Arctic charr (*Salvelinus alpinus* (L.)), an adequate host to *Gyrodactylus salaris* (Monogenea)

Anja Celine Winger

A dissertation for the degree of Philosophiae Doctor

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Spring 2009

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List of papers

The thesis is based on the following four papers. They will be listed in the text by their Roman numerals

I.   Winger, A. C., Kanck, M., Kristoffersen, R. & Knudsen, R. 2008. Seasonal dynamics and persistence of *Gyrodactylus salaris* in two riverine anadromous Arctic charr populations. *Environmental Biology of Fishes* **83**, 117-123

II. Winger, A. C., Kristoffersen, R., Siikavuopio, S. I. & Knudsen, R 2009. Experiments to test if allopatric *Salvelinus alpinus* (L.) are suitable year-round hosts of *Gyrodactylus salaris* (Monogenea). *Journal of Fish Biology* **74,** 1-11. doi:10.1111/j.1095-8649.2009.02215.x

III. Winger, A. C., Primicerio, R., Kristoffersen, R., Siikavuopio, S. I. & Knudsen, R. (2008) *Gyrodactylus salaris* infecting allopatric Arctic charr fry: an experimental study of host survival. *Journal of Fish Biology* **73**, 1-12

IV. Winger, A. C., Knudsen, R., Primicerio R. & Kristoffersen R. Infrapopulation dynamic and the role of transmission in the hyperviviparous *Gyrodactylus salaris* (Monogenea) (*Manuscript*)

Acknowledgements

Summary

The Arctic charr (*Salvelinus alpinus* (L.)) has a circumpolar distribution where it often lives in sympatry with Atlantic salmon (*Salmo salar* L.). The Monogenean parasite *Gyrodactylus salaris* is pathogenic to many Atlantic salmon stocks and its introduction into Norway has driven several Atlantic salmon populations to extinction. Previous studies have reported *G. salaris* infections in Arctic charr, and its role as long-term host to the parasite is highly important. In Troms County in northern Norway, two rivers are infected with *G. salaris*. These two rivers, the River Skibotnelva and the River Signaldalselva, are the northernmost *G. salaris* infected rivers in Norway. Both rivers contain Arctic charr and Atlantic salmon, but the natural salmon populations are regarded as extinct or close to extinction owing to the parasite. In order to eradicate the parasite, the River Skibotnelva has twice been treated with the plant poison rotenone. These treatments have failed, and an ongoing hypothesis is that *G. salaris* did survive on Arctic charr that escaped the rotenone treatment by staying in untreated ponds and marshes connected to the river.

In this thesis, the main aim is to illuminate the role of Arctic charr as a fully adequate long-term host to *G. salaris*. Both ecological and experimental studies have been undertaken in order to study the interaction between Arctic charr and *G. salaris*. The main results show that *G. salaris* has a seasonal occurrence, with an autumn high and a spring low, on Arctic charr in the two rivers Skibotnelva and Signaldalselva. These seasonal fluctuations are largely explained by temperature-dependent constraints on parasite reproduction. It is, however, likely that the immune response in individual hosts' also is a determining factor. It was found that Arctic charr in allopatry can sustain a *G. salaris* population during the five months of winter with low temperatures, and that newly-hatched charr fry are highly susceptible to the parasite. A population of *G. salaris,* introduced once to the host, did survive and reproduce on charr. Given that new susceptible hosts were added to the host population, the *G. salaris* population sustained and survived from early May until termination of the experiment in November. Significant mortality, owing to the parasite, was observed in newly hatched charr fry. Thus, *G. salaris* may have the ability to act as a selective force by increasing the resistance in infected charr populations. Further, we found that parasite transmission is frequent within the charr fry population during the entire colonisation process. The parasite metapopulation examined was at all times over-dispersed in the host population but, owing to transmission, the heaviest loads of parasites fluctuated among individual hosts over time.

Hence, transmission probably is an important process in determining the infrapopulation structure by reducing the over-dispersion of *G. salaris* between hosts, and may contribute to a prolonged period of growth of the parasite metapopulation.

G. salaris infections were registered for the first time in the rivers Skibotnelva and Signaldalselva in 1979 and 2000 respectively. It seems that Arctic charr in the River Skibotnelva are less susceptible than charr in the neighbouring River Signaldalselva. This is possibly an effect of parasite-induced mortality in the charr fry, and it could be that Arctic charr in the River Skibotnelva have, over the last thirty years, developed an increased resistance to *G. salaris*. Nevertheless, the parasite seems to survive and reproduce on Arctic charr in both rivers. We found that Arctic charr fry are highly susceptible and most probably play a crucial role in the parasites' lifecycle. There was evidence of a continuous transmission of parasites among infected charr fry. This factor is assumed to prolong the growth period of the parasite metapopulation, and to increases the number of parasites in the population. The thesis clearly demonstrates that Arctic charr have to be recognised as an adequate host to *G. salaris* and that not only Atlantic salmon, but also Arctic charr have to be removed completely from infected rivers in order to eliminate the parasite.

1. Introduction

1.1 General introduction

In 1975, *Gyrodactylus salaris* Malmberg, 1957 (Monogenea) was accidentally introduced to a fish farm on the west coast of Norway. It was introduced with parr from the Baltic strain of Atlantic salmon (*Salmo salar* L.) that were imported from Sweden for aquaculture production in Norway (Johnsen & Jensen, 1991). Then in 1978, a vehicle transporting salmon smolt to a salmon hatchery dumped infected salmon in the River Skibotnelva in northern Norway, and in 1979, for the first time, *G. salaris* was registered on salmonids in the river (Heggberget & Johnsen, 1982; Mo, 1994). It transpired that most Norwegian strains of Atlantic salmon are unable to mount a sufficient immune response to *G. salaris* (Bakke, 1991; Johnsen & Jensen, 1991; Johnsen *et al.*, 1999), and the introductions led to a devastating epidemic in the infected salmon populations. To provide insight into the invasion history of *G. salaris* in Norway, Hansen *et al.* (2003) analysed the cytrocrome oxidase I (COI) gene from 32 populations of *G. salaris* and its sibling species *G. thymalli* (infecting grayling (*Thymallus thymallus* (L.))). The genetics confirmed a multiple invasion of Norway by *G. salaris*, and the authors could divide the *G. salaris* populations in Norway into three well-defined clades with six haplotypes.

Compared with most other gyrodactylids, *G. salaris* has a rather wide host range (Bakke *et al.*, 2002; Harris *et al.*, 2004). In addition to salmon, it can survive and reproduce on five other salmonid species (Bakke *et al.*, 2002), and among them are Arctic charr (*Salvelinus alpinus* (L.)). Arctic charr have a circumpolar distribution where by they often live in sympatry with Atlantic salmon (Heggberget, 1981; Klemetsen, 1987). The two rivers, Skibotnelva and Signaldalselva, are the two northernmost *G. salaris* infected rivers in Norway. Both rivers are inhabited by sympatric populations of Arctic charr, Atlantic salmon and brown trout (*Salmo trutta* L.) (Heggberget, 1981; Klemetsen, 1987; Kristoffersen *et al.*, 2005; Knudsen *et al.*, 2007). Brown trout generally carry low numbers of *G. salaris* and are regarded as innately resistant (Bakke, 1991; Bakke *et al.*, 2002). As a direct consequence of the *G. salaris* epidemic, the salmon populations in the rivers Skibotnelva and Signaldalselva are regarded as extinct or close to extinction. Nevertheless, *G. salaris* is present in both rivers, where they infect both Arctic charr and the few remaining salmon (Mo, 1988; Kristoffersen *et al.*, 2005; Knudsen *et al.*, 2007; Robertsen *et al.*, 2007). This thesis focuses on the importance

of the interactions between *G. salaris* and Arctic charr in general and between Arctic charr fry and *G. salaris* in particular.

1.2 The genus Gyrodactylus

Gyrodactylids belong, according to Boeger & Kritsky (1993), to the class Monogenoidea. Monogeneans are hermaphroditic flatworms that are mainly external parasites of vertebrates, particularly fish. The order Gyrodactylidea consists of four families, whereas the family Gyroadctylidae consists of 30 genera, seven oviparous and 23 viviparous (hyperviviparous) (Bakke *et al.*, 2007). This not only makes them the most diverse genus within the family, but also one of the most diverse groups within the Monogenoidea in general (Boeger & Popazoglo, 1995). Hyperviviparity defines an animal that contains a developing embryo within its body cavity, which in turn contains another young embryo at an earlier stage of development (Cohen, 1977). This mode of reproduction is unique within the animal kingdom (Harris, 1993) and gives the gyrodactylids the possibility of rapid population growth on their host. Viviparous gyrodactylids have a direct lifecycle and, unlike all other Monogeneans, they have the ability to transfer to a new host at all times during their lifecycle. Studies on immunological processes in gyrodactylid-infected fish have demonstrated that most fish acquire immunological protection against gyrodactylids (Scott & Robinson, 1984; Bakke *et al.*, 1990; Cable *et al.*, 2000). This response has been shown to be non-specific (Lindenstrom & Buchmann, 2000), and it is assumed to start to inhibit parasite population growth at the onset of infection (Bakke *et al.*, 2002). The non-specific immune response from the host is, by some authors, assumed to be site-specific, forcing the parasite to migrate on its host away from immune-active sites (van Oosterhout *et al.*, 2007). A typical non-pathogenic gyrodactylid infection will, when introduced to a responding host, or host population, initially increase, whereupon the parasite population growth will decrease and become negative so that the infection will, after some time, be eliminated (Bakke *et al.*, 2007). However, studies on guppies (*Poecilia reticulata,* Peters 1859) has showed that fish that has been infected once, seem able to better control a secondary infection (Cable & van Oosterhout, 2007b). This means that some fish host are able to mount an acquired immune response, a response that must revolve around genes of specific immune responses, like the MHC gene. Recently it has been found that Baltic salmon can activate relevant genes in fin tissue when responding to *G. salaris* (Kania *et al.*, 2007), which in turn, makes the immune response depending on the fish's previous infection history.

