Variation in host preference within *Gyrodactylus salaris* (Monogenea): an experimental approach

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SUMMARY

The monogenean ectoparasite, *Gyrodactylus salaris* Malmberg, 1957, has had a devastating effect on wild Atlantic salmon (*Salmo salar*) since its introduction to Norway in the mid-1970s. In Lake Pålsbufjorden, southern Norway, upstream of the stretches of the River Numedalslågen with anadromous Atlantic salmon, a resident Arctic charr (*Salvelinus alpinus*) population has been reported to be infected with *G. salaris* which is viable in the absence of its normal host, the Atlantic salmon. Currently, there is no record of *G. salaris* infecting Atlantic salmon in the downstream sections of the River Numedalslågen. We studied experimentally the infectivity and reproductive capacity of *G. salaris* from Lake Pålsbufjorden on wild and hatchery-reared Atlantic salmon as well as on Arctic charr and rainbow trout (*Oncorhynchus mykiss*). Arctic charr and rainbow trout were moderately susceptible, whereas the Atlantic salmon stocks from River Numedalslågen and River Drammenselva were innately resistant to only slightly susceptible. Thus, the *G. salaris* from Arctic charr in Lake Pålsbufjorden is considered non-pathogenic to Atlantic salmon. This is the first observation of variation in host preference among Norwegian *G. salaris* populations. The observed differences in virulence between *G. salaris* populations could have important consequences for the international legislation and management of Atlantic salmon.

Key words: Atlantic salmon, *Salmo salar*, Arctic charr, *Salvelinus alpinus*, host specificity, infectivity.

INTRODUCTION

Monogenean gyrodactylids are ubiquitous ectoparasites on the skin and gills of teleost fishes (Bakke *et al*. 2002), with the most recent compilation listing 409 species (Harris *et al*. 2004). Although most *Gyrodactylus* species are considered non-pathogenic, this is not true for *Gyrodactylus salaris* Malmberg, 1957. After the first observation of *G. salaris* in Norway (Johnsen, 1978), this parasite has seriously hampered the natural, juvenile production of wild Atlantic salmon (*Salmo salar* L.) and caused great harm both in ecological and economical terms (Johnsen *et al*. 1999; Mo *et al*. 2004). Thus, it is not surprising that *G. salaris* is the best studied of the *Gyrodactylus* species.

Recent studies indicate that the original species description of *G. salaris* is no longer satisfactory for a proper management of gyrodactylosis. Ziętara and Lumme (2002) showed that the majority of *Gyrodactylus* species can be discriminated based on sequencing of the internal transcribed spacer (ITS) regions (ITS-1 and ITS-2) of the nuclear ribosomal DNA (rDNA). However, *G. salaris* cannot be differentiated from *G. thymalli* Žitnaň, 1960, parasitizing grayling (*Thymallus thymallus* L.) based on the ITS-1 and ITS-2 sequences (Cunningham, 1997). In fact, parasites from Atlantic salmon, grayling, and rainbow trout (*Oncorhynchus mykiss* (Walbaum)) share identical ITS-1 and ITS-2 sequences over a wide geographical range (Ziętara and Lumme, 2002). However, *G. salaris* and *G. thymalli* can be grouped into several well-supported clades based on the sequences of the cytochrome *c* oxidase subunit 1 (*cox1*) gene of the mitochondrial DNA (mtDNA) (Hansen *et al*. 2003, 2006; Meinilä *et al*. 2004). However, there was no support for the monophyly of either of the 2 species. Thus, groups based on host specificity or pathogenicity to Atlantic salmon, are paraphyletic with reference to *cox1* genealogy.

Despite *G. salaris* and *G. thymalli* being very closely related, their host-species preferences, as observed from laboratory infection experiments, are different (Soleng and Bakke, 2001; Bakke *et al*. 2002; Sterud *et al*. 2002). *G. salaris* is pathogenic to its host, the Atlantic salmon, whereas *G. thymalli* appears to be non-pathogenic to any known potential host (Soleng and Bakke, 2001; Bakke *et al*. 2002; Sterud *et al*. 2002).

The host preferences and specificity of *G. salaris* have been studied extensively (see Bakke *et al*. 2002) but, thus far, only little attention has been paid to possible variation in infectivity and reproductive potential among different populations of *G. salaris*.

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Nonetheless, Lindenstrøm *et al*. (2003) described a variant of *G. salaris* (Gx) exhibiting limited reproduction on Atlantic salmon. This variant, originating from a rainbow trout farm in Denmark, performed better on rainbow trout than on any of the experimentally infected Atlantic salmon stocks. A genetic characterization of this parasite based on the ITS region revealed 3 nucleotide substitutions (C<->T at position 276 in the ITS-1 and C<->T at position 911 and A<->T at position 1090 in the ITS-2) compared with the 'standard' *G. salaris* sequence (see Lindenstrøm *et al*. 2003).

