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Chapitre III

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Définitions

Autogène : Parasite complétant l'ensemble de son cycle de vie au sein d'un même milieu.

Allogène : Parasite qui transite à travers deux types d'écosystèmes durant son cycle de vie.

Cercaire : Deuxième stade larvaire des parasites digènes se déplaçant en eau libre.

Prévalence : Proportion ou pourcentage d'individus infectés dans une population.

Abondance : Nombre total de parasites retrouvés par individu.

Trématode digènes : Parasite nécessitant en général trois hôtes (primaire, intermédiaire, définitif) pour compléter son cycle de vie.

Chapitre I

Introduction

La présence et l'abondance d'un taxon parasitaire dans un écosystème dépendent de la capacité de celui-ci à soutenir tous les hôtes permettant à ce parasite d'accomplir son cycle vital. Plusieurs études ont montré que la prévalence et l'abondance parasitaire pouvaient varier à travers les systèmes aquatiques, suggérant une influence de l'environnement sur la capacité d'un parasite à accomplir son cycle de vie (Kennedy 1975; Kennedy 1978; Bailey et Margolis 1987). Une variété de facteurs, tant physico-chimiques (pH, conductivité; oxygène dissous; radiation ultra-violette (UV)) (Christensen et al. 1980; Pietrock et Marcogliese 2003; Studer et Poulin 2013), morphométriques (superficie du milieu, profondeur moyenne; Esch et al. 1986; Bailey et Margolis 1987; Marcogliese et Cone 1991) que biotiques (communauté ichthyenne; biomasse d'hôtes; Arneberg et al. 1998; Thielges et al. 2008; Lagrue et Poulin 2016) influençant la présence d'hôtes ainsi que la survie parasitaire ont déjà fait l'objet de différentes études (Figure 1). L'ensemble des effets étudiés dans le cadre de ce projet est détaillé dans les prochaines sous-sections.

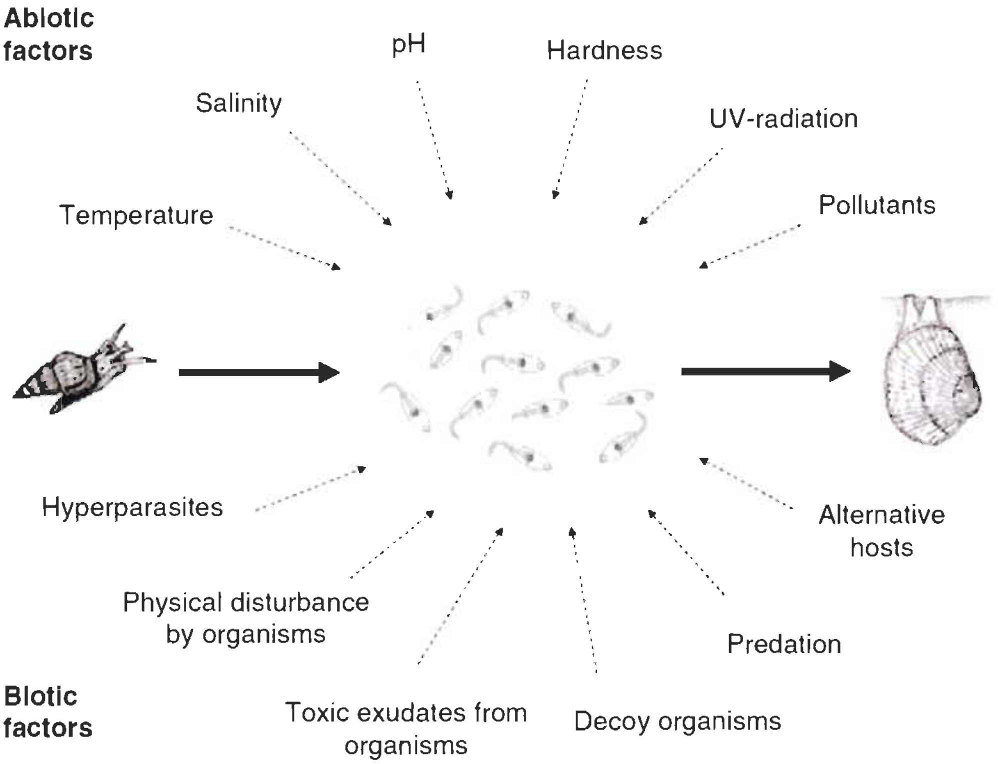

Figure 1: Ensemble des effets abiotiques et biotiques connus pour influencer la transmission d'un parasite en eau libre vers son hôte (tirée de Thielges et al. 2008).

1.1.1. Effet de la physico-chimie

Tel que mentionné précédemment, l'effet individuel de plusieurs facteurs physico-chimiques sur la capacité d'un parasite à accomplir son cycle de vie a déjà été bien étudié (Pietrock et Marcogliese 2003). Dans le cas de mon étude, nous nous sommes penchés sur l'effet de deux facteurs physico-chimiques qui seraient les plus susceptibles d'affecter la survie parasitaire en milieu naturel, soit le pH et la radiation UV. Par exemple, Christensen et al. (1980) ont montré qu'un pH acide (5 à 6) empêchait ou réduisait fortement le développement d'œufs de parasites comparativement à un pH neutre, limitant ainsi la présence de parasites dans les lacs plus acides. De plus, des milieux plus acides ont de la difficulté à supporter des populations élevées de gastéropodes, qui sont souvent des hôtes primaires de parasites trématodes (Hoffman 1999). La quantité d'ions calcium étant fortement réduite dans ces milieux, les gastéropodes ont de la difficulté à former leur coquille, et ultimement, sont quasi absents des milieux aquatiques acides (Marcogliese 2001).

L'autre facteur physico-chimique à l'étude dans le cadre de mon projet, la radiation ultraviolette, est déjà bien connu pour influencer la survie de plusieurs organismes invertébrés tels que les coraux, le zooplancton, les poissons ainsi que les amphibiens (Häder et al. 1998). Cependant, très peu d'études se sont concentrées sur l'effet de la radiation UV sur les parasites et les études réalisées jusqu'à ce jour ont été réalisées en laboratoire. Par exemple, Studer et Poulin (2013) ont montré un fort effet délétère de la radiation UV sur les cercaires du parasite *Maritrema noveazealandensis* (Figure 2). En contrôlant pour d'autres facteurs (température, salinité), ces derniers ont montré que la courbe de survie des cercaires passait d'un taux d'environ 40% en absence de rayonnement UV à un taux de survie d'environ 20% en présence de rayonnement UV, à l'intérieur d'une période de huit heures.

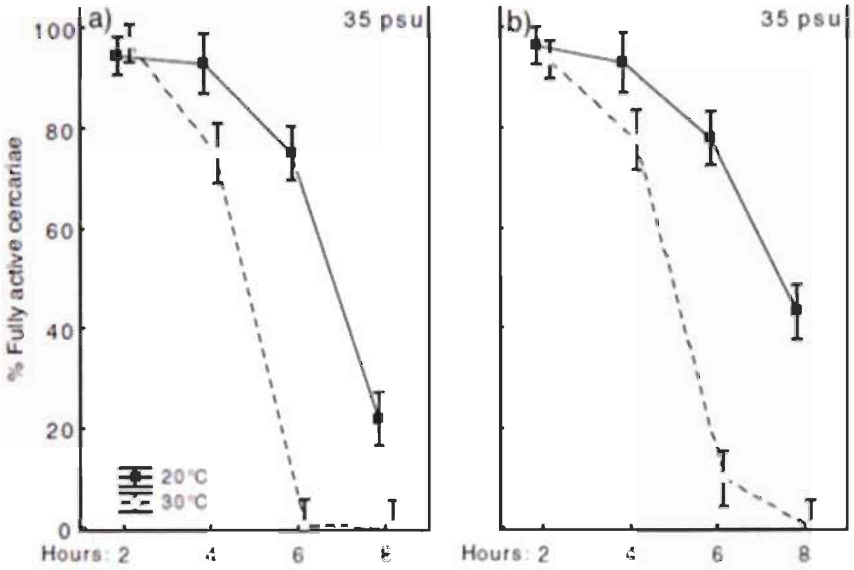

Figure 2 : Courbe de survie de cercaires du parasite *Maritrema noveazealandensis* a 20°C (ligne pleine) et 30°C (ligne pointillée) pour une même salinité. La figure de gauche représente un traitement avec radiation UV et celle de droite, un traitement sans radiation UV (tiré de Studer et Poulin 2013).

1.1.2 Effet de la morphométrie du milieu

La capacité d'un parasite à accomplir son cycle de vie est aussi influencée par les caractéristiques morphométriques d'un système. En effet, certaines études réalisées en milieu forestier ont démontré un impact direct de la superficie forestière sur la capacité d'un parasite à accomplir son cycle de vie. Par exemple, Allan et al. (2003) ont étudié l'effet de la fragmentation forestière sur le risque de transmission de la bactérie responsable de la maladie de Lyme, *Borrelia burgdorferi,* chez la tique. Ces derniers ont observé que les petites parcelles forestières contenaient plus de tiques infectées par la bactérie comparativement aux plus grandes parcelles. En milieu aquatique, Marcogliese et Cone (1991) ont observé que la profondeur moyenne des lacs avait une influence sur la faune parasitaire, les lacs moins profonds favorisant le contact entre l'ensemble des hôtes du cycle de vie d'un parasite. Ces auteurs ont aussi observé que la diversité parasitaire variait fortement en fonction de la taille des lacs, les lacs plus petits possédant moins d'espèces de parasites que les plus grands lacs.

