Lack of seasonal variation in the life-history strategies of the trematode *Coitocaecum parvum*: no apparent environmental effect

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SUMMARY

Parasites with complex life cycles have developed numerous and very diverse adaptations to increase the likelihood of completing this cycle. For example, some parasites can abbreviate their life cycles by skipping the definitive host and reproducing inside their intermediate host. The resulting shorter life cycle is clearly advantageous when definitive hosts are absent or rare. In species where life-cycle abbreviation is facultative, this strategy should be adopted in response to seasonally variable environmental conditions. The hermaphroditic trematode *Coitocaecum parvum* is able to mature precociously (progenesis), and produce eggs by selfing while still inside its amphipod second intermediate host. Several environmental factors such as fish definitive host density and water temperature are known to influence the life-history strategy adopted by laboratory raised *C. parvum*. Here we document the seasonal variation of environmental parameters and its association with the proportion of progenetic individuals in a parasite population in its natural environment. We found obvious seasonal patterns in both water temperature and *C. parvum* host densities. However, despite being temporally variable, the proportion of progenetic *C. parvum* individuals was not correlated with any single parameter. The results show that *C. parvum* life-history strategy is not as flexible as previously thought. It is possible that the parasite's natural environment contains so many layers of heterogeneity that *C. parvum* does not possess the ability to adjust its life-history strategy to accurately match the current conditions.

Key words: abbreviated life cycle, *Coitocaecum parvum*, life-history strategies, complex life cycle.

INTRODUCTION

Many taxa of parasites have evolved complex life cycles in which distinct developmental stages must go through a suite of different host species to complete their life cycle (Choisy *et al*. 2003; Parker *et al*. 2003; Lefebvre and Poulin, 2005*a*; Poulin, 2007). The evolution of such complex life-history strategies from simple one-host cycles is thought to offer several advantages for the parasites such as longer life span, greater body size, higher fecundity (Parker *et al*. 2003) or greater access to sexual partners (Brown *et al*. 2001). On the other hand, the life cycles of these species have become a series of unlikely events for which parasites have had to develop a range of adaptations to increase the likelihood of completion (Thomas *et al*. 2002; Poulin, 2007). In some systems, a radical strategy has evolved in which parasites skip one or several hosts from their life cycles (Combes, 2001; Poulin, 2007). The resulting decrease in the number of transmission steps in the cycle is likely to be the main benefit of the cycle's abbreviation; shorter life cycles should be easier to complete.

This strategy is widespread in trematode parasites; numerous species have independently evolved abbreviated life cycles (Font, 1980; Barger and Esch, 2000; Poulin and Cribb, 2002; Lefebvre and Poulin, 2005*b*). Why so many phylogenetically unrelated species have dropped one or two hosts from the typical 3-host trematode life cycle remains unclear in most cases. One hypothesis is that, in many systems, one host in the life cycle, often the vertebrate definitive host, is periodically absent. In these situations, life-cycle truncation should be favoured by selection. For example, in parasites using predation to reach their definitive host, seasonally low (or null) consumption rates of intermediate host prey by definitive host predators could drive the evolution of such alternative strategies (Poulin and Cribb, 2002).

The most frequent way in which trematode parasites abbreviate their life cycle is by adopting progenesis: following the infection of the second intermediate host, the parasite matures precociously and produces eggs by self-fertilization, most trematodes being hermaphroditic (Lefebvre and Poulin, 2005*b*). While in some species all individuals adopt the shorter life cycle, in other cases, only a certain proportion of the population uses the abbreviated

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route. Such developmental plasticity in life cycles could serve to increase the probability of completing the cycle (Davies and McKerrow, 2003). In species where life cycle abbreviation is facultative, one of the possible explanations for the co-existence of the two developmental strategies is that the shorter life cycle could be a conditional strategy adopted in response to variable environmental conditions (Poulin and Cribb, 2002; Poulin and Lefebvre, 2006). The normal cycle may be preferable under favourable conditions, when definitive hosts are abundant, while a switch to the abbreviated life cycle would be favoured when ecological conditions change. However, other environmental and host-related factors might influence life-history strategies in these species and the relative importance of each factor is also likely to vary over time (Lefebvre and Poulin, 2005*c*), but to which extent parasites perceive these variations and respond appropriately is unknown in a vast majority of species (Thomas *et al*. 2002; Poulin and Lefebvre, 2006).

