Parasitol Res (2017) 116:1773–1779
DOI 10.1007/s00436-017-5464-4

ORIGINAL PAPER

Behavioural modification of personality traits: testing the effect of a trematode on nymphs of the red damselfly *Xanthocnemis zealandica*

Antoine Filion[1] · **Clément Lagrue**[2] · **Bronwen Presswell**[2] · **Robert Poulin**[2]

Received: 27 February 2017 / Accepted: 26 April 2017 / Published online: 4 May 2017

Abstract Research on animal personality is increasingly demonstrating that individuals in a population are characterised by distinct sets of behavioural traits that show consistency over time and across different situations. Parasites are known to alter the behaviour of their hosts, although their role in shaping host personality remains little studied. Here, we test the effect of trematode infection on two traits of their host's personality, activity and boldness, in nymphs of the red damselfly *Xanthocnemis zealandica*. Genetic analyses indicate that the undescribed trematode species falls within the superfamily Microphalloidea. Results of laboratory behavioural tests indicate that the two behavioural traits are related to each other: bolder individuals also show higher levels of spontaneous activity than shy ones. However, parasite infection had no effect on either of these behaviours or on their repeatability over three separate testing sessions. Although our findings suggest that this trematode does not influence personality traits of the damselfly host, it remains possible that other standard personality traits not tested here (exploratory tendency, aggressiveness) are affected by infection.

Keywords Behavioural modification · Trematodes · Repeatability · Host-parasite interactions · Animal personality

Introduction

Recent studies on animal personality have revealed that each individual of a given species has a particular set of behavioural traits, repeatable under various conditions (Sih et al. 2004). At the same time, parasitological studies suggest that parasites play a big part in influencing the behaviour of an organism (Poulin 1994, 2010; Moore 2002; Poulin et al. 2005). Indeed, parasites have long been known to influence the behaviour of their host (Bethel and Holmes 1974; Moore 1984). This behavioural modification by the parasite can be direct, for example by influencing the nervous system of the host, or indirect, by altering subtle aspects of host development or physiology (Thomas et al. 2005). If adaptive, both types of behavioural modification involve changes in host phenotype that result in an increase in the parasite's fitness (Poulin 2010). In parasites with complex life cycles, behavioural manipulation by a parasite is more likely to occur in the intermediate host as a way of increasing trophic transmission, i.e. their susceptibility to predation by the final host (Lafferty 1999).

Diverse parasites from a wide range of taxa (protozoans, nematodes, trematodes, cestodes, acantocephalans) are known to induce a wide range of alteration to the behaviour of their host (Poulin 1995; Moore 2002). Some of the most interesting cases are found among trematodes, which provide striking textbook examples of both direct and indirect manipulation. A good example of a direct behavioural modification involves the digenean trematode *Dicrocoelium dendriticum*, which modifies the behaviour of its intermediate host (ant) by forcing it to climb to the top of a grass stalk and anchor itself there for hours at a time, allowing the parasite's final host, a sheep, to accidently graze upon the ant and thereby ingest the parasite as well (Lucius et al. 1980; Moore 2002). Another good example of an indirect behavioural modification is provided by the digenean trematode *Leucochloridium* sp. Sporocysts of this parasite modify the

✉ Robert Poulin
robert.poulin@otago.ac.nz

[1] Département des Sciences de l'environnement, Université du Québec à Trois-Rivières, C.P. 500, Trois-Rivières, Québec G9A 5H7, Canada

[2] Department of Zoology, University of Otago, PO Box 56, Dunedin 9054, New Zealand

phenotype of their intermediate host (terrestrial snail) by forming broodsacs within the eyestalks of the snail and causing them to pulsate with vibrant colour, making the snail more visible to a potential avian predator (Moore 2002).

Those two examples highlight the effect that trematode manipulators have on their hosts. Generally, in order to be successful in their manipulation, such parasites must alter not one but several traits in the host (Poulin 2013), meaning that parasite-induced behavioural modification must be studied in a comprehensive framework. As a matter of fact, Sih et al. (2004) argued that many behavioural traits of organisms are highly correlated and repeatable over time, making it important to study the behaviour of an animal across multiple situations and over time. This guideline was further developed by Réale et al. (2007), who established that the repeatability and consistency of behavioural traits, across individuals and over time, are defining characteristics of animal personality. Typically, personality is measured across five different traits: (1) shyness-boldness, (2) exploratory tendency, (3) activity, (4) sociability, and (5) aggressiveness (Réale et al. 2007).