Recently, Robertsen *et al*. (2006*b*) discovered a *Gyrodactylus* sp. parasitizing Arctic charr (*Salvelinus alpinus*) in Lake Pålsbufjorden, Buskerud County, southern Norway. This parasite was similar morphologically to *G. salaris*, and sequence data for the *cox1* revealed a haplotype which was identical to that found in *G. salaris* populations parasitizing Atlantic salmon in the neighbouring Rivers Drammenselva and Lierelva (Buskerud County) and Lærdalselva (Sogn og Fjordane County), as well as the rainbow trout in the Swedish Lake Bullaren (sequences from Hansen *et al*. 2003). Despite a difference of 1 nucleotide in the ITS-2 (G<->A at position 288) between *G. salaris* of the same *cox1* haplotype from Arctic charr and Atlantic salmon (Hansen *et al*. 2003), Robertsen *et al*. (2006*b*) concluded that this variant represented *G. salaris*.

The fish community in Lake Pålsbufjorden consists of Arctic charr, brown trout and minnow (*Phoxinus phoxinus*). Thus, this is the first observation of *G. salaris* maintaining a viable population in the absence of Atlantic salmon or other salmonids which are susceptible to *G. salaris* (see Jansen and Bakke, 1995; Bakke *et al*. 2002). Lake Pålsbufjorden drains into River Numedalslågen, in which the stretches holding anadromous Atlantic salmon end some 100 km downstream the outlet of the lake. Previously, observations of Arctic charr infected with *G. salaris* have been related only to river systems in which Arctic charr live in sympatry with Atlantic salmon which is also infected with *G. salaris* (see Mo, 1988; Knudsen *et al*. 2006, Kristoffersen *et al*. 2005). Arctic charr and rainbow trout (the latter species previously occurring in Lake Pålsbufjorden) are salmonid species susceptible to *G. salaris* from the River Lierelva (Bakke *et al*. 1990, 1991, 1996).

Gyrodactylosis of Atlantic salmon caused by *G. salaris* is a notifiable disease (OIE, 2004). Hence, a spread of the parasite downstream to salmon-populated, anadromous stretches of River Numedalslågen may have significant epidemiological and ecological consequences. Therefore, we studied the infectivity of *G. salaris* from Arctic charr from Lake Pålsbufjorden to Atlantic salmon and compared it with a known virulent *G. salaris* population from Atlantic salmon in the Lierelva/Drammenselva river complex (Bakke *et al*. 1990, 2002).

MATERIALS AND METHODS

Origin of the parasites

Adult Arctic charr infected with *G. salaris* were caught by overnight gillnetting in Lake Pålsbufjorden, Buskerud County and infected parr of Atlantic salmon were caught by electro-fishing in River Lierelva and the neighbouring River Drammenselva. The fish were transported alive to the laboratory in aerated plastic bags containing lake water. In the laboratory, the fish were kept in 200 l grey plastic tanks in charcoal-filtered tap-water (flow rate 2–4 l/min). Subsequently, the fish were anaesthetized in 0·04% chlorobutanol and killed by a blow to the head before the fins were cut off and examined for parasites in tap water under a stereomicroscope with fibre optic illumination. *G. salaris* from the adult Arctic charr were used directly, but *G. salaris* from the 2 stocks of Atlantic salmon parr were transferred to uninfected salmon parr and kept (in separate tanks) for >1 month prior to the start of the experiments to eliminate any accidental contamination with *G. derjavini*. As gyrodactylids are sensitive to host serum (see Buchmann, 1998; Harris *et al*. 1998), the water was changed several times between examinations for parasites and further experimental infection with the specimens. The *Gyrodactylus* specimens infecting Arctic charr and Atlantic salmon have been identified previously as *G. salaris* both by morphometry and sequencing of the ITS and intergenic spacer (IGS) regions of nuclear ribosomal DNA and the mitochondrial *cox1* (Robertsen *et al*. 2006*b*).

