Increased susceptibility of salmonids to the monogenean *Gyrodactylus salaris* following administration of hydrocortisone acetate

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SUMMARY

Gyrodactylus salaris infects numerous salmonid species, ranging from the fully susceptible (Norwegian strains of *Salmo salar*), through species which, though initially susceptible, eventually eliminate their infections (*Salvelinus alpinus* and *S. fontinalis*) to entirely resistant (*Salmo trutta*) species. Here we describe experiments in which *Salvelinus alpinus*, *S. fontinalis* and *Salmo trutta*, implanted with hydrocortisone acetate to simulate stress-induced immunosuppression, were challenged with *G. salaris*. With previously uninfected *Salvelinus fontinalis*, *G. salaris* infections on fish treated with hydrocortisone acetate grew larger, and for longer, than on sham-treated controls. A similar result was obtained with *S. trutta*. Patterns of infection on Arctic charr, *Salvelinus alpinus*, were more complex, because individual fish varied from susceptible to highly resistant. Fish were therefore initially infected with *G. salaris*, and the most highly resistant group of individuals identified and disinfected. After 6 months recovery from this primary infection, hydrocortisone acetate was administered to half the fish, and all were challenged with *G. salaris*. Parasite populations on the hydrocortisone-treated individuals were consistently larger than those on the sham-treated controls, exceeding 30 parasites per fish after 5 weeks, in comparison with less than 10 parasites per fish on controls. These results indicate that hydrocortisone administration can lead to enhanced gyrodactylid populations on a range of salmonids. This suggests that the response to *G. salaris* is mediated by the immune system, and that the spectrum of responses observed in different species are, at least in part, due to the same mechanism. At a practical level, stress-induced immunosuppression during handling and transport of cultured salmonids may prove an important factor in the dissemination of *G. salaris* between watersheds.

Key words: cortisol, *Salvelinus alpinus*, *Salvelinus fontinalis*, stress, immunosuppression.

INTRODUCTION

Gyrodactylus salaris Malmberg, 1957 is a significant pathogen of wild and managed populations of Atlantic salmon (*Salmo salar* L.) in rivers in Norway, along the Swedish west coast and along the Russian White Sea coast (Johnsen & Jensen, 1991; Alenäs, Malmberg & Carlstrand, 1998; Bakke & Harris, 1998). Experimental infections of susceptible salmonids are characterized by exponential parasite population growth, until the host is overwhelmed (Bakke, Jansen & Hansen, 1990*a*; Bakke & MacKenzie, 1993). Most gyrodactylids are, however, non-pathogenic, with an initial phase of rapid population growth giving way to a period of decline (Lester & Adams, 1974; Scott, 1982, 1985; Cusack & Cone, 1986), and eventually the parasites may disappear, or persist at a much reduced population size. This decline is probably host mediated, because there is evidence of memory (Scott & Robinson, 1984; Richards & Chubb, 1996), and the response depends upon host genotype (Madhavi & Anderson, 1985; Lyles, 1990; Jansen, Bakke & Hansen, 1991). Norwegian Atlantic salmon are therefore considered highly susceptible to *G. salaris*, although some stocks respond to infection at high parasite burdens (Bakke & MacKenzie, 1993; Jansen & Bakke, 1993), but there is a strong response in other salmonids, including Baltic salmon (Bakke *et al.* 1990*a*), brown trout, *Salmo trutta* L. (Jansen & Bakke, 1995), rainbow trout, *Oncorhynchus mykiss* (Walbaum) (Bakke, Jansen & Kennedy, 1991*a*), brook trout, *Salvelinus fontinalis* (Mitchill) (Bakke, Harris & Jansen, 1992*a*), lake trout, *S. namaycush* (Walbaum) (Bakke, Jansen & Grande, 1992*b*), and Arctic charr, *S. alpinus* (L.) (Bakke, Jansen & Harris, 1996). The much reduced response of Norwegian Atlantic salmon is therefore aberrant when compared both to other gyrodactylid host interactions, and to the interaction of *G. salaris* with other salmonids.