1.3 Gyrodactylus salaris *in Norway and Troms County*

In Norway, the authorities` attempts to eliminate the pathogen *G. salaris* from infected watercourses have been a mixed success (Johnsen *et al.*, 2008). So far, 46 rivers have been infected in Norway, and 35 out of these have been treated with the plant poison rotenone (http://hgic.clemson.edu/factsheets/HGIC1713.htm), with the result that 18 are now free from infection with *G. salaris.* According to the Norwegian Directorate for Nature Management, the yearly estimated economic loss owing to the infection in Norway is 200 to 250 million NKR. Lately, alternative treatment with acid aluminium has been tried, but so far without success (Poleo *et al.*, 2004; Johnsen *et al.*, 2008). Since the introduction of *G. salaris,* the River Skibotnelva has been treated with rotenone twice. The first time was in August 1988, but the parasite was again recorded on salmon in 1992. The second time was in the summer of 1995, but again infected salmon were discovered three years later. In 2000, *G. salaris* was also recorded on salmon in the neighbouring River Signaldalselva, and it was probably infected by fish migrating from the River Skibotnelva. The former river has never been treated in any way. The two watercourses are situated in the same fjord, Storfjorden, which constitutes the inner part of Lyngenfjorden. There are about 23 km between the two watercourses, and fish can easily move between them. In order to close the rivers to anadromous fish, the Norwegian Directorate for Nature Management (http://www.dirnat.no/content.ap?thisId=500013135) has suggested building long-term fish fences in both the River Skibotnelva and the River Signaldalselva. The fences will be used in combination with chemical treatment. The intention of using chemicals is to eradicate possible carriers of the parasite like fry, parr and stationary adults of susceptible hosts. It has, however, proved difficult to eradicate charr with chemicals, as mentioned, probably because they tend to stay in ponds, marshes and tributary streams connected to the rivers and may therefore escape the chemical treatment. From a management perspective, it is therefore a matter of some urgency to establish whether Arctic charr can act as a long-term host to *G. salaris* or not.

1.4 *What about* Gyrodactylus salaris *and Arctic charr?*

After the first rotenone treatment in the River Skibotnelva, Mo (1988) recorded Arctic charr infected with *G. salaris.* The high number of *G. salaris* on these charr was unexpected, and later Bakke *et al.*(1996) tested the susceptibility of anadromous (Hammerfest strain) and resident (Korrsjøen strain) Arctic charr to *G. salaris* (Haplotype F sensu Hansen *et al.* (2003))

infections in the laboratory. They found that *G. salaris* was able to survive and reproduce on juvenile anadromous charr for up to 280 days and that juvenile anadromous charr generally would mount an immune response towards the parasite, but that there was heterogeneity in susceptibility towards the worm within this charr population. Later, Bakke *et al.* (2002) classified the Hammerfest population of anadromous charr to be 40 % susceptible and 60 % innately resistant to a *G. salaris* infection (see Bakke *et al.* (2002) for definitions). Bakke *et al.* (1996) suggested that anadromous Arctic charr have the potential for carrying *G. salaris* between shoals of salmon parr in rivers where both species co-occur. Kristoffersen *et al.* (2005) once more reported high abundances of *G. salaris* on charr in the River Skibotnelva, and similar findings also applied to the River Signaldalselva (Knudsen *et al.*, 2004; Knudsen *et al.*, 2007). Therefore, several authors have suggested that Arctic charr is probably a suitable long-term host and that this species may be an important reservoir of *G. salaris* in its natural environment (Bakke *et al.*, 1996; Knudsen *et al.*, 2007; Robertsen *et al.*, 2007). Hence, it is likely that Arctic charr may act as a long-term host to *G. salaris*, and that the parasite survived the rotenone treatment in the River Skibotnelva by infecting charr which escaped treatment.

1.5 Testing a hypothesis

In order to test the above assumptions, several aspects of the interactions between *G. salaris* and Arctic charr have been studied in the present work. First, the seasonal occurrence of *G. salaris* on Arctic charr was investigated, as most previous investigations had been undertaken only in the autumn. The water temperature in the River Skibotnelva and the River Signaldalselva is close to 0°C from November to April and peaks at 12 to 14°C in
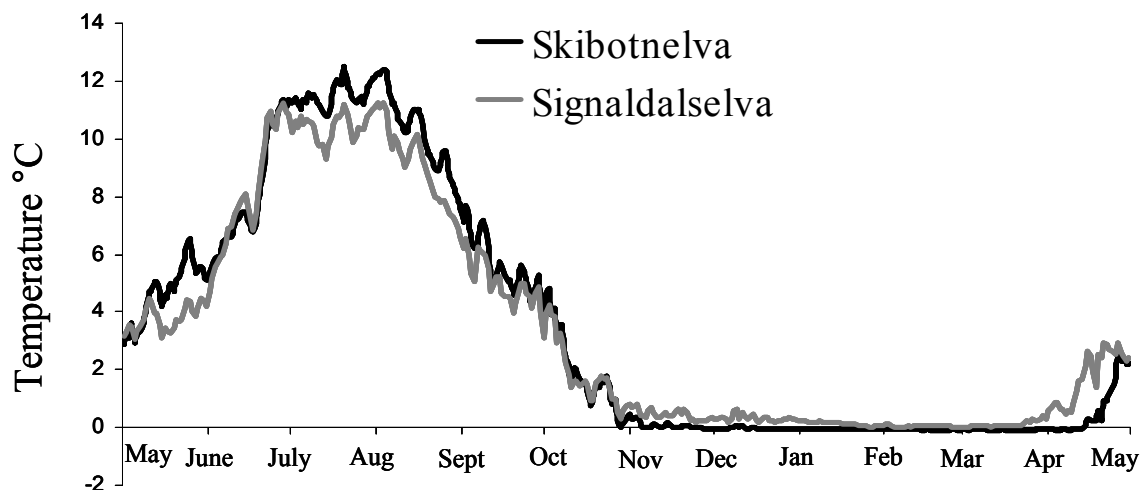
Figure 1
Mean daily water temperature in the rivers Skibotnelva and Signaldalselva in the period from May 2005 - May 2008.

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July/August (Fig. 1). The mean annual water temperature in the two rivers is 3·9°C ± 4·5 S.D. and 3·9°C ± 4·1 S.D., respectively. The temperature generally affects both birth and mortality processes of gyrodactylids and it is expected to generate seasonal variations in the population dynamics of *G. salaris* (Jansen & Bakke, 1991). If Arctic charr is an adequate long-term host to *G. salaris,* the parasite must be able to survive, on this species, on a whole-year basis, and it was unknown whether *G. salaris* infected charr occur throughout the year in these two rivers, as suggested above. Additionally, it was important to test whether Arctic charr in allopatry (i.e. in the absence of Atlantic salmon) could sustain a *G. salaris* population during the cold winter months. The reproduction, longevity and transmission of *G. salaris* (as in gyrodactylids in general (Scott & Nokes, 1984)) are temperature-dependent (Jansen & Bakke, 1991). Jansen & Bakke (1991) estimated the reproductive potential of *G. salaris* under different temperature regimes. They found a clear negative correlation between temperature and time between reproductions, and a negative correlation between the parasites' longevity and the number of offspring per worm. The temperature optimum for *G. salaris* reproduction was found to be between 6 and 13°C. Thus, during cold periods, the parasites have a prolonged life expectancy, but reproduce less frequently. Additionally, the rate of transmission of *G. salaris* between hosts is reduced during cold periods (Bakke *et al.*, 1990; Jansen & Bakke, 1991).

A third factor that might influence the lifecycle of *G. salaris* in these two rivers is its potential for infecting newly-hatched Arctic charr. The susceptibility of these potential hosts has never previously been examined. The annual new recruits are obviously naïve (never been exposed to *G. salaris*) to the parasite when they hatch. In the rivers Skibotnelva and Signaldalselva, Arctic charr fry hatch in mid-April when the temperature is between 0 and 2°C (Fig. 1). If the fry are susceptible, and hence become infected, they may play an important role in the seasonal dynamics of the *G. salaris* population, and may constitute a possible source of rapid increase in the parasite metapopulation (later referred to as a 'lifeline' to the parasite population) simply because they are numerous. In a *G. salaris* infected river, fish can, according to Bakke *et al.* (1992), become infected in four ways: (i) by live host to live host transmission, (ii) by contact with dead infected hosts, (iii) by detached parasites drifting in the water column, and (iv) by parasites attached to the substrate. Therefore, if the parasite is present in the river, and the fry are susceptible, it is likely that they become infected one way or another shortly after hatching. Soleng *et al.* (1999) showed that 6·4 % of caged Atlantic salmon (i.e. not in contact with fish in the river) placed in a *G. salaris* infected river became infected after 24 hours.

