**Effects of temperature on trematode-mediated impacts on a model amphipod host
species**

by

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ABSTRACT

I conducted laboratory experiments to assess the influence of temperature in the shedding of *Gynaecotyla adunca* trematode cercariae from its first intermediate host, the snail *Ilyanassa obsoleta*, and transmission of these cercariae to its second intermediate host, the amphipod *Corophium volutator*.

An increase in temperature resulted in a significant increase in cercariae emergence from snails over 24hr but not over 32 days and reduced the viability of the cercariae. Increase temperature did not impact the snails but was detrimental to the amphipods regardless of infection status.

Across 4 mudflats, I investigated the relationship between levels of trematode infection in snails, snail density, and surface temperatures in May and July 2010. Snail density and infection levels increased in the 4 mudflats with higher temperatures in July. The increases in infection levels was not linked to snail density but to temperature increments, indicating a possible climate change scenario.

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GENERAL INTRODUCTION

Climate change has been linked to increased disease in humans and wildlife. For example, the recent global decline on amphibians due to epidemics of chytrid fungus has been blamed on warming brought about by climate change (Pounds et al., 2006), and bleaching and death of corals around the world have also been attributed to severe warming periods (Harvell, et al., 1999). More recently, speculation on the possible changes and disruptions on host-parasite interactions due to climate change have been addressed (Poulin and Mouritsen, 2006; Brooks, 2007). However, empirical data on the effects of warmer temperatures on the transmission and viability of parasites (specially macroparasites like trematodes) and their infected hosts are recent and scarce (e.g. Studer et al., 2010). A main objective for this work was to study the short and long-term effects of temperature on the interactions of a trematode parasite with its hosts. Specifically, I studied the viability and transmission success of a trematode as well as viability of its infected and uninfected hosts at different temperature scenarios for set periods of time. For this study, I used the trematode *Gynaecotyla adunca* ((Linton, 1905), its first intermediate host the snail *Ilyanassa obsoleta* (Say, 1922), and its second intermediate host, the amphipod *Corophium volutator* (Pallas, 1766).

These trematodes have complex life cycles requiring several hosts for their completion. The general life cycle of these parasites is as follows (from Roberts and Janovy, 2004): the adults live in the guts of their final hosts (most often a vertebrate). They reproduce sexually producing hundreds or thousands of eggs, which are voided with the feces of the final hosts. A short-lived free-swimming infective form called *miracidium* hatch from the eggs and infects an adequate snail species (their first

intermediate host). In some species, the eggs are consumed by snails with the miracidia hatching and infecting them through their digestive system (trophic transmission). The infected snails are castrated, and the parasites will reproduce asexually to produce another short-lived free-living larval form called *cercaria* (plural: cercariae). The infected snails are effectively converted into “parasite factories”, shedding (depending on the species of parasite) hundreds or thousands of cercariae per day. The cercariae then search and penetrate their second intermediate host and form cysts called *metacercariae* inside them. The life cycle completes when second intermediate hosts are eaten by the final hosts (another example of trophic transmission). It is believed that some larval forms of the parasites influence the behaviour of their intermediate hosts (i.e. infected snails concentrate near the parasites’ next host, infected second hosts expose more to predatory final hosts) to facilitate the completion of their life cycles (McCurdy et al., 1999, 2000a, b).

Recently, local populations of *C. volutator* amphipods have been declining in some areas (e.g. Grande Anse mudflat in the Bay of Fundy, New Brunswick or NB [David Drolet, personal observations]), and in Northern Europe trematode parasites have been implicated in these declines (Jensen and Mouritsen, 1992; Bick, 1994). Studies conducted in the Baltic Sea region have shown that increased parasitism by the trematode *Maritrema subdolum* Jägerskiöld 1909 has a detrimental effect on the amphipods (Mouritsen and Jensen, 1997; Meissner and Bick, 1999), and that an increase in water temperature of ~4°C (predicted to occur in the next 50 years due to climate change [IPCC, 2007]) can have devastating effects on the local *C. volutator* populations in that region due to an increased output of larval trematodes from their snail hosts (Mouritsen et

al., 2005). In the Bay of Fundy, *C. volutator* is parasitized by the trematode *G. adunca*. The trematode uses the local mud snail *I. obsoleta* as its first intermediate host and shorebirds as their final host (McCurdy, 1999). Nothing was known about the emerging patterns of *G. adunca* from *I. obsoleta* snails in relation to temperature and their effects on the local populations of *C. volutator*. Thus the second main objective of the present work concerns whether an increase in temperature would also increase parasite-mediated impact (i.e. mortality) on *C. volutator*, a key species in the Upper Bay of Fundy (McCurdy, 1999). Whether increases in temperature would also increase parasite-mediated impacts on *C. volutator* or not also depends on the presence of first intermediate hosts (i.e. snails), I was therefore also interested in the possible effect of temperature on snail density and prevalence of infection levels by *G. adunca*.

Specific objectives

In chapter 1, I carried out a series of laboratory experiments to test the short and long term effects of temperature on the emergence of the parasite *G. adunca* from *I. obsoleta* snails, the long term viability of infected snails, the survivorship and swimming activity of the *G. adunca* cercariae, the infectivity of *G. adunca* towards the *C. volutator* amphipod (number of cercariae that penetrated the amphipod), and the survivorship of infected amphipods. Thus, the effects of temperature on all aspects of the transmission pathway of *G. adunca* from snails to amphipod, as well as the viability of the infected hosts, were investigated.

In chapter 2, I investigated the relationship between prevalence levels of infection by trematodes (including *G. adunca*) in *I. obsoleta* snails in the field and seasonal increases in temperature and snail density by performing 2 field samplings at 4 mudflats

in the Upper Bay of Fundy. The 2 field samplings were performed in May (mid spring) and in July (mid summer) to examine the effects that differences in temperature has –if any- on trematode infections of snails and snail densities. In this way I was using season as a surrogate for climate change. I also evaluated the possible effects of *G. adunca* on *C. volutator* populations in the field in relation to the prevalence levels of this parasite on the 4 mudflats.

CHAPTER 1

Effects of temperature on a model host-parasite system

INTRODUCTION

The fact that climate change is occurring is indisputable (IPCC, 2007), and it has brought serious effects on health of global ecosystems by increasing the incidence and severity of disease outbreaks (Daszak et al., 2000; Harvell et al. 2002; Pounds et al., 2006). However, the net effects of climate change on the interactions between trematode parasites and their hosts are understudied. It is believed that climate change will increase the impact of these parasites on their hosts because most studies focused on the cercarial stages have found a high output of these larval infective forms in the environment with increasing temperatures (Poulin, 2006); however, most of these studies were focused on short term effects, while long term effects of temperature not only on cercarial emergence but also on their hosts (infected and uninfected) have to be considered (Studer et al., 2010). For this study, we used the trematode *Gynaecotyla adunca* (Linton), its first intermediate host the snail *Ilyanassa obsoleta* (Say), and its second intermediate host, the amphipod *Corophium volutator* (Pallas) as our host-parasite system to elucidate the short and long term effects of temperature on the transmission success and viability of the parasite and hosts.

The amphipod, *C. volutator* is a small intertidal crustacean found from the Baltic Sea in Northern Europe to the North American coast from the Bay of Fundy to Maine (Wilson and Parker, 1996). It is also found in some regions of Japan (Omori and Tanaka,

1984). These crustaceans can reach a length of ~10 mm as adults although they will reach sexual maturity at ~5 mm (Matthews et al., 1992). In the Bay of Fundy they can reach densities as high as 50,000 individuals/m² (Peer et al., 1986) and form over 90% of the diet of local fish and migratory shorebirds (Gill and Daborn, 1981; Hicklin and Smith, 1984; McCurdy et al., 2005). Moreover, because of their high numbers, the amphipods are thought to stabilize the intertidal sediments in which they reside (Mouritsen et al., 1998). For all these reasons, they are considered a key species in the Bay of Fundy ecosystem (McCurdy, 2000).

Corophium volutator is parasitized by several species of trematodes in European and North American coasts (Mouritsen and Jensen, 1997; McCurdy et al., 2000a, b). In the Bay of Fundy, *C. volutator* is parasitized by *G. adunca*. The flatworm uses the local mud snail *I. obsoleta* as its first intermediate host and shorebirds as its final host (Rankin, 1940). Studies conducted in the Baltic Sea region suggest that increased parasitism by a trematode (i.e. *Maritrema subdolum* Jaegerskioeld) has a detrimental effect on the amphipods (Mouritsen and Jensen, 1997; Meissner and Bick, 1999), and that an increase of 4°C in water temperature could have devastating effects on the local amphipod populations in that region due to an increased output of larval trematodes from their snail hosts (Mouritsen et al., 2005). Nothing is known about exit patterns of *G. adunca* from *I. obsoleta* in relation to temperature and their effects on the local populations of *C. volutator*; hence the reason for this study.

The objective of the present work was to study the effects of temperature on: 1) the emergence of the parasite *G. adunca* from *I. obsoleta* snails in short (24 hr) and long-terms (32 days); 2) the long term viability of infected snails; 3) the survivorship and

swimming activity of the *G. adunca* cercariae; 4) the infectivity of *G. adunca* towards the *C. volutator* amphipod (number of cercariae that penetrated the amphipod) 3, 12, and 24 hr after emerging from snails, and 5) the survivorship of infected amphipods. A graphical representation of the experimental objectives is presented in Figure 1.1. If we found increased shedding, survivorship, activity, and penetration success of cercariae under higher temperatures, this would be evidence for temperature-associated increases in transmission and infectivity of the parasite. Higher rates of mortality of infected amphipods at higher temperatures would certainly be further evidence for temperature-associated increases in impacts of parasites on host populations, but even if mortality were the same for infected amphipods under low and high temperatures (but both higher than uninfected controls), more hosts infected by more parasites at higher temperatures would mean more parasite-induced mortality. The question is whether temperature effects on transmission, infectivity, and impact are *additive* or not.

