

INTERACTIONS BETWEEN TWO CONGENERIC GYRODACTYLIDS (*GYRODACTYLUS DERJAVINI* AND *G. SALARIS*) WITH DIFFERENT MICROHABITATS ON THEIR COMMON HOST RAINBOW TROUT, *ONCORHYNCHUS MYKISS*

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Background. The two gyrodactylid species *G. derjavini* and *G. salaris* (a special Danish rainbow trout form which is non-pathogenic to salmon) show similar predilection for their host rainbow trout and express similar population dynamics (but different microhabitat-preference) when occurring in single species infections. The present study elucidates the colonisation ability and site selection of the two parasites when occurring in mixed populations on the same host.

Materials and Methods. Fry of rainbow trout, *Oncorhynchus mykiss*, infected either with a total of 459 *G. derjavini* (12 fish) or a total of 405 *G. salaris* (12 fish) (approximately 35 parasites per fish) were maintained with a total of 12 uninfected fish for two weeks. The infection dynamics and dispersal of parasites among the three groups were recorded during the period. For localization of specific parasites in various microhabitats (fins or skin) two fish from each group were preserved weekly and their parasites mounted for species determination.

Results. Within the two-week study period, parasites spread from infected- to naïve rainbow trout, which obtained a parasite burden (293 parasites in 10 hosts) corresponding to the level of previously infected fish. *Gyrodactylus derjavini* demonstrated a higher colonisation ability especially on naïve fish but did also colonize previously *G. salaris* infected hosts. This species continued to exhibit predilection for both fins and body skin as microhabitats. In contrast, *G. salaris* was less mobile and less prone to colonise new hosts, especially infected ones. The parasite remained mostly in the preferred microhabitat (body surface) and colonised only fins to a much lower degree.

Conclusion. The results suggest the existence of specific microhabitats on the body and fins of rainbow trout, which are occupied by specific parasites. This will to some extent minimize negative interactions between the two parasites on a short-term basis (two weeks). The different microhabitats of *G. derjavini* (both fins and body) and *G. salaris* (primarily body skin) did only change slightly during the two-week study period. The few interactions between parasites and rainbow trout may partially be mediated by local host response mechanisms. It is indicated that the predilection of *G. salaris* for body surface microhabitats must be taken into account when sampling for surveys are being performed.

Keywords: *Gyrodactylus salaris*, *Gyrodactylus derjavini*, parasite, Monogenea, rainbow trout, *Oncorhynchus mykiss*, microhabitat, interaction

INTRODUCTION

A few studies have focused on interactions between different congeneric monogenean species when colonising their common host. For example, Paperna (1964) showed that the gill parasitic *Dactylogyrus extensus* was outcompeted by *D. vastator* on carp gills. Likewise, competitive exclusion of *Pseudodactylogyrus anguillae* by the congener *P. bini* on gills of eels was reported by Buchmann (1988). Interactions between skin-parasitizing gyrodactylids on

guppies were studied by Richards and Chubb (1996), who mainly focused on host responses as the main factor in this context. The basic elements responsible for these interactions may be direct physical contact between the congeners. Also emission of hostile compounds, deterring congeners, could theoretically explain exclusion of other species. Finally, due to the fact that many congeners occupy different microhabitats, it has been suggested that competitive exclusion is mediated by a general host response elicited by the

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parasites but affecting the congeners differently (Buchmann 1988). Rainbow trout, *Oncorhynchus mykiss*, is a good host for both *Gyrodactylus derjavini* (cf. Malmberg 1993, Buchmann and Uldal 1997) and for a special Danish farm form of *G. salaris*, which is non-pathogenic to Atlantic salmon (Jørgensen et al. 2007). The two congeners show a similar population increase on this host when occurring in single species infections but their microhabitat preferences differ. *G. derjavini* is mainly occupying the fins (Buchmann and Uldal 1997) whereas *G. salaris* to a large extent is found on the body surface although some fins can be found infected (Jørgensen et al. 2007). We have isolated the two parasite species and erected single species populations on rainbow trout in the laboratory. Therefore, it is possible to conduct a series of controlled studies in order to elucidate interactions between the two congeneric parasites on this host and to detect any changes of microhabitat selection in mixed infections. In this study we kept three groups of rainbow trout in the same fish tank and followed the dispersal of both parasite species and their colonisation potential on hosts previously infected with *G. derjavini*, fish previously infected with *G. salaris* or trout previously uninfected.