Some of those personality traits have recently been investigated in the light of parasite manipulation of intermediate hosts (Coats et al. 2010; Hammond-Tooke et al. 2012). For example, Coats et al. (2010) found no correlation between parasite infection level and behaviour of amphipod hosts, although infected individuals exhibited significantly weaker repeatability of behaviour than their uninfected counterparts. Behavioural repeatability can be decreased by parasites through pathology and lower energy reserves in infected hosts, causing them to act more erratically. Alternatively, lower repeatability of host behaviour could be part of an adaptive manipulation of host phenotype by the parasite. For example, lower repeatability may increase the chances of transmission by predation to a definitive host, if the infected intermediate host does not always react appropriately to a threat stimulus (Poulin 2013). Nevertheless, links between host manipulation by parasites and animal personality remain mostly unexplored.

The present study investigates the potential effect of parasite infection on two specific behavioural traits of the host, its activity (spatial distribution) and boldness (reaction to a threat). Our model system consists of an undescribed trematode parasite occurring as metacercariae in the aquatic nymph (juvenile) of the red damselfly *Xanthocnemis zealandica*, in a New Zealand stream. Our main goals were to (1) provide a genetic characterization and partial identification of the trematode, as well as confirm that birds are serving as definitive host, and (2) investigate the parasite-host relationship in a behavioural manipulation context by testing for alterations in two personality traits of the intermediate host. We hypothesised that if the parasite manipulates its damselfly host to increase predation by its bird definitive host, infected damselflies should be more active and bolder than uninfected ones. In addition, behavioural traits should be less repeatable (more erratic) in infected individuals than uninfected ones.

Methods

Sampling

Sampling took place on 17 February 2016 on the Waipori River (45° 95′ S, 170° 12′ E), south of Dunedin, South Island, New Zealand. A dip net was used to sample various kinds of substrate. Afterward, all materials were sorted using sieves and damselfly nymphs were retrieved, and returned to the laboratory in water from the collection site. To minimise size variation among tested individuals, only nymphs ≥5 mm in body length (caudal gills excluded) were used (Brodin and Johansson 2004). For the experiments, 48 individuals were individually placed in labelled 125-ml plastic containers, which were immersed in a larger tank filled with water from the collection site, and maintained at approximately 18 °C on a natural photoperiod. Individual containers were left open and their aperture was covered with 80-μm-sized mesh to allow regular water flow.

Before each experiment, damselfly nymphs were transferred into the experimental arena at least 24 h prior to the start of the test to allow acclimation (Brodin and Johansson 2004; Brodin 2008). Experiments took place between 14 and 21 March 2016, between 1400 h and 1700 h daily.

Tests of activity

For this experiment, damselflies were placed individually in a 170 mm × 120 mm × 68 mm-test arena with a grid of 1-cm squares marked on its front, to evaluate their spontaneous activity in the absence of stimulus. The test arena was filled with 300 ml of water from the collection site and each damselfly nymph was fed with zooplankton 24 h prior to each experiment to eliminate any possible food foraging behaviour. The exact position of a damselfly was recorded every 10 min for 140 min, according to the protocol of Brodin (2008). Damselflies were considered to have moved when their entire head was in another square. The data were compiled as the total number of squares visited, divided by the duration of the experiment, thus giving a number of centimetres moved per 10 min (Brodin and Johansson 2004; Brodin 2008). This test was repeated three times for each individual (except for two nymphs that died before the third test), with an interval of 2 days separating each repeat test, during which the individuals were returned to their original maintenance mesh-covered container within the larger tank.

Test of boldness

In this experiment, damselfly nymphs were subjected to a disturbance, and their response was recorded. Each nymph was tested in the same test arena right after the activity experiment, since shifting the individual to a new tank could cause unwanted stress (Réale et al. 2007). Each individual was lightly poked on the abdomen by a grass stalk to initiate a response to disturbance. We then measured the time lag between the cessation of movement immediately after the disturbance until the next movement (e.g. return to a normal state after the typical freezing behaviour of these nymphs). Damselfly nymphs that returned to a normal behaviour (e.g. crawling or swimming) within 120 s were classified as bold (quick resumption of movement), while those that remained still after 120 s (end of the monitoring period) were classified as shy. As for the previous experiment, this test was repeated three times for each individual with an interval of 2 days separating each repeat test.