For example, in the trematode *Coitocaecum parvum* (Opecoelidae), a common parasite of freshwater fish in New Zealand (MacFarlane, 1939; Holton, 1984*a*), both the normal 3-host route and a truncated life cycle can be observed synchronously in parasite populations. Eggs produced by adult worms inside the fish gut are released in fish faeces and hatch into free-swimming larvae (miracidia). Miracidia penetrate the snail *Potamopyrgus antipodarum* in which they multiply and develop into sporocysts. Sporocysts asexually produce cercariae, free-living larvae that enter the amphipod *Paracalliope fluviatilis* where they encyst as metacercariae in the body cavity. At this stage, metacercariae can either await ingestion by a fish, the common bully *Gobiomorphus cotidianus*, where they will mature and reproduce, or keep growing and reach maturity while still inside the amphipod. Progenetic individuals that reach maturity in the crustacean intermediate host reproduce by selfing (i.e. self fertilization; *C. parvum* is hermaphroditic) and lay eggs within their cyst in the amphipod's body cavity (Holton, 1984*b*; Poulin, 2001). Eggs produced by progenetic metacercariae remain enclosed in the cyst, and therefore the host, until amphipod death after which they hatch into larvae that are infective to the snail first host without the need to pass through the fish host. Such a mechanism allows the parasite to reproduce even if transmission to fish fails (Wang and Thomas, 2002). While the maintenance of both strategies in *C. parvum* populations suggests that they may have equal fitness payoffs over time (Poulin, 2001), the factors influencing the adoption of either one or the other strategy by individual parasites remain unclear, let alone the outcome of interactions between different factors.

Experiments under controlled conditions have shown that multiple factors can each influence the proportion of parasites adopting progenesis. First, rising temperatures, by either reducing the life expectancy of the amphipod host and/or increasing the growth rate of metacercariae, induced a higher level of progenesis in *C. parvum* kept under controlled conditions (Poulin, 2003). Second, chemical cues emanating from predators also influence the proportion of parasites adopting progenesis in laboratory experiments. *C. parvum* individuals infecting amphipods exposed to chemical cues from their fish definitive host (*G. cotidianus*) show lower rates of progenesis than metacercariae exposed either to odours produced by other predators that are not definitive hosts, or to no odour at all (Lagrue and Poulin, 2007). Therefore, rather than a fixed strategy, progenesis in *C. parvum* seems to be driven by local environmental cues. To a large extent, habitat characteristics are known to greatly influence parasite and host distributions (Krist *et al*. 2000; Marcogliese *et al*. 2001; Skirnisson *et al*. 2004). Although it is likely that all these factors affect the rate of selfing in natural populations of *C. parvum*, the more obvious cause would be a periodical unavailability of definitive hosts as transmission success is highly dependent on host density (Lefebvre and Poulin, 2005*c*; Hansen and Poulin, 2006). Shortages of suitable hosts can result from unpredictable environmental conditions or seasonal migrations (Holton, 1984*b*). For example, in the case of *C. parvum*, seasonal changes in the abundance of common bullies, i.e. definitive hosts, can be substantial owing to the periodicity of spawning and related ontogenic shifts in habitat use as juvenile fish develop (Rowe, 1994; Kattel and Closs, 2007). Progenesis in the second intermediate host may act as a reproductive insurance when definitive hosts are missing, and the proportion of parasites adopting progenesis in the population is likely to be related to the perceived opportunities of transmission. Still, temporal patterns of variation in progenesis rate remain to be documented and linked to the proximal factors likely to influence the adoption of progenesis in nature.

Here, we document the temporal variation of both the environmental factors known to influence the proportion of individuals adopting the shorter cycle in *C. parvum*, and the proportion of progenetic metacercariae in a natural population of parasites. We used our data to investigate several questions relating to the occurrence of progenesis in natural populations of *C. parvum*. First, is there any seasonal variation in the frequency of progenesis or is the proportion of progenetic individuals constant over time? Second, if temporal changes in the proportion of parasites adopting the abbreviated life cycle are observed, which external cues are correlated with the relative proportions of each life strategy? Third, what is the relative strength of the association between each factor and the frequency of progenesis?

Altogether, this study allows a first insight into the real plasticity of facultative life cycle abbreviation as a reproductive insurance in populations of parasites under natural conditions.

MATERIALS AND METHODS

A total of 12 monthly samples were collected between January and December 2007 in Lake Waihola, a shallow, coastal, eutrophic lake (mean depth of less than 2 m; Schallenberg and Burns, 2003), South Island, New Zealand. Samples were taken once a month from the same site at low tide and under similar weather conditions (windless and overcast) to reduce variations. Water temperature was recorded on each sampling occasion. Sampling was done along a 25 m × 2 m transect placed 3m from the shoreline. It was selected for the relative absence of macrophytes and the uniformity of the gravel substrate, making it a suitable habitat for snails, amphipods and fish, and a homogeneous sampling area. The 3 different hosts of *C. parvum* were all sampled on the same day each month.