The nature of any host response to gyrodactylids is basically unknown, but recent studies have indicated a possible role for the complement system (Moore, Kaattari & Olson, 1994; Harris, Soleng & Bakke, 1998; Buchmann, 1998) in resistance.

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Complement is also involved in resistance to other salmonid pathogens (Forward & Woo, 1996) and serum complement titres may correlate with resistance to a number of diseases (Røed *et al*. 1992; Hollebecq *et al*. 1995). Buchmann (1999) has summarized knowledge of the response to gyrodactylids, suggesting that responses are predominantly local, triggered by cytokine-mediated signalling between epidermal malphigian cells, mucous cells and cells of the immune system within the epidermis.

The stress hormone cortisol has been linked with disease susceptibility in salmonids (Pickering & Pottinger, 1989; Espelid *et al*. 1996), and Fevolden & Røed (1993) have shown that fish selected for a high cortisol response following confinement had reduced serum haemolytic activity due to complement. Cortisol titre also provides a link between stress and disease resistance in fish (Pickering & Pottinger, 1989; Wendelaar Bonga, 1997; Bakke & Harris, 1998), and stress predisposes fish to gyrodactylid infection (Khalil, 1970; Lester & Adams, 1974). Finally, Lindenstrøm & Buchmann (1998) have recently shown that dexamethasone, an immunosuppressant with similar action to cortisol, increases susceptibility of rainbow trout to *G. derjavini*.

Host specificity in *G. salaris* is believed to involve at least 2 mechanisms (Bakke *et al*. 1992*c*). Differences in susceptibility of *S. fontinalis* and *S. alpinus*, and of different strains of *S. salar*, may be due to differences in the immune response to the parasite (Bakke *et al*. 1992*c*), but the absolute failure of the parasite to establish on *S. trutta* is thought to be due to innate resistance (Jansen & Bakke, 1995). Cortisol administration, with its variety of immunosuppressive effects, can be used as a tool to probe the role of the immune system in determining susceptibility to *G. salaris*. Here we report on the effect of hydrocortisone acetate implants (Maule, Schreck & Kaattari, 1987; Carlson, Anderson & Bodammer, 1993) on growth of *G. salaris* populations on brown trout (*S. trutta*), brook trout (*S. fontinalis*) and Arctic charr (*S. alpinus*). These hosts were chosen because of the diversity of their responses to *G. salaris* in previous studies. *Salvelinus fontinalis* were generally susceptible with a highly repeatable induced response (Bakke *et al*. 1992*a*), with parasite populations growing to a size of approximately 40 individuals after 28 days before disappearing from the fish. *Salmo trutta*, on the other hand, was innately resistant to *G. salaris* (Jansen & Bakke, 1995). Few *G. salaris* attached to this host, and infections generally disappeared within 21 days. Finally, Arctic charr (*S. alpinus*) were chosen for their heterogeneity of response (Bakke *et al*. 1996). We first screened charr with a primary infection of *G. salaris*, selecting those which responded strongly. These fish, after a period of recovery, were then used for hydrocortisone acetate-induced immunosuppression experiments.

MATERIALS AND METHODS

Gyrodactylus salaris were collected from heavily infected salmon parr electrofished from the river Lierelva (Buskerud County, 40 km west of Oslo) and maintained at 12 °C in the laboratory for up to 1 month before experiments began. All fish were maintained in dechlorinated, charcoal filtered, continuously running (2 l/min) Oslo tapwater under constant dim illumination. Fish were fed unmedicated pellet food (Ewos). Basic experimental protocols, including infection and disinfection procedures, have been reported elsewhere (Bakke *et al*. 1991*a*; Bakke, Jansen & Hansen, 1991*b*). Individual experimental protocols varied and will be described separately. Parasite population size is expressed as abundance (Margolis *et al*. 1982), that is the mean parasite suprapopulation size calculated using all infrapopulations, including those containing zero parasites.

