material in suspension; 22 - 27°C (Dec.; Jan.; Feb.- summer); 23 - 27°C (Mar.; Ap.; May.- autumn) water temperature.	2 (summer; autumn)
III	Water column (50 - 170cm depth) without fixed vegetation. Only emerged fluctuant vegetation. Strong stream (?) and turbidity due to suspension of sediment and organic matter. 19 - 25°C (spring); 22 - 28°C (summer); 20-24°C (autumn); 10-16°C (winter) water temperature.	3 -7 (spring; summer; autumn; winter)

Table 3 - *Rhamdia quelen* size class limits (cm) obtained by Sturgers equation and by Cluster analysis. N = number of fish.

Class	Sturgers	N	Cluster	N
1	1.70 - 7.36	29	1.70 - 7.36	29
2	7.37 - 13.02	12	7.37 - 13.02	12
3	13.03 - 18.68	5	13.03 - 24.35	12
4	18.69 - 24.35	7	24.36 - 30.01	28
5	24.36 - 30.01	28	30.02 - 35.67	24
6	30.02 - 35.67	24	35.68 - 41.34	11
7	35.68 - 41.34	11	41.35 - 47.00	04
8	41.35 - 47.00	4	-	-

This was due to the easiness found by the cercariae invading the sub-opercular tissue, distributing from there to other body regions. Metacercariae of *C. marginatum* from *Loricariichthys platymetopon* (Loricariidae), has larger intensity of infection in the buccal cavity and opercle (Eiras et al., 1999). In Amazon, Thatcher (1991) reported the infection by *C. marginatum* on the gills of *Cichla ocellaris* (Cichlidae) and in *Crenicichla* sp (Cichlidae). The data of Thatcher (1991), Eiras et al. (1999) and Kalantan et al. (1987), however, were not obtained with rigorous host body subdivision, as done in this study.

Besides the body regions already mentioned, cysts of *C. complanatum* were also observed in the barbels, opercle, branchiostegal membranes, muscles, gills, palate, pharynx and eyes of *R. quelen*. Eiras et al. (1999) reported cysts in the ocular globule, which did not cause blindness. However, fish vision could be affected by the

parasite. This would facilitate the predation by birds (the main final host of *Clinostomum*).

Most of the Digenea cysts occurred in the trunk, followed by in the fins, usually with larger movement, as caudal and pectoral (Erasmus, 1972), which also contradicted the common belief that these regions were difficult to the cercariae penetration in the host. Eiras et al. (1999) found low infection in the caudal fin and they attributed this to the constant movements of the anal fin. The PeF, AF and CF regions in *R. quelen* showed the largest densities after H, entering inside of each host size class and the largest densities were in the fins with larger movement (Erasmus, 1972). Holmes (1973) found that parasites with active infection processes selected its site, which was also observed in cercariae of *C. complanatum* by Krull (1934) and Paperna (1980).

The fact that the density values of *C. complanatum* observed in the pairs of body regions H and PeF, CF and PF and P and AF were similar in all host

size classes suggested that a pattern of site occupation occurred when they had similar environmental conditions. Furthermore, the fact that among the adults, class 5 was the most frequent in the environment (Table 3) and showed the highest metacercariae density in all body regions, except for CAV (Table 4) indicated higher densities than those observed contributing to the host mortality. Hence in these hosts were not detected. It was therefore, possible to suggest that the parasitosis influenced the structure of the host population.

Different sizes of metacercariae of *C. complanatum* are found in *R. quelen*, which may point to several subsequent recruitment processes and a cumulative infection. This idea was reinforced by Esch et al. (1975), who found that the parasitic density could be increased by recruitment when a reduction of natural or acquired resistance, elimination of ecological or behavior barriers, and seasonal changes of environmental conditions occurred.

Different sizes of *C. complanatum* metacercariae found in *R. quelen* can be evidenced in subsequent recruitment processes and a cumulative infection.