Dissection, parasite recovery, and genetic analysis

All damselfly nymphs were measured (head width and body length—excluding caudal gills) and dissected. The parasites they harboured were counted, extracted, and placed in 99% ethanol for genetic analysis. For these analyses, 11 isolates of the metacercaria were characterised molecularly, ten encysted examples and one pre-encysted. Genomic DNA was extracted from ethanol-fixed isolates in 200 μl of a 5% suspension of Chelex® in deionised water and containing 0.1 mg/ml proteinase K followed by incubation at 56 °C for 5 h, boiling at 90 °C for 8 min, and centrifugation at 14,000 *g* for 10 min. The D2 domain of the large subunit of ribosomal DNA (28S) was amplified using primers T16 (5′ GAGACCGATAGC GAAACAAGTAC 3′) and T30 (5′ TGTTA GACTCCTT GGTCCGTG 3′) (Harper and Saunders 2001).

Polymerase chain reaction (PCR) amplifications were performed in 25-μl reactions containing 5 μl of extraction supernatant, 1× PCR buffer (16 mM $(NH_4)2SO_4$, 67 mM Tris-HCl at pH 8.8), 2 mM $MgCl_2$, 200 μM of each dNTP, 0.5 mM each primer, and 0.7 units BIOTAQ™ DNA polymerase (Bioline Ltd.). Thermocycling conditions used for amplification of the 28S region follow Blasco-Costa et al. (2010). PCR amplicons were purified prior to sequencing using exonuclease I and shrimp alkaline phosphatase enzymes (Werle et al. 1994). Amplicons were cycle-sequenced from both strands using PCR primers, employing BigDye® Terminator v. 3.1 Ready Reaction Cycle Sequencing Kit, alcohol-precipitated, and run on an ABI 3730XL Analyser (Applied Biosystems, Foster City, CA, USA). Contiguous sequences were assembled and edited using Bioedit v.7 (Hall 1999) and a representative sequence submitted to GenBank (accession number KY623662).

Newly generated sequences for the 28S rDNA together with published sequences of microphalloid species from Genbank were aligned using ClustalW implemented in MEGA v6 (Tamura et al. 2013). The extremes of the alignments were trimmed to match the shortest sequence prior to phylogenetic analyses. The 28S dataset (633 bp long) included 19 representative sequences of species of the superfamily Microphalloidea retrieved from GenBank. Three sequences of species belonging to the superfamily Plagiorchioidea and six of the Lepocreadioidea were included, with one sample of a gorgoderoid serving as outgroup on which the tree was rooted. The phylogenetic analysis was conducted in MEGA6 (Tamura et al. 2013) and inferred using the maximum likelihood method based on the Tamura-Nei model (tree not shown).

Data analysis

All statistical analyses were performed using the R software version 3.2.4 (R core team 2016). We used general linear mixed models with random effect using a gamma distribution (package: lme4) to analyse data on our two response variables while controlling for host body size. As mentioned above, activity values consisted of distance moved divided by the number of observations. However, since some nymphs did not move at all during the experiment, we had activity values equal to 0, which violates the assumptions of a gamma distribution. To fix this, we used body length of inactive nymphs to account for possible movements that would not have been recorded due to our experimental setup, and divided body length by the number of observations. This allowed us to respect the strictly positive values assumed by the gamma distribution while giving near-zero activity values for all inactive nymphs, with larger nymphs getting slightly higher values. The fixed effects of our activity model were the length of the individuals (scaled value), the presence of parasite (coded as dummy variable, i.e. present or absent), the boldness level (shy or bold), and whether the measure came from the first, second, or third repeat of the test. In this model, we used the shyness/boldness of individuals as a fixed effect since this parameter is known to influence activity level (Brodin 2008).

To test our other hypothesis (i.e. whether parasite influence boldness), we used a logistic model (package: lme4) to explain the variation observed in the second behavioural axis evaluated in this study, i.e. the shy-bold distinction. Our fixed effects for this model were the scaled body length of the individuals, whether the measure came from the first, second, or third repeat of the test (to check if repeat testing had an effect on the nymphs), and parasite presence. For all of our models, the test variable was split in two columns using a variance matrix and tests were separated using dummy variables combination (0:1 for test 1, 1:1 for test 2, and 1:0 for test 3), meaning that we had 144 data points in our data sheet (3 values for 48 individuals). We used individual ID as the random factor for our two models to account for idiosyncratic differences among individuals. We then used the dredge function (package: NuMIn) to evaluate every possible combinations of variables that could influence our response variables.

Finally, we selected the best models using the Akaike information criterion (AIC).

