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Recruitment and effects of *Discocotyle sagittata* (Monogenea) infection on farmed trout

Miguel Rubio-Godoy [a,*], Richard C. Tinsley [b]

[a] *Instituto de Ecología, A.C., km 2.5 ant Carretera a Coatepec, Xalapa, Veracruz, 91070, Mexico*
[b] *University of Bristol, School of Biological Sciences, Bristol BS8 1UG, United Kingdom*

Abstract

Farmed rainbow trout *Oncorhynchus mykiss* and brown trout *Salmo trutta* were monitored over 3 years for infection with the blood-feeding gill fluke *Discocotyle sagittata*. Parasite transmission is seasonal: new infections take place during summer/autumn, and transmission is generally negligible during winter/spring. There are 2 sources of infection for naïve fish-of-the-year: limited invasion when fish are in the raceways by riverborne larvae originating external to the farm; and internally, within the farm, when 0+ fish are transferred to ponds previously occupied by older cohorts of infected fish. Thereafter, infection levels continue to increase in rainbow trout primarily through transmission within the farm. Prevalence rose to 100% in 1+ fish by the end of their second summer. In *O. mykiss*, mean abundance reached 194 worms/host for 1+ fish (up to 489 worms/host) and 160 worms/host for 2+ fish. By contrast, in *S. trutta*, parasite prevalence never exceeded 85% and, after the first year's invasions, infection levels decreased over time: in 1+ and 2+ brown trout, parasite mean abundance was <4 (maximum 15) worms/host. We present evidence of the detrimental effects of *D. sagittata* on the host: high burdens are associated with pale gills, decreased body condition and host mortality. Parasite burdens become overdispersed during the warmer part of the year, as prevalence and mean abundance increase. However, the degree of parasite overdispersion decreases over winter; we cannot distinguish whether decreased aggregation is due to parasite losses from infected fish (including immune-mediated parasite mortality) or parasite-induced host mortality.
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Keywords: Monogenea; *Discocotyle sagittata*; Trout; *Oncorhynchus mykiss*; *Salmo trutta*; Epidemiology

1. Introduction

Several species of Monogenea have been implicated in the mortality of cultured fishes (Thoney and Hargis, 1991). Most documented cases of host pathology involve mucus- and epithelium-feeding monopisthocotylean monogeneans (Buchmann and Bresciani, 2006). However, several blood-feeding polyopisthocotylean monogeneans have been shown to induce gill damage and host mortality; examples include *Allobivagina* sp. infecting rabbitfish, *Siganus* sp., *Neoheterobothrium hirame* infecting Japanese flounder, *Paralichthys olivaceus*, *Zeuxapta seriolae* infecting amberjack, *Seriola dumerili* and kingfish, *Seriola lalandi*, *Sparicotyle chryso-*phrii infecting gilthead sea bream, *Sparus aurata*, and *Sciaenacotyle sciaenicola* infecting mulloway, *Argyrosomus japonicus* (Roberts, 1978; Paperna et al., 1984; Williams and Jones, 1994; Anshary et al., 2002; Montero et al., 2004; Sitjà-Bobadilla, 2004; Mansell et al., 2005; Hayward et al., 2007). *Discocotyle sagittata*, a polyopisthocotylean parasite of salmonid fishes, likewise causes mortality in farms in the Isle of Man (IoM) (Gannicott, 1997). Laboratory-based studies indicate a progressive decline of *D. sagittata* reproductive activity with decreasing temperature, affecting egg production, viability, development and hatching (Gannicott and Tinsley, 1997, 1998a,b). This led to the prediction that parasite transmission in IoM farms would be negligible over winter–spring (December–April) at water temperatures generally <10 °C. Conversely, it was predicted that transmission would be continuous between May and November, when higher temperatures would accelerate

* Corresponding author. Tel.: +52 228 842 1849x6208; fax: +52 228 818 7809.
  E-mail address: miguel.rubio@inecol.edu.mx (M. Rubio-Godoy).

reproductive rates. These effects would be expected to generate a pronounced seasonal cycle of infection within confined trout populations in fish farms (Rubio-Godoy and Tinsley, submitted for publication).

In these populations, there are 2 probable sources of *D. sagittata* infection: infected trout inhabiting the rivers feeding the farms (including wild fish and farm escapees), and already-infected fish maintained within the farm. Several characteristics of this infection indicate the potential for intense transmission in aquaculture, including: parasite longevity exceeding one year (Chubb, 1977), accumulation of burdens of several hundred worms/fish contributing to massive output of eggs into the environment (Gannicott, 1997), mass hatching of infective larvae under favourable conditions (Rubio-Godoy and Tinsley, 2002) and the greater susceptibility of farmed rainbow trout *Oncorhynchus mykiss* in comparison with the European natural host, brown trout *Salmo trutta* (Rubio-Godoy and Tinsley, 2004a).

This study describes the accumulation of *D. sagittata* infection in trout reared in farms subject to natural temperature fluctuations, assesses the extent to which transmission originates from sources external or internal to the farms, and evaluates the relationship between host condition factor and parasite burden.

