

Behavioural mechanisms underlying ‘specific’ host manipulation by a trophically transmitted parasite

Otto Seppälä^{1,2}, Anssi Karvonen³ and E. Tellervo Valtonen⁴

¹Eawag, Swiss Federal Institute of Aquatic Science and Technology, Dübendorf, Switzerland,

²ETH Zürich, Institute of Integrative Biology (IBZ), Zürich, Switzerland,

³Centre of Excellence in Evolutionary Research, Department of Biological and Environmental Science, University of Jyväskylä, Jyväskylä, Finland and

⁴Department of Biological and Environmental Science, University of Jyväskylä, Jyväskylä, Finland

ABSTRACT

Background: The larvae of the eye fluke, *Diplostomum pseudospathaceum*, emerge into the water from a snail and then infect a fish. Once in a fish, they travel to its eye lenses. The parasite infects its definitive host, a fish-eating bird, if the fish is eaten by the bird. Parasitized fish behave in ways that make them more susceptible to bird predation. Previous studies have suggested that this host manipulation is ‘specific’, as parasites do not increase the susceptibility of fish to non-host predators such as piscivorous fish. This suggests that eye flukes evolved to manipulate host fish as a strategy to enhance transmission.

Question: Do eye flukes change the behavioural traits of fish important in determining their vulnerability to non-host fish predators?

Hypotheses: Given the evidence of specific host manipulation in this system, the parasites (1) should not alter such traits, or (2) alterations in them should have opposite effects in determining the susceptibility of fish to piscine predation.

Organisms: The trematode parasite, *D. pseudospathaceum*, and its rainbow trout, *Oncorhynchus mykiss*, intermediate host.

Methods: A laboratory experiment to determine the effect of eye flukes on behavioural traits of fish that are part of their general anti-predatory tactics or specifically determine their susceptibility to piscine predation. We measured the following traits: activity, use of shelter, and escape response to underwater predator attacks.

Results: Parasites did not affect any of the behavioural traits examined.

Conclusion: *Diplostomum pseudospathaceum* manipulates only traits that specifically predispose fish to bird predators, not the traits important in determining their susceptibility to non-hosts.

Keywords: cataracts, *Diplostomum pseudospathaceum*, *Oncorhynchus mykiss*, parasite–host interactions, transmission, Trematoda.

INTRODUCTION

Parasites that are transmitted trophically (i.e. through predation) between hosts in their life cycles often alter their intermediate hosts' phenotype (e.g. behaviour, appearance), thereby predisposing hosts to increased risk of predation (reviewed by Moore, 2002). Since completion of the life cycle for an individual parasite is unlikely (e.g. Dobson *et al.*, 1992), such manipulation can give a selective advantage by enhancing parasite transmission (Rothschild, 1962; Holmes and Bethel, 1972). Recent studies, however, have emphasized that manipulation can be exploited also by predator species that are unsuitable next hosts for the parasites (Ness and Foster, 1999; Mouritsen and Poulin, 2003; Kaldonski *et al.*, 2008; Seppälä *et al.*, 2008a; but see Rossiter and Sukhdeo, 2011). This 'non-host predation' always leads to failure in parasite transmission, and because prey can be exposed to several non-host predator species in nature, it can significantly erode the adaptive value of manipulation. In some systems, however, parasites can avoid these costs with 'specific' host manipulation that predisposes parasitized hosts only to predation by target hosts (next host in the life cycle) but not to non-hosts (Levri and Lively, 1996; Seppälä *et al.*, 2006; Médoc and Beisel, 2009). In such systems, selective advantage of manipulation seems obvious. However, it is generally poorly understood by which mechanisms this specificity emerges (but see Levri and Lively, 1996; Médoc *et al.*, 2009). This is because parasites often alter general anti-predatory tactics of their hosts, which could predispose them to several different predator species.