Animal collection and dissections

Potamopyrgus antipodarum. Snail densities were determined at 2 different points along the transect using a surber net (mesh size 500 μm). This method allows the collection of organisms over a standardized surface (0·1 m^2). The mean density of the 2 samples was used to estimate overall snail density per square meter. All snails were returned to the laboratory, separated from any residual debris and fixed in 70% ethanol. They were then counted and dissected under a dissecting microscope; each snail was crushed using fine forceps and the presence of any parasite larvae was recorded.

Paracalliope fluviatilis. Amphipod densities were determined along with snail densities using a surber net and the overall density was calculated as the average of the 2 samples. Additional amphipods were captured by dragging a dip net (mesh size 500 μm) through the water column along the defined transect. All amphipods were separated by sample type, returned alive to the laboratory and kept in aerated lake water with strands of macrophytes (*Elodea canadensis*) as food source. Dissections and measurements were then completed within 48 h after sampling. Before examination, amphipods were killed in 70% ethanol and rinsed in distilled water to facilitate handling. This method kills the amphipod but not its internal parasites; therefore it has no effect on *C. parvum* measurements. All amphipods captured in surber nets were counted and dissected, and an extra 800 captured using the dip net were also examined. All amphipods were measured (total length). Their infection status was then assessed by dissection under the microscope.

Gobiomorphus cotidianus. Common bullies were sampled along the entire length of the transect using a seine net and push nets (mesh size 5 mm). The 25 meter transect was first enclosed by the seine net and the push nets were then used to capture the fish. Any remaining fish were captured when retrieving the seine net. All fish were counted and measured (total length) before a subsample of 30 (or all fish if less than 30 were captured) were haphazardly selected for dissection. The surplus fish were released along the study transect. Bullies kept for dissection were returned to the laboratory and maintained alive in aerated lake water and then dissected within 48 h. Fish were killed by decapitation before dissection. The digestive tract of each fish was opened and examined for parasites under a dissecting microscope.

Effects of water temperature on the mean density of snails, amphipods and fish were tested using General Regression Models (GRM) including both linear and non-linear functions. The effect of sampling date on the size of amphipods and fish was assessed using a one-way ANOVA. Amphipod and fish length were log-transformed to normalize the data.

Coitocaecum parvum. The prevalence of *C. parvum* in its 3 different hosts was determined every month. The intensity of infection (i.e. numbers of *C. parvum* individuals per infected host; Bush *et al.* 1997) was also determined in amphipods and fish. In amphipods, when *C. parvum* metacercariae were found, in addition to being counted and measured (length and width), they were recorded as 'normal' (non-egg producing worm) or 'progenetic' (egg-producing worm); eggs of progenetic worms were counted. These included both eggs released by the parasite in its thin-walled cyst and eggs still *in utero*. The body surface of metacercariae was then calculated and used as a surrogate for size. We used the formula for an ellipsoid, ($\pi LW/4$), where L and W are the length and width of the parasite.

Effects of host density, host mean size and water temperature on *C. parvum* prevalence and intensity in each host were tested using General Regression Models (GRM). Differences in the size of amphipods and fish with respect to infection status (*C. parvum* infected or uninfected) were assessed and tested using one-way ANOVAs.

Effects of fish number and water temperature on the proportion of progenetic metacercariae were tested using General Regression Models (GRM). Effects of sampling date on metacercarial size and egg production were tested using one-way ANOVA and the relation between host size, parasite size and egg production were assessed by General Regression Models (GRM).

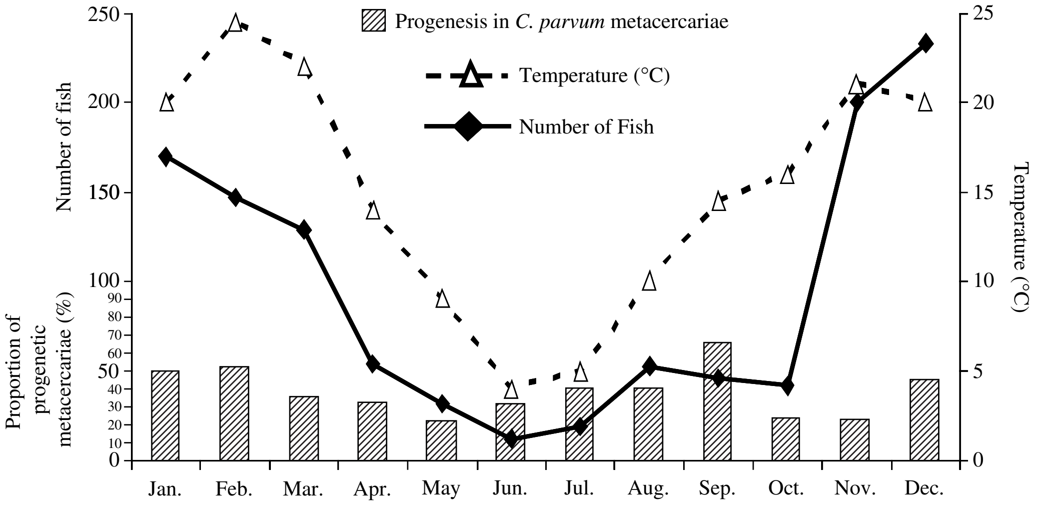

Fig. 1. Annual variation of water temperatures, number of fish (*Gobiomorphus cotidianus*) and proportion of progenetic metacercariae in *Coitocaecum parvum* population.