2. Materials and methods

2.1. Fish and study sites

Samples were obtained on the Isle of Man from 2 fish farms that experienced *D. sagittata*-related mortality during the 1990s (Gannicott, 1997). The farms receive water from 2 unconnected river systems. In both farms, fish year classes were kept separate throughout the study. Fish-of-the-year were kept in raceways receiving water directly from the river during their first 6–9 months, from hatching in winter until mid-to late summer. Then, in July–September, fish year classes were moved "downstream" within the farms: class of the year (0+) fish from the raceways to ponds previously occupied by 1-year old (1+) fish, which in turn were transferred to ponds where 2-year old (2+) fish had been kept, etc. During this process, fish from the same age class occupying different ponds are mixed; however, fish from different age classes are not mixed. Older fish were maintained in ponds receiving water from both the river and from other ponds located upstream in the circulation system; in both farms, water flows from ponds containing young fish to those harbouring older fish. Ponds in farm 1 are mud-bottomed, in farm 2 concrete-lined. Both farms produce rainbow trout (*O. mykiss*); farm 2 additionally rears brown trout (*S. trutta*). Rainbow trout and brown trout in farm 2 were held in separate systems; however, *S. trutta* received water from ponds higher up in the farm which contained old *O. mykiss*. Neither farm employed specific treatments against *D. sagittata* during the period of this study. Samples of both fish species were collected in early summer (late May) and late autumn (late November) in 1999, 2000 and 2001, during general fish health inspections carried out for the Department of Agriculture, Fisheries and Forestry (Isle of Man Government). The May and November sampling dates

Table 1
Discocotyle sagittata infection levels in farmed rainbow trout (*Oncorhynchus mykiss*) and brown trout (*Salmo trutta*) from the Isle of Man

Farm	Trout (year class)	May 1999	Nov 1999	May 2000	Nov 2000	May 2001	Nov 2001
		Mean parasite abundance / range (*n*; prevalence) determined during inspection in:					
1	Rainbow (1998)	12.3[a] / 2–33 (20; 100%)	193.8[a] / 12–489 (10; 100%)	159.7 / 60–255 (22; 100%)			
1	Rainbow (1999)		0.05[a] / 0–1 (20; 5%)	2.0[ab] / 0–9 (20; 65%)	30.5[b] / 4–100 (20; 100%)	14.1[c] / 4–38 (20; 100%)	122.3[c] / 7–506 (20; 100%)
1	Rainbow (2000)			0[a] (30; 0%)	0.2[ab] / 0–1 (20; 20%)	1.6[bc] / 0–10 (20; 60%)	69.7[c] / 22–169 (19; 100%)
2	Rainbow (1998)	0.7[a] / 0–3 (20; 45%)	32.7[a] / 12–51 (20; 100%)	31.9[b] / 5–71 (20; 100%)	99.3[b] / 11–246 (10; 100%)		
2	Rainbow (1999)		2.4 / 0–6 (20; 85%)	2.3[a] / 0–7 (20; 70%)	47.8[a] / 7–92 (20; 100%)	33.6[b] / 10–61 (20; 100%)	132.4[b] / 37–311 (20; 100%)
2	Rainbow (2000)			0[a] (30; 0%)	1.8[a] / 0–5 (20; 90%)	2.6[b] / 0–8 (20; 85%)	19.5[b] / 5–33 (20; 100%)
2	Brown (1998)	1.6 / 0–5 (20; 85%)	2.0[a] / 0–7 (20; 80%)	0.3[a] / 0–1 (20; 25%)			
2	Brown (1999)		6.6 / 0–23 (20; 80%)	3.0 / 0–10 (20; 75%)	3.2 / 0–15 (20; 70%)	1.2 / 0–4 (20; 55%)	
2	Brown (2000)			0[a] (30; 0%)	5.5[a] / 0–28 (20; 85%)	2.9[b] / 0–13 (20; 85%)	1.4[b] / 0–6 (20; 55%)

Fish of the year are enclosed in broken lines, 1+fish in continuous pale lines, and 2+ fish in bold lines. Equal superscript letters indicate statistically-significant differences in the mean parasite abundances determined for adjacent groups.

were designed to give maximum information on the annual transmission of *D. sagittata* (Rubio-Godoy and Tinsley, submitted for publication), including data on, respectively, a) parasites surviving over winter when transmission is negligible, and b) the outcome of the transmission season extending from early summer to late autumn. Fish were anaesthetised terminally with MS222, measured (fork length), weighed and dissected for general bacteriological/parasitological tests. Gill arches were removed and preserved in 10% formalin for later microscopic analysis.

2.2. Parasites

Preserved gill arches were examined under the dissecting microscope. The total number of parasites per left and right gill arch was recorded. Individual parasites were grouped in different (categorical) developmental (age) cohorts based on the number of pairs of clamps (p.c.) present on the haptor: cohorts range from 1 for freshly hatched worms with one p.c. to 4.5 for sexually mature worms with 4 p.c. containing eggs *in utero* (Rubio-Godoy and Tinsley, 2002).

2.3. Statistical analysis

Data were analysed with SPSS 10.0 for Macintosh. Parasite burdens reported represent the total worms/host recorded, since no significant difference was found between infection levels in the right and left gill arches (data not shown). Infection levels (abundance and intensity) considering the total number of worms/host and burdens of distinct developmental cohorts were described following Bush et al. (1997). Raw data were $\log_{10}(1+x)$ transformed prior to comparisons with ANOVA (General Linear Models) and significant differences between groups were detected with Tukey's honestly significant difference (HSD) test; the significance level was set at $P<0.05$. The frequency distribution of parasite intensities was described by the variance to mean ratio (V/M) or coefficient of dispersion (Bush et al., 2001). Kolmogorov–Smirnov (KS) tests were used to assess whether the observed distributions corresponded to four theoretical distributions: Normal, Poisson, uniform and exponential; only cases where a significant fit was found are reported. The coefficient of body condition K (Lemly and Esch, 1984) was calculated for each fish, following the formula:

$$K = \frac{100 \times \text{weight(g)}}{[\text{standard(fork)length(cm)}]^3}.$$

The association between the coefficient of body condition K and parasite intensity was plotted and its significance calculated with a Pearson's correlation.