MATERIALS AND METHODS

Collecting and keeping of experimental specimens

Ilyanassa obsoleta snails used for the experiments were collected haphazardly in Peck's Cove mudflat, near Upper Rockport, New Brunswick (45° 45' 3.98" N, 64° 29' 19.3" W) in Sept 29, 2009 and May 19, 2010, and in Starr's Point mudflat, near the community of Starr's Point, Nova Scotia (45° 7' 36.69" N, 64° 21' 58.37" W) in May 23, 2010. Both sites are located in the Upper Bay of Fundy. To determine if snails were infected by trematodes, they were individually placed overnight in plastic cups with seawater, after which the water was examined for cercariae using a dissecting scope.

Those snails parasitized by *G. adunca* cercariae were used for all the experiments; thus, all cercariae mentioned hereafter are referring to this trematode species.

Corophium volutator amphipods used for the experiments were collected at Peck's Cove mudflat during low tide (~100 m from shoreline) on May 19 and July 16, 2010 by gently sieving (0.85 mm mesh) the mud containing the burrowed amphipod. Only adults (>5 mm in body length) were used for experiments.

Snails and amphipods were housed in 5-gallon aquaria with filtered seawater at 17°C for 1.5 weeks at 16:8 hr light:dark regimen. Then they were transported for ~30 hr in coolers to Carleton University in Ottawa (~1300 km away from field sites) and housed in an environmental chamber (Conviron ® model C710, Winnipeg, Canada) with temperature and light regimen as above. The snails and amphipods were kept in 5-gallon aquaria with artificial salt water made with 40g of Instant Ocean © (Madison, WI) aquarium salt mix per liter of distilled water (according to salinity measurements by SAL-BTA salinity sensor connected to LabPro ® interface [Vernier Software and Technology, Beaverton, OR], 40 g /L = ~ 30 ppt, which is close to the salinity levels in the Upper bay of Fundy [Tee and Amos, 1991]). Snails were housed at 10-15 individuals per aquarium with water levels just above their shells (i.e. 15 to 20 mm). Snails were fed daily with ground, San Francisco Bay Brand ® (Newark, CA) freeze-dried brine shrimp, and their water was changed every 2 days. The amphipods were kept in aquaria with a thin layer of sediment and 4L of artificial salt water with dissolved 250 µL/L of Wardley's Small Fry Fish Food ® (Secaucus, NJ) as the feeding media and 100 µg/ml each of the antibiotics streptomycin sulfate and penicillin-G (Bioshop, Burlington, Canada). The antibiotics increase the survivorship of the amphipods (Pelletier and

Chapman, 1996). Half of the water volume was changed weekly with water containing the food and antibiotic. Snails and amphipods were kept for at least 2 weeks before they were used for experiments.

The temperatures used for the experiments were 12°C and 17°C, which are the average and average maximum water temperatures in the New Brunswick side of the Bay of Fundy during the summer months of July and August according to Fisheries and Oceans Canada (DFO, 2009). We also conducted experiments at 22°C, which is 5°C above 17°C and also the difference between 17°C and 12°C. Additionally, 5°C is one of the expected increases in global surface temperatures in the next 100 years (IPCC, 2007). Summer month temperatures were chosen because this is the time when most *I. obsoleta* snails are present and active in the intertidal mudflats (Cranford, 1986).

Experiment 1: Emergence of *G. adunca* parasites from *I. obsoleta* mud snails

The objective of this experiment was to study the effect of temperature on the emergence of *G. adunca* cercariae from *I. obsoleta* snails in short (24 hr) periods of time. Two replicates were conducted. One replicate used 37 snails from Starr's Point (collected on May 23, 2010); a second replicate used 14 snails from Peck's Cove (snails collected on Sept 29, 2009). Snails were first housed in cups with artificial salt water at 17°C for 24 hr, then divided randomly into two groups: one in cups with water at 12°C and the other at 22°C for another 24 hr. After each of the 24 hr, the water in their cups was collected individually to count the number of cercariae emitted by each snail. The samples of water were poured into a small Petri dish (28.5mm radius) that had the bottom divided into a grid of 9x9 mm squares. The cercariae were killed by adding several drops of Lugol's

solution (5% iodine and 10% potassium iodine dissolved in distilled water) to the water, and their numbers counted in one of the dish's randomly chosen squares under the dissecting scope. Before counting, the liquid was shaken carefully to spread the cercariae evenly in the dish. The final number of cercariae per snail was estimated as the total number of cercariae per grid times the total area of the Petri dish.

Experiment 2: Parasite survivorship and swimming activity

The objective of this experiment was to study the effect of temperature on the survivorship and swimming activity of the *G. adunca* cercariae. Fifty-eight and 56 newly-emerged cercariae (≤ 3 hr old) from 12 snails combined were put in small Petri dishes at 17°C and 22°C respectively, and inspected every 3 hours until the death of all the parasites. On each inspection the number of dead parasites was counted and the number swimming was assessed over 30 sec. A second replicate (with 61 parasites for 17°C and 65 for 22°C) was also conducted.

Experiment 3: infectivity of parasites at 17°C and 22°C

Experiment 3A: The objective of this experiment was to study the effect of temperature on the infectivity of ~ 3 hr-old *G. adunca* cercariae towards the *C. volutator* amphipod (number of cercariae penetrating the amphipod). Twenty-two and 21 amphipods were put individually in culture dishes (12 well flat bottom Multiwell™ tissue culture plate by Falcon ® [Lincoln Park, NJ], each well at 22mm in diameter and holding 5mL of liquid) and placed at 17°C and 22°C respectively. We controlled the number of cercariae used by pipetting 50 (± 5) ~ 3 hr-old cercariae (obtained from 20 infected snails exposed to 25°C

for 3 hr) into each dish. After 3 hr, the amphipods were anesthetized in carbonated water and mounted in Hoyer's medium (30g Gum Arabic, 20ml Glycerol, 200g Chloral Hydrate, all dissolved in 50ml of warmed distilled water), which clears the amphipods and renders the soft-tissue cercariae inside them visible without dissections (Figure 1.2). After 24-48 hr of being mounted, the amphipods were examined and the trematodes inside them counted using a compound microscope. Those that were already parasitized before the experiment (possessing an encysted metacercaria, which needs weeks to develop [McCurdy et al., 1999]) were removed from the analysis. A second replicate was done following the same procedure but with 22 amphipods at 17°C and 23 amphipods at 22°C. A few representative *C. volutator* containing either a metacercarial cyst or a newly penetrated cercaria were photographed with a digital camera (Moticam 1000 with Motic Images 2.0 software suite, Motic ®, Xiamen, China) attached to a compound microscope's eyepiece.

Experiment 3B: to determine whether age of cercariae might be a more important factor in the infection process, another set of experiments was done following the same procedures in experiment 3A but with *C. volutator* exposed to 12 and 24 hr-old cercariae respectively. The number of amphipods used was 48 per experiment (24 for each temperature treatment).

Experiment 4: Survivorship of infected amphipods at 3 temperatures

The objective of this experiment was to study the effect of temperature on the survivorship of infected amphipods. Ninety *C. volutator* were individually exposed to 50 (± 5) parasites in culture dishes (12 well plates) for 24 hr at 22°C, then placed

individually in test tubes with 5 cm of autoclaved mud (from the site where the amphipods were collected) and 20-30mL of artificial salt water containing food and antibiotics (as above). The amphipods were divided into 3 groups of 30 each and put at 3 different temperatures: 12°C, 17°C, and 22°C. The water was changed weekly with freshly prepared artificial salt water containing the food and antibiotics. Three control groups of 30 amphipods each were set up as above but exposed to water used to house non-parasitized snails for 24 hr instead of cercariae, then placed at the same temperatures.

The amphipods were checked once every day until their death, after which they were mounted in Hoyer's medium and checked for parasites as in experiment 3. Only a portion of the original number (i.e. 30 per group) of amphipods were used for the final analysis due to the number of amphipods already infected by possessing a large metacercarial cyst (those having several fibrous layers surrounding it [Figure 1.2 A]), those that were not infected after the exposure to the parasite, and those lost accidentally during the experiment (died suddenly after changing their water); thus, the final sample size for 12°C was 20 for infected amphipods and 15 for uninfected or controls, for 17°C was 18 for infected and 8 for controls, and for 22°C was 19 for infected and 15 for controls.

Experiment 5: snail survival and long-term cercariae shedding at different temperatures

The objective of this experiment was to study the effect of temperature on the emergence of *G. adunca* cercariae from *I. obsoleta* snails in a long (32 days) period of time as well as the long-term viability of infected snails. The snails used for this

experiment were those collected in Peck's Cove (NB) in May 19, 2010 and were not used in any of the above experiments. As such, they were kept in the laboratory at constant 17°C and 16:8 hr light:darkness regime for almost 8 months before the start of this experiment. To simulate (at least in part) their natural wintering and quiescent conditions, the temperature in the chamber was progressively turned down 2°C per day until 4°C and the daily light hours was diminished 30 min daily until a light:darkness regime of 8:16 hr. Snails were kept in these conditions for a month, then reverted progressively back to the original settings (i.e. 17°C and 16:8 hr light:darkness). A week later, snails were put in plastic cups of ~6 cm in diameter with just enough salt water to cover them, then placed either at 17°C or 22°C treatments for 34 days. Water was changed every day and they were fed with freeze-dried brine shrimp after each change; thus, the condition of the snails was checked daily. Twice a week, the water of the infected snails was collected and their cercariae numbers counted as above but not extrapolated to estimate the total cercariae shed per snail as in experiment 1; only the total amount in the 9x9 mm quadrat was used for analysis. After the 34 days, snails were placed individually in Petri dishes with their ventral surface up and their time to upright (or to “flip”) was recorded. The snails that flipped faster were taken as “healthier”. Thus, the final “flipping” trial was a factorial design with temperature (17°C and 22°C) and the condition (i.e. infected or uninfected) of the snails as the fixed factors.

A total of 67 infected (33 at 17°C and 34 at 22°C) and 59 uninfected or control (29 at 17°C and 30 at 22°C) snails were used for this experiment. Three infected snails (1 at 17°C and 2 at 22°C) died during the experiment and data generated by them were removed from the final analysis. Moreover, data from 4 snails (1 infected and 2 controls

at 17°C and 1 infected at 22°C) were removed from the flipping trials because of their flipping time above the 10 min mark (i.e. identified as outliers).