1.1.3 Effet de facteurs biotiques

La présence d'hôtes alternatifs dans un système influence négativement la prévalence et l'abondance d'un parasite dans un écosystème. Thielges et al. (2008) ont remarqué que les systèmes contenant des hôtes agissant comme « puits » réduisaient d'environ 70% la prévalence du parasite chez son hôte cible. De plus, Krakau et al. (2006) ont observé que l'abondance de parasite était réduite dans les systèmes comportant des espèces introduites. En étudiant un système où le trématode *Renicola roscovita* infecte normalement un bivalve sous forme de cercaire (hôte intermédiaire) et un oiseau sous sa forme adulte (hôte définitif), les auteurs ont observé que l'introduction d'un bivalve invasif réduisait l'abondance du parasite chez le bivalve indigène, expliquant cette différence par l'absence de prédation de l'hôte définitif sur l'hôte intermédiaire introduit (Figure 3), menant ultimement à une réduction du parasite dans le système.

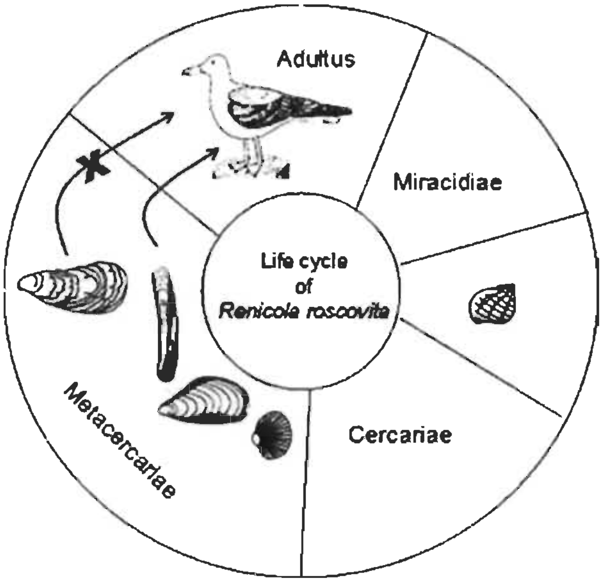

Figure 3 : Cycle de vie du parasite *Renicola roscovita* en absence d'hôte alternatif (ligne continue) et en présence d'un hôte alternatif (ligne coupée) (tirée de Krakau et al. 2006).

La biomasse d'hôtes dans un système est un autre facteur biotique clé dans le succès de la capacité d'un parasite à accomplir son cycle de vie. Par exemple, Arneberg et al. (1998) ont modélisé l'abondance d'un parasite en fonction de la densité d'hôtes disponible et ont observé que l'abondance parasitaire augmentait proportionnellement à la densité de sa population cible (voir figure 4). Plus concrètement, plusieurs exemples de la littérature ont confirmé un lien direct entre l'abondance de parasite et la densité d'hôtes en milieu aquatique. Par exemple, Meyrowitsch et al. (1991) ont étudié la transmission du stade libre du parasite *Echinostoma caproni* et ont observé que sa densité était directement associée à la densité de son hôte cible, dans leur cas un gastéropode aquatique. Lagrue et Poulin (2016) ont aussi observé une relation semblable entre la biomasse parasitaire et la biomasse de l'hôte du parasite, où la biomasse parasitaire était positivement corrélée avec la biomasse du poisson cible, *Gobiomorphus cotidianus*.

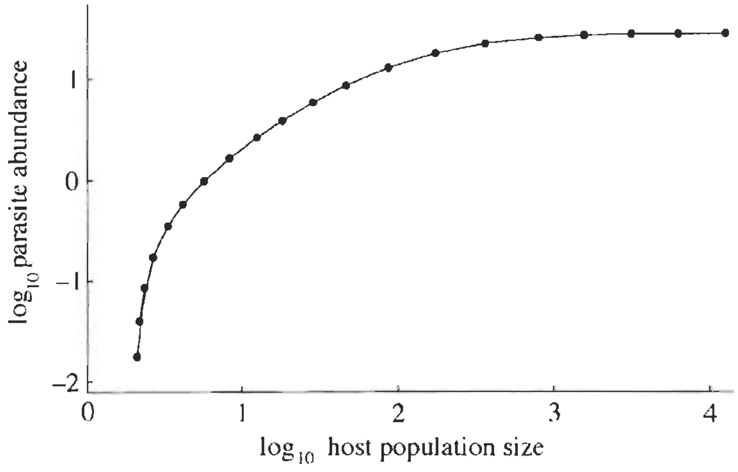

Figure 4 : Relation théorique de l'abondance d'un parasite en fonction de l'abondance de son hôte (tirée de Arnerberg et al. 1998).

1.2 Problématique

Les connaissances acquises jusqu'à ce jour dans ce domaine sont difficiles à transposer aux systèmes naturels puisque la plupart des études explorant l'effet de facteurs biotiques et abiotiques sur les parasites ont été réalisées sur un seul facteur ou en laboratoire, faisant en sorte que l'effet intégré de l'ensemble des facteurs présents en milieu naturel n'est probablement pas représenté (Morley et Lewis 2004). De plus, Kennedy (2009) a soutenu que nombre de recherches sur les parasites en milieu naturel restaient à faire puisque l'extrapolation des recherches effectuées en laboratoire au milieu naturel reste au mieux spéculative. Il serait donc pertinent de considérer un ensemble de facteurs biotiques et abiotiques dans un système naturel afin d'obtenir une vue plus intégrée de leur influence sur la prévalence et l'abondance des trématodes dans les milieux aquatiques.

L'objectif de la présente étude était donc d'investiguer les effets de plusieurs facteurs abiotiques et biotiques sur l'abondance et la prévalence de deux trématodes digènes, *Crepidostomum* et *Apophallus*, dans les lacs du bouclier laurentien. Ces deux genres ont été sélectionnés puisqu'ils représentent les deux stratégies de transmission les plus communes parmi le taxon des trématodes (Kennedy et Burrough 1978); *Apophallus* est un parasite allogène qui utilise un gastéropode comme hôte primaire, un poisson comme hôte intermédiaire et un oiseau aquatique comme hôte définitif alors que *Crepidostomum* est un parasite autogène qui utilise un gastéropode comme hôte primaire, un invertébré benthique (éphéméroptère ou écrevisse) comme hôte intermédiaire et un poisson comme hôte définitif (Hoffman 1999). Notre système d'étude est constitué d'un ensemble de lacs oligotrophiques ayant été colonisé par l'omble de fontaine (*Salvelinus fontinalis*) durant la période postglaciaire (Power 1980). Durant les dernières décennies, plusieurs pêcheurs utilisant des appâts vivants ont introduit une grande variété de poisson dans ces lacs, notamment le mulet à cornes (*Semotilus atromaculatus*) ainsi que le meunier noir (*Catostomus commersonii*) (Magnan 1988). Ces introductions ont eu pour effet de faire diminuer la biomasse et l'abondance de l'omble de fontaine de par la compétition pour les ressources benthiques, résultant ultimement en un déplacement de niche de l'omble de fontaine vers la ressource pélagique (Magnan 1988, Lachance et Magnan 1990a, b, Tremblay et Magnan 1991). Dans ce système, la compétition interspécifique pour la ressource benthique varie de nulle (lac contenant uniquement de l'omble de fontaine), intermédiaire (omble de fontaine et mulet à corne) a élevée (omble de fontaine avec mulet à corne et meunier noir) (Magnan 1988, Bourke et al. 1999). Dubois et al. (1996) ainsi que Bergeron et al. (1997) ont aussi trouvé que la communauté parasitaire de l'omble de fontaine changeait en présence de compétiteurs, passant d'une dominance par les parasites utilisant le zoobenthos vers ceux utilisant le zooplancton lorsque l'omble de fontaine interagissait avec le mulet à corne et/ou le meunier noir. Cependant, une grande variabilité dans l'abondance de parasite était observée dans les études précédentes, suggérant l'influence d'autres facteurs non étudiés. De plus, une autre étude menée dans ce milieu a montré que la richesse parasitaire variait en fonction de lac possédant différentes caractéristiques morphométriques (Bertrand et al. 2008). Cependant, comme seulement deux lacs avaient été échantillonnés lors de cette étude, ces résultats ne reflètent probablement pas l'étendue des conditions environnementales de cet écozone.

1.3 Objectifs et prédictions

L'objectif général de ce projet était d'explorer la relation environnement – parasite à l'échelle locale à travers un gradient de variables physico-chimiques, morphométriques et biotiques dans 22 lacs du bouclier laurentien en étudiant les variations d'abondance et de prévalence des deux genres parasitaire à l'étude. Nos principales hypothèses sont 1) l'abondance des deux parasites diminuera lorsque l'omble de fontaine interagit avec d'autres espèces de poisson et utilise les ressources pélagiques puisque les deux parasites utilisent des hôtes associés à la zone littorale (gastéropode aquatique et invertébrés benthiques); 2) les parasites seront moins abondants dans les lacs où la pénétration des rayons UV est plus importante (Studer et Poulin 2013) et/ou lorsque le pH est bas (Christensen et al. 1980, Marcogliese 2001); 3) Les deux parasites seront moins abondants dans les grands lacs profonds (Marcogliese et Cone 1991).

