Acquired resistance in rainbow trout against *Gyrodactylus derjavini*

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Abstract

Investigations were conducted on the host response in rainbow trout and the associated changes in mucous cell density during infection with the skin monogenean *Gyrodactylus derjavini*. Parasite populations increased on all naive hosts and peaked 4–5 weeks p.i. after which infection levels decreased. Introduction of naive fish into responding host populations resulted in heavy infections of the naive fish, whereas parasite expulsion continued in the responding host groups showing an acquired, non-sterile immunity. This non-sterile immunity lasted at least a month as these hosts were refractory to reinfection despite being exposed to a high infection pressure. Mucous cell hyperplasia was seen in some groups during the intermediary phase of infection, but at the termination of the study a significant depletion was evident. Passive immunization of naive host (with sera from immune hosts) did not confer protection. This indicates differences between host responses to *G. derjavini* compared to responses against other pathogens where such a passive immunity has been described.

Introduction

Infections with ectoparasitic monogeneans are known to induce partial protective host responses in many teleosts. Thus, infection of the European eel with the gill inhabiting monogeneans *Pseudodactylogyrus anguillae* and *P. bini* induces a relative acquired immunity to reinfection (Slotved & Buchmann, 1993). Teleosts are also known to mount host responses against most monogeneans belonging to the genus *Gyrodactylus*, as parasites seem to be actively expelled during later stages of infection (e.g. Lester & Adams, 1974; Scott, 1982; Cusack, 1986; Cone & Cusack, 1988; Bakke *et al*., 1992; Richards & Chubb, 1996; Buchmann & Bresciani, 1998; Lindenstrøm & Buchmann, 1998). Several studies have indicated the crucial importance of epidermal mucous cells in the response against gyrodactylid infections, and both the quantity of these cells (Lester, 1972; Heggberget & Johnsen, 1982; Cusack, 1986; Wells & Cone, 1990; Lindenstrøm & Buchmann, 1998; Sterud *et al*., 1998), as well as various humoral and cellular factors (Buchmann & Bresciani, 1998, 1999; Harris *et al*., 1997, 1998) seem to play a role in these skin reactions. The present study was conducted in order to characterize parasite population dynamics in trials, where naive fish were introduced into responding host populations and to correlate epidermal changes with infection intensities. Thus, mucous cell density was followed during the course of infection. Furthermore, it was tested if adoptive transfer of immune sera could confer protection against *Gyrodactylus derjavini*.

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Materials and methods

Fish

Parasite-free fry of rainbow trout *Oncorhynchus mykiss* (Walbaum, 1792) raised in a pathogen-free closed system with recirculated water were delivered by Fisher Fish, Zealand. The fish (mean body weight 1.4 g; SD 0.2 g) were acclimatized before the trials and were held in 200 l aquaria half-filled with aerated local tap water (pH 7.4–7.5, $NO_3^- < 10\ mg\ l^{-1}$, $NH_4^+ < 0.5\ mg\ l^{-1}$ and $NO_2^- < 0.075\ mg\ l^{-1}$). Three-quarters of the water volume was exchanged twice a week and the fish were fed a restricted diet of pelleted food equivalent to 6% of the biomass

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divided into two weekly feedings. All infection experiments, including parasite enumeration, were conducted in a thermostat-regulated room with a temperature of 11–12°C and a 12 : 12 h light–dark cycle.

Parasites

Parasites from a laboratory batch of *Gyrodactylus derjavini* Mikailov, 1975 were used in the infection experiments. This strain of *G. derjavini* was originally obtained from a Danish trout farm in the south-eastern part of Jutland (Paelebro Dambrug). The parasite population had been maintained in the laboratory for more than a year through serial passage on susceptible hosts infected by cohabitation.