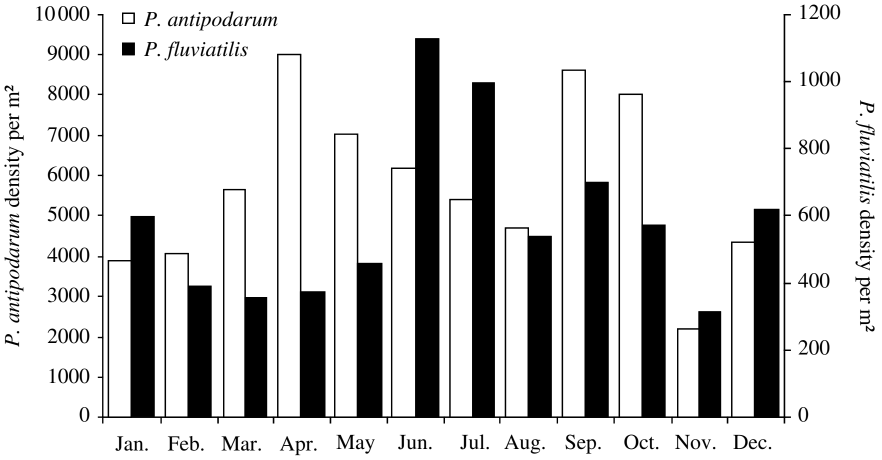

Fig. 2. Annual variation in snail (*Potamopyrgus antipodarum*) and amphipod (*Paracalliope fluviatilis*) densities.

RESULTS

As expected, water temperatures varied seasonally from 4 °C in early winter to 24·5 °C in late summer (Fig. 1). Although recorded temperatures were typical of this shallow coastal lake (Kattel and Closs, 2007), and remained within a suitable range for snails, amphipods and fish (McDowall, 1990; Quinn *et al.* 1994), the amplitude of variation was substantial (Fig. 1).

Potamopyrgus antipodarum

A total of 13 797 snails were dissected for this study. While highly variable, the snail density remained at a relatively high level all year (mean ± S.E.: 5749 ± 604 individuals per m^2). Densities ranged from around 2000 in late spring to almost 9000 individuals per m^2 in early autumn and early spring (see Fig. 2 for details). Although *P. antipodarum* densities were higher at intermediate temperatures (around 15 °C in April and September; Fig. 2) than at the extreme winter and summer temperatures, we found no significant relationship between water temperatures and snail densities ($r=0{\cdot}640$, $n=12$, $P=0{\cdot}093$; Fig. 3A); even summer temperatures remained well below the critical limit for *P. antipodarum* survival (Quinn *et al.* 1994).

Paracalliope fluviatilis

In total, 11 077 amphipods were measured and dissected for this study. Densities fluctuated from 315 to 1130 individuals per m^2 (Fig. 2; mean ± S.E. = 587 ± 73 individuals per m^2). Amphipod densities were significantly correlated with water temperatures ($r=0{\cdot}798$, $n=12$, $P=0{\cdot}0104$; Fig. 3B) with higher densities found during cold-water periods; water temperatures occasionally reached the critical values for amphipod survival during summertime (Quinn *et al.* 1994). Overall, we found no difference