We addressed this question using a fish eye fluke *Diplostomum pseudospathaceum* [Trematoda; called *Diplostomum spathaceum* in our earlier host manipulation studies but revealed to be *D. pseudospathaceum* using genetic analysis (Louhi *et al.*, 2010)]. Metacercariae of the parasite induce cataracts in the eye lenses of fish, which reduces their vision (Rushton, 1937, 1938; Karvonen *et al.*, 2004; Seppälä *et al.*, 2011). This alters the behaviour of the fish so that they move towards the water surface (Crowden and Broom, 1980; but see Seppälä *et al.*, 2004), and show reduced anti-predatory behaviour to aerial attacks (Seppälä *et al.*, 2004, 2008b). Moreover, parasites impair the ability of fish to adjust to the environment using cryptic coloration when observed from above (Seppälä *et al.*, 2005a). These effects predispose fish to simulated avian predation (birds are the definitive hosts of the parasite) (Seppälä *et al.*, 2004, 2005b). However, the susceptibility of fish to non-host piscivorous fish is not affected (Seppälä *et al.*, 2006).

To determine the behavioural mechanisms underlying the specificity of host manipulation by *D. pseudospathaceum*, we expanded the approach of our previous studies that examined parasite-induced alterations in traits specifically determining the susceptibility of fish to avian predation. In this study, we experimentally tested whether eye flukes alter general anti-predatory tactics of fish that affect their susceptibility to a broad range of predators (both target hosts and non-hosts) or traits that specifically determine their susceptibility to non-host predatory fish. We focused on the effects of parasites on fish activity (affects the likelihood of contact with ambush-type fish predators and detectability by both bird and non-host predators), use of shelter (protects against both bird and non-host predators), and escape response to a simulated attack by a predatory fish (affects the probability of capture in an attack by a non-host fish) (see Godin, 1997; Smith, 1997). Given previous evidence of specific host manipulation in this system (Seppälä *et al.*, 2006), we hypothesized that (1) the parasites do not affect the traits examined, or that (2) alterations in them have opposite effects in determining the susceptibility of fish to piscine predation. The latter is possible, for example, if parasitized fish are less capable in escaping predators and therefore reduce their activity and/or increase preference for shelter in order to avoid attacks.

METHODS

Study animals

We used juvenile rainbow trout, *Oncorhynchus mykiss*, in this study. From the variety of fish host species suitable for the parasite, we selected rainbow trout because they are highly susceptible to infection (Betterton, 1974) and are easy to maintain under laboratory conditions. We obtained the fish from a commercial fish farm. We recognize that the behaviour of farmed fish may not be fully comparable with that of free-living fish. However, wild fish are commonly infected with several parasite species (including *Diplostomum* eye flukes), which favours the use of farmed fish. Fourteen months before the experiment, we exposed randomly chosen fish to *D. pseudospathaceum* cercariae under laboratory conditions at a water temperature of 12.3°C. Since the metacercarial stages of the parasite are long-lived (Chappell *et al.*, 1994), and their complete development takes 1–2 months depending on water temperature (see Sweeting, 1974), this procedure ensured that the parasites were fully developed by the time of the experiment and capable of manipulating fish (Seppälä *et al.*, 2005b). We obtained parasite cercariae from 10 naturally infected *Lymnaea stagnalis* snails. We pooled all the cercariae released by the snails into one suspension, from which we estimated the cercarial density by taking twenty 1-ml samples. We conducted the parasite exposures using six tanks each containing 150 fish in 250 litres of water. Three randomly chosen tanks received an infection dose of 100 cercariae per fish. We chose this exposure dose, as our aim was to produce fish with infection intensities [intensity indicates the number of parasites in an infected host (Bush *et al.*, 1997)] high enough to induce possible effects but still corresponding to natural infections (see Valtonen and Gibson, 1997; Marcogliese *et al.*, 2001). We sham-exposed the fish in the remaining three tanks with water and retained them as control fish. During the exposures, water flow through the tanks was turned off, and we aerated the water using aquarium pumps. After 30 min of exposure, we turned the water flow on to flush off any cercariae remaining in the tanks. We then increased the water volume in each tank to 1385 litres. We maintained the fish under these conditions until the experiment and fed them daily with commercial fish pellets (Royal Plus, Raisio Feed Ltd., Raisio, Finland). It is important to note that the parasite can infect fish only as cercariae, i.e. parasitized fish cannot pass the infection to other individuals. We infected more fish than used in the experiment described below, because fish were also used in other studies (e.g. Seppälä *et al.*, 2008b).