Statistical analysis

All data were analyzed using SPSS v.19 for PC. Data were examined for normality; if deviating from normal distribution, they were transformed (Grafen and Hails, 2002) and tested for normality again, or non-parametric tests were used if data still deviated from normality after transformations.

RESULTS

Experiment 1: Emergence of *G. adunca* parasites from *I. obsoleta* mud snails

I used a Paired t-test to compare the mean number of cercariae from snails exposed to 17°C and 12°C and those exposed to 17°C and 22°C. The data were transformed by $\ln(n+1)$ to improve normality. An ANOVA test was not used to analyze the results because of the experimental set up (I set up the experiment with paired t-tests in mind), which was done to compensate for the low number of infected snails used for the experiment specially in replicate 2

For replicate 1, significantly more cercariae emerged at higher temperatures (17°C vs. 12°C: $t = 3.18$, $df = 19$, $P = 0.005$, $N = 20$; and, 17°C vs. 22°C: $t = -5.79$, $df = 16$, $P < 0.001$, $N = 17$). The back-transformed mean (and its lower and upper 95% confidence intervals) number of cercariae was 155.02 (97.49 - 246.15) for 12°C and 1,117.79 (712.37 - 1,753.61) for 17°C; and 360.41 (224.88 - 577.25) for 17°C and 6,633.24 (5,376.61 - 8,183.52) for 22°C (Figure 1.3A).

Replicate 2 also had significantly more parasites emerging at higher temperatures (17°C vs. 12°C: $t = 3.37$, $df = 6$, $P = 0.015$, $N = 7$; and, 17°C vs. 22°C: $t = -3.1$, $df = 6$, $P = 0.021$, $N = 7$). The back-transformed mean (and its lower and upper 95% confidence intervals) number of cercariae was 2.25 (1.25 - 3.71) for 12°C and 51.98 (19.49 – 136) for 17°C; and 70.52 (25.31 – 193.42) for 17°C and 1,175.15 (371.41 – 3,713.5) for 22°C (Figure 1.3B).

For both replicates, the number of cercariae emerging at 17°C was not different among the snails that were subsequently allocated to 12°C and 22°C 24 hr after (independent samples t-test on $\ln(n)$ transformed data, replicate one: $t = 0.32$, $df = 35$, $P = 0.75$, $N = 20$ vs. 17; replicate two: $t = -0.19$, $df = 12$, $P = 0.85$, $N = 7$ vs. 7; table 1.1).

Experiment 2: Parasite survivorship and swimming activity

In both replicates, cercariae started to die after 6-9 hr at 22°C and at 9-12 hr at 17°C (Figures 1.4A and 1.4B). Log Rank (Mantel-Cox) test was used to compare the survival distributions of the cercariae at 17°C and 22°C. Cercariae at 17°C survived significantly longer than those at 22°C in replicate 1 ($\chi^2 = 70.39$, $df = 1$, $P < 0.001$) as well as in replicate 2 ($\chi^2 = 57.4$, $df = 1$, $P < 0.001$) (Figures 1.4A and 1.4B). The average ($\pm$ SE) time at which 50% of cercariae died between the two replicates was 15.5 (± 0.9) hr at 22°C and 24.5 (± 3.9) hr at 17°C.

Gynaecotyla adunca cercariae swam in a circular motion and did not show a particular direction during swimming periods (e.g. toward light or darkness). They seem to be poor swimmers. The number of cercariae actively swimming (taken as a proxy for searching for the next host) decreased almost linearly at both temperatures (Figures 1.5A

and 1.5B). However, there were more parasites swimming at 17°C than at 22°C after 6 hr of emerging from the snails, and a few remained swimming beyond the 9 hr mark at 17°C while those at 22°C stopped swimming at this time. The time when all the cercariae stopped swimming coincided with the time they started to die (i.e. 9 to 12 hr).

Experiment 3: infectivity of parasites at 17°C and at 22°C

An independent-samples Mann-Whitney U test was carried out to compare the differences in the numbers of trematodes inside the amphipods at the 2 temperatures for the 3 infective experiments.

For experiment 3A (3hr-old cercariae), there was no significant difference in the number of parasites inside the amphipods at the two temperatures for both replicates; replicate 1: $Z=0.078$, $P= 0.938$, $N= 22$ (at 17°C) and 21 (at 22°C), replicate 2: $Z= -0.291$, $P= 0.771$, $N= 22$ (at 17°C) and 23 (at 22°C) (Figures 1.6A and 1.6B). The median (maximum and minimum range) number of cercariae inside the amphipods was 1 (0-3) for the 17°C treatment and 1 (0-9) for the 22°C treatment in replicate 1, and 1 (0-8) for the 17°C treatment and 2 (0-10) for the 22°C treatment in replicate 2 (Table 1.2, figure 1.6).

For experiments 3B (using 12 hr and 24 hr –old cercariae), only 2 to 3 out of 48 crustaceans per experiment were infected, all having one cercaria inside them (Table 1.2); this represents 4% to 6% infected crustaceans respectively compared to over 53% to 82% of crustaceans infected during experiment 3A (Table 1.2); thus, using older cercariae (≥ 12 hr) is not likely to yield interpretive results. There was no significant difference in

the number of cercariae inside the amphipods at 17°C and 22°C for these last 2 experiments (Table 1.2).

Most-recently penetrated cercariae were found in the hind pereopods of the amphipods, while encysted metacercariae were found in their body cavities (Figure 1.2); this combined with unpublished live observations, indicate that the parasite penetrate *C. volutator* through the connecting tissue of their appendages and migrate to their body cavity.

Experiment 4: Survivorship of infected amphipods at 3 temperatures

Logistic regression was used to compare the days to death for infected and control amphipods (groups 1 and 2 respectively). After the analysis, only the 12°C group had a significant difference in the number of days to death between infected and control (Wald [W]= 5.675, df=1, P=0.017, N= 20 for infected amphipods and 15 for uninfected or controls, figure 1.7A), while there was no significant difference for the 17°C (W= 1.223, df=1, P=0.269, N=18 for infected and 8 for controls, figure 1.7B) and 22°C groups (W= 1.363, df=1, P=0.243, N=19 for infected and 15 for controls, figure 1.7C). No definitive metacercarial cysts in the lab-infected amphipods were found at the end of the experiment. A comparison across the 3 temperature treatments (i.e. as in a factorial ANOVA analysis) was not carried out because each temperature treatment was conducted in a separate growth chambers (12°C and 17°C in Conviron ® growth chambers model 125L and 22°C at model C710); thus, a chamber effect might have taken place, which might affect the analysis.

Experiment 5: snail survival and long-term cercariae shedding at different temperatures

Two days into the long-term cercariae shedding trial, more cercariae emerged from snails at 22°C than those at 17°C; however, the numbers from both treatments dropped considerably to almost identical values on the 4th day of the trial, then afterwards and until the end of the trial at day 32, the snails at 22°C shed less than those at 17°C (Figure 1.8). Average total number of cercariae shed by individual snails for the 32 days was not significantly different for the 2 temperature treatments (independent samples t-test on square root-transformed data; $t=0.929$, $df=63$, $P=0.357$, $N=32$ for 17°C and 33 for 22°C). The back-transformed mean number of cercariae (and their lower and upper 95% confidence intervals) shed by snails for the 32 days are 59.44 (46.92 – 73.44) and 42.9 (31.92 – 55.5) for 17°C and 22°C respectively (Figure 1.9).

For the flipping trial, data were transformed by inverse of $(n+1)$ to satisfy the normal distribution. The inverse mean time (in min) for the snails to flip was compared at 17°C and 22°C for infected and uninfected snails. When controlling for snail condition (i.e. infection status of snails), temperature had a strong significant effect on the flipping time of the snails ($F_{1,3}=21.21$, $P<0.001$). Infection status of the snails had a slight significant effect on the flipping time of the snails when temperature was controlled for ($F_{1,3}=4.11$, $P=0.045$). However, there was no significant difference on the flipping time of the snails when temperature and condition of the snails were combined (temperature x snail condition: $F_{1,3}=0.179$, $P=0.673$).

The back-transformed means (and their lower and upper 95% confidence intervals) for the flipping times were 2.03 (1.78 - 2.33) and 1.08 (0.92 - 1.27) minutes for

infected snails at 17°C and 22°C respectively, and 1.5 (1.33 - 1.7) and 0.89 (0.79 - 1) minutes for control snails at 17°C and 22°C respectively (Figure 1.10). Overall, the snails that flip faster were the uninfected ones at 22°C. There was a significant difference between the average lengths of infected and uninfected snails (independent samples $t = 13.24$, $df = 121$, $P < 0.001$) with the mean ($\pm$ SE) length of infected and uninfected snails at 21.7 ± 0.2 and 18.6 ± 0.1 mm respectively.

DISCUSSION

The present results indicate that although *G. adunca* cercarial output from infected *I. obsoleta* snails increased from 7 to 124 times at increments of 5°C (i.e. 12°C to 17°C, and 17°C to 22°C, figure 1.3), the prospects for each cercaria of locating its amphipod secondary host might have been negatively affected at warmer temperatures by its shorter life span and swimming period (Figures 1.4 and 1.5). Moreover, the ability of the parasites to infect the amphipod was not improved at warmer temperatures (Figure 1.6). Thus, the only impact *G. adunca* might have on *C. volutator* amphipods in climate change scenarios (i.e. increase in temperatures) would be the increased number of cercariae emerging from the infected snails. From past studies (Mouritsen and Jensen, 1997; Meissner and Bick, 1999; Meissner and Schaarschmidt, 2000), *C. volutator* has been shown to be specially susceptible to the numerous cercarial attempts at infection by direct penetration and/or the migration of the parasites inside them, which might cause severe injury, lost of hemolymph, and stress leading to death of the amphipods. Thus, an increase in temperature might also increase parasite-mediated impact (i.e. mortality) on *C. volutator* in the Upper Bay of Fundy, on the sole account that increase temperatures

means higher numbers of cercariae to penetrate the amphipods during the parasites' first hours of lifespan, when they are most successful at infecting the amphipods (Table 1.2).

