Parasitol Res (2004) 92: 367–374
DOI 10.1007/s00436-003-1056-6

ORIGINAL PAPER

Miguel Rubio-Godoy · Richard C. Tinsley

Immunity in rainbow trout, *Oncorhynchus mykiss*, against the monogenean *Discocotyle sagittata* following primary infection

Received: 21 November 2003 / Accepted: 26 November 2003 / Published online: 22 January 2004

Abstract Rainbow trout (*Oncorhynchus mykiss*) were experimentally infected by continuous or single exposure with the monogenean *Discocotyle sagittata*. To determine whether immunity follows primary infection, fish were exposed to a secondary challenge by one of two modes: (1) primary infections were cleared with praziquantel (PZQ) and hosts re-infected with 100 oncomiracidia; (2) parasites were allowed to reach maturity and hosts super-infected with 100 oncomiracidia. Fish challenged after initial continuous exposure developed significant partial resistance to re-infection, carrying burdens 35% lower than controls. PZQ treatment controls demonstrated that the drug did not account for the protection observed. Single exposure did not significantly modify the outcome of secondary challenges. Super-infection experiments suggested that no concomitant immunity develops. No correlation was found between initial burdens and the outcome of secondary challenges in the same individual. Significantly elevated anti-*D. sagittata* antibodies were detected in infected fish, but there was no correlation between immunoglobulin levels and parasite burdens.

Introduction

The monogenean fluke *Discocotyle sagittata* (Leuckart, 1842) is a parasite of salmonid fishes. Temperature is the most important abiotic factor controlling parasite life cycle characteristics: egg production (Gannicott and Tinsley 1998a), hatching (Gannicott and Tinsley 1997), larval survival (Gannicott and Tinsley 1998b) and worm growth (Gannicott 1997) have optima between 13°C and 18°C. This produces a clear annual transmission cycle in temperate climates: virtually no new infections occur during the winter with temperatures <10°C, while transmission is continuous during the summer and into the autumn. Parasites are long-lived, with an estimated life span of 3–4 years (Paling 1965). The combination of these two factors (yearly transmission cycles and long-lived worms) results in different infection scenarios in fish farms affected by *D. sagittata* (Rubio-Godoy and Tinsley 2002). In early summer, synchronous hatching of eggs accumulated in ponds leads to mass infection of fish. Subsequently, during the summer and early autumn, egg production by established parasites leads to a continuous trickle infection of host populations. The mass (single) infection at the beginning of the annual transmission cycle is different for juvenile and older hosts: fish of the year are naive until this time when they experience a sudden input of infective larvae; in contrast, a proportion of older fish already harbour worms from previous years and the early summer exposure creates a secondary challenge. The continuous (trickle) exposure during summer adds to these burdens and constitutes a super-infection for hosts already harbouring established parasites.

Experimental single and trickle infections of rainbow trout, *Oncorhynchus mykiss*, are comparable in terms of parasite establishment rates (Rubio-Godoy and Tinsley 2002). *O. mykiss* develops effective partial immunity to *D. sagittata* following active immunisation (Rubio-Godoy et al. 2003). Humoral immunity may be involved in mediating protection, as a significant negative correlation was found between infection levels in vaccinated fish and antibody titres (Rubio-Godoy et al. 2003). The present study was undertaken to determine whether the experience of a primary *D. sagittata* infection under experimental conditions moderates invasion success in a secondary challenge.

M. Rubio-Godoy (✉) · R. C. Tinsley
School of Biological Sciences,
University of Bristol, Bristol, BS8 1UG, UK
E-mail: mrubiogodoy@yahoo.com
Tel.: +44-117-9287478
Fax: +44-117-9257374

Materials and methods

Fish

O. mykiss fingerlings were purchased from a parasite-free hatchery. Batches of 30–50 fish were maintained in 500 l tanks at 13°C and

fed commercial fish feed pellets. Animals were allowed at least 7 days to recover from transport-related stress before the experimental procedures.

Parasites

Farmed rainbow trout naturally-infected with *D. sagittata* from the Isle of Man (UK) were transported to Bristol and maintained at 13°C under a light:dark regimen of 12:12 h. Parasite eggs were collected by filtration of tank water through 125 μm nylon mesh, washed in dechlorinated water and incubated at 13°C under a 12L:12D photoperiod. After embryonation (ca. 28 days), egg batches hatched en masse in the first hour of the dark period (Gannicott and Tinsley 1997), allowing collection of recently emerged oncomiracidia. Under the dissecting microscope, batches of 100 active oncomiracidia were collected with a Pasteur pipette, placed in Petri dishes and used within 2 h of hatching.

Experimental infections

Fish were infected as described previously (Rubio-Godoy and Tinsley 2002). Briefly, fingerlings (ca. 20 cm long) were placed individually in infection tanks (ca. 10 l, 13°C) and exposed to 100 active oncomiracidia for 24 h, the first 12 h of which were in darkness. Afterwards, they were kept in 250 l tanks at 13°C until dissection. Experimentally infected fish were anaesthetised terminally with 0.05% MS222, bled by caudal vein puncture, weighed and measured; haematocrit (packed cell volume, PCV) was determined for each fish. Gills were removed and examined under a dissecting microscope and the number of parasites per gill arch was recorded. Parasites were grouped in different (categorical) developmental cohorts based on the number of pairs of clamps they had grown (Gannicott 1997); adult worms have four pairs of clamps and contain eggs visible through the tegument. Age cohort data were transformed into continuous variables by determining the mean number of pairs of clamps attained by worms recovered from individual fish (Rubio-Godoy and Tinsley 2002). This average, the Developmental Index, was calculated as follows:

$$\text{Developmental Index} = \frac{\sum(\text{worms recovered} \times \text{developmental cohort})}{\text{Total number of worms}}$$

