Trematode infection effects on survival and behaviour of *Littorina sitkana*

by

Mónica Ayala-Díaz
B.Sc., Universidad Autónoma de Baja California Sur, 2007

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Supervisory Committee

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Abstract

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Several parasites that require two or more hosts to complete their life cycles are known to manipulate host behaviour, enhancing their transmission to the next host. The intertidal snail, *Littorina sitkana*, is host to a diverse assemblage of parasites dominated by trematodes. Trematodes often use snails as first intermediate host and vertebrates as definitive host. Trematode infections can affect host behaviours such as dispersal and foraging. I identified four sites in Barkley Sound that varied in trematode prevalence and species richness. I measured dispersal of snails at these sites and in the laboratory to assess effects of trematode infection on behaviour. I measured feeding rate under laboratory conditions. Trematode effects lowered snail grazing activity at three of the four sites studied, suggesting trematode infection lowers feeding rate of *L. sitkana*, potentially affecting algal composition of the intertidal zone. Infected male snails travelled longer distances in some sites but shorter distances in others. There was an almost significant effect of trematode infection on vertical displacement of *L. sitkana* in the field. I estimated survival rates on each site through intensive capture-mark-recapture experiments. There was differential survival among sites, but no negative correlation between survival estimates and trematode prevalence.

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Dedication

This thesis is dedicated to my mother, Estela Díaz-Ledesma for her efforts, support and encouragement, and to my sister, Lucía Ayala-Díaz for always believing in me.

Chapter 1: Introduction

Until a couple of decades ago, parasites had been overlooked in the majority of ecological studies as they were thought to be an insignificant proportion of animal and plant populations and thus the effects of parasites on the ecology of their host were neglected. This perception changed when evolutionary and ecological studies pointed out that parasitism has evolved in the majority of clades and is very likely one of the most common life strategies in the world (Bush *et al.*, 2001). Recently, it has been proposed that a minimum of half of the species on Earth are parasites in at least one of their life stages (Rohde, 2012).

It has also been noted that some parasites can affect their host's evolutionary fitness by decreasing the reproductive success of their host, as several parasites have important effects on the process of sexual selection of their hosts (Clayton, 1991; Abbott and Dill, 2001), and some parasites castrate both male and female hosts within a population (Coustau *et al.*, 1993; Claereboudt and Bouland, 1994). By affecting the evolutionary fitness of their host, parasites shape both their host and their own evolutionary histories. Parasites evolve in response to evolutionary changes in the host, maintaining the host-parasite relationship (Ruiz, 1991; Ballabeni, 1995; Poulin and Thomas, 1999; Raeymaekers *et al.*, 2013). Host-parasite co-evolution is often referred to as an arms race and is widely interpreted under the "Red Queen" hypothesis (Van Valen, 1973; Dawkins and Krebs, 1979).

The biomass of parasitic species in some estuaries is greater than the biomass of the top predators present in the same habitat, suggesting that parasites are important drivers of energy flow in ecosystems (Kuris *et al.*, 2008). Ecosystems are formed by ecological communities, which are typically defined as an aggregation of different species sharing the same geographical space and interacting with each other and their environment. Host-parasite interactions can be studied as an ecological community, since an individual host has the potential to provide habitat and nutrition for different species of parasites (Bush *et al.*, 1997; Buckland-Nicks *et al.*, 2013). The interactions between species in a community can vary depending on the resources present in their habitat (or host), the food source

exploited by each species, and the required space for an individual to live and meet its nutritional requirements. A combination of interactions between parasites and their host with the host's environment shape the species richness of a parasite community. The idea that parasite community structure is shaped by interactions among parasite species, their host, and the host's environment, gave rise to the field of parasite ecology, which focuses on the study of parasite communities and the potential explanations for community structures observed within a host (Poulin, 1995). Most of the basic studies in parasite ecology describe the composition of macroparasite (i.e. multicellular organisms) communities in order to identify the role different parasite species play in the community, the interactions among individuals and species, and between parasites and their host (e.g., Ching, 1962; 1974; 1991; Ching *et al.*, 2000; Russell-Pinto *et al.*, 2006).

Hosts in nature provide habitat, but parasites that require multiple hosts to complete their life cycle must also be transmitted from one host to the next. The majority of parasites that require different hosts cannot survive in the ecosystem if one of their hosts is absent. Any loss of host species also leads to the loss of their parasites, which has led to some parasite species being proposed as ecological indicators of healthy ecosystems (Campião *et al.*, 2012; MacLeod and Poulin, 2012; Shea *et al.*, 2012; Shah *et al.*, 2013). Transmission of parasites between hosts often involves predation of one host by another, and as the biomass of parasites is greater than previously believed, some authors have proposed parasites be included in food webs that study energy flow and interspecific relationships within an ecosystem (Huxham *et al.*, 1995; Lafferty *et al.*, 2008; Byers, 2009).

Parasites compete for resources with their host, thus parasites have the potential to influence several different aspects of the ecology of their host (Freeland, 1983). For example, parasites can affect host population dynamics (Granovitch and Maximovich, 2013), by reducing host survival (Huxham *et al.*, 1993; Lafferty, 1993; Fredensborg *et al.*, 2005), altering host fecundity (Cheng *et al.*, 1973; Baudoin, 1975; Granovitch *et al.*, 2009), and altering host dispersal (Curtis, 1987; 2007; Sánchez *et al.*, 2007). Many aspects of host ecology affected by parasitic infections are clearly observable. In the early 1950s, ecologists noted a positive correlation between parasitic infection and susceptibility to predation in fish (Van Dobben, 1952). Two decades later, changes in

morphology and behaviour of intermediate hosts due to parasitic infection were tested and proposed as the mechanism leading to increased predation of parasitized hosts (Hindsbo, 1972; Bethel and Holmes, 1973; 1974; 1977). The alteration of host morphology and behaviour by parasites was then termed host manipulation. Host manipulation by parasites is any modification caused by a parasite in its host's phenotype that confers a fitness advantage to the parasite (Poulin, 2010). Under this definition, behavioural manipulation by parasites must lead the infected host to behave in a different way than uninfected individuals of the same population. This altered behaviour will enhance the transmission of the parasite, increasing the probability that the parasite will complete its life cycle, or reduce predation by non-host predators and thereby increasing the parasite's reproductive success (Levri, 1998; Poulin, 2010).

Host manipulation by parasites occurs extensively in nature. There are many examples of terrestrial, freshwater and marine organisms that display remarkable changes in morphology and/or behaviour when carrying a manipulative parasite (Poulin *et al.*, 1992; Roy, 1994; Poulin and Thomas, 1999). Manipulative parasites inhabit members of the majority of taxa in at least one of their life cycle stages, and some examples are widely known due to their complexity and effects on their host's ecology and evolution (see review in Moore, 2012). Sometimes host manipulation can confer certain characteristics beneficial to both the parasite and the host. This can occur when parasite development depends on temperatures that are beyond the optimum for uninfected hosts, resulting in broader temperature tolerance of the host (Bates *et al.*, 2011). Host manipulation can also increase host survival when encounters with some predators that are not suitable parasite hosts occur (Medoc and Biesel, 2008). Host manipulation can also alter host community dynamics by modifying host interactions intra- and interspecifically (Price *et al.*, 1986; Miura *et al.*, 2006). In some cases, manipulative parasites also reduce the reproductive output, feeding activity and competition ability of their host, thus having an effect in the community structure of their host (Minchella and Scott, 1991; Wood *et al.*, 2007). Host manipulation can potentially alter ecosystems when vulnerable hosts are manipulated to release the parasite in an environment where the host would normally be absent (Thomas and Poulin, 1998; Mouritsen and Poulin, 2003; Fenton and Rands, 2006). Parasite presence can result in a new food source for the predators in that environment, changing

the food web dynamics of the ecosystem (Sato *et al.*, 2011). When a parasite manipulates phenotypic characters of the host that are under selection, the parasite can bias selection of the host's genotype regulating those phenotypic traits and thus, have an important evolutionary effect on its host species (Poulin and Thomas, 1999).

Some of the most famous examples of host manipulation involve trematodes; parasitic flatworms with complex life cycles that require two or more hosts, i.e. one definitive host and one or more intermediate hosts (Esch *et al.*, 2002). *Dicrocoelium dendriticum* is a trematode that manipulates the behaviour of its second intermediate host, an ant, by encysting in the ant's suboesophageal ganglion. The mechanism for this manipulation is still not completely understood, but it has been proposed that at night, when the temperature around the ant drops, the encysted worm causes the ant to climb to the top of a grass blade, where the ant remains overnight (Manga-González *et al.*, 2001). The ant's altered behaviour increases the probability that sheep and/or cattle, the trematode's definitive host, will ingest the infected ant while grazing, completing the trematode's life cycle (Badie *et al.*, 1973). An example of morphological manipulation of the host involves another trematode, *Ribeiroia ondatrae*, which infects the developing hind limb bud system of tadpoles and causes deformities to the resulting adult frog (i.e. multiple hind limbs). One hypothesis explaining the adult frog deformities following tadpole infection with *R. ondatrae* suggests that multiple limbs are caused by the encystment of trematode larvae in the growing limb buds of tadpoles, resulting in mechanical disruption of the mitotic cells' arrangement located in the limb buds and affecting hind limb development (Stopper *et al.*, 2002). Another potential explanation for multiple hind limbs in frogs infected with *R. odatrae* is the release of biochemical substances (e.g. retinoic acid) in the limb bud region by trematode larvae which affect normal limb growth (Szuroczki *et al.*, 2012). Although the mechanism causing these deformities remains unclear, it has been observed that deformed frogs are more susceptible to predation by birds and reptiles, which are the definitive hosts of the trematode (Johnson *et al.*, 2001; 2002; Goodman and Johnson, 2011).

Most of the known trematode species worldwide (approximately 25 000 described) use snails as their first intermediate host (Esch *et al.*, 2001). Trematodes inhabit the digestive and reproductive glands of the snail, potentially causing severe damage to the host's

tissue that may have ecological and evolutionary consequences (Cheng *et al.*, 1973; Curtis, 1993; Levri, 1998; Davies and Knowles, 2001; Wood *et al.*, 2007). Trematodes that encyst in their snail host are trophically transmitted to the next intermediate host or the definitive host (typically birds or small mammals). However, usually the behavioural alteration of the snail host increases transmission of the trematode by facilitating its contact with the next intermediate host without involving predation but resulting in altered distribution of the snail host (Curtis, 1993; 2007).

Littorina sitkana Philippi, 1846 is a caenogastropod snail that occurs in high densities along the rocky intertidal shores of the northeastern Pacific Ocean, predominantly in mid- to high-intertidal (≈1-2.28 m) habitats (Rochette and Grand, 2004). *L. sitkana* is host to a variety of trematode parasites and infections can be very intense (hundreds of trematode larvae can be found inside one snail), making this snail an excellent study species for assessment of the local trematode community. This species of snail feeds primarily on seaweeds and microalgae by scraping their substrate (Voltolina and Sacchi, 1990) and prefer seaweeds from the genus *Ulva* (Van Alstyne *et al.*, 2009). Due to their high population densities, these snails can be important grazers of the intertidal communities and can modify the algal community structure by feeding preferentially on some seaweeds over others (Van Alstyne *et al.*, 2009). A decrease in seaweed consumption of snails infected with trematodes has been previously noted (Wood *et al.*, 2007; Clausen *et al.*, 2008) and due to the importance of herbivorous snails in the intertidal zone as seaweed community drivers, the study of potential effects of trematodes on snail foraging behaviour is necessary.

In this thesis I test the effects of trematode parasites on some aspects of their snail host ecology. I relate trematode community composition to survival, and aspects of distribution and foraging of *L. sitkana* in four different localities on the west coast of Vancouver Island, British Columbia, Canada (Fig. 1).

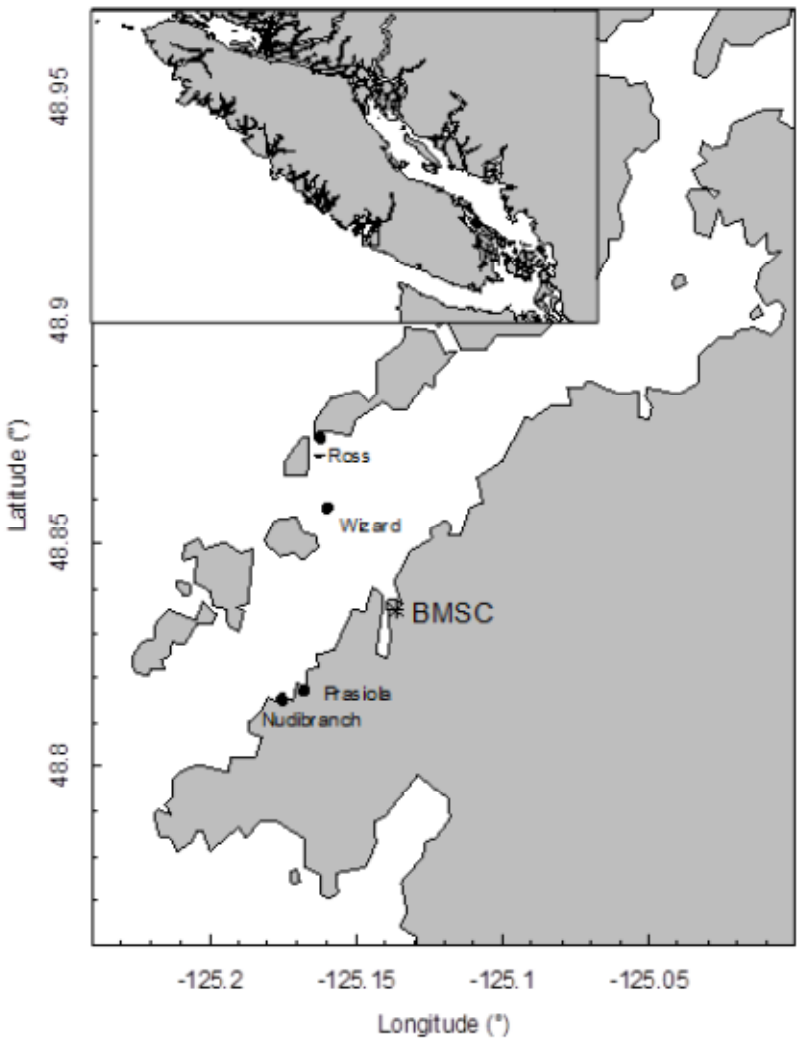

Figure 1. Map showing collection sites located in Barkley Sound and the Bamfield Marine Sciences Centre (BMSC), British Columbia. Vancouver Island.

Chapter 2: Survival related to trematode infection

Introduction

Several species of trematodes manipulate the behaviour of their intermediate host, resulting in increased parasite transmission (parasite transfer from one host to another within the same population) or dispersal (parasite transfer from one host to another of a different population) to other hosts (Dobson, 1988). In some cases, host manipulation by trematodes leads to an increase in predation risk for the first intermediate host by the second intermediate host or by the definitive host (Johnson *et al.*, 2001; Thomas and Poulin, 1998; Thomas *et al.*, 2010). Thus, if predation is the method by which trematodes get to the next host, and if behavioural manipulation occurs, intermediate host survival would be low when trematode prevalence is high.

Snails are often hosts to many phylogenetically unrelated parasites. For each of these parasites, both habitat and resources are determined solely by the snail host. Therefore, an individual snail host can be viewed as a community of parasites. As with any community, several types of interaction can occur among parasites, including competition, and cooperation (Thomas *et al.*, 1998a; 2010; Cezilly *et al.*, 2000; Poulin *et al.*, 2000; Mouritsen and Poulin, 2002; Thomas *et al.*, 2002; Poulin *et al.*, 2003; Haine *et al.*, 2005; Rigaud and Haine, 2005). The parasite community composition within a snail host can depend on interactions between parasite species (Lafferty, 1999), on the availability and species richness of co-occurring definitive hosts (Hechinger and Lafferty, 2005; Levakin *et al.*, 2013), and on the season when a study is conducted (Kube *et al.*, 2002; Faltynkova *et al.*, 2008).