Origin of the fish hosts

Experimental infections were performed on wild Atlantic salmon from River Numedalslågen, Buskerud County, southeastern Norway (1·4–8·2 g; 6–10 cm). The fish were caught by electro-fishing and brought to the aquarium in aerated plastic bags. All Atlantic salmon individuals from River Numedalslågen were tested genetically at the Norwegian Institute for Nature Research (NINA, Trondheim) for the possibility of brown trout (*Salmo trutta* L.) or Atlantic salmon × brown trout hybrids among them, which may interfere with the interpretation of results (see Bakke *et al*. 1999), but there was no evidence of these fishes. Due to low numbers and general problems acclimatizing wild fish to aquarium conditions, 1 experimental set-up included hatchery-reared Atlantic salmon (1·5–9·4 g; 6–11 cm) from River Drammenselva, Buskerud County, southeastern Norway. Since Arctic charr and rainbow trout have immigrated and been stocked, respectively, in Lake Pålsbufjorden, representatives of both species were included in the experiments. Hatchery-reared Arctic charr (2·3–9·2 g; 7–11·5 cm) originated from Tallvik

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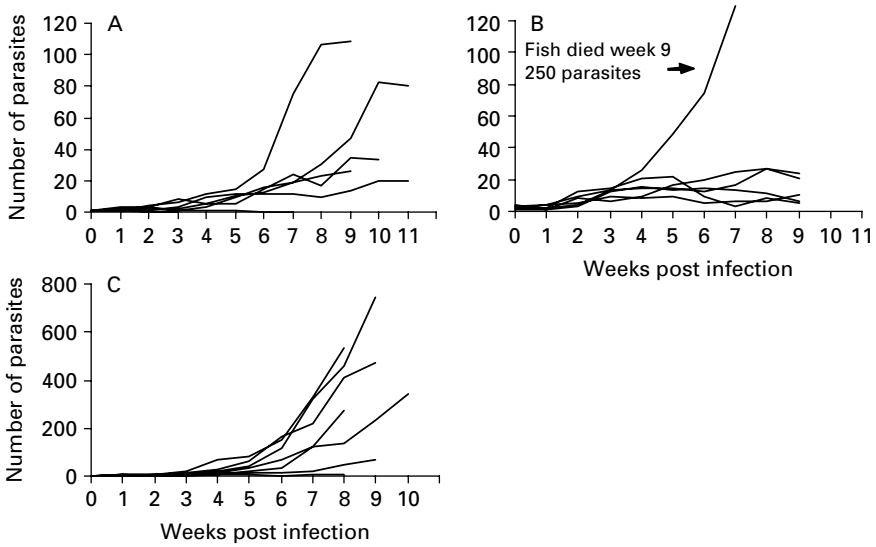

Fig. 1. (A–C) The course of *Gyrodactylus salaris* infection on individually isolated wild salmon from River Numedalslågen. The data are based on weekly counts of the parasites on the skin and fins of successfully infected fish. (A) Exp. I: infection with *G. salaris* from Arctic charr in Lake Pålsbufjorden. Hosts initially infected with 1 parasite ($n=9$ hosts). (B) Exp. I: infection with *G. salaris* from Arctic charr in Lake Pålsbufjorden. Hosts initially infected with 1–4 parasites ($n=6$ hosts). (C) Exp. II: *G. salaris* from Atlantic salmon in River Lierelva ($n=9$ hosts). The salmonid fish in Exps I and II were kept in separate tanks. Note the different scales of the ordinate.

settefiskanlegg, Alta, Finmark County, North Norway, and commercially reared rainbow trout (1·3–6·9 g; 5·5–9·5 cm) were supplied by Valdres ørretoppdrett, Røn, Buskerud County, southern Norway.

Infection experiments

The infection experiments were performed in the aquarium facilities in the Department of Zoology, Natural History Museum, University of Oslo. All fish were acclimatized for >1 month to the laboratory conditions prior to the commencement of the experiments. The fish were infected individually with 1–4 specimens (Experiment I, see below) or 1 specimen (Experiments II–V, see below) of *G. salaris*. For the initial infection with 1 parasite, the flukes were induced to attach to the tip of an insect pin before being moved to attach on to the bottom of a glass Petri dish. The parasites were then presented to the caudal fin of an anaesthetized fish, to which they frequently attached spontaneously. Extreme care was taken to prevent any contamination of parasites with host body fluids. For the initial infection with >1 *G. salaris*, the hosts were infected through the exposure to fins from infected, adult Arctic charr. After being infected, individual hosts were kept isolated in grey floating plastic boxes ($20\times10\times10$ cm) with wire-mesh bottoms to ensure a free flow of fresh water into the boxes. Infections were considered successful if 1 or more worms were present on the host 2 days post-infection (p.i.). The course of infection was monitored on a weekly basis (up to 11 weeks) by counting all of the parasites on the skin and fins of infected fish. For this examination, fish were anaesthetized in 0·04% chlorbutanol. The water temperature ranged from 7 to 12 °C during the experimental period. The fish hosts were under daily supervision, and dead fish were removed immediately.

Experimental protocol

Five different experiments were carried out.