Infection procedure

Two groups of 68 and 61 fish, respectively, were infected by exposing them in 10 l aquaria with free parasites as described in Buchmann & Uldal (1997). Six of these infection aquaria were used, each containing 175–445 parasites and 12–26 recipients. The mean parasite load per recipient were approximately equal in the six infection aquaria, ranging from 12 to 18. Fish were exposed to the detached parasites for 24 h. After the infection procedure, fish were pooled and transferred to two 200 l aquaria and constituted the before-mentioned experimental groups (A: N = 68; B: N = 61). The parasite populations were monitored weekly for a total of 70 days. After 34 days, when the infections of the host populations in both groups showed signs of decline, 30 naive fish were transferred to each fish tank for infection by cohabitation (host group Aa and Bb respectively). These fish were taken from two uninfected control groups of 60 fish each and had their adipose fin cut to differentiate them from the primed, responding fish. Parasite population dynamics were followed weekly in both primed (A and B) and non-primed (Aa and Bb) subgroups of hosts.

Monitoring of infection and site specificity

Parasite enumeration was carried out on anaesthetized fish (50 ppm MS222) using a dissecting microscope with sub-illumination (7–50 × magnification). The total number of parasites on each fish was recorded, and site specificity was assessed according to Buchmann & Uldal (1997). All fish in each subgroup of hosts were examined at each counting.

Sampling and fixation of fish

To assess the density of mucous cells in the epidermis, ten fish from each group were sampled at random. Sampling occurred at day 0, 34 and 64 (day 30 for introduced hosts) p.i. Fish were fixed in 10% phosphate-buffered formalin (pH 7.1). Fish were fixed up to three months before processing.

Mucous cell count

Determination of the density of the superficial epidermal mucous cells (goblet cells) was carried out on the caudal fin as this is an important microhabitat for *G. derjavini* (e.g. Buchmann & Uldal, 1997; Lindenstrøm & Buchmann, 1998). Mucous cells were stained by the Alcian Blue method according to Buchmann & Bresciani (1998). Briefly, fixed fins were rinsed for 10 min in distilled water followed by 25 min staining in 1% Alcian Blue in 3% acetic acid. Following a final wash of 25 min in distilled water, fins were mounted in Aquamount. Using a compound microscope (200 × magnification), mucous cells were counted in five zones of each 0.61 mm^2 successively placed from the edge of the caudal fin towards the tail peduncle. The density of mucous cells for each subgroup of hosts to the various day p.i. was thus expressed as the mean of 50 counts (five zones, ten fish).

Passive immunization

Immune sera were obtained from 12 rainbow trout (20–25 cm) infected with *G. derjavini* for more than 12 months. Blood samples were taken by caudal vein puncture without heparin. Sera was pooled and half of the volume was heat-inactivated at 44°C for 20 min (Sakai, 1992). Non-immune sera were obtained from naive rainbow trout (pooled from six fish) and was likewise heat treated in order to inactivate complement.

The trials included 45 rainbow trout (4–5 cm) in three groups of 15 fish each. Each fish received 0.1 ml serum by intraperitoneal injections under anaesthesia (80 mg ml^{-1} MS222) (Gudmundsdóttir & Magnadóttir, 1997). One group comprising 15 fish received native immune sera (with active complement) and were subsequently tagged by cutting off the lower part of the caudal fin. The second group, which was marked by cutting off the upper lobe of the caudal fin, contained 15 fish that were administered heat-inactivated immune sera (with heat-inactivated complement). The last group of 15 untagged fish received non-immune sera (with heat-inactivated complement) and served as a control group.

The three groups of fish were infected by cohabitation with 26 *G. derjavini*-infected fish in a 200 litre aquarium. These donor fish had 50% of their caudal fin cut off vertically so as to differentiate them from fish in the experimental groups. Infection levels in the three experimental groups were monitored as previously described. The first parasite enumeration was carried out at day 4 p.i. and thereafter infections were monitored at weekly intervals for a total of 32 days.