These factors may vary the number of interactions between parasite species sharing a host and have potentially differential effects on the ecology of the host. Since not all species of trematodes have the same effect on their snail host, it is important to establish the community structure of trematode fauna inhabiting snails in order to predict trematode effects on the ecology of its snail intermediate host (Thomas *et al.*, 1998a; 1998b). With this in mind, in this chapter I compare trematode fauna composition and infection prevalence among sites and sampling seasons.

Trematode parasites can decrease survival of snail intermediate hosts other than by increasing predation risk of the host. Some trematode parasites decrease heat tolerance of their snail host, making the host more susceptible to death during summer months (McDaniel, 1969; Huxham *et al.*, 1993). It has also been noticed that snails carrying trematode infections are less successful when oxygen and nutrients are limiting factors, suggesting that parasitized snails consume more oxygen and energy than uninfected snails (Sousa and Gleason, 1989; Fredensborg *et al.*, 2005). Some species of trematodes make their snail host more susceptible to certain herbicides and thus increase mortality of snails in the presence of such contaminant (Koprivnikar and Walker, 2011). Other authors have suggested snails infected with trematodes have lower survival rates due to tissue incompatibilities between parasite and host, as well as host tissue damage while the parasites feeds or during cercarial release (Minchella, 1985; Sorensen and Minchella, 2001). Immune responses to trematode infections are energetically costly and increased mortality in snails infected with trematodes may reflect excessive energy demand by parasites (Sandland and Minchella, 2003; Gorbushin and Iakovleva, 2008).

Survival of *Littorina sitkana, L. scutulata* and *L. plena* in Barkley Sound, BC has been previously studied to assess the role of predation risk on the distribution (Rochette and Dill, 2000) and mating behaviour (Koch *et al.*, 2007) of snails. Two methods are widely used in ecology for measuring survival of intertidal snails in the field. One method is the tethering approach, which consists of gluing a transparent nylon string to the shell of each snail, while the other end of the string is attached to substrate in the intertidal zone to prevent snail movement and/or escape (Behrens Yamada and Boulding, 1996; Rochette and Dill, 2000). A second method involves Mark-Release-Recapture (hereafter, MRR) experiments, where snails are collected and marked individually, released in the field, and recaptured after a determined time period (López-Rocha and Naegel, 2007; Kovach and Tallmon, 2010).

The tethering method is highly time consuming, limiting the number of animals that can be tested when working in intertidal zones, where the experimental set up must take place during the short time available between high tides (pers. obs.). Further, it is difficult to incorporate predator exclusion methods into tethering experiments with reliable results. Cages are typically used as predator exclusion devices with tethering

experiments, but when used in the intertidal zone, cage size (i.e. surface area) is limited due to the force of the waves cages must withstand. Similarly, the duration predator exclusion cages can be left out is restricted due to wave exposure (the longer the cages are left out in the intertidal zone, the greater the risk of being dislodged from the substrate), and sometimes differences in predation are non-detectable in short time periods (pers. obs.).

MRR techniques have some logistical advantages over tethering methods for experimentation in the intertidal zone, as snail marking can be done under laboratory conditions, avoiding time restrictions while working between tides. There is also no need for the addition of predator exclusion cages to detect differences on snail survival, thus time between recapture occasions can be longer, making differences in survival easier to detect. MRR experiments have been used to assess *L. irrorata*, *L. saxatilis* and *L. brevicula* dispersal or migration between habitats (Vaughn and Fisher, 1992; McCarthy *et al.*, 2000; Takada, 2003), growth and longevity of *Ilyanassa obsoleta* (Curtis, 1995) and distribution and survival of *L. sitkana* (Rochette *et al.*, 2003). However, the advantages MRR methods have on the logistics of experimentation in the field are counter-balanced by the uncertainty of the cause of differential survivorship and by the need for more complicated models to analyze the data obtained.

In the last fifteen years maximum likelihood models based on Jolly-Seber estimators have been extensively developed in order to analyze the kind of data obtained from MRR experiments. General maximum likelihood (ML) methods are based on probability theories and are used to both estimate parameters of a certain model that best explain a data set, and to test hypotheses about the parameters estimated (Carey, 1998). These ML techniques can be implemented for MRR data analyses but most researchers use special purpose estimation software such as the program MARK (White and Burnham, 1999).

Bayesian inference is also based on probability laws and can be used to analyze MRR data as well. The main difference between Bayesian inference and the ML approach is based on the way parameters are treated by the model while analyzing the data. While ML treats parameters as a fixed value that is the most likely for a data set after replicating fictitious data sets, Bayesian inference treats parameters as random variables with certain probability distribution given a data set, which is fixed and not replicated (Kéry, 2010).

and Nudibranch Point (48° 48' 53.73" N, 125° 10' 29.72" W). The other two sites were small Islets located in Barkley Sound: Ross Islet (48° 52' 26.13" N, 125° 9' 43.18" W) and Wizard Islet (48° 51' 29.25" N, 125° 9' 35.14" W). The sites located on mainland have similar abiotic components (e.g. wave exposure, temperature, salinity, vegetation and substrate) and are geographically close to each other (550 m apart). Therefore, these sites are expected to have similar biotic components (e.g. invertebrate and vertebrate species richness and abundance). Hereafter, I will refer to the combination of botic and abiotic components of a site as habitat composition of the locality. The Islets are located approximately 6 km away from the sites located on the mainland; they are separated by 1.78 km, have similar habitat composition between them, but differ in habitat composition to the sites located on mainland. Snail collections were made monthly from June to August 2011 and from March to September of 2012. After collection, all snails were transported within an hour to the laboratory at the Bamfield Marine Sciences Centre (BMSC) for processing as described below.

In the laboratory, snails were housed in lidded small square plastic containers (13 cm width X 9.5 cm height) with multiple holes on all sides and the lid. Containers were placed on sea water tables with constant sea water flow at approximately 10 ^{o}C. Snails were kept in this conditions for a maximum of 126 days prior to trematode infection evaluation. During this time, snails were fed *Ulva intestinalis ad libitum*. To ensure all trematode species were detected, I crushed the shell of each snail and removed reproductive and digestive glands from head and foot. As snails survive for several hours after the removal of digestive and reproductive glands, I placed the head and foot of each individual in a concentrated solution of Magnesium chloride (9% $MgCl_2$) immediately after gland removal to euthanize the snails. Digestive and reproductive glands of each snail were placed in filtered sea water and dissected carefully under a dissecting microscope. I then examined the contents of each snail's digestive and reproductive glands using an inverted microscope looking thoroughly through the entire sample. I identified all trematodes found morphologically to the lowest taxonomic level possible based on morphological descriptions from Ching (1963, 1991), James (1968), Yamaguti (1975), Saville *et al.* (1997) and Gorbushin and Shaposhnikova (2002).

The number of trematode species found in all the snails collected from each study site provide an estimate of the site's trematode species richness. I measured trematode presence at each site as the number of snails that had at least one species of trematode. Trematode prevalence is the percentage of snails infected with a particular species of trematode, and was determined as the number of snails infected with one or more individuals of each trematode species divided by the number of snails dissected in each sample (Bush *et al.*, 1997). This amount was multiplied by 100 and expressed as the percentage of the sampled snails that were infected with a particular trematode species. This estimate was calculated for each of the trematode species found at each site. Trematode richness, presence and prevalence were estimated from three different collection dates in 2011 performed at one month intervals. In 2012, I estimated the same indices eight times from collections made approximately every 20 days on each of the study sites. Differences in trematode presence (number of snails with at least one trematode versus number of snails with no trematodes) were analyzed using Pearson chi-square (χ^2) test of independence in program R (R Development Core Team, 2010). Species prevalence data were analyzed with linear regressions in software R (R Development Core Team, 2010). For each trematode species, I used trematode species as response variable coded as a matrix of number of successes (if the trematode species was present in a snail) and failures (if the trematode species was absent in a snail) and the interaction site:year as explanatory variable, unless indicated otherwise. I adjusted the explanatory variable contrasts to compare between years and among sites as necessary for each trematode species. These data were analyzed with the glm() function specifying binomial distribution and logit link function.

Snail Survivorship

On the same four study sites, I did a Mark-Release-Recapture (MRR) experiment from March to October 2012. At each site, I marked eight groups of snails with individually unique tags and had nine recapture occasions. The first capture occasion of each group of snails collected and marked matches the collection dates for the trematode community evaluation in 2012. Each collection of a new group of snails took place 2 days before a

recapture occasion. Each new group of marked snails was released on the same day as snails were censused for recaptures, immediately after each census was completed.

For each mark-recapture occasion at each site in 2012, I collected between 52 and 300 snails (> 7 mm shell height) and returned them to the laboratory for individual marking. Shell height of each snail was recorded as a measurement of snail size. Uniquely numbered all-weather paper tags were attached to the shell of each snail using super glue and then covered with clear nail polish. I tested this method before starting the experiments in the field and had 100% retention of the tags over 4 months in the laboratory. Each group of marked snails released at the same time is a cohort. To decrease the risk of underestimating survival due to tag loss, a coloured mark of nail polish was also applied on the outer apertural rim of the shell, using a different colour per cohort. After the nail polish was dry, snails were placed back in plastic containers on wet tables until released together at site of capture after recapture censuses were done. At each site, I used the same three tide pools for release of all cohorts; release pools at each site were located approximately 3 meters away from the area where snails were initially collected.

Twenty days after release, I returned to the site and searched thoroughly for marked snails along visual transects parallel to the waterline starting at the furthest place from the release point where marked individuals were sighted. Each recapture occasion was considered finished once no more snails were found in a radius of approximately 6 meters away from the release point. Each time a tagged individual was spotted, its tag number was recorded and the snail immediately replaced at the location in which it was found. At the end of each recapture occasion, the next cohort of 300 newly captured was released. Empty marked shells were noted as dead recoveries to improve survival estimates (Juillet *et al.*, 2010).

I recaptured ten snails alive but without a numbered tag or with illegible numbers on the tag (presumably attacked by crabs, as the tag damage resembled the effect of crab pincers) but with the coloured mark on their shell. As the capture histories of those individuals couldn't be followed anymore beyond that recapture occasion, they could be interpreted as dead snails in the analysis and survival estimates would be lower than true survival in the field. Therefore, before analyzing the data and to avoid underestimation of

survival, I matched the colour of the secondary mark on the shell to the cohort number the unidentified individual came from. I then removed randomly the same number of capture histories from the respective cohort containing only zeroes after the recapture occasion in which I found the snails with damaged or missing tags.

I analyzed live recapture data using a ML approach though the program MARK (White and Burnham, 1999) using Cormack-Jolly-Seber (CJS) models. Estimate precision was analyzed within MARK by plotting residuals and calculating the median ĉ Goodness Of Fit (GOF). To improve estimate precision, I removed the capture histories of dead individuals as suggested in the MARK manual (Cooch and White, 2011). I also used a Bayesian approach to estimate survival using the software WinBUGS (Lunn *et al.*, 2000). I fitted a state-space CJS model to analyze live recaptures as described by Royle (2008). Shell height of each snail was used as an individual covariate during the analysis to assess if body size has any effect on survival (Royle, 2008). State-space models were run from within the program R (R Development Core Team, 2010) using the R2WinBUGS package (Sturtz *et al.*, 2005), with 300,000 iterations of 3 chains, a burn in of 10,000 and 100 as thinning. The model outputs were analyzed using the R package Coda (Plummer *et al.*, 2006). Uninformative priors were used for all the estimates to avoid biased estimates.

As a control for survival of *L. sitkana* in the absence of predation, I followed survival of the snail samples used for assessing trematode communities (see above) and held under laboratory conditions. I counted the number of snails that survived for periods of 20 days to match the time snails were left in the field between recapture occasions. The proportion of snails that survived from each time period was then used to estimate mean survival. This information was compared to results from the MRR experiment conducted in the field.

Results

Trematode Species Richness and Prevalence

A total of 181 snails for 2011 (Table 1) and 922 snails for 2012 (Table 2) were dissected and their trematode community determined. Trematode presence differed significantly among sites in both years (2011: $\chi^2_3 = 63.67$, $P < 0.001$; 2012: $\chi^2_3 = 138.89$, $P < 0.001$) (Fig. 2). Wizard Islet had the highest trematode presence, while Ross Islet had the lowest presence among the four sites. Nudibranch and Prasiola Points have intermediary presence in comparison to Wizard and Ross Islets. Presence of trematodes did not differ significantly between years ($\chi^2_1 = 1.34$, $P = 0.25$) (Fig. 2).

Table 1. Mean shell height of *L. sitkana* per site and collection date dissected in 2011.

Site	Collection date	N snails dissected	Mean shell height (mm)
Prasiola	08-Jun	18	12.09
Nudibranch	22-Jun	14	11.70
Ross	11-Jul	14	12.63
Wizard	19-Jul	11	13.43
Prasiola	22-Jul	19	12.29
Nudibranch	25-Jul	18	13.84
Ross	30-Jul	8	12.13
Wizard	02-Aug	11	12.04
Prasiola	04-Aug	18	13.71
Nudibranch	06-Aug	17	13.67
Ross	11-Aug	13	11.62
Wizard	14-Aug	20	12.72

I identified at least six different taxa of trematodes based on morphological characteristics (Table 3). Prevalence of *Himasthla* sp. was significantly higher in 2011 than in 2012 at Nudibranch Point ($z_{35,28} = 3.61$, $P = 0.003$) and Wizard Islet ($z_{35,28} = 3.99$, $P < 0.001$), but there was no significant difference between years in Prasiola Point ($z_{35,28} = 0.78$, $P = 0.433$) and Ross Islet ($z_{35,28} = -1.02$, $P = 0.308$). Wizard Islet had the highest prevalence of *Himasthla* sp. when compared to the other three sites ($z_{35,28} = 8.01$, $P < 0.001$), Nudibranch Point had higher prevalence of this trematode species when compared to Prasiola Point and Ross Islet ($z_{35,28} = 2.86$, $P = 0.004$). Prasiola Point and

Ross Islet did not differ significantly on *Himasthla* sp. prevalence ($z_{35,28} = 1.86$, $P = 0.062$) (Fig. 3). *Himasthla* sp. was found as encysted metacercariae in the majority of their snail hosts in both years (Proportion of 1.0 in 2011 and 0.93 in 2012).

Table 2. Mean shell height of *L. sitkana* per site and collection date dissected in 2012.

