Full Length Research Paper

Spatial distribution of Monogenean and Myxosporidian gill parasites of *Barbus martorelli* Roman, 1971 (Teleostei: Cyprinid): The role of intrinsic factors

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The gill parasite fauna of *Barbus martorelli* is much diversified and this raises up the problem of biotope partitioning by these pathogens. The gill colonization analysis of these organisms as well as four distribution gradients: transversal, lateral, longitudinal and vertical was undertaken as a function of the host's side. The distribution of *B. martorelli* gill parasites did not reveal any asymmetry in function of the host's side. The transversal partitioning applied solely to *Dactylogyrus insolitus* and *Myxobolus barbi*. The lateral partitioning was inexistent except for *D. insolitus* which was mostly concentrated on the posterior hemibranch of arch II and *Myxobolus njinei* which colonized mostly the anterior face of the same arch. The longitudinal partitioning was observed only for *M. barbi* which mostly encysted into sectors which are more opened to respiratory water current. The vertical partitioning of Monogeneans was subject to the size of their sclerified haptorial pieces; therefore, *Dactylogyrus bopeleti* and *D. insolitus,* which are robust, accumulate mostly in the filamentary distal zone whereas *Dactylogyrus simplex* and *Dactylogyrus maillardi,* which are weaker, prefer the basal zone. The volume of ventilated water currents and certain intrinsic factors of these fish parasites may determine their spatial gill distribution.

Key words: *Barbus martorelli*, gill parasites, Myxosporidia, Monogenea, spatial distribution, intrinsic factors.

INTRODUCTION

The gills of fishes represent one of the biotope mostly exploited by different fish ectoparasites (Fernando and Hanek, 1976). In most cases, these pathogens showed a preference for specific sites of the gill apparatus of their host. Monogenea for example, can be specific for one side of the host, for some arches (or one of their faces), or for some parts of the gill filaments (Rohde, 1979). For Myxosporidia, trophozoites of the species of *Myxobolus* from *Notemigonus crysoleucas* develop in the distal half of a primary lamella (Cones and Wiles, 1985). On the same line, some *Myxobolus, Henneguya* and *Thelohannellus* species are characterized by strict tissue specificity and species showing affinity to the epithelium, connective tissue, cartilage or vascular tissue usually occur in a strictly defined location within the gill apparatus (Molnàr, 2002). Such restriction of microhabitat is interpreted in many ways: it could be due to variation of the relative volumes of water current passing over the different gills (Wiles, 1968; Paling, 1968; Smith, 1969; Suydam, 1971; Arme and Halton, 1972), or due to interspecific competition (Holmes, 1973). However for Monogenea, Rohde (1976, 1977, 1979), after observing the restriction of site in the absence of competitors suggested that narrow microhabitats increase the chances of Monogenea to mate. According to Kristian et al. (2006), the factors responsible for narrow microhabitat specificity are not clear. Since the works of Dogiel and colleagues in the 1940s, community studies have been undertaken in earnest by parasitologists and today, the spatial structure of parasites communities is a central theme of current parasite ecology. For the past three decades the spatial structure of parasites communities has been in the center of many ecological studies (Bashirellah and Rodriguez,

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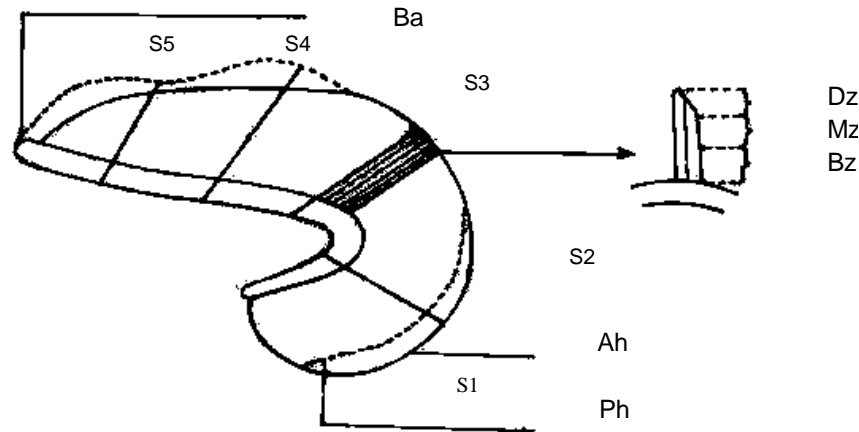
Figure 1. Schematic diagram of a gill cut out.
S1= Dorsal sector, S2 = medio – dorsal sector, S3 = median sector,
S4 = medio – ventral sector and S5 = Ventral sector
Ba = Bony arch
Dz = Distal zone, Mz = Median zone, Bz = Basal zone
Ah = Anterior hemibranch, Ph = Posterior hemibranch