Data processing and statistics

Infection levels were, according to Margolis *et al.* (1982), expressed as prevalence and abundance. Variance to mean ratios were likewise calculated. Microhabitat distribution was expressed as the percentage of the parasite population recorded in a particular site (Buchmann & Uldal, 1997) as well as the mean number of parasites in that location. Differences in abundance were tested with a Mann-Whitney U-test; however a Kruskall-Wallis test was applied to test for differences in abundance between the three experimental groups in the passive immunization trial. As mucous cell density in the experimental groups was not normally distributed, a Kruskall-Wallis test was applied. Dunn's multiple

comparison test was subsequently used in order to isolate groups that differed significantly.

Results

Parasite population dynamics

All fish were infected at the first examination on day 2 p.i. Infection prevalences of 100% were evident in all subgroups of hosts throughout the entire investigation period, except for a small decrease in group B at day 44 (90.1%) and 58 p.i. (91.9%). No differences in abundance between group A and B could be detected at day 2 p.i. (fig. 1). Parasite populations increased on all naive host groups and peaked within 4–5 weeks p.i., whereafter infection levels decreased. In the introduced naive host groups (Aa and Bb), the highest abundances were however already seen after approximately 3 weeks (day 58/24 p.i.) (see fig. 1). Significant differences in abundance between group A and B were observed at day 23 and 29 p.i. and from day 44 p.i. and forth ($P < 0.05$). Nevertheless, no significant difference between these groups could be detected at day 34 p.i. when subgroups Aa and Bb were introduced ($P > 0.05$ – see fig. 1). The introduction of these naive fish into responding host populations resulted in heavy infections in the naive fish, whereas the process of parasite expulsion continued in the primed fish in group A and B (fig. 1). These immune fish remained infected at a very low level, not only at the termination of the study, but also at an additional counting at day 111 p.i., where abundances of 10.9 and 11.4 were observed for group A and B respectively (data not shown). Significantly higher abundances were seen at all countings in group Aa when compared to group Bb ($P < 0.05$ – see fig. 1). In all host groups, the variance to mean ratio always exceeded unity and, on a temporal basis, reflected the pattern which was seen for the abundances (fig. 2). Mortality occurred in all groups but in comparable numbers in the various replicates. Thus, 14 fish died in group A compared to 16 in group B, whereas six hosts died in group Aa compared to seven in group Bb.

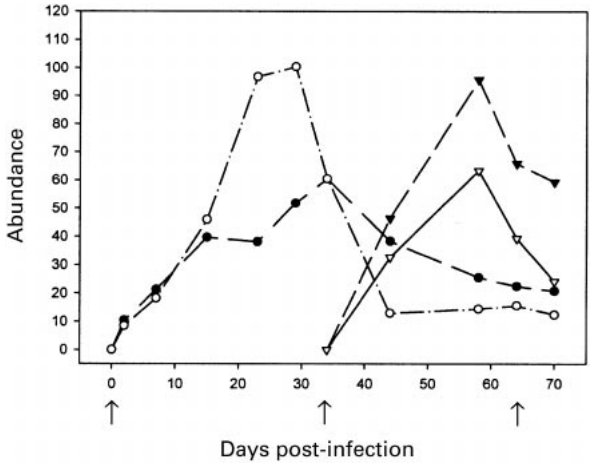

Fig. 1. Parasite abundance of *Gyrodactylus derjavini* infected rainbow trout. Group Aa (▼) and Bb (▽) (naive, non-primed fish) were introduced into responding host populations (group A (●) and B (○)) at day 34 p.i. Arrows indicate days were fish were sampled for epidermal investigations.

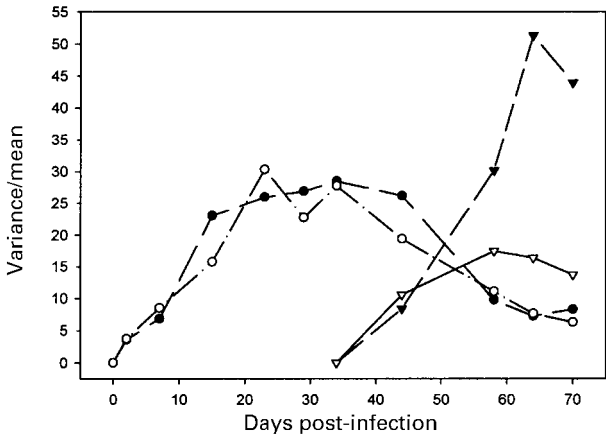

Fig. 2. Variance/mean ratios of *Gyrodactylus derjavini* infected rainbow trout. ●, Group A (primed); ○, group B (primed); ▼, group Aa (non-primed); ▽, group Bb (non-primed).

