Smelling the future: subtle life-history adjustments in response to environmental conditions and perceived transmission opportunities in a trematode

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SUMMARY

A number of parasites with complex life cycles can abbreviate their life cycles to increase the likelihood of reproducing. For example, some trematodes can facultatively skip the definitive host and produce viable eggs while still inside their intermediate host. The resulting shorter life cycle is clearly advantageous when transmission probabilities to the definitive hosts are low. *Coitocaecum parvum* can mature precociously (progenesis), and produce eggs by selfing inside its amphipod second intermediate host. Environmental factors such as definitive host density and water temperature influence the life-history strategy adopted by *C. parvum* in their crustacean host. However, it is also possible that information about transmission opportunities gathered earlier in the life cycle (i.e. by cercariae-producing sporocysts in the first intermediate host) could have priming effects on the adoption of one or the other life strategy. Here we document the effects of environmental parameters (host chemical cues and temperature) on cercarial production within snail hosts and parasite life-history strategy in the amphipod host. We found that environmental cues perceived early in life have limited priming effects on life-history strategies later in life and probably account for only a small part of the variation among conspecific parasites. External cues gathered at the metacercarial stage seem to largely override potential effects of the environmental conditions experienced by early stages of the parasite.

Key words: progenesis, life-cycle abbreviation, *Coitocaecum parvum*, environmental cues, complex life cycle.

INTRODUCTION

The life-history and transmission strategies of parasites show huge variation among different parasite species, even among closely related ones (Poulin, 1996; Morand and Sorci, 1998; Viney and Cable, 2011; Koehler *et al*. 2012). There also exists substantial variation among individual parasites of the same population, which can be interpreted in terms of adaptive plastic responses to immediate environmental conditions (Thomas *et al*. 2002). There is some empirical evidence that parasites can adjust their growth, reproduction, virulence or mode of transmission in a state-dependent manner, in apparent response to the physiological condition of the host, or to direct or indirect cues from the external environment (Tseng, 2006; Lagrue and Poulin, 2007; Seppälä *et al*. 2008; Badets *et al*. 2010). Matching life-history strategies to perceived opportunities for transmission and/or reproduction is within the neurosensory and developmental toolkit of parasites (Thomas *et al*. 2002), although the phenomenon remains poorly studied. In particular, it remains unclear whether cues related to future transmission opportunities that are perceived early in life can affect strategies manifested later in life.

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Here, we use the trematode *Coitocaecum parvum* as a model, to explore the ability of parasites to make adaptive plastic adjustments to their developmental and transmission strategies in response to external cues regarding the presence of hosts needed later in the life cycle. Like many trematodes, *C. parvum* has a typical three-host life cycle (Galaktionov and Dobrovolskij, 2003). Juvenile stages multiply asexually within the first intermediate host, the freshwater snail *Potamopyrgus antipodarum*. Infective stages, or cercariae, produced in this manner then leave the snail to seek and penetrate its second intermediate host, the amphipod *Paracalliope fluviatilis*. Within the amphipod, parasites encyst as metacercariae, and await ingestion by a fish definitive host, in which they grow into adult worms and reproduce sexually. Parasite eggs, released in fish feces hatch into ciliated larvae, which seek and infect snails, thus complete the life cycle. However, many individual *C. parvum* adopt an alternative life-history route, in which they mature precociously while still at the metacercarial stage within an amphipod, and initiate egg production by self-fertilization (trematodes are hermaphrodites; Holton, 1984). This phenomenon, known as progenesis, allows the parasites to bypass the need to reach a fish host, thus truncating the life cycle from three to two hosts (Poulin and Cribb, 2002). Three independent experiments conducted in

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previous studies, each following slightly different procedures, have established that fish chemical cues in the water surrounding amphipods determine which life-history route is adopted: the presence of fish cues triggers a dramatic shift to the 'normal' three-host cycle in the parasites within amphipods, whereas the absence of fish cues results in the adoption of progenesis and the abbreviated two-host cycle (Poulin, 2003; Lagrue and Poulin, 2007, 2009*a*). Fish chemicals indicate local fish presence, and thus a higher probability of transmission to the definitive host than in the total absence of fish cues. The mechanism by which parasites within amphipods indirectly detect fish chemicals probably involves enhanced host stress, as small crustaceans show stress in response to predator odours (Dodson, 1989; Kats and Dill, 1998). The net outcome is that the parasites adopt the transmission strategy best suited to immediate external conditions.

In the present study, we test two further hypotheses related to the ability of the trematode *C. parvum* to adjust to perceived transmission opportunities, this time with transmission cues presented to the parasite at an earlier stage of the life cycle. First, we hypothesize that the parasite can use information on the availability of second intermediate hosts to fine tune the release of cercariae from the snail, the first intermediate host. In many trematode species, cercarial emergence from snail hosts is triggered by abiotic cues, such as short-term increases in light or temperature, which may coincide with diurnal cycles in the activity or availability of the second intermediate hosts (e.g. Shostak and Esch, 1990; Lo and Lee, 1996; Fried *et al*. 2002; Fingerut *et al*. 2003). However, the possible effect of direct chemical cues from the second intermediate hosts themselves has rarely been investigated (see Mouritsen, 2002). We predict that *C. parvum* cercariae will be prompted to emerge from the snail host in greater numbers when chemical cues indicate the immediate availability of second intermediate hosts. We expect this response to be specific to cues from the amphipod *P. fluviatilis*, which is the second intermediate host, and not to cues from other aquatic arthropods living in sympatry.

