Primming of host resistance to protect cultured rainbow trout *Oncorhynchus mykiss* against eye flukes and parasite-induced cataracts

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In the present study, immunologically naive rainbow trout *Oncorhynchus mykiss* were experimentally exposed to a low-level *Diplostomum spathaceum* (Trematoda) infection to stimulate acquired resistance and, along with unexposed controls, were subsequently exposed to natural infection for 8 weeks. The priming of the host resistance, designed to simulate a procedure applicable in aquaculture, decreased the number of establishing parasites compared to untreated controls by the end of the experiment. This effect was slow and did not protect the fish against the parasite-induced cataracts. The results suggest that this type of priming of host resistance is probably inefficient in preventing the deleterious effects of *D. spathaceum* infection in aquaculture conditions.

Key words: diplostomiasis; fish farming; host–parasite interactions; parasite resistance; Trematoda.

INTRODUCTION

Aquaculture facilities are ideal environments for a variety of parasitic pathogens (Rintamäki-Kinnunen & Valtonen, 1996, 1997; Hakalahti & Valtonen, 2003), which thrive in high densities of susceptible hosts showing unnaturally high rates of transmission. Infections are generally controlled with chemicals, or treated with medication, which significantly reduce host mortality and deleterious effects of infection. These methods, however, are also expensive and cause secondary effects such as stress to the host organisms as well as environmental pollution. Consequently, alternative methods are being sought to combat parasitic diseases. One of such method is the stimulation of natural resistance in hosts (Ellis, 1988a), which could reduce the harmful effects of the parasite in subsequent infections. If infections are kept to a minimum with treatments, diseases may be eradicated before natural resistance

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Typically, this can maintain high susceptibility among the hosts and can be problematic especially in cases where these hosts are reared for introduction to the wild where they are exposed to a range of new infections. The possibility to protect fishes against the harmful effects of infection from the eye fluke Diplostomum spathaceum was explored through experimental priming of host resistance.

*Diplostomum spathaceum* is an ubiquitous parasite of freshwater fishes, which completes its life cycle by passing through three host species, an avian definitive host and snail and fish intermediate hosts. In fishes, parasite metacercariae infect the eye lenses and cause cataracts, a disease known as diplostomiasis, in an intensity-dependent manner (Shariff *et al.*, 1980; Karvonen *et al.*, 2004a). Cataracts, on the other hand, cause a range of secondary effects such as impaired growth (Karvonen & Seppälä, 2008) and increased susceptibility to predation (Seppälä *et al.*, 2005). The parasite is also found in aquaculture (Stables & Chappell, 1986; Field & Irwin, 1994; Karvonen *et al.*, 2006), where it causes significant local problems in terms of reduced growth and condition of fishes. It is known that the infection elicits a range of different types of immune responses in fishes (Chappell *et al.*, 1994), which reduce the rate of infection in subsequent exposures (Whyte *et al.*, 1990; Karvonen *et al.*, 2005). The role of host resistance in protecting the fishes against cataracts, however, has received very little attention although this is of key importance from an applied perspective. Indeed, most of the previous studies have focused on details of immune responses in laboratory conditions (Chappell *et al.*, 1994), while the relationships between resistance, infection and cataracts in natural or semi-natural exposure conditions have remained virtually unexplored (Karvonen *et al.*, 2004b).

The present study experimentally investigated the role of resistance of fishes in preventing *D. spathaceum* infections and associated cataracts. The aim was to simulate procedures that could be applied in priming of host resistance in aquaculture. Fishes were first exposed to a low-level *D. spathaceum* infection (i.e. natural priming of resistance) in early summer before the peak in natural parasite exposure. Second, the exposed fish, as well as unexposed controls, were placed in cages in a lake for natural exposure. Rainbow trout *Oncorhynchus mykiss* (Walbaum) was used as a model species as it is one of the most common fish species in aquaculture and also susceptible to infection and cataracts (Karvonen *et al.*, 2004a, 2005). It was postulated that the priming of the host resistance would reduce the number of parasites becoming established in the fish, as well as the coverage of the parasite-induced cataracts.

