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Susceptibility and resistance of minnows, *Phoxinus phoxinus* (L.) to *Gyrodactylus salaris* Malmberg, 1957 (Monogenea) under laboratory conditions

TOR A. BAKKE AND LAURA A. SHARP

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The host susceptibility and resistance of minnows, *Phoxinus phoxinus,* to *Gyrodactylus salaris* was examined under laboratory conditions. The following experiments were carried out: transmission rate was examined by exposure of uninfected minnows to infected living or dead salmon; «homing response» was examined using «two-choice» host tests; and *G. salaris* survival on minnows was examined using individual fish isolation or grouped fish isolation. The results of the experiments demonstrated low transference rates to minnows; the transmission rates to salmon were much higher. The parasite was not observed to reproduce on the minnows. The maximum duration of infection after isolation of minnows, individually or grouped, was two and four days, respectively. Minnows are not considered to represent any risk for *G. salaris* reproduction or dissemination.

Bakke, T. A. Zoological museum, University of Oslo, Sars gt. 1, 0562 Oslo 5, Norway.
L. A. Sharp. Department of Zoology, University of Glasgow, Glasgow, G12 8QQ, U.K.

INTRODUCTION

Minnows, *Phoxinus phoxinus* (L.) occur throughout most of Europe (Myllylä et al. 1983, Pethon 1985). In Norway, the wide distribution of minnows, including important salmon and trout rivers, is largely due to the more or less accidental translocation by man, as the species is used as live bait for fishing, although this is forbidden under Norwegian law (Borgstrøm 1973, Lien 1981). Nevertheless, the species has naturally colonized the south-east of Norway from the east, and the northern Norway, from the south (Borgstrøm 1973, Eggan & Johnsen 1983). Minnows are distributed in two river courses infected by *G. salaris* Malmberg, 1957 in south-eastern Norway, the Drammenselva and Lierelva rivers (Brittain et al. 1986, Økland 1990). From East-Norway, the species has a continuous distribution to the Baltic area, occurring also in brackish water (Myllylä et al. 1983). Drainage systems in the Baltic are also infected with *Gyrodactylus salaris* (see Malmberg 1988).

In the wild, *G. salaris* has been recorded on non-salmonid hosts, such as *Platichthys flesus* (L.) (Mo 1987). *Gyrodactylus* species on non-salmonid hosts, such as *G. arcuatus* Malmberg, which naturally infects three spined stickleback, *Gasterosteus aculeatus* (L.), has also been found on salmon in Norway (Tanum 1983). On the other hand, *Gyrodactylus* species, such as *G. macronychus* Malmberg and *G. aphyae* Malmberg, occuring naturally in high frequencies on minnows, have been observed on both brown trout and salmon, respectively, in the same river system (Mo 1983). In another river course, *G. phoxini* Malmberg occurred on salmon (Mo 1988). Generally, minnows are natural hosts for a large range of *Gyrodactylus* species (Malmberg 1970, Harris 1985).

As *Gyrodactylus salaris* has been reported to be disastrous for salmon parr in infected Norwegian rivers (see Heggberget & Johnsen 1982, Halvorsen & Hartvigsen 1989), the aim of this study was to investigate the potential of minnows as hosts for *G. salaris* propagation or dissemination in the wild. Thus, the attachment potential of *G. salaris* to minnows, the «homing response» of *G. salaris* in «two-choice» host tests including minnows and the susceptibility and resistance of minnows themselves to this ectoparasite, were tested under laboratory conditions.

MATERIAL AND METHODS

The Atlantic salmon, *Salmo salar* L. used, were of age 0+, the river Ims stock and hatchery reared.

The minnows, *Phoxinus phoxinus,* were caught on 20th July in the Akerselva river, Oslo, Norway. When collected, some of the minnows were found to be infected with *Gyrodactylus aphyae* (identification based on ammonium picrate-glycerol mounted specimens according to Malmberg (1970)). All the minnows were disinfected before the experiments were performed, by immersion in Actomar (1:10000). A total immersion time of 2.5 hours was found necessary to eliminate the infection. No harmful effects of the treatment to the minnows were observed.

A population of *G. salaris* was established in the laboratory from six wild, heavily infected, salmon from the river Drammenselva. Salmon exposed to this infection source were later used in the experiments.

The fish were acclimatized for approximately 10 days after disinfection and prior to the experiments. The water temperature was kept at 12.4±0.4°C. The fish were kept under continuous dim illumination and not fed during the experiments. The experiments were performed in three sizes of grey plastic tanks: large tanks (1.0 x 1.0 m, water depth 0.3 m); medium tanks (0.27 x 0.38 m, water depth 0.12 m); small tanks (medium tanks divided into two equal compartments using a wire netting and polystyrene divider). During isolation procedures, the medium and small sized tanks were floated in a large tank. The laboratory water was charcoal filtered, dechlorinated tap water. No special substrate was provided.