Although *G. salaris* has the potential to reduce infected Atlantic salmon populations, parasite-induced host mortality (PIHM) has never been examined in *G. salaris* infected Arctic charr. Bakke *et al.* (1996) reported mortality in juvenile charr, but as the experiment was not designed for testing mortality, it was unclear if this was a direct consequence of the infection or if the charr died from other factors related to the experiment. Recently, Cable & van Oosterhout (2007a) found that the size of guppies infected with *G. turnbulli*, Turnbull 1956 was a decisive factor determining the outcome of an infection. They found that larger fish appeared to carry high maximum parasite load resulting in severe mortality, whereas smaller-sized fish could eradicate the parasites before they reached a lethal intensity level. Other authors have suggested that fry are more vulnerable than older fish, so that mortality decreases with fry age (size) (Cusack & Cone, 1986). The above results suggest two different scenarios. According to Cable & van Oosterhout (2007b), the hosts will benefit from a *Gyrodactylus* infection early in life as they obtain acquired immunity, and thus can better control the infection later in life. The other scenario is that a host will suffer and perhaps die if infected early in life. We experimentally tested the PIHM, to establish if *G. salaris* causes additive mortality in newly-hatched charr fry.

Finally, the mechanisms controlling parasite meta- and infrapopulation dynamics in a juvenile Arctic charr population was studied experimentaly. As mentioned, Bakke *et al.* (1996) demonstrated heterogeneity in susceptibility towards *G. salaris* in a charr population (Hammerfest strain). To reveal individual differences in susceptibility to *G. salaris* among juvenile charr from the River Skibotnelva strain, an experiment was set up. Heterogeneity in the number of gyrodactylids harboured by teleost hosts is common and often associated with differences in host responses (Bakke *et al.*, 1990). Therefore, we expected the temporal change in parasite infrapopulations to coincide with the temporal change in the metapopulation. Further, it was expected that the parasite population would be over-dispersed between the hosts, so that a few fish would harbour the majority of the parasites throughout the experimental period. Owing, however, to the parasites` facility for continuous transmission, it was expected that transmission also could be an important factor controlling the infrapopulation dynamics.

2. Key questions of the thesis

In order to examine the potential of Arctic charr as an adequate host to *Gyrodactylus salaris,* six key questions have been formulated.

1. Is Arctic charr a long-term host to *G. salaris* in natural systems, and are the parasites found on Arctic charr in the rivers Skibotnelva and Signaldalselva, sustained on a whole-year basis (paper I)?

2. Can *G. salaris* survive on allopatric anadromous Arctic charr parr throughout a long winter period with low temperatures (paper II)?

3. Are newly-hatched Arctic charr fry susceptible to a *G. salaris* infection and is their role as a potential 'lifeline' to the parasite in the spring prominent (paper II)?

4. Can newly-hatched Arctic charr fry sustain a *G. salaris* population for a substantial time, making them important in the parasites` lifecycle (papers II, III and IV)?

5. Can *G. salaris* cause additive mortality in Arctic charr fry (paper III)?

6. To what extent does the gyrodactylids` facility for continuous transmission between hosts influence the infrapopulation dynamics of *G. salaris* in Arctic charr fry (paper IV)?

3. Summary of papers

3.1 Paper I

Seasonal dynamics and persistence of *Gyrodactylus salaris* in two riverine anadromous Arctic charr populations

A. C. WINGER, M. KANCK, R. KRISTOFFERSEN & R. KNUDSEN

This study is a three-year survey of the seasonal occurrence of *Gyrodactylus salaris* Malmberg 1957 infecting Arctic charr (*Salvelinus alpinus* (L.)) in the rivers Skibotnelva and Signaldalselva in northern Norway. Previous studies have reported that Arctic charr are infected with *G. salaris* in both rivers, and earlier experimental studies indicate that Arctic charr can act as a good host to the parasite. Unlike Atlantic salmon, which are highly susceptible to *G. salaris,* Arctic charr can display a wide range of host-responses to *G. salaris* infections. In 1979, the parasite was introduced to the River Skibotnelva, and the river has been treated with rotenone twice without success. It is probable that resident Arctic charr avoided the rotenone treatment in small tributary streams, and thus were the source of the repeated re-infection of this river. In the River Signaldalselva, *G. salaris* was recorded for the first time in 2000, and this river is still untreated. The main results from this survey demonstrated an evident seasonal dynamic in *G. salaris* infection in charr in the two rivers, and both the prevalence and intensity of infection fluctuated with the rise and fall in temperature in a similar manner in both rivers. The seasonal fluctuations are mainly explained as a response to temperature. The prevalence and mean intensity were, however, significantly lower in the River Skibotnelva than in the River Signaldalselva. The former river has been infected for 21 years longer than the latter, and thus charr in the River Skibotnelva may have developed a better resistance towards the parasite than charr from the River Signaldalselva. An alternative or additional hypothesis is that the Atlantic salmon (*Salmo salar* L.) population in the River Skibotnelva was less dense than in the River Signaldalselva, so the infection pressure was lower in the latter river. Finally, we generally found a lower prevalence and intensity of *G. salaris* in older than in younger charr, which can be explained by a difference

in parasite tolerance or immunity between the different age groups of charr owing to their different previous infection history. In conclusion, this survey supports the theory that remaining Arctic charr carrying *G. salaris* caused the return of the parasite in the River Skibotnelva after the two rotenone treatments.

Experiments to test if allopatric *Salvelinus alpinus* (L.) are suitable year-round hosts of *Gyrodactylus salaris* (Monogenea)

A. C. WINGER, R. KRISTOFFERSEN, S. I. SIIKAVUOPIO & R. KNUDSEN

This field experiment focuses on the ability of Arctic charr (*Salvelinus alpinus* (L.)) to act as a year-round host to *Gyrodactylus salaris* Malmberg 1957 (Monogenea), by following the infection dynamics of *G. salaris* infecting parr and fry of Arctic charr from the River Skibotnelva in northern Norway. The study had two different experimental designs. In both experiments, the fish were initially infected with *G. salaris* from Atlantic salmon (*Salmo salar* L.). The first, a field experiment, was intended to test whether *G. salaris* could survive on allopatric Arctic charr parr through a five-month-long winter with temperatures around 1°C. Of the 92 exposed charr parr, 90.2 % were infected with an average of 12.4 parasites. They were released in a closed (initially fish empty) brook during the ice-free season, ranging from November to the end of April. Approximately two-thirds of the released fish were re-captured and two out of these parr were infected with one and two live *G. salaris*. Second, we studied the susceptibility to *G. salaris* of newly-hatched Arctic charr fry. In the experiment, we used fry that were reared in the experimental brook and had never previously been exposed to *G. salaris*. We monitored the parasite metapopulation throughout the summer and autumn. Two replicas with high densities of fry were exposed to *G. salaris* once, and within two weeks all hosts were infected. The experiment showed that the parasite population persisted, and it was maintained for 155 days until the end of the experiment. At this time, the metapopulation had stabilised and was not declining, provided that new uninfected hosts were added once during the period. In conclusion, *G. salaris* is able to reproduce and survive on Arctic charr during periods of low water temperature. The results also indicate that in the spring, newly-hatched Arctic charr fry may act as a potential 'lifeline' for a stressed *G. salaris* population, as they are highly susceptible and capable of carrying relatively heavy loads of parasites over a substantial time interval. These results confirm the hypothesis that charr can sustain a *G. salaris* population on an annual basis.

Gyrodactylus salaris infecting allopatric Arctic charr fry: an experimental study of host survival

A. C. WINGER, R. PRIMICERIO, R. KRISTOFFERSEN, S. I. SIIKAVUOPIO & R. KNUDSEN

In this experimental study, we examined the possible lethal effects of the Monogenean ectoparasite *Gyrodactylus salaris* Malmberg 1957 infecting newly-hatched artificially-reared fry of Arctic charr (*Salvelinus alpinus* (L.)). Arctic charr are recognised as responding to *G. salaris*, but the effect in very young fish has not been tested. We infected newly-hatched charr with *G. salaris* from Atlantic salmon (*Salmo salar* L.) parr caught in the main river. Mortality in infected fry was compared with mortality in uninfected fry. About 95% of the fry became infected within the first week post-exposure, and at the end of the experiment (77 days) all fry carried the parasite. The mortality rate was significantly higher in the groups of infected fry than in the uninfected control groups. By the end of the experiment, approximately 30 % of the infected and 8 % of the uninfected fish had died. Thus, *G. salaris* had a pronounced negative effect upon fry survival and caused additive host mortality. Moreover, the concurrent temporal changes in parasite intensities and aggregation indicated that the parasite-induced host mortality was density-dependent. This was revealed by the concurrent fluctuations in the parasite metapopulation. Mortality was linked with increased mean intensities and decreasing aggregation ($s^2 \bar{x}^{-1}$) in the metapopulation. Additionally, we found signs of fin clamping and opaque corneas that might reduce the fitness of the fry, although these effects were not quantified. The present results demonstrate that *G. salaris* is a potential agent for significant mortality in Arctic charr fry and therefore may act as a selective force to increase resistance to *G. salaris* in infected charr populations.