Praziquantel treatment

Fish were anaesthetised with 0.05% MS222 and given p.o. (by gavage) 2–3 ml of a 1% w/v praziquantel (PZQ) solution in ethanol:olive oil (5:95 parts). Animals were observed until recovery from anaesthesia and placed in individual tanks (ca. 10 l, 13°C) for 24 h, then returned to bigger tanks. Water in the treatment tanks was filtered using a 125 μm sieve to recover the parasites shed. Preliminary experiments (data not shown) indicated the administration of 5% ethanol in olive oil without PZQ to anaesthetised fish did not result in significant worm dislodgement.

Experimental design

Fish were randomly allocated to six groups (each $n = 20$; Table 1) and infected by different exposure methods. Primary infection was either by single (S) experimental exposure of fish to 100 *D. sagittata* oncomiracidia, or by continuous (C) exposure to oncomiracidia for up to 1 month. Continuous exposure was achieved by placing naive fish in tanks where heavily infected fish had been kept for 6 months and therefore contained parasite eggs which hatched continuously during the following ca. 1 month. Fish infected by either mode of primary exposure were then subjected to secondary re-infection (RI) or super-infection (SI): the different groups are identified below by the mode of primary (S or C) and secondary (RI or SI) exposure; e.g., C-SI = continuous exposure + super-infection. Re-infection involved treating the fish with PZQ 3 months post-start of primary infection (months PPI) to clear the initial infection, allowing them to recover for 1 month, and re-infecting them with 100 oncomiracidia. Parasite intensities were recorded 1 month post-secondary challenge, which corresponds to 5 months PPI. Super-infection involved allowing primary infections to develop during 4 months, and then exposing fish to an experimental, secondary super-infection with 100 oncomiracidia; parasite intensities were recorded 1 month post-secondary challenge (5 months PPI). All fish treated with PZQ were tagged to allow correlation between primary and secondary infection intensities for individual hosts. Controls were naive fish infected with 100 oncomiracidia and dissected 1 month post-infection (p.i.). PZQ treatment controls were also naive and received PZQ 1 month prior to experimental infection with 100 oncomiracidia; these were dissected 1 month p.i. Before the end of the experiment, one fish was lost in both the control and S-SI groups, and two in each of the PZQ treatment and C-SI groups.

Antibody titres

Antibody titres were determined as described previously (Rubio-Godoy et al. 2003). Briefly, 96-well microtitre plates were coated with sonicated *D. sagittata* antigens. Fish sera were diluted serially (1:16 to 1:8,194), plated in duplicate, and incubated for 1 h under gentle agitation. Rabbit anti-salmon Ig (Buchmann and Pedersen 1994) were used as secondary antibodies and peroxidase-conjugated goat anti-rabbit Ig (Sigma) as tertiary antibodies. Enzyme substrate (*o*-phenylenediamine; Sigma) was added to the plates and these were incubated for 10–15 min. The enzymatic reaction was stopped by adding 3 M HCl, and optical density (OD) determined at 492 nm using an ELISA-reader. Each plate included two controls: wells with all antibodies and substrates except sample

Table 1 Experimental design. An*arrow* indicates that the fish were exposed to infective larvae for a month.*C-RI* Continuous exposure + reinfection, *C-SI* continuous exposure + superinfection, *PZQ*praziquantel, *S-RI* single exposure + reinfection, *S-SI* single exposue + superinfection, *UNK* exposed to unknown numbers of oncomiracidia for the period indicated by the arrow, *100* infected experimentally with 100 oncomiracidia, *D* dissection

	Group	*n*	Months					
			0	1	2	3	4	5
Controls	Control	20		100	D			
	PZQ control	20	PZQ	100	D			
Re-infection	Continuous exposure (C-RI)	20	UNK →			PZQ	100	D
	Single exposure (S-RI)	20	100			PZQ	100	D
Super-infection	Continuous exposure (C-SI)	20	UNK →				100	D
	Single exposure (S-SI)	20	100				100	D

material (primary antibodies) as negative controls (blanks), and sera obtained from naive fish. Values greater than twice the (blank) background absorbance were considered to be positive. ELISA was validated by means of the OD readings obtained from naive sera, which were the same in the different plates/assay lots.

Statistical analysis

Data were analysed with the statistical package SPSS for Windows 10.0. For each experimental group, the mean intensity (Bush et al. 1997) ± standard error of the mean (SE) are reported. One-way ANOVA (General Linear Models) was used to analyse the differences in $\log_{10}$-transformed worm burdens recovered in treatment groups, parasite developmental indices, antibody titres, and to validate ELISA tests; the significance level was set at $P < 0.05$. Tukey's test was used for post-hoc comparisons. Pearson correlations were calculated for the parasite burden-PCV relationship, and the association between primary and secondary infection intensities. A regression was calculated for the ratio log mean intensity/log variance determined for primary and secondary infections in all groups. The significance of the regressions was tested by ANOVA. Parasite burdens were tested for normality with Kolmogorov-Smirnov (KS) tests.