**MATERIALS AND METHODS**

**PRIMING OF HOST RESISTANCE**

A stock group of 1100 1 year-old *O. mykiss* (mean ± s.e. total length, *L*<sub>T</sub>, 188-63 ± 2.94 mm) was obtained from a fish farm. The farm used a ground water source, which ensured that the fish had no prior experience of eye flukes. For the exposure protocol, 400 fish were used, which were distributed to eight tanks, each with 50 fish and 250 l of water. Four randomly chosen tanks then received a dose of 30 *D. spathaceum* cercariae per fish (total 1500 cercariae tank<sup>-1</sup>) for 30 min. Freshly emerged (<4 h old) cercariae were extracted from five naturally infected *Lymnaea stagnalis* and mixed in one suspension (Karvonen *et al.*, 2003). This ensured that the fish were exposed to multiple parasite genotypes (Rauch *et al.*, 2005). Snails were collected from the location of the caging experiment. The other four tanks received sham exposure with water without cercariae. Water temperature was raised to 15° C
for the time of exposure and 48 h after to ensure parasite infection success. After this, fish were maintained in the tanks in 250 l of water for 26 days (14 June to 10 July) when the water temperature corresponded to natural lake temperature (average 11.8 °C). It is important to note that the development of resistance in fishes takes a few weeks (Chappell et al., 1994), which is why the exposure of the fish was conducted early in the season so that they would acquire resistance by the time the natural exposure began. The water for the tanks was taken from the basin of the lake, which ensured that the fish acquired no further infections. As a precaution, the incoming water was also filtered. After 26 days, the mean ± s.e. number of parasites in the exposed fish was 2.17 ± 0.38, whereas the controls had no parasites. First exposure to D. spathaceum is known to elicit a variety of immune responses, basically mobilizing both non-specific and specific branches of the immune system (Chappell et al., 1994). To capture a comprehensive coverage of these processes, no particular immunological variable from the fish was determined, but the number of parasites in the eye lenses was used as a comparative measure of acquired resistance between the treatment groups.

**CAGING EXPERIMENT**

On 10 July 2006, 300 randomly chosen fish (remaining 100 fish were used in other experiments), taken evenly from all tanks, were placed in Lake Konnevesi (62° N; 26° E) in three cages. Each cage received 50 treated fish and 50 control fish, which were marked by clipping the adipose fin from one, randomly selected group within each cage under light anaesthesia (0.01% MS-222; www.sigmaaldrich.com). Cages were 120 cm × 80 cm × 100 cm and had a mesh-size of 10 mm, which allowed D. spathaceum cercariae to pass (Karvonen et al., 2004b). The size of the cages was large enough not to restrict the movement of fish, but small enough to ensure homogeneous exposure between individual fish. Fish can recognize the presence of cercariae in the water and reduce the rate of infection by avoiding exposure (Karvonen et al., 2004b). The small size of the cages, however, eliminated such a possibility and corresponded to conditions in tanks and ponds of a fish farm where high numbers of fish are kept in a limited space. Cages were anchored in the shallow littoral zone of the lake so that they floated at the surface c. 50 cm from the bottom. Fish were fed with commercial fish pellets every second day. After 2 weeks, 10 treated and 10 control fish were taken from each cage. A corresponding number of new fish from the stock group, marked with a different fin mark, was introduced to the cages to keep the fish density constant. Fish were brought to the laboratory and killed with 0.01% MS-222. Coverage of parasite-induced cataracts was determined from both eyes of each fish using slit-lamp microscopy (Karvonen et al., 2004a) and scored using a categorical scale: 0, no cataracts; 1, cataracts covering <25%; 2, cataracts covering 25–50%; 3, cataracts covering 50–75%; 4, cataracts covering 75–100% of the lens horizontal area and 5, lens opaque and whitish. The thickness of the cataracts was not measured. Eyes were then examined for the number of D. spathaceum metacercariae. Mean cataract coverage and total parasite number for each fish was calculated as an average and sum for right and left eye respectively. Previous studies at the same location using the same fish species have indicated a strong relationship between cataract coverage and number of parasites in the lens (Karvonen et al., 2004a). The sampling was repeated every second week for 8 weeks (i.e. four sampling times) so that all remaining fish (20 treated fish and 20 control fish cage⁻¹) were examined by the end of the experiment.

The data were analysed using MANCOVA with parasite number and cataract coverage as response variables, and treatment group (treated or control), time under exposure and cage as fixed factors. Fish $L_T$ was used as a covariate. Data were log_{10} transformed to meet the assumptions of the analysis when necessary. It is important to note that different individuals were examined at each sampling time so that the data did not require a repeated-measures analysis. Furthermore, the fish exposed to D. spathaceum already had a few parasites when introduced to the cages, but as these numbers were very low (mean ± s.e. 2.17 ± 0.38), this was unlikely to affect the observed result. Parasite numbers determined from individual fish taken from the same cage did not represent completely independent observations in a statistical sense. This is unlikely, however, to have an effect in this study because: (1) parasite cercariae are produced continuously from the snails so that no dilution in the level of exposure could
have occurred; (2) fish were evenly distributed within the cages and therefore encountered the cercariae randomly; and (3) the infection cannot be transmitted directly between fish.