Site	Collection date	N snails dissected	Mean shell height (mm)
Prasiola	01-Mar	28	14.13
Nudibranch	24-Mar	30	14.62
Ross	30-Mar	29	12.10
Wizard	08-Apr	30	11.20
Prasiola	26-Apr	29	14.16
Ross	05-May	29	12.03
Wizard	08-May	30	13.27
Prasiola	12-May	29	14.52
Nudibranch	19-May	30	13.14
Ross	24-May	30	11.91
Wizard	26-May	30	13.47
Prasiola	01-Jun	30	14.14
Nudibranch	07-Jun	27	13.88
Ross	14-Jun	29	11.44
Wizard	16-Jun	29	14.36
Prasiola	21-Jun	28	14.61
Nudibranch	27-Jun	29	14.37
Ross	05-Jul	28	12.95
Wizard	05-Jul	27	14.52
Prasiola	11-Jul	30	14.38
Nudibranch	19-Jul	30	14.08
Ross	26-Jul	28	12.87
Wizard	26-Jul	26	14.25
Prasiola	31-Jul	30	14.50
Nudibranch	08-Aug	28	14.24
Ross	15-Aug	29	12.69
Wizard	15-Aug	30	13.37
Prasiola	20-Aug	30	15.12
Nudibranch	28-Aug	30	14.69
Ross	02-Sep	21	12.28
Wizard	02-Sep	30	12.75
Nudibranch	17-Sep	29	14.26

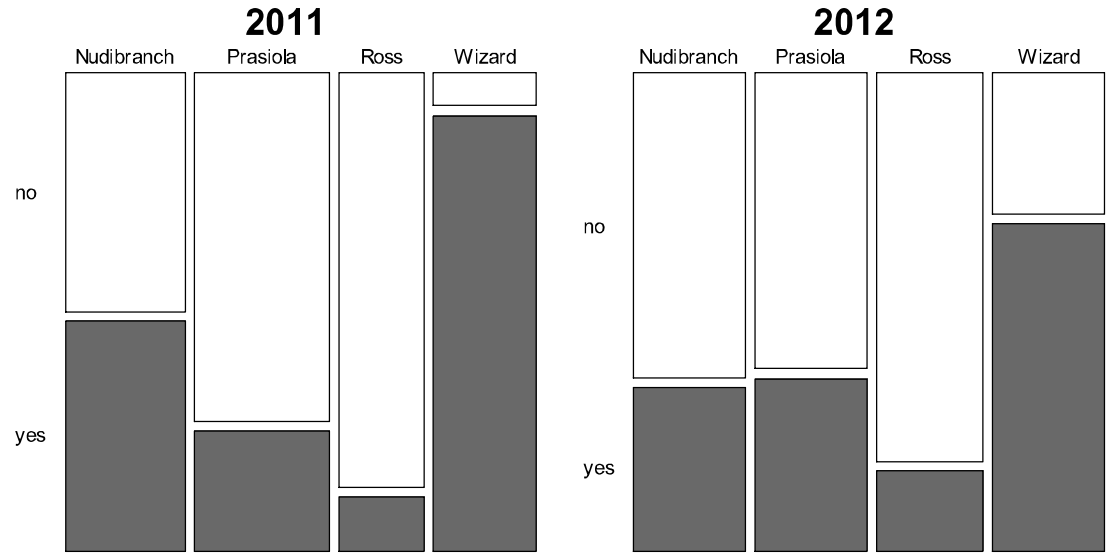

Figure 2. Trematode presence in *L. sitkana* on the four sites studied. Dark bars = Infected snails. Light bars = Uninfected snails. Bar width is proportional to sample size.

Table 3. Prevalence of trematode species (%) per site.

	Site	n	*Himasthla* sp.	*Maritrema laricola*	*Microphallus* sp.	U1	U2	U3
2011	Prasiola	55	16.36	0.00	1.82	7.27	0.00	0.00
	Nudibranch	49	32.65	18.37	16.33	0.00	0.00	0.00
	Ross	35	5.71	0.00	0.00	5.71	0.00	0.00
	Wizard	42	92.86	0.00	0.00	2.38	0.00	0.00
2012	Prasiola	234	18.80	9.40	9.40	0.43	0.00	0.00
	Nudibranch	233	15.02	2.58	16.74	0.86	0.43	0.43
	Ross	223	7.18	8.07	1.79	1.79	0.00	0.00
	Wizard	232	53.02	22.85	0.00	3.45	0.00	0.00

Maritrema laricola was found only at Nudibranch Point in 2011, thus prevalence comparison between years was possible for this site only. I used a proportion test (prop.test() function) in R for this comparison and the result showed significantly lower prevalence in 2012 for this trematode species ($\chi^2_1 = 10.49$, $P = 0.001$). Comparison among sites was possible for 2012, when Wizard Islet had the highest prevalence of *Maritrema laricola* from the four sites ($z_{22,19} = 4.32$, $P < 0.001$), while Nudibranch Point had the lowest prevalence for this trematode species when compared to Prasiola Point and Ross Islet ($z_{22,19} = -2.20$, $P = 0.028$). Prasiola Point and Ross Islet did not differ in prevalence of *Maritrema laricola* ($z_{22,19} = 1.05$, $P = 0.296$) (Fig. 3). This species was found always as sporocysts containing motile cercariae.

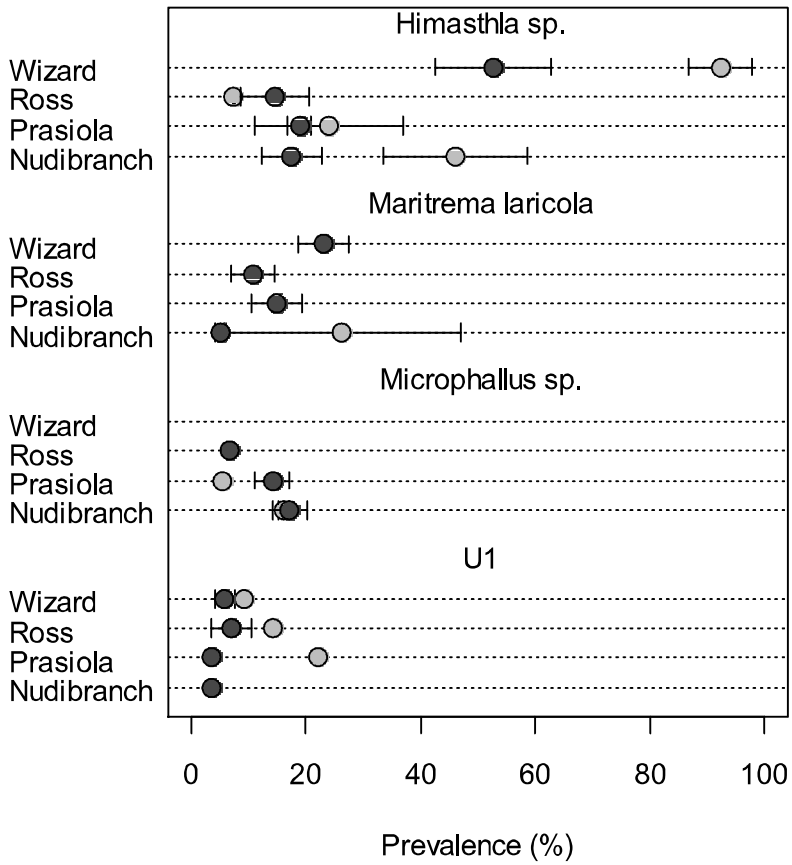

Figure 3. Mean trematode species prevalence in *L. sitkana* from the four sites studied. Light figures = 2011; Dark figures = 2012.

Microphallus sp. was not found in Wizard Islet in any of the years sampled, thus this site was excluded from the models. This trematode species was found in both years at Nudibranch and Prasiola Points, where statistical analysis suggested no significant variation in prevalence of *Microphallus* sp. between years (Nudibranch: $z_{17,14} = -0.14$, $P = 0.887$; Prasiola: $z_{17,14} = -1.05$, $P = 0.295$). There was no significant difference of *Microphallus* sp. prevalence between Nudibranch and Prasiola Points in 2011 ($z_{17,14} = 1.30$, $P = 0.193$). In 2012, this trematode had almost significantly lower prevalence at Ross Islet when compared to Nudibranch and Prasiola Points ($z_{15,13} = -1.76$, $P = 0.079$), while there was no significant difference of *Microphallus* sp. prevalence between Nudibranch and Prasiola Points ($z_{15,13} = 0.80$, $P = 0.426$) (Fig. 3). This species was found as sporocysts containing unencysted metacercariae in all their snail hosts.

In the trematode samples, I found undeveloped (i.e. empty) sporocysts, apparently dead cercariae without internal structures and a kind of cercaria that I could not identify with similar morphology to the apparently dead cercariae. All of these cases are grouped in Table 3 as U1. A second and third type of cercariae could not be identified to species (referred in Table 3 as U2 and U3), but they could be differentiated from U1, and from each other, based on morphological differences of shed cercariae. Both were only present at Nudibranch Point in 2012 with very low prevalence (Table 3). Due to their low prevalence, U2 and U3 were excluded from statistical analyses.

U1 was not found at Nudibranch Point in 2011 and was excluded from the analysis comparing between years. Prevalence of this trematode species was almost significantly higher in 2011 for Prasiola Point ($z_{9,4} = 1.82$, $P = 0.069$), but Ross and Wizard Islets did not show a significant difference of prevalence for this trematode species between years (Ross: $z_{9,4} = 0.86$, $P = 0.391$; Wizard: $z_{9,4} = 0.43$, $P = 0.669$). Prevalence of this trematode species was not significantly different among sites on any of the years studied (2011: $z_{9,4} = 0.08$, $P = 0.936$; 2012: $z_{8,5} = -0.55$, $P = 0.580$) (Fig. 3).

Results from a two-way ANOVA using year as blocking effect suggest that mean shell size of snails varied significantly among sites in both years ($F_{3,1092} = 162.33$, $P < 0.001$). In 2011 snails at Ross Islet were smallest (mean shell height ± SE of 11.77 mm ± 0.13), followed by the snails at Prasiola Point (12.7 mm ± 0.17), Wizard Islet (12.73 ± 0.14) and snails at Nudibranch Point were largest (13.44 mm ± 0.16). Snails from Ross Islet were again the smallest ones (12.28 mm ± 0.07) in 2012, followed by Wizard Islet (13.36 ± 0.09), Nudibranch Point (14.16 ± 0.06) whereas snails at Prasiola Point were largest (14.45 ± 0.07). The same model suggest that shell height of *L. sitkana* differed significantly between years ($F_{1,1092} = 108.24$, $P < 0.001$), snails being larger at the four sites in 2012 (Fig. 4).

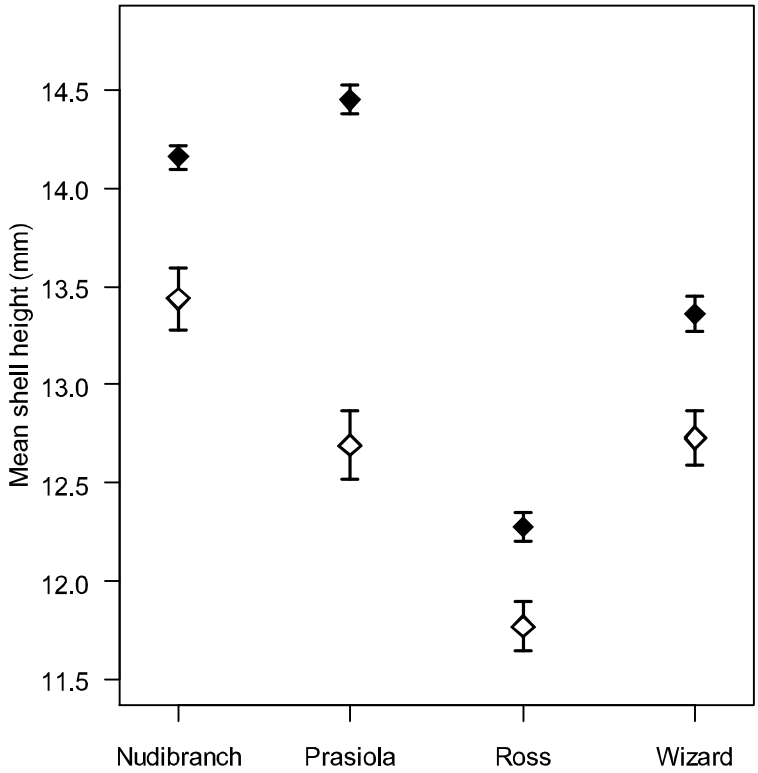

Figure 4. Mean shell height ± SE of *L. sitkana* from each collection site in the two years studied. Open symbols = 2011; Filled symbols = 2012.

Snail Survivorship

I used information from a total of 960 snails collected at the four sites and kept under laboratory conditions to estimate survival in a period of 8 months to compare these estimates with estimates obtained from programs MARK and WinBUGS. Mean survival estimates in the laboratory ranged from 0.990 to 0.995, where Wizard Islet and Nudibranch Point seem to have the highest survival, followed by Prasiola Point; while Ross Islet has the lowest survival among the four sites studied (Fig. 5).

A total of 8,772 individual encounter histories were analyzed from the four sites to obtain survival estimates of *L. sitkana* in the field. At each site, between 1,983 and 2,399 snails were released (mean shell height ranging from 10.3-12.3 mm). The number of snails recaptured alive at least once during the MRR experiment ranged from 1,355-1,683. The total number of snails recovered dead during the experiment ranged from 71-179 (Table 4).

Table 4. Total number of *L. sitkana* released, recaptured alive at least once during the experiment and recovered dead by the end of the MRR experiment in 2012.

Site	Snails released	Snails recaptured alive	Snails recovered dead	Mean shell height (mm)	SE shell height (mm)
Prasiola	2,399	1,670	147	12.3	0.02
Nudibranch	1,983	1,683	71	12.1	0.03
Ross	2,274	1,565	86	10.3	0.02
Wizard	2,116	1,355	179	10.8	0.03

I ran a series of models using program MARK to analyze live recaptures and dead recoveries at the same time (choosing the option 'Joint Live and Dead Encounters (Burnham)' in the model specification window of MARK), but there was severe overdispersion in the residuals from that model. Therefore, I used the Cormack-Jolly-Seber model (option 'Live Recaptures (CJS)' in model specification window of MARK) removing information from dead recoveries to improve normality in the residuals. Snail size was a factor of interest for snail survival due to expected predator preference for larger snails (Behrens Yamada *et al.*, 1998). Nevertheless, when information from shell height was added to the CJS analysis in MARK, residuals were overdispersed and results suggested that larger snails had higher survival than smaller snails. As previous research has shown that larger snails have lower survival than smaller snails, I compared observed and expected survival estimates mathematically (as described in Chapter 11 of the MARK manual) to test estimate accuracy. In this comparison, observed estimates were higher than expected values and larger snails seemed to have lower survival than smaller snails, as suggested on the literature. As these results were clearly inaccurate, I ran the simplest CJS model in MARK excluding the effects of size on snail survival. *L. sitkana* at Nudibranch Point had the highest survival among the four sites, followed by Prasiola Point and Ross Islet; whereas Wizard Islet is the site with lowest survival among the sites studied here (Fig. 5).

The state-space CJS model using WinBUGS allowed me to add information about snail size as individual covariate to survival estimation. The pattern of results from this model is similar to the estimates obtained from MARK. Nevertheless, MARK seems to overestimate survival in Ross and Wizard Islets when compared to estimates obtained

through WinBUGS (Fig. 5). Snail size had a negative effect on snail survival at Prasiola Point and Ross and Wizard Islets, but a weak positive correlation between these variables at Nudibranch Point was observed (Table 5).

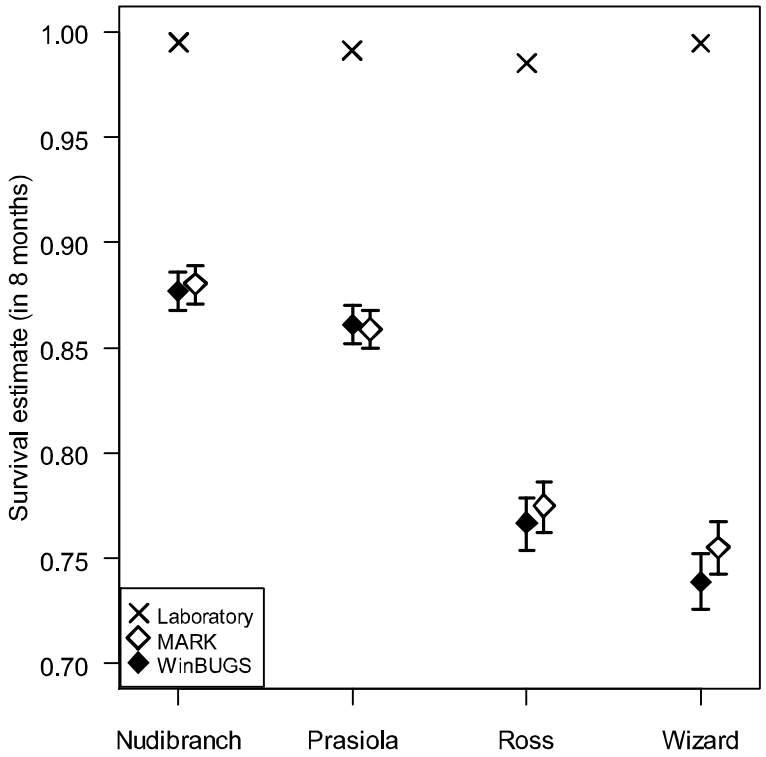

Figure 5. Mean survival estimates ± Credible (CRI) and Confidence Intervals (CI) of *L. sitkana* from each collection site in 2012. Bayesian estimates and CRIs calculated using WinBUGS; Maximum likelihood estimates and CIs calculated with MARK.