Second, we hypothesize that exposure to chemical cues from fish definitive hosts while the parasites are still within the snail first intermediate host, will prime them to later adopt the three-host life cycle when they reach an amphipod second intermediate host. In contrast, exposure to water lacking cues from fish hosts should prime the parasites to later adopt progenesis and a truncated two-host life cycle. In addition to the influence of cues present at one life stage affecting development at that same life stage, we thus expect an ontogenetic time lag: cues perceived when inside the first intermediate host affecting later development inside the next host. Generally, progenesis is a highly plastic strategy, with no genetic basis (Lagrue and Poulin, 2009*b*), and influenced by many environmental cues that *C. parvum* uses to preferentially adopt one or the other strategy when in its amphipod intermediate host (Poulin, 2003; Lagrue and Poulin, 2007, 2009*a*; Lagrue *et al*. 2009). However, it also requires much growth by metacercariae in amphipod hosts (Lagrue and Poulin, 2007). Therefore, initiating development towards precocious maturation immediately following infection of the amphipod, based on information acquired earlier (i.e. in the snail first intermediate host) and indicating a low probability of reaching a fish, should maximize parasite fitness. To the best of our knowledge, there have been no previous attempts to test for such effects of external cues on life-history strategies across ontogenetic life stages of parasites.

Our aim is therefore to test for adaptive plasticity in life-history responses of juvenile parasites to cues from their next host and second-next host, i.e. cues that convey information about future transmission opportunities. Specifically, we test: (i) whether exposure of *C. parvum* within snail hosts to cues from amphipod second intermediate hosts trigger a greater immediate emergence of cercariae than exposure to plain water or water containing cues from non-host arthropods; and (ii) whether exposure of *C. parvum* within snail hosts to cues from fish definitive hosts primes the parasites to subsequently adopt a three-host life cycle, while non-exposed control parasites instead later adopt progenesis in the amphipod host. Because ambient temperature also greatly affects *C. parvum* growth in amphipods (Poulin, 2003), we include temperature as an additional factor, to determine whether early priming by fish *vs* no-fish cues while in the snail host is strong enough to overcome the influence of a more immediate abiotic factor. Overall, our study tested whether external cues perceived early in life may affect life-history strategies later in life.

MATERIAL AND METHODS

Animal collection

Naturally infected snails (*P. antipodarum*) were collected among macrophytes in Lake Waihola, South Island, New Zealand (46°01′14·1″S, 170°05′05·8″E) using dip nets (500 μm mesh size). A large stock of infected snails was obtained by selectively choosing individuals that displayed an altered shell shape, a sure sign of infection by *C. parvum* (Lagrue *et al*. 2007). The three crustacean species (*P. fluviatilis, Austridotea annectens* and *Tenagomysis chiltoni*) and the definitive fish host (*Gobiomorphus cotidianus*) used to prepare scented water for the different experiments described below were also captured in Lake Waihola using dip nets. Uninfected

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P. fluviatilis amphipods were collected in the Waitaki River, South Island, New Zealand (44°55′ 51·9″S; 171°05′55·5″E) as natural *C. parvum* infection levels are very low, but amphipods are highly susceptible to *C. parvum* at this site, thus increasing the efficiency of experimental infections while almost eliminating the risk of using already infected individuals (Lagrue *et al*. 2016). A previous study showed that amphipod host origin (Lake Waihola *vs* Waitaki River) did not affect body size or life-history strategy of *C. parvum* metacercariae and the use of amphipods collected in a different location from the parasite should thus not affect the results of the present experiments (Lagrue *et al*. 2016). Snails, fish and crustaceans were held separately by species in stock tanks, kept alive in aerated water from their respective sampling sites and maintained at room temperature (16 ± 1 °C) until required for experiments. Invertebrates were provided with macrophytes (*Elodea canadensis*) and plankton for food, while fish were fed commercial fish pellets.

Short-term effects of invertebrate chemical cues on cercarial shedding by snail hosts

Naturally infected snails (*P. antipodarum*) were haphazardly selected from the stock tank and transferred to 24-well plates. Each well contained a single snail and 500 µL of either aged lake water (control) or aged lake water scented with amphipod (*P. fluviatilis*), isopod (*A. annectens*) or mysid shrimp (*T. chiltoni*) chemical cues. These invertebrate species were selected to represent a variety of crustacean orders and for their contrasting interactions with *C. parvum; P. fluviatilis* is the main intermediate host for *C. parvum, A. annectens* is a non-host benthic isopod and potential predator of *C. parvum* cercariae, whereas *T. chiltoni* is a non-host pelagic planktivore.

Scented water was prepared by maintaining 30–35 isopods, 200–225 amphipods or 45–50 mysid shrimps haphazardly selected from the stock tanks into separate 150 mL containers filled with aged lake water. The different numbers of individuals for the different species represent similar biomasses per volume of water, and thus likely similar concentrations of chemical cues. In crustacean, chemical cues are released through urine and we simply made the assumption that similar biomasses of crustacean would produce comparable amounts of urine and thus chemical signals (Breithaupt and Thiel, 2010). The exact nature of chemical cues released by the different crustacean species are unknown but likely differ and these potential differences could be used by the parasite to adjust cercarial release (Breithaupt and Thiel, 2010). A fourth tank was left with only water (control) to standardize the treatment. Animals were left in the containers for 20 h, removed and returned to their respective stock tanks. The different types of scented water were used immediately.

Snails were incubated at 25 °C under constant light, conditions known to induce cercarial release (Hay *et al*. 2005). Wells were checked regularly over a 24 h period; cercariae shed by the snails were counted and removed every time to avoid counting these twice. After 24 h, snails were measured (total shell length), dissected to confirm their infection status and the number of sporocysts in each snail counted to control for the potential effects of snail size and sporocyst numbers on observed cercarial production.