ANOVA was used to compare the coefficient of body condition of rainbow trout between fish farms.

3. Results

Levels of *D. sagittata* infection found for *O. mykiss* and *S. trutta* in the 2 fish farms studied between 1999 and 2001 are shown in Table 1. Fish-of-the-year sampled in early summer (late May), i.e. 4–6 months after hatching, were not infected (Table 1). By late autumn (late November) of their first year, 0+ rainbow trout had become infected: some hosts only carried freshly-invaded worms with 1 p.c. (e.g., class of 2000 fish at farm 1; Fig. 1), others exclusively harboured worms with 4 p.c. (e.g., class of 1999 fish at farm 1; Fig. 1), and some had worms in different stages of development, from freshly-invaded to adult parasites (e.g., fish classes of 1999 and 2000 at farm 2; Fig. 1). Parasite populations of 0+ rainbow trout in both farms (except class of 1999 fish from farm 2) had variance to mean ratio (V/M) close to unity and fitted perfectly to a Poisson distribution (Figs. 2 and 3).

Infection levels increased significantly over the warmer part of the year (between May and November) in 1+ and 2+ *O. mykiss*. Mean parasite abundance varied from year to year, and also between farms (Table 1): by November, 1+ fish reached 100% prevalence after mean worm abundance increased between 8- and 47-fold compared to the previous May; in 2+ fish, mean abundance further increased between 3- and 9-fold in the same period. Infection levels in 1+ rainbow trout were generally higher at farm 1 than at farm 2: the highest mean intensities (±SE) recorded in November were 193.8±37.33 worms/host (with up to 489 worms/host) and 47.8±4.39 worms/host (up to 92 worms/host), respectively; the difference is also reflected in the greater degree of parasite dispersion and correspondingly higher V/M found in farm 1 (Fig. 2) than in farm 2 (Fig. 3). Burdens in 2+ rainbow trout in May were significantly higher in farm 1 (mean intensity 76.9±82.58 worms/host) than in farm 2 (32.8±15.56 worms/host; $P=0.001$); there was no significant difference in infection levels in November between both farms (Table 1). 2+ class of 1998 *O. mykiss* sampled in May 2000 in farm 1 had a mean intensity of 159.7±13.95 worms/host, which is higher than any 2+ fish sampled in November; these fish had very pale gills when inspected, suggestive of severe anaemia. During the
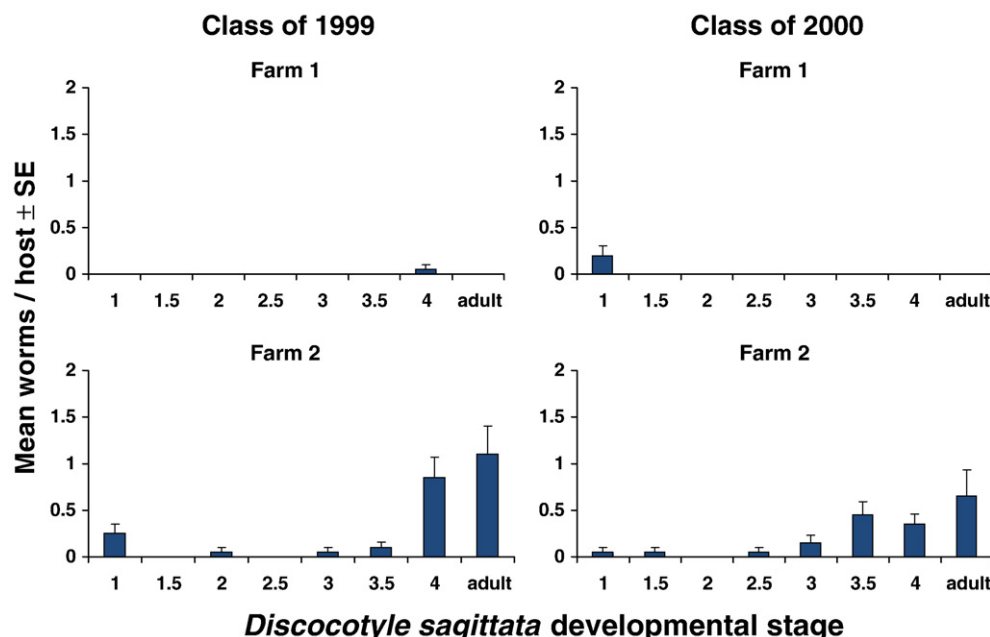