Another three cages were placed to the same location in the lake on 15 May 2006, each with 100 unexposed fish. These fish were used to follow the timing of parasite transmission. Samples of 10 fish were taken from each cage every 2 weeks for 16 weeks (i.e. eight sampling times) so that the remaining fish were examined at the same time at the termination of the other experiment. Each time, a corresponding number of new fish from the stock group (fin clipped) were placed into cages to keep the fish density constant. Fish were fed and dissected for parasites, as described earlier. \( \log_{10} (x + 1) \) transformed data were analysed for differences between the sampling times using one-way ANOVA followed by Tukey’s multiple comparisons.

**ETHICAL NOTE**

Resistance of fish to *D. spathaceum* was induced using an experimental exposure of fish to the parasite cercariae and the cercarial dose of this exposure was based on earlier experience of the system (Karvonen et al., 2003). The level of infection in the fish after the caging ranged from 0 to 130 corresponding to infection intensities observed in wild fish populations (Valtonen & Gibson, 1997; Marcogliese et al., 2001). For example, Chappell (1969) recorded >200 metacercariae from an individual three-spined stickleback *Gasterosteus aculeatus* L., and Wootten (1974) found 550 metacercariae from an individual *O. mykiss*. Mortality of fish was low (15 fish from a total of 600) during the experiment. All fish appeared healthy, and no alterations in behaviour, feeding or growth were observed. The experiments used a total of 600 fish (as well as fish from the original stock group used in keeping the fish density constant), but more fish were initially exposed for purposes of other experiments. All fish were killed with an overdose of 0.01% MS-222, a commonly used fish anaesthetic. The experiment was carried out with permission from the Lab-Animal Care and Use Committee of the University of Jyväskylä (license number 8/27.3.2006).

**RESULTS**

Parasite numbers increased significantly in fish during caging in the lake, but there was no difference in parasite acquisition between the treatment groups [Table I and Fig. 1(a)]. When analysed separately for the sampling times, however, parasite numbers were lower in treated fish in the end of the experiment (*t*-test, *P* > 0.05).

### Table I. Result of MANCOVA on \( \log_{10} (x + 1) \) transformed numbers of *Diplostomum spathaceum*, and cataract coverage, in initially exposed and control *Oncorhynchus mykiss* caged in a lake for a period of 8 weeks. Fixed factors: status, exposed or control; time, time under exposure (2, 4, 6 and 8 weeks) and cage, cages 1, 2 and 3. Fish total length (\( L_T \)) was used as a covariate.

<table>
<thead>
<tr>
<th>Source</th>
<th>Pillai’s trace</th>
<th>d.f.</th>
<th>Error d.f.</th>
<th>( F )</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Status (S)</td>
<td>0.0002</td>
<td>2</td>
<td>267</td>
<td>0.033</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Time (T)</td>
<td>0.850</td>
<td>6</td>
<td>536</td>
<td>66.092</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cage (C)</td>
<td>0.025</td>
<td>4</td>
<td>536</td>
<td>1.680</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>( L_T )</td>
<td>0.021</td>
<td>2</td>
<td>267</td>
<td>2.796</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>S × T</td>
<td>0.032</td>
<td>6</td>
<td>536</td>
<td>1.475</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>S × C</td>
<td>0.035</td>
<td>4</td>
<td>536</td>
<td>2.392</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>T × C</td>
<td>0.048</td>
<td>12</td>
<td>536</td>
<td>1.101</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>S × T × C</td>
<td>0.087</td>
<td>12</td>
<td>536</td>
<td>2.022</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>
Figs. 1. Mean ± s.e. [n = 30 fish (control and exposed) except for 8 weeks when n = 60 fish (control and exposed)] (a) number of Diplostomum spathaceum and (b) coverage of parasite-induced cataracts in eyes of Oncorhynchus mykiss kept in three cages in a lake and studied for infection and cataracts every second week for a period of 8 weeks (□ control fish (no prior exposure); ■ fish exposed to D. spathaceum before the experiment. **, statistical significance (P < 0.01).

d.f. = 116, P < 0.01). There were no differences in parasite numbers between the groups at other sampling times (t-test, P > 0.05 for all). The pattern of infection was also similar between the cages (Table I). Coverage of cataracts also increased with time, but the pattern of cataract development did not differ either between the treatment groups [Table I and Fig. 1(b)]. On average, cataracts covered c. 75–100% of the lens area (mean 3.8 on the five-step scale) in both treatment groups in the end of the experiment. The $L_T$ of fish increased from 201.78 ± 2.46 to 222.2 ± 1.37 mm during the experiment, but $L_T$ had no effect on the pattern of infection (Table I).