Experimental design

We conducted the experiment in four tanks (bottom: 190 × 190 cm; water depth: 15 cm; colour: dark green). We used a shallow water depth as it enables observations from above (see below) and subsequent two-dimensional spatial analysis without loss of measurement accuracy (Barber and Huntingford, 1996; Seppälä *et al.*, 2008b). We illuminated the tanks with fluorescent tubes (True Light 36 W) placed 280 cm above the water surface. We set the light intensity to 200 lux measured from the water surface and adjusted the lighting so that all parts of the tanks received similar light intensity. We set the flow of water through the tanks to 10 litres per minute. To measure the activity and escape responses of fish, we used tanks with water only (Fig. 1). To measure the use of shelter by fish, we stood whole and halved bricks (26 × 12.5 × 5.5 cm and 13 × 12.5 × 5.5 cm, respectively) on their long sides along one half of each tank, thus providing shelter for fish (Fig. 1). During the experiment,

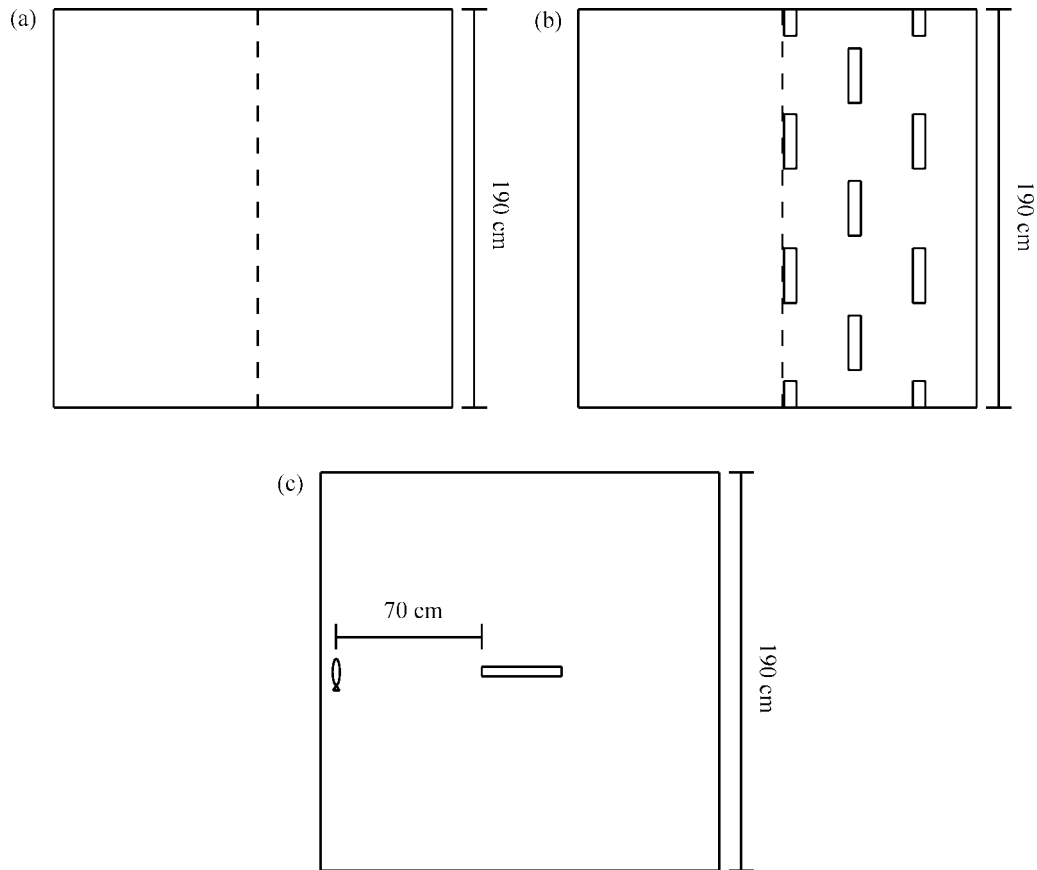


Fig. 1. Overhead schematic view of the experimental arenas used to measure (a) activity [number of times fish crossed a centre line (dashed line) of a tank in 60 min], (b) use of shelter [proportion of time spent unexposed (on the side with bricks; rectangles indicate the locations of the bricks) in 60 min], and (c) response to underwater predator attack [rectangle indicates the location of the simulated predator (length: 35 cm; diameter: 5 cm) before the attack] by fish.

water temperature ranged between 13.7°C and 16.9°C, corresponding to the natural temperature fluctuations at the time of the study.