Long-term effects of definitive host cues and water temperature on the production and size of C. parvum *cercariae*

Coitocaecum parvum-infected snails were taken from the stock tank and haphazardly separated into 44 groups of ten individuals (440 snails in total). Eleven groups of snails were assigned to each of four treatments (110 individuals per treatment) in a two-by-two design using control *vs* fish-scented water and two different temperatures (18 or 22 °C). Snails were maintained in 200 mL plastic containers (one group of ten snails per container) half-filled with 100 mL of aged lake water and strands of macrophytes (*E. canadensis*) for food. Fish scented water was prepared every second day following a similar protocol as for invertebrates (Lagrue and Poulin, 2007): 2·5 L of aged lake water were placed in each of two identical 5 L plastic tanks and four definitive host fish (*G. cotidianus*) haphazardly chosen from the stock tank were added to one of the tanks. Note that the study was carried out in accordance with Otago University animal ethic rules and regulations for husbandry, care and use of laboratory animals.

The second tank was left with only water (control) to standardize the treatment. Fish were kept overnight and removed in the morning. Scented water was immediately used and added to the containers housing the naturally infected snails. Every 2 days, 50 mL of water was removed from each snail container with a plastic syringe and replaced with either control or fish conditioned water. The same treatment was applied to a given container for the whole experiment. Half of the snail containers maintained at 18 °C received control water and half received fish water, the same applied for snails maintained at 22 °C. Treatments were applied for 5 weeks prior to the following experiments to allow *C. parvum* larvae produced before the beginning of the treatment to be shed by the snails. This delay thus ensured that all cercariae used in our experiments had been produced by sporocysts exposed to our controlled conditions (personal observations). Water and temperature treatments were maintained until snail dissections.

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To test for the effects of the presence (fish treatment) or absence (control) of chemical cues from *G. cotidianus*, the fish definitive host, and temperature on *C. parvum* cercarial production, snail groups were transferred into Petri dishes and incubated for 24 h at 4 °C above their acclimation temperatures (22 and 26 °C for snails maintained at 18 and 22 °C, respectively). Petri dishes were checked regularly and cercariae counted and removed every time to avoid counting the same individuals several times. The same protocol was used on two occasions, 1 week apart, to obtain two replicate measures per snail group.

To test for potential effects of treatment (fish *vs* control) and temperature (18 or 22 °C) on *C. parvum* larval size, snails were incubated a third time a week later and a subsample of the cercariae released was used for measurements (345 individuals; at least 80 per treatment and 5 per group of snails). A digital picture of each cercaria was taken using an Olympus DP12 Microscope Digital Camera System connected to a stereo-microscope; cercariae were previously heat-killed to standardize their state of contraction/relaxation. Photographs were also standardized by using a fixed magnification (×100) and focus, so that the edge of the cercaria was sharp on the picture. Each cercaria was measured (length and width) using the ImageJ software (Wayne Rasband, NIH, USA). Their body surface was then determined and used as a surrogate for body size. This was done using the formula for an ellipsoid, $(\pi LW)/4$, where L and W are the length and width of the parasite. Snails used in this experiment were subsequently used in the next experiment (see below).

Delayed effects of external cues at the cercarial stage on C. parvum *metacercariae developmental strategy*

Potential effects of fish chemical cues and water temperature on the life-history strategy, size and fecundity of metacercariae were tested using experimental infections. Snails from the previous experiment were used as a source of cercariae in controlled infections. Uninfected amphipods were obtained by inspecting each amphipod sampled in the Waitaki River under a microscope and discarding all individuals that showed any sign of infection, i.e. an opaque mass in the body cavity corresponding to a metacercaria (Lagrue and Poulin, 2007). Because host condition (age, size, sex) can influence larval helminth growth and life strategy in their intermediate hosts, we used only male *P. fluviatilis* amphipods of similar size (Parker *et al*. 2003; Lagrue *et al*. 2016).

Cercariae of *C. parvum* were obtained from snails under controlled conditions to ensure that the cercariae used to experimentally infect amphipods were freshly released and, therefore, more probable to penetrate the amphipod. Snails used in the previous experiment were also used here; at this point, snail hosts had experienced the treatment protocol of fish water and control water for 8 weeks. Snails were transferred to Petri dishes filled with 10 mL of filtrated lake water. Snails were then incubated 4 °C above the temperature they were maintained under in the previous experiment (i.e. 22 or 26 °C) for 20 min under constant light. The Petri dishes were then screened under a microscope and the cercariae found were transferred to 500 μL Eppendorf tubes using a 20 μL micropipette. A single *C. parvum* cercaria was placed in each tube with 5 μL of filtrated river water and an amphipod was then added. Amphipods were left in the tube along with the parasite for 5 h, a time after which unsuccessful cercariae stop moving and die. A total of 384 amphipods were exposed to a *C. parvum* cercaria; 96 amphipods for each of the four snail host treatments used in the previous experiment (fish *vs* control, 18 or 22 °C). Surviving amphipods were then separated based on the treatment to which their source snails were exposed, and placed in plastic containers filled with 500 mL of aged and aerated river water; strands of macrophytes (*E. canadensis*) were added for food. Aged water consisted of water collected in the Waitaki River at least 1 week before the experiment to allow any chemical cue to deteriorate (Poulin, 2003). At that time, amphipod survival was about 80% and there was no difference in survival among treatments (Fisher's exact tests, all $P > 0{\cdot}05$). In total, 151 amphipods received cercariae shed by snails from the control treatment (74 and 77 from snails maintained at 18 and 22 °C, respectively) and 161 from the fish treatment (81 and 80 at 18 and 22 °C, respectively).