Table 5. Mean intercept (a) and slope (b) of correlation between survival estimates and shell height of *L. sitkana* obtained through WinBUGS. Data from field MRR experiment in 2012.

Site	a	SD a	b	SD b
Prasiola	3.245	0.426	-0.116	0.034
Nudibranch	1.499	0.414	0.038	0.035
Ross	1.878	0.379	-0.066	0.037
Wizard	1.963	0.249	-0.085	0.023

Recapture estimates from MARK and WinBUGS were almost identical for the four sites studied, suggesting estimate reliability (Fig. 6).

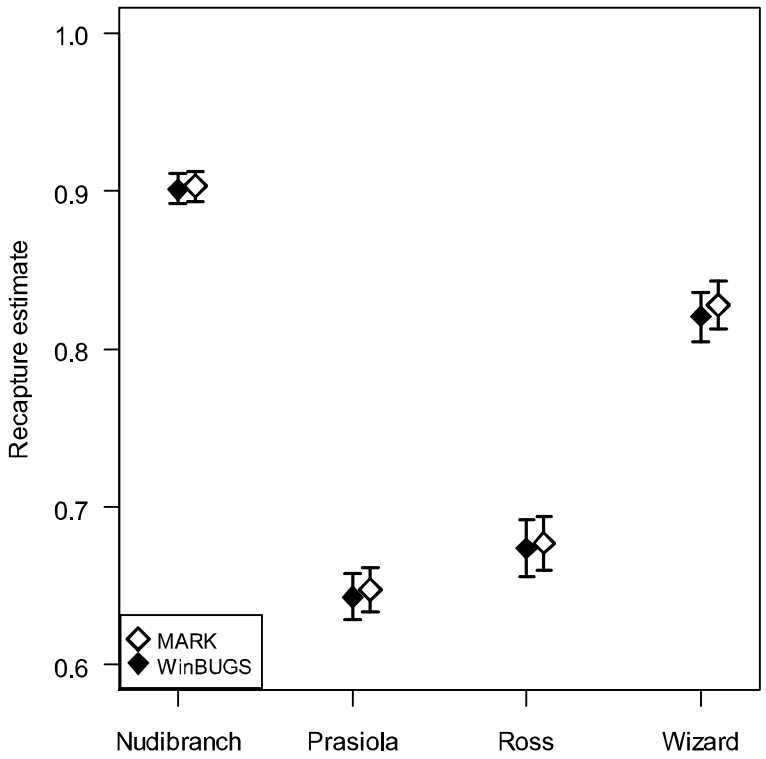

Figure 6. Mean recapture estimates ± Credible (CRI) and Confidence Intervals (CI) of *L. sitkana* from each collection site in 2012. Bayesian estimates and CRIs calculated using WinBUGS; Maximum likelihood estimates and CIs calculated with MARK.

Discussion

Survival

Results from MRR experiments show that snail survival varies among the four sites studied here. Patterns of survival and recapture estimates are the same from both statistical inference modes, suggesting estimate reliability. Survival estimates were slightly different between MARK and WinBUGS. While survival estimates from Prasiola and Nudibranch Points were practically identical, estimates from Ross and Wizard Islets were slightly higher from MARK than from WinBUGS. The addition of snail size as an individual covariate was the most important difference while running models in both programs. Although effects of size on survival of *L. sitkana* are very small in the four sites, the addition of information accounting for snail size to the models improved model fit and most likely improved survival estimate precision. The data used in both programs was also different. While capture history information from dead individuals was removed

from the analysis in MARK, those capture histories were kept as never recaptured again after being recovered dead while running WinBUGS. This could lead to survival overestimation in MARK when compared to WinBUGS, due to apparent lower recapture probabilities when all the capture histories are analyzed in the latter program, producing more conservative survival estimates.

In general, survival and recapture estimates for *L. sitkana* were high at the four sites, suggesting that populations of this snail are stable in Barkley Sound, B.C., and offering a plausible explanation for the high densities noted in previous research in the same geographical area (approximately 500 individuals/m^2) (Rochette and Dill, 2000). The confidence and credible intervals were narrow in survival and recapture estimates, suggesting high estimation precision and increasing estimate reliability.

There is a clear difference in survival between sites located on the mainland and sites on the Islets. Several biotic and abiotic factors can affect snail survival in the field and will be discussed separately starting from the original question for this project: Does trematode infection lower survival rates of intermediate snail hosts? It has been previously noted that trematode infection lowers survival of snail hosts (Huxham *et al.*, 1993; Fredensborg *et al.*, 2005); nevertheless, my results might be harder to interpret. My results are somewhat in agreement with this idea when compared under natural conditions. Prasiola and Nudibranch Points have medium trematode presence and also medium survival estimates, whereas Wizard Islet has both the highest trematode presence and lowest snail survival estimates from the four sites studied in the field. However, Ross Islet has the lowest trematode presence but the second lowest snail survival estimates, contrary to the highest survival estimate expected if trematode infection was the sole cause for lower snail survival under natural conditions. It is also important to note that survival under laboratory conditions was very high in samples from all sites, contradicting the idea of a decrease in snail survival caused directly by trematode infection as seen in previous research (Fredensborg *et al.*, 2005).

Trematodes are capable of altering snail intermediate host behaviour presumably making snails more susceptible to predation and thereby increasing trematode transmission to their definitive host (McCarthy *et al.*, 2000). Differences in survival between laboratory and natural conditions observed here could be explained if trematodes

influence behavioural traits of their snail host and make them somehow more susceptible to predators. In a different part of this project, I saw some evidence of behavioural changes that could increase predation on *L. sitkana*, but results are not ubiquitous (details in chapter 3). From these results, it seems that some other factor present in the natural habitat of *L. sitkana* has a larger effect on snail survival than trematode infection and its consequences in their snail intermediate host.

Snail survival in the intertidal zone can also be affected by wave action, as larger snails are more susceptible to dislodgement by force of crashing waves (Boulding and Van Alstyne, 1993). Dislodged snails are believed to be less likely to return to favourable locations in the intertidal zone or more susceptible to predation by subtidal fauna and, can therefore be presumed to be dead (Miller *et al.*, 2007). This hypothesis combined with information obtained here that mean shell height of *L. sitkana* was smaller on the Islets than on the mainland, might offer a plausible explanation for lower survival of *L. sitkana* at Ross and Wizard Islets if larger snails are constantly being removed from the population at those sites. However, studies using *L. keenae* suggest snails dislodged from the rocky shore frequently succeed in returning to previous intertidal heights in the same location, suggesting that dislodgement by wave action is not a strong force driving snail survival in the rocky intertidal (Miller *et al.*, 2007). More information is necessary in order to understand the importance of wave action in dislodgement and posterior survival of *L. sitkana* in the sites studied here.

Competition for resources with conspecifics might also be important for survival of snails in the field (Chapman, 1997). In general, Prasiola and Nudibranch Points have more coverage of macroalgae of the genus *Fucus* sp. than the Islets (pers. obs.). Some species of the genus *Fucus* are used as primary food resource by littorinid snails from intertidal habitats and might play an important role on snail survival (Kozminsky, 2013). This explanation seems plausible as food resources were available at all times in the laboratory while in the field this resource might be less available to the snails, helping to explain higher snail survival under laboratory conditions. Other species of macroalgae are supplied to the four sites when seaweed washes up to the intertidal zone. During the sampling season, I observed *Ulva lactuca* among other green and brown algae washed up sometimes in my experimental tide pools. However these macroalgae wash-ups were

observed a few times only, providing extra food resources to the Islets in a more irregular fashion than the attached *Fucus* that grows more commonly on mainland sites.

Related to resource availability, population density has an important effect on snail survival in the field (Kozminsky, 2013). If food resources are scarce, intraspecific competition increases and survival decreases. When food availability is higher, intraspecific competition decreases and survival increases. Snail samples in the laboratory were maintained at a constant density with unlimited food availability, and it seems likely that these conditions are impossible to maintain in nature where recruitment and food availability are constantly shifting. Both food availability and population density combined might explain the higher survival in the laboratory when compared to the field. Nevertheless, it is necessary to conduct further research comparing food availability and population densities among the four sites studied in this project to find out the importance these factors have on survival of *L. sitkana*.

Temperature and desiccation are important stressors of higher intertidal organisms and can lower their survival, as organisms remain exposed for hours between high tides. Increased desiccation is expected when higher temperatures occur. Shade provided by vegetation or geological components can help retain moisture in substrate surfaces by preventing direct sunshine from overheating the substrate (Kuczyńska and Moorkens, 2010). Vegetation and geological components differ between mainland and the Islets. If these differences cause temperature and humidity to vary between the Islets and mainland, this could help to explain the survival differences I found during this study. Without measurements of substrate temperature and humidity it is not possible to test this hypothesis and pertinent data are missing from this study. Therefore, research regarding differences in temperature and desiccation probabilities among the four sites studied here is encouraged.

Predators have a direct effect on survival of littorinid snails (Behrens Yamada and Boulding, 1996; Behrens Yamada *et al.*, 1998). Shore crabs (*Hemigrapsus nudus*) and red rock crabs (*Cancer productus*) are the main known predators of *L. sitkana* and both are present in my study sites. The presence of trematode metacercariae in the majority of snails infected with trematodes, suggest that shorebirds are also important predators of *L. sitkana*, but bird feeding experiments are necessary to prove this. Potential differences

between mainland and Islet habitats on crab and bird abundance might explain the higher survival found on Prasiola and Nudibranch Points when compared to the Islets. I did not collect information on crab or bird abundance in any of my study sites, but I collected empty tagged shells with distinctive marks of crab pincers (peeled shells) and bird beak damage as evidence of crab and bird predation. However, while recording live recapture and dead recovery data, I did not make a distinction between individuals killed by predatory crabs, birds or dead for another cause, and this precluded me from comparing frequency of attacks among the four sites studied. It is impossible to properly test the effects of predation on snail survival using an MRR approach, as cause of death of individuals is often unknown and peeled/damaged shells are not recovered in sufficient numbers to provide enough information about the proportion of snails killed by predation alone. Nevertheless, a study on predatory crab abundance on the four sites studied here would help to correlate predator abundance with snail survival and test the hypothesis proposed here.

Crevices in rocks and empty barnacle tests provide protection against temperature, desiccation, wave exposure and predation to intertidal snails (Boulding and Van Alstyne, 1993; Behrens Yamada and Boulding, 1996; Catesby and McKillup, 1998; Kovach and Tallmon, 2010). The rocky intertidal zone where my research took place is very similar between sites located on mainland but differs greatly when compared to the rocky intertidal present in the Islets. Prasiola and Nudibranch Points have almost no slope and several tide pools are arranged close to each other where snails can relocate to find a suitable microhabitat. In contrast, rocks at Ross and Wizard Islets are more irregular, slopes are very pronounced and tide pools are farther from each other, potentially affecting snail relocation in a suitable microhabitat and possibly lowering snail survival probabilities. However, I could see more crevices between the rocks at the Islets than on mainland sites and this might increase snail survival of smaller snails in the Islets. This could help to support the hypothesis suggested while discussing size differences (see below) that larger snails have less probability of surviving in the Islets, as larger snails might be unable to fit in the crevices and therefore be more susceptible to heat, desiccation, predators and wave action.

Trematode species richness and prevalence

Results from this study show that trematode species richness and prevalence differ among sites geographically close to each other. The most surprising difference was observed between Ross and Wizard Islets, which showed the lowest and highest trematode prevalence, respectively, of the four sites. Trematode species richness was highest at Nudibranch Point and lowest at Wizard Islet in both years. It is an accepted idea that trematode species richness in snail intermediate hosts is linked to species richness of definitive hosts co-occurring in the same habitat (Hechinger and Lafferty, 2005; Faltynkova *et al.*, 2008; Levakin *et al.*, 2013). I did not make a census of potential definitive hosts on any of the four sampling sites, but due to similarities in their abiotic characteristics, I expected the Islets to be more similar to each other than to sites located on mainland. It is not possible to know the causes for my results without conducting appropriate vertebrate abundance studies correlating trematode species richness with potential definitive hosts.

Trematode prevalence is related to abundance of definitive hosts in a locality (Fredensborg *et al.*, 2006) rather than to species richness of definitive hosts. If vertebrate abundance is smaller at Nudibranch Point than at Wizard Islet, this could explain the pattern of my results. On Wizard Islet I found sea gulls and oystercatchers (known definitive hosts for trematode species I found in my study) in high numbers during my sampling period, potentially explaining the high trematode prevalence observed on that site. In lower numbers, I observed mink, harlequin ducks and crows occasionally at Wizard Islet, which could also be used as definitive hosts by trematodes. All these vertebrates could contribute to trematode presence and prevalence at Wizard Islet, and the relatively low vertebrate species richness I observed at this site is in accordance with the low trematode species richness I present here.

Low species richness in trematode communities can be caused by competition among trematode species (Leung and Poulin, 2011). Some species of trematodes develop a larval stage –called rediae– capable of ingesting other trematode species or individuals from the same species but different colonies (Hechinger *et al.*, 2011). This larval behaviour increases reproductive success of trematode species that develop rediae by reducing competition with other trematode species (Lloyd and Poulin, 2012). This kind of direct

competition among trematode larval stages might reduce species richness inhabiting intermediate hosts (Hechinger *et al.*, 2011). Thus, direct ingestion of competing species might be an additional explanation for the low trematode species richness found at Wizard Islet, where *Himasthla* sp. was the most prevalent species. The genus *Himasthla* develops rediae and only one *L. sitkana* individual with rediae also hosted another trematode species at Wizard Islet in both years.

Nudibranch Point had the highest trematode species richness and this could be related to highest definitive host species richness. I did not see many birds or other vertebrates while sampling at Nudibranch Point, but I saw some gulls, crows, loons and other small song birds as this site is next to forest. This proximity to the forest might provide the intertidal zone with higher species richness of suitable trematode definitive hosts. However, a census of vertebrate species is necessary in order to confirm this hypothesis.

Snail size

Snail size differed among the four sites during this study, and in general, snails from sites located on the mainland are larger than snails from the Islets. Larger snails are typically preferred by predators (Behrens Yamada *et al.*, 1998) and this could lead to differential size distributions in populations with differential predator distributions (Rochette and Dill, 2000). This hypothesis cannot be tested with my results as I did not make a census of predatory crabs or birds to compare among sites and further research is necessary to test if predatory crabs and birds are more abundant in the Islets than at sites located on mainland.

Larger snails are older, and it is possible that older snails are more susceptible to some environmental stressors (e.g. temperature, humidity). If this proves true, older/larger snails would be less common on sites with more extreme environmental conditions, such as the Islets studied here. Larger snails are more easily dislodged from their substrate (Boulding and Van Alstyne, 1993) due to increased surface area and drag. Ross and Wizard Islets seem to have higher wave exposure than the sites located on the mainland, and snail dislodgement by wave action could help to explain the shorter shell heights observed at both Islets. Although the sites located on the mainland were visibly more protected from direct sunshine and wave action than were sites on the Islets, I did not

measure temperature, desiccation or wave force to make comparisons among sites. Thus field experiments on snail susceptibility to temperature, desiccation and dislodgement due to wave action in the four study sites used here are required to test these hypotheses.