Effects of hydrocortisone acetate-induced immunosuppression on primary G. salaris *infections on brook trout*

Naive brook trout (*S. fontinalis*, mean weight 15·1 g, mean fork length 11·9 cm) were obtained from Syrtveit fish farm (Aust-Agder County), and routinely disinfected with formalin (1 : 4000 for 1 h). After a period of acclimation, they were divided into 4 groups of approximately 50 fish, which were placed in grey plastic tanks (1 × 1 m, 0·3 m water level). Each group formed 1 experimental treatment. One group received hydrocortisone acetate implants. Hydrocortisone acetate (Sigma) was dissolved in warmed coconut oil to a final concentration of 20 mg/ml. The oil was then injected (100 μl per fish) into the body cavity of fish anaesthetized with 0·04 % chlorbutanol. Fish were returned to the experimental tanks, where they quickly recovered. A second group of brook trout (sham controls) received coconut oil-implants alone. A third group received injections of 100 μl of sterile saline to control for handling and injection protocols, while a final group was not handled. After a further 7 days acclimation, all fish were experimentally infected with *G. salaris* attached to pieces of salmon fin (see Soleng *et al*. 1999). After 48 h to allow infection, the fish were returned to the experimental arena, and monitoring of infections began. Parasite burdens were monitored weekly on anaesthetized fish (15 fish chosen at random from each of the 4 groups). All parasites visible on the external surface were counted using a stereomicroscope. Fish were returned to the tanks, where they quickly recovered. The experiment continued for 3 months.

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Effects of hydrocortisone acetate-induced immunosuppression on primary G. salaris *infections of brown trout*

Migratory trout (*S. trutta*, mean weight 3·9 g, mean fork length 7·2 cm), river Lierelva stock, were obtained from DOFA hatchery (Buskerud County), disinfected and acclimatized in the laboratory for about 50 days. Batches of fish were implanted with hydrocortisone acetate in coconut oil ($n = 24$), or with coconut oil alone ($n = 24$), as described above. After a further week of acclimatization, fish were infected with *G. salaris* attached to pieces of salmon fin, and after 24 h returned to the experimental tanks. Infections were monitored every 7 days for 6 weeks (15 fish chosen at random from each of the 2 groups).

Effect of hydrocortisone acetate-induced immunosuppression on secondary G. salaris *infections of Arctic charr*

Anadromous Arctic charr (*S. alpinus*, mean weight 2·3 g, mean fork length 6·6 cm), River Alta stock, were obtained from Talvik hatchery (Finnmark County), disinfected and acclimatized in the laboratory for 70 days. They were then infested with *G. salaris* using the methods described above and individually isolated in mesh cages (0·38 × 0·27 m, 0·12 m waterlevel) as described by Bakke *et al.* (1996). Infections on individual fish were followed for 5 weeks, after which the charr were divided into 3 groups (susceptible, moderately resistant, highly resistant), based upon the behaviour of their *G. salaris* infections. After 7 further days (total period of infection 6 weeks), all fish were disinfected by placing them in a 1:4000 formalin solution for 1 h. The highly resistant fish were kept for 28 weeks to ensure full recovery from their primary infections before use in subsequent experiments. They were fed *ad libitum*, growing to a mean weight of 6·9 g and a mean length of 9·3 cm. The fish were then divided into 2 groups. One group was implanted with hydrocortisone acetate in coconut oil as described ($n = 22$), while the other group received coconut oil alone ($n = 15$). Fish were returned to the tanks for 1 week, prior to infection with *G. salaris* as described above. Infections were then monitored every 7 days for 6 weeks (15 fish chosen at random from each of the 2 groups).