Moreover, my results also indicate that the higher-than-normal temperatures might have a detrimental effect on *G. adunca*. Many previous studies (e.g. see review by Poulin, 2005 and references therein) have found that most cercariae tend to emerge from their snail hosts in numbers much higher than the expected values from an increase in the physiological processes of the snails at higher temperatures (using the so-called Q_{10} value of physiological measurements), and the present results were not different. Temperature has a direct influence on the increased output of *G. adunca* cercariae from *I. obsoleta* snails. However, the cercariae that were exposed to the higher temperatures survived shorter periods of time, which might indicate a faster depletion of their energy reserves at higher temperatures (Young et al., 1984; Pechenik and Fried, 1995; Mouritsen, 2002), thus limiting the window of opportunity to complete their life cycles. This might indicate that *G. adunca* cercariae are more active at higher temperatures in their search for the next host (e.g. swimming longer intervals and faster) and subsequently contacting their next potential hosts more often (e.g. Evans, 1985); however, we did not quantify any of these scenarios. Irrespectively, the number of *G. adunca* cercariae found penetrated inside *C. volutator* amphipods was not different between 17°C and 22°C for both replicates, meaning at least for this system, higher temperatures do not mean higher transmission success of the parasites. Yet, *G. adunca* cercariae might still be having more contacts and attempts at penetrating *C. volutator* at higher temperatures (either by being more active or being present in higher numbers) but just failing at the last penetration and

infection step, which might be counterproductive to the parasite since as mentioned above, *C. volutator* is highly susceptible to the injuries during infection.

In contrast to experiment 1 where infected snails exposed to different temperatures for short period of time (i.e. 24 hr) shed significantly different amounts of cercariae, results from experiment 5 indicate that 2 days after the initial peak in the number of cercariae emerging from infected snails, the numbers dropped sharply and remained low for both the 17°C and 22°C temperature treatments during the remaining 30 days of the trial (Figure 1.8). Most surprisingly, those infected snails at 22°C shed consistently lower numbers of cercariae than those at 17°C after 8 days into the trials (although the difference in mean number of cercariae emerged between the 2 temperature treatments for the 32 days was not significantly different [figure 1.9]). It appears that once the snails have settled into their respective temperatures, their cercariae emerged in consistently low quantities, which indicates that abrupt changes (e.g. sudden temperatures change or being moved into novel and smaller environments such as their holding cups) might play an important role in triggering the exit of cercariae. This differs from a similar experiment. Studer et al. (2010) found that *Zeacumantus subcarinatus* snails infected by the trematode *Maritrema novaezealandensis* kept at 25°C for 8 weeks shed increasingly higher numbers of cercariae per week than infected snails at other lower (i.e. 16 and 20°C) and higher (i.e. 35°C) temperatures. However, when the snails were exposed to ~5°C higher for 24hr, their cercaria numbers also increased sharply, which in this case is similar to my results.

Moreover, after the 34 days of the long term cercarial shedding trials, *I. obsoleta* snails seemed to be healthier and more active at 22°C than at 17°C as indicated by their

faster flipping time (Figure 1.9), indicating that this temperature is within their tolerance levels. However, being infected did have a slight significant effect on their flipping time (i.e. health) as uninfected control snails flipped faster at all the temperature treatments (i.e. 17 and 22°C) (Figure 1.9). Nevertheless, the infected snails were larger than those uninfected ones, indicating that infected snails were older than uninfected ones. Hence, it is also possible that uninfected snails flipped faster than infected ones on the account of them being younger and therefore healthier and has no relation to trematode parasitism. However, this is difficult to assess since in most cases, the infected snails collected in the field are larger than uninfected ones (e.g. Curtis 1997; unpublished data).

From the perspective of *C. volutator*, an increase in the temperature seems detrimental to them regardless of being infected or not. It is only at normal average water temperatures (i.e. 12°C, DFO) that being infected had a noticeable impact on them (Figure 1.5A). This is especially visible during the first days of experiment 4 where there were high mortality rates in the infected and uninfected amphipods at 17°C and 22°C (Figures 1.5B and 1.5C). Similar results were found by McCurdy et al. (1999) where infected *C. volutator* females survived for shorter periods of time than uninfected ones presumably at room temperature (though the exact temperature was not indicated); however, they also found that those with higher parasitemia (higher number of metacercariae) survived longer, which the authors attributed to the females halting their reproductive effort and possibly allocating more resources towards survival. All *C. volutator* used for the present study were reproductively mature adults (i.e. >5mm in body length); however, none were found carrying eggs or a brood, and if they had, they might have released their young before their use in experiments. Thus, I expect that there

were no energy reserves from reproduction that would have been available for relocation, explaining the discrepancies between McCurdy et al. (1999) and this study. In any case, the high mortality patterns in *C. volutator* at higher-than-average temperatures (i.e. $>12^{\circ}\text{C}$) from the present work also fit with other laboratory experiments where *C. volutator* grew best at low to mid teen temperatures (Peters and Ahlf, 2005; David Drolet, unpublished data) and field observations of high mortality rates during summer heat waves in the Upper Bay of Fundy mudflats (personal observations). One possible answer as to why infected amphipods at normal average temperatures (i.e. 12°C) died sooner might be due to the fact that trematode infections might undermine the general health of the amphipods or act as a stressor, which makes them more likely to being preyed upon in the field, thereby improving the chances of the parasite to be transmitted to their final bird hosts. Similar results were found on another trematode-amphipod system. Studer et al. (2010) found that temperature had a direct impact on the mortality of the amphipod *Paracalliope novizealandiae*, and that at temperatures of $\geq 30^{\circ}\text{C}$, the survival rate of the amphipods was greatly diminished irrespective of being infected by the trematode *M. novaezealandensis*. However, they also found (though they did not offer an explanation) that those amphipods infected at 20°C to 25°C survived slightly longer than those uninfected ones, which is different from the present results. Perhaps, stopping the reproductive investment and diverting their resources towards survivorship might have increased the life span of the infected amphipods at those temperatures as in McCurdy et al. (1999).

It is also telling that none of the laboratory-infected amphipods had *G. adunca* metacercaria cysts after the end of experiment 4. This might be explained by the fact that

over 90% of the infected amphipods at 17°C and all at 22°C died within 2 weeks after the start of the experiment (Figures 1.7B and 1.7C) and *G. adunca* metacercariae form cysts after 15 days inside the amphipod at room temperature (McCurdy et al., 1999). However, 20% of infected amphipods survived for over 20 days at 12°C and still did not develop metacercarial cysts. Only a single laboratory-infected individual (not used for the present data analysis) kept at 12°C for over six months was found to contain 11 young cysts (>5 times more than the usual number found in the field) inside its body cavity after it died. This indicates that *G. adunca* metacercariae might develop faster at higher temperatures although more work is needed to fully settle this issue.

The present results indicate that transmission of *G. adunca* cercariae to *C. volutator* amphipods might only occur during low tide periods in the spring and summer months in the Upper Bay of Fundy mudflats. The *I. obsoleta* snails, first hosts to the *G. adunca* cercariae, are present in the mudflats only during spring and summer months (Cranford, 1986; McCurdy et al. 2000). The cercariae are poor swimmers, emerging from infected snails in high numbers at higher than average water temperatures in the region (i.e. >12°C, figure 1.1) and in abrupt changes in environmental conditions (results from experiment 5); thus, for all these reasons, emerging at low tide increases their chances of success in finding their next host. Consequently, the pools of standing water in the mudflats during low tide periods, where the water can reach higher than average temperatures in daytimes (personal observations), should be indispensable in the transmission of *G. adunca* to *C. volutator*. These are also the places where *I. obsoleta* snails like to congregate during low tide (Drolet et al. 2009 and references therein). Accordingly, the water in these tide pools might suffer from evaporation during diurnal

hours in the summer because of the sun, which in turn affects salinity levels in the pools. The effect of salinity coupled with temperature in the emerging and behavioral patterns of *G. adunca* cercariae and their effect on *C. volutator* should also be addressed in the future for a more comprehensive picture of host-parasite interactions between *C. volutator* and *G. adunca*. Nevertheless, results from this work suggest no clear and consistent effect of temperature on parasite mediated impact of *G. adunca* on its second intermediate host.

The present results were obtained from laboratory experiments. One can argue that the laboratory conditions are not the same as natural field conditions, and that the results obtained here will not be applicable to the field. This argument is probably accurate to a certain degree; laboratory conditions will never be the same as in the field and the best a researcher can do is to best replicate the natural conditions in the lab. I believe I have done this with my specimens by providing them with living conditions that were similar to these found in the Upper Bay of Fundy in the summer (e.g. 16:8 light:dark hours, temperature of 17°C, best quality artificial salt water mixture that approximates to natural sea water). However, some features that I was not able to reproduce in the laboratory and which might have confounded my results are the actual tide cycles found in the field, the actual chemical and physical composition of the water found in the area (e.g. detritus, agricultural and settlement run-offs), the strong sunlight (including UV), and abrupt changes in the local weather patterns. How these factors can alter the parasite transmission patterns and their effects on the hosts is still unclear due to the lack of experiments in the field, which should be addressed in the future if this host-parasite system is to be understood fully.

Table 1.1. Testing for differences in the number of cercariae emerging from snails after first day of exposure to 17°C for both replicates in Experiment 1. After first day at 17°C, snails in both replicates were separated in equal numbers and moved to temperature treatments below (12°C) and above (22°C) the original temperature treatment. See material and methods for more details.

Replicate	Subsequent assignment	N	Mean*	Back-transformed mean	t [†]	df [†]	P [†]
1	12°C	17	6.61	742.48	0.32	35	0.75
	22°C	20	6.39	595.86			
2	12°C	7	3.85	46.99	0.19	12	0.85
	22°C	7	4.14	62.8			

*After data have been transformed by $\ln(n)$

[†]From the Independent samples t-test on transformed data

Table 1.2. Laboratory-induced infections of *Corophium volutator* at two temperature treatments (17°C and 22°C) using 3, 12, and 24 hr old cercariae.