The following three experiments were carried out:

(Expt. 1) Transmission rate: exposure of uninfected minnows to *Gyrodactylus salaris* infected salmon, both living and dead, for a total of three days. On DAY 0, 15 uninfected minnows (mean weight 2.5 g, range 0.8—4.6 g; mean length 6.4 cm, range 4.2—7.9 cm) were placed in a medium tank with 6 heavily infected (>500 parasites on each) live salmon parr. After recording the parasite burden on DAY 1, 10 randomly selected minnows were used further. They were directly placed together with five heavily infected dead salmon parr (>500 parasites on each) in a medium tank, one end of which was covered with black sheeting. The bodies of the salmon were placed under the covered area. Both modifications enhanced conditions for transmission. The results of Expt. 1, which was carried out in duplicate, are pooled in Table 1.

Table 1. Pooled results of two identical experiments to elucidate the transmission potential of *G. salaris* from heavily infected (>500 parasites on each) *Salmo salar,* dead or alive, to uninfected minnows, *Phoxinus phoxinus,* when kept together in medium tanks. The same salmon and minnows were used during the three days (DAY 1—3) of exposure. During days 2 and 3, the tank was shaded at one end and the infected salmon were killed.

Number of salmon (condition)	DAY 1 12 (living)	DAY 2 10 (dead)	DAY 3 10 (dead)
Number of minnows	30	20	20
Mean intensity (Range)	1.0 (0—2)	1.5 (0—7)	3.5 (0—14)

(Expt. 2) «Homing response» of the parasite: using «two-choice» host tests. The attachment preference of minnows was examined by exposing uninfected salmon (mean weight 3.3 g, range 2.4—4.8; mean length 6.5 cm, range 5.8—7.4) and uninfected minnows (mean weight 3.6 g, range 2.9—4.9 g; mean length 7.0 cm, range 5.6—7.8 cm) to live salmon parr infected with *G. salaris* in small tanks for 24 hours. The results of two identical tests are pooled in Table 2.

(Expt. 3) Duration of infection: assessing change in parasite infrapopulations on minnows when isolated after exposure to an infection source, both individually and grouped. The *G. salaris* infected minnows used were taken directly after termination of Expt. 1 (Table 1), and individually isolated (minnow size: mean weight 3.6 g, range 2.1—4.6 g; mean length 6.9 cm, range 5.4—7.9 cm) in small tanks or grouped (minnow size: mean weight 1.8 g, range 0.8—3.0 g; mean length 5.7 cm, range 4.2—7.3 cm) in a large tank.

The parasite burden was assessed by making direct counts on the fish in a petri dish containing laboratory water, following anaesthetization with a 0.04% chlorbutanol solution. Previous pilot experiments have shown

that the parasites on the host show no visible harm from the chlorbutanol at the above concentrations and periods of use.

RESULTS

Transmission of *G. salaris* to minnows

The pooled results of two identical transmission experiments demonstrate that very few *G. salaris* transferred to minnows during the 24 hour period of exposure (Table 1, DAY 1). In addition, improving the conditions for transfer did not subsequently significantly increase the parasite burden on the minnows (Table 1, DAYS 2, 3).

«Homing response» of *G. salaris*

When given *G. salaris* on infected salmon parr, a «two-host» choice for transfer (Table 2) demonstrated that after 24 hours the infection was significantly higher on the initally uninfected salmon than on the uninfected minnows. (Parasite burden on the originally infected salmon after the tests: mean intensity 152, range 21—750.)

Duration of infection on isolated minnows

After isolation of infected minnows from the infection source, the parasites persisted on the minnows for only two and four days, on grouped and individually isolated fishes, respectively (Table 3).

DISCUSSION

Scott & Robinson (1984) demonstrated that guppies, *Poecilia reticulata* Peters, challenged 1 to 2 weeks after an initial infection, were less suitable hosts for *G. turnbulli* Harris. This resistance appeared to be lost after 4—6 weeks. Lester & Adams (1974) found a period of 4 weeks in the *Gasterosteus aculeatus — G. alexanderi* Mizelle & Kritsky, interaction. Such a phenomenon has to our knowledge not been observed for interspecific *Gyrodactylus* relations. Furthermore, the minnows were infected with *G. aphyae* (and probably other *Gyrodactylus* species), when collected, and disinfected 10 days prior to the present experiments. This indicates that the fish were not in a resistance stage, even to *G.*

Table 2. Results of the «homing response» of *G. salaris* in the «two-choice» host infection experiments. Two identical tests were run, each with 5 uninfected salmon, *S. salar,* and 5 uninfected minnows, *Phoxinus phoxinus,* exposed to 5 infected live salmon (adipose clipped), all kept together in medium tanks for one day.