Microhabitat distribution

During the initial phase of infection, caudal, anal, pectoral and pelvic fins served as important microhabitats in all host groups. As the infection progressed, a distinct decrease in the proportion of parasites parasitizing anal, pectoral and pelvic fins was evident. During the same period, the caudal fin became a more important microhabitat, and from week 1 to week 3 p.i. the proportion of the parasite population observed in this locality increased from 10–15% to 50% in both group A and B (data not shown). In the terminal phase of infection, the corneal surface was covered by a significant proportion of the parasites. From harbouring around 5% of the parasite population in the first week, up to 20% could be observed at this locality at 5–6 weeks p.i.

Mucous cell density

No significant differences in the uninfected controls were seen over time (fig. 3; $P = 0.405$). A highly significant difference in the mean number of mucous cells among the various groups was observed ($P < 0.001$). Dunn's multiple comparison test revealed a significantly higher mucous cell density in group A at day 34 p.i. compared to both corresponding controls ($P < 0.05$). Thirty days after the introduction of the naive host groups (day 64/30 p.i.), one of these groups (Aa) had a significantly higher number of goblet cells compared to the uninfected control group 2 ($P < 0.05$) – but not control group 1 ($P > 0.05$) (fig. 3). At this time, the remaining groups (A, B and Bb) exhibited a significantly lower number of mucous cells compared to both controls ($P < 0.05$ – see fig. 3). Thus an approximately 50% reduction in the mean number of mucous cells could be observed in later stages of infection.

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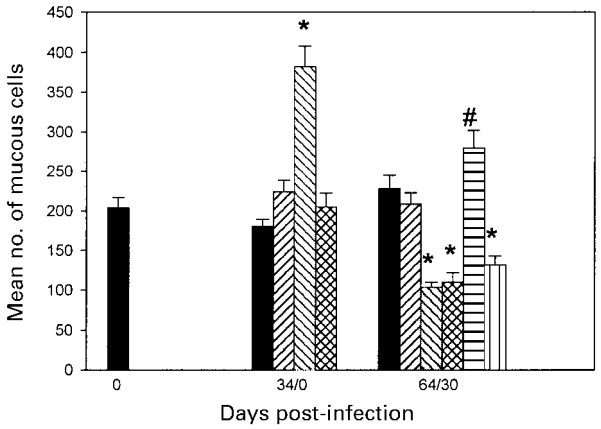

Fig. 3. Mucous cell density of caudal fins in rainbow trout infected with *Gyrodactylus derjavini*. Bars represent the mean of 50 countings. Error bars show S.E. Numbers behind slashes represent days post-infection for the introduced groups. ■ Uninfected, control 1; ▨ uninfected, control 2; ▧ infected group A (primed); ▩ infected group B (primed); ▤ infected group Aa (non-primed); ▥ infected group Bb (non-primed). *Significantly different from both uninfected groups. #Significantly different from uninfected control group 2.

Passive immunization

Infection prevalences were 100 in all groups injected with serum from day 11 p.i. and forth. No significant differences were observed at any time between the three groups during the experimental period (P ranging from $P = 0.603$ (day 4 p.i.) to $P = 0.900$ (day 11 p.i.)). Infection levels increased in all three groups and attained highest values at the termination of the study (fig. 4).