1992; Dzika, 1993; Gutierrez and Martorelli, 1994; Dzika, 1999; Molnar and Szèke, 1999; Nie, 2000; Lo and Morand, 2001; Simkova et al., 2002; Matéjusovà, 2003; Gonzalez and Poulin, 2005; Kristan et al., 2006; Turgut et al., 2006).

At Nkolfoulou (Cameroon, Central Africa), the gills of *Barbus martorelli* Roman, 1971 (Cyprinid – Teleostean) are colonized by eight species of Monogenea: *Dactylogyrus insolitus*, *D. bopeleti*, *D. simplex*, *D. maillardi*, *Dogielius martorelli*, *Dogielius* sp. (Birgi and Lambert, 1987), *Gyrodactylus* sp., Polystomatidae larva (Tombi and Bilong-Bilong, 2004) and two species of Myxosporidia: *Myxobolus barbi* and *Myxobolus njinei* (Fomena et al., 1985). The study of the distribution of core and secondary species of this component community in the host population in relation to sex, age, and fish size was done by Tombi and Bilong-Bilong (2004) ; that of heterogeneity of gill system and the dynamics of parasite populations of this cyprinid was demonstrated by Bilong-Bilong and Tombi (2004, 2005).

Owing to the high parasite species richness of *B. martorelli* and the abundance of some species such as *M. barbi* and *M. njinei* (Tombi and Bilong-Bilong, 2004), the aim of this work is to examine the spatial structure of gill infracommunities of the host to reveal the mechanisms that determine the coexistence of these organisms on the gill apparatus.

MATERIALS AND METHODS

A total of 558 *Barbus martorelli* individuals Individuals were captured using a 1cm by 1cm mesh gillnet from May 1998 to August 1999 in the Foulou watercourse in Nkolfoulou locality (3° 53'N, 11° 34'E, Cameroon, Central Africa). Host sampling, transportation, dissection and parasites collection methods were

according to Tombi and Bilong-Bilong (2004). A minimum of 30 fishes were collected monthly except the months of February and March when less than 30 fishes were collected. The fishes captured were immediately fixed in 5% formalin and transported to the laboratory were the size and sex of each specimen was determined. The gills from both sides were dissected and examined with an optical microscope. Myxosporidia cysts present on the filaments or on the bony arch were counted. The Monogeneans were collected and mounted on slides in a drop of hematoxylin eosin. The different species were identified using a microscope magnification of 40 and 100X respectively for Monogenea and Myxosporidia. The microhabitat of each parasite species was delimited according to Rohde (1977) with some modifications (Figure 1). Gill arches from each side of the fish were numbered I – IV from the anterior gill arch to the posterior (transversal gradient). An anterior hemibranch was distinguished from a posterior one for each arch (lateral gradient). Each hemibranch was divided into five approximately equal regions dorsoventrally: sectors I, II, III, IV, V (longitudinal gradient), and each gill filament into three equidistant zones: basal, median and distal (vertical gradient). The bony part of the gill arch was also divided into five sectors. The number and location of individual parasites were noted. For a given fraction of an infracommunity (filamentary, zonal and sectorial levels), the terms rate or percentage of infection and parasite load were used respectively in the place of parasite prevalence and intensity. These two latter parameters were defined according to Margolis et al. (1982), while population, infrapopulation, community, infracommunity and component community were defined according to Combes (1995). The χ^2 test was used to compare the percentages, and the Student t test and ANOVA were used to compare the mean parasite loads of two and many samples (Sokal and Rolf, 1981; Schwartz, 1984). The degree of security retained was 95%.