After 4 weeks, all surviving amphipods were killed in 70% ethanol, measured (body length) and dissected. There was no difference in amphipod survival among treatments at the time of dissection (Fisher's exact tests, all $P > 0{\cdot}05$; Table 1). Any live *C. parvum* metacercaria recovered from amphipod hosts was removed from its cyst (all live metacercariae are encysted at 4 weeks post-infection), killed in 70% ethanol and immediately rinsed in distilled water, measured (length and width) under a compound microscope, and recorded as 'normal' (non-egg-producing metacercaria) or 'progenetic' (egg producing; Table 1); in the case of progenetic parasites, eggs were also counted. The body surface of each parasite was then determined and used as a surrogate for body size as described previously for cercariae. Dead and melanized cercariae found in amphipod hosts were also recorded and treated as successful infection but failed development; uninfected amphipods represented failed infections where the cercaria did not reach the host body cavity. Snails used in this experiment and the previous one were measured (total shell length) and dissected to confirm their infection status; at this

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Table 1. Detail of the number (*N*) of amphipod hosts alive (surviving) and infected at the end of the experiment and of the number (*n*) of *Coitocaecum parvum* metacercariae that successfully infected the amphipods in the two treatments (control *vs* fish) and under the two temperature regimes (18 or 22 °C) under which snails used to obtain cercariae for experimental infections were maintained.

		Amphipod host		*C. parvum* metacercariae		
Treatment	T°C	*N* surviving	*N* infected	*n* normal	*n* progenetic	*n* dead
Control	18	24	14	1	9	4
	22	25	17	4	13	0
Fish	18	23	15	6	7	2
	22	24	18	7	8	3

The outcome of infection is divided into three categories, 'normal' for non-egg producing and 'progenetic' for egg-producing metacercariae. Cercariae that reached the body cavity of the amphipod but were subsequently killed and melanized by the host were recorded as dead.

point, snail hosts had been exposed to treatments for 8 weeks. The number of sporocysts in each snail was also counted to control for the potential effects of snail size and sporocyst numbers on cercarial production.

Statistical analyses

The potential effects of different invertebrate chemical cues on short-term larval output (i.e. the number of cercariae shed by snails) were tested using a generalized linear model (GLM) with Poisson distribution and a log link function. Scented water treatment was used as the categorical predictor (control, amphipod, isopod or mysid), and snail size and sporocyst number as continuous predictors. Snail size and sporocyst number were included in the model to control not only for possible differences in these variables among the four treatments but also for potential effects of these variables on cercarial production. The response variable (larval output) was considered as count data.

When testing for potential effects of fish host chemical cues and temperature on cercarial production, snails were kept and shed in groups, so it was not possible to include individual snail size and sporocyst numbers as continuous predictors in the model to control for these. However, there was no difference in snail size [two-way analysis of variance (ANOVA); treatment, $F_{1,386} = 0{\cdot}69$, $P = 0{\cdot}410$; temperature, $F_{1,386} = 0{\cdot}82$, $P = 0{\cdot}366$; treatment × temperature, $F_{1,386} = 0{\cdot}09$, $P = 0{\cdot}759$] or sporocyst numbers (two-way ANOVA; treatment, $F_{1,386} = 3{\cdot}23$, $P = 0{\cdot}073$; temperature, $F_{1,386} = 0{\cdot}92$, $P = 0{\cdot}338$; treatment × temperature, $F_{1,386} = 0{\cdot}037$, $P = 0{\cdot}847$) among snails exposed to fish scented or control water (treatment) or maintained at different temperature (18 or 22 °C). Potential differences observed in cercarial output could thus not be due to differences in snail size or sporocyst numbers among treatments. Furthermore, the two dates at which snails were shed could not be used as repeated measures because some snails died between the two dates. Instead, we split these data into two replicates and included shedding date as a categorical predictor and the number of live snail per group was used as a continuous predictor in the model to control for different snail numbers among groups.

Potential long-term effects of fish definitive host chemical cues and temperature on larval output (i.e. the number of cercariae shed by snails) were tested using a GLM with normal distribution and a log link function. Treatment (fish *vs* control), temperature (18 or 22 °C) and date (first or second) were used as categorical predictors. Number of snails per group was used as a continuous predictor. The response variable (larval output) was considered as count data. Effects of treatment (fish *vs* control) and temperature, and their potential interaction, on *C. parvum* cercariae body area ($mm^2 \pm$ S.E.) were tested using a two-way ANOVA with the cercarial size (log-transformed) used as the dependent variable.

Effects of treatment and temperature on the infection success, survival and strategy (normal or progenetic) of *C. parvum* metacercariae were tested using Fisher's exact test; the proportions of parasites in each category were compared in a pairwise manner. There was no difference in the body size of surviving amphipods between treatments (fish *vs* control, three-way ANOVA, $F_{1,88} = 1{\cdot}25$, $P = 0{\cdot}266$), temperature (18 or 22 °C, $F_{1,88} = 1{\cdot}49$, $P = 0{\cdot}225$) or infection status (infected or uninfected, $F_{1,88} = 1{\cdot}51$, $P = 0{\cdot}222$). Amphipod size was thus not included in the following analyses. Effects of treatment (fish *vs* control), temperature and parasite strategy (normal or progenetic) on the size of metacercariae were tested using a three-way ANOVA with the body area (log-transformed) of the parasite used as the dependent variable; dead and melanized cercariae were not included in the analysis. Finally, we tested the effects of treatment and temperature (categorical predictors) and parasite size (continuous predictor) on egg output (Log-transformed) using

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an analysis of covariance (ANCOVA); only parasites that had produced at least one egg were included in this model.

