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The susceptibility of three-spined stickleback (*Gasterosteus aculeatus*), nine-spined stickleback (*Pungitius pungitius*) and flounder (*Platichthys flesus*) to experimental infections with the monogenean *Gyrodactylus salaris*

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Abstract. Three-spined stickleback (*Gasterosteus aculeatus* L.), nine-spined stickleback (*Pungitius pungitius* (L.)) and flounder (*Platichthys flesus* (L.)) are widespread teleosts, which all have behaviours involving migration between freshwater and brackish/sea water environments. Their importance in dispersal of the freshwater monogenean *Gyrodactylus salaris* Malmberg, 1957, which causes heavy losses of Atlantic salmon (*Salmo salar* L.) parr in infected Norwegian rivers, was tested indirectly by their susceptibility and resistance to the parasite in laboratory experiments. *Gyrodactylus salaris* attached to the three fish species, but no parasite reproduction was observed. The infections were eliminated after a maximum of 3 days on flounder, 6 days on nine-spined stickleback, and 8 days on three-spined stickleback. Thus these fishes are innately resistant to *G. salaris*, and are therefore of no importance concerning the population dynamics of *G. salaris* in freshwater systems. However, attachment of parasites indicates that these fish species may function as transport hosts, and theoretically play a part in the dispersal of *G. salaris* in nature.

The freshwater monogenean *Gyrodactylus salaris* Malmberg, 1957 is well known for its devastating effects on Atlantic salmon (*Salmo salar* L.) populations in Norway (cf. Johnsen and Jensen 1991, Mo 1994). By the end of 1994 the parasite had been reported from a total of 38 rivers along the Norwegian coastline in addition to the same number of hatcheries (Soleng and Bakke 1995).

The susceptibility of both non-salmonid and salmonid fish species to *G. salaris* has been examined previously (see Bakke et al. 1992, 1996) in order to study the potential host range and dispersal potential of the parasite. Earlier, Johnsen and Jensen (1986) found the distribution of *G. salaris* in Norway to be related to stocking of fish from infected hatcheries, but also the possibility of natural dispersal by migration of infected fish through fjord systems has been hypothesized (Anonymous 1982, Johnsen and Jensen 1986, Lund and Heggberget 1992). This latter is supported by Soleng and Bakke (1997) and Soleng et al. (1998) who examined the salinity tolerance and dispersal potential of *G. salaris* on salmon.

Mo (1987) found two flounders (*Platichthys flesus* (L.)) in the River Litledalselva in western Norway infected with 2 and 3 specimens of *G. salaris*. Flounder is a common fish species in fjords and estuaries in Norway, especially in brackish water, and they frequently ascend rivers and migrate long distances upstream (Pethon 1985). Both estuaries and freshwater environments are reported as important nursery and feeding areas, especially for immature individuals (e.g., Ladiges 1935, Summers 1979, 1980). The widespread three-spined stickleback (*Gasterosteus aculeatus* L.) consists of both euryhaline anadromous and freshwater resident populations. Most populations of the *trachurus* type, which possess a complete row of lateral bony plates (Münzing 1963, Aneer 1973), is anadromous (Wootton 1976). Fish that overwinter in sea water migrate to fresh (or brackish) water environments in spring, and following breeding, back to the sea in the autumn (Wootton 1976). The *leiurus* type, which possess only few (or no) lateral plates in the anterior region of the body (Münzing 1963, Aneer 1973), is mainly resident in fresh water, but this is not absolute (Wootton 1976). The *semiarmatus* type, with lateral plates on both the pectoral and caudal part of the body, is said to be a hybrid between the two former types (Aneer 1973), and is found as both freshwater resident and anadromous populations (Wootton 1976). The nine-spined stickleback (*Pungitius pungitius* (L.)) has a more limited distribution in Norway than the three-spined stickleback, and is more restricted to fresh and brackish water, seldom being found in pure sea water (Pethon 1985). The salinity tolerance of nine-spined stickleback is lower than that of the three-spined stickleback, but it is, however, still considerable (Heuts 1943, Nelson 1968). As with the three-spined stickleback, the salinity tolerance of the nine-spined stickleback is lowest during the breeding season when they are found only in fresh or brackish water (Wootton 1976). Some populations of

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nine-spined sticklebacks also overwinter in the sea (Wootton 1976).

In order to assess the possible role of flounder, three- and nine-spined sticklebacks in dispersal of *G. salaris*, we examined the susceptibility and resistance of these fish species to the parasite in laboratory experiments.

MATERIALS AND METODS

The flounders and three-spined sticklebacks were caught with a beach seine in a brackish water area (salinity 18 ‰) at the inner part of Bunnefjorden near Oslo, Norway, while the nine-spined sticklebacks were caught in a small freshwater stream draining into the same area. All fishes were caught in the end of August 1995. All three-spined sticklebacks were of the *trachurus* type with well developed lateral bony plates (see Münzing 1963, Aneer 1973).