The parasitic density in the second intermediate hosts, as *R. quelen* to *C. complanatum*, could be increased by recruitment when a reduction of natural or acquired resistance, elimination of ecological or behavior barriers, and seasonal changes of environmental conditions occurred (Esch et al., 1975). The benthonic condition of *R. quelen* (Bizerril, 1998, Bizerril and Lima, 2000) can also facilitate the cercarial recruitment of *C. complanatum* (Eiras et al., 1999).

The density differences in the host size classes may be due to the several sizes of corporal surfaces, (Elliott and Russert, 1949, Kalantan et al., 1987). The consistently similar density values of *C. complanatum* observed in the pairs of body regions H and PeF, CF and PF and P and AF in all the host size classes suggested that the differences in the host location in the environment had little influence on the body regions colonized by the metacercariae. However, as shown in Table 2, it was possible to conclude that as environmental segregation by the different host size classes occurred, the density differences could not be influenced exclusively by body size.

Table 4 - Comparison of *Clinostomum complanatum* density between size classes in the body regions (letters without parenthesis in the same column) and between body regions in the host size classes (letters in parenthesis in the same line) of *Rhamdia quelen*. H - Head; DF - Dorsal fin; AdF - Adipose fin; P - Peduncle; CF - Caudal fin; AF - Anal fin; PF - Pelvic fin; PeF - Pectoral fin; CAV- Abdominal cavity.

Size classes	Density by body region									
	H	DF	AdF	P	CF	AF	PF	PeF	CAV	
1	2 G (A)	1 E (A)	0 F (A)	0 E (A)	1 F (A)	0 E (A)	0 F (A)	0 G (A)	0 D (A)	0 (A)
2	24 F (A)	4 E (BC)	0 F (C)	0 E (C)	5 F (BC)	1 E (C)	2 F (C)	9 F (B)	4 C (BC)	4 (BC)
3	259 E (A)	30 D (C)	9 E (D)	14 D (D)	46 E (C)	77 D (B)	37 E (C)	77 E (B)	2 CD (E)	2 (E)
4	1605 B (A)	410 B (D)	136 C (F)	178 A (E)	546 CB (C)	754 BA (B)	414 B (D)	1002 B (A)	28 A (G)	28 (G)
5	2344 A (A)	559 A (D)	224 A (E)	169 A (F)	624 A (D)	804 A (C)	567 A (D)	1330 A (B)	8 BC (G)	8 (G)
6	1208 C (A)	289 C (D)	181 B (E)	99 B (F)	302 C (D)	409 B (C)	268 C (DE)	533 C (B)	23 AB (G)	23 (G)
7	1001 D (A)	263 C (B)	49 D (E)	39 C (E)	138 D (D)	204 C (C)	177 D (C)	276 D (B)	12 B (F)	12 (F)

When values are followed by different letters in the same line or in the same column $P < 0,05$.

