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Antibodies against *Discocotyle sagittata* (Monogenea) in farmed trout

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ABSTRACT: The relationship between *Discocotyle sagittata* intensities and host length, weight and specific anti-parasite antibody titres was studied in 3 year-classes of farmed rainbow trout *Oncorhynchus mykiss* and brown trout *Salmo trutta* at the end of the annual transmission cycle. Antibody titres were significantly higher in infected farmed fish than in naïve controls, indicating that infection elicits immunoglobulin production. No correlation was found between host size and parasite burdens, nor between infection intensities and antibody titres.

KEY WORDS: Trout · *Oncorhynchus mykiss* · *Salmo trutta* · Monogenea · *Discocotyle sagittata* · Antibodies

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Discocotyle sagittata is a monogenean parasite infecting freshwater salmonid fishes with a wide distribution in the northern hemisphere (Williams & Jones 1994). In common with other monogeneans (Thoney & Hargis 1991), *D. sagittata* occurs at low prevalence and intensity in wild fish, but can reach pathogenic levels of infection in farmed fishes. Temperature is the main abiotic factor affecting its development, which is virtually arrested with water temperatures <10°C (Gannicott & Tinsley 1997, 1998a,b). This results in clear annual transmission cycles: no infections occur during the winter, while transmission is continuous during the summer and until late autumn. Knowledge of *D. sagittata* biology enabled the establishment of a dependable experimental infection system (Rubio-Godoy & Tinsley 2002). In laboratory trials, rainbow trout were partially protected against controlled challenges following immunisation with *D. sagittata* extracts, and a significant negative correlation was found between antibody levels and parasite numbers recovered from immunised fish (Rubio-Godoy et al. 2003). This study aimed to determine whether infection under farm conditions induced the production of specific anti-parasite antibodies.

Materials and methods. Fish: Fish examined for the present investigation were raised in a fish farm in the Isle of Man, British Isles, where different year-classes and fish species were kept in separate ponds. Fish were weighed and measured after anaesthesia (MS-222), exsanguinated via the caudal vein, and the gills dissected and preserved in formalin for later analysis. In a survey at the end of the yearly transmission cycle in late November 2001, 20 rainbow trout *Oncorhynchus mykiss* from each of the 0+, 1+ and 2+ yr classes were sampled; brown trout *Salmo trutta* samples consisted of 17 × 0+, 20 × 1+ and 10 × 2+ yr class fish. Naïve fish used as controls came from a parasite-free farm in Denmark.

Infection levels: To calculate the mean parasite intensity (Bush et al. 1997), *Discocotyle sagittata* were recovered from the gills under a dissecting microscope. Parasite development was determined by counting the number of clamps present on the haptor; a mean developmental index (DI) was calculated for worms from individual fish, and mean DI are reported for each fish year-class (Rubio-Godoy & Tinsley 2002). DI values range from 1 for freshly attached worms with 1 pair of clamps, to 4.5 for sexually mature worms with 4 pairs of clamps.

Antibody titres: Blood samples were allowed to clot overnight at 4°C and sera were frozen (–70°C) until processed. Antibody titres were determined by ELISA, as described previously (Rubio-Godoy et al. 2003). Briefly, sonicated worms were used as antigenic source and each plate included 2 controls: blank wells (no primary antibodies from farmed fish) and sera from naïve fish, which were the same, irrespective of the year-class analysed. Sera were diluted serially up to a factor of 2¹³ (1:8194) and analysed using rabbit anti-salmon immunoglobulin (Buchmann & Pedersen 1994) as secondary, and peroxidase-conjugated goat anti-rabbit immunoglobulin [Sigma] as tertiary antibodies; values

greater than 2 times the (blank) background absorbance were considered to be positive. Titres are reported as the 2-fold dilution factor beyond which no response was detected. Results were validated against optical density readings at 492 nm obtained from naïve sera, which were the same in the different plates/assay lots (Rubio-Godoy et al. 2003).

Statistics: Worm intensities were compared with Kruskal-Wallis tests. Antibody titres and ELISA tests were analysed by 1-way ANOVA plus Tukey's post-hoc tests. Pearson correlations between parasite intensity and antibody titres, fish weight and length were calculated. Calculations were carried out with SPSS for Windows 10.0; the significance level was set at $p < 0.05$.

Results. Fish host and *Discocotyle sagittata* infection-characteristics are shown in Table 1. Prevalence of infection was $>50\%$ in all cases, and the mean intensities were significantly different between fish year-classes. The mean parasite DI calculated for each fish year-class shows the majority of parasites were at or approaching sexual maturity (value 4.5); however, the DI range found (1 to 4.5) indicates that worms from all developmental stages were present. Fig. 1 illustrates the specific anti-*D. sagittata* immunoglobulin titres determined for individual fish, as well as the correlation between these and parasite intensities. Mean antibody titres (detectable 2-fold dilution factors) \pm SE for different year-classes (0+, 1+ and 2+) were as follows: 0+ rainbow trout (RT), 8.0 ± 0.48 ; 1+ RT, 9.4 ± 0.53 ; 2+ RT, 8.7 ± 0.57 ; 0+ brown trout (BT), 8.8 ± 0.49 ; 1+ BT, 10.6 ± 0.67 ; 2+ BT, 7.7 ± 0.52 . ELISA tests were validated by ANOVA, demonstrating that OD readings from naïve fish were comparable in all plates. Most fish presented higher antibody titres than naïve fish (mean titre \pm SE of 5.8 ± 0.40); the mean titres determined for farmed fish were all significantly different from naïve controls. 1+ BT antibody titres were the highest overall, and significantly different from all other groups except 1+ RT. No significant correlation was found between fish length/weight and parasite intensity and antibody titre. The parasite intensity–antibody titre correlation was also not significant in all fish year-classes.