Infrapopulation dynamic and the role of transmission in the hyperviviparous *Gyrodactylus salaris* (Monogenea)

A. C. WINGER, R. KNUDSEN, R. PRIMICERIO & R. KRISTOFFERSEN

In this study, we aimed to quantify the differences in host responses by following the parasite infrapopulations on individually tagged fish. In the experiment, Arctic charr fry (*Salvelinus alpinus* (L)), reared in an experimental brook connected to the River Skibotnelva, were used. The fry were individually tagged and they had never previously been exposed to *G. salaris.* The fry were infected with *Gyrodactylus salaris* Malmberg 1957 from Atlantic salmon (*Salmo salar* L.) caught in the main river, and the infection was monitored on each individual fish for 68 days. The temporal change in the infrapopulations was modelled with a generalised additive model describing the nonlinear relationship in the data. The model described a temporal dynamic similar to other gyrodactylid/host dynamics where the infrapopulation sizes, generally increased, flattened out and decreased. This was consistent and applied to the great majority of the fish. The infrapopulation dynamics were analysed by estimating the start value, N_0, the rate of initial parasite infrapopulation increase, r, and the peak infection, K. No significant relationship was found between N_0 and K and between r and K. A strong negative correlation was found, however, between r and N_0. This indicate that the parasites move from densely to sparsely infected hosts. It is notoriously difficult, however, to identify and differentiate between the different processes determining infrapopulation growth, and therefore the variance in the data was analysed. This suggested that parasite transmission between hosts occurs frequently, leading to large fluctuations in the infrapopulations over short time periods. The parasite's facility for continuous transmission is assumed to prolong the longevity of the parasite population within the host population and to increase the number of parasites at peak infection.

4. Discussion

4.1 Arctic charr as long-term host to Gyrodactylus salaris *in natural systems*

During the survey of the two rivers Skibotnelva and Signaldalselva (paper I), we found that the metapopulations of *G. salaris* on Arctic charr exhibited clear and similar seasonal changes in the two rivers, with a peak in occurrence during late summer and autumn, and a decline during the winter period. In the spring, we did not record any G. *salaris* on the charr in the River Skibotnelva, while in the River Signaldalselva the charr were infected with only low prevalence and intensities of the parasite (paper I). Later however, *G. salaris* has been recorded on both charr parr and smolts during springtime in the River Skibotnelva (Roar Kristoffersen, personal communication).

Several authors have reported seasonal dynamics in freshwater gyrodactylids, and generally, the dynamics are related to seasonal changes in water temperature. As the temperature regime changes with latitude, so does the seasonal dynamic (see Bakke *et al.*, 2007 and references therein), and it seems that gyrodactylids, during certain parts of the season, do experience poor population growth owing to unfavourable temperatures. Although the seasonal fluctuations are mainly explained as temperature-dependent alterations in the reproductive rate of *G. salaris* (Jansen & Bakke, 1991), both transmission of parasites and increased immune responses will influence the reproductive rate of *G. salaris* as well. It has been showed that transmission of parasites between hosts is reduced during low temperatures (Soleng *et al.*, 1999), and low transmission may hamper the reproductive potential of the parasites, as continuous transmission will increase it (Boeger *et al.*, 2005) (paper IV). Additionally, it is likely to assume, at least under similar condition as presented here (paper I), that most hosts become infected during summer and autumn, and the number of naïve hosts are therefore strongly reduced during the autumn and winter (paper I). Acquired immune

responses from the hosts will thus, inhibit parasite population growth (Buchmann & Lindenstrom, 2002).

In more temperate regions (between 30°N and 70°N), it is normal to observe two peaks in the population size of freshwater gyrodactylids during the year, one in the springtime and one in the autumn (see Bakke *et al*., 2007 and references therein). Generally, it is assumed that it is the high number of newly-hatched fry emerging in the spring, combined with increasing temperatures, that induce this first bloom (Bakke *et al.*, 2007). In our survey, we found that the charr fry (0+) generally exhibited higher prevalence than did the older charr (paper I), and our further experimental studies revealed that the charr fry are highly susceptible to *G. salaris* (papers II, III and IV). We did not, however, find a bloom during springtime. This lack of spring bloom observed in the River Skibotnelva and the River Signaldalselva can be explained by several factors. First, the number of salmon is strongly reduced during winter (Knudsen *et al.*, 2007), and this may reduce the total number of *G. salaris* in the river. Second, the number of *G. salaris* on the charr parr is strongly reduce during this period (paper II). Third, the temperature does not start to rise considerably before late June or July (Fig. 1).

During summer the intensity and prevalence of *G. salaris* increased and peaked in the autumn (paper I). This is probably caused by changes in host density and immunity. During late spring, the few salmon fry emerge from the gravel, approximately two months later than the charr do. The charr fry start to disperse more rapidly during this period and additionally, during summer and autumn, adult anadromous fish enter the river to spawn. These events together increase the number of susceptible fish in the rivers, increase the transportation of *G. salaris* upstream and thus most probably increase the parasite's possibility for transferring to new hosts. The immigration of anadromous fish will in it self perhaps also influence the behaviour of the resident fish population, so that crowding could be a result. Crowding can

lead to stress (Tort *et al.*, 1996a; Tort *et al.*, 1996b; Rotllant *et al.*, 1997), which generally is a immunosuppressant. Therefore, it is likely that the parasite bloom in the autumn (paper I) is partly caused by increased parasite reproduction as a result of the temperature optima in August (see Fig.1) (Jansen & Bakke, 1991), partly caused by changes in the fish host densities and species composition, and maybe also by stress-related immunosuppressant in the infected host populations.

Conclusion: The results from the survey (paper I) clearly indicate that Arctic charr do act as a long-term host to *G. salaris* in the rivers Skibotnelva and Signaldalselva, but to test the hypothesis further, several new questions were addressed.

4.2 Arctic charr in allopatry

We found that young Arctic charr could sustain a *G. salaris* population throughout the six months of cold winter in a small brook without Atlantic salmon present (paper II).

Whether or not this result is representative for the Arctic charr population under natural conditions in the rivers Skibotnelva and Signaldalselva is still uncertain, as it is impossible to test. However, all previous studies on anadromous Arctic charr and *G. salaris* support this possibility. Experimental studies have revealed that *G. salaris* can reproduce and survive for several weeks on this host (Bakke *et al.*, 1996; Harris *et al.*, 2000; Olstad *et al.*, 2007) (papers II, III and IV), and several surveys have reports of *G. salaris* infected anadromous charr in the wild (Mo, 1988; Kristoffersen *et al.*, 2005; Knudsen *et al.*, 2007). Additionally, Robertsen *et al.* (2008) found *G. salaris* infections on resident charr in the salmon-free part of the watercourse, Numedalsvassdraget, in southern Norway. The haplotype found on this charr was slightly different from haplotype F found on Atlantic salmon in southern Norway (Hansen *et al.*, 2003; Robertsen *et al.*, 2007). Olstad *et al.* (2007) tested the strain infecting the resident charr on different salmon populations and found, surprisingly, that

it was non-pathogenic to Atlantic salmon. Robertsen *et al*. (2007) suggest that the genetic difference between *G. salaris* from Numedalsvassdraget and *G. salaris* haplotype F is the result of adaptation to the ecology of Arctic charr. It is possible that *G. salaris* can adapt to the ecology of the Arctic charr populations in the rivers Skibotnelva and Signaldalselva as well. The outcome of such an adaptation is of course difficult to predict. A similar scenario to the one reported from the Numedalsvassdraget is not, however, altogether unlikely.

Conclusion: The result from this experiment (paper II) indicates that allopatric Arctic charr from the River Skibotnelva have the ability to act as year-round host to *G. salaris*.

4.3 Arctic charr fry and Gyrodactylus salaris

When testing the susceptibility of newly-hatched Arctic charr fry from the River Skibotnelva to a *G. salaris* infection, we found that all of the fry quickly got infected when exposed to the parasites (paper II) and that the parasites was successfully established (as defined by Scott & Robinson, 1984) on all the fry. We found that the parasite population increased rapidly, in defiance of low temperatures (papers II, III & IV), and it is reasonable to assume that this could happen in the wild as well.

Furthermore, our experiments showed that Arctic charr fry could sustain *G. salaris* infections from early May to November given an input of new susceptible hosts (paper II). Additionally, we found that one batch of Arctic charr fry could sustain the parasites for more than two months without input of hosts or parasites (paper III and IV). During the field survey in the rivers Skibotnelva and Signaldalselva, we found a higher prevalence and mean intensity in charr fry (0+) compared with older charr parr (1+ and 2++) (paper I).

As most of the infected charr are immature, the distribution pattern of parasites among hosts is not directly related to the sex of the fish, as showed elsewhere (Robertsen *et al.*, 2008). Instead, it may be that the parasites are distributed in relation to the hosts age and

previous infection history. Studies on guppies have revealed that hosts do benefit significantly from a previous gyrodactylid infection (Cable & van Oosterhout, 2007b). This is because the fish have developed an acquired immunity to the parasite, and this acquired immunity protects them during a second infection. This implies that acquired immunity contributes to gyrodactylid resistance (Scott, 1985; Cable & van Oosterhout, 2007b). If this also applies to charr, it means that while charr fry have to rely entirely on their innate immune response, most parr on the other hand, have mounted an acquired immune response towards *G. salaris*, owing to previous infections (Lester & Adams, 1974; Richards & Chubb, 1996). The observed decrease in intensity and prevalence from charr fry to older charr parr (paper I) may thus be explained by an acquired immunity in the charr parr.

Conclusion: These results confirm that newly-hatched Arctic charr fry are susceptible to a *G. salaris* infection. Compared with older charr (Bakke *et al.*, 1996), the fry may be more vulnerable and highly susceptible to a *G. salaris* infection (papers II, III and IV). It is, however, difficult to establish fully the role of Arctic charr fry as potential 'lifelines' to the parasites in the River Skibotnelva in the spring. Nevertheless, the fry are highly susceptible and quickly become infected, the experiments suggest that they do play a prominent role in the parasite's lifecycle since they, together with the few existing juvenile salmon, are the most susceptible hosts in the river.