Results

Primary infection

All fish challenged by continuous or single exposure developed primary infections; following this, they were randomly assigned to groups exposed to 100 oncomiracidia by either re-infection after PZQ treatment or super-infection (Table 1). PZQ treatment resulted in worms being dislodged with the respiratory current within minutes of application. No adult parasites were found at the time of dissection of fish that received PZQ, pointing to the treatment being 100% effective. Continuous exposure resulted in mean primary burdens of 81.8 ± 7.04 worms/fish ($n = 40$), while a mean intensity of 17.0 ± 1.80 worms/fish was found in single-infected hosts ($n = 39$). No significant differences in primary infection intensities were found between re-infected (Fig. 1) and super-infected groups (Fig. 2), despite the fact that burdens in the first group were determined after PZQ treatment 3 months PPI and those in the latter at the time of dissection 5 months PPI (Table 1). Figure 1 illustrates the relationship between primary and secondary parasite burdens determined for individual re-infected fish; worms from the two infection episodes were not concurrent on fish, but the graph shows the lack of correlation between primary and secondary intensities. In fish subjected to continuous exposure, primary burdens were mainly composed of adult parasites but included a range of developmental cohorts encompassing some juvenile worms [developmental index (DI) 4.2 ± 0.04]. In contrast, single-infected fish predominantly harboured stage 4 parasites (DI 4.0 ± 0.04). DI were significantly greater in the group subjected to continuous infection ($F_1 = 13.554$; $P = 0.001$), reflecting the differences in the periods of original exposure to oncomiracidial invasion. In

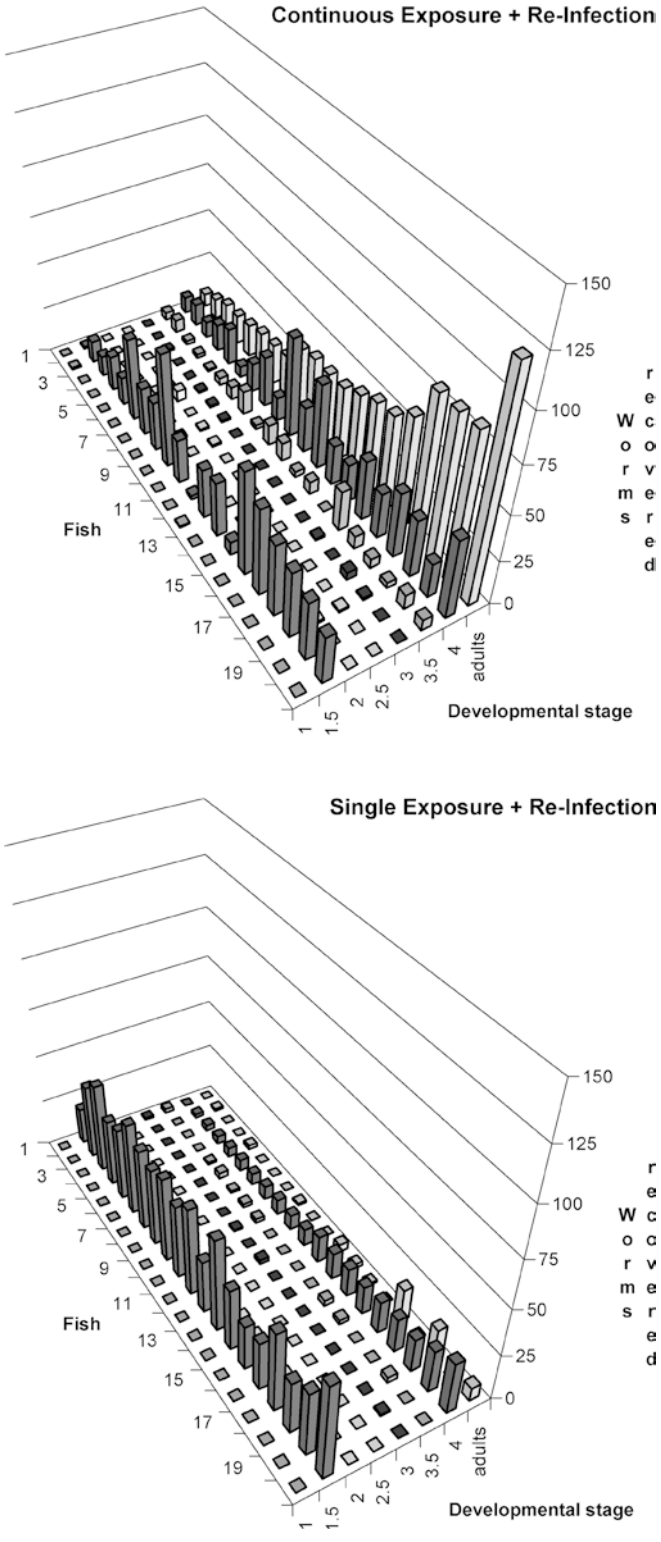

Fig. 1 Primary and secondary infection intensities of *Discocotyle sagittata* in fish re-infected after initial continuous or single exposure. Older worms (≥2.5 pairs of clamps) derive from the primary infection, whilst juveniles (≤ 2 pairs of clamps) represent the outcome of the secondary challenge. Burdens are shown for individual fish; there was no significant correlation between the primary and secondary intensities