RESULTS

Infections on brook trout

Initial abundance on brook trout ranged from 16·5 (range 8–45) on the coconut oil-injected controls to 32·6 (range 7–62) on the uninjected controls, with a prevalence of 100 % in all 4 groups (Fig. 1). After 7 days, abundance varied from 18·1 (range 7–37) in coconut oil-injected controls up to 29·5 (range 9–58) in hydrocortisone acetate-treated fish (Fig. 1). Prevalence remained at 100 % until 21–28 days after infection, when it began to decline in all except the hydrocortisone acetate-treated fish. The weekly abundance failed to increase on any host group except the hydrocortisone acetate-treated fish, and infections declined steadily from day 7 onwards, reaching a mean of less than 2 parasites per fish by the end of the experiment. The hydrocortisone acetate-treated fish, however, showed a radically different pattern of parasite population growth, differing significantly ($P < 0·05$, paired t-test on $\log_{10}$ transformed data) from that on control groups from 14 days onwards until the end of the experiment. On hydrocortisone acetate-treated fish the population grew slowly for up to 35 days, reaching a mean of 44·2 (range 0–59) parasites per fish. Subsequently the infection on these fish declined, but still remained significantly larger ($P < 0·05$, paired t-test on $\log_{10}$ transformed data) than that on control fish until the end of the experiment.

Infections on brown trout

Brown trout proved less susceptible than brook trout at the start of the infection, and carried burdens of 5–9 parasites when the experiment began (prevalence 100 % in both groups). On the coconut oil-implanted controls, abundance declined throughout, reaching 0·5 (range 0–4) parasites per fish at the end of 6 weeks (Fig. 2). On hydrocortisone acetate-treated fish, however, a different pattern was noted. On these fish abundance increased for the entire period of the experiment, and finally attained 16·3 (range 2–72) parasites per fish. This difference in pattern was significantly different from that of the control fishes from 14 days until the end of the experiment ($P < 0·05$, paired t-test on $\log_{10}$ transformed samples). The prevalence declined throughout the experiment in the coconut oil-implanted controls (13·3 % at the end), while it varied between 83·3 and 100 % in the hydrocortisone acetate-treated fish (100 % at the end).

Infections on Arctic charr

The initial infections showed a heterogeneity of response similar to that described by Bakke *et al.* (1996), with fish being classified as fully susceptible (infection grew initially but was then limited by a host reaction); moderately resistant (infection failed to grow but did not decline substantially); or highly resistant (infection declined throughout). The trajectories of these initial infections on naive fish are

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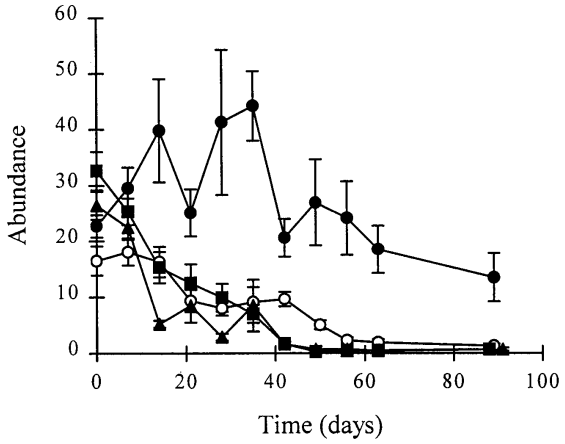

Fig. 1. Course of infection of *Gyrodactylus salaris* on Brook trout (*Salvelinus fontinalis*) following implantation with hydrocortisone acetate in coconut oil (●), with coconut oil alone (○), or following injection with saline (▲). Control fish (■) were not injected. Mean abundance, bars indicate standard error.