We measured each behavioural trait from 12 individual parasitized and 12 individual control fish (we used different fish for each trait). We chose this sample size because it is similar to that used in our previous studies revealing phenotypic changes in traits that predispose fish to bird predators (Seppälä *et al.*, 2004, 2005a, 2005b). At each round of the experiment, we measured one of the behavioural traits from two parasitized fish and from two control fish. We took the fish evenly from all storage tanks, and randomly assigned them to the experimental tanks. We allowed the fish to recover from the transfer for 10 h before the experiment. To assess the activity of fish, we counted the number of times they crossed the centre line of the tank (Fig. 1a) in 60 min. To estimate the use of shelter by fish, we measured the proportion of time fish spent unexposed (on the side of the tank with bricks; Fig. 1b) in 60 min. To measure the reaction of fish to simulated attack by

a piscivorous fish, we pushed a 35-cm long circular cylinder (diameter: 5 cm) attached to a long stick from the centre of the tank towards the fish when they were motionless next to the edge of the tank (distance between the fish and the simulated predator before attack: 70 cm; duration of the attack: 1 s; Fig. 1c). We recorded the distance (± 1 cm) between the fish and the cylinder when the fish reacted to the attack by escaping. We measured all traits from recordings taken using JVC TK-C1380 Colour Video Cameras placed above each tank.

After the experiment, we killed the fish with an overdose of 0.01% MS 222 (Sigma Chemical Co., St. Louis, MO). We determined the coverage of parasite-induced cataracts from both eyes using a Kowa Portable Slit Lamp SL-14 microscope (Wall and Bjerkås, 1999; Karvonen *et al.*, 2004) and a subjective scale, where 0 = no cataracts, 1 = cataracts covering less than 25% of the lens horizontal area, 2 = cataracts covering 25–50%, 3 = cataracts covering 50–75%, 4 = cataracts covering 75–100%, and 5 = cataracts covering 100% of the lens horizontal area. We did not consider the thickness of the cataracts. We counted the *D. pseudospathaceum* metacercariae by dissecting the lenses (all metacercariae were alive at the time of the dissection as they were moving), and measured the length (± 1 mm) and mass (± 0.1 g) of each fish. All fish exposed to cercariae became parasitized, the mean intensity being 41.3 parasites per fish (range: 12–74). This caused cataract formation in the lenses, and its coverage varied between <25% and 100% (median: 75–100%). In addition, 54% of the control fish had a low-level *D. pseudospathaceum* infection (range: 1–4; mean: 1.9 parasites per fish) obtained at the fish farm, but no cataracts were observed. The average body length and mass $\pm 95\%$ CI of fish was 246 ± 3.6 mm and 183 ± 9.1 g, respectively. One fish died during the recovery period, and we could not measure the response to simulated predator attack from one fish. We excluded these individuals from the data. We conducted the experiment with permission of the Lab-Animal Care and Use Committee of the University of Jyväskylä.

Statistical analyses

To assess whether parasitized and control fish differed in their behaviour, we analysed the variation in all measured behavioural traits using analyses of covariance (ANCOVAs). In the analyses, we used infection status of fish (parasitized, control) as a fixed factor, and fish length and mass as covariates. We reduced the above models by dropping the covariates with statistically non-significant effects (we always dropped the most non-significant covariate first). We also calculated the proportion of total variance explained by infection status of fish (η^2) for each behavioural trait. Assumptions of the all analyses were fulfilled, and we performed them using IBM SPSS v.19.0 (SPSS Inc., Chicago, IL) statistical software.