Exp. I (*Fig. 1A and B*). Wild Atlantic salmon from River Numedalslågen were each infected with *G. salaris* from Lake Pålsbufjorden. Originally, 21 fish were attempted to be infected with 1 *G. salaris* each. Due to the low number of fish available and low establishment of infection, hosts not successfully infected were attempted to be re-infected. Also, 8 fish were initially infected with ⩾1 parasite, as described above (hosts were previously naïve). The experiment was conducted during 11 weeks.

Exp. II (*Fig. 1C*). Wild Atlantic salmon ($n=13$) from River Numedalslågen were each infected with 1 *G. salaris* from River Lierelva, as a control group for Exp. I. The *G. salaris* in River Lierelva is a known pathogen of Atlantic salmon (see Johnsen *et al.* 1999). This experiment was conducted for 10 weeks in a separate tank in parallel with Exp. I.

Exp. III (*Fig. 1A–C*). Hatchery-reared Atlantic salmon from Drammenselva (duplicated, $n=18$ and 17), Arctic charr ($n=18$) from Alta and rainbow trout ($n=18$) from Røn were each infected with 1 *G. salaris*

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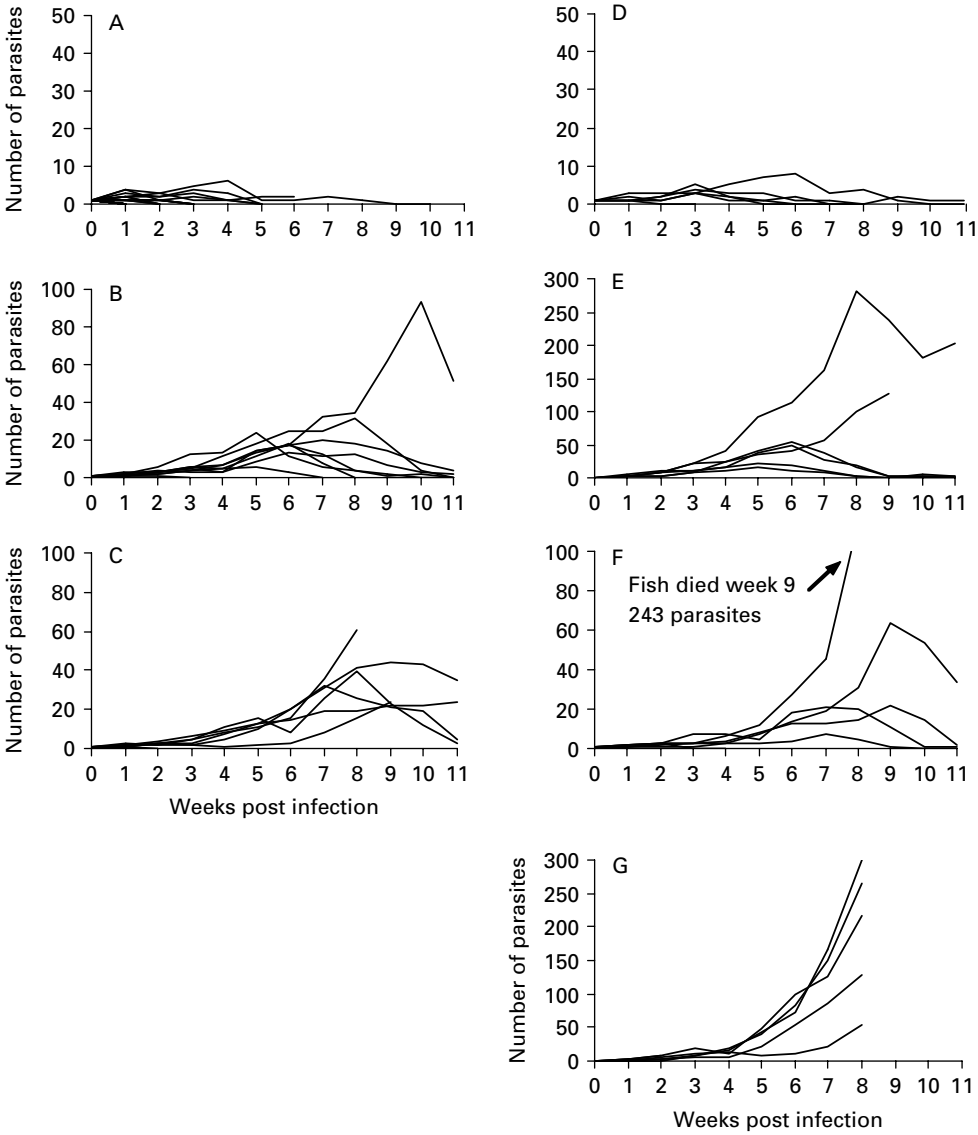