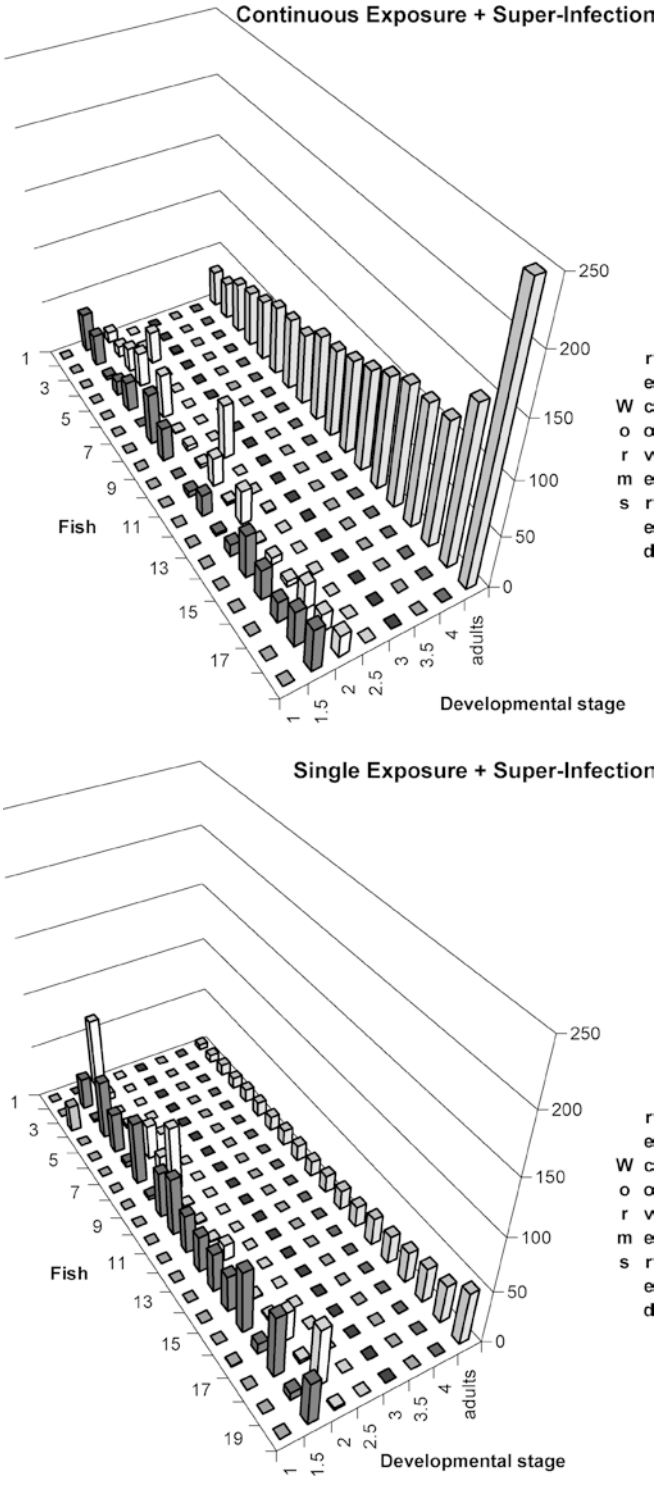

Fig. 2 Primary and secondary infection intensities of *Discocotyle sagittata* in fish super-infected after initial continuous or single exposure. Adult worms derive from the primary infection, whilst juveniles (≤ 2.5 pairs of clamps) represent the outcome of the secondary challenge; no significant correlation was found between the primary and secondary intensities

super-infected fish, the primary and secondary infection cohorts could be readily separated by their degree of development 5 months PPI: primary infections were entirely composed of adult parasites in both continuous and single-infected fish (Fig. 2).

Experimental re-infection

One month after experimental re-infection (5 months PPI) with 100 oncomiracidia, fish previously exposed to continuous exposure had mean burdens of 27.8 ± 3.88 juvenile worms/fish (C-RI; $n = 20$), while those initially single-infected harboured 38.0 ± 2.10 juvenile worms/fish (S-RI; $n = 20$; Figs. 1, 3); this difference was significant ($F_1 = 10.577$; $P = 0.002$). Control fish had 42.4 ± 1.18 worms/fish 1 month p.i., while PZQ treatment controls harboured 44.3 ± 2.44 worms/fish (Fig. 3). Parasite development in both re-infection groups was uniform, the majority of worms having grown 1.5 pairs of clamps. No significant correlation was found between the intensities recorded for individual hosts in primary and secondary infections (Fig. 1). Secondary burdens differed in their degree of overdispersion: S-RI fish had intensities in the range 19–52 worms/host and a variance/mean (V/M) ratio of 2.32, C-RI in the range 1–62 worms/host and a V/M ratio of 10.82.

Experimental super-infection

One month following experimental exposure to 100 oncomiracidia, juvenile parasite intensity (representing the secondary challenge) was comparable in super-infected groups: 5 months PPI, C-SI fish ($n = 18$) had 40.8 ± 2.96 juvenile worms/host, and S-SI fish ($n = 19$) 49.0 ± 3.14 juvenile worms/host (Figs. 2, 3). Primary infection intensities have already been described; no significant correlation between initial and secondary burdens was detected. The majority of secondary infection parasites had grown 1.5–2 pairs of clamps 1 month p.i., but two C-SI fish harboured mainly worms with 2.5 pairs of clamps (Fig. 2). The mean DI attained by juvenile parasites in the C-SI group (1.8 ± 0.06) was slightly lower than in the S-SI group (2.0 ± 0.12), but the difference was not significant. Secondary infection burdens in super-infected fish were overdispersed, as illustrated by intensity ranges and V/M ratios: S-SI fish ranged from 26 to 76 worms/host with a V/M ratio of 3.82, C-SI ranged between 12 and 62 worms, with a V/M ratio of 3.85.