Experiment number	Trial	Age of cercariae (hr) [*]	Temperature treatments	Percent <i>C. volutator</i> infected (n/N) [†]	Overall Percent <i>C. volutator</i> infected (n/N) [†]	Median (range) of cercariae inside <i>C. volutator</i>	Z [‡]	P [‡]
Experiment 3A	1	3hr	17°C	54.5% (12/22)	53.5% (23/43)	1 (0-3)	0.08	0.94
			22°C	52.4% (11/21)		1 (0-9)		
	2	3hr	17°C	86.4% (19/22)	82.2% (37/45)	1 (0-8)	0.29	0.77
			22°C	78.3% (18/23)		2 (0-10)		
Experiment 3B	-	12hr	17°C	0% (0/24)	6.3% (3/48)	0	1.77	0.08
			22°C	12.5% (3/24)		0 (0-1)		
	-	24hr	17°C	8.3% (2/24)	4.2% (2/48)	0 (0-1)	1.77	0.08
			22°C	0% (0/24)		0		

* Age as hours after exiting from snails

[†]“N” is the total number of individuals in the experiment; “n” is the number of infected individuals

[‡]From the Independent samples Mann-Whitney U test

Figure 1.1. Graphical representation of the experiments from present study (drawings of snails and amphipod from McCurdy et al., 1999; cercaria from Rankin, 1940)

Figures 1.2 A and B. *Corophium volutator* parasitized by *Gynaecotyla adunca* mounted in Hoyer's medium. (A) Arrowhead indicates metacercaria cyst in the amphipod's pereopod. (B) Cercaria in the amphipod's pereopod; St= stylus.

Figures 1.3 A and B. Experiment 1 replicates 1(A) and 2 (B): emergence of *Gynaecotyla adunca* parasites from *Ilyanassa obsoleta* mud snails at 3 temperatures. Paired t-tests were used to compare the mean number of cercariae from each temperature treatment after the data was transformed ($\ln [n+1]$). There were significant differences on the number of cercariae between 12°C and 17°C, and 17°C and 22°C on both replicates. Error bars represent ± 1 S.E. Black points on graph are the back-transformed means.

Figures 1.4 A and B. Percent survival curve of cercariae hours after emerging from snails. Log Rank test was used to compare the survival distributions of the cercariae at 17°C and 22°C; there were significant differences on the survival distributions between both temperatures on replicates 1 (A) and 2 (B).

Figures 1.5 A and B. Number of cercariae swimming in 30 sec at intervals of 3 hr after emerging from snail hosts. (A) Replicate 1, (B) replicate 2.

Figures 1.6 A and B. Laboratory-induced infection intensities (number of parasites inside) of *Corophium volutator* amphipods at 17°C and 22°C. Independent-samples Mann-Whitney U test was carried out to compare the differences in the numbers of trematodes inside the amphipods at the 2 temperatures; there were no significant differences on the number of cercariae inside the amphipods for replicates 1(A) and 2(B). Values above box plots are those above the 1.5 interquartile range of the third quartile. The parasites used were 3 hr-old cercariae.

Figures 1.7 A, B and C. Percent death in days of infected (treatment) and uninfected (control) *Corophium volutator* amphipods at 12°C, 17°C and 22°C. Logistic regression was used to compare the days to death for infected and control amphipods for the 3 temperatures. There was a significant difference for the treatment and control amphipods in the days leading to their death at 12°C (A), but not between treatment and controls for 17°C (B) and 22°C (C).

Figure 1.8. Long term *Gynaecotyla adunca* cercarial emergence patterns from *Ilyanassa obsoleta* snails at 17°C and 22°C. Error bars represent ± 1 S.E.

Figure 1.9. Average number of *Gynaecotyla adunca* cercariae emerging from *Ilyanassa obsoleta* snails for 33 days at 17°C and 22°C. Error bars represent ± 1 S.E. There was no significant difference on the mean number of cercariae emerged at the 2 temperature treatments (data were square root-transformed before statistical analysis). Black dots on graph are the back-transformed means.

Figure 1.10. Mean time to flip (min) of *Gynaecotyla adunca*-infected and uninfected *Ilyanassa obsoleta* snails after 34 days in 17°C and 22°C. The flipping time was transformed ($1/[n+1]$) and analyzed in a 2-way ANOVA with temperature treatments (17°C and 22°C) and snail conditions (i.e. infected or uninfected) as the fixed factors. There were significant differences in the flipping time of the snails when either condition or temperature was controlled for but no significant difference when temperature and condition of the snails were combined (temperature x snail condition). Error bars represent ± 1 S.E. Black dots on graph are the back-transformed means.

CHAPTER 2

The relation of temperature, snail density, and prevalence of infection by trematodes in the Upper Bay of Fundy

INTRODUCTION

In the past decades, the effect of temperature, specifically the effects of increments on temperature brought about by climate change scenarios on human and wildlife pathogens, has been a topic of much discussion and study (Harvell et al., 2002; Patz et al., 2005; Ostfeld, 2009). As mentioned earlier, most of these discussions and studies were concerned with microparasites (i.e. unicellular parasites) such as bacteria and malaria and not much on macroparasites (i.e. multicellular parasites) such as trematodes. However, recently the interests on the latter and their effects on their hosts have increased steadily, resulting in papers conjecturing a probable negative effect on the hosts (e.g. Mouritsen et al., 2005; Poulin and Mouritsen, 2006; Mas-Coma et al., 2009). This hypothesis has catapulted at least one study (i.e. Studer et al. 2010) on the net effects of temperature increase on a host-parasite system, where they not only studied the parasite, but also the whole of the transmission process from its first intermediate host to its second intermediate host and the viability of both hosts.

I was also interested on the proposed negative effects of increases in temperature on host populations, and as Studer et al. (2010) I studied the effects of increases in temperature on the transmission success of a trematode parasite to its second intermediate hosts and the viability of all the players involved (i.e. parasite, first and second

intermediate hosts [chapter 1]). I specifically investigated the effects that temperature had on the trematode *Gynaecotula adunca* (Linton), its first intermediate host, the snail *Ilyanassa obsoleta* (Say) and its second intermediate host, the amphipod *Corophium volutator* (Pallas) (Chapter 1). I found no evidence for increase *G. adunca* infection levels in the amphipods at higher temperatures and little –if any- evidence on the infection-induced mortality of amphipods at these higher temperatures (Chapter 1); thus, I do not expect to see temperature-mediated increases in parasitic impacts on the amphipod host populations. Moreover, the viability of snail hosts was not affected by trematode parasitism at higher temperatures (Chapter 1).

However, even if there were strong effects from the laboratory studies, it is still important to ask questions pertinent to field scenarios, such as what the likelihood is that warming mudflats will house more snails, or mudflats with high number of snails will also harbour higher number of trematode (including *G. adunca*) infections? All trematode species castrate their snail hosts (e.g. Hechinger et al., 2009) and might influence the behavior and reproductive success of their second intermediate hosts (e.g. McCurdy et al. 1999). Thus, these parasites might influence –if not limit- snail and second intermediate host (for this study, *C. volutator* amphipods) populations in the field, which might be exacerbated if the parasite presence is intensified in warmer scenarios. Therefore, questions such as how snails and second intermediate host populations will be affected by higher presence of trematode parasites in the field in warmer scenarios are important and should be addressed accordingly.

To answer some of these questions, we examined 4 mudflats in the Upper Bay of Fundy (where our host-parasite system occurs) for May and July for population density

of adult *I. obsoleta* snails (i.e. number of snails/m²), prevalence levels of infection by trematodes (specially by *G. adunca*) in these populations, as well as surface temperature between spring (May) and summer (July) months. The objective for this chapter was to examine if increases in temperature (in this case due to change in seasons) also increase snail density and with it their trematode infections. If an increase in infections by trematode species is found at warmer temperatures and mudflats, populations of their first intermediate hosts (i.e. snails) might be greatly affected and consequences to their second intermediate hosts will also be significant. As mentioned above, the second intermediate host for my study is the amphipod *C. volutator*, which is parasitized by *G. adunca*; thus, I will also be focusing on the prevalence levels for this trematode between the seasons and mudflats.

MATERIALS AND METHODS

Snail sampling

Ilyanassa obsoleta snails were collected at 4 intertidal mudflats in the Upper Bay of Fundy in May and in July. Name and locations of the mudflats as well as date of collections are in Table 2.1. Snails were collected haphazardly and vertically (i.e. perpendicular to the low water line) along the mudflats following the ebb tide. Only snails of ≥ 10 mm in shell length were collected because of the very low incidence of infection in juvenile snails (Curtis, 1997; McCurdy et al., 2000). Snails were transported live back to a laboratory at Mount Allison University in Sackville (NB) where they were dissected to determine infection status (i.e. infected or not infected). For those snails infected by trematodes, the species (specially *G. adunca*) were recorded.

Snail densities and mudflat surface temperature data

Due to logistic and time constraints, I did not measure mudflat surface temperatures and snail densities during my snail collections. Instead, these data were obtained from a large unpublished database collected and assembled by a colleague and fellow graduate student Travis Gerwing from the University of New Brunswick, who has agreed to share the data with me. Mr. Gerwing measured the surface temperatures and snail densities on May 31 and July 14 for Grande Anse, June 1 and July 15 for Peck's Cove, June 1 and July 16 for Starr's Point, and June 1 and July 14 for Moose Cove (all in 2010); thus, these measurements were 13-14 days after my collections on May and 0-3 days after my collections on July. He measured adult snail (>10mm in length) density by counting the number of snails in 1x1m quadrats on 2 vertical transects (i.e. perpendicular to the low water line) in each mudflat following the ebb tide; each transect had 20 quadrats for a total of 40 per mudflat on each month. Mudflat surface temperature was measured once in 8 randomly chosen 1x1m quadrats on the 2 vertical transects (4 quadrats per transect) on each mudflat at each month.

Statistical analysis

All the data were analyzed using SPSS v.19 for PC. First, data were examined for normality. If deviating from the normal distribution, data were transformed and tested for normality again, or non-parametric tests were used if still deviating from the normal distribution after transformations (Grafen and Hails, 2002).