Fig. 2. (A–G) The course of *Gyrodactylus salaris* infection from Arctic charr in Lake Pålsbufjorden (A–F) and from Atlantic salmon in River Drammenselva (G) on individually isolated salmonids, in separate tanks (A–C, Exp. III and G, Exp. V) and in 1 common tank (D–F, Exp. IV), infected with 1 parasite each. The data are based on weekly counts of the parasites on the skin and fins of the successfully infected fish. (A) *G. salaris* on Atlantic salmon ($n=13$) of the Drammenselva stock. (B) *G. salaris* on Arctic charr ($n=9$) of the Tallvik stock. (C) *G. salaris* on rainbow trout ($n=7$) from Valdres commercial farm. (D) *G. salaris* on Atlantic salmon ($n=6$) of the Drammenselva stock. (E) *G. salaris* on Arctic charr ($n=6$) of the Tallvik stock, (F) *G. salaris* on rainbow trout ($n=6$) of the Valdres stock. (G) *G. salaris* on Atlantic salmon ($n=6$) of the Drammenselva stock. Note the different scales of the ordinate.

from Arctic charr from Lake Pålsbufjorden and kept in 3 separate tanks.

Exp. IV (*Fig. 2D–F, Table 1*). Hatchery-reared Arctic charr ($n=6$), rainbow trout ($n=6$) and Atlantic salmon ($n=6$) were each infected with 1 *G. salaris* from Arctic charr from Lake Pålsbufjorden and kept individually in the same tank in small boxes.

Exp. V (*Fig. 2G*). Hatchery-reared Atlantic salmon ($n=6$) from the same stock as used previously (River Drammenselva) were included as a control group and each was infected with 1 *G. salaris* from River Drammenselva. The *G. salaris* in River Drammenselva is a known pathogen to Atlantic salmon (see Johnsen *et al.* 1999). This experiment was conducted simultaneously with Exp. III.

Statistical test

In an attempt to deduce the analytical power of the timing of the peak-points along the trajectories of infection intensities, we tested whether the peak points were locally statistically significant. A statistical method was employed for all host-parasite combinations, testing whether pairwise consecutive sampling points were equal or not. Tests for significant differences between pairwise similar

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sampling points (week) in Exps III and IV were conducted for each of the 3 host species, in order to reveal any effects of the experiments being carried out in separate tanks. Additionally, we tested for statistically significant differences along the trajectories of infection intensity between experimental groups of salmon and their respective controls (Exp. III *vs*. Exps V and IV *vs*. Exp.V). In all statistical tests, the method used was Kruskal-Wallis one-way analysis of variance, run in SYSTAT, ver. 10 (©SPSS Inc.).

RESULTS

The establishment success of *G. salaris* from Arctic charr in Lake Pålsbufjorden on Atlantic salmon from Rivers Numedalslågen and Drammenselva as well as on Arctic charr and rainbow trout was limited. Total average successful establishments from Exps III–IV, were 38·0, 48·5 and 30·5% on Atlantic salmon, Arctic charr and rainbow trout, respectively. Unexpectedly, the pathogenic *G. salaris* from Drammenselva established successfully on Atlantic salmon from River Drammenselva (Exp. V) in only 33·5% of all transfer attempts of the parasite. A number of fish died during the run of the experiments. The cause of death is unknown, but is assumed to not be related to *Gyrodactylus* infection, as numbers of parasites on dead fish were relatively low (maximum number of parasites registered on a dead host: 250).

Exp. I (*Fig. 1A, B*)

In total, 37 attempts were made to infect Atlantic salmon from River Numedalslågen with *G. salaris* from Lake Pålsbufjorden. Nine infections (24·3%) were successful. Of these 9 infections, 3 were successful on a first attempt, whereas 5 needed 2 and 1 needed 3 attempts. Reproduction was observed for 8 (88·9%) of the 9 successfully infected fish; this includes 2 host fish with parasite reproduction limited to 1 birth (Fig. 1A). The highest observed intensities from this experimental set-up were 109 and 82 parasites before apparently levelling off. The maximum intensity of infection on the other fish did not exceed 40 parasites. Four host fish were able to eliminate the infection during the experimental period. Attempts were made to infect Atlantic salmon from River Numedalslågen with >1 *G. salaris* from Lake Pålsbufjorden (once for each host). Six of 8 fish (75·0%) were successfully infected. Reproduction of *G. salaris* was observed on all successfully infected fish. However, the infrapopulations did not exceed 20 parasites, except on 1 fish with an intensity of 250 parasites at week 9 (Fig. 1B). None of these infections were eliminated during the course of the experiment.