RESULTS

Short-term effects of invertebrate chemical cues on cercarial shedding by snail hosts

There was an overall weak but significant positive relationship between snail size and sporocyst numbers ($r = 0{\cdot}188$, $P < 0{\cdot}0001$). However, there was a small but significant difference in mean snail size among treatments (ANOVA, $F_{3,431} = 9{\cdot}89$, $P < 0{\cdot}0001$); snails exposed to *T. chiltoni* chemical cues were slightly smaller ($2{\cdot}74 \pm 0{\cdot}03$; shell length in mm ± S.E.) than in all other treatments [Fisher's least significant difference (LSD), all $P < 0{\cdot}05$; $2{\cdot}88 \pm 0{\cdot}03$, $2{\cdot}92 \pm 0{\cdot}03$, $2{\cdot}91 \pm 0{\cdot}02$ for the control, amphipod and isopod treatment, respectively]. There was no difference among the other three treatments (Fisher's LSD, all $P > 0{\cdot}05$). There was also a significant difference in the mean number of sporocysts per snail (± S.E.) among treatments (ANOVA, $F_{3,431} = 3{\cdot}137$, $P = 0{\cdot}025$). However, in contrast to snail size, this was due to a higher number of sporocysts in snails from the isopod treatment ($21{\cdot}5 \pm 1{\cdot}0$) than in the control and amphipod treatment (Fisher's LSD, both $P < 0{\cdot}05$; $18{\cdot}3 \pm 1{\cdot}1$, $17{\cdot}8 \pm 0{\cdot}8$ for the control and amphipod isopod treatment, respectively). There was no difference between the mysid shrimp treatment ($19{\cdot}7 \pm 0{\cdot}9$) and any other treatment (Fisher's LSD, all $P > 0{\cdot}05$). We thus kept both continuous predictors in the model.

Infected snail size had a significant effect on cercarial production (GLM, $F_{1,429} = 24{\cdot}67$, $P < 0{\cdot}0001$); there was a positive relationship between snail size and the number of *C. parvum* cercariae released during the experiment ($r = 0{\cdot}253$, $P < 0{\cdot}0001$). The number of sporocysts per snail also had a small but significant effect on cercarial production (GLM, $F_{1,429} = 4{\cdot}73$, $P = 0{\cdot}044$); the number of cercariae released was positively correlated with sporocyst numbers ($r = 0{\cdot}136$, $P = 0{\cdot}0044$). Finally, there was no overall effect of the treatment on cercarial output (GLM, $F_{3,429} = 0{\cdot}778$, $P = 0{\cdot}506$; Fig. 1).

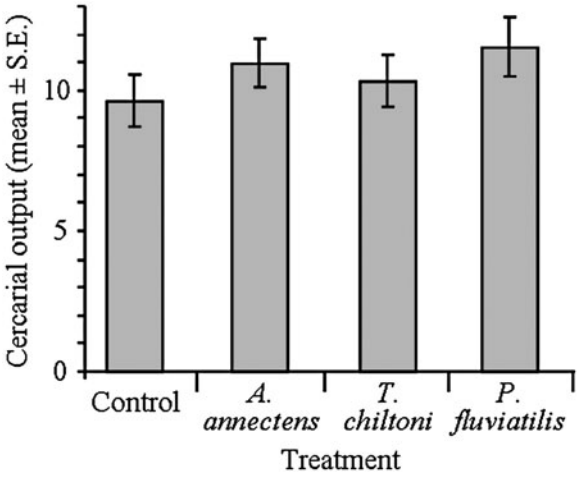

Fig. 1. Cercarial output (mean number of cercariae released per individual snail ± S.E.) of *Coitocaecum parvum*-infected snails (*Potamopyrgus antipodarum*) exposed to different chemical cues (i.e. treatment).

Long-term effects of definitive host cues and water temperature on the production and size of C. parvum *cercariae*

As expected, the number of cercariae shed in each group of snail increased significantly with the number of snails in the group but shedding date had no effect on cercarial output (GLM, $F_{1,79} = 1{\cdot}13$, $P = 0{\cdot}288$). Cercarial output was significantly influenced by the treatment (control *vs* fish) to which the snails were exposed (GLM, $F_{1,79} = 5{\cdot}1$, $P = 0{\cdot}025$; Fig. 2A); snails in the control treatment produced fewer cercariae than their counterparts in the fish treatment [mean (±S.E.) number of cercariae produced per date per snail = $6{\cdot}17 \pm 0{\cdot}44$ and $7{\cdot}68 \pm 0{\cdot}49$, respectively]. Cercarial production was also significantly influenced by water temperature (GLM, $F_{1,79} = 17{\cdot}8$, $P < 0{\cdot}0001$; Fig. 2A); snails maintained at 18 °C produced more cercariae than snails maintained at 22 °C ($8{\cdot}18 \pm 0{\cdot}36$ and $5{\cdot}67 \pm 0{\cdot}50$, respectively). Finally, there was no significant interaction among any of the factors included in the GLM (all $P > 0{\cdot}05$).

A total of 245 cercariae were measured and their body area calculated. There was no effect of the treatment, control *vs* fish, on the mean size of *C. parvum* cercariae (ANOVA, $F_{1,341} = 1{\cdot}4$, $P = 0{\cdot}245$). However, cercariae produced by snails maintained at 22 °C were significantly larger than those produced at 18 °C (ANOVA, $F_{1,341} = 19{\cdot}7$, $P < 0{\cdot}0001$). There was also a significant interaction between the two factors (ANOVA, $F_{1,341} = 14{\cdot}8$, $P = 0{\cdot}0001$). There was no size difference between cercariae produced at 18 or 22 °C in the fish treatment (Fisher's LSD, $P = 0{\cdot}683$, $n = 78$ and 89, respectively; Fig. 2B). In contrast, cercariae shed by snails maintained at 18 °C in the control treatment were significantly smaller than cercariae produced in all other treatments (Fisher's LSD, all $P < 0{\cdot}05$, $n = 82$; Fig. 2B). Cercariae produced at 22 °C by snails in the control treatment were larger than in all other treatment combinations (Fisher's LSD, all $P < 0{\cdot}05$, $n = 96$; Fig. 2B).