MATERIALS AND METHODS

Fish. Rainbow trout *Oncorhynchus mykiss* (body length 6–7 cm) hatched from disinfected eggs from the Fousing Trout Farm (Jutland, western part of Denmark) were used. Fish were fed commercial pelleted trout feed (Biomar, Denmark) (1 percent biomass per day) following stocking as first feed fry in the laboratory system.

Parasites. Laboratory strains of *Gyrodactylus derjavini* Mikailov, 1975 (cf. Lindenstrøm and Buchmann 2000) and *Gyrodactylus salaris* Malmberg, 1957 (cf. Jørgensen et al. 2007) on rainbow trout were used.

Fish tanks. Fish were kept in a temperature-controlled room (12–13°C) in 120-L fish tanks with pathogen-free recirculated water (50% municipal water and 50% deionised water) for 6 months prior to the experiment. Recirculation was achieved by internal biofilters (Eheim, Germany). Two thirds of the water was replaced twice a week. Water was aerated (oxygen saturation 90%–100%) and the levels of ammonia, nitrites, and nitrites were measured regularly (Merck Aquacant, manufactured by Merck, Germany).

Experimental design. Three groups of fish (each comprising 12 specimens) were used. One group was uninfected

(naïve fish), while another group was infected during three weeks of cohabitation with three *Gyrodactylus derjavini*-infected fish, whereby they obtained a mean intensity of 34 parasites per fish. The third group of trout was infected in a similar way but by cohabitation with three *Gyrodactylus salaris*-infected fish whereby the fish became infected with a mean intensity 38 parasites per fish. The three fish groups (a total of 36 fish) were then placed together in one large aquarium (volume 120 L) and the infection was subsequently monitored for the following two weeks.

In order to differentiate fish following mixing minor incisions in the tail fin were done before experimental start. Thus, *G. derjavini* infected fish were cut (1/8) in the upper tail fin. *G. salaris* infected fish were cut likewise in the lower tail fin and the uninfected fish left uncut.

Parasitological investigation. An overall state of the infection was determined on day 0, 7, and 14 by anaesthetising all fish (36, 28, and 19 rainbow trout, respectively) with MS 222 (50 mg · L⁻¹) and counting the number of gyrodactylids (no species differentiation possible at low-power microscopy) in different microhabitats using a dissection microscope (magnification 7–40×) according to Buchmann and Uldal (1997). In order to determine the specific identity of the gyrodactylids, two fish were taken out from each group at day 0, 7, and 14. The fish were preserved in 70% ethanol. The parasites from the different microhabitats (fins and body) were isolated, mounted in ammonium picrate glycerine, and finally studied under a compound microscope (40–1000× magnification) for species determination based on morphological criteria.

RESULTS

The initial, one-species populations of *Gyrodactylus salaris* (on 12 trout) and *Gyrodactylus derjavini* (on 12 trout) comprised 405 and 459 parasites, respectively (Table 1). The infected fish were then placed together with 12 uninfected trout and two weeks later 293 parasites (both parasite species) were recorded on 10 previously naïve hosts concomitant with a significant decrease of populations on the previously infected fish (Table 1). It was indicated that a number of *G. derjavini* moved from its original host to the other host groups and in particular—to the previously uninfected hosts. However, a subpopulation of this species remained also on the originally infected fish. Thus, 459 *G. derjavini* parasites were recorded on 12 fish at start of the experiment and a total of 103 *G. derjavini* parasites on

Table 1

Total number of gyrodactylids (both *Gyrodactylus salaris* and *Gyrodactylus derjavini*) (no specific differentiation made by low power dissection microscopy) on fins and body of three groups of rainbow trout (uninfected, *Gyrodactylus salaris* infected or *Gyrodactylus derjavini* infected at the start of the experiment) during three weeks

	Number of gyrodactylids found		
	Day 0	Day 7	Day 14
Fish originally infected by <i>Gyrodactylus salaris</i>	405 (12)	297 (6)	124 (3)
Fish originally infected by <i>Gyrodactylus derjavini</i>	459 (12)	343 (10)	268 (6)
Fish originally uninfected	0 (12)	143 (12)	293 (10)

Number of fish examined given in parentheses.

two fish from the same group of fish were isolated at the end of the experiment. During this process, the previously uninfected fish became colonised and two of these fish harboured a total of 60 *G. derjavini* in week 2 (Table 2). Population parameters, such as birth rate, mortality rate, and survival were not recorded for the gyrodactylids and it was therefore not possible to differentiate between old parasites and newly produced offspring. Fish previously infected with *G. salaris* became colonised with fewer *G. derjavini*. Although ending up with 97 parasites (both species) on two hosts only 28 out of these parasites were *G. derjavini* (Table 2).