Exp. II (*Fig. 1C*)

In the control group with wild Atlantic salmon from River Numedalslågen infected with *G. salaris* from River Lierelva, 9 of the 18 (50·0%) salmon were successfully infected. In this tank, reproduction was observed on all but 1 of the successfully infected salmon. All but this single salmon with a maximum of 6 parasites during the experimental period, were highly susceptible. The maximum number of parasites ranged from 70 to 742 by week 10 (Fig. 1C).

Exp. III (*Fig. 2A–C*)

Of the 18 hatchery-reared Atlantic salmon, 13 (72·2%) were each infected successfully with 1 *G. salaris* from Lake Pålsbufjorden (Fig. 2A). Reproduction was observed for 6 (46·2%) of these 13 fish, including 2 fish with reproduction limited to 1 birth. The parasite numbers increased for up to 4 weeks p.i. The highest intensity of infection observed was 6 parasites. All 13 *G. salaris* infrapopulations were eliminated by 8 weeks. One infected salmon died during the experiment. Of the 18 Arctic charr, 9 (50%) were successfully infected (Fig. 2B). Of these 9 fish, reproduction of *G. salaris* from Lake Pålsbufjorden was observed on 8 (88·9%). The peak intensity of infection was 93 parasites. The parasite infrapopulations increased in number from 5 to 10 weeks p.i. On 6 of the 9 (66·7%) successfully infected Arctic charr the parasite infrapopulations were eliminated during the experimental period of 11 weeks. Seven of the 18 (38·9%) rainbow trout were successfully infected with 1 *G. salaris* from Lake Pålsbufjorden (Fig. 2C). Reproduction was observed for 6 (85·7%) established infections. The peak intensity of infection was 61 parasites. An established infection was not eliminated during the course of the experiment. The parasite infrapopulations increased in number in 4 rainbow trout 7 weeks p.i. Two of the rainbow trout died during the experiment.

Exp. IV (*Fig. 2D–F, Table 1*)

The course of the infection of *G. salaris* from Lake Pålsbufjorden on individually isolated, hatchery-reared Atlantic salmon from Drammenelva ($n=6$), Arctic charr ($n=6$) and rainbow trout ($n=6$) in a common tank is shown in Fig. 2D–F. One salmon was considered to be innately resistant; for the others, the infection was eliminated by week 9. During the experimental period of 11 weeks, 2 Arctic charr and 1 rainbow trout eliminated their infections. The time of peak for the intensity of infection varied between host species: on salmon, 3–6 weeks p.i. (Fig. 2D), on Arctic charr and rainbow trout, 5–8 and 7–9 weeks p.i., respectively (Fig. 2E and F). The maximum numbers of parasites during the entire

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Table 1. Mean, median, maximum (Max) and minimum (Min) observations and number of infected fish (*n*) in weekly counts on Atlantic salmon, Arctic charr and rainbow trout successfully infected with *Gyrodactylus salaris* from Arctic charr in Lake Pålsbufjorden

The fish were initially infected with 1 parasite and subsequently kept individually isolated in a common tank (Exp. IV). These data are based on weekly counts of the parasites on the skin and fins of the fish.

	Week:	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Salmon	Mean	1·0	1·5	1·8	3·6	2·6	3·0	3·7	2·0	2·5	1·5	1·0	1·0
	Median	1·0	1·0	2·0	3·0	2·0	2·0	2·0	2·0	2·5	1·5	1·0	1·0
	Max	1	3	3	5	5	7	8	3	4	2	1	1
	Min	1	1	1	3	1	1	1	1	1	1	1	1
	n	6	6	5	5	5	4	3	2	2	2	1	1
Arctic charr	Mean	1·0	3·3	6·3	13·2	22·2	40·7	47·8	50·3	70·3	61·8	47·8	70·0
	Median	1·0	3·0	7·0	11·0	20·0	37·0	45·0	32·5	16·5	2·5	4·5	4·0
	Max	1	5	10	22	41	93	114	161	282	237	180	204
	Min	1	2	2	7	11	15	11	8	2	1	2	2
	n	6	6	6	6	6	6	6	6	6	6	4	3
Rainbow trout	Mean	1·0	1·5	2·5	3·4	4·6	7·0	15·2	21·0	37·2	68·2	23·3	12·3
	Median	1·0	1·5	3·0	3·0	4·0	7·0	14·0	19·0	20·0	22·0	15·0	2·0
	Max	1	2	3	7	7	12	27	45	115	243	54	34
	Min	1	1	1	1	3	3	4	7	5	1	1	1
	n	6	6	6	5	5	5	5	5	5	5	3	3

experimental period were 8, 282 and 243 for Atlantic salmon, Arctic charr and rainbow trout, respectively. One Arctic charr and 1 rainbow trout died during the experiment.