Discussion

The observed increase in infection levels within the first few weeks post-infection followed by a more or less distinct decrease, confirms earlier studies of this particular parasite–host system (Buchmann & Uldal, 1997; Andersen & Buchmann, 1998; Lindenstrøm & Buchmann, 1998). Many other *Gyrodactylus*/fish host systems show similar patterns (e.g. Lester & Adams, 1974; Scott, 1982; Cusack, 1986; Cone & Cusack, 1988; Bakke *et al*., 1992; Richards & Chubb, 1996). The observed infection pattern is ascribed to host responses against *Gyrodactylus*. This view-point is supported by the fact that immunosuppressed hosts seem less well equipped to combat *Gyrodactylus* infections (Harris *et al*., 1997; Lindenstrøm & Buchmann, 1998). The present study adds further evidence to the assumption that rainbow trout respond against *G. derjavini*, as the introduction of naive fish into responding host populations resulted in heavy infections of naive fish, whereas primed fish remained infected at a very low level. Despite the fact that the primed fish were exposed to high infection pressures during the last weeks of the study, no increase in infection levels could be detected in these immune fish.

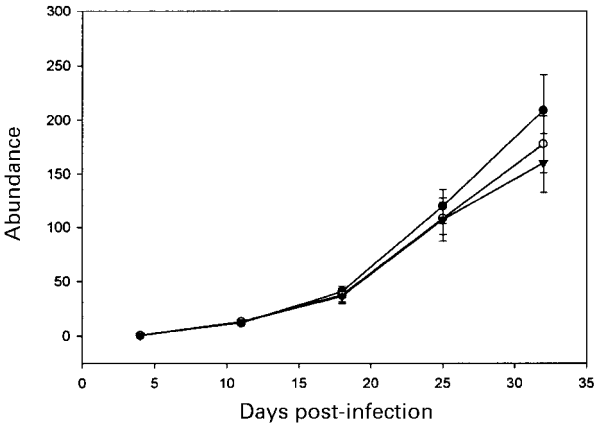

Fig. 4. Parasite abundance of *Gyrodactylus derjavini* infected rainbow trout passively immunized with decomplemented non-immune sera (●) and immune sera with (▼) and without (○) complement. Error bars represent S.E.

No changes in the density of mucous cells were observed in uninfected fish whereas the mean number of mucous cells changed significantly during infection with *G. derjavini*. Thus, at day 34 p.i. a marked mucous cell hyperplasia was evident in group A compared to uninfected fish. A similar goblet cell hyperplasia was observed in group Aa after 30 days of infection (64/30 p.i.). Nevertheless, this initial hyperplasia was replaced at the termination of the experiment by a significant depletion in three of the four experimental groups, constituting an approximately 50% reduction in mucous cell density. Infection with *G. derjavini* in rainbow trout has earlier been shown to induce a depression in mucous cell density of a similar magnitude (40%) (Lindenstrøm & Buchmann, 1998) corresponding to infection with *G. colemanensis* (Wells & Cone, 1990) and *G. salaris* (Sterud *et al*., 1998). The present study shows that the density of mucous cells is rather plastic, and can change rapidly during infection. The present data document that proliferation and exhaustion of mucous cells is a coupled process as originally suggested by Pottinger *et al*. (1984). Such a connection has also been observed by Urawa (1992) in chum salmon infected with *Icthyobodo necator*. It is possible that infections with gyrodactylids and other ectoparasites induce goblet cell proliferation eventually leading to hyperplasia. During heavy and/or prolonged/sustained infections, depletion of mucous cells in epidermis could be an effect of the continuing discharge of mucus from these cells as a means to combat infection. Buchmann (1999) has suggested the involvement of interleukin-like factors (especially IL-1) in this process, as IL-1 is known to have a pronounced effect on fish epidermis (Balm *et al*., 1995) and in mammals evidently serves as a mucus secretagogue (Cohan *et al*., 1991). If so, the exhaustion of the epidermis could be related to the magnitude of the injury exerted by the gyrodactylids – and thus to the infection intensity. Interestingly, the density of parasites on caudal fins from fish in group A, which showed goblet cell hyperplasia at day 34 p.i., only attained values half as high as in group B, where no increases in mucous cell density could be detected. Thus, low or intermediate infections could lead to the mucous

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cell hyperplasia seen in group A, whereas high infections due to the greater associated injury seem to initiate an exhaustion of goblet cells, which could account for the lack of increase in these cells as observed in group B. The depletion of mucous cells evident in both groups at the termination of the study could be ascribed to the prolonged effect of the parasites.