Conclusions

Despite the variation in trematode prevalence among the four sites studied here, there is no conclusive evidence of a trematode effect on snail survival under natural or laboratory conditions. Trematode species found in this study do not appear to negatively affect the health of *L. sitkana*, as infected snails kept under laboratory conditions have higher survival than snails studied in the field. Trematode infection on its own cannot explain the lower survival on both Islets when compared to mainland sites. Snail survival in natural conditions is likely influenced by several factors, and information from all the possible environmental stressors that might impact snail survival is necessary in order to understand survival differences found in this project. Some potential environmental components that might be important to bear in mind for future research on the topic are wave exposure, resource availability and interspecific competition, temperature, desiccation, predation, geological composition and vegetation cover.

Chapter 3: Behaviour related to trematode infection

Introduction

The effect of parasites on intermediate host movement and distribution is one of the most commonly studied forms of host behavioural manipulation. For example, uninfected cockles living in intertidal mudflats are always buried in sediment, while cockles of the same population infected with the trematode *Curtuteria australis* lay exposed above the sediment (Thomas and Poulin, 1998). In the amphipod *Gammarus pseudolimnaeus*, uninfected individuals form groups, an anti-predator behaviour, whereas amphipods infected with the acanthocephalan parasite *Corynosoma* sp. are less likely to aggregate in the presence of fish predator cues (Lewis *et al.*, 2012). Almost all of the described trematode species use snails as their first intermediate host (Esch *et al.*, 2001). Previous work on behaviour in marine snails has shown that trematode infections can lead to spatial variation in the distribution of snail hosts; infected animals spend longer periods out of the water than uninfected ones (Curtis, 1987; 1990).

Changes in snail distribution due to trematode infection are believed to facilitate trematode transmission to their second intermediate host or the definitive host. Enhanced trematode transmission can take place both through predation on the snail host or by facilitation of the contact between swimming cercariae leaving the snail and the trematode's next host. The method of transmission depends on the type of life cycle of the trematode species infecting a snail host. Studies using *Littorina littorea* and *L. saxatilis* showed that snails carrying mature trematode infections (i.e. shedding cercariae or having encysted metacercariae) move less downward than uninfected individuals, but move further upward when tide level rises (Lambert and Farley, 1968; McCarthy *et al.*, 2000). These differences in exposure behaviour appear to increase proximity between gulls and snails shedding cercariae, and to make snails having encysted metacercariae more susceptible to predation by gulls, potentially facilitating the parasite's transmission (Lambert and Farley, 1968; Williams and Ellis, 1975; Poulin *et al.*, 1998; McCarthy *et al.*, 2000). In the marine snail *Batillaria cumingi*, snails carrying mature *Cercaria batillariae* infections move to areas lower in the intertidal zone (Miura *et al.*, 2006). This

suggests that snails shedding cercariae are manipulated by *Cercaria batillariae* to remain most of the time underwater and enhance the encounter between swimming cercariae and its next intermediate host, a fish (Miura *et al.*, 2006). In this case, behavioural manipulation increases transmission of this trematode species to the next host without involving predation of the first intermediate host.

Trematode infections in marine snails can also affect the energy requirements of their host, thus altering the grazing rate of herbivore snails. For example, infected snails tend to decrease their grazing rate in comparison to uninfected individuals (40% and 65% less algae consumption according to Wood *et al.*, 2007 and Clausen *et al.*, 2008 respectively). The grazing rate of a snail may also vary with size, gender and reproductive condition (Levri and Lively, 1996).

Littorina sitkana is a caenogastropod snail that occurs in high densities along the rocky intertidal zone on the West Coast of the Pacific Ocean. It feeds mainly on seaweeds and microalgae by scraping substrate (Voltolina and Sacchi, 1990) and feeds preferentially on seaweeds from the genus *Ulva* (Van Alstyne *et al.*, 2009). Due to their high densities, *L. sitkana* can be an important grazer species of intertidal communities, potentially driving algal community structure by feeding preferentially on some seaweeds over others (Van Alstyne *et al.*, 2009). Alteration of feeding behaviour due to parasites thus has the potential to affect intertidal community structure.

One possible explanation for reduced grazing rates of infected snails is that trematodes often stop host reproduction (a process known as parasitic host castration). Trematodes can stop snail host reproduction directly by feeding on snail gonads, or by releasing proteins that inhibit gonadotropic hormones necessary for snail fecundity (Baudoin, 1975; Schallig *et al.*, 1992). Trematodes can castrate their snail host indirectly as well, by diverting necessary resources and decreasing the available energy that would otherwise be allocated for reproduction (Baudoin, 1975; Cheng *et al.*, 1973). Another proposed hypothesis for low grazing activity in marine snails is that trematode infection can slow or stunt the growth of their snail host (Wood *et al.*, 2007), reducing the energy demand that is used for growth in uninfected snails (Clausen *et al.*, 2008). However, it is difficult to distinguish cause from effect in this case, as trematode infection might be consuming resources otherwise allocated to somatic growth and therefore causing slow snail growth

(Minchella, 1985). Trematodes typically infect the digestive and reproductive glands of their snail host, and some species of trematodes feed directly on their snail host tissue (e.g. *Himasthla* sp.), damaging it (Esch *et al.*, 2002). The damage caused by trematodes to the snail digestive tissue has also been proposed as the cause of lower algae consumption in marine snails because it can reduce the efficiency of food processing by the snail (Wood *et al.*, 2007).

Here, I use both field and laboratory experiments to test the hypotheses that movement and feeding behaviours of infected *L. sitkana* differ from those of uninfected snails. Host manipulation depends on the species of trematode present in the host (Thomas *et al.*, 1998a), and some manipulative parasites do not manipulate their host behaviour until they reach maturity and are ready for transmission to the next host (McCarthy *et al.*, 2000, Thomas *et al.*, 2010). Further, parasites may reduce the risk of predation by the wrong (non-host) predator, as opposed to increasing transmission to appropriate hosts, through host behavioural change to avoid predation (Tierney *et al.*, 1993; Levri, 1998; Medoc and Beisel, 2008). Thus, I also assess the influence of trematode species and infection stage on snail movement and feeding rate by quantifying prevalence, species richness and maturity of trematodes inhabiting *L. sitkana* and I relate each of these measures to individual snail movement and algal consumption.

Methods

To measure movement and feeding behaviours of *L. sitkana*, I collected a total of 562 snails (>7 mm shell height) by searching on the rocky intertidal zone of four sites located on the West Coast of Vancouver Island, B.C., Canada (Fig. 1). Two of these sites were located on the mainland of Barkley Sound: Prasiola Point (48° 49' 1.14" N, 125° 10' 4.42" W) and Nudibranch Point (48° 48' 53.73" N, 125° 10' 29.72" W). The other two sites are small islets located in Barkley Sound: Ross Islet (48° 52' 26.13" N, 125° 9' 43.18" W) and Wizard Islet (48° 51' 29.25" N, 125° 9' 35.14" W). I collected snails at each site in two years: from June to August 2011 and from March to October of 2012. After collection, snails were transported within an hour to the laboratory at the Bamfield Marine Sciences Centre (BMSC). Snails were housed in lidded plastic square containers (13 cm width X 9.5 cm height) with multiple holes on all sides and lid that were placed

on sea tables with constant sea water flow (at approximately 10 ^{0}C). *Ulva intestinalis* was provided as a food source *ad libitum*.

After movement and feeding trials, the trematode community of each snail was quantified. To assess trematode community composition of *L. sitkana* correctly, and to ensure all trematodes present were detected, I crushed the shell of each snail and separated reproductive organs and digestive glands from head and foot. As snails survive for several hours after the removal of digestive glands and reproductive organs, I placed the head and foot of each individual in a concentrated solution of magnesium chloride (9% $MgCl_2$) immediately after gland removal to euthanize the snails. Digestive glands and reproductive organs of each snail were placed in filtered sea water and dissected carefully under a dissecting microscope. I then thoroughly examined the contents of each snail's digestive glands and reproductive organs using an inverted microscope to look through the entire sample. I identified all trematodes to the lowest taxonomic level possible based on morphological descriptions from Ching (1963, 1991), James (1968), Yamaguti (1975), Saville *et al.* (1997) and Gorbushin *et al.* (2002). Trematode infections in snails were considered mature if motile cercariae or encysted metacercariae were found and immature if only sporocysts or rediae without motile cercariae were seen.

Movement field experiment

After completion of a separate mark-recapture experiment in October 2012, I randomly selected 100 individually marked snails from the approximately 900 (mean n ± SE: 893 ± 128 snails) recaptured at each of the four sites. At each study site, I placed the 100 snails together in an identified position in one tidal pool. After 5 to 9 days (access to sites was weather-dependent), I measured the straight-line distance moved by each uniquely marked snail from the release point with a measuring tape (distance travelled). I also measured the elevation of the recapture point relative to the release point (vertical displacement) using a flexible tube water level. Finally, I noted the direction of the movement of each snail in relation to the ocean (i.e. towards, away from, or parallel to the ocean). After snail movement measures had been taken, each snail was collected and transported to the laboratory at the BMSC for dissection and trematode infection assessment as above.

Both distance travelled and vertical displacement data were analyzed with linear models using the function lm() included in the stats package of R software version 2.12.1 (R Development Core Team, 2010). The linear models included site, infection status, snail sex and size as explanatory variables. Interactions among variables that showed no significant effect were removed from the fully parameterized linear models and results interpreted afterwards.

I tested the hypothesis that snails are more likely to move away from the water if infected with trematodes. Sample sizes varied among sites because the frequency of infection varied among sites. Therefore, movement direction data from each site were analyzed separately as categorical variables in relation to trematode infection status (i.e. infected and uninfected snails) using permutation (1000 iterations) Pearson's chi-square (χ^2) tests of independence included in the R package coin (Hothorn *et al.*, 2008). Snail movement from Prasiola Point was measured before deciding to include direction of movement as a factor of interest; therefore, direction data are missing from Prasiola Point and this site could not be included in the analysis. It was not possible to test whether parasite identity affected snail movement for this part of the project because three of the four trematode species were present in fewer than 10 hosts. Infection maturity could not be analyzed as only a few immature infections ($n < 5$) were detected.

Movement laboratory experiment

Laboratory movement studies were conducted with snails collected from all four sites in both years. I took snails to the laboratory within an hour of collection and marked them uniquely by painting a number on their shells with white nail polish. Data on shell height, gender and collection locality were carefully recorded for further analyses. These experiments were conducted without prior knowledge of infection status. It is impossible to accurately tell infected from uninfected snails apart prior to dissection (Curtis and Hubbard, 1990).

I measured the response of snails to rising tides by placing a sample of snails (mean n ± SE: 15 ± 1 for 2011; 30 ± 0 for 2012) in a glass aquarium (40 cm L x 20 cm W x 26 cm H) with a painted grid (1 cm x 1 cm) on the bottom and walls to track individual snail movements. A total of eight samples of *L. sitkana* from Prasiola Point, six samples from

Nudibranch Point and seven samples from each of Ross and Wizard islets were used in these experiments. I placed snails from one sample randomly at separate grid points at the start of the experiment and recorded this starting grid reference for each snail as the starting point. Subsequently, the grid reference for each snail was recorded every 15 minutes.

The snails were left to acclimate in the tank without water for an hour. To simulate the rising tide after the acclimation period, water height in the tank was increased every hour by adding enough sea water to the aquarium to reach the heights of: 1, 2, 5, 10 and 15 cm marked on the walls of the tank. This allowed me to measure movement response from each snail to the rising tide. For each sample of snails, a replicate trial was run on two consecutive days. All of the trials and their replicates for all the samples started at 9:00 h and ended at 18:00 h.

Distance travelled and vertical displacement of each snail were considered separately. For distance travelled I calculated straight-line distance based on the x-y coordinate grid made on the bottom of the tank. For vertical displacement I considered only change in the vertical position of the snail regardless of its position in the x-y plane. For each snail, I calculated total distance travelled and vertical displacement as the sum of distance moved during each 15 minute interval. To combine information from the two consecutive movement trials, I calculated the mean total distance travelled and mean total vertical displacement per snail in a sample and used that information in the analysis. Data for distance travelled and vertical displacement in the laboratory were analyzed in the same way as movement data collected in the field (see above). Trematode presence and prevalence variation at field sites was not significant; therefore years were combined for analyses. To test for potential differences in snail movement due to different trematode species, a second analysis using only snails infected with trematodes considered trematode species effects using distance moved as the response variable, and snail size, snail sex, and trematode species as explanatory variables. Maturity of infection could not be used as an explanatory variable due to insufficient sample size.

Snails from 2011 were dissected after movement trials to determine their trematode community as described above. Snails from movement trials in 2012 were dissected after

they had been used for feeding trials, within a maximum of 5 days after completion of the movement trials (see below).

Feeding experiment

A total of 381 snails (>7 mm shell height) from the four study sites were used in these trials. The majority of snails used in this experiment (n = 354) were also tested for movement in the laboratory for 2012; the remaining snails (n = 27) were sampled from Nudibranch Point on June 2012 to equalize the number of collection dates from each of the four sites. Snails were divided into 13 groups of 29 ± 1 for each trial. During trials, each snail was housed individually in a 100 mm diameter x 15 mm high Petri dish. A 35 mm diameter opening made in both the bottom and lid of the Petri dish was covered with 0.5 mm plastic screening to allow sea water flow but prevent snail escape. Petri dish lids were secured to bottoms with rubber bands.

Pieces of *Ulva intestinalis* (250.07 ± 0.16 mg after blotted dry) collected from the beaches between Prasiola and Nudibranch Points were provided to each snail following the procedure described by Van Alstyne *et al.* (2009). Modified Petri dishes containing snails and algae were placed in a wet sea table (all the Petri dishes were placed together so they had the most similar environmental conditions possible) with a constant flow of sea water at approximately 10 ^{0}C for 5 days. For each trial, four Petri dishes with algae only were also placed in the sea table as controls.

At the end of 5 days, the remaining *U. intestinalis* from each dish was blotted dry and weighed to estimate the amount of algae consumed by the snails (initial algae mass - final algae mass), correcting for the gain or loss of algae mass obtained from the controls. For each snail, I recorded shell height, gender and its site of origin to assess whether feeding behaviour varies with any of these factors. As with the movement experiments, feeding experiments were conducted without previous knowledge of infection status while trials were run.

After movement and feeding experiments, all snails were dissected for identification of trematode infection as described above. Infection status, trematode species present and maturity of infection were noted for each individual as categorical variables. Data were analyzed with multiple linear regression models using the function lm() in the stats

package of R (R Development Core Team, 2010). Linear models used for testing potential effects of trematode infection on feeding behaviour of *L. sitkana* included amount of algae consumed as the response variable and site, infection status, snail size and sex as explanatory variables. Interactions among variables that showed no significant effect were removed from the fully parameterized linear model and results interpreted afterwards. Subsequently, infected snails were used to fit a model that considered amount of algae consumed as the response variable, trematode species present, snail size and sex as explanatory variables to consider potential effects of trematode species on snail feeding behaviour. Only infected snails were used in these models since uninfected individuals would have nonexistent values for trematode species. As trematode richness and prevalence differ among sites, each site was analyzed separately in this case. As with movement data, insufficient sample sizes for snails infected with trematodes of different life stages precluded testing effects of maturity of trematode infection on feeding behaviour.

I tested the hypothesis that larger snails are more likely to be infected with trematodes by applying exact two-sample permutation t -tests using the function 'oneway_test' included in the coin package (Hothorn *et al.*, 2008) of R (R Development Core Team, 2010). Permutation tests give more precise estimates when sample sizes differ between the two groups compared, as is the case between uninfected and infected snails in this study. Data were analyzed separately for each study site as trematode presence varies among these.