4.4 Gyrodactylus salaris *induced mortality in Arctic charr fry*

The experiment set up to test whether *G. salaris* imposes mortality in newly-hatched charr indicated that the fry experienced a significant additive mortality owing to the parasite infections (paper III). Additionally, we found that the mean intensity increased during the experiment, and temporal fluctuations in both intensity and aggregation ($s^2\, \bar{x}^{-1}$) suggested that the mortality was density-dependent.

As the mortality is generally high in the first life stages of salmonids (Elliot, 1994), it is difficult to know if the observed parasite-induced host mortality (PIHM) is also additive under natural conditions (paper III). Nevertheless, the hosts that survive a *G. salaris* infection by evolving resistance toward the parasite will, if surviving to maturation, pass its genes on. In this way, the ability to evolve resistance towards *G. salaris* will act as a selective force to increase the resistance to *G. salaris* in the Arctic charr population over the generations by imposing an alteration in the gene pool. This line of thinking could be useful in understanding the differences found in intensity and prevalence of *G. salaris* on the Arctic charr in the rivers Skibotnelva and Signaldalselva (paper I).

It seemed like the host with the highest parsite burden died from infections with *G. salaris* (paper III). In comparison, it could appear as the parasites "escaped" before reaching lethal numbers on the hosts, in the study on the older fry (also naïve) (paper IV). Some sort of host response, which was better developed in the older charr fry, seems the most likely explain for this difference in survival between newly hatched and older fry. It is therefore likely to assume that newly-hatched fry are more vulnerable to a *G. salaris* infection compared with older fry.

Conclusion: Newly-hatched Arctic charr fry from the River Skibotnelva experience additive mortality owing to infections with *G. salaris* under experimental conditions. The mortality was density-dependent. This implies that young charr may die as a direct effect of an early infection with the parasite. Generally, such PIHM could act as a selective pressure, altering the gene pool of any infected host populations, making them less susceptible.

4.5 Infrapopulation dynamics

Through monitoring the infrapopulation dynamics of *G. salaris* in two-month-old individually tagged Arctic charr it was found, as expected, that the parasite intensities initially

increased, where after the population growth decreased and started to decline. It was, however, impossible to classify the different hosts as either susceptible or resistant, as the parasites appeared to move frequently between hosts in a random fashion, and it was concluded that the infrapopulation dynamics were largely controlled by continuous transmission (paper IV).

There are several possible mechanisms behind the transmission of gyrodactylids between hosts. Transmission could be caused directly by host immune response (Bakke *et al.*, 2007), or as a means of preliminary escape from host immune response (Boeger *et al.*, 2005) or even as a means of avoidance of inbreeding (Olstad *et al.*, 2006).

We found that the size of all infrapopulations peaked after approximately 54 days independent of the individual parasite load. This could mean that immune response in the hosts is controlled by time rather than by the individual host's parasite load. This is contrary to the suggestion by van Oosterhout (2007) that the immune response in the hosts is dependent on the intensity of gyrodactylids on their skin. Additionally, several studies have shown that hosts tend to dispose of a gyrodactylid infection faster when isolated than when grouped (Scott & Anderson, 1984; Bakke *et al.*, 1991; Bakke *et al.*, 1996; Boeger *et al.*, 2005). Boeger *et al.* (2005) explain this by transmission, and state that temporary evasion of host responses to parasitism by host transfer apparently would augment the abilities of the parasite to colonise new host resources. Further, they suggest that during the switching of hosts, parasites may rapidly conquer a new (host) resource, but subsequently avoid (by fleeing) developing host resistance in the new host (Boeger *et al.*, 2005). Boeger *et al.* (2005) use this model to describe how continuous transmission may be responsible for the greater relative rate of diversification demonstrated by viviparous gyrodactylids. This model may also be used, however, to explain how transmission can prolong the parasite's longevity within a host population. As mentioned earlier, another mechanism behind transmission could be to

enhance avoidance of inbreeding, since it has been observed that the majority of transferring

parasites have given birth once (Harris *et al.*, 1994; Olstad *et al.*, 2006).

4.5.1 Infrapopulation development of *Gyrodactylus salaris* on grouped versus individual hosts

In an attempt to elucidate the difference in gyrodactylid population growth on grouped

versus isolated host, the mean infrapopulation growth of *G. salaris* estimated from paper IV

was compared with the mean infrapopulation growth of *G. salaris* estimated from two

computer-simulated charr (see below) under two different temperature regimes. The data on

the fry from paper IV were used in an individually-based computer model (IBM) developed

by van Oosterhout *et al.* (2007). This model was developed to simulate the infection dynamics

of gyrodactylid parasites and the immune response of naïve hosts. According to van

Oosterhout *et al.* (2007), the model will accurately predict the infection dynamics of

gyrodactylids infecting guppies and salmonid hosts by the use of small number of input

variables (see Box 1). The model estimates the population dynamic for separate

infrapopulations of gyrodactylids, and does not include the option that the interaction between

hosts may increase the number of parasites in the different infrapopulations owing to the

possibility of continuous transmission of parasites between hosts (paper IV). Therefore, the

model can be used to simulate the infection pattern on isolated hosts and compare the result

from the simulations with the observed data from paper IV (see Box 1). The results from

running the model showed that the two simulated infrapopulations of *G. salaris* from the two

gestation regimes (Sim 1 and Sim 2; Fig. 2) reach peak infection before the peak in

infrapopulation of *G. salaris* in the observed experimental charr population (paper IV).

Further, it was found that the parasites in all infrapopulations from paper IV was sustained

approximately twice as long on the hosts as in the simulated fish. The prolonged lifetime and

the high number of parasites at peak infection estimated from the real data may be explained as a direct effect of transmission.

Conclusion: Both the hypothesis of Boeger *et al.* (2003; 2005) and Olstad *et al.* (2006) explain how continuous transmission can be genetically promoted in gyrodactylids. The hypothesis of Boeger *et al.* (2003; 2005) is, however, the only one that tries to explain how transmission in gyrodactylids can prolong the longevity of a parasite population within a host population as a direct effect of escaping host response. Although the exact reason for transmission may still be unclear, the parasite's facility for continuous transmission may apparently contribute to increased parasite survival and prolonged longevity of the parasite metapopulation.

BOX 1

Infrapopulation development of *Gyrodactylus salaris* on live grouped versus modelled individual hosts

In accordance with van Oosterhout *et al.* (2007), the different input variables required to do a simulation in the IBM model was estimated. The total length (L_T) and the circumference of the infected charr from paper IV were used to construct the foraging area for the parasites. Second, the gestation time (the time between births) for the first- and secondborn gyrodactylid was required to estimate the population growth. The last input needed was the initial number of parasites (N_0) and duration of the infection period (for more details see van Oosterhout *et al.* (2007)).

The mean L_T (50·4 mm) was measured on the day the experiment was terminated, and the circumference was measured on a small sample of ethanol-conserved fry (measuring on average 21 mm including two extra mm to correct for loss during conservation). Two computer simulations were undertaken with two different input values. We used the gestation regime at 6·6°C (Sim 1) and at 13·0°C (Sim 2) for *G. salaris* haplotype F (Hansen *et al.*, 2003) estimated by Jansen & Bakke (1991). The initial number of parasites (N_0) was set at ten, which equals the mean N_0 for the whole experimental population in paper IV. The mean result of 30 simulations for each gestation regime (Sim 1 and Sim 2) is presented in Fig. 2. These simulations were compared with the infection dynamics for all charr in paper IV (presented as the mean of all infrapopulation changes over time). The first part of the IBM shows a good fit with the empirical data on *G. salaris* from Arctic charr fry (Fig. 2). From paper IV it seems that the *G. salaris* haplotype B (Hansen *et al.*, 2003) infecting Arctic charr has a reproduction rate that lies between the two values estimated by Jansen & Bakke (1991). Within the two simulated charr populations, the parasites are eradicated within 36 to 47 days, while the experimental hosts sustained the parasite population during the 68 days of the experiment. Additionally, the parasite intensity at peak infection was twice that of the modelled hosts.

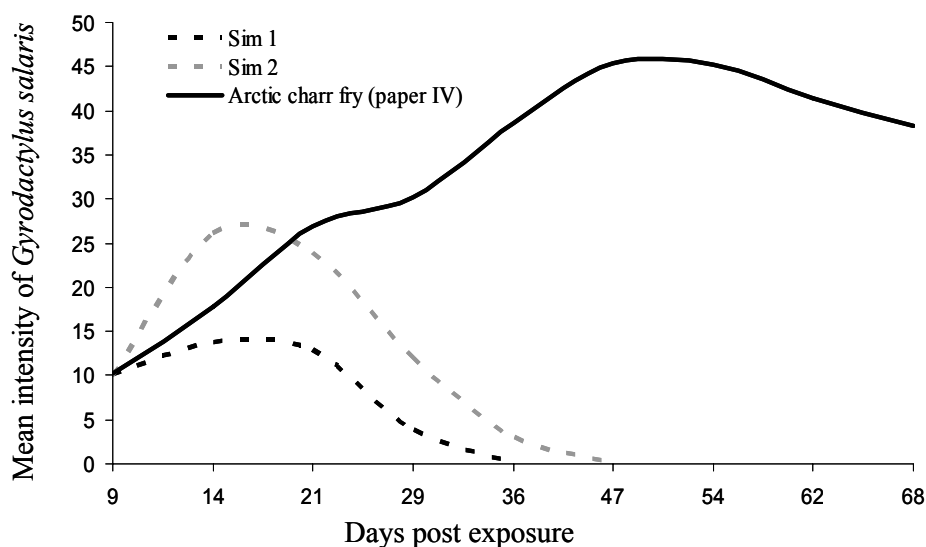

Figure 2
Mean intensity of *Gyrodactylus salaris* on Arctic charr fry in experiment (paper IV),
compared with two simulated fish host at 6·6°C (Sim 1) and at 13·0°C (Sim 2) (each fish
has been simulated 30 times) during a 68 days long experiment

4.6 Live charr to live charr transmission

Finally, an experiment was conducted to elucidate the importance of infected live charr as a source of *G. salaris* infections to other uninfected conspecifics (Box 2). The parasite intensities on the donor fish were low, and thus low intensities were found on the exposed charr as well.