Discussion. The present study provides evidence for the occurrence in farmed trout of high antibody titres against the gill-inhabiting ectoparasite *Discocotyle sagittata*. Similarly, antibodies have been detected in carp against the monogenean *Dactylogyrus vastator* (Vladimirov 1971), in tiger puffer against *Heterobothrium okamotoi* (Wang et al. 1997), and in eels against *Pseudodactylogyrus bini* (Buchmann 1993) and *P. anguillae* (Mazzanti et al. 1999). In contrast, no elevated immunoglobulin levels were found in *Leiostomus xanthurus* infected with *Heteraxinoides xanthophilis* (Thoney & Bureson 1988), Japanese flounder harbouring *Neobenedeniagirellae* (Bondad-Reantaso et al. 1995), nor in rainbow trout infected with *Gyrodactylus derjavini* (Buchmann 1998). In *Oncorhynchus mykiss*, immunisation with *D. sagittata* extracts conferred partial protection and elicited the production of significant titres of antibodies; these correlated negatively with parasite burdens following controlled exposure, suggesting they may mediate protection (Rubio-Godoy et al. 2003).

In this study, elevated specific anti-parasite immunoglobulin titres were found in the 3 year-classes of both rainbow and brown trout. Despite quite different parasite burdens, antibody titres were comparable between rainbow and brown trout of the same age class, but no correlation was found between infection intensity and immunoglobulin levels in individual fish. Blood samples were obtained in November, towards the end of the annual transmission season. This probably facilitated the assessment of humoral immunity, because (1) parasite exposure would have been maximal, with invasion occurring continuously for the previous 6 mo since late May/early June; and (2) during summer, fish would have experienced optimal temperatures favouring the development of humoral immunity (Alcorn et al. 2002). Continuous transmission throughout the summer was confirmed in this survey by the presence of parasites in all stages of development. Field records (Rubio-Godoy & Tinsley unpubl.) indicate that fish-of-the-year (0+ fish) harbour no parasites at the beginning of the annual transmission cycle

Table 1. Descriptive statistics of farmed rainbow trout *Oncorhynchus mykiss* (RT) and brown trout *Salmo trutta* (BT) infected with *Discocotyle sagittata*. DI = developmental index calculated for parasites from individual fish

Fish year-class	n	Mean length (cm \pm SE)	Mean weight (g \pm SE)	Prevalence of infection (%)	Mean parasite intensity \pm SE	Mean DI (range)
0+ RT	20	14.6 \pm 0.3	39.1 \pm 2.2	85	2.5 \pm 0.3	3.8 (1–4.5)
1+ RT	20	27.0 \pm 0.3	220.6 \pm 8.5	100	19.5 \pm 1.6	3.7 (1–4.5)
2+ RT	20	37.3 \pm 0.4	690.1 \pm 29.5	100	132.4 \pm 17.0	2.7 (1–4.5)
0+ BT	17	13.4 \pm 0.4	30.4 \pm 2.5	88	11.0 \pm 2.6	3.4 (1–4.5)
1+ BT	20	27.8 \pm 0.4	256.2 \pm 12.3	55	2.5 \pm 0.4	2.6 (1.5–4.5)
2+ BT	10	42.6 \pm 0.8	1026.9 \pm 63.1	100	34.1 \pm 7.1	3.2 (1–4.5)