Overall comparisons

Secondary infection levels in the C-RI group were ca. 35% lower than control burdens, and post hoc analysis demonstrated that C-RI intensity was significantly different from that of all other groups (Fig. 3; $F_5 = 6.921$; $P < 0.0001$). Intensities in the control and PZQ treatment control groups were comparable, as were these to both super-infected and to S-RI hosts. Control fish

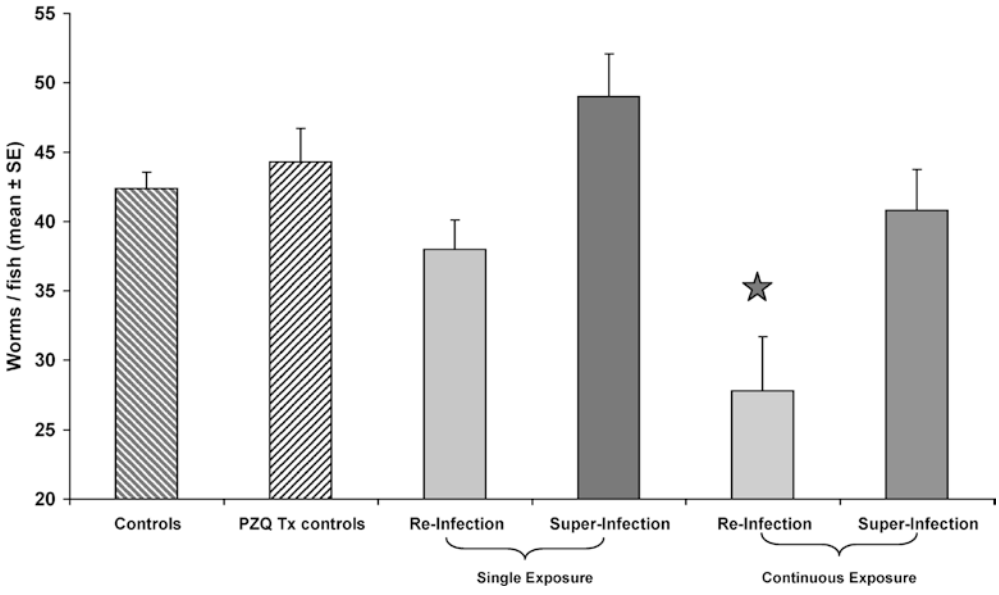

Fig. 3 Mean parasite burdens found 1 month post-infection in experimental groups after controlled challenge with 100 *Discocotyle sagittata* oncomiracidia. The *star* denotes the only group significantly different from all other groups (Tukey's test)

exhibited normal parasite distributions at the time of dissection (KS; $P = 0.954$). As indicated by V/M ratios > 1, secondary infection parasite distributions were overdispersed. The log mean/log variance ratio calculated with pooled primary and secondary infection data gave a tight, significant linear relationship with slope $\pm$ SE $= 2.32 \pm 0.280$ ($r^2 = 0.90$; $P < 0.0001$).

No correlation was found between the number of juvenile parasites recovered and PCV values at the time of dissection. However, a significant negative correlation was observed between adult worm burdens and PCV (data not shown; $n = 82$; $F_1 = 15.239$; $P < 0.0001$); r^2 values were highest in the super-infected groups, where primary parasite populations were 5 months old and still present at the time PCV was determined.

Parasite development for secondary infection worms differed significantly among groups, with a mean DI of 1.9 ± 0.069 in super-infected and 1.5 ± 0.002 in re-infected fish 1 month after experimental exposure ($F_1 = 36.140$; $P < 0.0001$). Mode of exposure during primary infection (continuous or single exposure) did not significantly modify parasite development (as determined 3 and 5 months PPI).

Antibody titres

Anti-*D. sagittata* antibody titres were determined for PZQ treatment controls and C-RI fish, the only group exhibiting significant partial protection. As shown in Fig. 4, both groups had comparably high antibody titres in the vicinity of 1:4,096 (2^{12} titre); these did not differ significantly. Naive fish had detectable anti-parasite antibodies up to a mean titre of $2^{5.75}$. There was no significant correlation between parasite burdens and immunoglobulin levels detected for individual fish in either group; this was the case for both primary and secondary intensities in re-infected hosts (Fig. 4).

Discussion

This investigation demonstrates that continuous (trickle) primary infection of rainbow trout with *D. sagittata* confers partial immunity against re-infection. In contrast, no significant protection was detected following initial parasite invasion through single (mass) exposure, nor against super-infection. Protected fish had secondary challenge burdens ca. 35% lower than controls, after initial infection had been cleared with PZQ. Protection is not likely to arise from residual PZQ affecting the parasites, since control and PZQ treatment control fish had comparable burdens after experimental challenge, suggesting that the drug had been completely eliminated by the time of infection. Indeed, PZQ administered orally to fish leads rapidly to a peak concentration in plasma which starts declining 48 h after application, and the drug is not detectable 120 h post-administration (Kim et al. 2001). PZQ induces spastic musculature paralysis, probably releasing the grip of the clamps and resulting in the rapid worm dislodgement observed. The drug has been shown to be effective in treating various monogenean infestations of fish (Schmahl and Mehlhorn 1985).

