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Microhabitats Preference of *Cichlidogyrus berrebii*, *C. kothiasi* and *C. pouyaudi* (Monogenea: Ancyrocephalidae) on the Gills of *Tylochromis jentinki* from Ebrié Lagoon, Côte d'Ivoire

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Abstract: Background Objectives: The microhabitat preference of the three congeneric monogenean species *Cichlidogyrus berrebii* Pariselle & Euzet, 1994, *C. kothiasi* Pariselle & Euzet, 1994 and *C. pouyaudi* Pariselle & Euzet, 1994 on the gills of *Tylochromis jentinki* (Steindachner, 1894) was conducted. Methods: Eighty-seven specimens were sampled from February 2016 to January 2017. After fish euthanasia, each gill arch was removed and examined to quantify the number of parasites and their distribution on the gills. Results: Results were analyzed with regard to: general occurrence, bispecific infections and monospecific infections of the parasites in the *T. jentinki* population. Both parasites have the same affinity for the both sides of the fish. Gill arches I, II, dorsal, medial segments, distal were preferred by *C. berrebii*, gill arches II, III, medial, ventral segment, distal part by *C. kothiasi* and gill arch II, dorsal segment distal part by *C. pouyaudi*. The coexistence of these three monogenean species on the same fish does not induce a change in their respective distribution. With such low parasite presence, the role of intraspecific or interspecific competition appears to have little influence on the distribution of monogeneans. However, these specific preferences might be influenced by the interaction of several factors such as differences in the water current over parts of the gill surface, parasite density, as well as ecological and morphological differences between monogenean species.

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1. Introduction

Modern ecology has highly emphasized on the importance of parasites as study models of structure and organization of communities [1]. Ectoparasites such as monogeneans figure among these pathogens. These organisms are considered to be among the most-specific parasites in fish, commonly found on fins, body skin, gills chambers, buccal cavity, cornea and nostrils of their host [2].

Most species of monogeneans are restricted not only to a particular host but also to a particular body part of the host [3]. The microhabitat preference of monogeneans has been investigated by several authors [4-8]. The effect of these gill preferences is not clear, and many host and environmental factors could be involved [9]. Site specificity in monogeneans may result from physico-chemical requirements [10], variations in water current over the gill surface or differences in the area between the gills arches [11], avoidance of interspecific competition [12], reinforcement of reproductive barriers and enhancement of the chance to mate [13].

Tylochromis jentinki (Steindachner, 1895), typically estuarine fish of West Africa [14] is commercially important exploited by artisanal fishermen in some Ivorian water bodies and represents an important food source that supports many small communities through both fishing and aquaculture [15]. This fish species has been shown that to be the host of monogenean parasites. In Côte d'Ivoire, despite its obvious importance, no ecological studies (in terms of site preference) of gill monogenean parasites of this Cichlid are nonexistent. To date, the limited data are available for monogenean systematic studies [16-17]. On this view, the aim of this study is to investigate microhabitat and relationship of this fish species' gills parasites.

2. Material and Methods Host collection

A total of 87 *Tylochromis jentinki* were captured from February to November 2016. Fish were caught using gillnet by local fishermen from Ebrié lagoon located at 5°16'-5°21'N and 4°14'-4°23' E. Once out of the water, the fish individuals' gill arch apparatus was dissected and investigated from both sides then stored in ice (0°C) and transported to the laboratory for parasitological analysis.

Parasite collection

The gills arches were carefully separated and numbered I to IV from anterior to posterior. Each arch was divided into three gill segments: dorsal, medial and ventral ; two gill areas: proximal and distal (Figure 1). All monogeneans were collected one by one from each sector separately under an Olympus SZ 60 light microscope and the exact location of the parasites was recorded before removal. Monogeneans parasites were cleared in ammonium picrate-glycerine [18] and identified based on their haptor (hamuli, connective bars and hooklets) and reproductive organs (copulatory organ and vaginal armament) according to [17].



Figure 1. Division of branchial arch: 1-2-3 Distal part; 4-5-6 Proximal part.

Statistical analysis

Prevalence (%) and mean intensity were use as defined by [19]. Kruskal-Wallis tests were used to test the significance of the differences in the number of parasites between the dorsal, medial and ventral segments. The differences in the parasite numbers between the proximal and distal parts, left and right sides, and gill arches were tested using the Mann Whitney *U*-test. Differences of p < 0.05 were considered significant.

Statistica 7.1. and Microsoft Excel software were used for the analysis of various data.