Quantitative as well as qualitative changes in mucus and goblet cells are known to be involved in the expulsion of some mammalian gastro-intestinal helminth infections (e.g. Miller & Nawa, 1979; Castro & Harari, 1982; Fujino & Fried, 1993; Ishikawa *et al*., 1994) and differences in the intestinal responses have been found between high and low responder animals during primary infection (Manjili *et al*., 1998). Studies in mammals indicate the direct involvement of the immune system, as goblet cell hyperplasia can be adoptively transferred by immune lymphocytes (Miller & Nawa, 1979). The precise mechanism responsible for mucous cell hyperplasia during infection is presently unknown, but, at least in mammals, seems to be attributable to the cytokine profile secreted from leukocytes (McKenzie *et al*., 1998).

A passive immunization trial was conducted in the present study, but it was not possible to confer naive fish even partial resistance to infection with *G. derjavini*. No attempt to detect or characterize the existence of specific antibodies was done in the present study, but previous studies could not detect specific *G. derjavini*- antibodies in serum from infected rainbow trout (Buchmann, 1998b). Specific anti-monogenean antibodies are seldomly reported in the literature, but have been described in infections with *Dactylogyrus* (Vladimirov, 1971), *Pseudodactylogyrus* (Buchmann, 1993) and *Heterobothrium* (Wang *et al*., 1997). Furthermore, it is not known if antibodies against *Gyrodactylus* are allocated to mucosal surfaces. Nevertheless, these passive immunization trials have shown that the non-sterile immunity elicited by rainbow trout infected with *G. derjavini* cannot be achieved by transfer of sera from immune to naive hosts, which is in contrast to reports of successful passive immunization of fish against bacterial (Viele *et al*., 1980; Gudmundsdóttir & Magnadóttir, 1997) and viral (Houghton & Ellis, 1996) infections using comparable protocols. Although it cannot be excluded that the transferred components had a too short survival time in the recipients to affect infection, the findings seem to suggest that the elimination of *G. derjavini* is not primarily attributed to a systemic humoral component. Combined with the quantitative and qualitative (Buchmann, 1999) alterations seen in the epidermis of *Gyrodactylus*-infected fish, these observations further strengthen the viewpoint that localized epidermal responses play a crucial role in the elimination of gyrodactylid infections. The emerging knowledge from *in vitro* studies, showing the extreme sensitivity of *Gyrodactylus* to killing by the alternative complement pathway (Buchmann, 1998b; Harris *et al*., 1998), the colonization of *G. derjavini* by macrophages and subsequent killing of the parasite by macrophage-derived complement and ROMs (Buchmann & Bresciani, 1999), strongly suggest the involvement of the non-specific effector arm of the fish immune system against *Gyrodactylus* infections.

References

Andersen, P.S. & Buchmann, K. (1998) Temperature dependent population growth of *Gyrodactylus derjavini* on rainbow trout. *Journal of Helminthology* **72**, 9–14.

Bakke, T.A., Harris, P.D. & Jansen, P.D. (1992) The susceptibility of *Salvelinus fontinalis* (Mitchill) to *Gyrodactylus salaris* Malmberg (Platyhelminthes, Monogenea) under experimental conditions. *Journal of Fish Biology* **41**, 499–507.

Balm, P.H.M., Lieshout, E. van, Lokate, J. & Wendelaar Bonga, S.E. (1995) Bacterial lipopolysaccharide (LPS) and interleukin 1 (IL-1) exert multiple physiological effects in the tilapia *Oreochromis mossambicus* (Teleostei). *Journal of Comparative Physiology B* **165**, 85–92.

Buchmann, K. (1993) A note on the humoral immune response of infected *Anguilla anguilla* against the gill monogenean *Pseudodactylogyrus bini*. *Fish and Shellfish Immunology* **3**, 397–399.