Examination of the fish from the other three cages (following temporal dynamics of parasite transmission) indicated significant changes in the parasite transmission with time [one-way ANOVA on log$_{10}$ transformed data: d.f. 1 = 5, d.f. 2 = 217, P < 0.001 (the first two sampling times with no infections were excluded)]. Transmission began in mid-June with increasing water temperature, peaked in mid-July and decreased in August when parasite numbers in fish reached a plateau (Tukey’s multiple comparisons: $P > 0.05$ between the last three sampling times; Fig. 2). This indicates that the time when the treated and control fish groups were introduced into the lake (10 July) corresponded to the time of the highest parasite exposure. The $L_T$ of these fish
Fig. 2. Mean ± s.e. number (n = 30 fish, except week 16 when n = 75 fish) of Diplostomum spathaceum in eyes of Oncorhynchus mykiss (■) kept in three cages in a lake and studied for infection every second week for a period of 16 weeks showing the timing of the parasite transmission. | Water temperature in the lake during the experiment. Increased from 188.63 ± 2.94 to 222.17 ± 1.85 °C during the experiment. Of the total of 300 fish, 15 died during the caging and were excluded from the data.

DISCUSSION

A key question in disease prevention in aquaculture concerns the role of infections in stimulating natural resistance in the host; more specifically, if infections and their deleterious effects could be reduced by allowing some infection instead of rearing animals in virtually infection-free conditions. In the present study, this question was explored in the D. spathaceum and O. mykiss interaction by experimentally exposing the fish to a low-level infection before their introduction to natural infection conditions. These simulated conditions could be applied in fish farming by priming the resistance of the fish in early summer before the peak in natural parasite exposure. Contrary to expectations, acquired resistance had only a minor effect on the parasite numbers, which became evident at the end of the experiment. This had no effect on cataract intensities, however, which were high in both treatment groups, suggesting that the acquired resistance was insufficient to prevent the deleterious effects of infection.

Although the result can be affected by a range of factors, the small effect of acquired resistance on the parasite numbers is nevertheless surprising considering the previous evidence (Whyte et al., 1990; Karvonen et al., 2005). For example, earlier results from this system have shown that immunologically naive O. mykiss are highly susceptible to infection and that acquired resistance following a low-level infection can lower the infection rate by 90% in a single high-dose experimental exposure in the laboratory (Karvonen et al., 2005). The pattern of exposure in nature, however, is very different and may impose a different type of challenge for the fish immune system compared with single high-level exposure. In general, the relationships between the pattern of exposure and function of the immune system.
have received very little attention so, at present, this explanation remains speculative. It is also possible that the exposure procedure applied here did not elicit a maximal resistance in fish by the time of their introduction to cages although it had an effect on the parasite numbers at the end of the experiment. First, this may be related to water temperature during the experimental exposure (Ellis, 1988b), which was lower than the summer temperatures in fish farms. In terms of feasibility of the priming of the resistance in aquaculture conditions (note that the resistance takes a few weeks to develop), it would have to be conducted early in the season before the start of natural transmission following the increase in water temperature. Second, the efficiency of acquired resistance could also be affected by the parasite dose so that higher doses could yield stronger host response (Ellis, 1988b; LaPatra et al., 2000). In this system, higher doses would also result in higher initial parasite numbers in fish and subsequent cataract formation following the exposure procedure itself. This makes such procedures impractical.

In tanks and ponds of a fish farm, fishes generally have very limited opportunities to avoid exposure to *D. spathaceum* if cercariae are present in the water. These conditions were simulated in this study by keeping the fish in a restricted space. In an earlier study (Karvonen et al., 2004b), *O. mykiss* with a low-level initial infection were shown to develop intensive cataracts under such conditions. In that study, comparisons with uninfected controls could not be made. Fish had also gained the initial infection at a fish farm in uncontrolled conditions with varying doses, which may have affected the degree and variation in resistance at population and individual levels. The same study, however, also demonstrated that fish can react to the presence of cercariae and move away from the source of infection (Karvonen et al., 2004b), which may significantly reduce the degree of exposure and compensate apparently weak protection from resistance alone. Prevention of such behaviour may still provide the most plausible explanation for the low efficiency of the acquired resistance observed in the present study. This would suggest that any type of priming of fish resistance against the parasite may be of little significance in preventing the parasite-induced cataracts in fish farms, which emphasizes the importance of reduction in level of parasite exposure.

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References