RESULTS

There were no preferences in the distribution of parasite species (Monogenea and Myxosporidia) between the left
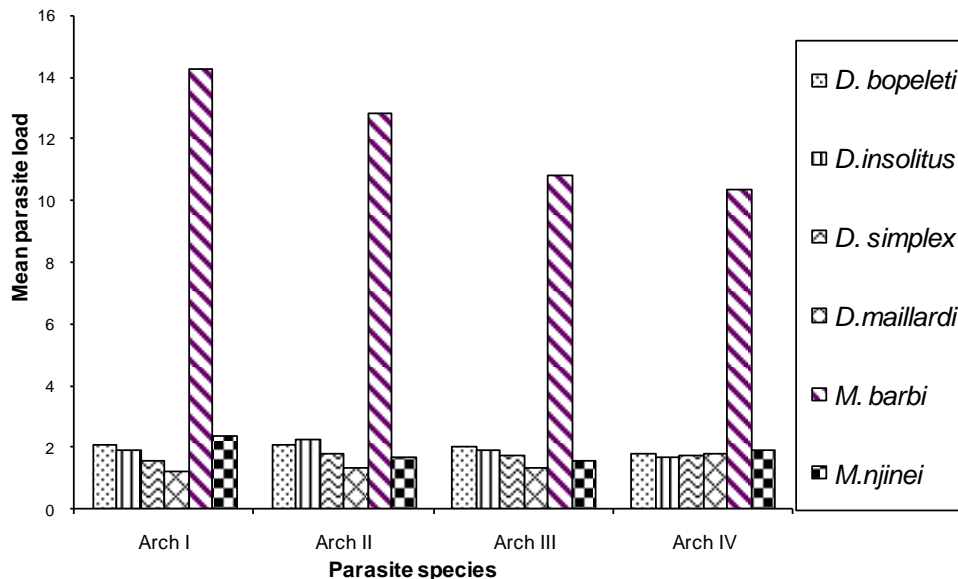
Figure 2. Mean parasite load as a function of gill arch.

Table 1. Comparison of parasitism between hemibranches of same arch (t value).

	Arch			
Parasite species	**Arch I**	**Arch II**	**Arch III**	**Arch IV**
D.bopeleti	0.74	0.85	0.72	0.58
D.insolitus	0.13	2.86*	0.21	1.09
D.simplex	1.14	0.70	0.26	0.70
D.maillardi	1.05	0.098	0.20	1.03
M.barbi	1.16	0.56	0.53	1.44
M.njinei	0.36	2.45*	1.03	0.42

* = Significant test.

and right sides of *B. martorelli* (P > 0.05). At the arch level and for all parasite species, the mean loads seemed to reduce antero - posteriorily (Figure 2). The Monogenea *D. insolitus* was more concentrated on arch II (P < 0.05) than on the three others where no significant difference was observed (P > 0.05). For the Myxosporidia *M. barbi*, its average cyst load reduced progressively without any significant difference between the two given consecutive arches. Our observations also showed that arch IV was always less colonized by almost all the parasite species. Irrespective of the gill arch, almost all the parasite species equally colonized the anterior and posterior hemibranches, except for *D. insolitus* whose load was higher (P < 0.05) in the posterior hemibranch/arch II and for *M. njinei* which mostly encysted on the anterior than the posterior face of the bony part/arch II (Table 1). *M. barbi* was lightly (P > 0.05) encysted on the posterior hemibranches, except for arch I. For the distribution of

parasites on the longitudinal gradient (Figure 3a - d), only *M. barbi* showed differences in intersectorial colonization of arches II to IV (P < 0.05). The vegetative form of this *Myxobolus* was more abundant in the medio – ventral sector S4 of arch II and on the median sector S3 of arch III (P < 0.05). Its load decreased progressively towards the dorsal and ventral extremities. On arch IV the gill filaments of the median S3 and medio - ventral S4 sectors harbored more cysts of *M. barbi* (P < 0.05) than the other areas which were less infected. At the filament level, a total of 189,302 zones of each type were examined, but only 3.13, 2.14 and 0.95% for the distal, median and basal zones, respectively, were occupied.