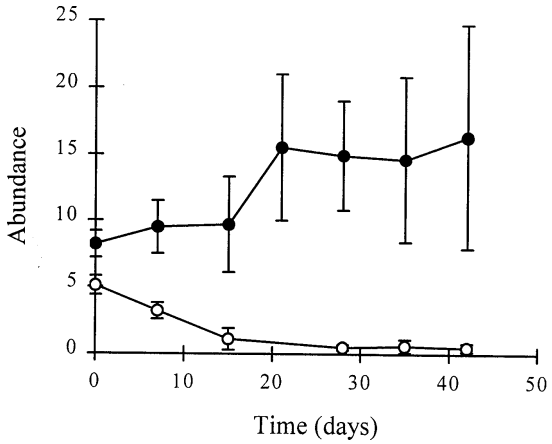

Fig. 2. Course of infection of *Gyrodactylus salaris* on brown trout implanted with hydrocortisone acetate in coconut oil (●) or with coconut oil alone (○). Mean abundance, bars indicate standard error.

shown in Fig. 3A–C. The highly resistant fish were chosen for further experimentation (Fig. 3C). The course of the secondary infection on these previously highly resistant fish in the presence and absence of hydrocortisone acetate is shown in Fig. 3D. The initial parasite burdens ranged between 30 and 40 parasites per fish, and after a short period of initial increase (<14 days) the infections declined. However, the rate of decline was much slower in the hydrocortisone acetate-treated fish than in the coconut oil-implanted control fish, and the difference in the course of infection was significantly different from that on control fishes from 14 days until the end of the experiment ($P < 0{\cdot}05$, paired t-test on $\log_{10}$ transformed samples). By the end of the experiment, after 6 weeks, the abundance on coconut oil-implanted controls had declined to 4·4 (range 0–14) parasites per fish, while remaining at approximately 35 (range 5–175) on hydrocortisone acetate-treated fish (Fig. 3B). The prevalence remained at 100% throughout the experiment in the hydrocortisone acetate-treated fish, while in the coconut oil-implanted controls prevalence declined to 80·0% at the end.

DISCUSSION

Hydrocortisone acetate implants led to elevated *G. salaris* populations in all 3 host species used in this work. In brook trout, treatment of fish with hydrocortisone acetate was the only treatment having this effect, while untreated, saline injected and coconut oil-implanted fish showed a similar pattern of elimination of parasite infections over a period of several weeks. Growth of the *G. salaris* population did not take place in the control fish groups. On both brook trout and Arctic charr, the effect of hydrocortisone acetate implantation was not clearly noticeable during the intial growth phase of the infection, but became progressively more apparent after the infection began to decline. On brown trout, the parasite population failed to grow on sham-implanted fish, and behaved similarly to that observed by Jansen & Bakke (1995). On hydrocortisone acetate-implanted fish, however, *G. salaris* grew substantially, achieving populations of up to 16 parasites per fish after 42 days. This represents most unusual behaviour for *G. salaris* on this host strain, which normally fails to support parasite population growth (Jansen & Bakke, 1995). Similar results, of improved parasite survival and population growth, were also noted for *G. derjavini* on rainbow trout treated with the dexamethasone (Lindenstrøm & Buchmann, 1998), which mimics some of the actions of cortisol.

Hydrocortisone acetate and dexamethasone are immunosuppressants which increase susceptibility to parasite disease (Pickering & Pottinger, 1989; Bakke & Harris, 1998). The results observed in this work, and by Lindenstrøm & Buchmann (1998) are likely to be due to immunosuppression, confirming that gyrodactylid population growth is normally limited by aspects of the host immune response. The nature of this response remains unknown, but both Buchmann (1998) and Harris *et al*. (1998) have shown susceptibility to complement in serum and mucus which, in the case of *G. salaris*, is physiologically relevant (Harris *et al*. 1998) and may be part of the normal response. Several authors (Sunyer *et al*. 1995; Tort *et al*. 1996*a*, *b*) have noted the down regulation of complement activity by stress, suggesting a possible mechanism for the action of hydrocortisone acetate in reducing the intensity of the host response against *G. salaris*. However, cortisol also has a variety of other actions on the immune system of fishes, including modification of leucocyte distribution, dynamics and function (Wendelaar