Chapitre II

Alternative hosts identity has a stronger effect than abiotic factors on trematode transmission in brook charr

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Abstract

Biotic and abiotic factors are both invoked to explain the large variations observed in the prevalence and abundance of parasites in aquatic ecosystems. However, these factors are usually considered one at a time in laboratory and field studies, and we have no knowledge of their potential interplay in natural systems. Therefore, it is important to analyze the effects of multiple environmental factors together to get an integrated view of their influence on the prevalence and abundance of parasites. To address this question, we selected two genera of digenean trematode parasites, *Crepidostomum* and *Apophallus*, representing two different transmission strategies of digenean trematodes; infection via consumption of infected prey (*Crepidostomum*) and infection by direct penetration of the skin (*Apophallus*). This study was conducted in 22 Canadian Shield lakes exhibiting orthogonal gradients of biotic (fish species richness) and abiotic (morphometry, physico-chemical factors) variables. We analyzed skin and intestinal parasites in brook charr (*Salvelinus fontinalis*) to quantify their prevalence and abundance. Our results show that fish species richness and total fish biomass are the main drivers of parasite abundance and prevalence in these lakes, with *Crepidostomum* being positively correlated with the presence of other fish species, whereas *Apophallus* is negatively associated with both fish species richness and biomass. Among the abiotic variables, only lake size influenced *Apophallus*, with individuals from larger lakes exhibiting higher infections levels than those in smaller lakes. Our results suggest that the interaction between parasites and the presence of alternative hosts is the main driver of parasites transmission success in these lakes.

Keyword: species interactions, parasite cycle, fish biomass, fish community, lake morphometry, interspecific competition

Introduction

The survival of parasitic organisms depends ultimately on the capacity of natural systems to sustain all hosts needed to complete their life cycle. Numerous experimental studies have shown that many factors could influence both the survival and infectivity of aquatic parasites (Christensen et al. 1980, Shostack 1993, Studer and Poulin 2013). However, these results are difficult to extrapolate to natural systems since they were obtained either in controlled conditions or only by looking at one factor at a time in natural conditions. In this context, only tentative hypotheses about their potential effects in the wild can be suggested (Morley and Lewis 2004). Similarly, Kennedy (2009) emphasized that most research on fish parasites has yet to be done in the field since extrapolations based on laboratory findings are at best speculative. Studying parasites in the wild can prove difficult since many environmental factors could mask the effects of variables selected from laboratory studies (Prah and James 1977, Morley et al. 2003). Although many studies acknowledge that local environmental factors could play a role in the ability of the parasite to complete their life cycle (Kennedy 1975, Kennedy 1978, Bailey and Margolis 1987), many were unable to find consistent patterns of parasite transmission in natural conditions (Kennedy 2009). Hartvisgen and Kennedy (1993) hypothesized that parasite transmission was probably influenced by multiple biotic and abiotic factors, suggesting that one must consider multiple variables to study parasites and their transmission in a comprehensive way. Morley and Lewis (2004) also suggested that biotic factors were as important as abiotic factors when considering transmission success of parasites in a natural habitat.

The effect of biotic factors on parasite transmission is often overlooked when studying parasites in natural systems. Nevertheless, an increasing body of literature has shed light on their importance for the ability of parasites to reach their definitive host. For example, Thielges et al. (2008) proposed that six biotic factors could be key drivers of cercariae transmission success: 1) hyperparasitism (parasitism on parasites), 2) physical disturbance by other organisms, 3) toxic exudates from either hosts or other organisms, 4) sink hosts (i.e. an organism acting as a decoy for the parasite), 5) predation and 6) alternative hosts. While these factors highlight many potential threats that parasites face when trying to complete their life cycle, only some of them have been investigated in natural systems. For example, Arneberg et al. (1998) reviewed the literature on parasite infection in mammals in natural conditions and found that the host population density is directly correlated with its associated parasite abundance and prevalence. Even though this study was done on terrestrial parasites, trematodes in aquatic systems face similar challenges during the completion of their life cycle. In a study on an aquatic snail, Meyrowitsch et al. (1991) showed that the density of the target host population, drives the susceptibility of infection by the free-living stage of trematode parasites. This conclusion was supported by Lagrue and Poulin (2016), who observed that the population biomass of the fish host *Gobiomorphus cotidianus* was positively correlated with parasite biomass in aquatic systems.

The effect of abiotic factors on parasite transmission has been studied to a great extent in laboratory. For example, Christensen et al. (1980) experimentally showed that reduced pH hindered parasite egg development. Moreover, Marcogliese and Cone (1997) showed that parasites diversity was strongly associated with pH, with more acidic aquatic system having less diversity (especially of digenean trematodes) than more neutral systems. Other physico-chemical factors, such as UV radiation, could play a role in parasite transmission. Studer and Poulin (2013) observed a reduced survival in cercariae of the parasite *Maritrema noveazealnadensis* when exposed to UV radiation, leading to a decrease in the parasite infection capacity. However, the effects of these physico-chemical factors on parasite transmission are difficult to assess in natural systems, especially with digenean parasites, since they are usually long-lived in their fish host (Chubb 1979). In this context, other abiotic factors, such as lake morphometry, were investigated in the light of parasite transmission: since they are more stable factors, they can integrate long-term infection patterns. Marcogliese and Cone (1991) suggested that the physical characteristics of lakes, mainly mean depth, may in part influence parasite transmission, because shallow areas would enhance the long-term contact between hosts and parasites. They also suggested that lake area could be determinant for parasite assemblage, with small lakes harbouring fewer parasite species than larger ones.

The goal of the present study was to investigate the effects of both biotic and abiotic factors on the abundance and prevalence of two genera of digenean trematodes, *Crepidostomum* and *Apophallus,* in Canadian Shield lakes. These two genera represent the two most common patterns of transmission among the trematode taxa (Kennedy and Burrough 1978): *Apophallus*, mainly *Apophallus imperator* (Margolis and Arthur 1979), is an allogenic parasite, using a gastropod as primary host, a fish as intermediate host and a bird as definitive host (Hoffman 1999) while *Crepidostomum*, mainly *Crepidostomum farionis* and *Crepidostomum cooperi* (Margolis and Arthur 1979, Dubois et al. 1996), is an autogenic parasite, using an aquatic snail as primary host, a benthic invertebrate (mayflies, crayfish) as intermediate host and a fish as definitive host (Hoffman 1999). The two selected parasites differ in how they infect charr: one by consumption of infected prey

(*Crepidostomum*) and the other by direct penetration of the skin (*Apophallus*). Our system consists of oligotrophic lakes colonized by brook charr (*Salvelinus fontinalis*) during the post-glaciation period (Power 1980). Over the last century, bait fishers have introduced various species such as creek chub (*Semotilus atromaculatus*) and white sucker (*Catostomus commersonii*) to many Canadian Shield lakes (Magnan 1988). These introductions have affected both the relative abundance and biomass of brook charr through competition for benthic invertebrates, ultimately resulting in a shift from benthic to pelagic resources (Magnan 1988, Lachance and Magnan 1990 a, b; Tremblay and Magnan 1991). In this system, the level of interspecific competition varies from none (allopatric brook charr lakes), to intermediate (brook charr with creek chub lakes), and to high (brook charr with creek chub and white sucker lakes) (Magnan 1988, Bourke et al. 1999). Dubois et al. (1996) and Bergeron et al. (1997) also found that the parasite community shifted from zoobenthos-transmitted parasites to zooplankton-transmitted parasite in lakes where brook charr was in sympatry with creek chub or creek chub and white sucker. However, a large variability in parasite count was also observed across lakes hosting similar fish communities, suggesting the influence of other factors. A previous study in this area found that parasite species richness and abundance varied between two lakes with different morphometric characteristics (Bertrand et al. 2008), but the lakes studied may not reflect the range of environmental conditions of this ecozone.

Here, we explore the environment-parasite relationship at the local scale among gradients of biotic and abiotic factors in 22 Canadian Shield lakes by looking at the prevalence and abundance of two genera of brook charr parasites. Our main hypotheses are: i) the abundance of both parasites is expected to decrease when brook charr interact with other fish species because both parasites use hosts that are mainly found in the littoral zone (aquatic snails and benthic invertebrates) and charr shift their feeding habit from benthic to pelagic prey in sympatry with chub and sucker (Fig. 1) ; ii) parasites should be less abundant in lakes were the UV radiation penetration is deeper (Studer and Poulin 2013) and/or where pH is lower (Christensen et al. 1980); and iii) both genera of parasites will be less abundant in large lakes (Marcogliese and Cone 1991).

Methods

Study area and fish sampling

The study was conducted in the Mastigouche (46° 40′ N, 73° 30′ W) and the Saint-Maurice (47° 05′ N, 73° 15′ W) wildlife reserves, and in La Mauricie National Park of Canada (46°45′ N, 73°08′ W) from June to August 2012, 2013 and 2014. We sampled nine allopatric brook charr lakes, six brook charr with creek chub lakes and seven brook charr with creek chub and white sucker lakes. Each lake was sampled only once. Brook charr were caught in both the littoral (< 3m) and pelagic (> 4m) zones of the lakes using four Alaska trap nets (Neilsen and Johnson 1983) per lake (two for each zone). The traps were set between 18:00 and 6:00, during four to five days. For each lake, we sampled 60 brook charr in each zone. Each fish was measured (fork length), weighed, sexed and photographed using a Kaiser reproduction stand (model RA-2). The digestive tract was preserved in a 4% formalin solution for laboratory analysis (Neilsen and Johnson 1983). Due to logistic constraints, samples from only 11 lakes sampled in 2014 were available for the *Crepidostomum* analysis while all 22 lakes were available for *Apophallus* analysis.