As for the repeatability in behaviour, we used the intraclass correlation coefficient (ICC) to estimate the repeatability in the behavioural response of the damselflies across our three tests. We used the ICC package to extract ICC estimates and 95% confidence intervals from our data (Wolak et al. 2012). We then used a Student's *t* test to verify if the repeatability observed for the two behaviours evaluated was significantly different between infected and uninfected individuals.

Results

Overall, 20 of the 48 (42%) damselfly nymphs were infected by metacercariae. Infection level ranged from one to three metacercariae per individual, with the majority of the infected damselflies (12 out of 20) infected by only one parasite. A total of 34 metacercariae were recovered from the 20 infected damselflies. Only one of these metacercariae was not fully encysted, possibly indicating a recent infection; however, its host was retained in the analyses as it also harboured a fully encysted metacercaria. We classified 31 individual hosts out of 48 (64.5%) as bold since they had a quick resumption of movement in at least two tests. We decided to use only one body size measurement (body length) in our model since the two measurements taken (body length and head width) were highly correlated ($R^2 = 0.8684$).

Genetic characterization of the trematode

All 11 isolates of metacercariae were genetically identical for their 28S sequences, notwithstanding a single dimorphic site. The sequence was used in a BLASTn search (http://blast.ncbi.nlm.nih.gov/) on GenBank, revealing no close match, but a general relationship with sequences from members of the superfamily Microphalloidea.

A tree (not shown) based on the 28S sequences available on Genbank was inferred, and the species assigned to higher taxa as designated in Tkach et al. (2003), Cribb et al. (2003) and Olson et al. (2003). Support for the relevant relationships was poor in this tree, but the specimen was placed either as a sister genus to *Microphallus* or as sister clade to representatives of the families Prosthogonimidae and Pleurogenidae. The latter two families contain species that regularly use Odonata (damselflies) as second intermediate hosts. A conservative identification therefore places these specimens in the superfamily Microphalloidea. Although they do not belong to any of the currently known and described microphalloids from New Zealand (*See* Presswell et al. 2014), this partial identification indicates that the parasite's definitive host is a bird, in this case, a species of waterfowl.

Activity

We selected the best model to explain variation in the activity of the damselfly nymphs using differences in AIC (ΔAIC), meaning that we chose the model including only one fixed effect, the boldness of the individuals (Table 1, Fig. 1). We did not retain the model including boldness and parasite presence because, even if it is not significantly different from the selected model (ΔAIC = 1.9), it is not the most parsimonious model.

The only significant fixed effect in our model, the boldness of individuals, returned a negative effect on activity of the nymphs (effect size = −0.077, SE = 0.032), meaning the bold individuals were significantly more active than shyer ones (Figs. 1 and 2). Infection by metacercariae had no effect on activity levels. There was no correlation between the activity of the individuals and their size (Fig. 2); however, large bold

Table 1 Selection of the best-fitting mixed model to explain two response variables using the Akaike information criterion (AIC). All of those models include nymph body length as a covariate and the individuals tested as a random factor

Model	Model complexity	Variables in models	AIC	ΔAIC
Activity	Without interactions	Null	830.01	4.69
		Boldness	825.32	0
		Parasites	831.82	6.5
		Repeat test number	832.85	7.53
	With interactions	Boldness + parasites	827.22	1.9
		Boldness + repeat test	828.16	2.84
		Parasites + repeat test	834.67	9.35
		Boldness + parasites + repeat test	830.06	4.74
Boldness	Without interactions	Null	59.6	0
		Parasites	63.1	3.6
		Repeat test number	65.3	5.7
	With interactions	Parasites + repeat test	66.5	6.9