Delayed effects of external cues at the cercarial stage on C. parvum *life strategy*

The proportion of amphipods successfully infected by *C. parvum* through experimental exposure did

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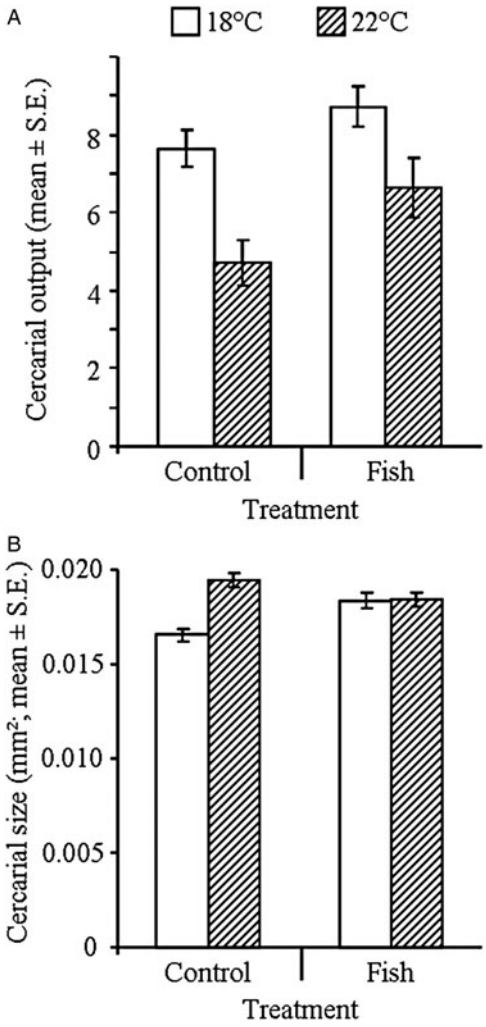

Fig. 2. A/Cercarial number (mean daily number of cercariae shed per individual snail ± S.E.) and B/cercarial size (mean body surface ± S.E. in mm^2) produced by *Coitocaecum parvum* infected snails (*Potamopyrgus antipodarum*) maintained under the four different treatment (control *vs* fish) and temperature (18 or 22 °C) combinations.

not differ among treatment and temperature combinations (Fisher's exact tests, all $P > 0{\cdot}05$; Table 1). In the control treatment, parasite survival (i.e. the proportion of surviving metacercariae among successful infections) was significantly higher in cercariae from snails maintained at 22 °C than at 18 °C (Table 1 and 2A). Although similar trends were noticeable between parasites from control snails maintained at 22 °C and parasites from the fish treatment, at 18 and 22 °C, these differences were not significant (Table 1 and 2A). There was no significant difference among treatment–temperature combinations in the proportion of progenetic metacercariae although the proportion of progenetic individuals was slightly lower among parasites originating from snails from the fish treatment (Table 1 and 2B).

Parasite body size was not affected by the temperature at which snails serving as source of cercariae were maintained (18 or 22 °C; ANOVA, $F_{1,47} = 0{\cdot}202$, $P = 0{\cdot}655$; Table 3A). Parasite body size was significantly influenced by the treatment; metacercariae originating from cercariae produced by snails from the control treatment were slightly larger than those from the fish treatment (ANOVA, $F_{1,47} = 5{\cdot}68$, $P = 0{\cdot}021$; Table 3A). Parasite strategy also had a significant effect on the size of *C. parvum* metacercariae (ANOVA, $F_{1,47} = 39{\cdot}1$, $P < 0{\cdot}0001$); progenetic individuals were significantly larger than non-egg-producing individuals (Table 3B and C). There was no significant interaction among any of the three predictors (all $P > 0{\cdot}05$). Generally, egg output increased significantly with parasite size (ANCOVA, $F_{1,32} = 22{\cdot}6$, $P < 0{\cdot}0001$; Fig. 3). When controlled for parasite size, treatment (fish *vs* control) also had a small but significant effect on egg output (ANCOVA, $F_{1,32} = 6{\cdot}03$, $P = 0{\cdot}020$); progenetic metacercariae originating from cercariae released by snails from the control treatment produced more eggs than their counterparts originating from snails from the fish treatment (Table 3C, Fig. 3). Finally, there was no effect of temperature on egg output (ANCOVA, $F_{1,32} = 0{\cdot}042$, $P = 0{\cdot}839$; Table 3C, Fig. 3), and no significant interaction between the two categorical predictors (treatment × temperature, $F_{1,32} = 0{\cdot}108$, $P = 0{\cdot}745$).

Table 2. Results of Fisher's exact tests for pairwise comparisons of the proportion of A/dead and melanized *Coitocaecum parvum* metacercariae and B/progenetic parasites among surviving metacercariae in amphipod hosts among treatment–temperature (T°C) combinations

A/Treatment–T°C combinations compared	χ^2	*P* value
Control-18 °C *vs* Control-22 °C	5·58	0·018
Control-18 °C *vs* Fish-18 °C	1·02	0·311
Control-18 °C *vs* Fish-22 °C	0·65	0·419
Control-22 °C *vs* Fish-18 °C	2·42	0·120
Control-22 °C *vs* Fish-22 °C	3·10	0·078
Fish-18 °C *vs* Fish-22 °C	0·07	0·790
B/Treatment–T°C combinations compared	s^2	*P* value
Control-18 °C *vs* Control-22 °C	0·76	0·382
Control-18 °C *vs* Fish-18 °C	3·49	0·061
Control-18 °C *vs* Fish-22 °C	3·71	0·054
Control-22 °C *vs* Fish-18 °C	1·70	0·193
Control-22 °C *vs* Fish-22 °C	1·89	0·169
Fish-18 °C *vs* Fish-22 °C	0·00	0·978

DISCUSSION

Progenesis and life-cycle abbreviation in the second intermediate host allow *C. parvum* to adjust its life strategy to environmental changes. Progenesis is preferentially adopted when chemical cues from

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Table 3. Number (N), size (mm^2) and egg output of *Coitocaecum parvum* metacercariae recovered during dissection of the experimentally infected amphipods for A/all metacercariae, B/normal and C/progenetic individuals only.