This protocol was approved by the Animal Care Committee at the Université du Québec à Trois-Rivières (permit #MAU-2012-1593, 2013-05-15-046-04-S-P and 2014-04-29-031-04-S-P)

Parasite analysis

Brook charr of at least 18 cm in fork length were selected to maximize the chances of observing *Crepidostomum*. We randomly selected 20 fish per lake (10 for each zone), yielding a total of 220 fish. The intestine and stomach of each individual was dissected under a binocular microscope and *Crepidostomum* parasites were counted. For *Apophallus*, we analyzed 2080 pictures of fish from the 22 lakes. For each picture, we drew a rectangle from the caudal peduncle to the opercula and counted black spot appearing on the skin which are signs of infections by the metacercaria of *Apophallus* parasites in the same area for each fish using the image analysis software image J v1.49 (Schneider et al. 2012). Data were corrected for fish length (see Results section).

Lake morphometry and physico-chemical variables

A vertical profile of water physico-chemistry was done on each lakes to measure pH, dissolved oxygen (mg*L^{-1}), conductivity (μS*cm^{-1}) and temperature (°C) using a YSI multi-parameters probe (model 556 MPS). We also sampled water to measure the dissolved organic carbon (mg*L^{-1}) following the persulfate oxidation method (Guillemette and del Giorgio 2011). We used DOC as a proxy of UV attenuation because these two variables are highly correlated in oligotrophic lakes (Morris et al. 1995). We estimated lake area (ha) and mean depth (m) from maps provided by the Société des établissements de plein air du Québec (SÉPAQ) and La Mauricie National Park. Since both parasites use hosts that are mainly found in the littoral zone of lakes (aquatic snails and benthic invertebrates), we calculated the proportion of the littoral zone for each lakes (i.e., littoral zone volume / total volume). The littoral zone was defined as the zone where the depth varied between 0 and 3 m (see supplementary materials for more details).

Data analysis

We used a principal components analysis (PCA) (vegan package; Oksanen et al. 2016) to visualize the gradients of lake morphometry as well as the biotic and physico-chemical variables measured during sampling (Fig. 2). PCA allowed us to confirm the independent gradients of biotic and abiotic variables in the data (see Results section). We found that the mean total fish biomass was partially confounded with the fish community, because allopatric brook charr lakes also have a lower mean total fish biomass (not shown in the PCA). The range of mean total fish biomass varied from: 1.043 ± 0.084 kg/trap (mean ± SE) in allopatric brook charr lakes to 2.340 ± 0.206 kg/trap in brook charr / creek chub lakes and to 4.736 ± 0.469 kg/trap in brook charr / creek chub / white sucker lakes. However, we used the two variables in our analysis (although in separate models) because the presence of a given species in the fish community might affect parasite transmission independently of fish biomass. Another PCA was done for the 11 lakes included in the *Crepidostomum* dataset (not shown here). We found that morphometric variables (lake area and mean depth) and the total fish biomass were highly correlated, with large and deep lakes harbouring more complex communities and higher total fish biomass, reducing the possibility to fully disentangle the role of some factors in this dataset.

We used general linear mixed models (GLMM; see details below) to investigate the influence of environmental and biotic factors using lake as a random effect factor since we were working on fish individuals nested in the lakes (Zuur et al. 2009a). Fish fork length (log-transformed standardized values) was included as a covariable in all models to account for parasite accumulation over time (Dogiel et al. 1958, Des Clers 1991, Grutter and Poulin 1998). We used a GLMM (lme4 package; Bates et al. 2015) with a negative binomial distribution to model the abundance of *Crepidostomum*. The biotic fixed effects were total mean fish biomass (log-transformed standardized values) and fish community (coded as a dummy variable; reference category: lakes with brook charr only), whereas the abiotic fixed effects were lake area (log-transformed standardized values), lake mean depth (log-transformed standardized value), proportion of littoral zone (logit-transformed value) and pH. We did not used the DOC as a fixed effect in *Crepidostomum* models because the transmission between the intermediate and definitive host is not during a free-living stage and cannot be affected by UV radiation. We were unable to analyze the prevalence data on *Crepidostomum* since almost all fish from all lakes were infected by this parasite (see supplementary materials) and models failed to converge.

We used a GLMM (glmmADMB package; Fournier et al. 2012) with a zero-inflated negative binomial distribution to model the abundance of *Apophallus*, since 51% of fish did not have this parasite. We selected this distribution to take into account the overdispersion of our data and to explain the overabundance of zeros, thus avoiding the production of biased estimates of parameters and associated standard error (Zuur et al. 2009; Zuur et al. 2010). A set of candidate models was built using different combinations of variables such as the proportion of littoral zone (logit-transformed value), lake mean depth (log-transformed standardized value) and lake area (log-transformed standardized value) for abiotic variables and fish community (reference category: lakes with brook charr only) and mean total fish biomass (i.e., mean number of fish caught per trap; log-transformed standardised values) for biotic variables (see details in Table 1).

We used a binomial distribution in a GLMM (glmmADMB package; Fournier et al. 2012) to model *Apophallus* prevalence. We used the same predictor variables as we used in the *Apophallus* abundance models.

We selected the best model using the Aikake information criterion corrected for small samples (AICc) (Burnham and Anderson 2001; see Table 1), with models being in the ΔAICc range of two being considered as equivalent. All statistical analyses were performed using R software version 3.2.4 (R core team 2016).

Results

The two first axes of the PCA explained 53% and 21% of the variation, respectively. The first axis was mainly related to morphometric variables (i.e., proportion of the littoral zone, lake area, and mean depth) whereas the second axis was mainly related to pH (Fig. 2). The convex hull polygons showed large overlap between fish communities, indicating that each fish community shared similar environmental gradients (Fig. 2).

Overall, 1024 fish out of 2080 (49%) were infected by *Apophallus* (1 to 837 parasite metacercaria per fish) and 197 fish out of 220 (90%) were infected by *Crepidostomum* (1 to 620 parasites per fish). We did not find any relationship between physico-chemical variables and either the abundance or the prevalence of either genera of parasite (Table 1).

Crepidostomum abundance

The best model explaining the variation of *Crepidostomum* abundance included the fish community and fish length (Table 1). While the intermediate level of competition showed a marginal negative effect on *Crepidostomum* abundance (effect size = - 0.961, SE = 0.624), there is a positive effect with the presence of a strong competitor (white sucker) on the abundance of this parasite (effect size = 1.604, SE = 0.697) (Fig. 3). There is also a positive effect of brook charr size on *Crepidostomum* abundance (effect size = 0.184, SE = 0.082). No other model represented a plausible alternative to the selected model (ΔAICc > 4 in all cases; Table 1)

Apophallus abundance

The best model explaining the variation of *Apophallus* abundance included fish length (effect size = 0.501, SE = 0.036), lake area (effect size = 1.089, SE = 0.318) and fish community. The introduction of an intermediate competitor had a marginal negative effect (effect size = -1.247, SE = 0.860) and that of a strong competitor had a significant negative effect (effect size = -2.852, SE = 0.827) (Fig. 3). The model including the mean total fish biomass was also good for explaining variation in *Apophallus* abundance (Table 1). However, except for the change between the biotic variables (mean total fish biomass and fish community; see Table 1), the other fixed effects (lake area and fish length) had a similar effect on *Apophallus* abundance (see supplementary material).

Apophallus prevalence

The best model explaining the probability of infection by *Apophallus* includes fish length (effect size = 0.337, SE = 0.074), mean fish biomass (effect size = -1.823, SE = 0.493), and lake area (effect size = 1.108, SE = 0.445) (Fig. 4).

Discussion

Our study shows that the prevalence and abundance of both *Apophallus* and *Crepidostomum* are mainly driven by fish community and biomass, with *Apophallus* being also driven, albeit to a lesser degree, by lake area. The lack of effect by physico-chemical factors on both parasites could be due to the restricted range of these variables in our study system.

The abundance of *Crepidostomum* in brook charr is mainly associated with the presence of white sucker. Even though white sucker consumes benthic resources more efficiently and thus decreases brook charr use of the littoral zone (Magnan 1988,Tremblay and Magnan 1991), our results shows that *Crepidostomum* is more abundant in brook charr in these lakes, and therefore in the aquatic system. This means that this parasite could be able to infect a larger proportion of benthic invertebrates when brook charr lives in sympatry with white sucker. However, logistic constraints impeded us to study all hosts involved in *Crepidostomum* life cycle, so we cannot state unequivocally that this interpretation is correct. Since *Crepidostomum* infect fish via consumption of infected benthic invertebrate, it seems that the adult stage of this parasite is able to infect and reproduce in the white sucker, meaning that white sucker could act as an alternative host for this parasite and its presence could lead to an increase in the total parasite population. The parasite checklists of Margolis and Arthur (1979) and previous studies in this system (Dubois et al. 1996, Bergeron et al. 1997) corroborate our results by showing that *Crepidostomum* seems to be able to colonize the intestinal track of white sucker but not that of creek chub.