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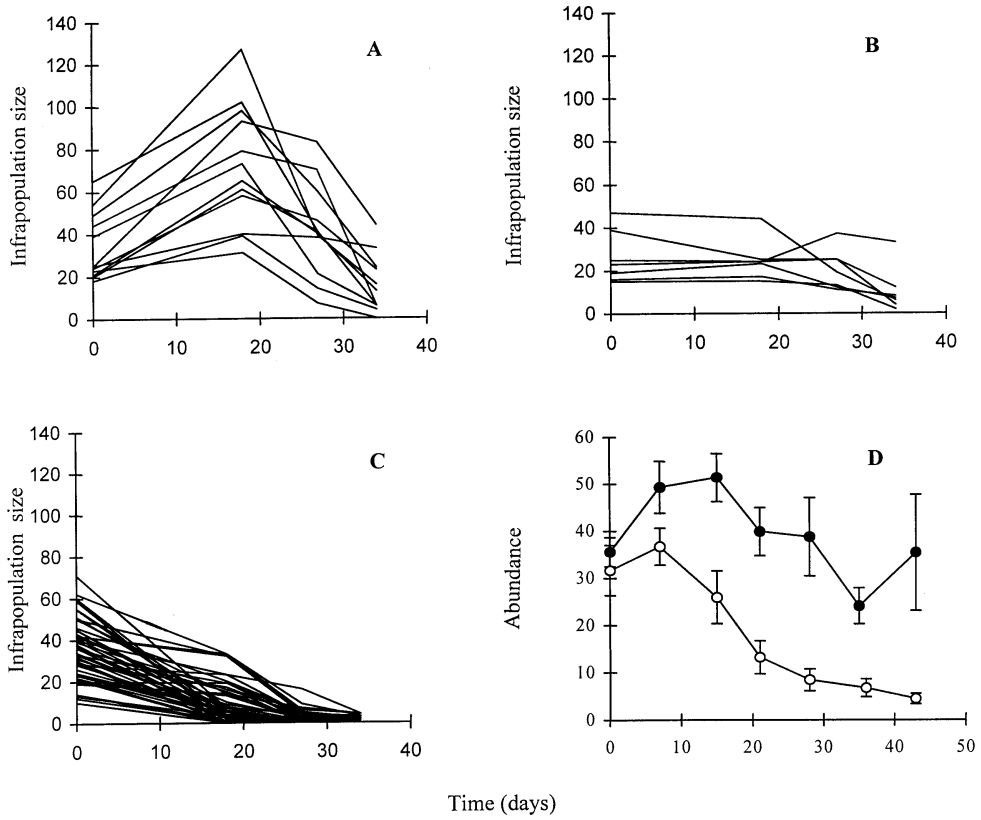

Fig. 3. Course of *Gyrodactylus salaris* infection on Arctic charr. (A–C) Results of primary infection to screen charr for susceptibility to *G. salaris*. (A) Individual trajectories of susceptible fish ($n = 12$). (B) Individual trajectories of moderately resistant fish ($n = 7$). (C) Individual trajectories of highly resistant fish ($n = 52$). (D) Pattern of challenge infection on fish from group (C) (highly resistant), 6 months after primary infection, in fish which were first implanted with hydrocortisone acetate in coconut oil (●), or with coconut oil alone (○). Values in (D) represent mean abundance, bars indicate standard error.

Bonga, 1997) and can influence skin structure and function through its role as a mineralocorticoid hormone (Wendelaar Bonga, 1997). Further experimental work is therefore needed to fully dissect the mode of action of cortisol in increasing susceptibility to *G. salaris*. In particular, our lack of data on the serum concentration of cortisol in implanted fishes means that further experiments using natural sources of stress (e.g. crowding) to elevate blood cortisol will be important in understanding the relevance of cortisol-induced immunosuppression in controlling susceptibility to gyrodactylids.