RESULTS

Prevalence of infection in snails

The total number of snails collected and numbers infected by *G. adunca* and other trematode species are in table 2.2. The prevalence of infection (in %) by *G. adunca* and other trematodes species can be seen in table 2.3 and figure 2.1. The overall (all trematode species combined) prevalence levels for May was relatively low for the mudflats in New Brunswick with 13.76% and 7.39% for Grande Anse and Peck's Cove respectively, while the prevalence levels for the Nova Scotian mudflats were relatively high with 78.01% and 42.66% of snails infected at Moose Cove and Starr's Point respectively. In July, prevalence levels of infection increased at all sites except Moose Cove, where it fell from 78.01% to 33.33%, a drop of 44.68% (Table 2.3, figure 2.1).

Snails infected with *G. adunca* in May were relatively low in all mudflats except Starr's Point where 22.38% of snails were infected by this parasite. In July, the levels of snails infected by *G. adunca* increased in all mudflats except Moose Cove where no snails were found to be infected by this parasite (Table 2.3, figure 2.1). Also, only 1 (out of 141) snail was parasitized by *G. adunca* at Moose Cove in May, indicating the near absence of this parasite species in this mudflat. Overall at least 12 different species (based on distinct morphological disparities) of trematodes were found to be parasitizing *I. obsoleta* snails on all the mudflats combined.

Snail Densities

The statistics for the density of the snails (number of snails per m²) for May and July are in table 2.4. There were relatively few snails per m² in May on all the sites except

Starr's Point, where it had the most number of snails per m² not only in this month but also in July (Table 2.4). However, the number of snails increased significantly more in July for all sites except in Peck's Cove, where the increase was not significant (Table 2.5).

Mudflat surface temperature

The average ($\pm$ S.E.) mudflat surface temperature (in °C) for May and July are in table 2.6. The temperatures increased significantly in July for all the sites (Table 2.7).

Relationship between mudflat surface temperature, snail density, and trematode infection levels

Summary data on the mudflat surface temperature, snail density, and trematode infection levels in snails (including by *G. adunca*) are in table 2.8 and in figure 2.2. With increases in temperature from May to July in all the mudflats, both snail density and prevalence of infection levels increased as well, except in Peck's Cove where density did not change significantly (Table 2.5), and in Moose Cove where infection levels decreased by 44.68% (Table 2.8, figures 2.1 and 2.2).

In May, trematode infections was highest in Nova Scotian mudflats, which were also warmer (Table 2.8); however, snail density in this month was not as strongly associated to warmer mudflats (Table 2.8). In July, both snail densities and infection levels did not show a strong relationship with temperature (Table 2.8). Prevalence of infection levels was not linked strongly with snail densities (figure 2.2). For example prevalence of infection increased by 9.61% at Peck's Point from May to July but the snail

density did not change for this mudflat, whereas snail density increased significantly in Moose Cove but the infection levels in its snail populations dropped 44.68% (Table 2.8, figure 2.2).

DISCUSSION

Relation of temperature, snail density, and prevalence levels of infection

As mentioned earlier, the main objective of this chapter was to examine if increases in temperature also increase snail densities and trematode infections in the field. Indeed temperatures in all the mudflats examined increased significantly from May to July and with these increases, prevalence of infection levels in snails (including those infected by *G. adunca*) and snail densities increased in July for most of the mudflats as well (Tables 2.3, 2.4, 2.6, 2.7, and 2.8). Snails tend to be most active in late spring and summer and migrate from subtidal to intertidal areas for breeding and growth during these seasons (Scheltema, 1964; Cranford, 1986); therefore, it is not surprising that snail numbers increased in July. However, snail infection levels also increased on almost all the mudflats with the higher temperatures in July. Infection levels do not seem to be related to snail density since some mudflats had relatively low snail densities and still had high prevalence of infection levels (e.g. Moose Cove, Table 2.8, figure 2.2). This indicates that trematode-infected snails (including those infected by *G. adunca*) appear at –if not prefer- warmer climates and might be independent of snail densities. These results might indicate that in climate change scenarios (e.g. higher average temperatures in the spring and summer) there will be a higher number of infected snails in the field, which

might affect the population of the snails (due to castration) and second intermediate hosts (e.g. *C. volutator*).

Prevalence levels of infection of *Gynaecotyla adunca* in the field

In all sites (except Moose Cove), *G. adunca* prevalence also increased in July, ranging from a 0.7% increase in Starr's Point to an 8.8% in Peck's Cove (Table 2.3, figure 2.1). This increase is likely due to an increase in surface temperature in this month (Table 2.6). This parasite exits in greater numbers at temperatures $>17^{\circ}\text{C}$ (Chapter 1); thus, temperatures in the high teens to twenties might trigger the exit of larval forms (i.e. cercariae) of this parasite looking to complete their life cycles on the next host (i.e. *C. volutator*). However, warmer temperatures do not necessarily improve the probabilities of individual cercariae to complete their life cycle (Chapter 1).

Yet, the prevalence levels of *G. adunca*-infected snails for all the mudflats on both months were relatively low compared to other trematode species. For example, the maximum prevalence of *G. adunca*-infected snails was seen in Starr's Point in July with 23.08%, while snails infected by the trematode *Lepocreadium setiferoides* (Miller and Northup) reached 73.05% at Moose Cove in May (data not shown). Thus, *G. adunca*-infected snails do not reach overwhelming numbers in all the sampled mudflats as seen in other trematode species. This plus the fact that *G. adunca* cercariae are not more successful at completing their life cycles at warmer temperatures (chapter 1), lead me to believe that the impact of this trematode species on the populations of *C. volutator* is negligible.

It is more likely that the *I. obsoleta* snails themselves (trematode-infected or not) are affecting much more gravely the populations of *C. volutator*. The snails compete directly with *C. volutator* for food and grazing territory and are known to displace the amphipods (Connor and Edgar, 1982; Feller, 1984; Drolet et al., 2009). Moreover, *I. obsoleta* snails have been observed to actively hunt and eat *C. volutator* (Michael Coffin, personal observations).

Effect of trematodes on snail populations

As mentioned earlier, trematodes castrate the infected snails (e.g. Hechinger et al., 2009), turning them into “parasite factories”. These infections last for the entire lifespan of the snails (Curtis, 1997) which means that all the infected snails have zero fitness after infection. Also, in most cases, large adult snails (likely in prime fertile stage) are most likely to be infected than younger snails (Curtis, 1997; experiment 5 results in chapter 1). Thus, if prevalence of infection is excessively high, snail population turnover (number of next-generation snails) can be greatly affected. In this study, trematode-infected snails almost reach 50% of the population in some mudflats (i.e. Starr’s Point and Grande Anse in July; table 2.3, figure 2.1) and over 78% in Moose Cove in May, which might seriously affect the snail population levels in these sites in the future. In addition, the high incidences of snails infected by *L. setiferoides* in Moose Cove (73.05% of sampled snails in May and 21% in July, data not shown) might exert a considerable pressure on the annelid worms and flounder fish populations (second intermediate and final hosts respectively) and should be studied in the future because of the economic importance of these hosts (Martin, 1938).

Peculiarities of Moose Cove mudflat

Moose Cove was the only mudflat examined that did not fit into the pattern of the other 3 mudflats. The prevalence of infection level decreased drastically from 78.01% in May to 33.33% in July, a drop of 44.68% (Table 2.3, figure 2.1) even though snail densities and surface temperatures increased significantly in this mudflat from May to July (Tables 2.5 and 2.7). This drop in infection levels in Moose Cove remains a puzzle. However, one possible explanation for this is that the great majority of infected snails in this site (93.64% in May and 63.64% in July) is parasitized by *L. setiferoides*, a trematode that infects annelid worms as its second intermediate host and flounder fish as its final host (Martin, 1938). McCurdy et al (2000) have shown that *L. setiferoides* influences the dispersal patterns on infected *I. obsoleta* snails at Starr's Point in July. Thus, it is possible that snails infected by *L. setiferoides* prefer cooler water temperatures from May and migrate back to the subtidal zone. However, nothing is known about the effect of temperature on snails infected by this parasite. Moreover, snails infected by *L. setiferoides* were also present at Grande Anse and Starr's Point, and their numbers increased in July (data not shown). Another possible explanation is that the drop in infection levels might be a chance event for this particular season. It is also interesting to point out that *G. adunca*-infected snails were almost absent in Moose Cove (completely absent in July, table 2.2), which might indicate that very few –if at all- shorebirds infected with this parasite (source of infective stages for snails) visit the area, although more yearly surveys would need to be conducted to verify this.

Conclusion

In summary, an increase in temperature from May to July correlated with an increase in snail densities and trematode infections in almost all of the mudflats. The increase in trematode infections in the warmer season might indicate a similar scenario in a potential increase in ambient temperature due to climate change. This increase in trematode infections might affect snail populations and the second intermediate hosts.

Table 2.1. Sampling mudflat sites on the Upper Bay of Fundy.

Mudflats	Collecting Dates		Location	Coordinates
Grande Anse	May 18, 2010	July 17, 2010	New Brunswick	45° 48' 49.4" N, 64° 30' 6.2" W
Peck's Cove	May 19, 2010	July 16, 2010	New Brunswick	45° 45' 3.98" N, 64° 29' 19.3" W
Starr's Point	May 23, 2010	July 13, 2010	Nova Scotia	45° 7' 36.69" N, 64° 21' 58.37" W
Moose Cove	May 24, 2010	July 14, 2010	Nova Scotia	45° 17' 38.5" N, 63° 48' 33.41" W

Table 2.2. Number of snails collected and dissected, including their infection status by site and sampling month

		May				July			
Snail condition		Grande Anse	Peck's Cove	Moose Cove	Starr's Point	Grande Anse	Peck's Cove	Moose Cove	Starr's Point
Not parasitized		94	188	31	82	56	205	132	98
	<i>G. adunca</i> only	2	15	1	32	5	40	0	45
	Other spp.*	11	0	108	24	35	2	66	47
Parasitized	2+ spp.†	2	0	1	5	0	0	0	5
	Total‡	15	15	110	61	40	42	66	97
Overall total§		109	203	141	143	96	247	198	195

*Snails parasitized by all trematode species other than *G. adunca*.