Our results show that both the abundance and prevalence of *Apophallus* are driven by total fish biomass and lake area. Creek chub and white sucker could act as sink hosts for *Apophallus* since few brook charr exhibiting infection were found in sympatry with these fish species: this is in agreement with our predictions (Fig. 1). Since *Apophallus* infects fish by direct penetration of the skin in the second stage of its life cycle, these results could be explained by the low specificity of trematode cercariae to their intermediate host. Previous studies found that free-living trematodes tend to infect a wide range of species (Conn et al. 1994, Krakau et al. 2006, Kelly et al. 2009). All three studies found that introduced species had a negative impact on the parasitic abundance of native species, with introduced species acting as sinks for the parasite. Evans and Gordon (1983) also showed that sink intermediate hosts can protect more suitable hosts by reducing the overall level of parasite transmission within a system. In our system, the presence of both creek chub and white sucker was associated with a reduction in the prevalence and abundance of *Apophallus* in brook charr.

An alternative explanation of the strong decline of the abundance of *Apophallus* in lakes where brook charr live in sympatry with white sucker could be due to a shift from littoral to pelagic resources caused by interspecific competition (Magnan 1988, Tremblay and Magnan 1991; Fig. 1). This could result in a reduction of the encounter probability between brook charr and *Apophallus* cercariae emerging from their snail host in the littoral zone, leading to a decrease in successful infection.

Our results also suggest that larger lakes harbour more *Apophallus* parasites, with a higher proportion of infected fish with increased lake size. This result could be related to habitat selection by aquatic birds. In our system, previous observations showed that one aquatic bird involved in the life cycle of *Apophallus*—the common loon (*Gavia immer*)—prefers to breed and feed in larger lakes (Desgrange and Darveau 1985, Masse 2000). This suggests that higher abundances of aquatic birds in large lakes would lead to an increase of potential definitive host for this parasite, and thus to an increased abundance and prevalence in its intermediate host. This interpretation is supported by Marcogliese and Cone (1991), who showed that small ponds were impoverished in terms of parasite species when compared to large lakes. It is also possible that the relationship observed between lake morphometry and parasite abundance is related to lake stability, with large deep lakes being less prone to quick variations of environmental factors (Gorham 1964).

In conclusion, our results clearly show that parasite prevalence and abundance can be explained by the independent contribution of abiotic (lake morphometry) and biotic (fish community composition and biomass) factors despite the complexity of the natural systems. More interestingly, the two parasite species show contrasting responses to fish community or fish biomass according to the transmission mode of the parasite (i.e., autogenic vs. allogenic), suggesting that host identity is key to understand our results. More specifically, we suggest that alternative hosts can boost the total host population abundance in the case of *Crepidostomum* (i.e., autogenic parasite) whereas they act as a sink in the case of *Apophallus* (i.e., allogenic parasite). Further studies should include all hosts involved in the life cycle of these parasites in order to better characterize the mechanisms involved in the introduction of definitive and alternative hosts of parasites along the trophic chain in lakes.

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Tables

Table 1. Corrected Akaike Information Criterion (AICc) for all tested models. ΔAICc represents the difference between the actual AICc value and the AICc value of the best model. All models included fish length as a covariate and lake as random effect.

Tested variable	Fixed effect	AICc	ΔAICc
Crepidostomum abundance	Proportion of littoral zone	2068.69	8.18
	Lake area	2067.96	7.45
	pH	2066.66	6.15
	Fish biomass	2065.00	4.49
	Mean depth	2066.55	6.04
	Fish community	2060.51	0
Apophallus abundance	pH	8252.9	11.7
	DOC	8252.72	11.52
	Proportion of littoral zone	8251.7	10.5
	Mean depth	8251.58	10.38
	Community	8248.68	7.48
	Proportion of littoral zone + Fish community	8247.7	6.5
	Mean depth + Fish community	8247.5	6.3
	Lake area	8246.58	5.38
	Proportion of littoral zone + Fish biomass	8244.6	3.4
	Mean depth + Fish biomass	8244	2.8
	Fish biomass	8243.76	2.56
	Lake area + Fish biomass	8241.86	0.66
	Lake area + Fish community	8241.2	0

Apophallus prevalence	pH	1636.47	18.11
	DOC	1636.16	17.8
	Proportion of littoral zone	1635.6	17.24
	Mean depth	1635.53	17.17
	Fish community	1630.62	12.26
	Lake area	1629.94	11.58
	Proportion of littoral zone + Fish community	1629.7	11.34
	Mean depth + Fish community	1629.6	11.24
	Proportion of littoral zone + Fish biomass	1622.9	4.54
	Mean depth + Fish biomass	1622.3	3.94
	Fish biomass	1621.99	3.63
	Lake area + Fish community	1620.64	2.28
	Lake area + Fish biomass	1618.36	0

Figures captions

Figure 1: Life cycle of the autogenic parasite *Crepidostomum* and the allogenic parasite *Apophallus*. Numbers refer to potential impacts of interspecific competition: (1) decrease in the use of littoral zone by brook charr, (2) use of the benthic resource by other fish species and (3) transmission pathway of both parasites that could hinder or boost the abundance and prevalence of both parasites in brook charr.

Figure 2: Principal component analysis (PCA) of the study lakes based on the physico-chemical and morphometric variables included in the models. Fish communities are represented by convex hull polygons: allopatric lakes (no competition; blue), lakes with brook trout and creek chub (intermediate level of competition; yellow) and lakes with brook trout, creek chub and white sucker (high level of competition; green). Note that we did not include fish biomass in this ordination since it is redundant information with fish community. Letters refer to lakes (see Table A1 for details).

Figure 3: Prediction of *Crepidostomum* (black) and *Apophallus* (white) abundance (number of parasites per fish) according to the fish community. Predictions are derived from the best models (see Table 1) and adjusted for mean fish length (20.6 cm and 22.3 cm for *Apophallus* and *Crepidostomum* respectively) and total lake area (40.2 ha for *Apophallus*). The bars represent the 95% confidence intervals.

Figure 4: Probability of infection by *Apophallus* in brook charr according to total fish biomass (g / net) adjusted for mean fish length (20.6 cm) and total lake area (40.2 ha). The tick bars on the X axis represent the lakes and the gray zone represent the 95% confidence interval.

Figure 1:

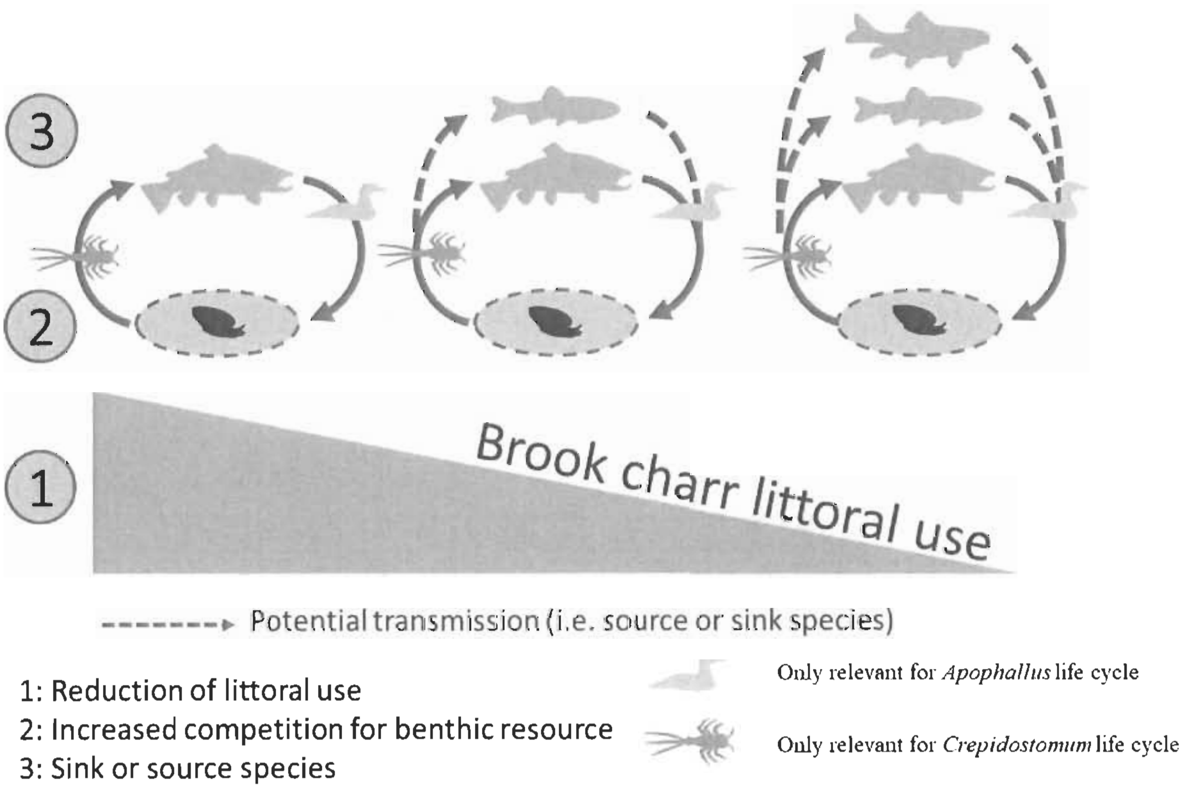

3
2
1
Brook charr littoral use
Potential transmission (i.e. source or sink species)
1: Reduction of littoral use
2: Increased competition for benthic resource
3: Sink or source species
Only relevant for *Apophallus* life cycle
Only relevant for *Crepidostomum* life cycle