3. Results

A total of 87 *Tylochromis jentinki* were examined, 76 of which (87.36%) were infected by *Cichlidogyrus berrebii*, 51 (58.62%) by *C. kothiasi* and 23 (23.43%) by *C. pouyaudi*. A total of 3004

specimens of monogenean were recorded on the gills of this fish host including respectively 2104, 642 and 258 individuals of these species. The overall mean intensity for these parasite species was found to be 27.68, 12.58 and 11.21, respectively.

General occurrence of the Parasites

The distribution of *Cichlidogyrus berrebii*, *C. kothiasi* and *C. pouyaudi* on the gills of *Tylochromis jentinki* in general occurrence was analysed (Table 1).

Of the 87 dissected fish, 76 were infected with *C. berrebii* (prevalence = 87.36%). A total of 2104 *C. berrebii* were recorded. The differences were not found to be significant between the number of this monogenean species on the left and right set of the gill arches (p = 0.73 > 0.05). Gill arches I (36.45%) and II (38.64%) were preferred compared to the other

two (p = 0.03 < 0.05). A significant greater number of *C. berrebii* occurred on the dorsal (46.15%), medial segments (43.35%) and distal parts gill arches (80.94%) (p < 0.05). The anterior hemibranch was more colonized than the posterior one (p = 0.004 < 0.05).

Among 87 specimens of *T. jentinki* sampled, 51 were infected with *C. kothiasi* (prevalence = 58.62%). These infected fish harbored 642 individuals of this monogenean species. This parasite did not show a left or right side preference (p = 0.83> 0.05). This parasite was more abundant on the gill arches II (18.85%) and III (37.85%) (p = 0.01 < 0.05). This species preferred medial (45.02%) and ventral segment (39.25%) and distal part of the gill arches (80.69%) (p < 0.05). *C. kothiasi* showed more affinity for the anterior hemibranch (p = 0.021 < 0.05).

Of 87 examined fish, 23 were infected by *C.* pouyaudi (prevalence = 26.44%). A total of 258 individuals of this species were recorded. No significant differences were noticed in the distribution of this parasite between the right and left sides (p = 0.1 > 0.05). This parasite showed more affinity for the gill arch II (40.70%), dorsal segment (67.05%), distal part (73.64%) (p < 0.05) and the posterior hemibranch (p = 0.001 < 0.05) was observed.

 Table 1. General occurrence of Cichlidogyrus berrebii, C. kothiasi and C. pouyaudi on the gill of Tylochromis jentinki.

	C. berrebii 76 27.68±11.5		C. kothiasi 51 12.58±8.2		C. pouyaudi		
Number of infected host					23 11.21±5.4		
Mean intensity±SD							
	Number	%	Number	%	Number	%	
Right side	1041	49.48	318	49.53	126	48.84	
Left side	1063	50.52	324	50.47	132	51.16	
Gill arch I	767	36.45	121	18.85	67	25.97	
Gill arch II	813	38.64	243	37.85	105	40.70	
Gill arch III	346	16.44	222	34.58	54	20.93	
Gill arch IV	178	8.46	56	8.72	32	12.40	
Dorsal segment	971	46.15	101	15.73	173	67.05	
Medial segment	912	43.35	289	45.02	62	24.03	
Ventral segment	221	10.50	252	39.25	23	8.91	
Proximal part	401	19.06	124	19.31	68	26.36	
Distal part	1703	80.94	518	80.69	190	73.64	
Anterior hemibranch	1401	66.59	402	62.62	100	38.76	
Posterior hemibranch	703	33.41	240	37.38	158	61.24	

Bispecific infections

Bispecific infections of *T. jentinki* were investigated with *Cichlidogyrus berrebii-C. kothiasi* and *C. berrebii-C. pouyaudi* combinations (Table 2).

Of 87 dissected fish, 37 were simultaneously parasitized by *C. berrebii* and *C. kothiasi* (prevalence = 43.67%). In these hosts, 1164 *C. berrebii* and 492 *C. kothiasi* were recorded. Most individuals of *C. berrebii* were located on gill arches I (39.18%), II (40.46%), medial (43.99%) and ventral segment (46.65%) and distal part (82.30%) (p < 0.05). This

species preferred the anterior hemibranch (p = 0.0053 < 0.05). *Cichlidogyrus kothiasi* preferred gill arches II (35.98%), III (34.15%), medial (41.46%) and ventral segment (40.04%) and distal part (80.08%) (p < 0.05). The anterior hemibranch was more colonized than the posterior one (p = 0.0041 < 0.05).