A/All metacercariae			Body size (mm^2)		Egg output	
Treatment	T°C	*N*	Mean	S.E.	Mean	S.E.
Control	18	10	0·138	0·014	57·6	11·8
	22	17	0·166	0·021	65·1	12·6
Fish	18	13	0·103	0·017	28·1	9·5
	22	15	0·086	0·011	20·3	7·8
B/Normal metacercariae			Body size (mm^2)			
Treatment	T°C	*N*	Mean	S.E.		
Control	18	1	0·058	–		
	22	4	0·069	0·009		
Fish	18	6	0·048	0·008		
	22	7	0·047	0·009		
C/Progenetic metacercariae			Body size (mm^2)		Egg output	
Treatment	T°C	*N*	Mean	S.E.	Mean	S.E.
Control	18	9	0·147	0·012	64·0	11·1
	22	13	0·196	0·021	85·2	11·6
Fish	18	7	0·151	0·014	46·4	6·8
	22	8	0·120	0·005	29·3	5·9

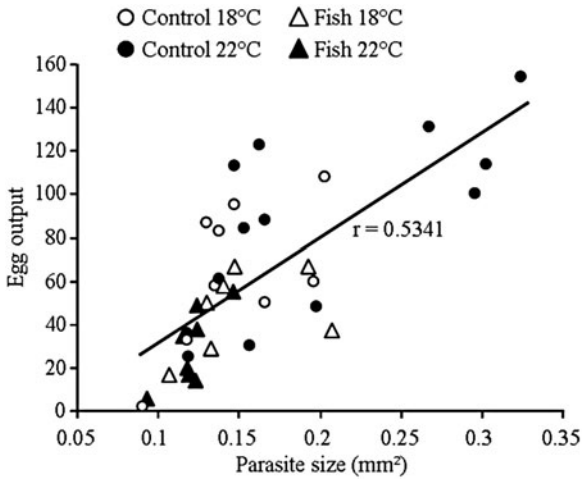

Fig. 3. Relationship between parasite size (body surface in mm^2) and egg output (number of eggs produced by each individual at the time of dissection) by progenetic *Coitocaecum parvum* metacercariae in their amphipod second intermediate host *Paracalliope fluviatilis*. Cercariae used to experimentally infect the amphipods originated from infected snails maintained under the four treatment (control *vs* fish)–temperature (18 or 22 °C) combinations detailed above the graph. The line of best fit and coefficient of correlation shown on the figure are for all *C. parvum* individuals.

the definitive host are absent or when water temperature increases during the metacercarial stage (Poulin, 2003; Lagrue and Poulin, 2007). It is thus possible that similar environmental cues could be used by the parasite earlier in the life cycle, in the snail first intermediate host. Information on host availability could be used by the parasite as a trigger for the shedding of cercariae or have a priming effect on the preferential adoption of either of the two alternative life-history strategies (normal or progenetic) at the metacercarial stage.

Generally, cercarial production was affected by snail host size, and thus space and resources available to the parasite (Graham, 2003; Thieltges *et al*. 2008), and sporocysts numbers. Sporocysts are the cercarial producing units, each of which contain and release only a few cercariae at a time (McCarthy *et al*. 2002). It is thus logical that the more sporocysts in a snail, the more cercariae can be produced and released. However, when controlled for snail size and sporocyst number, we found that chemical cues from crustacean intermediate host and non-host species did not influence cercarial output by *C. parvum* in the snail first intermediate host. Although snail hosts exposed to control, untreated water shed slightly fewer cercariae than snails exposed to water containing cues from the amphipod intermediate host *P. fluviatilis*, there was no clear effect of crustacean chemical cues on cercarial production (Fig. 1). *Coitocaecum parvum* may either be unable to detect directly the presence of suitable or unsuitable crustacean hosts in the immediate vicinity of its snail host, or this information is simply not used. Alternatively, the presence of crustaceans may not induce any response or stress, physiological or behavioural, in the snail host and thus be undetectable by the parasite; *C. parvum* likely uses these indirect cues from its host to evaluate environmental conditions (Poulin, 2003; Lagrue and Poulin, 2007).

In contrast, chemical cues from the definitive host fish did induce an increase in cercarial production in snail first intermediate hosts (Fig. 2A). Although fish are not an appropriate target host for cercariae, it is possible that fish presence induces stress in snail hosts as *G. cotidianus* feeds heavily on *P. antipodarum* (Rowe *et al*. 2001). Whether the parasite uses this information as cues about transmission opportunities later in its life cycle or simply as the potentially imminent death of its snail host cannot be determined here but in either scenario, an increase in cercarial production is likely the optimal strategy. Cercarial production was also influenced by water temperature, a common trend in trematode parasites (Poulin, 2006). Rising temperatures most often result in an increase in cercarial production (Mouritsen, 2002; Poulin, 2006). However, we found here that snails maintained at the highest experimental temperature produced significantly fewer cercariae, regardless of the

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treatment (control *vs* fish; Fig. 2A). Although increased metabolic activity of the snail host, and thus the greater energy made available to the parasite, at higher temperatures is often linked to an increase in cercarial production, several factors may counteract these effects. For example, snail host size is a strong limiting factor for cercarial production; smaller snail species, such as *P. antipodarum* (4–6 mm adult shell length; Holomuzki and Biggs, 2006), may offer limited extra resources to their parasites, even when their metabolism increases with higher ambient temperatures (Poulin, 2006). The thermal regime experienced by both the trematode parasite and snail host in nature may also constrain any physiological responses to increasing temperatures. Here, we used temperatures (18 and 22 °C) that are within the natural range (4–24 °C) but in the higher bracket of seasonally recorded water temperatures at the sampling site (Lagrue and Poulin, 2008*a*). Snail hosts and the parasite may thus have been exposed to experimental temperatures at the extreme end or even beyond their natural optimum, thus inducing a decline rather than an increase in cercarial production as a result of thermal stress.