D. bopeleti and *D. insolitus* never occupied the basal zone but were more frequent in the distal one. *D. simplex* and *D. maillardi* did not colonize the distal zone (Table 2). *M. barbi* exploited the entire filamentous zone and its cystic load reduced progressively and significantly (P <
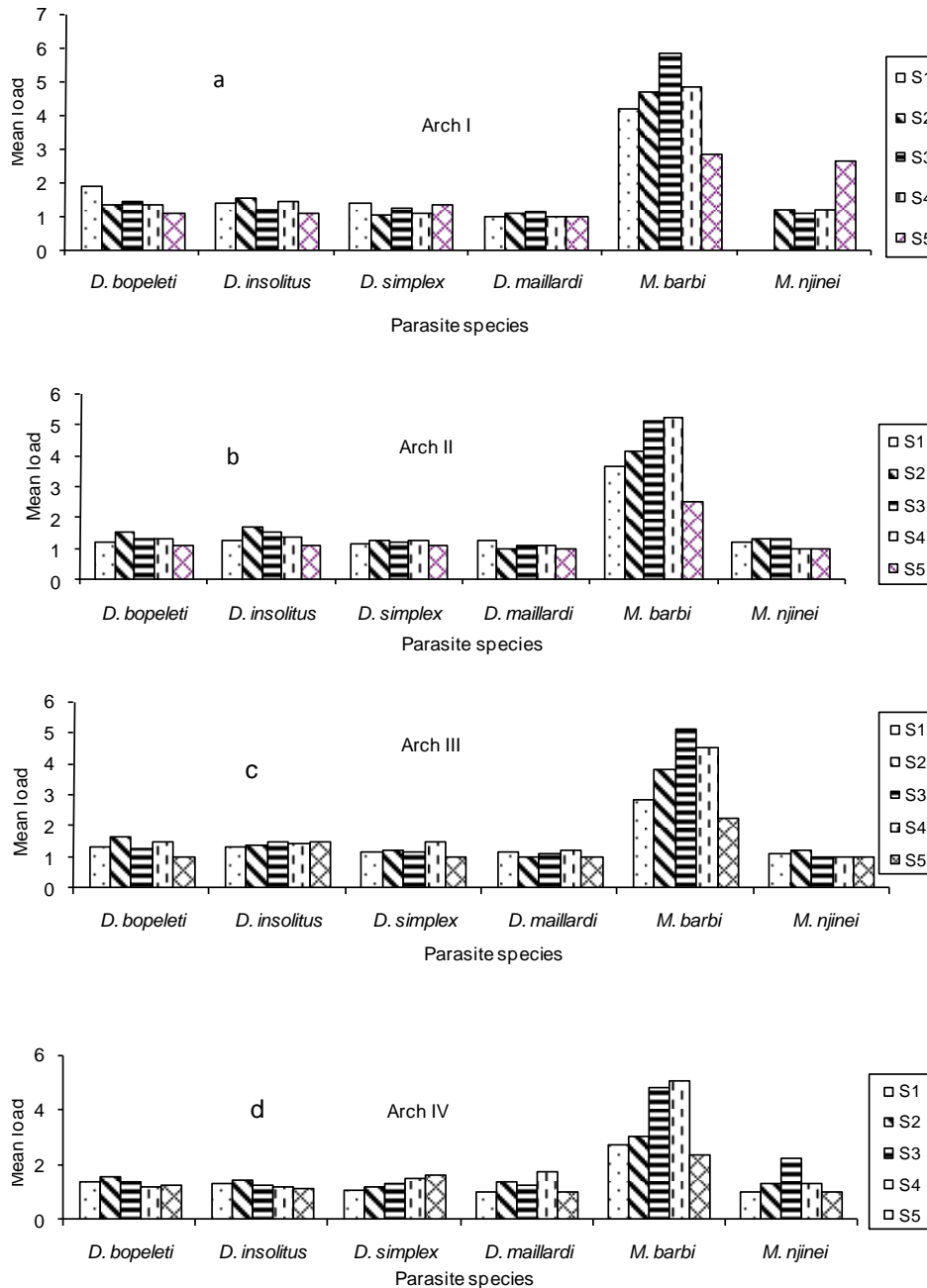
Figure 3 (a – d). Parasite mean load as a function of sector.
S1= Dorsal sector, S2 = medio – dorsal sector, S3 = median sector,
S4 = medio – ventral sector and S5 = Ventral sector

0.05) from the distal part towards the basal one (Tables 2 and 3).

DISCUSSION

This study has shown that some parasite species have affinity for certain sites of the *B. martorelli* gill system. No distinction in term of the left or right side exploitation was

observed. Similar results were obtained by Roubal (1981); Euzet and Sanfilippo (1983); Buchmann (1993); Gutierrez and Martorelli **(**1994); Bilong-Bilong (1995); Dzika (1999); Chapman et al. (2000); Lo and Morand (2001); Baker et al. (2005); Kristan et al. (2006) and Turgut et al. (2006). On the contrary, Hendrix (1990) found an asymmetrical distribution of *Bothrithrema bothi* (Monogenea) on *Scophtalmus aquosus* (Bothidae). This phenomenon was interpreted by Bilong-Bilong (1995) to

Table 2. Zonal infection rate (%) of different parasite species.