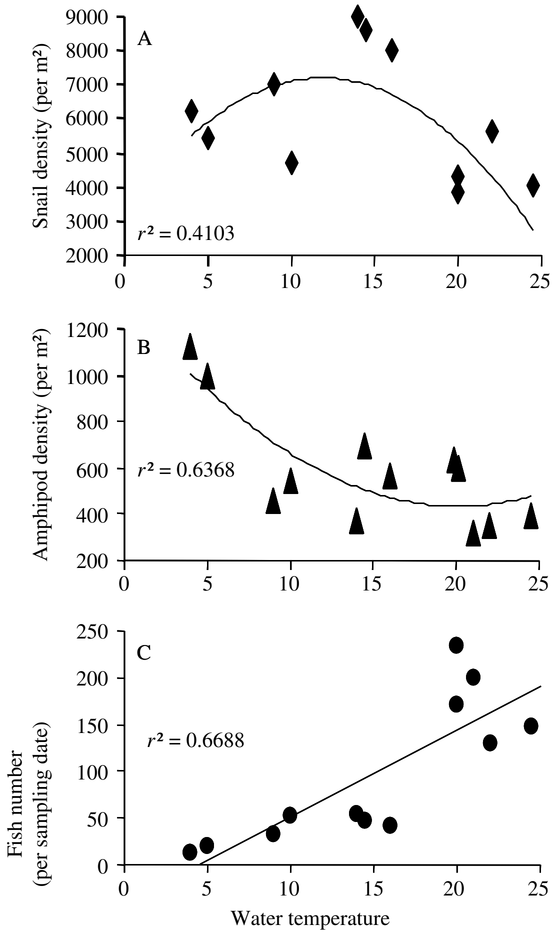

Fig. 3. Relationship between water temperatures and densities of *Coitocaecum parvum* hosts for (A) *Potamopyrgus antipodarum*, (B) *Paracalliope fluviatilis* and (C) *Gobiomorphus cotidianus* across 12 monthly samples. Lines of best fit and coefficients of determination are shown on each figure.

in amphipod size between sampling dates (ANOVA, $F_{11,11\,053}=1{\cdot}0$, $P=0{\cdot}418$).

Gobiomorphus cotidianus

In total, 1137 fish were captured and measured during the course of the study and 331 were dissected. *G. cotidianus* numbers varied dramatically throughout the sampling period from 12 fish captured in June up to over 230 in December (Fig. 1; mean number ± S.E. = 94·8 ± 22·2). Fish numbers were significantly correlated with water temperatures ($r=0{\cdot}818$, $n=12$, $P=0{\cdot}0012$; Fig. 3C), *G. cotidianus* preferring temperatures between 18·7 and 21·8 °C (Richardson *et al.* 1994). The mean size (± S.E.) of captured fish was 31·1 ± 0·2 mm and varied on average from 25·6 ± 0·4 mm in February to 35·4 ± 0·4 mm in December. Sampling date had a significant effect on fish length (ANOVA, $F_{11,1140}=34{\cdot}7$, $P<0{\cdot}0001$). Juvenile fish, < 30 mm in length, were particularly abundant from January to April, being the fry of the larger adult fish sampled from September to December that spawned during spring and early summer (Stephens, 1982).

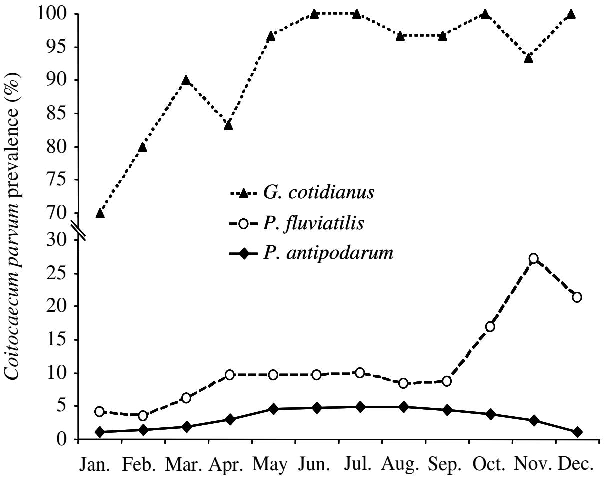

Fig. 4. Annual variation in the prevalence of *Coitocaecum parvum* in its three hosts: snail (*Potamopyrgus antipodarum*), amphipod (*Paracalliope fluviatilis*) and fish (*Gobiomorphus cotidianus*).

Coitocaecum parvum

C. parvum was the most abundant parasite infecting the snail *P. antipodarum* (mean prevalence = 3·4%). Two other trematode species were found: some snails contained either metacercarial cysts of *Microphallus* sp. ($n=140$; 1·0%), a common bird parasite (Levri *et al.* 2005), or sporocysts of another unidentified trematode ($n=88$; 0·6%). *C. parvum* prevalence in the snail population rose gradually from 1% in summer to 5% in winter (Fig. 4). Prevalence was not linked to snail density ($r=0{\cdot}450$, $n=12$, $P=0{\cdot}142$) but was negatively correlated with water temperature ($r=0{\cdot}863$, $n=12$, $P=0{\cdot}0003$).