Alternatively, there may be a trade-off between the number of cercariae produced and their size (McCarthy *et al*. 2002). Here we found, in the control treatment, that although snails maintained at the highest temperature produced fewer cercariae, these were on average significantly larger (Fig. 2B); hinting at a trade-off between number and size of *C. parvum* larvae produced in the first intermediate host. The production of larger cercariae is more costly given that resources for production are limited and a decrease in shedding rate is common due to the trade-off between number and size (Loker, 1983; Thieltges *et al*. 2008). Cercariae do not feed and can only use their glycogen stores to find and infect a suitable host (Xu *et al*. 1994; Fried *et al*. 1998). Producing larger cercariae can thus be advantageous, especially at higher temperature when metabolic rates, and thus energy consumption, are higher (Lawson and Wilson, 1980; Pechenik and Fried, 1995). However, this trade-off between number and size of *C. parvum* cercariae was not observed in snails exposed to fish chemical cues (Fig. 2). In the fish treatment, snails shed cercariae of similar body size regardless of the temperature at which they were maintained even though snail hosts produced more cercariae at 18 °C (Fig. 2). By inducing an increase in cercarial production, fish chemical cues may reduce the parasite's ability to modulate the amount of energy allocated to each larva; cercarial size may reach an optimum in terms of size (i.e. energy reserve) *vs* number produced. The decrease in cercarial production between 18 and 22 °C in the fish treatment is possibly due to increased metabolic/thermal stress in the snail host and reduction in the energy available to *C. parvum* to produce cercariae without risking killing its host (Frank, 1996).

Larval size did not seem to have any effect on the probability that cercariae successfully penetrated the amphipod host. However, larger cercariae had a higher survival rate post-infection and were less likely to be killed and melanized by the host. High-energy storage may provide larger cercariae with more energy to combat the host immune system early post-infection while producing the protective cyst typically surrounding *C. parvum* metacercariae. Within each cercaria, resources must be partitioned between host-seeking, infection, survival and subsequent developmental functions (McCarthy *et al*. 2002). As expected, progenetic metacercariae were significantly larger than normal individuals. More interestingly, while the temperature at which snails were maintained had no effect on *C. parvum* metacercariae strategy or size in their amphipod second intermediate host, treatment (control *vs* fish) had a small but significant effect on the size of *C. parvum*. Metacercariae originating from snails maintained in the control treatment were larger than those from the fish treatment, potentially indicating a priming effect of early life cues on parasite growth. However, potential priming effects of early environmental conditions in the first intermediate host on parasite size and life-history strategy may eventually be largely overridden by the actual, current transmission probabilities, or lack thereof, experienced by the parasite in the amphipod second intermediate host. *Coitocaecum parvum* metacercariae have been shown to use the more immediate cues available in their amphipod second intermediate host to adaptively adjust their life-history strategy (Lagrue and Poulin, 2007, 2008*b*). Alternatively, amphipod host origin (i.e. different from that of the parasite) could have influenced parasite life-history strategy. However, a previous study showed that this is not the case and the results observed here cannot be due to the different origins of host and parasite (Lagrue *et al*. 2016).

Usually, egg production increases with parasite size in progenetic *C. parvum* metacercariae (Lagrue and Poulin, 2007), and we found a similar trend in the present study. While temperature had no effect on egg production, we found a small but significant priming effect of the treatment (control *vs* fish) in which *C. parvum* infected snails were maintained. Progenetic metacercariae originating from cercariae released by snails from the control treatment produced more eggs, for a given metacercarial size, than those from the fish treatment (Table 3, Fig. 3). Whether higher egg output in metacercariae originating from the control treatment was due to higher daily fecundity or earlier progenesis, and thus more time to produce additional eggs, compared with *C. parvum* individuals from the fish

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treatment cannot be determined here. However, because we used amphipod hosts of similar size and thus offering comparable resources to the parasite, it is probable that the difference in egg production observed was due to metacercariae from the control treatment adopting progenesis earlier than those from the fish treatment (Lagrue and Poulin, 2009*a*). Priming effects resulting from the absence of definitive hosts, and thus low transmission opportunities, in the environment of early life stages of the parasite (i.e. sporocysts in the snail first intermediate host) on the life history of later stages (i.e. metacercariae) are thus possible, albeit seemingly limited.

Overall, our results and those of previous studies show that trematode parasites, including *C. parvum*, are able to operate life strategy adjustments in response to environmental cues and perceived transmission opportunities (Lagrue and Poulin, 2007, 2008*b*, 2009*a*). However, the present study, combined with results from previous work, indicates that cues from the current and immediate environment should largely override potential priming effects of information gathered previously in the life cycle by earlier stages of the parasite. However, experiments presenting contradicting cues as different times in the parasite's life cycle would be needed to illustrate a true override. Generally, changes in life-history strategies observed in parasites capable of progenesis and life-cycle abbreviations are likely the results of multiple factors, including environmental cues gathered early in their life cycle.

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