Fig. 1. Mean *Discocotyle sagittata* abundance±SE for individual developmental cohorts from 0+ rainbow trout (*Oncorhynchus mykiss*) sampled in November.
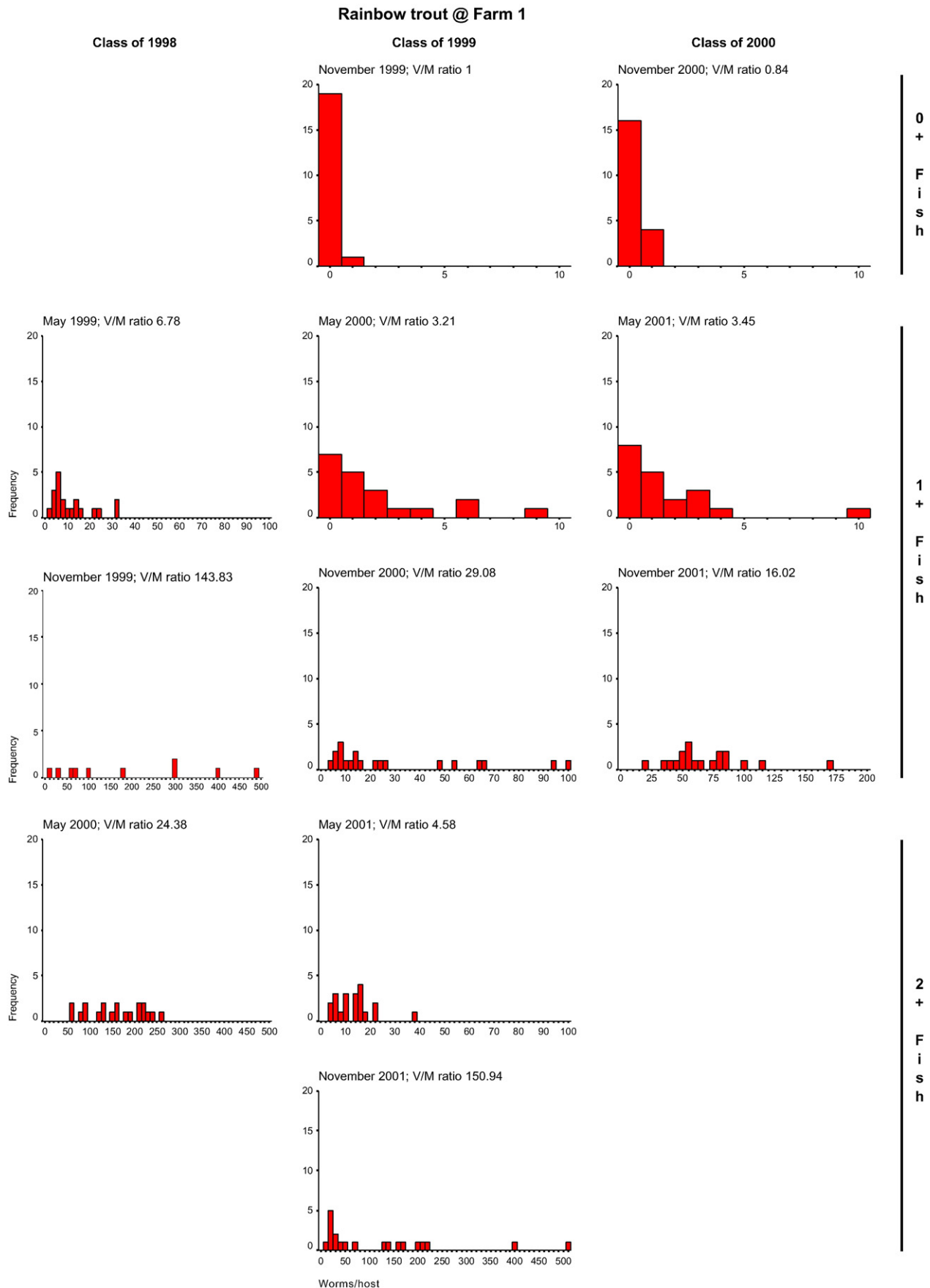
Rainbow trout @ Farm 1