†Snails parasitized by 2 or more species (including those co-parasitizing the snails with another trematode species).

‡Total number of snails parasitized including *G. adunca*.

§Total number of snails sampled and dissected.

Table 2.3. Prevalence levels of infection (%) and their 95% confidence intervals (CI) of *I. obsoleta* snails infected by *G. adunca* and all other trematode species according to mudflat and month of sampling.

	May				July			
	Grande Anse	Peck's Cove	Moose Cove	Starr's Point	Grande Anse	Peck's Cove	Moose Cove	Starr's Point
Overall prevalence*	13.76	7.39	78.01	42.66	41.67	17.00	33.33	49.74
Lower 95% CI[†]	7.91	4.19	70.27	34.43	31.68	12.54	26.77	42.52
Upper 95% CI[†]	21.68	11.89	84.55	51.19	52.18	22.28	39.90	56.97
<i>G. adunca</i> prevalence[†]	1.83	7.39	0.71	22.38	5.21	16.19	0.00	23.08
Lower 95% CI[†]	0.22	4.19	0.02	15.83	1.71	11.83	0.00	17.36
Upper 95% CI[†]	6.47	11.89	3.89	30.10	11.74	21.39	0.00	29.63

*Prevalence of infection by all trematode species including *G. adunca*.

[†]Prevalence of infection by *G. adunca* only.

*The Upper and lower 95% confidence intervals (CI) from Clopper and Pearson, 193

Table 2.4. Snail density (number of snails/m²) of all the sampled mudflats according to month. N= 80 1m x 1m quadrats per mudflat per month

Sites	May					July				
	Minimum Value	1 st quartile	Median	3 rd quartile	Maximum Value	Minimum Value	1 st quartile	Median	3 rd quartile	Maximum Value
Grande Anse	0	0	0.5	8	18	0	1	13	25.75	66
Moose Cove	0	0	0	1	9	0	0.75	5	10	51
Peck's Cove	0	0	0	0	5	0	0	0	1	32
Starr's Point	0	3.75	7.5	20.25	176	0	40.75	127.5	191.25	317

Table 2.5. Differences on the snail density (number of snails/m²) of the 4 sampled mudflats between May and July

Sites	Statistical test	N	F	P
Grande Anse	Mann-Whitney U	80	3.862	<0.001
Moose Cove	Mann-Whitney U	80	4.393	<0.001
Peck's Cove	Mann-Whitney U	80	0.891	0.373
Starr's Point	Mann-Whitney U	80	5.058	<0.001

Table 2.6. Average ($\pm$ SE) mudflat surface temperature ($^{\circ}$ C) on the 4 mudflats sampled for May and July

	May		July	
	Mean	$\pm$ S.E.	Mean	$\pm$ S.E.
Grande Anse	9.14	0.55	18.79	0.23
Moose Cove	11.01	0.34	19.81	0.14
Peck's Cove	9.84	0.13	23.34	0.35
Starr's Point	11.69	0.13	23.6	0.89

Table 2.7. T-tests comparing the difference of the average mudflat surface temperature of the 4 sampled mudflats between May and July.

Sites	t value	df	P
Grande Anse	-16.193	14	<0.001
Moose Cove	-23.891	14	<0.001
Peck's Cove	-35.723	14	<0.001
Starr's Point	-13.258	14	<0.001

Table 2.8. Mudflat surface temperature, snail density, and trematode infection levels including *G. adunca* in the 4 mudflats. Mudflats were positioned from coolest to warmest in each month.

	May (Cooler to warmer mudflat)				July (Cooler to warmer mudflat)			
Mudflats (Province)	Grande Anse (NB)	Peck's Cove (NB)	Moose Cove (NS)	Starr's Point (NS)	Grande Anse (NB)	Moose Cove (NS)	Peck's Cove (NB)	Starr's Point (NS)
Mean Temperature (°C)	9.14	9.84	11.01	11.69	18.79	19.81	23.34	23.6
Snail density (median snail number/m ²)	0.5	0	0	7.5	13	5	0	127.5
<i>G. adunca</i> prevalence*	1.83%	7.39%	0.71%	22.38%	5.21%	-	16.19%	23.08%
Overall Prevalence [†]	13.76%	7.39%	78.01%	42.66%	41.67%	33.33%	17%	49.74%

*Prevalence of snail infected by *G. adunca* only.

[†]Prevalence of snail infection by all trematode species including *G. adunca*.

Figure 2.1. Prevalence of infection levels on *Ilyanassa obsoleta* snails parasitized by trematodes including *Gynaecotyla adunca* in May and July, 2010. Error bars are the upper and lower 95% confidence intervals (Clopper and Pearson, 1934).

Figure 2.2. Relationship between prevalence of trematode infection levels in *Ilyanassa obsoleta* snails and snail densities in May and July 2010 at 4 mudflats in the Upper Bay of Fundy. July surface temperatures were significantly warmer than those in May on all the mudflats.

SUMMARY AND CONCLUSIONS

The net result in chapter 1 indicates that increases in temperature affected negatively the transmission success of the trematode *Gynaecotyla adunca* cercariae to the amphipod *Corophium volutator*. Although the trematode emerged in significantly higher numbers at warmer temperatures, its survivorship and swimming period (i.e. time on active search for next host) decreased, which very likely reduced its transmission success. More importantly, the infectivity or penetration success of the parasite to its amphipod host did not change significantly between low (17°C) and high (22°C) temperatures. Moreover, although the viability of the infected *Ilyanassa obsoleta* snail hosts was not affected at higher than average temperatures (i.e. 22°C), the viability of the amphipod hosts was affected at $\geq 17^\circ\text{C}$. Regardless of infection status, the amphipods died sooner at 17°C and 22°C than at 12°C. It was only at 12°C that infection status had a negative effect on the survivorship of *C. volutator*. Overall this indicates that at least for this host-parasite system, an increment in temperatures from climate change is likely to have negative effects not only on the second intermediate hosts but also on the parasites, with disruptions of the host-parasite relationship. Thus, my results do not support the hypothesis of enhanced parasitemia and host population crashes due to parasitism by trematode parasites in future climate change scenarios (e.g. Mouritsen et al., 2005; Poulin and Mouritsen, 2006). Studer et al. (2010) found similar results in another host-parasite system. Their results indicate that extreme increases in temperature also enhanced the probabilities of host-parasite disruptions and lower the viability of second intermediate hosts independent of infection status.

However, there still exist a possibility of *G. adunca* affecting *C. volutator* populations in warmer temperatures due to the significantly larger emergence of cercariae from infected snails, potentially increasing the burden on amphipods nearby. Nonetheless, I believe this burden on the amphipod population at large is very low because of the reduced life span and motility of the parasites. Moreover, these increased parasite outputs would only occur in the inherently-small tide pools during low tides (due to the aquatic lifestyle of the cercariae), thus, limiting these occurrences to little spots in the large mudflats and would not affect the overall amphipod populations.

Furthermore, field surveys in chapter 2 indicate that the prevalence levels of infection by *G. adunca* in the field, even increasing in the warmer month of July, were relatively low at most sites (almost completely absent in Moose Cove), thus, it is very unlikely that *G. adunca* will have an impact on the *C. volutator* amphipod populations in the field. Other factors such as snail competition (Drolet et al., 2009) and increased heat (chapter 1) are more likely to impact the amphipod populations.

Results from Chapter 2 indicated that an increase in seasonal temperatures from May to July also increase snail density and parasite infections. Increase in snail numbers are due to seasonal migrations of the snails toward the intertidal zone (Scheltema, 1964; Cranford, 1986), and it is not strongly associated with trematode infections. However, the increase in trematode-infected snails in July indicates that trematode-infected snails (including those infected by *G. adunca*) appear at warmer climates. These results might be an indication that there will be a higher number of infected snails in the field in climate change scenarios (e.g. higher average temperatures in the spring and summer),

which might affect the populations of the snails and second intermediate hosts (e.g. *C. volutator*).

It is also important to note that results from chapter 2 show that mudflats in the Upper Bay of Fundy are very dissimilar among each other in terms of trematode infections. Mudflats such as Moose Cove, where *G. adunca* is almost absent, can serve as refugia for *C. volutator* free of trematode infections for other mudflats that had the unlikely event of an amphipod population crash due to the parasite.

Another much discussed topic on the effects of climate change in the spread of infectious diseases is the northward shift in the geographical distribution of the pathogens in a warmer environment (e.g. Lafferty, 2009 and references therein). This phenomenon is also applicable to trematodes (Brooks and Hoberg, 2007). Trematode species infecting *I. obsoleta* snails in the southern regions in the U.S. (and not found in the Bay of Fundy) can spread northward by following the distribution of their snail hosts, thus, potentially increasing the number of trematode species in the Upper Bay of Fundy. Also, host switch events can take place in climate change scenarios (Brooks and Hoberg, 2007). These features can produce dire consequences to the potential second and final hosts in the northern region. However, comparisons of trematode species numbers from Woods Hole area (MA, U.S.A.) (Stunkard, 1983) to the surveys from the present work (chapter 2) shows there are more species found in the Upper Bay of Fundy than in the southern area (~12 vs. 8 species). Nevertheless, the survey conducted by Stunkard was done before 1983 (over 28 years ago), and it is possible that the trematode species have shifted northward during this time. It is also possible that the Upper Bay of Fundy area is a natural hot spot of trematode species diversity for *I. obsoleta* snails. More surveys need

to be conducted in southern and northern regions to elucidate this and potential consequences of trematode parasitism to the local biodiversity.