RESULTS

The length of fish affected only their use of shelter (ANCOVA: $F_{1,20} = 4.509$, $P = 0.046$), and the mass of fish did not affect any of the measured behavioural traits. Therefore, we dropped the non-significant covariates from the final statistical models. Parasitized fish did not differ from the control fish in any of the measured behavioural traits [AN(C)OVAs: activity: $F_{1,22} = 0.077$, $P = 0.783$; use of shelter: $F_{1,20} = 0.091$, $P = 0.766$; response to underwater predator attack: $F_{1,21} = 0.111$, $P = 0.742$; Fig. 2]. For activity and escape response to underwater predator attack, variation among fish individuals was high and infection status explained only 0.35% and 0.53% of their total variance, respectively. For use of shelter,

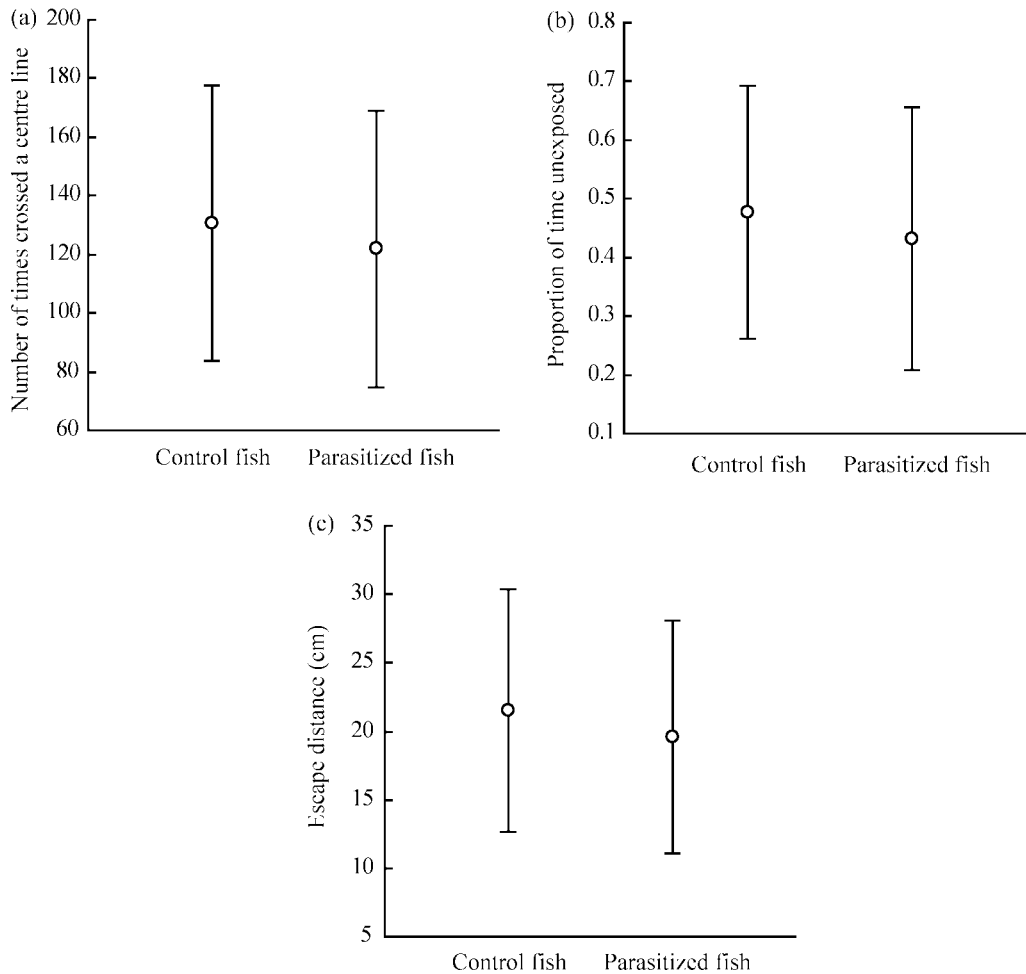


Fig. 2. (a) Activity (number of times fish crossed a centre line of a tank; mean $\pm$ 95% CI), (b) use of shelter (proportion of time unexposed; length-adjusted mean $\pm$ 95% CI), and (c) response to underwater predator attack (escape distance; mean $\pm$ 95% CI) for parasitized and control fish.

variation among individuals was extremely high, as some individuals showed almost complete preference and some almost complete avoidance of shelter in both parasitized and control groups. Infection status of fish explained 0.37% of the total variance in this trait.