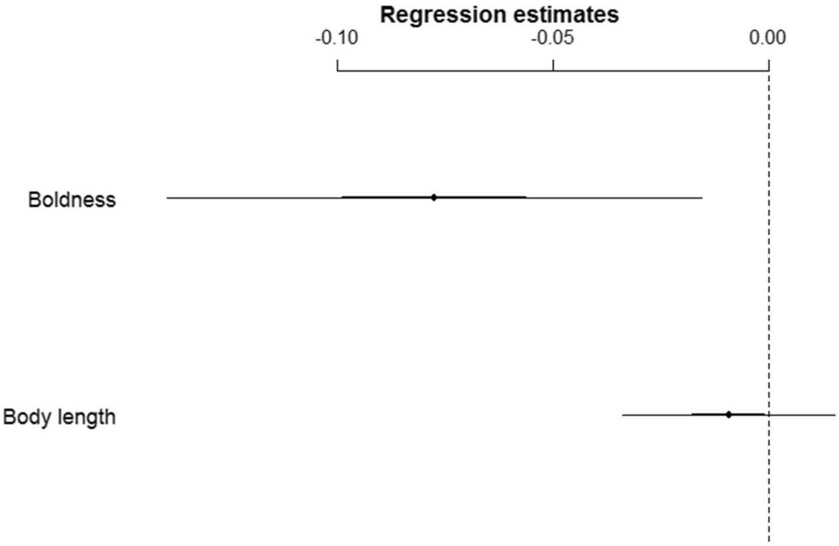

Fig. 1 Regression estimate for all fixed effects in the activity model with 95% error bars in the GLMM model that was retained (AIC = 825.32). A regression estimate different from 0 (including *error bars*) indicates that the effect is significant in the model

individuals (slope = 0.109) were more active than large shy individuals (slope = −0.033).

Boldness

We did not detect any significant effect in models for this response variable. No model was able to explain variation in boldness among nymphs (Table 1). Infection by metacercariae had therefore no effect on the risk-taking tendencies of the damselfly nymphs.

Repeatability of behaviours

There was no significant difference between infected and uninfected individuals with respect to the repeatability of both behaviours evaluated (Fig. 3). Repeatability estimates we obtained for both activity (difference of means = −0.0288, p = 0.8744) and the time taken to recover from a threat stimulus (difference of means = 0.1105, p = 0.8286) were similar for uninfected and infected individuals.

Discussion

Alterations in host behaviour induced by parasites have been reported across a wide range of host-parasite interactions (Moore 2002; Thomas et al. 2005; Poulin 2010), and they have recently been implicated as possible determinants of the key traits shaping animal personalities (Poulin 2013). Here, we tested for an effect of trematode metacercariae on two key personality traits, activity level and boldness, in nymphs of the damselfly *X. zealandica*. Both should be related to predation risk from any type of aquatic predator. Our results indicate that the two traits are linked, i.e. boldness affects activity levels, but that neither the expression of the traits themselves nor their repeatability over time was affected by parasite infection.

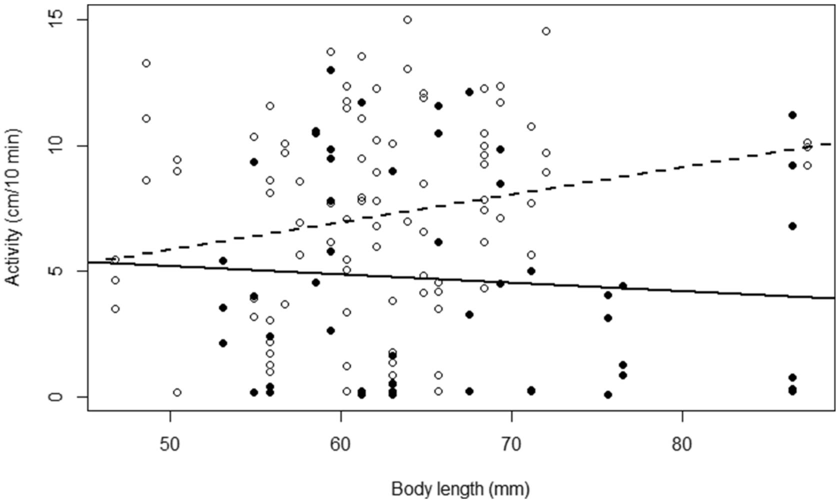

Fig. 2 Activity level for all 144 activity measurements (cm/10 min) related to the body length of the individuals. The *dashed line* represents the bold individuals (N = 31, *open circles*) while the *solid line* represents the shy individuals (N = 17, *black circles*)