Figure 2:

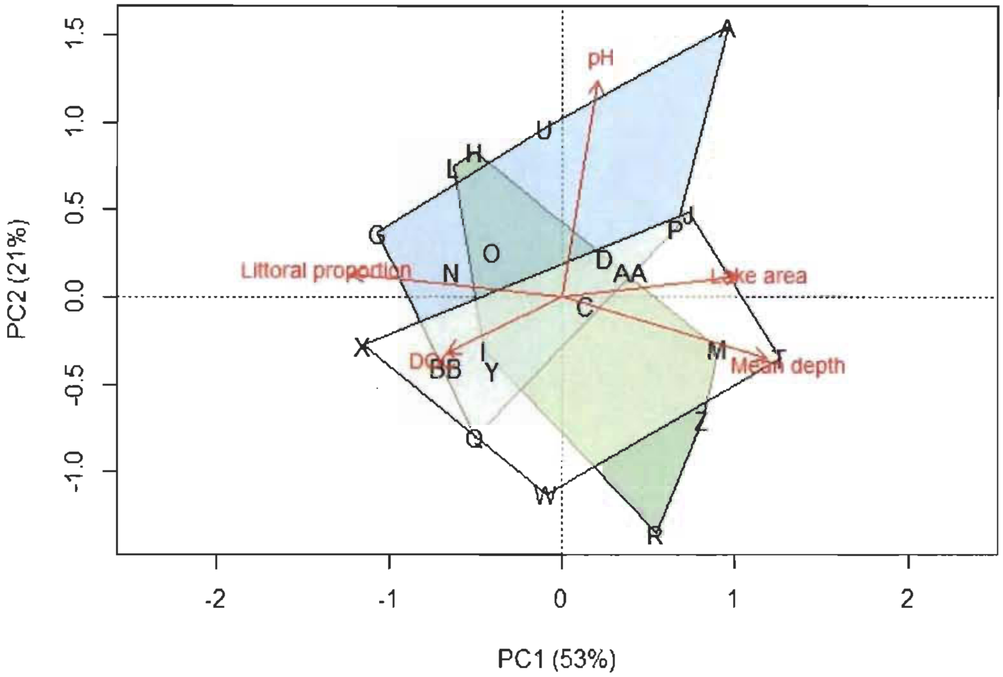

PC2 (21%)
1.5
1.0
0.5
0.0
-0.5
-1.0
PC1 (53%)
-2
-1
0
1
2
pH
Littoral proportion
Lake area
DOC
Mean depth
A
U
H
L
G
O
N
D
AA
P
J
C
X
I
BB
Y
M
T
Q
Z
W
R

Figure 3:

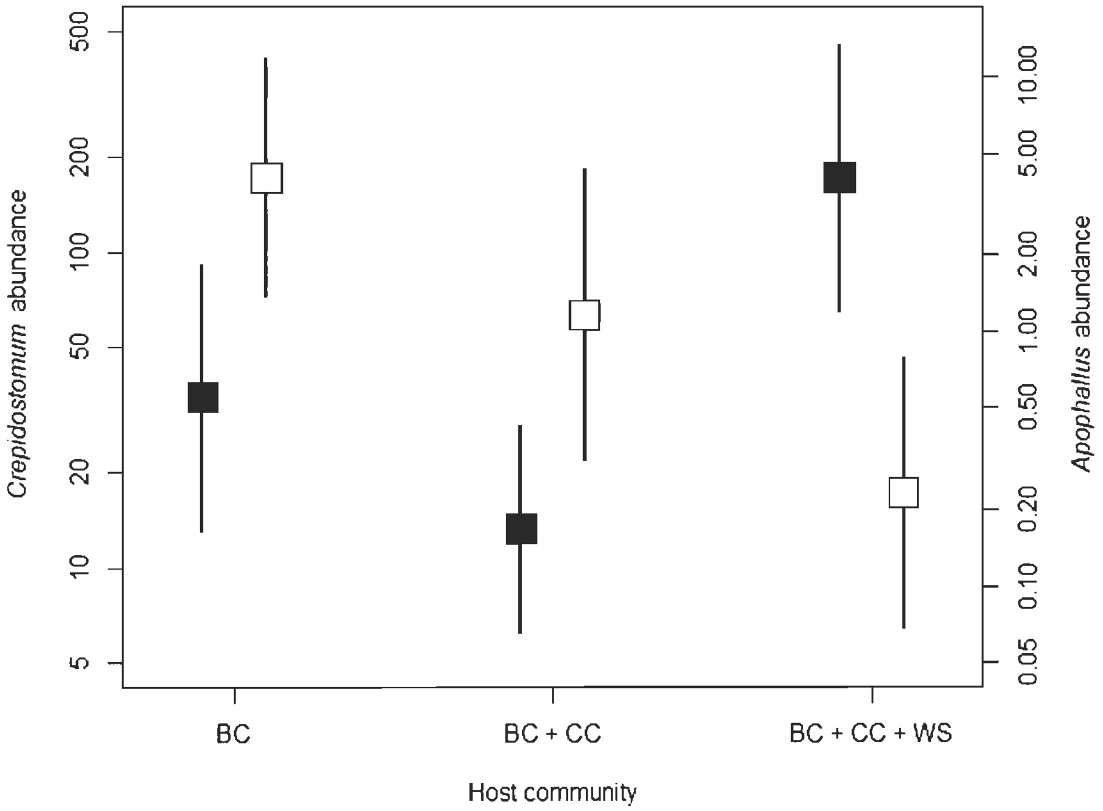
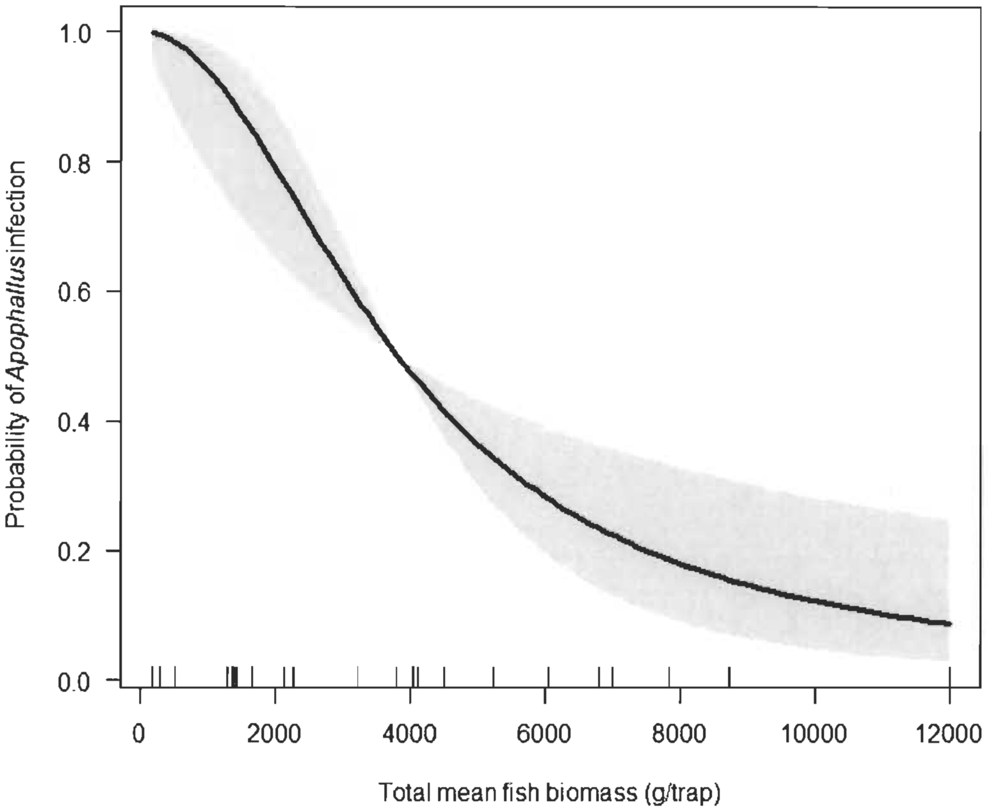

Crepidostomum abundance
500
200
100
50
20
10
5
BC
BC + CC
BC + CC + WS
Host community
Apophallus abundance
10.00
5.00
2.00
1.00
0.50
0.20
0.10
0.05

Figure 4:


1.0
0.8
0.6
0.4
0.2
0.0
Probability of *Apophallus* infection
0
2000
4000
6000
8000
10000
12000
Total mean fish biomass (g/trap)

Supplementary material

Table A1. Prevalence (%) and abundance (mean number of parasite per fish) of *Crepidostomum* and *Apophallus* in the study lakes. N: number of brook charr analysed. SE: standard error. Letters in parentheses after lake names indicate lake identities in Fig 2 (PCA).