Fig. 2. Frequency distribution and variance to mean (V/M) ratio calculated for *Discocotyle sagittata* infection in rainbow trout (*Oncorhynchus mykiss*) in farm 1.
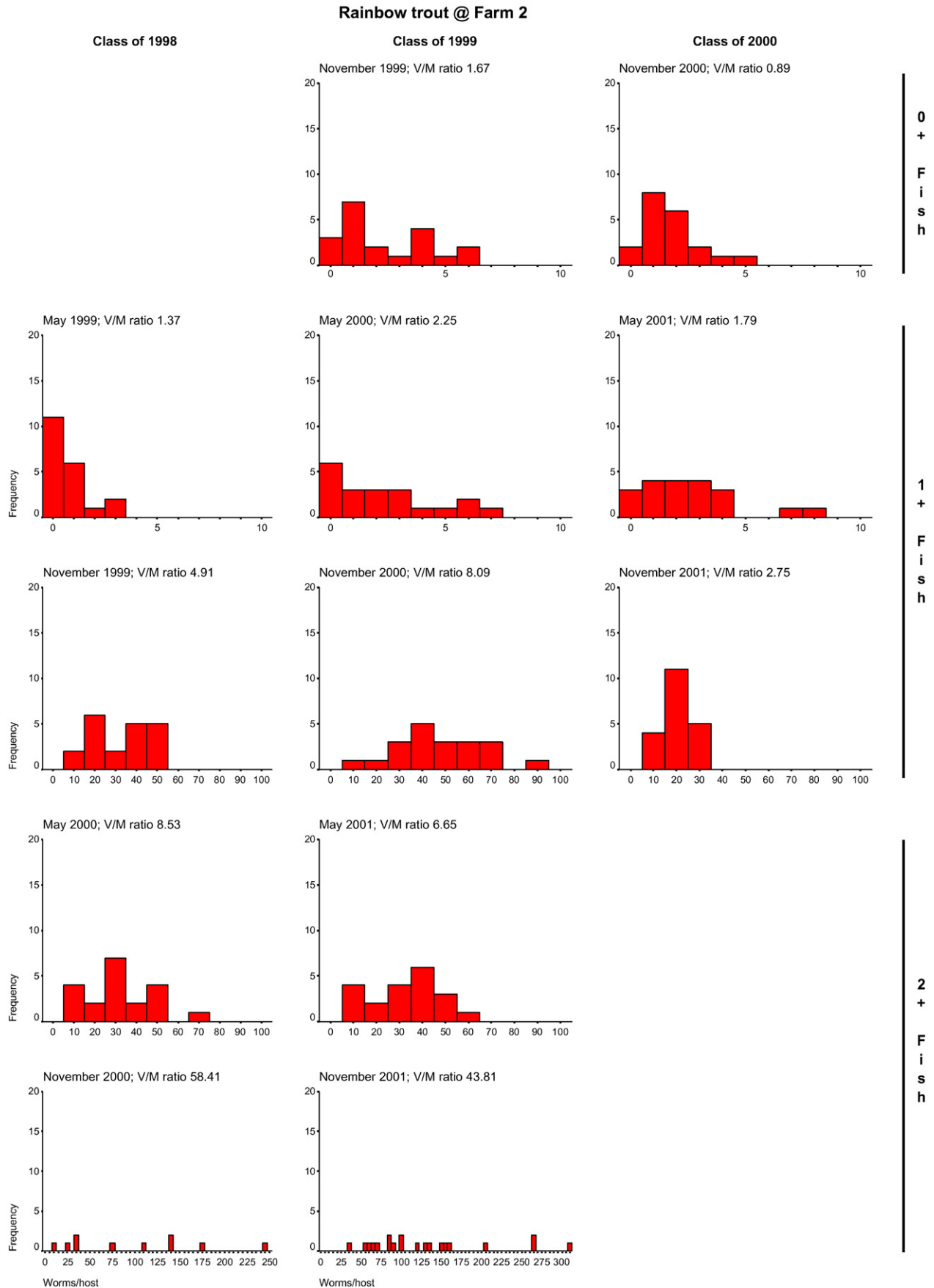
Fig. 3. Frequency distribution and variance to mean (V/M) ratio calculated for *Discocotyle sagittata* infection in rainbow trout (*Oncorhynchus mykiss*) in farm 2.

following months, these heavily-infected fish suffered mass mortality. Typically, deaths were associated with respiratory distress and coincided with elevated water temperatures (when dissolved oxygen concentrations would be reduced). Surviving fish were culled by the farm manager because of their poor condition, resulting in the loss of the entire year class of thousands of fish.

A significant negative correlation was found between worm intensity and the coefficient of body condition K of 2+ *O. mykiss* (Correlation significance: May $r^2=0.1326$, $P<0.0001$; November $r^2=0.0162$, $P=0.036$; Fig. 4). The coefficient of body condition K was significantly higher in farm 2 than in farm 1 in both May and November samples (1.18 ± 0.016 cf. 1.01 ± 0.015; 1.32 ± 0.021 cf. 1.16 ± 0.023, respectively; $P<0.0001$).

Mean parasite abundance increased significantly between May and November in all *O. mykiss* year classes. In contrast, when comparing November samples with those from the following May, in some cases, mean abundance was not significantly different, while in others limited but significant increases were detected (Table 1). Older fish exhibited non-significant declines in infection levels. Nonetheless, some of these declines were accompanied by a loss of the heaviest burdens and a corresponding decrease in the degree of parasite aggregation: for instance, during winter 1999–2000, in class of 1998 rainbow trout at
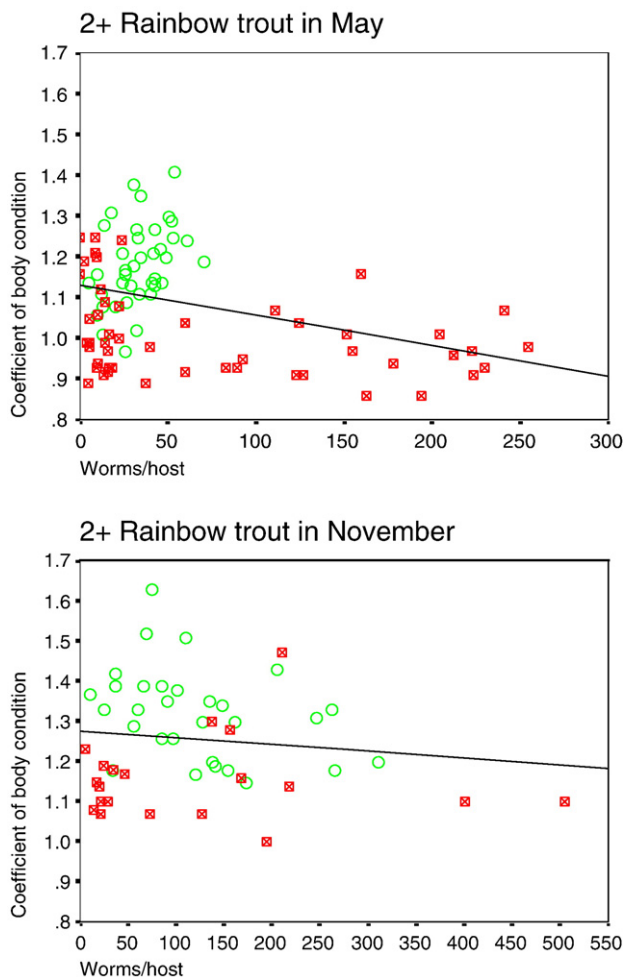