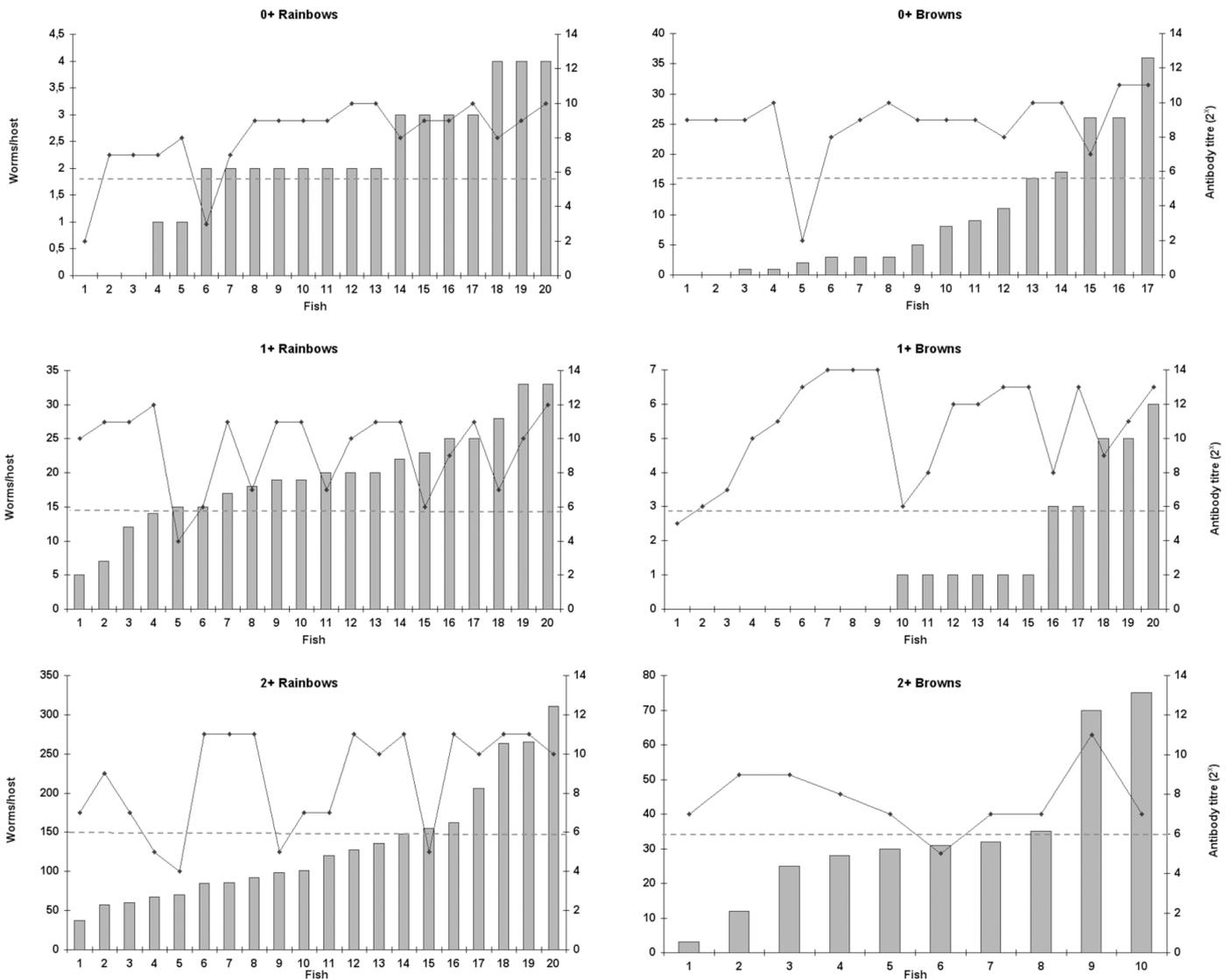


Fig. 1. Correlation between *Discocotyle sagittata* burdens and parasite-specific antibody titres (reciprocal dilution factors) in individual rainbow trout *Oncorhynchus mykiss* (rainbows) and brown trout *Salmo trutta* (browns) from different year-classes (0+, 1+, 2+). Dashed line across graphs represents the mean antibody titres detected in naïve controls. Note that the antibody titre axis is the same for all graphs, but the intensity axis differs

in May, and present ca. 85% prevalence of *Discocotyle sagittata* infection when sampled in November, suggesting most fish would have been exposed to parasites during their first summer. Significant antibody titres found in 0+ fish indicate that their production can be elicited in response to natural infection, despite relatively low parasite intensities (2.5 and 11 worms per infected host for rainbow and brown trout, respectively). However, parasite intensity towards the end of the transmission season may only represent a fraction of the overall antigenic stimulus potentially created by the exposure of fish to infective larvae. Older fish likewise presented high immunoglobulin levels, with the

overall highest titre (dilution factor 14) found in 1+ BT bearing no worms (almost half of the sample; Fig. 1). Nonetheless, considering the absence of a significant correlation between parasite burdens and antibody titres, and the fact that we only analysed one time point of the dynamic host-parasite interaction, the precise role of immunoglobulin titres had cleared previous infections, or were never infected. However, given that almost all fish became infected under the maintenance regime at the farm, it is most likely that older fish found uninfected had eliminated their worm burdens. It must be emphasised

that, in this study, parasite intensities do not necessarily reflect differences in immune-mediated control of worm numbers, because rainbow and brown trout were kept in different ponds and may have been exposed to distinct infective pressures.

Absolutely parasite-free naïve fish have detectable antibodies against *Discocotyle sagittata*, which could be due to naturally occurring immunoglobulin with low affinity to this parasite. Alternatively, cross reactivity to other parasites might account for detectable anti-parasite immunoglobulin levels. Nonetheless, no helminth parasites other than *D. sagittata* were found over a 3 yr study in the farm where the fish came from. Moreover, fishes from the 3 year-classes analysed had significantly higher immunoglobulin titres than naïve controls, demonstrating that, under farming conditions, rainbow and brown trout do produce antibodies against *Discocotyle sagittata*.

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