			Apophallus			*Crepidostomum*	
Lake	**Fish community (w/o BC)**	**N**	**Prevalence (%)**	**Abundance (mean ± SE)**	**N**	**Prevalence (%)**	**Abundance (mean ± SE)**
Adam (U)	-	119	1.681	0.017 ± 0.012	20	85	21.2 ± 5.536
Baie cobb (C)	-	50	100	59.320 ± 7.372	-	-	-
Baie onze îles (A)	-	33	100	53.152 ± 6.063	-	-	-
Bourassa (P)	PE	119	100	25.292 ± 5.545	-	-	-
Brise (L)	CC; PE; SA	55	16.364	0.182 ± 0.059	-	-	-
Bucheron (G)	-	120	24.167	0.333 ± 0.064	-	-	-
Camp (I)	CC; PE; SA	69	21.740	0.290 ± 0.075	-	-	-
Chute noire (N)	PE	113	84.071	11.637 ± 1.400	-	-	-
Corneille (O)	CC; PE; SA	88	29.546	0.523 ± 0.123	-	-	-
Coteau (Q)	PE	118	70.339	3.034 ± 0.518	20	100	69.3 ± 19.395
Cutaway (H)	CC; PE; SA	102	13.726	0.235 ± 0.074	-	-	-
Dorval (V)	CC; PE; SA; CI; RC; other cyprinids	-	-	-	6	100	294.167 ± 85.868
Gauthier (S)	MM; PE; SA	-	-	-	20	90	17.8 ± 8.584
Lézard (M)	CC; PE; SA	9	33.333	1.444 ± 1.094	-	-	-
Longue vue (Y)	SA	120	12.5	0.150 ± 0.040	20	95	41.25 ± 13.292
Maréchal (D)	-	59	100	20.203 ± 2.975	-	-	-
Marmotte (X)	SA	120	7.5	0.092 ± 0.031	20	85	24.9 ± 9.199
Marshall (W)	SA	118	100	130.246 ± 12.857	20	95	14.9 ± 5.381
Oudiette (R)	CC; PE; SA	98	23.470	0.429 ± 0.121	20	100	265.65 ± 41.131
Plouf (Z)	CC; PE; PP; SA	111	8.108	0.081 ± 0.0.026	20	100	192.2 ± 45.028
Shawinigan (T)	LC; MM; PE; SA	118	73.729	3.729 ± 0.778	20	100	104.65 ± 15.411
Simpson (BB)	-	121	95.041	14.347 ± 2.061	20	95	30.8 ± 6.077
Thibert (AA)	SA	100	46	1.250 ± 0.185	20	40	1.45 ± 0.560
Visons (J)	PE; SA	120	55	1.442 ± 0.290	-	-	-

Legend: PE = *Phoxinus eosinus*; CC = *Catostomus commersonii*; SA = *Semotilus atromaculatus*; LC = *Luxilus cornutus*; MM = *Margariscus margarita*; PP = *Pimephales promelas;* CI = *Culaea inconstans;* RC = *Rhynyctis cataractae*

Table A2: Physico-chemical, morphometric and biotic variables measured for the study lakes. Letters in parentheses after lake names indicate lake identities in Fig 2 (PCA).

Lake	Area (ha)	Mean depth (m)	Littoral zone proportion (%)	pH	Doc	Total mean fish biomass (g/trap)
Adam (U)	14	5.24	13	6.95	3.33	1650.99
Baie Cobb (C)	61.7	5.88	8	6.38	8.43	286.03
Baie onze îles (A)	135	7.2	7	7.78	4.01	181.71
Bourassa (P)	55.9	7.26	8	6.47	3.56	1424.74
Brise (L)	39.4	3.59	23	6.68	6.06	8713.40
Bucheron (G)	9.9	3.9	18	6.73	9.24	2274.77
Camp (I)	11.1	5.82	13	5.95	5.95	6787.48
Chute noire (N)	17	3.8	18	5.98	4.68	1367.20
Corneille (O)	23.1	4.78	17	6.28	5.14	6041.62
Coteau (Q)	31	4.72	16	5.26	7.09	3230.94
Cutaway (H)	40.2	3.38	15	6.87	6.72	7837.25
Gauthier (S)	36.7	8.62	9	7.30	NA	5331.64
Lézard (M)	110	7.83	5	6.03	5.6	6990.95
Longue vue (Y)	28.2	5.44	16	5.74	6.67	4031.63
Maréchal (D)	103.4	5.18	10	6.31	5.84	508.16
Marmotte (X)	14	2.97	27	5.42	5.51	4115.38
Marshall (W)	41	6.39	10	5.17	7.91	4487.36
Oudiette (R)	44	8.59	7	4.76	4.42	3777.26
Plouf (Z)	60	8.82	5	5.59	4.88	11984.79
Shawinigan (T)	60	11.7	4	6.02	3.77	5227.10
Simpson (BB)	30	4.24	26	5.41	5.38	1280.38
Thibert (AA)	43	5.79	8	6.06	3.5	2124.23
Visons (J)	74	7.54	6	6.88	4.47	1375.81

Chapitre III

Conclusion générale

3.1. Contextualisation

Plusieurs facteurs physico-chimiques, morphométriques et biotiques sont connus pour affecter la capacité d'un parasite à accomplir son cycle de vie. Cependant, peu d'études se sont penchées sur les effets intégrés de multiples facteurs environnementaux, autant abiotiques que biotiques, sur les parasites en milieu naturel.

L'objectif de mon projet de recherche a donc été d'étudier un ensemble de facteurs physico-chimiques (pH, radiation UV), morphométriques (profondeur moyenne, superficie) et biotiques (communauté ichthyenne, biomasse d'hôtes) pour avoir une vue intégrée de leurs effets conjugués sur l'abondance et la prévalence parasitaire en milieu aquatique naturel.

3.2. Principaux résultats

Nos résultats montrent que la prévalence et l'abondance de *Crepidostomum* et d'*Apophallus* sont clairement influencées par la communauté ichthyenne ainsi que par la biomasse d'hôtes disponible, et à un plus faible niveau par la taille du lac pour *Apophallus* seulement. L'absence d'effets physico-chimiques sur les deux parasites pourrait être due à la faible variabilité de ces variables à travers notre système d'étude.

L'abondance du parasite *Crepidostomum* est fortement influencée par la présence du meunier noir dans le système, étant beaucoup plus abondant dans l'omble de fontaine lorsque celle-ci est retrouvée en sympatrie avec ce compétiteur. L'abondance et la prévalence du parasite *Apophallus* sont négativement influencées par la biomasse de poisson ainsi que par la communauté ichthyenne, les ombles de fontaines capturées dans les lacs ayant un plus fort degré de compétition interspécifique montrant moins de signes d'infection par le parasite comparativement aux lacs où l'omble de fontaine est en allopatrie.

Le principal facteur abiotique ayant un effet sur la capacité du parasite allogène a été la superficie du lac : les ombles de fontaine capturés dans les plus grands lacs étaient significativement plus infectés par la métacercaire du parasite *Apophallus* que dans les plus petits lacs.

3.3 Conclusion

La capacité d'un parasite à accomplir son cycle de vie peut être altérée par plusieurs facteurs abiotiques (Marcogliese et Cone 1991; Pietrock et Marcogliese 2003; Studer et Poulin 2013) et biotiques (Arneberg et al. 1998; Thielges et al. 2008; Lagrue et Poulin 2016). Jusqu'à présent, peu d'études s'étaient intéressées à l'effet intégré de ces facteurs sur la transmission d'un parasite en milieu naturel. Nos résultats montrent que les deux principales stratégies de transmission des parasites trématodes digènes sont influencées en fonction d'un gradient de compétition interspécifique ainsi qu'à travers des systèmes aquatiques possédant des caractéristiques différentes.

Mon étude pourrait donc servir d'outil de gestion du territoire pour des pourvoyeurs voulant ensemencer des systèmes naturels. En connaissant mieux la dynamique des populations parasitaires selon la communauté ichthyenne, il sera maintenant plus facile de sélectionner les lacs attrayants pour les pêcheurs.

À plus large échelle, mon étude a permis de comprendre les patrons environnementaux influençant la transmission de parasites ayant des cycles de vie complexe (trois hôtes) dans les lacs oligotrophes. Les résultats obtenus pourraient donc être transposés vers un ensemble de systèmes aquatiques afin de mieux comprendre les patrons de transmissions d'autres parasites autogènes et allogènes.

Au niveau appliqué, les résultats obtenus pourraient servir de bases de réflexion pour l'utilisation de parasites comme bio-indicateur de l'utilisation des ressources chez l'omble de fontaine. Comme le parasite *Crepidostomum* utilise principalement des hôtes retrouvés en zone littorale des lacs, son utilisation comme traceur à long terme du comportement alimentaire de l'omble de fontaine sera maintenant possible puisque nous avons mis en lumière ses principaux patrons de transmission en milieu naturel.

Nous sommes conscients que cette étude est principalement exploratoire puisque nous n'avons observé que deux genres de parasites au sein d'un de leurs hôtes. Malgré cela, nous montrons qu'il existe des patrons clairs de transmission de parasites trématodes digènes variant autant en fonction de facteurs biotiques qu'abiotiques dans les lacs du bouclier laurentien. Des études futures devraient se concentrer sur l'étude de l'ensemble des hôtes de chacun des genres parasitaires étudiés afin d'avoir une vue plus intégrée des effets des facteurs en cause sur la transmission parasitaire en milieu naturel.