Fig. 4. Correlation between the coefficient of body condition and *Discocotyle sagittata* burdens recovered from 2+ rainbow trout (*Oncorhynchus mykiss*) in May ($n=50$) and November ($n=90$). Notes: Farm 1 = crossed squares; Farm 2 = open circles.

farm 1, mean abundance ($\pm$SE) decreased from 193.8 ± 37.33 worms/host (with up to 489 worms/host) to 159.7 ± 13.95 worms/host (with up to 255 worms/host) and the V/M ratio fell from 143.83 to 24.38; a similar decline was observed in winter 2000–2001 in class of 1999 rainbow trout at farm 1 (Fig. 2). Some fish-of-the-year populations exhibited significant increases in mean parasite abundance over the cold season, indicating that a limited number of invasions can take place during this period. In farm 1, 0+ class of 1999 and 2000 trout had almost negligible infection levels (mean abundance 0.05 ± 0.05 worms/host, prevalence 5%; abundance 0.2 ± 0.09 worms/host, prevalence 20%, respectively; Table 1) when sampled in November, but these had increased significantly by May (mean abundance 2.0 ± 0.56 worms/host, prevalence 65%; abundance 1.6 ± 0.53 worms/host, prevalence 60%, respectively); early summer burdens mainly contained worms with ≥3 p.c. (data not shown).

The pattern of *D. sagittata* infection in brown trout is markedly different from that in rainbow trout kept concurrently in the same farm. In November, new infections in 0+ *S. trutta* were on average 2–3 times higher than on 0+ *O. mykiss*: in class of 1999 fish, 0+ brown trout had 6.6 ± 1.44 worms/host while 0+ rainbow trout had 2.4 ± 0.44 worms/host; 0+ class of 2000 brown trout had 5.5 ± 1.76 worms/host and 0+ rainbow trout 1.8 ± 0.28 worms/host (Table 1). Mean worm abundances in 0+ brown trout decreased slightly but not significantly between November and the following May; however, parasite burdens were more overdispersed in November than in May (Fig. 5). In contrast to infection of *O. mykiss*, *D. sagittata* populations in 1+ *S. trutta* remained stable or declined during summer, despite the fish being infected in May (Table 1) and continuous exposure to larvae from the upper ponds containing infected rainbow trout: in the classes of 1998 and 1999, mean parasite abundance was not significantly different in May and the following November and the degree of parasite aggregation more or less doubled over summer (Fig. 5); in class of 2000 brown trout, a significant decline in parasite abundance was mirrored by a decrease in V/M ratio. During the second winter, *D. sagittata* levels decreased further in brown trout: in class of 1999 fish, mean abundance fell from 3.2 ± 0.89 worms/host (with up to 15 worms/host; 70% prevalence) to 1.2 ± 0.29 worms/host (up to 4 worms/host; 55% prevalence); in class of 1998 *S. trutta*, mean abundance diminished significantly from 2.0 ± 0.42 worms/host (up to 7 worms/host; 80% prevalence) to 0.3 ± 0.10 worms/host (1 worm/host; 25% prevalence). Thus, in both 1+ and 2+ *S. trutta* mean parasite abundance tended to be less than 4 worms/host (maximum intensity 15 worms/host). This contrasts markedly with data from equivalent age classes of *O. mykiss* in the same farm: mean abundance in 2+ rainbow trout sampled in May was ca. typically 33 worms/host with maximum burdens of 71 worms/host; 2+ rainbow trout sampled in November harboured ca. 110 worms/host, with up to 311 worms/host (Table 1). In May samples, the coefficient of body condition K was significantly higher in brown trout compared to rainbow trout of the same age class (1+ fish: 1.18 ± 0.011 cf. 1.09 ± 0.017; 2+ fish: 1.25 ± 0.015 cf. 1.18 ± 0.016, respectively); no significant difference was detected in November.