All fishes were gradually acclimated to active-charcoal filtered dechlorinated laboratory fresh water in grey plastic tanks (1 x 1 x 0.30 m) during a period of 24 hours. Prior to the experiment the fishes were disinfected with a 0.009% formaldehyde solution for one hour, and further acclimated for one week to the experimental conditions at 12.5°C (range 12.4-12.6°C).

Twenty-one flounders (mean length 3.8 cm, range 2.9-5.5 cm; mean weight 0.9 g, range 0.3-3.5 g), 42 three-spined sticklebacks (mean length 5.9 cm, range 5.1-6.6 cm; mean weight 1.7 g, range 1.1-2.6 g) and 42 nine-spined sticklebacks (mean length 4.1 cm, range 3.4-4.8 cm; mean weight 0.5 g, range 0.3-0.7 g) were experimentally infected with *G. salaris* in small plastic boxes (0.38 x 0.27 x 0.12 m). This was accomplished by exposing 21 uninfected fish to four living heavily infected salmon parr and the fins from three heavily infected salmon parr per box for 24 hours. The *G. salaris* strain used originated from wild salmon parr caught in the River Lierelva, Buskerud County, approximately one month prior to the start of the experiments.

After being infected, all fishes were individually isolated in plastic boxes (0.17 x 0.11 x 0.05 m) with mesh bottoms floating in grey plastic tanks. A total of 21 fish was used in each large fish tank which had a continuous flow of water (2 l/min). Aquarium filters (Fluval 403 without filter medium; 1200 l/hour) circulated the water. To prevent fish escapees a transparent lid was placed over the boxes, while a semi-transparent lid was placed over the fish tanks as shelter. The experiments were performed under constant dim illumination, and the fishes were not fed. We used 10 hatchery reared Atlantic salmon parr (1^+) of the River Lierelv stock as control fish. These fish had not previously been exposed to any *Gyrodactylus* infection, and had been routinely disinfected against ectoparasites and fungus in the hatchery. They were experimentally infected by exposure to fins from only one heavily infected salmon parr for 24 hours.

All fishes were anaesthetized with 0.04 % chlorobutanol in petri-dishes, and later examined under a stereo-microscope in laboratory water each day. The number of *G. salaris* was assessed by counting the parasite specimens on the skin and fins (not gills, olfactory organs, opercular and oral cavity). Anaesthesia may be stressful to the parasites. Harris et al. (1994) stressed, however, that the mortality and fecundity schedules of *G. salaris* derived experimentally by Jansen and Bakke (1991) are close to those occurring in natural infections, despite the complications of frequent anaesthesia.

Eight three-spined sticklebacks died during the first day of the experiment, and are not included in the results.

The statistical tests used are in accordance with Zar (1984), and the level of significance was 5 % (α = 0.05). All parasitological terms used follow the definitions given in Margolis et al. (1982).

RESULTS

Gyrodactylus salaris transmitted to every single fish during the experimental infection procedure of 24 hours (Table 1). There were, however, significant differences in the initial infection between the three tested fish species (Kruskal-Wallis test; $P < 0.001$). The number of *G. salaris* was significantly higher on the nine-spined stickleback compared to the three-spined stickleback (Tukey test; $P < 0.001$) and the flounder (Tukey test; $0.02 < P < 0.05$). Also the flounder were significantly more infected than the three-spined stickleback (Tukey test; $0.005 < P < 0.01$). However, no parasite reproduction was observed on any of these fish species. The infections were eliminated after a maximum of 3 days on flounder, 6 days on nine-spined sticklebacks and 8 days on three-spined sticklebacks (Table 1). However, during the same experimental period the infection increased continuously on all 10 control salmon parr (Table 1).

DISCUSSION

Gyrodactylus salaris was considered specific to Atlantic salmon by Malmberg (1957). Later laboratory studies have shown that *G. salaris* has a relatively broad spectrum of hosts (Bakke et al. 1992, 1996). Furthermore, in nature, *G. salaris* has been found on Atlantic salmon, rainbow trout (*Oncorhychus mykiss* (Walbaum)) (Mo 1988a), Arctic charr (*Salvelinus alpinus* (L.)) (Mo 1988a,b, Jørgensen and Kristoffersen 1992), brown trout (*Salmo trutta* L.) (Tanum 1983, Malmberg 1988, Malmberg and Malmberg 1991, Johnsen and Jensen 1992) and flounder (Mo 1987).