Exp. V (*Fig. 2G*)

In the control group with hatchery-reared Atlantic salmon from Drammenselva infected with *G. salaris* from Drammenselva, 6 of the 18 (33·3%) salmon were successfully infected. In this tank, reproduction was observed on all 6 infected salmon. The salmon were highly susceptible to the infection, and the size of the parasite infrapopulations increased continuously, albeit at different rates on individual fish (Fig. 2G). One salmon died during the experiment.

Substantial variation in susceptibility was recorded among the host individuals. The time lapse to an acquired response could not be specifically determined, as there were no statistically significant differences in mean parasite intensity between any 2 consecutive monitoring points for any of the infection trajectories in Exps I–V (Kruskal-Wallis one-way analysis of variance, $P>0{\cdot}05$). The influence of a 'tank effect' might be present, as the parasite intensities of infection for Arctic charr were significantly different ($P<0{\cdot}05$) in the weeks 1–6 between Exps III and IV. In similar tests for Atlantic salmon and rainbow trout, there were no significant differences ($P>0{\cdot}05$). Infection intensity among salmon in Exp. I was significantly different (Kruskal-Wallis one-way analysis of variance, $P<0{\cdot}05$) from the control in Exp. II from week 5 and throughout the experiment. Infection intensity among salmon in Exp. III was significantly different (Kruskal-Wallis one-way analysis of variance, $P<0{\cdot}05$) from the control in Exp. V from week 2 and throughout the experiment. For Exp. IV, the infection trajectory was significantly different ($P<0{\cdot}05$) from the control group in Exp. V from week 3.

DISCUSSION

The high pathogenicity of *G. salaris* from the Rivers Lierelva and Drammenselva on Atlantic salmon from the same rivers is well documented (see e.g. Johnsen *et al.* 1999). By contrast, *G. salaris* from Arctic charr in Lake Pålsbufjorden displayed (in general terms) a very restricted reproduction on hatchery reared Atlantic salmon parr from Drammenselva and also on wild Atlantic salmon parr from Numedalslågen, despite individual variation. Therefore, we consider *G. salaris* from Arctic charr in Lake Pålsbufjorden to be non-pathogenic to Atlantic salmon, at least from Drammenselva and Numedalslågen. The apparent differences in susceptibility between the 2 experimental groups, most likely reflect the fact that the former were hatchery-reared, whereas the latter from Numedalslågen represented a wild population, and thus would probably have been more stressed under laboratory conditions. This *G. salaris* population was not pathogenic to the Arctic charr or the rainbow trout stocks, although both appeared susceptible and suitable hosts. Based on these findings, the susceptibility of the 3 host species tested may be summarized as follows: Atlantic salmon was slightly susceptible, whereas Arctic charr and rainbow trout were moderately susceptible.

In recent years, a particular region of *cox1* (~800 bp) has been sequenced for *G. salaris* and *G. thymalli* from >30 different geographical populations in Europe. A number of haplotypes have

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been identified (Hansen *et al*. 2003, 2006; Meinilä *et al*. 2004) which group into 3–6 distinct clades. The sequence data supported 3 independent introductions of *G. salaris* into Norway (Hansen *et al*. 2003). Robertsen *et al*. (2006*b*) reported that the *cox1* sequence of *G. salaris* on Arctic charr in Lake Pålsbufjorden was the same as that of *G. salaris* recovered previously from Atlantic salmon from the Rivers Drammenselva, Lierelva and Lærdalselva in Norway as well as on rainbow trout from a hatchery in the Swedish Lake Bullaren (annotated clade III, haplotype F by Hansen *et al*. 2003). Additional sequence data for the ribosomal IGS and comparative morphometry of the haptoral hard parts of the parasites also identified this parasite as *G. salaris* (see Robertsen *et al*. 2006*b*).

Lindenstrøm *et al*. (2003) reported a *G. salaris* variant (Gx) from farmed Danish rainbow trout, which was non-pathogenic to Atlantic salmon in infection experiments. This observation may raise the question as to the relatedness of Gx and the *G. salaris* discovered in Lake Pålsbufjorden. However, the ITS sequence of the *G. salaris* variant described by Lindenstrøm *et al*. (2003) differs by 3 nucleotide substitutions from the *G. salaris* consensus sequence. Robertsen *et al*. (2006*b*) reported a single nucleotide substitution in the ITS-2 for the *G. salaris* parasites from Arctic charr in Lake Pålsbufjorden; this equates to a total of 4 nucleotide substitutions when the ITS sequences of Gx are compared with those of *G. salaris* from Lake Pålsbufjorden. More recently, another Danish *G. salaris*, which also had a low pathogenicity to salmon, has been discovered (Thomas Jørgensen and Kurt Buchmann, personal communication). It seems that *G. salaris* which are non-pathogenic to Atlantic salmon may occur more frequently than previously thought.