4. Discussion

Transmission of *D. sagittata* in farmed trout exhibits a clear seasonality, with invasion maximised during the warmer part of the year and almost negligible infection over winter (Rubio-Godoy and Tinsley, submitted for publication). In the IoM and other temperate European climates, water temperature is below 10 °C for almost half of the year, and transmission is significantly inhibited during this period. When permissive temperatures allow
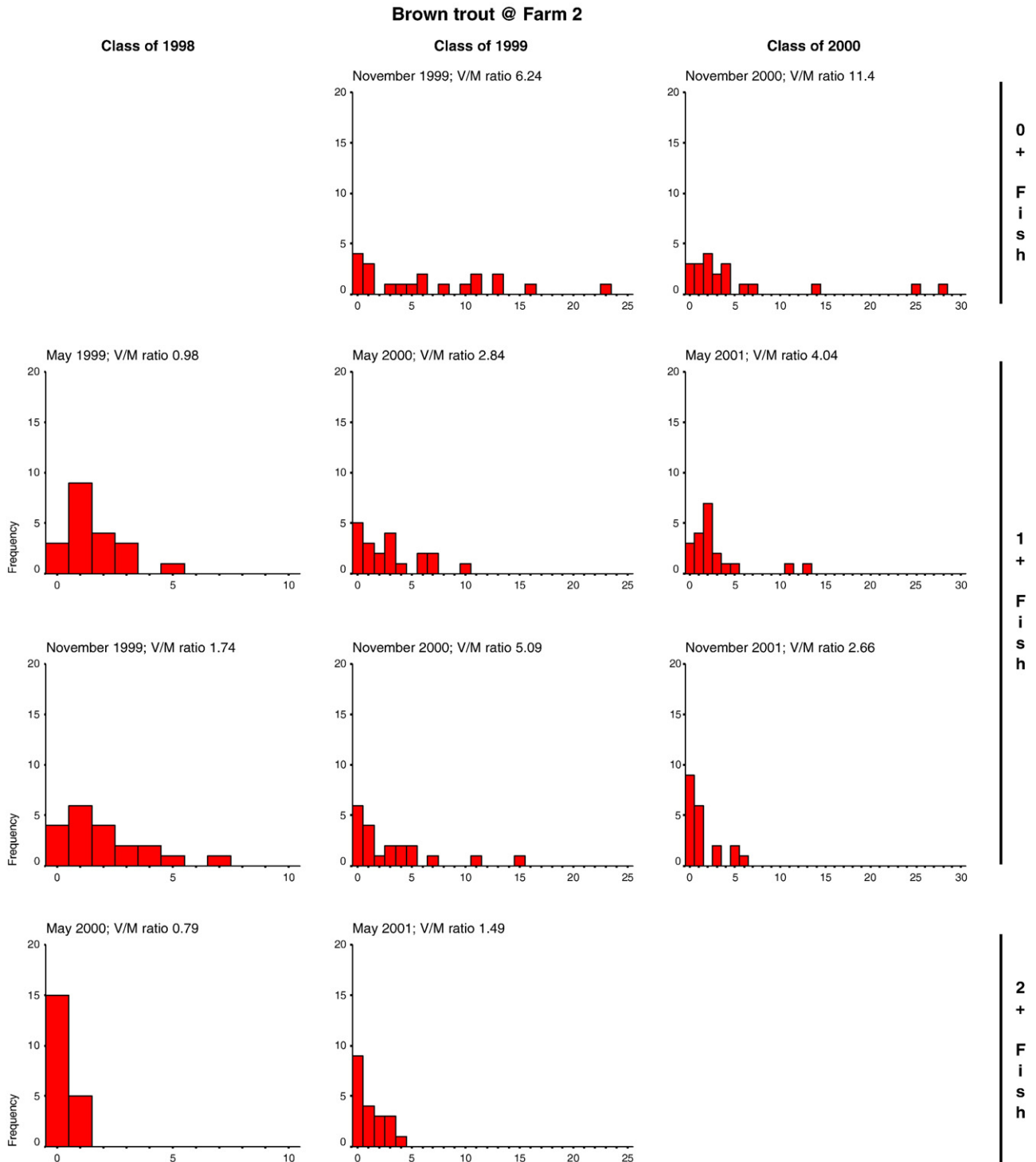
Fig. 5. Frequency distribution and variance to mean (V/M) ratio calculated for *Discocotyle sagittata* infection in brown trout (*Salmo trutta*) in farm 2.

transmission, there are two sources of invasion for naïve 0+ fish (both rainbow trout and brown trout). There may be limited infection from larvae in the river water that irrigates the raceways; these infective stages may originate from infected wild fish or escapees from the farms themselves. This is shown in farm 1 (class of 1999 *O. mykiss*, November 1999), with 1 worm present on a total of 20 fish (Table 1, Fig. 2). Invasion early in the transmission season may produce infections reaching maturity

within the first year and the adult worms may then contribute to further transmission within the 0+ populations. The second source of invasion begins when fish are transferred into rearing ponds in mid-late summer. In this case, transmission is derived from eggs deposited by the cohorts of older, infected fish previously maintained in the ponds. These events are illustrated by Fig. 1: at farm 1 in November 1999, 0+ rainbow trout carry very low levels of maturing (4 p.c.) worms originating from early summer infection

when kept in the raceways, but have not yet experienced further infection. In November 2000, there is no evidence of this raceway infection (no juvenile or adult worms) but the population shows the start of invasion (1 p.c. worms, suggesting invasion shortly before inspection) originating after the transfer to the ponds. In farm 2 however, November 1999 samples show relatively greater infection originating from the period in the raceways (invasion from riverborne infective stages external to the fish farm): 0+ rainbow trout carried a mean of 0.85 and 1.1 worms/host of 4 p.c. and adult worms, respectively (Fig. 1). These samples show the start of new invasions (0.25 worms/host with 1 p.c.) that probably originated from eggs laid by adult worms on these fish. A similar pattern is shown by 0+ rainbow trout in November 2000 (Fig. 1): 0.35 worms/host with 4 p.c. and 0.65 adult worms/host were recorded, and new invasions are illustrated by 0.05 worms/host with 1 and 1.5 p.c. Invasions of 0+ rainbow trout in both farms were probably random events, considering parasite populations (except class of 1999 fish from farm 2) had variance to mean ratio (V/M) close to unity and presented a Poisson distribution. These samples show that transmission may originate both external and internal to the fish farms and that, once infected, the younger cohorts of fish (0+) may begin to transmit within their population during their first summer. Thereafter, infection levels continue to increase within each pond population and also within the farm with water circulation from pond to pond. In contrast to the potential two annual generations of *D. sagittata* in farmed trout in the IoM, only 1 parasite generation per year occurs in wild whitefish (*Coregonus acronius*) in an arctic lake, where the transmission season is shorter (Valtonen et al., 1990). However, climate warming may extend the seasonal window for transmission and could lead to an increase of parasite populations and disease outbreaks in both farmed and wild host populations, as has been documented in northern ecosystems for sheep infected with protostrongylid nematodes (Jenkins et al., 2006).