The overall prevalence of *C. parvum* in its second intermediate host was 11·2%, a 3·3-fold increase from its prevalence in the snail first intermediate host. Two other helminth species were found infecting *P. fluviatilis*: the acanthocephalan *Acanthocephalus galaxii* (prevalence = 1·7%) and the trematode *Microphallus* sp. (prevalence = 9·6%). Some amphipods were infected by different species simultaneously: 154 individuals (1·4%) harboured *C. parvum* and *Microphallus* sp. in co-infection, 31 (0·3%) had both *A. galaxii* and *C. parvum*, and 10 individuals (0·1%) were infected by a combination of the 3 species. *C. parvum* prevalence in *P. fluviatilis* fluctuated from 3·5% to 27·1% over the year (Fig. 4). Prevalence was not influenced by either amphipod density ($r=0{\cdot}124$, $n=12$, $P=0{\cdot}700$), water temperature ($r=0{\cdot}121$, $n=12$, $P=0{\cdot}709$) or prevalence in the snail first intermediate host ($r=0{\cdot}029$, $n=12$,

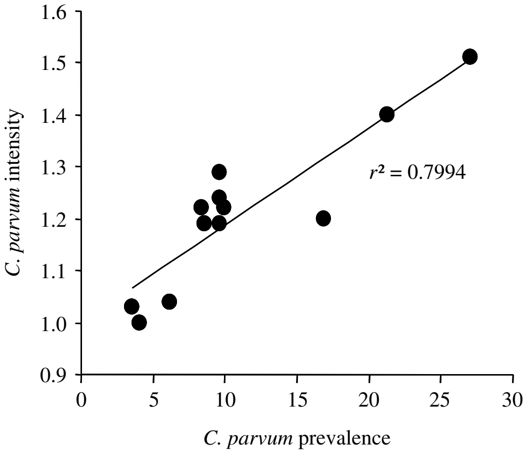

Fig. 5. Relationship between *Coitocaecum parvum* prevalence and mean intensity (i.e. number of *C. parvum* individuals per infected host) in the amphipod host *Paracalliope fluviatilis* across 12 monthly samples. Line of best fit and coefficient of determination are shown on the figure.

$P = 0{\cdot}928$). In general, *C. parvum*-infected amphipods were significantly larger than uninfected ones (mean length ± s.e. $= 3{\cdot}15 \pm 0{\cdot}01$ and $2{\cdot}93 \pm 0{\cdot}01$ mm respectively; ANOVA, $F_{1,11\,075} = 218{\cdot}1$, $P < 0{\cdot}0001$) but there was no significant correlation between the mean amphipod size in the population and *C. parvum* prevalence in individual samples ($r = 0{\cdot}135$, $n = 12$, $P = 0{\cdot}675$). The number of metacercariae per infected amphipod ranged from 1 to 6 (mean ± s.e. $= 1{\cdot}3 \pm 0{\cdot}0$) and was positively correlated with the prevalence of *C. parvum* in the amphipod population ($r = 0{\cdot}894$, $n = 12$, $P < 0{\cdot}0001$; Fig. 5).

C. parvum prevalence in its fish definitive host (*G. cotidianus*) fluctuated between 70 and 100% (Fig. 4), and was generally high (mean prevalence = 89·7%). This represents an 8-fold increase from the prevalence in amphipods and 26·4-fold compared to the snail first intermediate host. The acanthocephalan *A. galaxii* was also found in a small number of fish ($n = 13$, 3·9% prevalence). Overall, although parasites were recovered from fish of all sizes, *C. parvum*-infected fish were significantly larger than uninfected ones (mean length ± s.e. $= 33{\cdot}6 \pm 0{\cdot}4$ and $30{\cdot}2 \pm 0{\cdot}3$mm respectively; ANOVA, $F_{1,329} = 9{\cdot}01$, $P = 0{\cdot}0029$) and *C. parvum* prevalence was positively linked to the average fish size in each sample ($r = 0{\cdot}721$, $n = 12$, $P = 0{\cdot}0367$; Fig. 6A). *C. parvum* prevalence in amphipods was not related to the prevalence of infection in fish ($r = 0{\cdot}485$, $n = 12$, $P = 0{\cdot}110$). Intensity of infection (i.e. number of *C. parvum* individuals per infected fish) was highly variable and ranged from 1 to 69 worms (mean intensity ± s.e. $= 6{\cdot}9 \pm 0{\cdot}5$). Fish size had a significant effect on the intensity of infection with larger fish harbouring on average more parasites both across individuals ($r = 0{\cdot}416$, $n = 297$, $P < 0{\cdot}0001$) and across samples ($r = 0{\cdot}949$, $n = 12$, $P < 0{\cdot}0001$; Fig. 6B). Both intensity and prevalence of *C. parvum* in amphipods also had a weak but significant effect on the infection intensity in fish ($r = 0{\cdot}594$ and $0{\cdot}601$, $n = 12$, $P = 0{\cdot}041$ and $0{\cdot}039$ respectively).