In the experiments we report here, primary infection was allowed to persist ≥3 months at 13°C before super-infection or clearance prior to re-infection. This period is sufficient for the majority of worms to mature sexually (Gannicott 1997) and, presumably, for host immune mechanisms to recognise the parasite, as evidenced by the detection in rainbow trout of significant levels of specific anti-parasite antibodies 1 month p.i. with *D. sagittata* (Rubio-Godoy et al. 2003). Despite similarly prolonged initial infections, single exposure did not confer resistance against any form of secondary challenge. Likewise, more pronounced immune responses against nematodes were documented

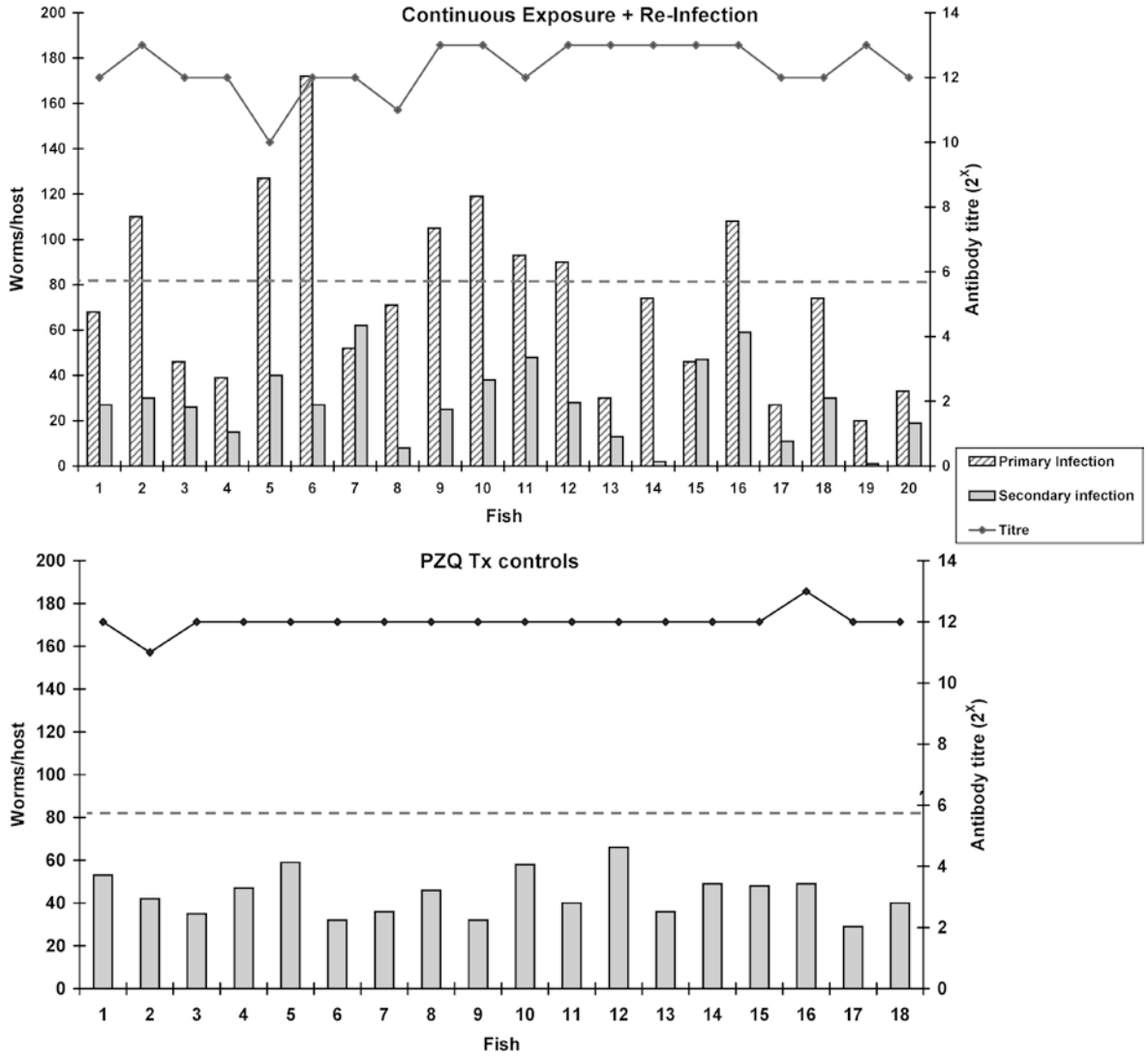

Fig. 4 Correlation between *Discocotyle sagittata* burdens and parasite-specific antibody titres in individual fish. The *dotted line* across the graphs represents the mean antibody titre detected in naive controls

following trickle rather than single infections with the nematodes *Cooperia oncophora* in calves (Van Diemen et al. 1996), *Trichostrongylus colubriformis* in lambs (Stankiewicz et al. 1996, Emery et al. 1999), and *Trichuris suis* in pigs (Pedersen and Saeed 2001), manifested as a reduction in worm establishment via incoming larvae. These different immune outcomes may be related to the antigenic dose experienced by the hosts; high antigen concentration reached via massive single infection may lead to tolerisation or to an allergic reaction and no protective immunity. In the work we report, we cannot rule out that the infective dose in the single exposure group was insufficient to elicit the effective response observed in continuous exposure (C-RI) fish. Similarly, low *Haemonchus contortus* doses did not elicit protection in sheep (Benítez-Usher et al. 1977), while trickle exposure with elevated numbers of *H. contortus* (Emery et al. 2000) and *T. colubriformis* (Emery et al. 1999) resulted in significant immunity to re-infection.

The super-infection experiments we report show no evidence of concomitant immunity developing against incoming secondary parasites. On the contrary, despite comparable infection levels in re-infected and super-infected hosts, a certain degree of facilitation could be proposed to have taken place in super-infections because parasite development was significantly higher in this group when compared to re-infections. Direct competition for attachment sites between primary and super-infection parasites is unlikely, considering that *D. sagittata* apparently invade their hosts passively with the respiratory current and maturing worms migrate from initial invasion sites to preferred regions on the gills (Rubio-Godoy and Tinsley 2002). Migration would make establishment locations available to new recruits, potentially decreasing competition for physical space and favouring growth.