It has previously been argued (e.g. Bakke *et al.* 1992*c*) that several mechanisms regulate the pattern of specificity observed in *G. salaris*. For example, brown trout were considered innately resistant (Jansen & Bakke, 1995) because they failed to support parasite population growth, whereas resistance in *Salvelinus* was considered induced and due to the host response (Bakke *et al.* 1992*a*, *b*, 1996). The current work has shown impairment of the response following hydrocortisone acetate administration in both hosts, suggesting that the spectrum of observed response to *G. salaris*, from innate resistance (brown trout and some Arctic charr) through acquired resistance (brook trout, Arctic charr, rainbow trout and Neva strain of salmon) to susceptibility (Ims strain of Atlantic salmon: unpublished results) may be mediated through a single mechanism. Further work on the nature of the response to *G. salaris* in normal and immunosuppressed salmonids may help to clarify this problem.

Stress is a generalized immunosuppressant which elevates blood cortisol (Henderson & Garland, 1980; Barton & Iwama, 1991), impairs immune function (Barton & Iwama, 1991; Carlson *et al.* 1993) and predisposes to disease (Pickering, 1989; Pickering & Duston, 1983; Pickering & Pottinger, 1989). The source of the stress is unimportant (Bakke & Harris, 1998), and environmental stressors such as habitat modification, unfavourable temperature, hypoxia, sediment loads and pollution (Schreck, 1981; Wedemeyer & McLeay, 1981), dietary (Blazer & Wolke, 1984) or social (Noakes & Leatherland, 1977; Ejike & Schreck, 1980) stressors, or handling (Sunyer *et al.* 1995) and crowding (Tort *et al.* 1996*a*, *b*; Rotllant *et al.* 1997), can all lead to elevated plasma cortisol accompanied by immunosuppression. The evidence that crowding can lead to immunosuppression may be particularly relevant in the case of the *G. salaris* epidemic in Norway. *G. salaris* is primarily a parasite

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of salmon parr, which live in schools and are territorial, each fish defending a home area against other parr. The shoals are age structured, containing up to 5 age classes in the north of Norway. Crowding may therefore stress, and immunosuppress, the subordinate parr in these shoals (Pickering & Stewart, 1984; Wendelaar Bonga, 1997). Although *G. salaris* was almost certainly introduced into Norway in the early 1970's (Mo, 1994), high stocking densities (Halvorsen & Hartvigsen, 1989) may have contributed to stress levels, and therefore to the magnitude of the epidemic. Introduction of hatchery-reared fish into waters containing a wild population is known to influence dominance hierarchies (Kalleberg, 1958; Symons, 1971) and could conceivably result in reduced performance because of the increased stress attributable to artificial, elevated densities and social pressures (Schreck, 1981). Future management should therefore take account of potential stress levels in the affected fish. An additional implication arising from this present work concerns the importance of immunosuppression when transshipping salmonids. The experiments on the host specificity of *G. salaris* (Bakke & Sharp, 1990; Bakke *et al*. 1990*a*, 1991*a*, *b*, 1992*a*, *b*, 1996; Bakke, Jansen & Brabrand, 1990*b*; Jansen & Bakke, 1995; Soleng & Bakke, 1998) were performed under controlled laboratory conditions minimizing stress. Management information, for example the risk of spreading the pathogen on rainbow trout (Bakke *et al*. 1991*a*) but not on brown trout (Jansen & Bakke, 1995) has been derived from these studies. However, transshipment is itself stressful (Sunyer *et al*. 1995), and our results suggest that *G. salaris* could infect any salmonid if levels of stress experienced are sufficient to induce immunosuppression. Extreme care should therefore be taken when transporting all salmonid species to ensure that *G. salaris* is not transferred to any currently unaffected watercourses with immunosuppressed hosts.

Immunosuppression is also evident in smolting and maturing salmonids when the natural level of plasma cortisol increases and the fish become more sensitive to stressors (Richards & Pickering, 1978; Pickering & Christie, 1980), and Buchmann (1997) has reported enhancement of *G. derjavini* population growth following administration of testosterone to the host, suggesting that sexual maturation may also increase susceptibility to gyrodactylids. Experimental studies of gyrodactylid specificity on salmonids tend to utilize immature hosts, and it is important to consider the potential impact of other life-history stages on the epidemiology of these parasites.

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