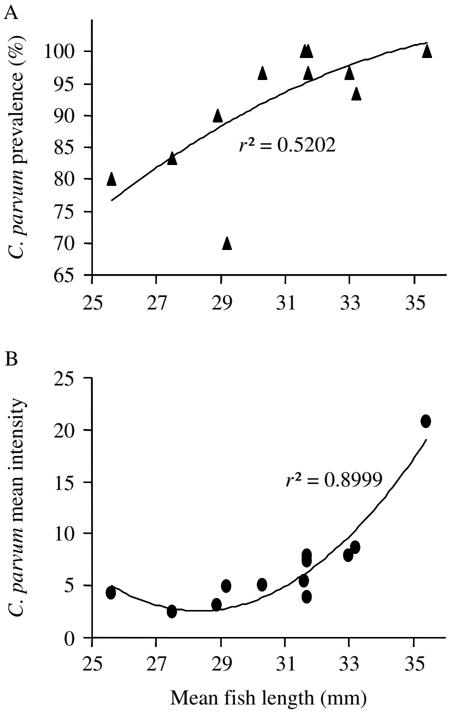

Fig. 6. Relationship between mean fish length and (A) *Coitocaecum parvum* prevalence, and (B) mean intensity (i.e. number of *C. parvum* individuals per infected fish) across 12 monthly samples. Lines of best fit and coefficients of determination are shown on each figure.

Progenesis in Coitocaecum parvum

Competition between parasites sharing the same host is likely to influence life-history strategies and growth of individual parasites (Dezfuli *et al.* 2001; Thomas *et al.* 2002; Parker *et al.* 2003; Lagrue and Poulin, 2007). Here, we wanted to assess the effects of environmental factors on *C. parvum* life-history strategy. Therefore, because of the possibly confounding effects of within-host competition, *C. parvum* metacercariae found co-infecting their amphipod host with other parasite larvae, conspecific and/or heterospecific, were excluded from the following analyses.

Overall, 799 *C. parvum* metacercariae were found in a single infection, among which 284 (35·5%) had produced eggs (progenetic metacercariae). Most importantly, the proportion of progenetic metacercariae in *P. fluviatilis* varied between sample dates from 22·8% to 65·5%. Still, no clear seasonal pattern was noticeable (Fig. 1). Furthermore, despite showing clear seasonal variation, neither water temperatures nor definitive host numbers seemed to have a significant effect on the proportion of progenetic metacercariae ($r=0{\cdot}239$ and $0{\cdot}164$, $n=12$, $P=0{\cdot}455$ and $0{\cdot}611$ respectively).

Progenetic individuals were, on average, larger than normal ones (mean body size ± S.E. = $0{\cdot}158 \pm 0{\cdot}002$ and $0{\cdot}053 \pm 0{\cdot}001$ mm^2 respectively; ANOVA, $F_{1,797}=2140{\cdot}2$, $P<0{\cdot}0001$) but there was no effect of sampling date on the size of normal and progenetic metacercariae (ANOVA, $F_{11,775}=1{\cdot}349$, $P=0{\cdot}192$). There was also a slightly significant effect of amphipod host size on the body area of progenetic metacercariae ($r=0{\cdot}188$, $n=285$, $P=0{\cdot}0015$). Progenetic worms had produced on average (± S.E.) $109{\cdot}8 \pm 6{\cdot}4$ eggs (range 1 to 471). Sampling date did not affect egg production (ANOVA, $F_{11,272}=0{\cdot}277$, $P=0{\cdot}227$) but the number of eggs produced by each worm was significantly correlated with body size ($r=0{\cdot}800$, $n=285$, $P<0{\cdot}0001$), a trend also present in laboratory reared *C. parvum* metacercariae (Lagrue and Poulin, 2007).

DISCUSSION

Progenesis and life-cycle abbreviation could offer *Coitocaecum parvum* a flexible life strategy enabling it to accurately respond to environmental changes. This strategy is often seen as a reproductive insurance when definitive hosts are scarce or absent (Lefebvre and Poulin, 2005*c*). In fact, previous studies have shown that laboratory-reared *C. parvum* metacercariae grow faster and adopt preferentially the shorter life cycle when chemical cues from the definitive host are absent (Poulin, 2003; Lagrue and Poulin, 2007). Thus, definitive host abundance and proportion of progenetic metacercariae should be negatively correlated in natural populations of the parasite. Here, the abundance of *G. cotidianus*, *C. parvum*'s definitive host, showed seasonal fluctuations of great amplitude. This variability should be large enough to trigger a seasonal life-strategy switch in the parasite, from the classical 3-host cycle to the abbreviated cycle. However, we found no relation between the abundance of fish definitive hosts and the proportion of progenetic metacercariae.

Another factor known to influence *C. parvum* life-history strategy is water temperature (Poulin, 2003). By increasing the mortality rate of the amphipod host, rising temperatures reduce the window of parasite transmission to the definitive host. Consequently, *C. parvum* metacercariae in laboratory amphipod hosts accelerate their development and are more likely to reach early maturity, while still inside the amphipod, in response to rising temperatures. Water temperatures recorded during our study fluctuated seasonally and amphipod densities varied accordingly, meaning that rising temperature also increased amphipod mortality rates in natural populations. Still, there was no clear effect of temperature on the proportion of progenetic metacercariae. Overall, although we detected large variations in water temperature and host densities, these did not influence the proportion of progenesis in the parasite population in its natural environment. Thus, there is no evidence from our results for adaptively flexible life-history strategies in *C. parvum*.