As snail sex was a variable of interest in the analysis of my data, I calculated the sex ratio of my samples as percentage of females by dividing the number of females by the total number of individuals in each sample and multiplying this quantity by 100. I refer to a female-biased sample if the total number of females exceeds the 50% (i.e. sex ratio 1:1) of each sample.

Results

Movement field experiment

A total of 311 snails (11.44 ± 0.006 mm shell height) were recaptured from the four sites and their distance travelled and vertical displacement relative to the release point

were noted. To improve model fit, distance travelled by *L. sitkana* was transformed to its square root value. Additional transformations were applied to the data that improved normality of the residuals better than the square root transformation. However, results were unchanged independent of type of transformation applied, and square root transformation has more biological relevance. A significant 3-way interaction between site, infection status and sex was present (Table 6). Uninfected male snails travelled larger distances than uninfected females and infected males in Nudibranch Point (Fig. 7). Infected male and female snails travelled further than uninfected male and females at Prasiola Point (Fig. 7). Infected males travelled longer distances than uninfected females in Ross Islet (Fig. 7). At Wizard Islet, infected male snails travelled less than uninfected males and both infected and uninfected female snails (Fig. 7). A significant 2-way interaction between site and snail size was found (Table 6). Larger snails travelled longer distances than smaller individuals at Nudibranch Point, Ross Islet and Wizard Islet. However, larger snails seem to travel shorter distances than smaller snails in Prasiola Point (Fig. 8).

Table 6. *Field distance travelled*: Significance levels of linear model with significant interactions for distance travelled by *L. sitkana* in the field in 2012.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
site	3	413.64	137.88	19.10	< 0.001	***
infected	1	3.58	3.58	0.50	0.482	
snail sex	1	1.11	1.11	0.15	0.696	
snail size	1	35.98	35.98	4.98	0.026	*
site:infected	3	43.01	14.34	1.99	0.116	
site:snail sex	3	96.98	32.33	4.48	0.004	**
site:snail size	3	65.21	21.74	3.01	0.031	*
infected:snail sex	1	30.29	30.29	4.20	0.041	*
site:infected:snail sex	3	66.17	22.06	3.05	0.029	*
Residuals	291	2101.10	7.22			

*** Significant value at the <0.001 level
** Significant value at the 0.001 level
* Significant value at the 0.01 level

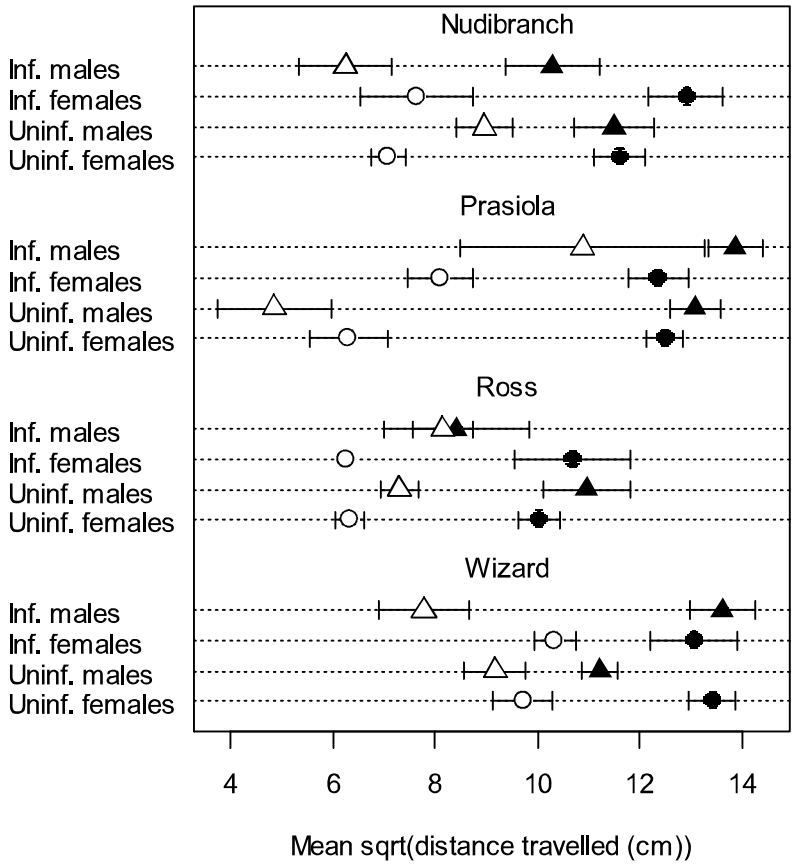

Figure 7. ***Field and laboratory distance travelled*****: Mean distance travelled ± SE of** ***L. sitkana*** **representing significant 3-way interaction among collection site, trematode infection (Inf. = infected, Uninf. = uninfected, as determined post experiment) and snail sex (male, female). Filled figures = Data combined from laboratory experiments in 2011 and 2012. Open figures = Data collected from the field in 2012. Distance data transformed to their square root value.**

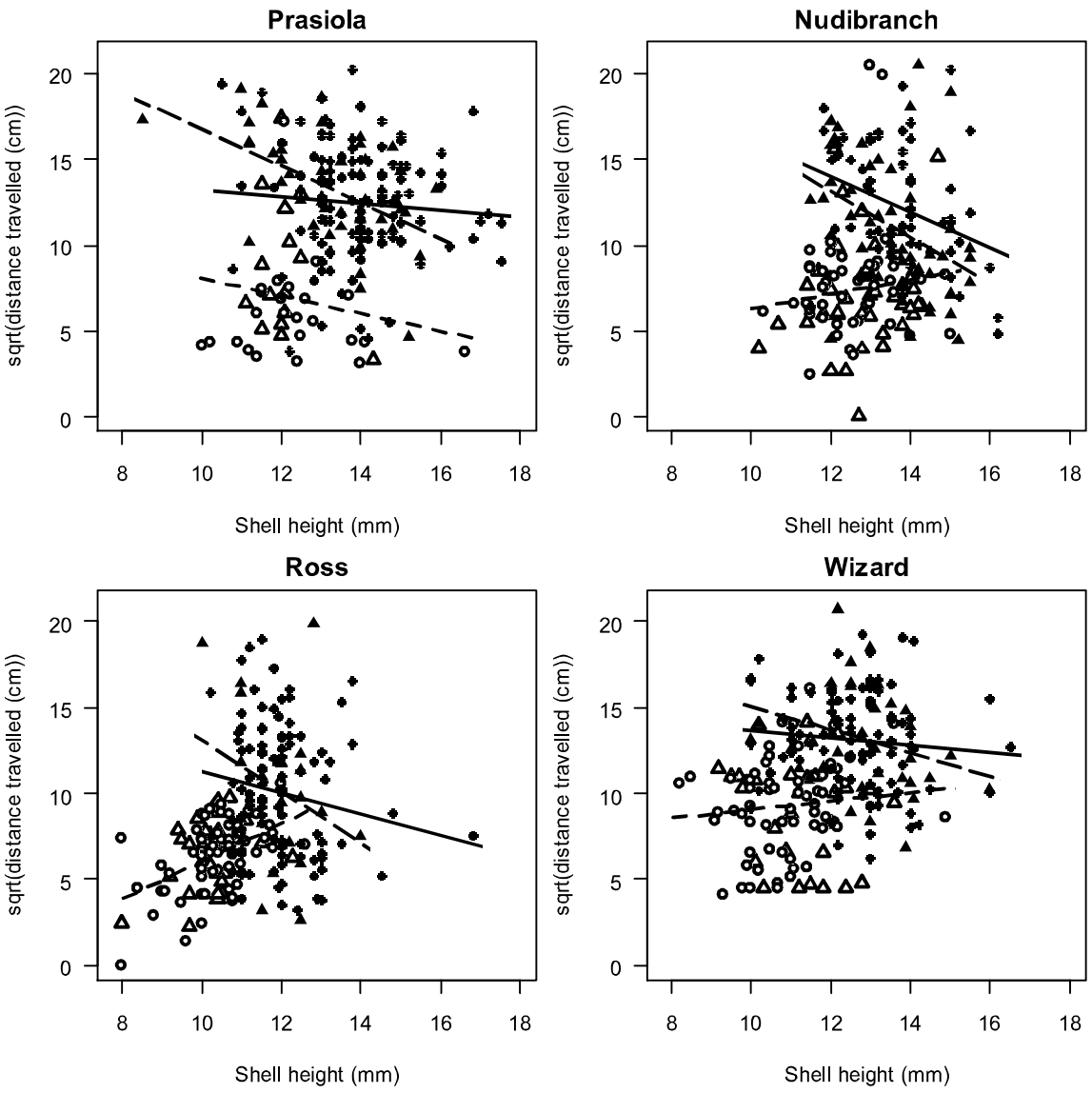

Figure 8. ***Field and laboratory distance travelled*****: Correlation between distance travelled and shell height of *L. sitkana* per site representing significant 2-way interaction between collection site and snail size (field and laboratory); significant 2-way interaction between snail sex and size (laboratory). Open circles = females (field); Open triangles = males (field); Filled circles = females (laboratory); Filled triangles = males (laboratory). Short-dashed regression line = males and females combined (Field data: sex had no significant effect on distance travelled); Solid regression line = females (laboratory); Long-dashed line = males (laboratory). Data combining information from both years studied (laboratory); 2012 only (field). Distance data transformed to their square root value.**

For vertical displacement, data were transformed using sqrt(|distance|) to correct for negative values and following the same criteria applied to distance travelled data

(described above). After square root transformation, negative signs were restored to keep track of displacement direction. Vertical displacement covered by *L. sitkana* differed significantly among the four sites (Table 7). Snails at Nudibranch Point displaced the least vertically when compared to the other three sites (Fig. 9). Infection status on its own was lightly important on vertical displacement of snails (Table 7; Fig. 9). Infected males at Prasiola Point displaced further vertically than uninfected males, whereas infected females moved further vertically than uninfected females at Wizard Islet. Snail sex (Table 7) and shell height (Table 7) did not significantly affect vertical displacement of *L. sitkana* in the field.

Table 7. *Field vertical displacement*: Significance levels of linear model with significant interactions for vertical displacement of *L. sitkana* in the field in 2012.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
site	3	42.86	14.29	6.96	< 0.001	***
infected	1	5.74	5.74	2.80	0.096	·
snail sex	1	4.09	4.09	1.99	0.159	
snail size	1	0.17	0.17	0.08	0.771	
Residuals	304	624.42	2.054			

*** Significant value at the <0.001 level
· Significant value at the < 0.10 level

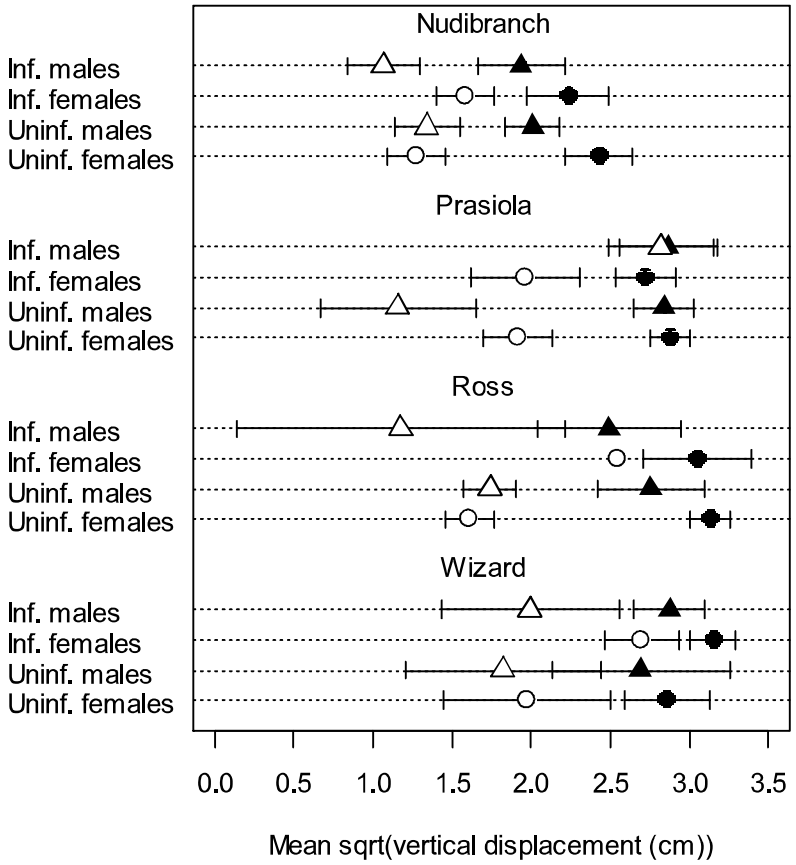

Figure 9. ***Field and laboratory vertical displacement*****: Mean vertical displacement ± SE of *L. sitkana* showing non-significant interactions among collection site, trematode infection (Inf. = infected, Uninf. = uninfected, as determined post experiment) and snail sex (male, female). Filled figures = Data combined from laboratory experiments in 2011 and 2012. Open figures = Data collected from the field in 2012. Laboratory displacement data transformed to their square root value. Field displacement data transformed using sqrt(|distance|); negative signs replaced after transformation.**

I could not reject the hypothesis of independence between movement direction and snail infection status in any of the sites tested (Nudibranch Point: $\chi^2_2 = 0.64$, P = 0.79; Ross Islet: $\chi^2_2 = 1.53$, P = 0.49; Wizard Islet: $\chi^2_2 = 3.50$, P = 0.19; Fig. 10).