Halvorsen and Hartvigsen (1989) suggested a list of possible *scenarios* regarding *G. salaris* in Norway. One of these *scenarios* was that *G. salaris* is endemic in Norway. Currently, the introduction of different pathogenic *G. salari*s strains to Norway by several routes is unquestioned. Hansen *et al*. (2003) proposed at least 3 independent introductions on the basis of *cox1* sequence data. However, the proposal of endemism of a benign *G. salaris* parasitizing an inland population of Arctic charr cannot be rejected, but historical events strongly support, also in this case, the hypothesis of a relatively recent introduction of the species. Originally, Arctic charr was introduced into the Numedalslågen river system from Lake Tinnsjøen (Aass, 1970), a lake in which Arctic charr infected with *G. salaris* has not yet been found (Robertsen *et al*. 2006*a*). However, rainbow trout has been introduced directly into several lakes connected to the Numedalslågen river system, including Lake Pålsbufjorden in the early 1960s. The rainbow trout originated from Denmark but came to Pålsbufjorden *via* various Norwegian hatcheries and farms (Per Aass, personal communication). As *G. salaris* has been shown previously to have a relatively wide host range among salmonids (Bakke *et al*. 2002) and host switches are considered common for species of *Gyrodactylus* (see Ziętara and Lumme, 2002; Huyse and Volckaert, 2002, 2005), it is reasonable to assume that *G. salaris* has been transferred to Arctic charr in this area through infected rainbow trout. Subsequently, the *G. salaris* may have adapted to the particular ecology of Arctic charr after rainbow trout disappeared from the lake (Robertsen *et al*. 2006*b*). *G. salaris* in the Rivers Drammenselva and Lierelva has also most likely been introduced *via* rainbow trout but, in this case, from Sweden (Mo, 1991; Hansen *et al*. 2003). Both *G. salaris* populations have the *cox1* haplotype F which is commonly found on rainbow trout in farms throughout Fennoscandia (Meinilä *et al*. 2004).

Employing *cox1* sequence data sets, several well supported clades of both *G. salaris* and *G. thymalli* (based on mitochondrial haplotypes) have been described, but there is no support for the monophyly of all haplotypes of either species (Hansen *et al*. 2003, 2006; Meinilä *et al*. 2004). However, host preference and host specificity vary significantly between at least some of the populations of *G. salaris* and *G. thymalli* (see Soleng and Bakke, 2001; Bakke *et al*. 2002; Sterud *et al*. 2002). Sterud *et al*. (2002) considered these biologically significant differences important when concluding that *G. salaris* and *G. thymalli* should remain as 2 distinct species. The present results indicate that the *G. salaris* population in Lake Pålsbufjorden is non-pathogenic to Atlantic salmon, and, importantly, Arctic charr and rainbow trout seem to be more suitable as hosts. The present results indicate that host specificity is no longer a striking argument for considering *G. salaris* and *G. thymalli* as 2 species. Previous experiments (unpublished data) indicate that *G. thymalli* from grayling, the preferred host of *G. thymalli*, can also reproduce on Arctic charr; the reciprocal experiment of testing *G. salaris* from Arctic charr on grayling needs to be conducted.

Gyrodactylosis is probably one of the most important threats to wild European Atlantic salmon populations, and *G. salaris* is listed by the 'Office International des Épizooties' (OIE) in the Aquatic Animal Code (OIE, 2004). Several countries have taken precautionary measures to avoid the introduction of the worm. Accordingly, the European Commission (EC) has restricted the import of salmonids that are susceptible to *G. salaris* to areas with an equivalent health status (Peeler *et al*. 2006). In Norway, comprehensive and expensive counter measures, such as, for example, rotetone treatment programs for entire river systems, have been launched to control epidemics and to eliminate *G. salaris* infection from wild salmon. The present observations of differing host preferences and

reproduction potential between different populations of *G. salaris* to Atlantic salmon is important for the design and implementation of management strategies and international legislation. Of paramount importance is that the identification of *G. salaris* based on any presently used molecular markers or morphology alone is not sufficient for a diagnosis of gyrodactylosis. Another key aspect in a management context is the apparent ease with which species and strains closely related to *G. salaris* undergo host switching with subsequent reproduction. Frequent host switches could ultimately be an avenue for a significant expansion of the geographical range of pathogenic variants of *G. salaris*.

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