Buchmann, K. (1998a) Some histochemical characteristics of the mucous microenvironment in four salmonids with different susceptibilities to gyrodactylid infections. *Journal of Helminthology* **72**, 101–107.

Buchmann, K. (1998b) Binding and lethal effect of complement from *Oncorhynchus mykiss* on *Gyrodactylus derjavini* (Platyhelminthes, Monogenea). *Diseases of Aquatic Organisms* **32**, 195–200.

Buchmann, K. (1999) Immune mechanisms in fish skin against monogenean infections – a model. *Folia Parasitologia* **46**, 1–9.

Buchmann, K. & Bresciani, J. (1998) Microenvironment of *Gyrodactylus derjavini* on rainbow trout *Oncorhynchus mykiss*: association between mucous cell density in skin and site selection. *Parasitology Research* **84**, 17–24.

Buchmann, K. & Bresciani, J. (1999) Rainbow trout leucocyte activity: influence on the ectoparasitic monogenean *Gyrodactylus derjavini*. *Diseases of Aquatic Organisms* **35**, 13–22.

Buchmann, K. & Uldal, A. (1997) *Gyrodactylus derjavini* infections in four salmonids: comparative host susceptibility and site selection of parasites. *Diseases of Aquatic Organisms* **28**, 201–209.

Castro, G.A. & Harari, Y. (1982) Intestinal epithelial membrane changes in rats immune to *Trichinella spiralis*. *Molecular and Biochemical Parasitology* **6**, 191–204.

Cohan, V.L., Scott, A.L., Dinarello, C.A. & Prendergast, R.A. (1991) Interleukin-1 is a mucus secretagogue. *Cellular Immunology* **136**, 425–434.

Cone, D.K. & Cusack, R. (1988) A study of *Gyrodactylus colmanensis* Mizelle and Kritsky, 1967 and *Gyrodactylus salmonis* (Yin and Sproston, 1948) (Monogenea) parasitizing captive salmonids in Nova Scotia. *Canadian Journal of Zoology* **66**, 409–415.

Cusack, R. (1986) Development of infections of *Gyrodactylus colemanensis* Mizelle and Kritsky, 1967 (Monogenea) and the effect on fry of *Salmo gardneri* Richardson. *Journal of Parasitology* **72**, 663–668.

Fujino, T. & Fried, B. (1993) *Echinostoma caproni* and *E. trivolvis* alter the binding of glycoconjugates in the intestinal mucosa of C3H mice as determined by lectin histochemistry. *Journal of Helminthology* **67**, 179–188.

Gudmundsdóttir, B.K. & Magnadóttir, B. (1997) Protection of Atlantic salmon (*Salmo salar* L.) against an experimental

infection of *Aeromonas salmonicida* ssp. *Achromogenes*. *Fish and Shellfish Immunology* **7**, 55–69.

Harris, P.D., Soleng, A. & Bakke, T.A. (1997) Cortisol induced immunosuppression renders brook trout (*Salvelinus fontinalis*) susceptible to *Gyrodactylus salaris* infection. *Bulletin of the Scandinavian Society for Parasitology* **7**, 70.

Harris, P.D., Soleng, A. & Bakke, T.A. (1998) Killing of *Gyrodactylus salaris* (Platyhelminthes, Monogenea) mediated by host complement. *Parasitology* **117**, 137–143.

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

Houghton, G. & Ellis, A.E. (1996) Pancreas disease in Atlantic salmon: serum neutralisation and passive immunisation. *Fish and Shellfish Immunology* **6**, 465–472.

Ishikawa, N., Horii, Y. & Nawa., Y. (1994) Inhibitory effects of concurrently present 'normal' *Nippostrongylus brasiliensis* worms on expulsion of 'damaged' worms and associated goblet cell changes in rats. *Parasite Immunology* **16**, 329–332.