DISCUSSION

The costs of host manipulation due to risk of non-host predation have received wide interest in recent parasite–host research. Host manipulation is often exploited by predator species that are unsuitable next hosts for the parasites (Ness and Foster, 1999; Mouritsen and Poulin, 2003; Kaldonski *et al.*, 2008; Seppälä *et al.*, 2008a; but see Rossiter and Sukhdeo, 2011), which can erode the adaptive value of manipulation. In some host–parasite interactions, however, manipulation is known to be ‘specific’, predisposing parasitized hosts only to predation by

target hosts but not to non-hosts (Levri and Lively, 1996; Seppälä *et al.*, 2006; Médoc and Beisel, 2009). This is surprising, because many of the reported parasite-induced phenotypic alterations could be expected to increase host susceptibility to predation not only by target hosts but also by other species (but see Levri and Lively, 1996; Médoc *et al.*, 2009).

In the present study, we examined the behavioural mechanisms underlying the specificity of host manipulation by *D. pseudospathaceum* (Seppälä *et al.*, 2006). The parasite induces strong phenotypic alterations (Crowden and Broom, 1980; Seppälä *et al.*, 2004, 2005a, 2008b) that predispose host fish to predation by avian target hosts (Seppälä *et al.*, 2004, 2005b). Since manipulation is most likely caused by impaired vision of fish (Seppälä *et al.*, 2005b), the ability of parasitized fish to avoid predators could be expected to decrease in general, not only in the case of avian predators. When we examined the effects of the parasite on traits that are part of the general anti-predatory tactics of fish (activity, use of shelter) or specifically determine their susceptibility to piscivorous fish (escape response to a simulated attack by a predatory fish), we found that none of the traits examined was affected by the parasite. This is surprising but may be explained by the ability of fish to use other sensory mechanisms to explore their environment and to avoid underwater predator attacks. For example, the lateral line system is very effective in detecting hydrodynamic pressure differences in the environment (reviewed by Montgomery *et al.*, 1995). Thus, the relative role of vision in determining the susceptibility of fish to piscine predators may be small when compared with the other senses. In contrast, vision is likely to be very important in avoidance of avian predators, as it enables detection of approaching birds before they plunge into the water. Therefore, it is possible that reducing the vision of fish by locating in the eyes and inducing cataracts can be highly advantageous for *D. pseudospathaceum* by specifically predisposing the fish to predation by target hosts.

Variation among individuals was very high in all behavioural traits examined, and infection status of fish explained only 0.35–0.53% of the total variance in them. This suggests that eye flukes have only a weak effect on these traits, and the observed variation results from other factors. Interestingly, fish did not show any preference for sheltered areas of the tanks (i.e. the side of the tank with bricks; Fig. 2b). This is surprising, because the use of shelter could effectively give protection against a wide variety of different predators. However, low preference for shelter in juvenile rainbow trout was reported in another study in which preference for shelter was found only in certain populations during periods of cold water (Reeves *et al.*, 2010). As our experiment was conducted during the summer months (water temperature ranged between 13.7°C and 16.9°C), our results are in line with those findings. These results suggest that use of shelter may not be an important trait for predator avoidance in this system.

It is important to note that we were unable to use control fish that were all completely free of eye flukes. This is because the fish originated from a fish farm where they had been reared in lake water, which resulted in low-level infections (range: 1–4 parasites per fish) in some control individuals. It has been suggested also that low parasite intensities could affect the vision of threespine sticklebacks (Owen *et al.*, 1993). Our earlier results, however, indicate that in rainbow trout high infection levels that result in strong cataracts are required to alter fish behaviour so that their susceptibility to predation increases (Seppälä *et al.*, 2005b). Therefore, because the control fish used in this study did not have any cataracts, it is unlikely that the observed infections would have modified their behaviour. However, we could not test this statistically because individuals carrying parasites were unevenly distributed among the different traits examined, thus leading to highly unbalanced data for such tests.

In conclusion, our results suggest that *D. pseudospathaceum* eye flukes do not affect the behavioural traits of fish that could be important in determining their susceptibility to piscine predators. Thus, the observed specificity of host manipulation (Seppälä *et al.*, 2004, 2005b, 2006) is likely to be driven by impairment of fish vision, which is a crucial sensory mechanism against aerial predators (Seppälä *et al.*, 2005b), while the infection does little or no harm to other senses that can be used to avoid non-host underwater attacks. Therefore, host manipulation may provide a highly selective advantage for the parasite in terms of increased transmission success in the life cycle in this system.