Interestingly, we also found that fish abundance was strongly and negatively correlated with water temperature. Since these two factors have opposite effects on the frequency of progenesis in laboratory experiments (Poulin, 2003; Lagrue and Poulin, 2007), it is possible that their effects counteract each other in the natural environment and that the proportion of progenetic metacercariae is affected by a combination of both factors. This would explain the total lack of seasonal pattern in the proportion of progenetic metacercariae in the amphipod host. However, it is impossible to determine which factor is more influential from our results and it might not account for all the variation observed in the frequency of progenesis.

Other factors could play subtle but significant roles in explaining the variation in proportions of progenetic individuals. For example, fish size, in addition to fish density, may indirectly affect *C. parvum* life-history strategy. Larger fish consume more prey than smaller fish, and are also likely to focus their predation efforts on larger prey. While the amphipod *P. fluviatilis* is consumed by *G. cotidianus* of all sizes in Lake Waihola and represents 80% of the total prey volume of the fish, the more abundant juvenile fish (<30 mm total length) also tend to selectively eat small amphipods (<2 mm in size; Wilhelm *et al*. 2007). Here, we found that infected amphipods were significantly larger than their uninfected conspecifics. Consequently, they are also on average much larger ($3{\cdot}15 \pm 0{\cdot}01$ mm) than those preyed upon by small bullies. Because juvenile *G. cotidianus* dominate the fish population almost all year round, no matter how abundant fish are, parasite transmission might be as unlikely as if fish were absent.

For the parasite, there is a major problem associated with predation by adult bullies. Common bullies usually die after their second spawning season (Stephens, 1982). Thus, large bullies that consume more infected amphipods, and therefore concentrate a large part of the parasite population, are also old fish with a reduced life expectancy. This phenomenon is reflected in our study by the sharp drop in *C. parvum* prevalence and fish size between December and January. The parasite is therefore faced with the double risk of either not finding a fish definitive host, whether because they are absent or too small, or reaching an old host with a short life expectancy. Quantitatively, reproductive output may still be lower in metacercariae producing eggs by progenesis inside the small-bodied amphipod host than in individuals reproducing in the larger vertebrate host. However, the risk of not reaching the definitive host, or reaching an old, short-lived host, could tip the balance back in favour of the progenetic strategy, used as a reproductive insurance.

Water temperature is also likely to have other effects on *C. parvum*. For example, high temperatures are known to increase the production and release of cercariae by trematode-infected snails (Hay *et al.* 2005; Fredensborg *et al.* 2005). When temperatures rose, the increase in cercarial release coupled with the decrease in amphipod densities translated into higher *C. parvum* prevalence in amphipod hosts, and thus in an increase in *C. parvum* intensity in fish definitive hosts. The increase in prevalence in amphipods was also linked to a higher number of parasites per host, and thus, an increase in within-host competition between *C. parvum* metacercariae. Within-host competition, being likely to affect metacercarial size (Thomas *et al.* 2002; Lagrue and Poulin, 2007, 2008), may consequently affect *C. parvum* life-history strategy.

Previous laboratory studies showed that definitive host densities and water temperature independently influence *C. parvum* life-history strategy. However, as documented herein, these two environmental factors are also linked and their variations clearly correlated. Complex interactions between simple environmental parameters can offset any adaptive strategic choice by parasites. It is often suggested that the maintenance of the two life-history strategies in *C. parvum* populations could simply be due to overall equal fitness payoffs, depending on temporally variable developmental conditions and probability of transmission (Poulin, 2001). However, the parasite environment contains so many layers of heterogeneity that, despite being able to perceive some environmental variation (Poulin, 2003; Lagrue and Poulin, 2007), it appears that *C. parvum* cannot efficiently adjust its life-history strategy to such a complex environment. Parasites have no other options but to make the best of whatever host and environment they find themselves in (Thomas *et al.* 2002). Although the trematode *C. parvum* potentially possesses, through its alternative developmental strategies, the ability to make the best of very bad situations, like the total absence of definitive hosts, this parasite does not seem to be able to accurately adapt to changing environmental conditions. Our results show that neither of the environmental parameters tested in this study are guiding the adoption of a particular life-history strategy in *C. parvum*. Although previous laboratory studies showed that these factors can independently affect *C. parvum* life history, the combination of multiple parameters encountered by the parasite in its natural environment could be too complex for individual *C. parvum* to accurately adjust their developmental strategy.

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