Lester, R.J.G. (1972) Attachment of *Gyrodactylus* to *Gasterosteus* and host response. *Journal of Parasitology* **58**, 717–722.

Lester, R.J.G. & Adams, J.R. (1974) *Gyrodactylus alexanderi*: reproduction, mortality and effect on its host *Gasterosteus aculeatus*. *Canadian Journal of Zoology* **52**, 827–833.

Lindenstrøm, T. & Buchmann, K. (1998) Dexamethasone treatment increases susceptibility of rainbow trout, *Oncorhynchus mykiss* (Walbaum), to infections with *Gyrodactylus derjavini* Mikailov. *Journal of Fish Diseases* **21**, 29–38.

Manjili, M.H., France, M.P., Sangster, N.C. & Rothwell, T.L.W. (1998) Quantitative and qualitative changes in intestinal goblet cells during primary infection of *Trichostrongylus colubriformis* high and low responder guinea pigs. *International Journal for Parasitology* **28**, 761–765.

Margolis, L., Esch, G.W., Holmes, J.C., Kuris, A.M. & Schad, G.A. (1982) The use of ecological terms in parasitology. *Journal of Parasitology* **68**, 131–133.

McKenzie, G.J., Bancroft, A., Grencis, R.K. & McKenzie, A.M.J. (1998) A distinct role for interleukin-13 in Th2-cell-mediated immune responses. *Current Biology* **8**, 339–342.

Miller, H.R.P. & Nawa, Y. (1979) *Nippostrongylus brasiliensis*: intestinal goblet-cell response in adoptively immunized rats. *Experimental Parasitology* **47**, 81–90.

Pottinger, T.G., Pickering, A.D. & Blackstock, N. (1984) Ectoparasite induced changes in epidermal mucification of the brown trout, *Salmo trutta* L. *Journal of Fish Biology* **25**, 123–128.

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.

Sakai, D.K. (1992) Repertoire of complement in immunological defence mechanisms of fish. *Annual Review of Fish Diseases* **2**, 223–247.

Scott, M.E. (1982) Reproductive potential of *Gyrodactylus bullatarudis* (Monogenea) on guppies (*Poecilia reticulata*). *Parasitology* **85**, 217–236.

Slotved, H.C. & Buchmann, K. (1993) Acquired resistance of the eel, *Anguilla anguilla* L., to challenge infections with gill monogeneans. *Journal of Fish Diseases* **16**, 585–591.

Sterud, E., Harris, P.H. & Bakke, T.A. (1998) The influence of *Gyrodactylus salaris* Malmberg, 1957 (Monogenea) on the epidermis of Atlantic salmon, *Salmo salar* L., and brook trout, *Salvelinus fontinalis* (Mitchill), experimental studies. *Journal of Fish Diseases* **21**, 257–263.

Urawa, S. (1992) Epidermal responses of chum salmon (*Oncorhynchus keta*) fry to the ectoparasitic flagellate *Icthyobodo necator*. *Canadian Journal of Zoology* **70**, 1567–1575.

Viele, D., Kerstetter, T.H. & Sullivan, J. (1980) Adoptive transfer of immunity against *Vibrio anguillarum* in rainbow trout, *Salmo gairdneri* Richardson, vaccinated by the immersion method. *Journal of Fish Biology* **17**, 379–386.

Vladimirov, V.L. (1971) The immunity of fishes in the case of dactylogyrosis. *Parasitologiya* **5**, 51–58 (in Russian). English translation, Parasitology, Riverdale 1971, **1**, 58–68.

Wang, R., Kim, J-H., Sameshima, M. & Ogawa, K. (1997) Detection of antibodies against the monogenean *Heterobothrium okamotoi* in Tiger puffer by ELISA. *Fish Pathology* **32**, 179–180.

Wells, P.R. & Cone, D.K. (1990) Experimental studies on the effect of *Gyrodactylus colmanensis* and *G. salmonis* (Monogenea) on the density of mucous cells in the epidermis of fry of *Oncorhynchus mykiss*. *Journal of Fish Biology* **37**, 599–603.

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