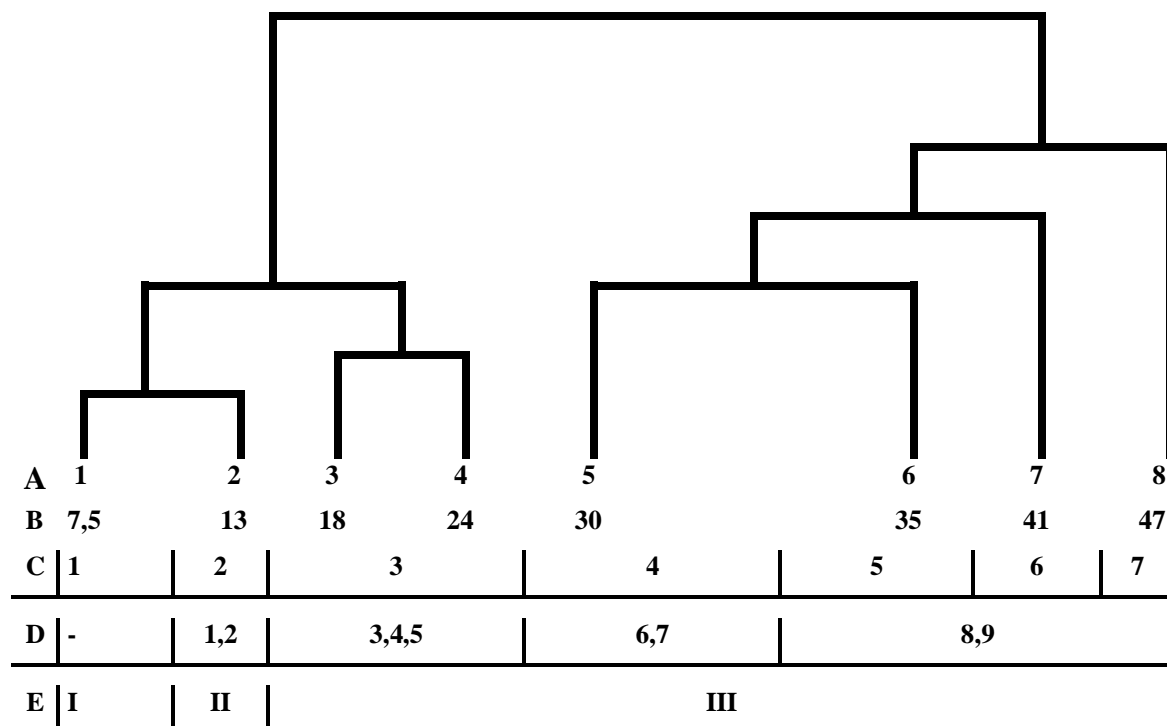


Figure 2 - Dendrogram for size classes (cm) of *Rhamdia quelen* and their relationships. A- Size class obtained by Sturgers equation; B- Size class upper limits obtained by Sturgers equation; C-Distribution of the size classes obtained by cluster analysis; D - Distribution using features shown in Table 1; E - Distribution using features shown in Table 2.

The corporal volume of the parasites could condition the colonization pattern, and this could be a more determining parameter than indexes such as the density (George-Nascimento *et al.*, 2002), but the observations in this study did not agree this. Previous studies have determined the specificity of parasite site of infection and the possibility of discarding parts to minimize fish carcass losses in the industry (Overstreet, 1977; Amato *et al.*, 1990). The high densities and non-specificity of infection site for metacercariae of *C. complanatum* in *R. quelen* make it possible to point that the selective discarding of parts of the fish carcass is not feasible.

RESUMO

A densidade de metacercárias de *Clinostomum complanatum* por regiões do corpo entre classes de comprimento de *Rhamdia quelen* foram comparadas. As classes de comprimento do hospedeiro foram definidas por análises de agrupamentos usando peso e comprimento total

como atributos. Estes valores foram relacionados com características do ambiente e dados biológicos disponíveis sobre o hospedeiro. O dendrograma com sete agrupamentos foi o escolhido. O corpo do hospedeiro foi dividido em nove regiões para as necropsias: Cabeça (H); Nadadeiras, Peitoral (PeF); Dorsal (DF); Adiposa (AdF); Caudal (CF); Anal (AF) e Pélvica (PF); Pedúnculo caudal (P); Cavidade Abdominal (CAV). Em todas as classes de comprimento do hospedeiro os pares de regiões H e PeF, CF e PF e P e AF mostraram densidades similares (teste do X^2). Hospedeiros com 30-36 cm mostram maior densidade de parasitas em todas as regiões exceto na CAV. Peixes com menos de 11 cm não mostram diferenças significativas na densidade de parasitas nas regiões do corpo. Nas demais classes, H é a região mais infectada, seguida por PeF. Os resultados sugerem que as variáveis ambientais têm pouca importância sobre o processo de colonização por metacercárias.

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