DISCUSSION

The number of fish examined for specific parasites in specific microhabitats is relatively low due to the time-consuming method of mounting individual parasites for precise diagnosis. Thus, a total of 447 individual slides (245 with *Gyrodactylus salaris* and 202 with *Gyrodactylus derjavini*) were prepared and measured in order to investigate the presented infection dynamics. Likewise, the study period was limited to 14 days. However, despite the low number of fish examined, some general differences between the congeners appeared and may provide a useful basis for further, extended analyses. *G. derjavini* exhibited a superior

Table 2

Number of *Gyrodactylus salaris* and *Gyrodactylus derjavini* in specific habitats of fish from the three fish groups during three weeks; the number given is the total number of specific parasites (*Gyrodactylus salaris* or *Gyrodactylus derjavini*) on skin or fins in a subsample of two fish from each group for each week

		Number of gyrodactylids found					
		Day 0		Day 7		Day 14	
		Gs	Gd	Gs	Gd	Gs	Gd
A	Fins	48	0	16	15	19	22
	Body	15	0	87	4	50	6
B	Fins	0	49	0	33	2	40
	Body	0	54	4	19	4	63
C	Fins	0	0	1	42	3	41
	Body	0	0	0	5	4	19

A, group originally infected by *Gyrodactylus salaris* (2 fish examined each week).

B, group originally infected by *Gyrodactylus derjavini* (2 fish examined each week).

C, group originally uninfected (2 fish examined each week).

Gs, *Gyrodactylus salaris*.

Gd, *Gyrodactylus derjavini*.

G. salaris parasites did not show the same ability to move to other host groups during the two-week study period. The originally uninfected fish were colonised by a total of 7 *G. salaris* in two fish in week 2 (Table 2). Fish previously infected by *G. derjavini* harboured 6 *G. salaris* parasites (two fish). When the parasites were assigned to different microhabitats it was noted that the caudal fin was the preferred site for *G. derjavini* with a mean number of parasites per fin between 6 and 12 from week 0 to week 2 (in fish previously infected with *G. derjavini*) and up to 21 *G. derjavini* per caudal fin in previously uninfected fish (data not shown). Fish previously infected with *Gyrodactylus salaris* received *G. derjavini* parasites mainly on the caudal fin (6 parasites) where *Gyrodactylus derjavini* outnumbered *Gyrodactylus salaris*. The latter species was (except for some parasites on the caudal fin) found to prefer the body surface (mostly dorso-caudal and ventro-caudal positions) but also to some extent dorso-cranial and ventro-cranial microhabitats on the body surface. The few *G. salaris*, moving to other hosts, colonised the posterior part of the fish body and the caudal fin of naïve fish. Also, the previously *Gyrodactylus derjavini*-infected fish received fewer *G. salaris* individuals in these parts (data not shown).

colonisation ability compared to *G. salaris*, which appeared more sedentary. The naïve fish seemed to be more susceptible compared to previously infected fish, which suggests that the host response of rainbow trout towards *G. derjavini* plays a role in this population dynamics (Lindenstrøm and Buchmann 2000). *G. salaris* appeared to possess a reduced colonisation ability and the extensive dispersal of gyrodactylids in general (only detected by a low-power microscope without species identification) was likely due to *G. derjavini* spreading. This was confirmed by the subsequent identification of the 447 parasites (recovered from 18 sampled fish and mounted in ammonium picrate) at high magnification. Even naïve fish seemed to receive only a few *G. salaris* although in slightly higher numbers than infected fish. The *G. derjavini* parasites did not seem to avoid *G. salaris* parasites in particular as judged from the fact that *G. derjavini* readily colonised fins with *G. salaris* parasites present. Therefore the infection dynamics in this system seems to be ruled both by colonisation activity and host responses. Similar observations were done by Paperina (1964) and Buchmann (1988) who found that congeneric gill parasites behaved differently and were affected differently by the host response. Likewise, Richards and Chubb (1996) studying two congeneric gyrodactylids on guppies

showed that the host responses of guppies affected not only the species eliciting the response but also the congener.