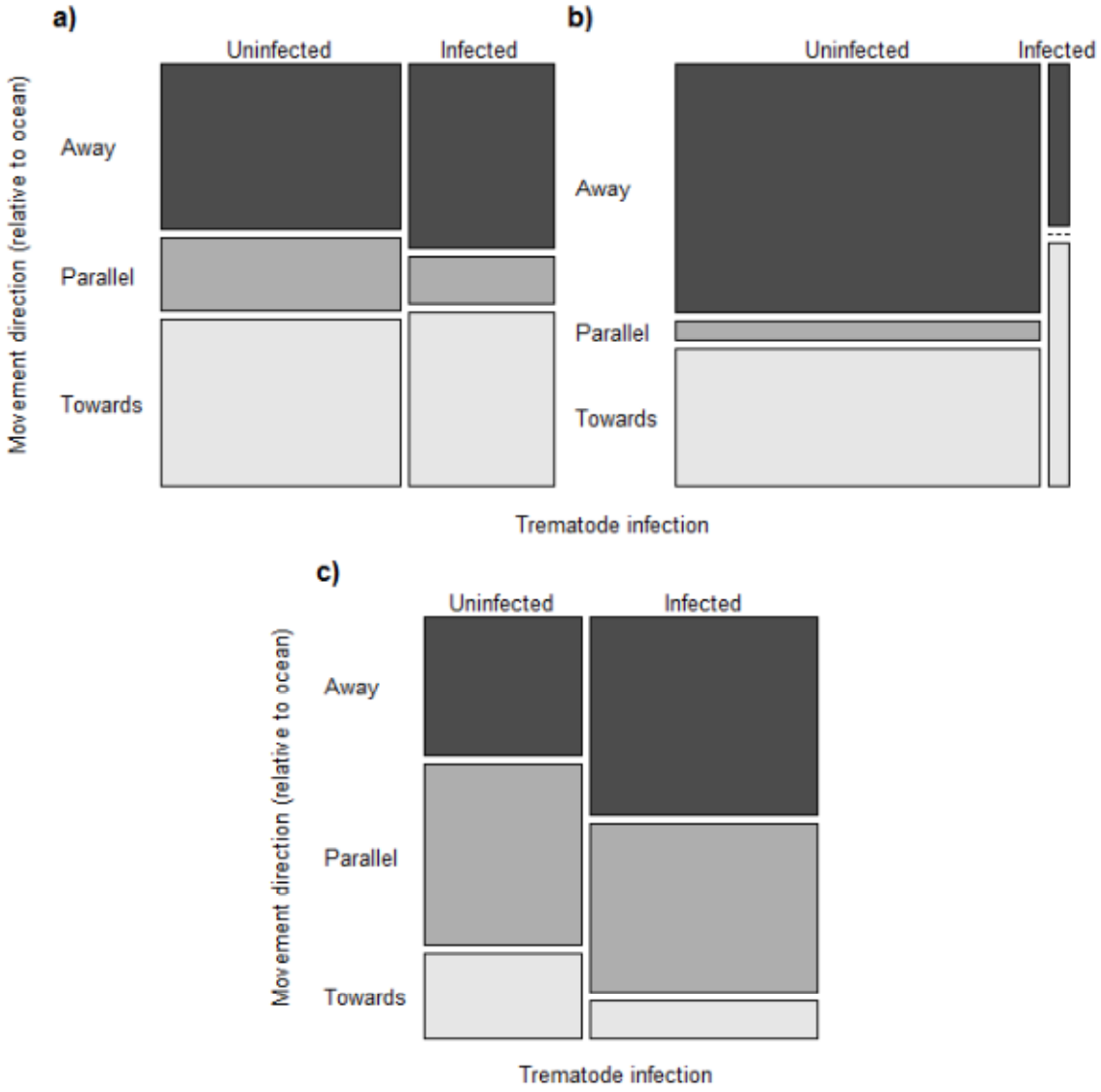

Figure 10. ***Field movement direction*****: Direction of movement relative to the ocean of *L. sitkana* according to trematode infection. a) Nudibranch Point; b) Ross Islet; c) Wizard Islet. Direction data are missing from Prasiola Point because snail movement was measured at that site before deciding to record direction. Data collected in 2012. Bar width is proportional to sample size.**

Movement laboratory experiment

A total of 181 snails (12.72 ± 0.09 mm shell height) collected in 2011 and 354 snails (13.22 ± 0.08 mm shell height) collected in 2012 were tested for movement patterns in the laboratory. Trematode species prevalence did not differ between years for snails used in movement trials; therefore, information of prevalence from both years was combined for further analysis. Six morphological trematode species were identified in snails from

both years, but two of those species were seen only in one snail, having prevalence < 0.01%. Prevalence of the four remaining species did not differ significantly among the four sites studied (Table 8). However, prevalence of *Himsathla* sp. is relatively high at Wizard Islet (Fig. 11). Trematode species richness differed among sites; Prasiola and Ross Islet had the four most prevalent trematode species whereas Nudibranch Point and Wizard Islet had three of the most prevalent trematode species (Fig. 11).

Table 8. ***Trematode prevalence by site*****: Significance levels of linear model for prevalence of the main four trematode species found at the four study sites. Data combined from movement trials under laboratory conditions with samples from 2011 and 2012.**

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
site	3	6.27	2.09	0.58	0.641
trematode species	3	17.37	5.79	1.61	0.254
Residuals	9	32.26	3.58		

Data from distance travelled of *L. sitkana* in the laboratory were transformed using square root values to improve normality. Overall, snails used for laboratory trials travelled longer distances than snails tested in the field. A significant 3-way interaction among site, infection status and snail sex occurred for distance travelled of *L. sitkana* (Table 9). Infected female snails travelled further than uninfected females, infected males and uninfected males at Nudibranch Point (Fig. 7). Infected males travelled longer distances than infected females and uninfected females at Prasiola Point (Fig. 7). Infected males traveled shorter distances than uninfected males at Ross Islet (Fig. 7). Uninfected males travelled the shortest distances when compared to uninfected females, infected males and infected females at Wizard Islet (Fig. 7). The 2-way interaction between snail sex and snail size was also significant (Table 9). Larger male snails travelled shorter distances than smaller males in the four sites (Fig. 8). Large females and small female snails from Prasiola Point and Wizard Islet travelled similar distances, whereas larger females travelled shorter distances than small females from Nudibranch Point and Ross Islet when tested under laboratory conditions (Fig. 8).

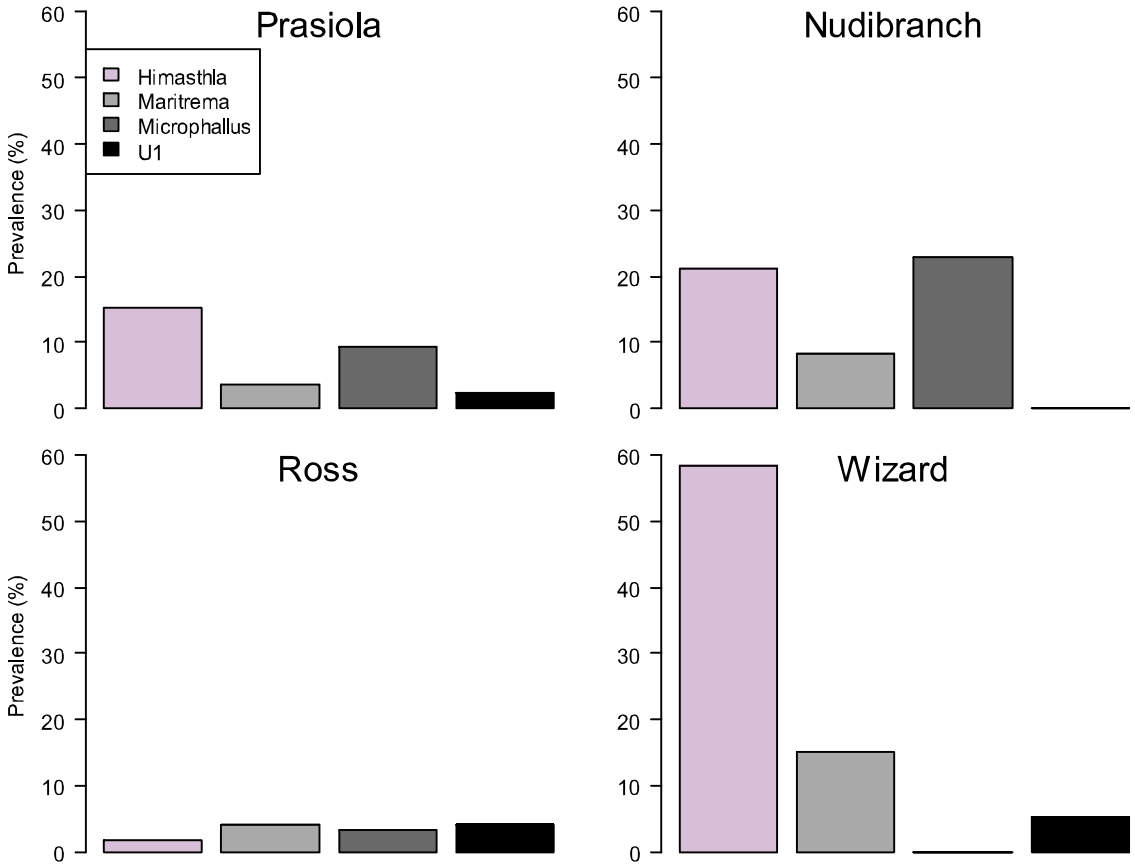

Figure 11. Laboratory trematode species prevalence: Prevalence (%) of all trematode species inhabiting *L. sitkana*. Data from movement experiments combined from both years studied.

Table 9. *Laboratory distance travelled (infected and uninfected snails)*: Significance levels of linear model with significant interactions for distance travelled by *L. sitkana* under laboratory conditions. Data combined for 2011 and 2012.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
site	3	692.10	230.69	20.76	< 0.001	***
infected	1	1.20	1.25	0.11	0.738	
snail sex	1	0.00	0.01	0.001	0.974	
snail size	1	296.50	296.55	26.68	< 0.001	***
site:infected	3	21.80	7.27	0.65	0.581	
site:snail sex	3	50.00	16.66	1.50	0.214	
site:snail size	1	3.10	3.12	0.28	0.597	
infected:snail sex	1	65.70	65.65	5.91	0.015	*
site:infected:snail sex	3	85.30	28.45	2.56	0.054	·
Residuals	517	5745.50	11.11			

*** Significant value at the <0.001 level
* Significant value at the 0.01 level
· Significant value at the < 0.10 level

Data for vertical displacement of *L. sitkana* in the laboratory were transformed directly to their square root value to improve normality and model fit as there were no negative values in this case. Snails displaced further vertically when tested in the laboratory than under natural conditions. The interaction between site and snail size was significant (Table 10), with the sites Nudibranch Point and Wizard Islet both showing a positive relationship between vertical displacement and snail size, while the other sites did not (Fig. 12). Snail sex had an almost significant effect on vertical displacement of *L. sitkana* (Table 10). Uninfected female snails displaced further vertically than uninfected males at Nudibranch Point, but not at Prasiola Point, Ross Islet or Wizard Islet (Fig. 9). Infection status (Table 10) did not significantly affect vertical displacement of *L. sitkana* in the laboratory (Fig. 9).

Table 10. ***Laboratory vertical displacement (infected and uninfected snails)*****: Significance levels of linear model with significant interactions for vertical displacement of *L. sitkana* under laboratory conditions. Data combined for 2011 and 2012.**

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
site	3	55.34	18.45	13.27	< 0.001	***
infected	1	0.19	0.19	0.13	0.715	
snail sex	1	5.08	5.08	3.66	0.056	·
snail size	1	7.41	7.41	5.33	0.021	*
site:snail size	3	18.14	6.05	4.35	0.005	**
Residuals	525	730.06	1.39			

*** Significant value at the <0.001 level
** Significant value at the 0.001 level
* Significant value at the 0.01 level
· Significant value at the < 0.10 level

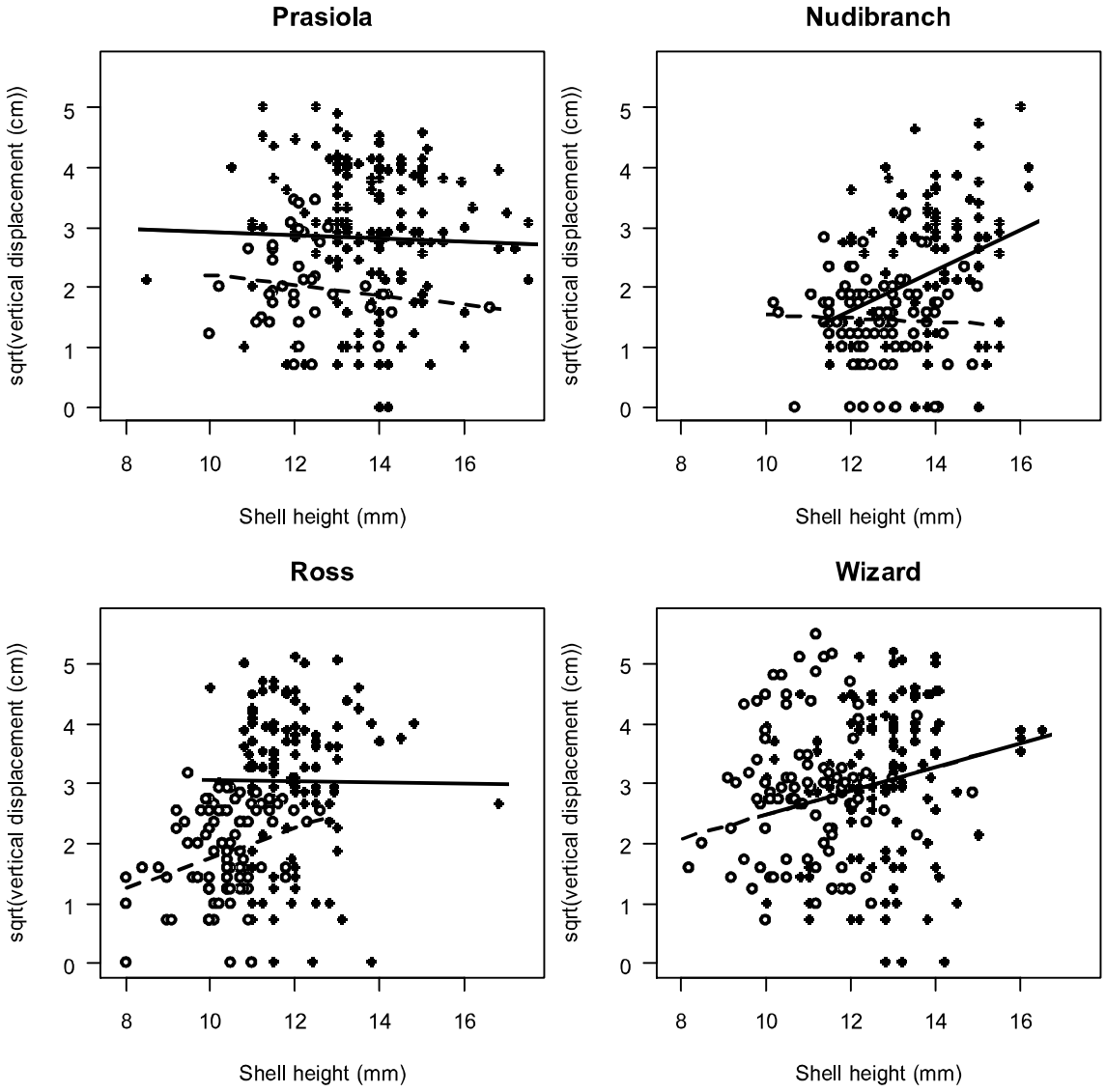

Figure 12. Field and laboratory vertical displacement: Correlation between vertical displacement and shell height of *L. sitkana* representing significant 2-way interaction between collection site and snail size (laboratory). Open circles = laboratory data; Filled circles = field data. Solid regression line = laboratory data; Short-dashed regression line = field data. Data combined from both years (laboratory); 2012 only (field). Displacement data transformed to their square root value.

Linear models used to test potential effects of trematode species on distance travelled by snails revealed a significant effect of snail size and snail sex on distance travelled for infected snails in Nudibranch Point, as indicated in analysis on all snails (Table 11).

However, trematode species did not significantly affect distance travelled by infected snails at any site (Table 11; Fig. 13).