The rate at which parasites moved between hosts was similar to what was found in paper IV. However, in paper IV the charr fry were infected with *G. salaris* from dead Atlantic salmon and 95 % of the fry was infected after ten days' post-exposure. In the current experiment, 76 % of the fry became infected during the same time interval. The temperatures were approximately the same. Although these two experiments are not directly comparable, this experiment does suggest that transmission between live charr is efficient. Additionally, Soleng *et al.* (1999) found that in a small population of uninfected salmon, the number of salmon that became infected with *G. salaris* after being exposed to live *G. salaris* infected salmon for 24 hours (in the laboratory) was 2·0 % at 4·7°C and 4·9 % at 12·2°C. In the current study (Box 2), we found that in a small population of uninfected charr, the number of charr that became infected with *G. salaris* after being exposed to *G. salaris* infected live charr was 4·4 % after 12h and 8·8 % after 36 h (Table I) at approximately 6°C.

Conclusion: The transmission of *G. salaris* from live charr to live charr is as efficient, if not more, as the transmission from live salmon to live salmon. Further, these results clearly substantiate the potential that Arctic charr have as whole-year host to *G. salaris* and how well Arctic charr may spread *G. salaris* to other hosts.

BOX 2

Live host to live host transmission in Arctic charr fry

Live charr to live charr transmission of *G. salaris* was experimentally tested. In the experiments in papers II, III and IV, charr have been exposed to dead salmon parr from the River Skibotnelva which are heavily infected with *G. salaris*. As the intensities in wild charr are generally low, and the potential of transmission from these charr was unknown, salmon was used to ensure infection in all previous experiments. If, however, Arctic charr in the wild can maintain *G. salaris* in the absence of salmon, as hypothesised in this thesis, the transmission from live infected charr to live uninfected charr must occur and be efficient. To test this, an experiment with a design almost identical to that reported in paper IV was carried out. We used two experimental units (EU) constructed of plankton cloth with a size of 100μm. This plankton cloth is so fine that it is unlikely that the parasites will drift through it (not tested). Naïve charr fry cohabited with charr fry that carried *G. salaris* infections for about 30 to 40 days prior to the experiment. The mean intensity was 26·3 parasites (Fig. 3, Table I). The experiment lasted for 61 days. The parasites were counted on the donor fish before they were introduced to the EU with uninfected fish, so the metapopulation size could be controlled at all times.

There were two replicas with initially 10 and 11 uninfected fry. Two donor fry were introduced into both EU. A repeated measure ANOVA was used to test for difference in intensities between the two EU during the experiment. No significant differences were found (p = 0·98), so the two replicas were treated as one group. After four days, half of the uninfected fish were infected and after 33 days all the fry were infected (Fig. 3, Table I).

Table I
Mean intensity, prevalence (%), aggregation ($s^2\bar{x}^{-1}$), min and max number of *Gyrodactylus salaris* for the 21 individually marked Arctic charr fry (initially uninfected) and the four donor fry during 61 days of the experiment

|	Days	0	12h*	36h*	4	7	10	15	18	23	29	33	36	44	47	50	54	57	61
Initially uninfected fry	Mean intensity	0	0·1	0·3	0·7	1	1·5	2·8	3·4	4	6·3	8·3	9	12·1	12·2	13·2	12·5	13·8	13
	Prevalence (%)	0	9·5	19·1	47·6	57·1	76·2	85·7	95·2	90·5	94·5	100	100	100	100	100	100	100	100
	Aggregation	0	1	1·5	1·1	1·2	1	1·4	1·7	2·3	2·5	3·1	2·7	4·5	4·4	4·6	5·2	4·6	5·1
	Min	0	0	0	0	0	0	0	0	0	0	2	2	2	2	1	1	3	2
	Max	0	1	2	3	3	4	7	9	14	13	18	18	28	23	25	28	27	26
Donor fry	Mean intensity	26·3	23·5	25·5	23·3	26·5	29·5	26·8	23·5	26	24·8	28	24·8	24	26	26	25	26·5	19·8
	Prevalence (%)	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
	Aggregation	4·7	4·6	4	2·1	3·7	5·3	1·4	3·5	2·6	10·5	16·2	15·1	20·5	21·9	29·3	33·3	27·9	20
	Min	11	11	14	15	15	16	20	13	17	9	11	10	5	3	3	2	3	4
	Max	35	33	35	30	37	44	34	34	34	45	57	52	56	58	66	67	65	48
	Tot. numb. of *G. Salaris*	105	96	108	107	126	149	165	166	187	212	261	252	302	311	329	313	341	300

* Hours

BOX 2
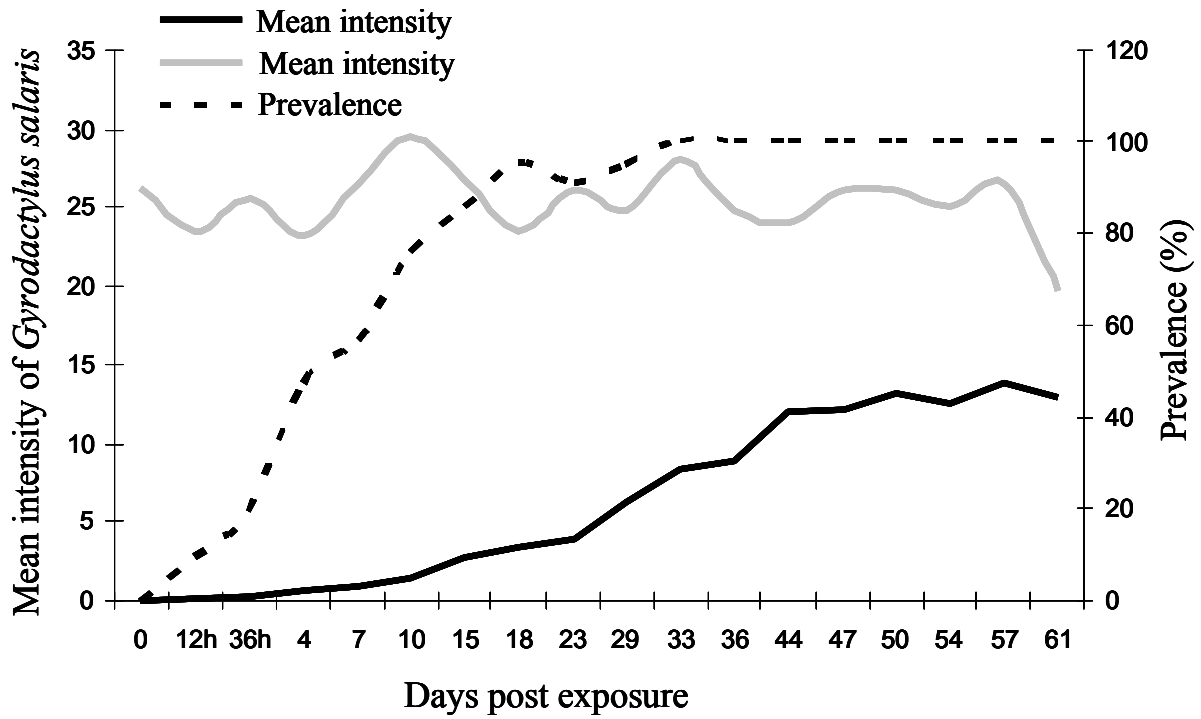
Figure 3
The left hand side of the Y-axis display the mean intensity of *Gyrodactylus salaris* on initially uninfected Arctic charr fry (black) and on the donor fry (grey). The right hand side of the graph indicate the prevalence (%) of the initially uninfected charr fry during the 61 days of the experiment

5. Implications for management

In northern Scandinavia and Lapland, both anadromous and resident Arctic charr are widely distributed. They inhabit streams and lakes connected closely or otherwise, to each other. The *G. salaris* that infect charr and salmon in the rivers Skibotnelva and Signaldalselva was first described in the Swedish Torne River (Fig. 4) and is referred to as haplotype B (Hansen *et al.*, 2003). This haplotype of *G. salaris* is also infecting other Baltic salmon in

Figure 4
Map describing the distribution of Gyrodactylus salaris in northern Norway, Sweden and Finland. The names are listed below, R for River and L for Lake. 1. Skibotnelva (R) 2. Signaldalselva (R) 3. Gálggojávri (L) 4. Goldajávri (L) 5. Kilpisjärvi (L) 6. Reisaelva (R) 7. Altaelva (R) 8. Kautokeino watercourse 9. Poroujärvi (L) 10. Lätäseno (R) (R) 11. Könkääelven (R) 12. Muonionjoki (R) 13. Lake Torne (L) 14. Torneelven (R). The infected regions are 1, 2, 9, 10, 11, 12 & 14 (See Antilla et. al. 2008)

rivers closely connected to several Norwegian watercourses (Anttila *et al.*, 2008) (Fig. 4). The *G. salaris* infected Swedish rivers are inhabited by only small populations of Arctic charr. However, resident charr populations are present in most lakes connected to these rivers. In Norway, only a few examinations of charr in lakes in the upper part of the rivers Skibotnelva and Signaldalselva have been undertaken. These examinations have not revealed any gyrodactylids (Kristoffersen *et al.*, 2005), with one exception: T. A. Mo (unpublished) discovered one gyrodactylid on charr above the natural fish hindrances in the River Skibotnelva. This specimen was, however, lost before the species was identified. The resident charr in the rest of Lapland have not been examined for *G. salaris* infections, and it is as yet unknown whether these charr are able to carry *G. salaris*. From knowledge gained from the present and other studies (Bakke *et al.*, 1996; Robertsen *et al.*, 2007; Robertsen *et al.*, 2008), it is likely to assume that both anadromous and resident Arctic charr constitute a possible source and target of *G. salaris* infection. The fish populations in lakes and streams in Norwegian, Swedish and Finnish Lapland are thus under constant threat of a *G. salaris* epidemic. It will have disastrous consequences for the Atlantic salmon population in northern Norway if, for instance, the parasite should enter the River Altaelva. Therefore, a thorough examination of the resident charr populations regarding their ability to sustain *G. salaris* is required.