The significant negative correlation found between adult worm intensities and PCV demonstrates the capacity for *D. sagittata* to cause anaemia in heavily

infected fish. This accords with the inverse correlation found between worm burdens and haematocrit in farmed rainbow trout (Gannicott 1997).

Natural immunity against monogeneans has been reported in amphibians and fish. Comprehensive, long-term field studies of polystomatid monogeneans infecting anuran amphibians consistently show low prevalence and intensity of infection (Tinsley 1995). As proposed by Tinsley and Jackson (2002), host immunity must partially account for the uniform low infection levels detected in host-parasite systems varying significantly in their ecology. One of the most striking examples is the comparably low prevalence and intensity of infection found for *Protopolystoma xenopodis*, a parasite of the aquatic *Xenopus laevis* transmitted virtually year-round, and for *Pseudodiplorchis americanus*, infecting the desert toad *Scaphiopus couchii*, which is transmitted during less than 24 h each year (Tinsley and Jackson 2002). Long-term, lab-based experiments demonstrated that primary infection with *P. xenopodis* can elicit strong, long-lived protective immunity against re-infection in *X. laevis* (Jackson and Tinsley 2001). Even though the *Xenopus* immune system has almost all the components well-documented in mammals, the exact mechanisms involved in protection have not been elucidated. In fish, non-specific and specific immunity has been reported against monogeneans. For instance, complement from salmonid fishes has been demonstrated to bind and kill gyrodactylids (Buchmann 1998, Harris et al. 1998) and *D. sagittata* (Rubio-Godoy et al. 2004). Rainbow trout have been shown to be able to acquire resistance to *Gyrodactylus salaris* (Bakke et al. 1991) and *G. derjavini* (Lindenstrøm and Buchmann 2000). Recently, it was established that primary *G. derjavini* infection elicits IL-1β expression in rainbow trout skin (Lindenstrøm et al. 2003), stressing the importance of the mucosal component in the teleost immune system. Specific responses include immunoglobulin detected in tiger puffer after *Heterobothrium okamotoi* infection (Wang et al. 1997), and in eels against *Pseudodactylogyrus bini* (Buchmann 1993) and *P. anguillae* (Mazzanti et al. 1999). In contrast, no antibodies were detectable after *Heteraxinoides xanthophilis* (Thoney and Burreson 1988), *Neobenedenia girellae* (Bondad-Reantaso et al. 1995), and *G. derjavini* (Buchmann 1998) infection. These examples add to the impression that fish immunity to monogenean parasites is almost certainly multifactorial and combinatorial in nature, hence the difficulty of correlating protection with one particular immune component.

Previous studies have indicated that single and trickle infection of fish with *D. sagittata* resulted in similar burdens under experimental conditions (Rubio-Godoy and Tinsley 2002). The present work suggests that trickle infection can lead to significant partial protection against this monogenean. In contrast to vaccinated rainbow trout that apparently control worm numbers via specific anti-*D. sagittata* immunoglobulin (Rubio-Godoy et al. 2003), no significant correlation was found in the present study between parasite intensities and antibody titres. Moreover, the lack of a significant association between primary and secondary infection characteristics implies that individual resistance may not be absolutely dependent on innate host traits but probably also involves parasite characteristics and stochastic events during infection. A similar lack of correspondence between primary and secondary infection characteristics in individual hosts was observed in *X. laevis* infected with *P. xenopodis* (Jackson and Tinsley 2001), where full-sib animals were used and therefore the influence of the genetic component of susceptibility was reduced to a minimum. In this report, worm burdens were normally distributed in control fish but overdispersed in challenged groups and this provides an indication of host immunity developing in response to primary infection. Log variance/log mean ratios >1 could indicate host heterogeneity in the ability to kill parasites by immune or other types of response (Anderson and Gordon 1982). This work demonstrates that rainbow trout can develop partial immunity following experimental infection; the relevance of this in trout hatcheries affected by *D. sagittata* is hard to assess, considering that fish often accumulate lethal levels of infection. Further work should determine whether fish develop effective immunity under farming (and natural) conditions, whether host defences are overwhelmed by the massive and continuous input of parasites, and whether monogenean parasites can modulate or suppress host responses.

Acknowledgements We are grateful to several people who contributed to this work: John Ballard and Ken Jervis (Isle of Man) for access to infected fish; Kurt Buchmann and Jens Sigh (Royal Veterinary and Agricultural University, Denmark) for their help and facilities to determine antibody titres. Praziquantel was kindly donated by Bayer (UK). M.R.G. is supported by postgraduate scholarships from the Mexican National Council for Science and Technology (CONACYT) and Universities UK. Experiments complied with the UK Animals (Scientific Procedures) Act 1986.