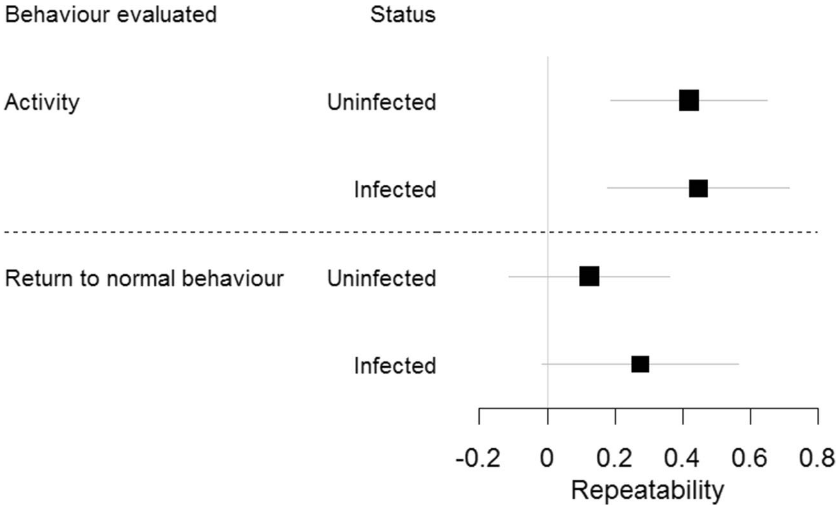

Fig. 3 Repeatability of the two behaviours evaluated (activity and return to a normal behaviour after a disturbance) shown separately for uninfected ($N = 28$) and infected ($N = 20$) damselflies nymphs. *Error bars* represent 95% confidence intervals of the ICC

Genetic identification of the metacercaria places it in the superfamily Microphalloidea. Sequencing of further genetic markers may achieve greater taxonomic resolution, although at present there are few comparable sequences available in the public domain. Regardless of the exact identity of this parasite, it is most likely transmitted to an avian definitive host. There are indeed several waterfowl species present at the site where the damselfly nymphs were collected. Trematode metacercariae have been shown to alter various behaviours in arthropod intermediate hosts, including insects, that in some cases may facilitate their trophic transmission to the definitive host (e.g. Lucius et al. 1980; Helluy 1983; Webber et al. 1987; McCurdy et al. 1999; Leaphart and Zelmer 2017; *See* Moore 2002). However, the metacercariae in our study had no influence on the two behaviours investigated.

One possibility may have to do with the timing of infection. Many juvenile parasites require a certain period of development inside their intermediate host before they trigger behavioural changes (Bethel and Holmes 1974; Urdal et al. 1995; Robb and Reid 1996). The damselfly nymphs used in the present study were collected in the second half of the austral summer. We do not know when they acquired metacercariae. Indeed, one metacercaria was not fully encysted, suggesting that some infections were perhaps very recent, with the parasites not sufficiently developed to induce changes in host behaviour. Alternatively, the ability to alter host behaviour may be intensity-dependent, and the low infection levels we observed (1–3 metacercariae per host, usually only 1) may not have been sufficient to affect the host. Also, our experimental set-up may not have been adequate to detect very subtle changes in behaviour, since behavioural changes may only become manifest under specific environmental conditions. Finally, it is also possible that the trematode species in our study is simply incapable of host behaviour manipulation, as this ability is not expected to have evolved or be expressed in all parasite species (Poulin 2010).

Our results also indicate that the metacercariae had no influence on the repeatability of the nymphs' behaviour. The consistency and repeatability of behavioural traits are central features of animal personality, contributing to the maintenance of inter-individual variability in animal populations (Sih et al. 2004; Réale et al. 2007). A previous study has shown that trematode metacercariae from the superfamily Microphalloidea can disrupt the repeatability of behaviours in arthropod intermediate hosts (Coats et al. 2010). However, the repeatability values we obtained were high for activity levels when compared to the results from a meta-analysis by Bell et al. (2009). Indeed, when examining 24 studies that analysed the repeatability of activity levels across a wide range of taxa, Bell et al. (2009) found that the average value of repeatability was around 0.25 for this behaviour, well below the value of 0.43 we obtained for our sample. Therefore, certain behaviour traits in our damselfly nymphs may be too hard-wired to be easily manipulated by parasites.

Although our study found no evidence for an influence of trematode metacercariae on the behaviour of *X. zealandica* nymphs, we have found an association between the two behaviours investigated: bolder nymphs that resume normal activity quickly following a threat stimulus are also generally more active than shy ones. This finding corroborates earlier research on the behaviour of damselfly nymphs (Brodin 2008). The apparent lack of effect of infection on nymph behaviour will need to be further evaluated, in particular by examining behavioural axes other than activity and boldness, and perhaps under more realistic conditions of predation threat once the life cycle of the trematode is elucidated.

Acknowledgment We thank Pierre Magnan and the Natural Sciences and Engineering Research Council of Canada (NSERC) CREATE-ÉcoLac grant for logistical support and funding. We also thank Olwyn Friesen and Riku Rinnevalli for field and laboratory support.

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