Of 87 examined fish, 16 were infected with only *C. berrebii-C. pouyaudi* (18.39%). In these hosts, 342 *C. berrebii and* 183 *C. pouyaudi* were recorded. *C. berrebii* was more abundant on the gill arches I (38.30%), II (37.43%), dorsal (41.52%) and

medial (39.77%) segments and distal part (69.88%) (p < 0.05). *C. berrebii* showed more affinity for the anterior hemibranch (p = 0.0011 < 0.05). The monogenean *C. pouyaudi* mostly preferred the gill

arch II (48.09%), dorsal (55.74%) segment, distal part (66.12%) (p < 0.05) and the posterior hemibranch (p = 0.0004 < 0.05).

	C. berrebii 38 30.63±4.5		C. kothiasi 38 12.94±15		C. berrebii 16 21.37±2.5		C. ponyaudi 16 11.44±8	
Number of infected host								
Mean intensity±SD								
	Number	%	Number	%	Number	%	Number	%
Right side	586	50.34	244	49.59	162	47.37	89	48.63
Left side	578	49.66	248	50.41	180	52.63	94	51.37
Gill arch I	456	39.18	94	19.11	131	38.30	44	24.04
Gill arch II	471	40.46	177	35.98	128	37.43	88	48.09
Gill arch III	153	13.14	168	34.15	60	17.54	41	22.40
Gill arch IV	84	7.22	53	10.77	23	6.73	12	6.56
Dorsal segment	512	43.99	91	18.50	142	41.52	102	55.74
Medial segment	543	46.65	204	41.46	136	39.77	57	31.15
Ventral segment	109	9.36	197	40.04	64	18.71	24	13.11
Proximal part	206	17.70	108	21.95	103	30.12	62	33.88
Distal part	958	82.30	394	80.08	239	69.88	121	66.12
Anterior hemibranch	800	68.73	300	60.97	210	61.4	132	38.6
Posterior hemibranch	364	31.27	192	31.03	132	38.6	210	61.4

 Table 2. Distribution of Cichlidogyrus berrebii, C. kothiasi and C. pouyaudi on the gill of Tylochromis jentinki in bispecific infection.

Monospecific infections

The distribution of *Cichlidogyrus berrebii*, *C. kothiasi* and *C. pouyaudi* on the gills of *Tylochromis jentinki* in monospecific infections was also examined (Table 3).

Of 87 hosts fishes sampled, 22 hosts (prevalence = 25.28%) were infected with only C. berrebii, 9 hosts (10.34%) with only C. kothiasi and 5hosts (5.74%) with only C. pouyaudi. A total of 598 C. berrebii, 98 C. kothiasi and 50 C. pouyaudi were recorded in these infections. There was no statistically significant difference in the number of each species between the left and right side of the host (p = 0.81 > 0.05). C. berrebii predominantly occurred on the gill arches II (35.79%), I (30.10%), dorsal (53.01%), medial (38.96%) segments and distal part (84.62%) (p < 0.05). The anterior hemibranch was more colonized than the posterior one (p = 0.022 < 0.05).C. kothiasi settled in gill arches II (37.76%) and III (41.84%) more frequently. This species was more concentrated on medial (46.94%), ventral segment (38.78%) and distal part (77.55%) (p < 0.05) and the anterior hemibranch (p =0.01 < 0.05). C. pouvaudi was more frequently found

on gill arch II (51.92%) while the gill arches I, III and IV were the least infected (p = 0.01 < 0.05). Dorsal segment and distal part were most occupied by this parasite (p < 0.05). This species showed more affinity for the posterior hemibranch ((p = 0.008 < 0.05).

The numbers of *C. berrebii*, *C. kothiasi* and *C. pouyaudi* between bispecific and monospecific infections were also examined. The numbers of *C. berrebii* showed no statistically significant difference between bispecific infections with *C. kothiasi*, bispecific infections with *C. pouyaudi* and monospecific infections (p = 0.17 > 0.05). There was no statistically significant difference in the numbers of *C. kothiasi* between bispecific infections (p = 0.42 > 0.05). There were no statistically significant differences in the numbers of *C. pouyaudi* between bispecific infections (p = 0.42 > 0.05). There were no statistically significant differences in the numbers of *C. pouyaudi* between bispecific infections with *C. berrebii* and monospecific infections (p = 0.72 > 0.05).

C. berrebii, *C. kothiasi* and *C. pouyaudi* were recorded in simultaneously infections in only 6 fish hosts but were not taken into account because of the low sample size.