The present results indicate that *G. salaris* can attach to the skin of three-spined sticklebacks, nine-spined sticklebacks and flounders. After exposure to infection the nine-spined stickleback and the flounder were found to be more heavily infected than the three-spined stickleback. It is known that gyrodactylids transmit preferentially to bottom-dwelling fish species, and to fish which have access to the bottom, compared to fish suspended in the water column (Hoffman and Putz 1964, Parker 1965, Bakke et al. 1990, 1991). In the present experiments the three-spined sticklebacks were prone to rest on the substratum only with the two ventral spines touching the bottom, in contrast to

Table 1. Course of infection of *Gyrodactylus salaris* on experimentally infected three-spined stickleback (*Gasterosteus aculeatus*), nine-spined stickleback (*Pungitius pungitius*), flounder (*Platichthys flesus*) and Atlantic salmon (*Salmo salar*).
n - number of fish; prev. - prevalence (%); Abund. - abundance; Day 0 - start of the experiment after 24 hours exposure to infected salmon.

Day	Three-spined stickleback (n=34)		Nine-spined stickleback (n=42)		Flounder (n=21)		Atlantic salmon (n=10)	
	Number infected (prev.)	Abund. (range)	Number infected (prev.)	Abund. (range)	Number infected (prev.)	Abund. (range)	Number infected (prev.)	Abund. (range)
0	34 (100.0)	5.9 (1-22)	42 (100.0)	30.6 (2-108)	21 (100.0)	10.7 (5-21)	10 (100.0)	25.4 (5-53)
1	21 (61.8)	2.0 (0-20)	25 (59.5)	5.4 (0-40)	11 (52.4)	0.8 (0-3)	10 (100.0)	29.6 (6-66)
2	10 (31.3)	1.1 (0-18)	11 (26.2)	0.8 (0-11)	1 (4.8)	0.1 (0-2)	10 (100.0)	33.8 (9-75)
3	5 (14.8)	0.6 (0-13)	5 (11.9)	0.2 (0-3)	0 (0)	0 (0)	10 (100.0)	40.2 (14-90)
4	5 (14.8)	0.3 (0-5)	2 (4.8)	0.05 (0-1)			10 (100.0)	48.4 (20-109)
5	5 (14.8)	0.3 (0-3)	1 (2.4)	0.02 (0-1)			10 (100.0)	56.8 (24-128)
6	2 (6.0)	0.1 (0-3)	0 (0)	0 (0)			10 (100.0)	64.4 (24-147)
7	2 (6.0)	0.1 (0-2)					10 (100.0)	70.2 (24-165)
8	0 (0)	0 (0)					10 (100.0)	76.5 (25-176)

the nine-spined sticklebacks and the flounders which rested on the substratum with the body. Direct substrate contact of the host promotes indirect transfer of detached parasites from the substratum, as well as increasing the contact rate with the bottom-dwelling salmon parr (and the cut-off fins during the experimental infection procedure). The difference in parasite attachment between the fish species may, however, also be due to stochastic varia-tion in the number of *G. salaris* specimens used for exposure during the 24 hours infection procedure.

The present experiments demonstrate that all three host species might become infected by *G. salaris* when migrating into rivers harbouring infected Atlantic salmon, as observed by Mo (1987) concerning flounder, and thus potentially function as transport hosts. However, no parasite reproduction was observed, and the short period (3-8 days) until elimination of the parasites demonstrates that the three fish species are innate resistant to *G. salaris*. The factors that efficiently prevent prolonged attachment and reproduction of *G. salaris* on these, and other innate resistant fish species, are at present unknown. However, results by Harris et al. (1998) indicate that complement may be a factor in host resistance to *G. salaris*. In the present study, the three- and nine-spined sticklebacks were infected with a relatively low number of gyrodactylids on the body surface when caught. There may be a possibility for cross-immunity interactions with *G. salaris* in accordance with the results presented by Richards and Chubb (1996) who demonstrated that the primary host response of guppies (*Poecilia reticulata* Peters) to *Gyrodactylus* was not species-specific. As the infection increased continuously on the control salmon during the experimental period, water chemistry was not jugded to have influenced our present results.

The present results are in accordance with previous laboratory experiments on the susceptibility and resistance of other non-salmonids to *G. salaris*, such as the minnow (*Phoxinus phoxinus* (L.)) (Bakke and Sharp 1990), European eel (*Anguilla anguilla* (L.)) (Bakke et al. 1991), roach (*Rutilus rutilus* (L.)), brook lamprey (*Lampetra planeri* (Bloch)), and perch (*Perca fluviatilis* L.) (Bakke et al. 1990), where parasite attachment but no reproduction were observed, leading to parasite elimination after 2 to 8 days. Although of no significant importance concerning the population dynamics of *G. salaris* in freshwater systems, the flounder, three- and nine-spined stickleback may function as potential transport hosts. They may therefore theoretically play a part in dispersal of *G. salaris* in nature.

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