The governmental plans to eradicate *G. salaris* is based on the use of fish fences in the mouth of both the rivers Skibotnelva and Signaldalselva and in the upper regions above the River Signaldalselva (Lake Goldajávri, where the river drains east; Fig. 4). If these fences are to work, all potential long-term hosts have to be eradicated from the rivers. Arctic charr from lakes in the upper regions can constantly migrate downstream. If the resident Arctic charr populations in the upper regions (see above) are susceptible to *G. salaris*, they could hamper

the effect of fish fences in the rivers Skibotnelva and Signaldalselva by constantly providing the systems with susceptible hosts.

Studies on Arctic charr migration between the rivers Skibotnelva and Signaldalselva in the Fjord Storfjorden have revealed that anadromous charr do travel between these rivers (Rikardsen, personal communication). The majority of the migratory fish migrate from the inner parts of the fjord, Signaldalselva, towards Skibotnelva located further out in the fjord. One adult charr has been recorded as swimming the 23 km from Signaldalselva to Skibotnelva in under 24 hours (Rikardsen & Winger, unpublished data). According to Rikardsen *at al*. (2007), Arctic charr spend 50 % of their time at a depth between 0 and 1 m, and usually no deeper than 3 m in the colder parts of the fjord, during sea migration. Soleng & Bakke (1997) reported that *G. salaris* survived and reproduced on salmon in brackish water up to 5 ‰ salinity, and that the survival was negatively correlated with temperature. In the inner parts of the fjord, the salinity and temperature are low, and therefore Arctic charr may have the potential to carry the parasite between the rivers. Thorstad *et al*. (2001) suggested that the River Signaldalselva was infected with anadromous fish from the River Skibotnelva, and later Kristoffersen *et al*. (2005) suggested that this was indirectly caused by the rotenone treatment, as the parasite infection bloomed in the River Skibotnelva when the river was restocked after treatment. Descending Arctic charr smolts were examined in the River Skibotnelva in late May 2007. Out of 30 charr smolt, three were infected with *G. salaris* with a mean intensity of 2·7 parasites (Kristoffersen, unpublished data). On the basis of this, both smolts as well as adult charr must be considered as a possible source of *G. salaris* dissemination. Therefore, it is important to treat both the two rivers Skibotnelva and Signaldalselva (and other possibly infected small rivers in the area) simultaneously, as the parasite may have the ability to spread through the fjord with migrating charr.

6. Future perspectives

The fact that the River Skibotnelva has been infected with *G. salaris* for 25 years longer than the River Signaldalselva in itself constitutes an excellent opportunity to examine the co-evolution of host and parasites. The two rivers are similar with regard to both abiotic and biotic factors, they belong to the same fjord system, and the two charr populations are probably closely related. By comparing the response to *G. salaris* in these two charr populations, it may be possible to determine whether 25 years of *G. salaris* infections have altered the genetics of the infected charr population as suggested above (paper III). Further, it is possible to compare infections with the local *G. salaris* strain from the River Skibotnelva with other strains of *G. salaris* on naturally uninfected anadromous Arctic charr populations. Such comparisons would perhaps illuminate alterations in the parasite's adaptation to the ecology of Arctic charr.

The fin clamping and opaque corneas in *G. salaris* exposed charr registered in the current studies could influence the life history of the Arctic charr (paper III). The extent of these deformities needs to be quantified, and the effects should be examined more closely. It is likely that surviving a *G. salaris* infection at a young age could have implications later in the life span. Possibly, these fish may experience poor growth, which again could prevent or postpone smoltification (Elliot, 1994) and thus contribute to an increased fraction of resident individuals within the charr population. This could in the next instance lead to a denser charr population in the river and thus a higher density of parasites. Experimental tests on the physical consequences of *G. salaris* infections are therefore highly relevant.

Additionally, I aim to use the data from this thesis in combination with future experimental data to construct a mathematical framework describing *G. salaris/*host interactions, involving both Arctic charr and Atlantic salmon, in the Skibotnelva and/or the Signaldalselva. Such a model could be used to generate a better general understanding of gyrodactylid epidemiology and also have important implications for the management of *G. salaris* infected rivers.

7. References

Anttila, P., Romakkaniemi, A., Kuusela, J. & Koski, P. (2008). Epidemiology of *Gyrodactylus salaris* (Monogenea) in the River Tornionjoki, a Baltic wild salmon river. *Journal of Fish Diseases* **31**, 373-382. 10.1111/j.1365-2761.2008.00916.x

Bakke, T. A. (1991). A review of the inter- and intraspecific variability in salmonid hosts to laboratory infections with *Gyrodactylus salaris* Malmberg. *Aquaculture* **98**, 303-310.

Bakke, T. A., Cable, J. & Harris, P. D. (2007). The Biology of Gyrodactylid Monogeneans: The "Russian-Doll Killers". *Advances in Parasitology* **64**, 161-376.

Bakke, T. A., Harris, P. D. & Cable, J. (2002). Host specificity dynamics: observations on gyrodactylid monogeneans. *International Journal for Parasitology* **32**, 281-308.

Bakke, T. A., Harris, P. D., Jansen, P. A. & Hansen, L. P. (1992). Host specificity and dispersal strategy in gyrodactylid monogeneans, with particular reference to *Gyrodactylus salaris* Malmberg (Platyhelminthes, Monogenea). *Diseases of Aquatic Organisms* **13**, 63-74.

Bakke, T. A., Jansen, P. A. & Hansen, L. P. (1990). Differences in the host-resistance of Atlantic salmon, *Salmo salar* L, stocks to the Monogenean *Gyrodactylus salaris* Malmberg, 1957. *Journal of Fish Biology* **37**, 577-587.

Bakke, T. A., Jansen, P. A. & Harris, P. D. (1996). Differences in susceptibility of anadromous and resident stocks of Arctic charr to infections of *Gyrodactylus salaris* under experimental conditions. *Journal of Fish Biology* **49**, 341-351.

Bakke, T. A., Jansen, P. A. & Kennedy, C. R. (1991). The host specificity of *Gyrodactylus salaris* Malmberg (Platyhelmintes, Monogenea): susceptibility of *Oncorhynchus mykiss* (Walbaum) under experimental conditions. *Journal of Fish Biology* **39**, 45-57.

Boeger, W. A. & Kritsky, D. C. (1993). Phylogeny and a revised classification of the monogenoidea Bychowsky, 1937 (Platyhelminthes). *Systematic Parasitology* **26**, 1-32.

Boeger, W. A., Kritsky, D. C. & Pie, M. R. (2003). Context of diversification of the viviparous Gyrodactylidae (Platyhelminthes, Monogenoidea). *Zoologica Scripta* **32**, 437-448.

Boeger, W. A., Kritsky, D. C., Pie, M. R. & Engers, K. B. (2005). Mode of transmission, host switching, and escape from the red queen by viviparous gyrodactylids (monogenoidea). *Journal of Parasitology* **91**, 1000-1007.

Boeger, W. A. & Popazoglo, F. (1995). Neotropical Monogenoidea .23. Two new species of *Gyrodactylus* (Gyrodactylidae) from a cichlid and an Erythrinid fish of southeastern Brazil. *Memorias Do Instituto Oswaldo Cruz* **90**, 689-694.

Buchmann, K. & Lindenstrom, T. (2002). Interactions between monogenean parasites and their fish hosts. *International Journal for Parasitology* **32**, 309-319.

Cable, J., Harris, P. D. & Bakke, T. A. (2000). Population growth of *Gyrodactylus salaris* (Monogenea) on Norwegian and Baltic Atlantic salmon (*Salmo salar)* stocks. *Parasitology* **121**, 621-629.

Cable, J. & van Oosterhout, C. (2007a). The impact of parasites on the life history evolution of guppies (*Poecilia reticulata*): The effects of host size on parasite virulence. *International Journal for Parasitology* **37**, 1449-1458.

Cable, J. & van Oosterhout, C. (2007b). The role of innate and acquired resistance in two natural populations of guppies (*Poecilia reticulata*) infected with the ectoparasite *Gyrodactylus turnbulli. Biological Journal of the Linnean Society* **90**, 647-655.

Cohen, J. (1977). *Reproduction*. London: Butterworths.

Cusack, R. & Cone, D. K. (1986). *Gyrodactylus salmonis* (Yin and Sproston, 1948) parasitizing fry of *Salvelinus fontinalis* (Mitchill). *Journal of Wildlife Diseases* **22**, 209-213.