ACKNOWLEDGEMENTS

We thank J. Iso-Tuisku for assistance in the laboratory, and D.P. Benesh and M.L. Rosenzweig for comments on the manuscript. Konnevesi Research Station provided the facilities for the experiment. The study was funded by the Emil Aaltonen Foundation (O.S.) and the Centre of Excellence in Evolutionary Research (Academy of Finland and University of Jyväskylä) (A.K.).

REFERENCES

- Barber, I. and Huntingford, F.A. 1996. Parasite infection alters schooling behaviour: deviant positioning of helminth-infected minnows in conspecific groups. *Proc. R. Soc. Lond. B*, **263**: 1095–1102.
- Betterton, C. 1974. Studies on the host specificity of the eye fluke, *Diplostomum spathaceum*, in brown and rainbow trout. *Parasitology*, **69**: 11–29.
- Bush, A.O., Lafferty, K.D., Lotz, J.M. and Shostak, A.W. 1997. Parasitology meets ecology on its own terms: Margolis *et al.* revisited. *J. Parasitol.*, **83**: 575–583.
- Chappell, L.H., Hardie, L.J. and Secombes, C.J. 1994. Diplostomiasis: the disease and host–parasite interactions. In *Parasitic Diseases of Fish* (A.W. Pike and J.W. Lewis, eds.), pp. 59–86. Dyfed: Samara Publishing.
- Crowden, A.E. and Broom, D.M. 1980. Effects of eye fluke, *Diplostomum spathaceum*, on the behaviour of dace (*Leuciscus leuciscus*). *Anim. Behav.*, **28**: 287–294.
- Dobson, A.P., Hudson, P.J. and Lyles, A.M. 1992. Macroparasites: it's a wormy world. In *Natural Enemies: The Population Biology of Predators, Parasites and Diseases* (M.J. Crawley, ed.), pp. 329–348. Oxford: Blackwell Scientific.
- Godin, J.-G.J. 1997. Evading predators. In *Behavioral Ecology of Teleost Fishes* (J.-G.J. Godin, ed.), pp. 191–236. Oxford: Oxford University Press.
- Holmes, J.C. and Bethel, W.M. 1972. Modification of intermediate host behaviour by parasites. In *Behavioural Aspects of Parasite Transmission* (E.U. Canning and C.A. Wright, eds.), pp. 123–149. London: Academic Press.
- Kaldonski, N., Perrot-Minnot, M.-J., Motreuil, S. and Cézilly, F. 2008. Infection with acanthocephalans increases the vulnerability of *Gammarus pulex* (Crustacea, Amphipoda) to non-host invertebrate predators. *Parasitology*, **135**: 627–632.
- Karvonen, A., Seppälä, O. and Valtonen, E.T. 2004. Eye fluke-induced cataract formation in fish: quantitative analysis using an ophthalmological microscope. *Parasitology*, **129**: 473–478.
- Levri, E.P. and Lively, C.M. 1996. The effect of size, reproductive condition, and parasitism on foraging behaviour in a freshwater snail, *Potamopyrgus antipodarum*. *Anim. Behav.*, **51**: 891–901.
- Louhi, K.-R., Karvonen, A., Rellstab, C. and Jokela, J. 2010. Is the population genetic structure of complex life cycle parasites determined by the geographic range of the most motile host? *Infect. Genet. Evol.*, **10**: 1271–1277.
- Marcogliese, D.J., Dumont, P., Gendron, A.D., Mailhot, Y., Bergeron, E. and McLaughlin, J.D. 2001. Spatial and temporal variation in abundance of *Diplostomum* spp. in walleye