In 1+ and 2+ rainbow trout in both farms, parasite abundance increased significantly over the summer and there were no statistically significant differences when contrasting samples from November and the following May. Increases in abundance during summer were always accompanied by increases in the degree of parasite overdispersion. In some instances, parasite abundance decreased non-significantly over winter, accompanied by reductions in the degree of parasite overdispersion. Decreases of the V/M ratio of infections could arise through 3 mechanisms: 1) transfer of fish within the farm diluting parasite populations; 2) parasite-induced host mortality due to pathogenicity; and 3) parasite mortality due to host immunity. In this study, the first alternative can be ruled out, but it is impossible to distinguish between the remaining two, as no precise fish mortality records were kept in the farms.

D. sagittata can have detrimental effects on its hosts, as indicated by 3 types of evidence. First, studies in the field in the IoM (Gannicott, 1997) and in the laboratory (Rubio-Godoy and Tinsley, 2004b) have demonstrated a significant negative correlation between parasite intensity and haematocrit. Although no haemoglobin levels were determined in the present study, class of 1998 rainbow trout sampled in farm 1 in May 2000 harbouring heavy *D. sagittata* infections (mean 159.7 worms/

host; range 60–255) had very pale gills, suggesting they were anaemic. Other polyopisthocotylean monogeneans have been implicated in anaemia-induced host mortality: e.g., *Allobivagina* sp. infecting rabbitfish, *Siganus* sp., *N. hirame* infecting Japanese flounder, *P. olivaceus, Z. seriolae* infecting amberjack, *S. dumerili* and kingfish, *S. lalandi, S. chrysophrii* infecting gilthead sea bream, *S. aurata*, and *S. sciaenicola* infecting mulloway, *A. japonicus* (Paperna et al., 1984; Anshary et al., 2002; Montero et al., 2004; Sitjà-Bobadilla, 2004; Mansell et al., 2005; Hayward et al., 2007). The second detrimental effect relates to body condition. In this study, a significant negative correlation was found between the coefficient of body condition *K* and parasite burdens. Similarly, a negative association was found between the body condition of juvenile olive flounder, *P. olivaceus* caught off the coast of Japan and the density of naturally-acquired *N. hirame* (Shirakashi et al., 2006). The third evidence of detrimental effects is host mortality. During this study, the severe effects of heavy infection were demonstrated by the loss of one entire year class of rainbow trout (class of 1998 fish from farm 1 dying during the summer of 2000). Deaths were a direct result of parasite pathology or resulted from the culling of remaining fish considered to be in too poor condition to survive. Gannicott (1997), working in the same farms in the IoM, also documented *D. sagittata*-related host mortality in the 1990s. In addition to anaemia, host mortality may be brought about by tissue damage, considering that *D. sagittata* and other polyopisthocotyleans have been reported to induce serious gill damage (Roberts, 1978; Buchmann et al., 2004). The host response in infected gills includes increased mucus production, epithelial hyperplasia, loss of lamellar structure, clubbing or fusion of gill filaments, haemorrhage, aneurysms and secondary invasion by bacteria or fungi; these pathological changes lead to a reduction or total cessation of gas exchange (Williams and Jones, 1994). Similar pathological changes have recently been shown in European sea bass, *Dicentrarchus labrax*, following infection by the monopisthocotylean *Diplectanum aequans* (Dezfuli et al., 2007).

This study documents the markedly different pattern of *D. sagittata* infection in rainbow trout and brown trout kept in parallel within the same farm. 0+ brown trout in farm 2 become infected by riverborne infective larvae whilst on the raceways. However, in contrast to rainbow trout in both farms, *D. sagittata* infection does not appear to thrive on *S. trutta*: in the 3 fish cohorts studied, parasite burdens (Table 1) and their degree of aggregation (Fig. 5) tended to decline over time, despite the presence of adult worms on the fish and the continuous exposure to larvae from upstream ponds. This may be a reflection of the different susceptibility of rainbow trout and brown trout to *D. sagittata* (Rubio-Godoy and Tinsley, 2004a), as well as the species-specific difference in the capacity of complement to kill oncomiracidia (Rubio-Godoy et al., 2004).

This study demonstrates that *D. sagittata* infection can be acquired from river water, and that management practices (transfer of young fish to ponds previously occupied by older fish) and the typical design of fish farms (water flowing downstream from pond to pond, as determined by water availability) favour the increase of infection levels as fish grow. We provide

evidence that the levels of *D. sagittata* infection attained under farming conditions are detrimental and can result in host mortality.

Acknowledgements

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