Parasite species	Zone		
	Distal	**Median**	**Basal**
D. bopeleti	80.02	19.98	0
D. insolitus	88.99	11.01	0
D. simplex	0	17.48	82.52
D. maillardi	0	30.82	69.18
M. barbi	44.04	37.69	18.26

be linked to host morphology; for Dessoutter (1992), Bothidae has a flat and dissymmetrical body. According to Rohde (1993), parasite preference for a given site of a fish body is not strange, given the strongly asymmetrical body shape of certain gill parasites particularly Monogenea. This remark in our opinion concerns especially Polyopisthocotylea; it is the case of *Gotocotyla acanthura* and *Pyragraphorus hollisae* parasites of *Trachinotus ovatus* (Mahdhi and Belghyti, 2006).

In *B. martorelli,* the transversal partitioning is observed only for *D. insolitus* (Monogenea) and for *M. barbi* (Myxosporidia). The infrapopulations of *D. insolitus* concentrated much on the second arch. This behavior can be explained by the direct mode of infection of Monopisthocotylea which is done in two steps: attachment on the skin and migration towards the microhabitat (Euzet and Combes, 1998). *D. insolitus* often exploits areas which may relate to increased oxygen availability. In fact, Paling (1968) showed in salmonid that most of the respiratory current flows over the second and the third pairs of gills, less flows over the first pair on each side and least of all across the most posterior pairs of gill. Chapman et al. (2000) suggested that the parasite may select the second gill arch to place itself in an area of maximal laminar flow in the gill. Other preferences for some gill arches are known; for example Bagge and Valtonen (1999) showed that *Dactylogyrus nanus* and *D. micracanthus* colonized much of the second arch of *Rutilus rutilus* (Cyprinidae). In this same host, Koskivaara et al. (1991) found that *D. suecicus* and *D. similis* were more abundant on the middle arches II and III, while *D. fallax* colonized much of the first two anterior gill arches. In *Anguilla anguilla*, *Pseudodactylogyrus anguillae* was more frequent in median arches, while *P. bini* occupied the first three arches (Dzika, 1999). Adams (1986) found that the infestation rate of the median arches II and III was much higher for all the Monogenea and Myxosporidia parasites of *Fundulus kansae*. In this work, the mean parasite load of the vegetative form of *M. barbi* reduced in the antero – posterior direction. This result suggests that the life cycle of this protozoan involves alternation with an actinosporean form (Kent et al., 2001) which is vehiculated by the respiratory water current. The spore fixes first on the anterior side of the gill and less and less

on the posterior side (Cassier et al., 1998).

For the lateral distribution partitioning, *D. insolitus* exploited mostly the posterior hemibranch (arch II). This model tries to confirm the mode of infestation of gill arch by Monopisthocotylea (Euzet and Combes, 1998): after fixation on the skin and migration through the opercular opening, *D. insolitus* reaches first the posterior hemibranch of the preferential arch. For *Myxobolus njinei,* the high infection rate of the anterior face of the bony part of the second gill tries to confirm that colonization of the host is done by free actinospores vehiculated by the respiratory water current. *M. barbi* (or *M. njinei*) concentrated its cysts, very much, on the anterior hemibranches (or face) except for arch I; this gradient similar to that of hemibranch surfaces (Bilong-Bilong and Tombi, 2004) seems to reinforce the idea of the host infestation by actinospores transported by respiratory water current. Cases of preferential exploitation of some hemibranches are equally known, for example that of the gill Monogenea of Pimelodidae (Gutierrez and Martorelli, 1999).

In this work, the longitudinal partitioning appeared not to be significant. Lo and Morand (2001) also did not found sector preferences. In the literature, some species of Monogenea attach essentially to the sectors which are more exposed to the respiratory water current (S2, S3, S4) (Gerasev and Staravoitov, 1988; Hendrix, 1990; Bilong-Bilong, 1995; Yang et al., 2006), while other especially colonize the dorsal S1 or ventral S5 sectors (Buchmann, 1993; Yang et al., 2006; Madhi and Belghyti, 2006). Due to the fact that the Myxosporidia *M. barbi* exploited more the medio - ventral S4 (arch II), median S3 (arch III), median and medio - ventral (arch IV), sectors which are more exposed to the respiratory water current, it is thought that its presporogonic development requires aerobic conditions. Presently, microhabitats studies of gill protozoa remain rare.