	C. berrebii 22 27.18±4.5		C. kothiasi 9 10.88±11		C. pouyaudi		
Number of infected host					5 10±2.5		
Mean intensity±SD							
	Number	%	Number	%	Number	%	
Right side	293	49.00	47	47.96	23	44.23	
Left side	305	51.00	51	52.04	27	51.92	
Gill arch I	180	30.10	12	12.24	9	17.31	
Gill arch II	214	35.79	37	37.76	27	51.92	
Gill arch III	133	22.24	41	41.84	12	23.08	
Gill arch IV	71	11.87	8	8.16	4	7.69	
Dorsal segment	317	53.01	14	14.29	31	59.62	
Medial segment	233	38.96	46	46.94	13	25.00	
Ventral segment	48	8.03	38	38.78	8	15.38	
Proximal part	92	15.38	22	22.45	12	23.08	
Distal part	506	84.62	76	77.55	40	76.92	
Anterior hemibranch	400	66.89	68	69.39	9	8	
Posterior hemibranch	198	33.11	30	30.61	41	82	

 Table 3. Distribution of Cichlidogyrus berrebii, C. kothiasi and C. pouyaudi on the gill of Tylochromis jentinki in monospecific infection.

4. Discussion

This study revealed that the exploitation of both sides of the gill system of *Tylochromis jentinki* by all monogenean species doesn't show a significant difference. Various authors have found the same observation. This is the case of [20] with *Anguilla anguilla*, [7] with *Sarotherodon melanotheron* and [21] with *Tilapia zillii*. The bilateral symmetry of *T. jentinki* associated to that of its monogeneans could justify a similar exploitation of both sides of this fish species [22]. According to [23], this symmetry was due to the equal chances for infection of both sides with the monogenean eggs.

In this work, \overline{C} . *berrebii* predominantly occurred on gill arches I and II, more *C. kothiasi* located on gill arches II and III and *C. pouyaudi* was more frequently found on the gill arch II. In general, arch IV has been least parasitized. This corresponds with the finding of other authors [24, 6-7, 25, 21]. Some authors tried to explain the cause of this preference. According to [26], the strongest water current passes through these parts of the gill system, favours the convenient conditions for these monogenean species settlement. [27] stated that parasite distribution over the arches is highly affected by respiratory current flow rate distribution. The same reasoning may well apply to the spatial distribution patterns of *Cichlidogyrus* spp. observed

in the current study. Furthermore, for surveys monogenean parasites exhibited a preference for the medial position of the gill (arches II and III) as well as the anterior face of the gill. This finding has been confirmed by several other studies [28-29]. This is once again most probably the result of water flow and water pressure on the dispersion of parasites during the respiration process [24]. The median preference arches may also be related to the large colonized surfaces that they offer to parasites [30]. It also appeared that, the posterior arch (arch IV) was least infected. This was due to the fact that this arch has the smallest colonized surfaces area and the lowest number of gill filaments as compared to the first three gill arches [31-32]. In fact, it was shown that the variation of host filaments decreased significantly from arch I towards arch IV.

Many monogenean species show a preference for specific parts of the gill apparatus of their hosts. In this study, *C. berrebii* was attached essentially to the dorsal and medial segments. *C. kothiasi* was mostly found on medial and ventral segments and *C. pouyaudi* was more frequently found on dorsal segment. These three species were more concentrated on the distal parts of gills. Previous studies were mainly focused on the monogenean preference for the different part of the gill [33-34, 7, 25]. Such studies showed that monogenean species attach essentially to sectors which are more exposed to the respiratory water current. The localization of C. berrebii, C. kothiasi and C. pouyaudi on distal parts may reflect a preference of these species for a site in which the water flow is maximal. In fact, according to [35], more water passes over the distal halves of the filaments than over the proximal ones. The preference of monogenean studied to sector exposed to more water currents could be to get exposed to more aerated conditions as it is always over dispersed with high density. It was shown that the size of the haptoral armature plays an important role in determining the preferences of site. Thus, species with relatively strong anchors occupy zones of high water movement, while species with weaker armature prefer proximal region where the force of ventilation current is generally less. In this study, the strong haptorial armature of C. berrebii, C. kothiasi and C. pouyaudi suggest their preferential attachment to distal-dorsal, distal-median and distal-ventral parts of the gills, which are more exposed to respiratory water current. It is therefore clear that, to coexist the species of this guild have adopted to share their space resource. Such aggregation of individuals of each parasite species on specific zone suggests the absence of an intraspecific competition [20]. The restriction of the niche facilitates species coexistence and opportunities to mate [36, 30]. However, according to [37], the exact explanation of site selection by the monogeneans remains enigmatic. Despite sharing the space resource by the three species studied, there is some mutual tolerance between them as their respective niches tend to overlap indicating the absence of interspecific competition. For [38], a polyparasitism cannot conduct to competition as long as there is still space available.

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