- (*Stizostedion vitreum*) and white suckers (*Catostomus commersoni*) from the St. Lawrence River. *Can. J. Zool.*, **79**: 355–369.
- Médoc, V. and Beisel, J.-N. 2009. Field evidence for non-host predator avoidance in a manipulated amphipod. *Naturwissenschaften*, **96**: 513–523.
- Médoc, V., Rigaud, T., Bollache, L. and Beisel, J.-N. 2009. A manipulative parasite increasing an antipredator response decreases its vulnerability to a nonhost predator. *Anim. Behav.*, **77**: 1235–1241.
- Montgomery, J., Coombs, S. and Halstead, M. 1995. Biology of the mechanosensory lateral line in fishes. *Rev. Fish Biol. Fish.*, **5**: 399–416.
- Moore, J. 2002. *Parasites and the Behaviour of Animals*. New York: Oxford University Press.
- Mouritsen, K.N. and Poulin, R. 2003. Parasite-induced trophic facilitation exploited by a non-host predator: a manipulator's nightmare. *Int. J. Parasitol.*, **33**: 1043–1050.
- Ness, J.H. and Foster, S.A. 1999. Parasite-associated phenotype modifications in threespine stickleback. *Oikos*, **85**: 127–134.
- Owen, S.F., Barber, I. and Hart, P.J.B. 1993. Low level infection by eye fluke, *Diplostomum* spp., affects the vision of three-spined sticklebacks, *Gasterosteus aculeatus*. *J. Fish Biol.*, **42**: 803–806.
- Reeves, G.H., Grunbaum, J.B. and Lang, D.W. 2010. Seasonal variation in diel behaviour and habitat use by age 1 + Steelhead (*Oncorhynchus mykiss*) in Coast and Cascade Range streams in Oregon, U.S.A. *Environ. Biol. Fish.*, **87**: 101–111.
- Rossiter, W. and Sukhdeo, M.W.K. 2011. Exploitation of asymmetric predator–prey interactions by trophically transmitted parasites. *Oikos*, **120**: 607–614.
- Rothschild, M. 1962. Changes in behaviour in the intermediate hosts of trematodes. *Nature*, **193**: 1312–1313.
- Rushton, W. 1937. Blindness in freshwater fish. *Nature*, **140**: 1014.
- Rushton, W. 1938. Blindness in freshwater fishes. *Nature*, **141**: 289.
- Seppälä, O., Karvonen, A. and Valtonen, E.T. 2004. Parasite-induced change in host behaviour and susceptibility to predation in an eye fluke–fish interaction. *Anim. Behav.*, **68**: 257–263.
- Seppälä, O., Karvonen, A. and Valtonen, E.T. 2005a. Impaired crypsis of fish infected with a trophically transmitted parasite. *Anim. Behav.*, **70**: 895–900.
- Seppälä, O., Karvonen, A. and Valtonen, E.T. 2005b. Manipulation of fish host by eye flukes in relation to cataract formation and parasite infectivity. *Anim. Behav.*, **70**: 889–894.
- Seppälä, O., Karvonen, A. and Valtonen, E.T. 2006. Host manipulation by parasites and risk of non-host predation: is manipulation costly in an eye fluke–fish interaction? *Evol. Ecol. Res.*, **8**: 871–879.
- Seppälä, O., Valtonen, E.T. and Benesh, D.P. 2008a. Host manipulation by parasites in the world of dead-end predators: adaptation to enhance transmission? *Proc. R. Soc. Lond. B*, **275**: 1611–1615.
- Seppälä, O., Karvonen, A. and Valtonen, E.T. 2008b. Shoaling behaviour of fish under parasitism and predation risk. *Anim. Behav.*, **75**: 145–150.
- Seppälä, O., Karvonen, A. and Valtonen, E.T. 2011. Eye fluke-induced cataracts in natural fish populations: is there potential for host manipulation? *Parasitology*, **138**: 209–214.
- Smith, R.J.C. 1997. Avoiding and deterring predators. In *Behavioral Ecology of Teleost Fishes* (J.-G.J. Godin, ed.), pp. 163–190. Oxford: Oxford University Press.
- Sweeting, R.A. 1974. Investigations into natural and experimental infections of freshwater fish by the common eye-fluke *Diplostomum spathaceum* Rud. *Parasitology*, **69**: 291–300.
- Valtonen, E.T. and Gibson, D.I. 1997. Aspects of the biology of diplostomid metacercarial (Digenea) populations occurring in fishes in different localities of northern Finland. *Ann. Zool. Fenn.*, **34**: 47–59.
- Wall, T. and Bjerkås, E. 1999. A simplified method of scoring cataracts in fish. *Bull. Eur. Assoc. Fish Pathol.*, **19**: 162–165.