Elliot, M. J. (1994). *Quantitative Ecology and the Brown Trout*. New York: Oxford University Press Inc.

Hansen, H., Bachmann, L. & Bakke, T. A. (2003). Mitochondrial DNA variation of *Gyrodactylus* spp. (Monogenea, Gyrodactylidae) populations infecting Atlantic salmon, grayling, and rainbow trout in Norway and Sweden. *International Journal for Parasitology* **33**, 1471-1478.

Harris, P. D. (1993). Interactions between Reproduction and Population Biology in Gyrodactylid Monogeneans - a Review. *Bulletin Francais De La Peche Et De La Pisciculture*, 47-65.

Harris, P. D., Jansen, P. A. & Bakke, T. A. (1994). The population age structure and reproductive biology of *Gyrodactylus salaris* Malmberg (Monogenea). *Parasitology* **108** 167-173.

Harris, P. D., Shinn, A. P., Cable, J. & Bakke, T. A. (2004). Nominal species of the genus *Gyrodactylus* von Nordmann 1832 (Monogenea : Gyrodactylidae), with a list of principal host species. *Systematic Parasitology* **59**, 1-27.

Harris, P. D., Soleng, A. & Bakke, T. A. (2000). Increased susceptibility of salmonids to the monogenean *Gyrodactylus salaris* following administration of hydrocortisone acetate. *Parasitology* **120**, 57-64.

Heggberget, T. G. (1981). Habitat selection and segregation of parr of Arctic charr (*Salvelinus alpinus*), brown trout (*Salmo trutta*) and Atlantic salmon (*Salmo salar* L.) in two streams i North Norway. In *Biology of the Arctic charr: Proceedings of the*

International Symposium on Arctic charr (Burns, L. J. B., ed.). The University of Manitoba, Winnipeg: The University of Manitoba Press, Winnipeg.

Heggberget, T. G. & Johnsen, B. O. (1982). Infestations by *Gyrodactylus* sp. of Atlantic salmon, *Salmo salar* L., in Norwegian rivers. *Journal of Fish Biology* **21**, 15-26.

Jansen, P. A. & Bakke, T. A. (1991). Temperature-dependent reproduction and survival of *Gyrodactylus salaris* Malmberg, 1957 (Platyhelminthes: Monogenea) on Atlantic salmon (*Salmo salar L.*). *Parasitology* **102**, 105-112.

Johnsen, B. O., Brabrand, Å., Jansen, P. A., Teien, H. C. & Bremset, G. (2008). Evaluering av bekjempelsesmetoder for *Gyrodactylus salaris*. DN, Direktoratet for naturforvaltning (Rapport fra ekspertgruppe).

Johnsen, B. O. & Jensen, A. J. (1991). The *Gyrodactylus* story in Norway. *Aquaculture* **98**, 289-302.

Johnsen, B. O., Møkkelgjerd, P. I. & Jensen, A. J. (1999). The parasite *Gyrodactylus salaris* on salmon parr in Norwegian rivers, status report at the beginning of year 2000. NINA Oppdragsmelding (in Norwegian, English summary). 1-129

Kania, P., 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.

Klemetsen, A. (1987). Røye. In *Fisk i ferskvann : økologi og ressursforvaltning / redaktører: Reidar Borgstrøm og Lars Petter Hansen*, pp. 79-87. Oslo: Landbruksforl.

Knudsen, R., Adolfsen, P., Sandring, S., Kristoffersen, R., Rikardsen, A. & Siikavoupio, S. (2007). The suitability of anadromous Arctic charr as host and vector of the monogenean *Gyrodactylus salaris*. *Ecology of Freshwater Fish* **16**, 99-104.

Knudsen, R., Rikardsen, A., Kristoffersen, R., Sandring, S. & Siikavoupio, S. (2004). Registrering av *Gyrodactylus* spp. på fiskesamfunnet i Signaldalselva og Kitdalselva i Troms 2003. NINA Oppdragsmelding (817) (In Norwegian, English summary). 1-24

Kristoffersen, R., Rikardsen, A., Winger, A. C., Knudsen, R. & Adolfsen, P. (2005). Registrering av *Gyrodactylus* spp. på fiskesamfunnet i Skibotnelva i Troms 2004. NINA Rapport (63) (in Norwegian, English summary). 1-27

Lester, R. J. & Adams, J. R. (1974). A simple model of a *Gyrodactylus* population. *International Journal for Parasitology* **4**, 497-506.

Lindenstrom, T, & Buchmann, K (2000). Acquired resistance in rainbow trout against *Gyrodactylus derjavini*. *Journal of Helminthology* **74**, 155-160.

Mo, T. A. (1988). *Gyrodactylus*-undersøkelsene av fisk i forbindelse med rotenonbehandlingen av Skibotnelva i august 1988. Universitetet i Oslo (in Norwegian). 1-14

Mo, T. A. (1994). Status of *Gyrodactylus salaris* problems and research in Norway. Vetrenary Institute of Oslo

Olstad, K., Cable, J., Robertsen, G. & Bakke, T. A. (2006). Unpredicted transmission strategy of *Gyrodactylus salaris* (Monogenea : Gyrodactylidae): survival and infectivity of parasites on dead hosts. *Parasitology* **133**, 33-41.

Olstad, K., Robertsen, G., Bachmann, L. & Bakke, T. A. (2007). Variation in host preferencen within *Gyrodactylus salaris* (Monogenea): an experimental approach. *Parasitology* **134**, 589-597. 10.1017/S0031182006001715

Poleo, A. B. S., Schjolden, J., Hansen, H., Bakke, T. A., Mo, T. A., Rosseland, B. O. & Lydersen, E. (2004). The effect of various metals on *Gyrodactylus salaris* (Platyhelminthes, Monogenea) infections in Atlantic salmon (*Salmo salar*). *Parasitology* **128**, 169-177.

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

Rikardsen, A. H., Diserud, O. H., Elliot, M. J., Dempson, B., Sturlaugson, J. & Jensen, A. J. (2007). The marine temperature and depth preferences of Arctic charr (*Salvelinus alpinus*) and sea trout (*Salmo trutta*), as recorded by data storage tags. *Fisheries Oceanography* **16**, 436 - 447.

Robertsen, G., Hansen, H., Bachmann, L. & Bakke, T. A. (2007). Arctic charr (*Salvelinus alpinus*) is a suitable host for *Gyrodactylus salaris* (Monogenea, Gyrodactylidea) in Norway. *Parasitology* **134**, 1-11.

Robertsen, G., Olstad, K., Plaisance, L., Bachmann, L. & Bakke, T. A. (2008). *Gyrodactylus salaris* (Monogenea, Gyrodactylidae) infections on resident Arctic charr (*Salvelinus alpinus*) in southern Norway. *Environmental Biology of Fishes* **83**, 99-105. doi: 10.1007/s10641-007-9228-3

Rotllant, J., Pavlidis, M., Kentouri, M., Adad, M. E. & Tort, L. (1997). Non-specific immune responses in the red porgy *Pagrus pagrus* after crowding stress. *Aquaculture* **156**, 279-290.

Scott, M. E. (1985). Dynamics of challenge infections of *Gyrodactylus bullatarudis* Turnbull (Monogenea) on guppies, *Poecilia reticulata* (Peters). *Journal of Fish Diseases* **8**, 495-503.

Scott, M. E. & Anderson, R. M. (1984). The population dynamics of *Gyrodactylus bullatarudis* (Monogenea) within laboratory populations of the fish host *Poecilia reticulata*. *Parasitology* **89**, 159-194.

Scott, M. E. & Nokes, D. J. (1984). Temperature dependent reproduction and survival of *Gyrodactylus bullatarudis* (Monogenea) on guppies (*Poecilia reticulata*). *Parasitology* **89**, 221-227.

Scott, M. E. & Robinson, M. A. (1984). Challenge infections of *Gyrodactylus bullatarudis* (Monogenea) on guppies, *Poecilia reticulata* (Peters), following treatment. *Journal of Fish Biology* **24**, 581-586.

Soleng, A. & Bakke, T. A. (1997). Salinity tolerance of *Gyrodactylus salaris* (Platyhelminthes, Monogenea): laboratory studies. *Canadian Journal of Fisheries and Aquatic Sciences* **54**, 1837-1845.

Soleng, A., Jansen, P. & Bakke, T. A. (1999). Transmission of the monogenean *Gyrodactylus salaris*. *Folia Parasitologica* **46**, 179-184.

Thorstad, E. B., Johnsen, B. O., Forseth, T., Alfredsen, K., Berg, O. K., Bremset, G., Fjellstad, H.-P., Grande, R., Lund, E., Myhre, K. O. & Ugedal, O. (2001). Fiskesperrer som supplement eller alternativ til kjemisk behandling av vassdrag infisert med *Gyrodactylus salaris*. DN-utredning (in Norwegian). 1-66

Tort, L., Gomez, E., Montero, D. & Sunyer, J. O. (1996a). Serum haemolytic and agglutinating activity as indicators of fish immunocompetence: Their suitability in stress and dietary studies. *Aquaculture International* **4**, 31-41.

Tort, L., Sunyer, J. O., Gomez, E. & Molinero, A. (1996b). Crowding stress induces changes in serum haemolytic and agglutinating activity in the gilthead sea bream *Sparus aurata*. *Veterinary Immunology and Immunopathology* **51**, 179-188.

van Oosterhout, C., Potter, R., Wright, H. & Cable, J. (2007). Gyro-scope: An individual-based computer model to forecast gyrodactylid infections on fish hosts. *International Journal for Parasitology* **38**, 541-548.

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