The host response could be induced by the both mechanical and chemical injuries caused by anchors/marginal hooklets and/or adhesive compounds (Whittington et al. 2000) or wounds inflicted by pharyngeal pressure and enzymatic action (Malmberg 1993). These response mechanisms in the host are not fully elucidated. Studies by Buchmann et al. (2004) could not detect host antibody reactions in infected salmon against *G. salaris* antigens but recent work (Lindenstrøm et al. 2003) indicated that other immune factors such as the cytokine interleukin 1 beta (IL-1beta) was expressed in skin of rainbow trout infected by *G. derjavini*. In addition, Kania et al. (2007) recorded expression of a number of immune genes (IFN-gamma, Mx, MHCI) in fins of salmon infected by *G. salaris*. This supports the notion that several immune factors interact in these host responses against gyrodactylids.

Generally the microhabitats occupied by the two congeners did not change markedly during the present investigation conducted over a short study period. *G. derjavini* parasites remained both on the fins and to some extent on the body of the fish. In contrast, *G. salaris* was mainly detected on the body surface of the host. The different preference for microhabitats of the two congeners is not readily explained but may be related to different immune evasion mechanisms of the two species.

The different site selection detected is noteworthy and may have some influence on monitoring programmes and surveys in countries where salmonid fins are being screened systematically for *G. salaris* infection. Thus, in the UK, fins of salmonids are sampled regularly in order to document the absence of the *Gyrodactylus salaris* parasite (Peeler and Thrush 2004). The present work suggests that the body of rainbow trout harbour a considerable part of the *G. salaris* population, which is in accordance with studies by Jørgensen et al. (2007). This parasite species will therefore probably be sampled at lower frequency due to the current sampling practice, which is based on fin examination only. Hence, the probability of detecting this particular type of *G. salaris* on rainbow trout will be unnecessarily low and raise questions of the suitability of the sampling method.

REFERENCES

Buchmann K. 1988. Interactions between the gill-parasitic monogeneans *Pseudodactylogyrus anguillae* and *P. bini* and the fish host *Anguilla anguilla*. Bulletin of the European Association for Fish Pathologists **8**: 98–100.

Buchmann K., Uldal A. 1997. *Gyrodactylus derjavini* infections in four salmonids: comparative host susceptibility and site selection of parasites. Diseases of Aquatic Organisms **28**: 13–22.

Buchmann K., Madsen K.K., Dalgaard M.B. 2004. Homing of *Gyrodactylus salaris* and *G. derjavini* (Monogenea) on different hosts and response post-attachment. Folia Parasitologica **51**: 263–267.

Jørgensen T.R., Larsen T.B., Jørgensen L.G., Bresciani J., Kania P.W., Buchmann K. 2007. Characterisation of a low pathogenic form of *Gyrodactylus salaris* from rainbow trout. Diseases of Aquatic Organisms **73**: 235–244.

Kania P.W., Larsen T.B., Ingerslev H.C., Buchmann K. 2007. Baltic salmon activates immune relevant genes in fin tissue when responding to *Gyrodactylus salaris* infection. Diseases of Aquatic Organisms **76**: 81–85.

Lindenstrøm T., Buchmann K. 2000. Acquired resistance in rainbow trout against *Gyrodactylus derjavini*. Journal of Helminthology **74**: 155–160.

Lindenstrøm T., Buchmann K., Secombes C.J. 2003. *Gyrodactylus derjavini* infection elicits IL-1 β expression in rainbow trout skin. Fish and Shellfish Immunology **15**: 107–115.

Malmberg G. 1993. Gyrodactylidae and gyrodactylosis of salmonidae. Bulletin Français de la Pêche et de la Pisciculture **328**: 5–46.

Paperna I. 1964. Competitive exclusion of *Dactylogyrus extensus* by *Dactylogyrus vastator* (Trematoda, Monogenea) on the gills of reared carp. Journal of Parasitology **50**: 94–98

Peeler E.J., Thrush M.A. 2004. Qualitative analysis of the risk of introducing *Gyrodactylus salaris* into the United Kingdom. Diseases of Aquatic Organisms **62**: 103–113.

Richards G.R., Chubb J.C. 1996. Host responses to initial and challenge infections, following treatment of *Gyrodactylus bullatarudis* and *G. turnbulli* (Monogenea) on the guppy (*Poecilia reticulata*). Parasitology Research **82**: 242–247.

Whittington I.D., Cribb B.W., Hamwood T.E., Halliday J.A. 2000. Host specificity of monogenean (platyhelminth) parasites: a role for anterior adhesive areas? International Journal for Parasitology **30**: 305–320.

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