LITERATURE CITED

- Bick, A. 1994. *Corophium volutator* (Corophiidae: Amphipoda) as an intermediate host of larval digenea –an ecological analysis in a coastal region of the Southern Baltic. *Ophelia*, 40: 27-36.
- Brooks, D. R., and E. P. Hoberg. 2007. How will global climate change affect parasite–host assemblages? *Trends in Parasitology*, 23: 571-574.
- Clopper, C. J. and E. S. Pearson. 1934. The use of confidence or fiducial limits illustrated in the case of the binomial. *Biometrika*, 26: 404-413.
- Coffin, M. R. S., D. Drolet, D. J. Hamilton, and M. A. Barbeau. 2008. Effect of immersion at low tide on distribution and movement of the mud snail, *Ilyanassa obsoleta* (Say), in the upper Bay of Fundy, eastern Canada. *Journal of Experimental Marine Biology and Ecology*, 364: 110–115.
- Connor, M.S., and R. K. Edgar. 1982. Selective grazing by the mud snail *Ilyanassa obsoleta*. *Oecologia*, 53: 271–275.
- Cranford, P.J., 1986. Behaviour and ecological importance of a mud snail (*Ilyanassa obsoleta*) population in a temperate macrotidal estuary. *Canadian Journal of Zoology*, 66: 459–466.
- Curtis, L. A., 1997. *Ilyanassa obsoleta* (Gastropoda) as a host for trematodes in Delaware estuaries. *Journal of Parasitology*, 83: 793-803.
- Drolet, D., M. A. Barbeau, M. R. S. Coffin, and D. J. Hamilton. 2009. Effect of the snail *Ilyanassa obsoleta* (Say) on dynamics of the amphipod *Corophium volutator* (Pallas) on an intertidal mudflat. *Journal of Experimental Marine Biology and Ecology*, 368:189–195.

- Evans, N. A. 1985. The influence of environmental temperature upon transmission of the cercariae of *Echinostoma liei* (Digenea, Equinostomatidae). *Parasitology*, 90: 269-275.
- Feller, R.J. 1984. Dietary immunoessay of *Ilyanassa obsoleta*, the eastern mud snail. *Biological Bulletin*, 166: 96–102.
- Fisheries and Oceans Canada (DFO). Table of water temperatures in the bay of Fundy, New Brunswick, Canada. http://www2.mar.dfo-mpo.gc.ca/science/ocean/coastal_temperature/coastal_temperature.html
[Accessed on July 20, 2009]
- Gillmurray, M. C., and G. R. Daborn. 1981. Feeding relations of the Atlantic silverside *Menidia menidia* in the Minas Basin, Bay of Fundy. *Marine Ecology Progress Series*, 6: 231-235.
- Grafen, A., and R. Hails. 2002. *Modern Statistics for the life sciences*. Oxford University Press, New York, New York, 368 p.
- Harvell, C. D., C. E. Mitchell, J. R. Ward, S. Altizer, A. P. Dobson, R. S. Ostfeld, M. D. Samuel. 2002. Climate Warming and Disease Risks for Terrestrial and Marine Biota. *Science*, 296: 2158-2162.
- Hechinger, R. F., K. D. Lafferty, F. T. Mancini III, R. R. Warner, and A. M. Kuris. 2009. How large is the hand in the puppet? Ecological and evolutionary effects on body mass of 15 trematode parasitic castrators in their snail host. *Evolutionary Ecology*, 23: 651–667.

- Hechinger R. F., A. C. Wood, and A. M. Kuris. 2010. Social organization in a flatworm: trematode parasites form soldier and reproductive castes. *Proceedings of The Royal Society B*, 278: 656 – 665.
- Hicklin, P. W., and P. C. Smith. 1984. Selection of foraging sites and invertebrate prey by migrating Semipalmated sandpipers, *Calidris pusilla* (Pallas), in Minas Basin, Bay of Fundy. *Canadian Journal of Zoology*, 62: 2201-2210.
- IPCC (Intergovernmental Panel on Climate Change). 2007. Climate change 2007: the physical science basis. Summary for Policymakers: contribution of working group I to the fourth assessment report of the Intergovernmental Panel on Climate Change. <http://ipcc.ch/> [Accessed on July 7, 2011].
- Jensen, K. T., and K. N. Mouritsen. 1992. Mass mortality in two common soft-bottom invertebrates, *Hydrobia ulvae* and *Corophium volutator*- the possible role of trematodes. *Helgolander Meeresunters*, 46: 329-339.
- Koprivnikar, J., and R. Poulin. 2009. Effects of temperature, salinity, and water level on the emergence of marine cercariae. *Parasitological Research*, 105: 957-965.
- Lafferty, K. D. 2009. The ecology of climate change and infectious diseases. *Ecology*, 90: 888-900.
- Martin, W. E. 1938. Studies on trematodes of Woods Hole: The life cycle of *Lepocreadium setiferoides* (Miller and Northup), Allocreadiidae, and the description of *Cercaria cumingiae* n. sp. *Biological Bulletin*, 75: 463- 474.
- Mas-Coma, S., M. A. Valero, and M. D. Bargues. 2009. Climate change effects on trematodiasis, with emphasis on zoonotic fascioliasis and schistosomiasis. *Veterinary Parasitology*, 163: 264–280.

- Matthews, S. L., J. S. Boates, and S. J. Walde. 1992. Shorebird predation may cause discrete generations in an amphipod prey. *Ecography*, 15: 393-400.
- Meissner, K., and A. Bick. 1999. Mortality of *Corophium volutator* (Amphipoda) caused by infestation with *Maritrema subdolum* (Digenea, Microphallidae) –laboratory studies. *Diseases of Aquatic Organisms*, 35: 47-52.
- Meissner, K., and T. Schaarschmidt. 2000. Ecophysiological studies of *Corophium volutator* (Amphipoda) infested by microphallid trematodes. *Marine Ecology Progress Series*, 202: 143-151.
- McCurdy, D. G. 1999. Impacts of parasites on reproductive behaviour and fitness of the intertidal amphipod *Corophium volutator* (Pallas). Ph. D. Thesis. Carleton University, Ottawa, Ontario, Canada. X p.
- McCurdy, D. G., M. R. Forbes, and J. S. Boates. 1999. Testing alternative hypothesis for variation in amphipod behaviour and life history in relation to parasitism. *International Journal of Parasitology*, 29: 1001-1009.
- McCurdy, D. G., M. R. Forbes, and J. S. Boates. 2000a. Male amphipods increase their mating effort before behavioural manipulation by trematodes. *Canadian Journal of Zoology*, 78: 606-612.
- McCurdy, D. G., M. R. Forbes, and J. S. Boates. 2000b. Spatial distribution of the intertidal snail *Ilyanassa obsoleta* in relation to parasitism by two species of trematodes. *Canadian Journal of Zoology*, 78: 1137-1143.
- McCurdy, D. G., M. R. Forbes, S. P. Logan, D. Lancaster, and S. I. Mautner. 2005. Foraging and impacts by benthic fish on the intertidal amphipod *Corophium volutator*. *Journal of Crustacean Biology*, 25: 558-564.

- Moller, L. F., and H. U. Riisgard. 2006. Filter feeding in the burrowing amphipod *Corophium volutator*. Marine Ecology Progress Series, 322: 213-224.
- Mouritsen, K. N., and K. T. Jensen. 1997. Parasite transmission between soft-bottom invertebrates: temperature mediated infection rates and mortality in *Corophium volutator*. Marine Ecology Progress Series, 151: 123-134.
- Mouritsen, K. N., L. T. Mouritsen, and K. T. Jensen. 1998. Change of topography and sediment characteristics on an intertidal mud-flat following mass-mortality of the amphipod *Corophium volutator*. Journal of Marine Biology Assessment, 78: 1167-1180.
- Mouritsen, K. N., D. M. Tompkins, and R. Poulin. 2005. Climate warming may cause a parasite-induced collapse in coastal amphipod populations. Global Change Ecology, 146: 476-483.
- Omori, K., and M. Tanaka. 1984. Life history trait changes among four seasonal generations of the mud flat amphipod, *Corophium volutator*. Physiological Ecology of Japan, 21: 89-114.
- Ostfeld, R. S. 2009. Climate change and the distribution and intensity of infectious diseases. Ecology, 90: 903–905.
- Patz, J. A., D. Campbell-Lendrum, T. Holloway, and J. A. Foley. 2005. Impact of regional climate change on human health. Nature, 438: 310-317.
- Peer, D. L. 1986. Life history and reproductive biology of the *Corophium volutator* (Crustacea: Amphipoda) and the influence of shorebird predation on population structure in Chignecto Bay, Bay of Fundy, Canada. Netherlands Journal of Sea Research, 20: 359-373.

- Pelletier, J. K., and J. W. Chapman. 1996. Use of antibiotics to reduce variability in amphipod mortality and growth. *Journal of Crustacean Biology* 16: 291-294.
- Peters, C. and Ahlf, W. 2005. Reproduction of the estuarine and marine amphipod *Corophium volutator* (Pallas) in laboratory for toxicology testing. *Chemosphere*. 59: 525-536.
- Poulin, R. 2006. Global warming and temperature-mediated increases in cercarial emergence in trematode parasites. *Parasitology*, 132: 143-151.
- Poulin, R., and K. N. Mouritsen. 2006. Climate change, parasitism and the structure of intertidal ecosystems. *Journal of Helminthology*, 80: 183-191.
- Pounds, J. A., M. R. Bustamante, L. A. Coloma, J. A. Consuegra, M. P. L. Fogden, P. N. Foster, E. La Marca, K. L. Masters, A. Merino-Viteri, R. Puschendorf, S. R. Ron, G. A. Sánchez-Azofeifa, C. J. Still, and Bruce E. Young. 2006. Widespread amphibian extinctions from epidemic disease driven by global warming. *Nature*, 439: 161-167.
- Roberts, L. S., and J. Janovy Jr., 2004. Gerald D. Schmidt & Larry S. Roberts' Foundations of parasitology. Boston : McGraw-Hill, 7th ed., pp: 207-233.
- Studer, A., D. W. Thieltges, and R. Poulin. 2010. Parasites and global warming: net effects of temperature on an intertidal host-parasite system. *Marine Ecology Progress Series*, 415: 11-22.
- Stunkard, H. W. The marine cercariae of the Woods Hole, Massachusetts region, a review and revision. *Biological Bulletin*, 164: 143-162.

- Wilson Jr., W. H., and K. Parker. 1996. The life history of the amphipod, *Corophium volutator*: the effects of temperature and shorebird predation. *Journal of Experimental Marine Biology and Ecology*, 196: 239-250.
- Zar, J. H. 1984. *Biostatistical Analysis*. 2nd ed. Prentice Hall, Englewood Cliffs, New Jersey. 718p.