	Expt. I		Expt. II		Total	
Parasite burden	Salmon	Minnow	Salmon	Minnow	Salmon	Minnow
Mean intensity (Range)	46.2 (34—60)	0.6 (0—1)	58.0 (28—94)	2.2 (1—4)	51.4 (28—94)	1.4 (0—4)

Table 3. Results of the experiments on duration of infection of isolated (n= 7) and grouped (n = 11) minnows, *Phoxinus phoxinus,* with *G. salaris* (mean intensity, range in brackets), after removal from the infection source. The minnows were taken directly from Expt. 1(Tab. 1, DAY 3; DAY 3 in Tab. 1 is Day 0 in Tab. 3). The minnows were subsequently kept isolated in small tanks (medium tanks divided into two compartments of equal volume).

	DAY					
Minnow situation	1	2	3	4	5	6
Isolated	6.7 (2—14)	1.4 (0—3)	0.3 (0—2)	0.1 (0—1)	0.1 (0—1)	0 —
Grouped	1.8 (1—4)	0.5 (0—2)	0.1 (0—1)	0 —	— —	— —

aphyae, when used. Delayed cross-immunity interactions was accordingly not judged to be a factor of significance in these experiments.

The experimental water temperature is within the optimum temperature range for *G. salaris* reproduction (Jansen & Bakke 1990) and also suitable for transmission to transport hosts (Bakke et al. 1991). Nevertheless, the attachment frequency of *G. salaris* to minnows was found to be very low and no subsequent development or reproduction of the attached specimens was observed. Even experiments which forced the two species to live in a limited space and the use of dead salmon as the infection source, enhancing searching and transference behaviour of the parasite (Scott 1985), did not substantially increase the attachment rate of *G. salaris.*

The transfer of for example *G. phoxini* from minnows to salmon, may be explained on basis of indirect transmission of dislodged minnow parasites instead of direct transmission. Also the presence of *G. arcuatus* and *G. aphyae* on salmon, naturally infecting threespined sticklebacks and minnows, respectively (Mo 1983, Tanum 1983), may be caused by this indirect method of transfer resulting from the bottom dwelling behaviour of the salmon.

The «two-choice» host tests demonstrated a marked preference for attachment to salmon, instead of minnows. This difference is more pronounced than previous results for *G. salaris* transfer to the resistant European eel, *Anguilla anguilla* (L.) (Bakke et al. 1991). Also the resistant brook lamprey, *Lampetra planeri* (Bloch) (Bakke et al. 1990) permitted a higher parasite attachment frequency. However, both species are bottom dwellers, which may at least partly, explain the relatively higher attachment frequency of *G. salaris* to this host species in relation to minnows. Hoffman and Putz (1964) have also reported a higher level of infection among fish having access to the bottom of a trough.

The minnow is widely distributed in streams and rivers with stony bottoms where as adults, they form shoals in shallow areas, often in slow-flowing parts (Tack 1941, Frost 1943, Jacobsen 1979). This behaviour contrasts with that of the territorial bottom dwelling salmon parr, which display a preference for habitats with higher water velocities (Heggenes & Saltveit 1990). This behavioural differences diminishes the opportunity for natural contact between minnows and salmon, and also between minnows and the bottom substrate, decreasing the possibility of indirect transmission of dislodged *G. salaris* to minnows, or direct transmission from infected dead salmon.

G. salaris persisted on the minnows for only two to four days after isolation, approximately the same as the maximum life span of the parasite, dislodged from the host (Mo 1987). Hence factors, at present unknown, efficiently prevent prolonged attachment and reproduction of the parasite. The transmission window thus seems very narrow. Transported *G. salaris* also would have to populate a new area to the density required for further transfer to salmonids. Accordingly, the very restricted duration of low level *G. salaris* infections on minnows, demonstrates that minnows are unlikely to represent any danger as regards dissemination and translocation of the parasite.

So far, results indicate that all the non-salmonid hosts examined, brook lamprey, perch, roach (Bakke et al. 1990), European eel (Bakke et al. 1991) and minnow, are resistant to infection with *G. salaris.* However, attachment of the parasite is increased if the recipient fish species has a behavioural pattern allowing for frequent bottom contact. Further parasite dispersal on such potential transport hosts is subsequently dependent on the vagility and migratory behaviour of the specific host species.

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