References

Anderson RM, Gordon DM (1982) Processes influencing the distribution of parasite numbers within host populations with special emphasis on parasite-induced host mortalities. Parasitology 85:373–398

Bakke TA, Jansen PA, Kennedy CR (1991) The host specificity of *Gyrodactylus salaris* Malmberg (Platyhelminthes, Monogenea): susceptibility of *Oncorhynchus mykiss* (Walbaum) under experimental conditions. J Fish Biol 39:45–57

Benítez-Usher C, Armour J, Duncan JL, Urquhart GM, Gettinby G (1977) A study of some factors influencing the immunisation of sheep against *Haemonchus contortus* using attenuated larvae. Vet Parasitol 3:327–342

Bondad-Reantaso MG, Ogawa K, Yoshinaga T, Wakabayashi H (1995) Acquired protection against *Neobenedenia girellae* in Japanese flounder. Fish Pathol 30:233–238

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

Buchmann K (1998) Binding and lethal effect of complement from *Oncorhynchus mykiss* on *Gyrodactylus derjavini* (Platyhelminthes: Monogenea). Dis Aquat Org 32:195–200

Buchmann K, Pedersen K (1994) A study on teleost phylogeny using specific antisera. J Fish Biol 45:901–903

Bush AO, Lafferty KD, Lotz JM, Shostak AW (1997) Parasitology meets ecology on its own terms: Margolis et al. revisited. J Parasitol 83:575–583

Emery DL, McClure SJ, Davey RJ, Bendixsen T (1999) Induction of protective immunity against *Trichostrongylus colubriformis* in neonatal Merino lambs. Int J Parasitol 29:1037–1046

Emery DL, McClure SJ, Davey RJ (2000) Protection of Merino lambs against *Haemonchus contortus* by trickle infection of neonates. Parasitol Int 49:165–170

Gannicott AM (1997) The biology of *Discocotyle sagittata* (Monogenea) infecting trout. PhD thesis, University of Bristol, Bristol

Gannicott AM, Tinsley RC (1997) Egg hatching in the monogenean gill parasite *Discocotyle sagittata* from the rainbow trout (*Oncorhynchus mykiss*). Parasitology 114:569–579

Gannicott AM, Tinsley RC (1998a) Environmental effects on transmission of *Discocotyle sagittata* (Monogenea): egg production and development. Parasitology 117:499–504

Gannicott AM, Tinsley RC (1998b) Larval survival characteristics and behaviour of the gill monogenean *Discocotyle sagittata*. Parasitology 117:491–498

Harris PD, Soleng A, Bakke TA (1998) Killing of *Gyrodactylus salaris* (Platyhelminthes, Monogenea) mediated by host complement. Parasitology 117:137–143

Jackson JA, Tinsley RC (2001) *Protopolystoma xenopodis* (Monogenea) primary and secondary infections in *Xenopus laevis*. Parasitology 123:455–463

Kim KH, Kim CS, Kim JW (2001) Depletion of praziquantel in plasma and muscle tissue of cultured rockfish *Sebastes schlegeli* after oral and bath treatment. Dis Aquat Org 45:203–207

Lindenstrøm T, Buchmann K (2000) Acquired resistance in rainbow trout against *Gyrodactylus derjavini*. J Helminthol 74:155–160

Lindenstrøm T, Buchmann K, Secombes CJ (2003) *Gyrodactylus derjavini* infection elicits IL-1β expression in rainbow trout cells. Fish Shellfish Immunol 15:107–115

Mazzanti C, Monni G, Varriale AMC (1999) Observations on antigenic activity of *Pseudodactylogyrus anguillae* (Monogenea) on the European eel (*Anguilla anguilla*). Bull Eur Assoc Fish Pathol 19:57–59

Paling JE (1965) The population dynamics of the monogenean gill parasite *Discocotyle sagittata* Leuckart on Windermere trout, *Salmo trutta* L. Parasitology 55:667–694

Pedersen S, Saeed I (2001) Acquired immunity to *Trichuris suis* infection in pigs. Parasitology 123:95–101

Rubio-Godoy M, Tinsley RC (2002) Trickle and single infection with *Discocotyle sagittata* (Monogenea: Polyopisthocotylea): effect of exposure mode on parasite abundance and development. Folia Parasitol 49:269–278

Rubio-Godoy M, Sigh J, Buchmann K, Tinsley RC (2003) Immunization of rainbow trout *Oncorhynchus mykiss* against *Discocotyle sagittata* (Monogenea). Dis Aquat Org 55:23–30

Rubio-Godoy M, Porter R, Tinsley RC (2004) Evidence of complement-mediated killing of *Discocotyle sagittata* (Platyhelminthes, Monogenea) oncomiracidia. Fish Shellfish Immunol (in press)

Schmahl G, Mehlhorn H (1985) Treatment of fish parasites. 1. Praziquantel effective against Monogenea (*Dactylogyrus vastator, Dactylogyrus extensus, Diplozoon paradoxum*). Z Parasitenkd 71:727–737

Stankiewicz M, Cabaj W, Pernthaner A, Jonas W, Rabel B (1996) Drug-abbreviated infections and the development of immunity against *Trichostrongylus colubriformis* in sheep. Int J Parasitol 26:97–103

Thoney DA, Burreson EM (1988) Lack of a specific humoral antibody response in *Leiostomus xanthurus* (Pisces: Sciaenidae) to parasitic copepods and monogeneans. J Parasitol 74:191–193

Tinsley RC (1995) Parasitic disease in amphibians: control by the regulation of worm burdens. Parasitology 111:S153–178

Tinsley RC, Jackson JA (2002) Host factors limiting monogenean infections: a case study. Int J Parasitol 32:353–365

Van Diemen PM, Ploeger HW, Nieuwland MGB, Rietveld FW, Parmentier HK (1996) Recognition of low molecular weight *Cooperia oncophora* antigens after primary and trickle infection of calves with third-stage infective larvae. Int J Parasitol 26:1305–1310

Wang G, Kim JH, Sameshima M, Ogawa K (1997) Detection of antibodies against the monogenean *Heterobothrium okamatoi* in tiger puffer by ELISA. Fish Pathol 32:179–180