In the vertical gradient of *B. martorelli* gill parasites distribution, *D. bopeleti* and *D. insolitus* occupied the second and third distal halves of the gill filaments and especially the distal zone, while *D. simplex* and *D. maillardi* were attached essentially to the second and third proximal halves of the primary filaments. The size of the gripi (hamuli or hooks) explains the preferences of site. In fact, the presence of relatively robust gripi in *D.*

Table 3. Zonal mean load of different parasite species.

Parasite species	Zone			
	Distal	Median	Basal	Test
D.bopeleti	1.13 ± 0.47	1.08 ± 0.35	0	t = 1.70
D.insolitus	1.18 ±0.44	1.06 ± 0.25	0	t = 3.86*
D.simplex	0	1.02 ± 0.13	1.06 ± 0.23	t = 2.20
D.maillardi	0	1.08 ± 0.34	1.03 ± 0.16	t = 1.02
M.barbi	1.30 ± 0.87	1.23 ± 0.61	1.11 ± 0.	F = 12.26*

* = Significatif test.

bopeleti and *D. insolitus* (Birgi and Lambert, 1987) enables them to live in the zones of high water move-ment, while *D. simplex* and *D. maillardi* with weaker gripi (Birgi and Lambert, 1987) are protected from this high water current. According to Ramasamy et al. (1985), the force of the ventilation current is considered to be less in the proximal region of primary lamilla than in the distal region due to the presence of the bony arch. Other Monogenea prefer hidden basal zone, this is the case with *Herorapta papoua* parasite of *Chorinemus tol* (Rohde, 1980), *Pseudodactylogyrus anguillae* parasite of *Anguilla anguilla* (Buchmann, 1993; Dzika, 1999), and *Onchobdella aframae* parasite of *H. fasciatus* (Bilong Bilong, 1995). Other parasites are known to fix especially on the distal zone which is more exposed to respiratory water current, for example: *Pseudodactylogyrus bini* parasite of *Anguilla anguilla* (Buchmann, 1993; Dzika, 1999) and species of the genus *Cichlidogyrus* parasites of *H. fasciatus* (Bilong Bilong, 1995). Also, some para-sites occupy preferentially the median zone of filament; this is the case of *Ligophorus parvicirrus* parasite of *Liza ramada* (Euzet and Sanfilippo, 1983) and *Benedenia* sp. parasite of *Cephalopholis argus* (Lo and Morand, 2001). In this work, the protozoa *M. barbi* colonized the three filamentous zones, its load reducing from the free extremity towards that which is attached to the bone. This type of study up till the present moment has retained less attention.

At certain degree, the segregation of Monogenean species parasite of *B. martorelli* is done on the vertical partitioning and on the base of haptorial phenotype as for non congeneric parasite of Cichlidae *Hemichromis fasciatus* (Bilong-Bilong, 1995). This spatial niche restriction of *Dactylogyrus* may facilitate locating mate. The parasite load of the different infracommunities remains weak and thus most niches remain vacant. The hypothesis of competition therefore seems questionable. In fact, interspecific competition (even intraspecific) for space only takes place in the situation of massive host infestation (Ramasamy et al., 1985). Latter authors supported this fact in addition the tendency that gill parasite species have to select different microhabitats can reduce the possibility of interspecific competition. Moreover, Buchmann (1989) and Koskivaara and Valtonen (1991) argued that plurispecific infection can not lead to

competition if there is enough space and resources. Price (1980) also considered that once vacant niches exist for parasites, interspecific interactions will appear rarely. Rohde (1979) showed that many potential niches for ecto-parasites of fish are empty, and because of this, competitive exclusion can not take place. Although Buchmann and Lindenstrom (2002) have recently added that the exact explanation of site selection by Monogenea remains enigmatic. Our work seems to indicate that intrinsic factors (haptorial phenotypes for Monogeneans and physiological requirements for Myxosporidia) play an important role in the site selection.

It is therefore especially on the vertical distribution partitioning (or zonal) that Monogenea parasites of *B. martorelli* segregate on the base of their haptorial phenotype. The case of Myxosporidia need much studies (Table 3).

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