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Annexe

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

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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, nor 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 phenotype of their intermediate host (terrestrial snail) by forming broodsacs within the eyestalks of the snail and causing them to pulsate with vibrant color, 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, is a defining characteristic 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 behavior 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 parasite 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 hypothesized 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 17th 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 material was sorted using sieves and damselfly nymphs were retrieved, and returned to the laboratory in water from the collection site. To minimize 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 125ml 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 um sized mesh to allow regular water flow.

Before each experiment, damselfly nymphs were transferred into the experimental arena at least 24 hours prior to the start of the test to allow acclimation (Brodin and Johansson 2004; Brodin 2008). Experiments took place between 14th and 21st March 2016, between 14h00 and 17h00 daily.

Tests of activity

For this experiment, damselflies was placed individually in a 170mm × 120mm × 68mm test arena with a grid of 1cm squares marked on its front, to evaluate their spontaneous activity in the absence of stimulus. The test arena was filled with 300ml of water from the collection site and each damselfly nymph was fed with zooplankton 24h prior to each experiment to eliminate any possible food foraging behaviour. The exact position of a damselfly was recorded every 10 minutes for 140 minutes, 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 minutes (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 seconds were classified as bold (quick resumption of movement), while those that remained still after 120 seconds (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, eleven 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 GACTCCTTGGTCCGTG 3′) (Harper & 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 follows Blasco-Costa et al. (2009). 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 (633bp 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 1 to 3 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 eleven 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, 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 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 (Figure 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 *Xanthocnemis 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.

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 is a central feature 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 metacercarie on the behaviour of *Xanthocnemis 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.

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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	Models 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

Figure captions

Figure 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.

Figure 2 Activity level for all 144 activity measurements (cm / 10min) 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).

Figure 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.

Figure 1:

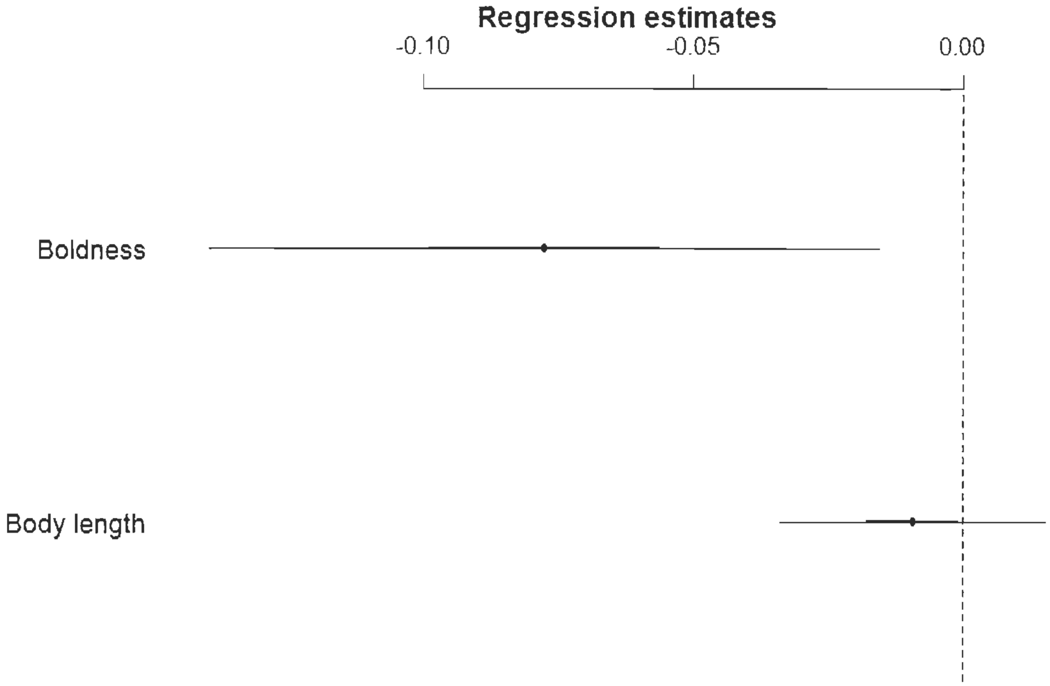


Figure 2:

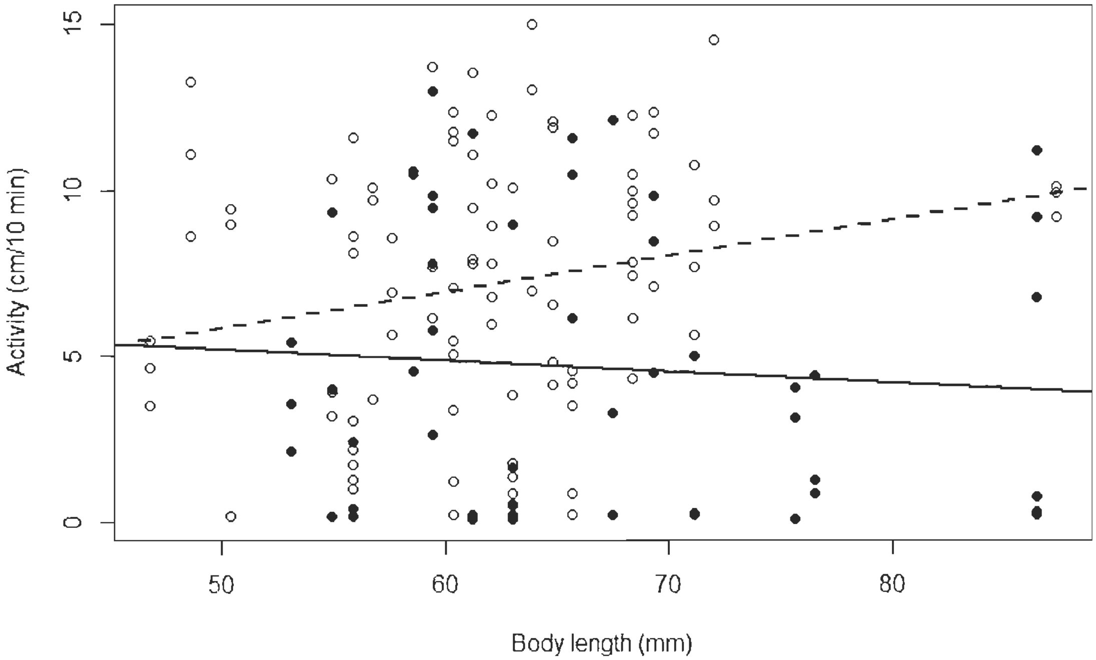
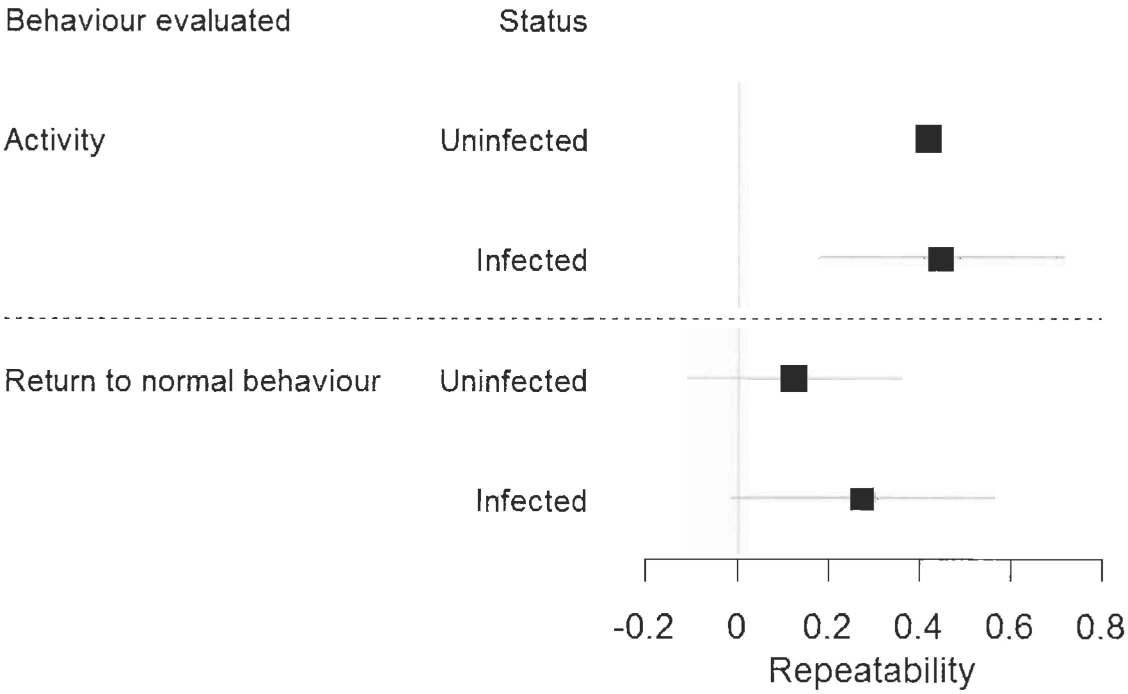


Figure 3:


Behaviour evaluated
Status
Activity
Uninfected
Infected
Return to normal behaviour
Uninfected
Infected
-0.2
0
0.2
0.4
0.6
0.8
Repeatability