Table 11. ***Laboratory distance travelled (infected snails only)*****: Significance levels of linear models for distance travelled of infected *L. sitkana* using trematode species as explanatory variable. Data from laboratory experiments from both years of study.**

		Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Prasiola Point	snail size	1	5.23	5.23	0.68	0.413	
	trematode species	4	54.27	13.57	1.78	0.151	
	snail sex	1	17.25	17.25	2.26	0.140	
	Residuals	43	328.64	7.64			
Nudibranch Point	snail size	1	77.88	77.88	4.84	0.034	*
	trematode species	5	75.88	15.18	0.94	0.464	
	snail sex	1	78.79	78.79	4.90	0.033	*
	Residuals	40	643.47	16.09			
Ross Islet	snail size	1	7.30	7.29	0.73	0.414	
	trematode species	3	24.78	8.26	0.82	0.510	
	snail sex	1	11.92	11.92	1.19	0.301	
	Residuals	10	100.27	10.03			
Wizard Islet	snail size	1	12.40	12.40	1.31	0.255	
	trematode species	4	73.84	18.46	1.95	0.109	
	snail sex	1	1.14	1.14	0.12	0.729	
	Residuals	91	860.46	9.46			

* Significant value at the 0.01 level

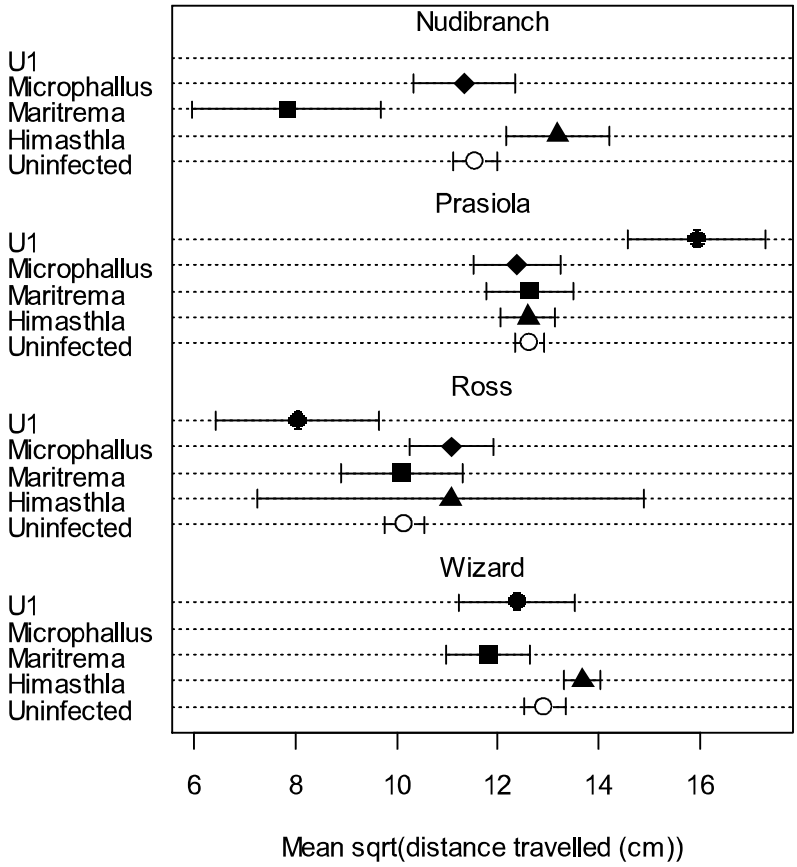

Figure 13. Laboratory distance travelled: Mean distance travelled ± SE of *L. sitkana* according to trematode species. Data combined from both years studied. Distance data transformed to their square root values.

The vertical displacement of *L. sitkana* from Prasiola Point in the laboratory revealed interactions between snail size and trematode species, as well as snail size and sex (Table 12). Snails infected with *Microphallus* sp. displace further vertically when compared with uninfected snails and snails infected with the other trematode species present at Nudibranch Point. Snails carrying *Maritrema laricola* infections displaced furthest vertically at Wizard Islet (Table 12; Fig. 14). This suggests that *Microphallus* sp. and *Maritrema laricola* have an effect on vertical displacement of *L. sitkana* at those sites.

Table 12. ***Laboratory vertical displacement (infected snails only)*****: Significance levels of linear models for vertical displacement of infected *L. sitkana* using trematode species as explanatory variable. Data from laboratory experiments from both years of study.**

		Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Prasiola Point	snail size	1	0.01	0.01	0.01	0.928	
	trematode species	4	8.42	2.11	2.63	0.049	*
	snail sex	1	0.02	0.02	0.03	0.862	
	snail size:trematode species	4	18.78	4.70	5.87	0.001	***
	Snail size:snail sex	1	5.28	5.28	6.61	0.014	*
	Residuals	38	30.37	0.80			
Nudibranch Point	snail size	1	8.47	8.47	6.98	0.012	*
	trematode species	5	22.72	4.54	3.75	0.007	**
	snail sex	1	0.33	0.33	0.27	0.605	
	Residuals	40	48.51	1.21			
Ross Islet	snail size	1	0.06	0.06	0.05	0.825	
	trematode species	3	3.57	1.19	1.03	0.421	
	snail sex	1	2.51	2.51	2.17	0.172	
	Residuals	10	11.55	1.15			
Wizard Islet	snail size	1	7.63	7.62	6.21	0.015	*
	trematode species	4	21.28	5.32	4.33	0.003	**
	snail sex	1	0.27	0.27	0.22	0.638	
	Residuals	91	111.74	1.23			

*** Significant value at the <0.001 level
** Significant value at the 0.001 level
* Significant value at the 0.01 level

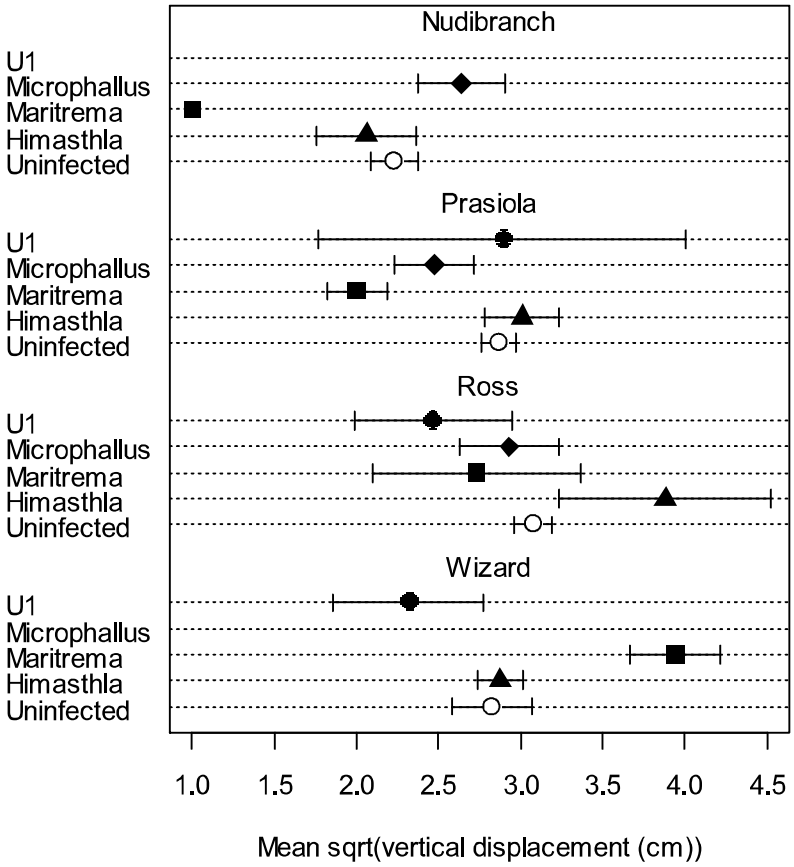

Figure 14. Laboratory vertical displacement: Mean vertical displacement ± SE of *L. sitkana* according to trematode species. Data combined from both years studied. Displacement data transformed to their square root values.

Feeding experiment

A total of 381 snails (13.27 ± 0.08 mm shell height) were used in this section, all of them collected at the four sites in 2012. Data from feeding trials were transformed using square root to improve normality. Significant 2-way interactions between site and snail size, and site and snail sex were present (Table 13). Larger snails consume more algae than smaller snails at Prasiola and Nudibranch Points, but not at Ross and Wizard islets (Fig. 15). Female snails feed more than males at Wizard Islet, whereas male snails eat more algae than females at Prasiola Point (Fig. 16). Infection status was lightly important for the amount of algae consumed by *L. sitkana* under laboratory conditions (Table 13). Infected snails tend to eat less algae than uninfected snails in Prasiola and Nudibranch Points as well as uninfected females in Ross Islet (Fig. 16).

Table 13. ***Feeding behaviour (infected and uninfected snails)*****: Significance levels of linear models with significant interactions for** ***U. intestinalis*** **consumed by** ***L. sitkana*** **under laboratory conditions in 2012.**

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
site	3	113.59	37.86	8.51	< 0.001	***
infected	1	14.22	14.22	3.20	0.075	·
snail sex	1	0.22	0.22	0.05	0.824	
snail size	1	37.67	37.67	8.47	0.004	**
site:snail sex	3	79.06	26.36	5.92	< 0.001	***
site:snail size	3	79.79	26.60	5.98	< 0.001	***
Residuals	368	1637.30	4.45			

*** Significant value at the <0.001 level
** Significant value at the 0.001 level
· Significant value at the < 0.10 level

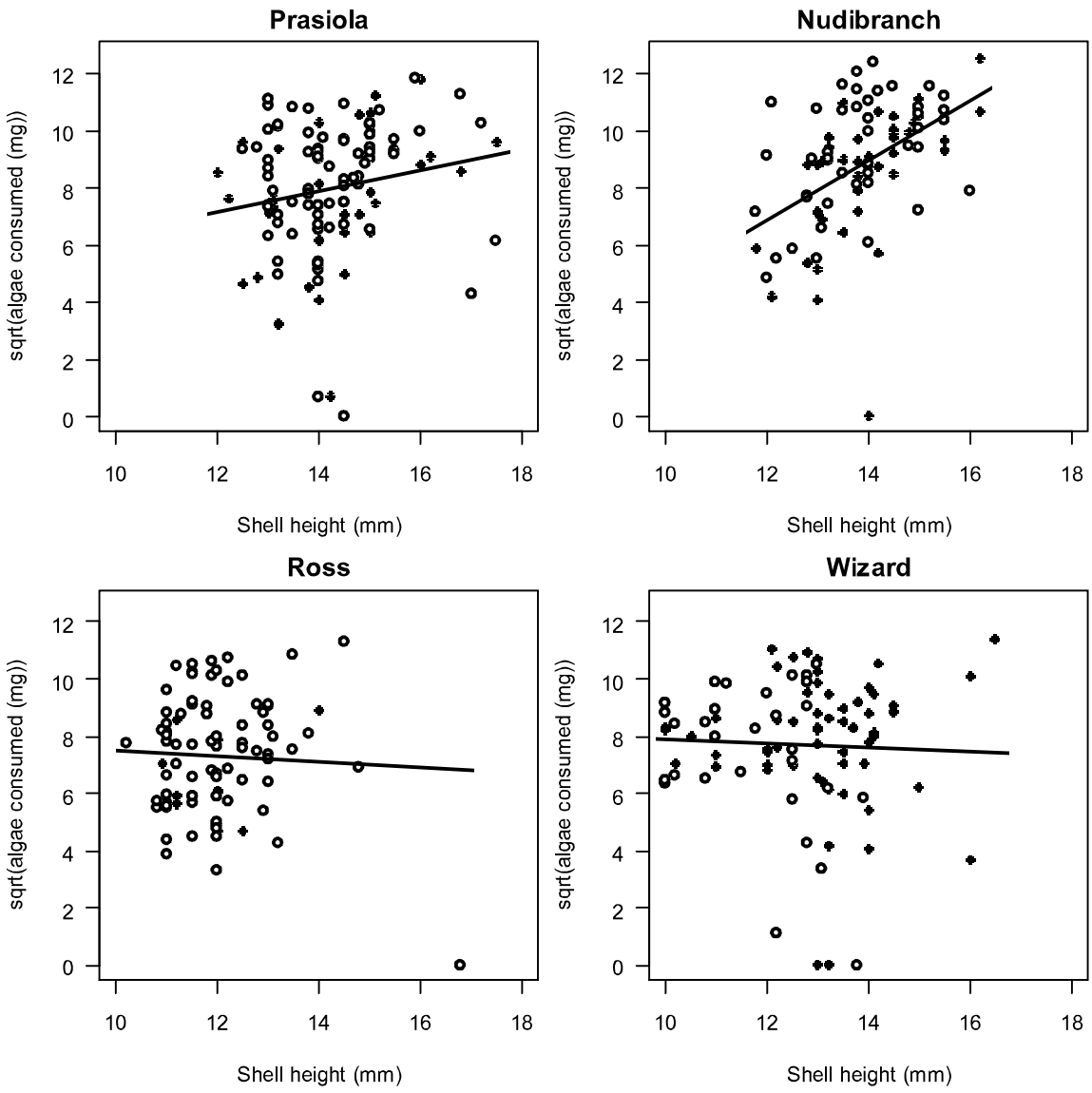

Figure 15. ***Feeding behaviour*****: Correlation between** ***U. intestinalis*** **consumed and shell height of** ***L. sitkana*** **per collection site representing significant 2-way interaction between these variables. Filled circles represent infected snails; open circles represent uninfected snails. Data from laboratory experiments in 2012. Amount of** ***U. intestinalis*** **consumed transformed to its square root value.**

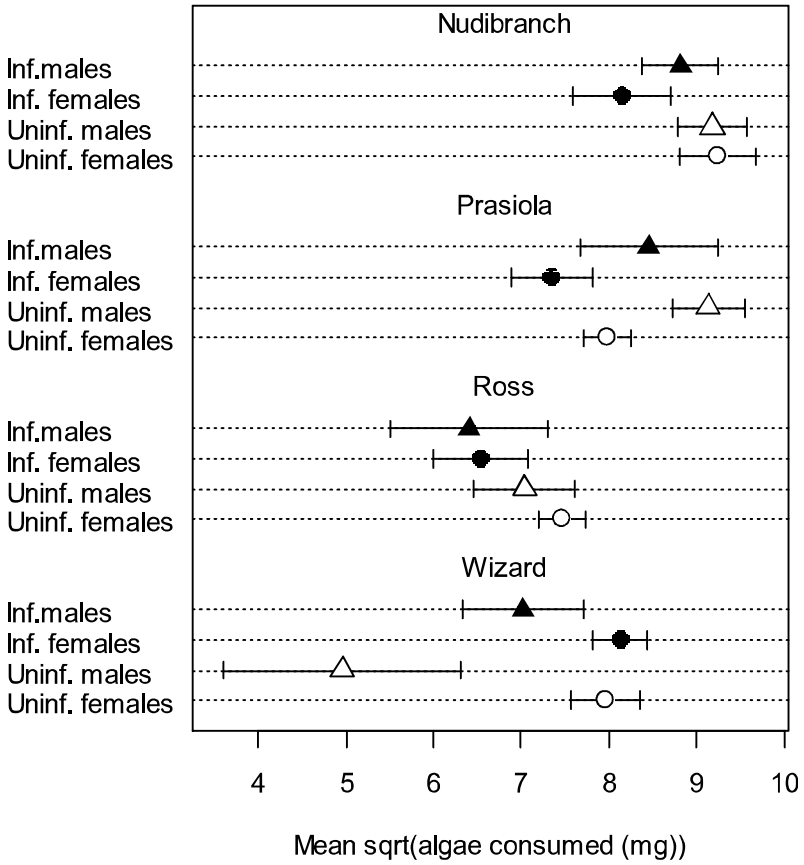

Figure 16. Feeding behaviour: Mean algae consumed ± SE by *L. sitkana* representing 2-way interaction between collection site and snail sex, and the almost significant effect of trematode infection. Data from laboratory experiments in 2012. Amount of algae consumed transformed to its square root value.

Trematode species had no effect on the amount of algae consumed by *L. sitkana* (Table 14; Fig. 17). As expected based on the above analysis, both Prasiola and Nudibranch Points showed a significant effect of snail size on the amount of algae consumed by *L. sitkana* under laboratory conditions (Table 14; Fig. 15).

Table 14. ***Feeding behaviour by site (infected snails only)*****: Significance levels of linear models for *U. intestinalis* consumed by infected *L. sitkana* using trematode species as explanatory variable. Data from laboratory experiments from 2012.**

		Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Prasiola Point	snail size	1	25.73	25.73	4.74	0.037	*
	trematode species	3	8.68	2.89	0.53	0.663	
	snail sex	1	11.01	11.01	2.03	0.165	
	Residuals	30	162.69	5.42			
Nudibranch Point	snail size	1	75.49	75.49	17.99	< 0.001	***
	trematode species	4	4.35	1.09	0.26	0.902	
	snail sex	1	3.51	3.51	0.84	0.367	
	Residuals	34	142.65	4.20			
Ross Islet	snail size	1	1.10	1.10	0.61	0.460	
	trematode species	2	11.07	5.53	3.08	0.110	
	snail sex	1	0.46	0.46	0.26	0.629	
	Residuals	7	12.58	1.80			
Wizard Islet	snail size	1	0.03	0.03	0.01	0.938	
	trematode species	3	2.50	0.83	0.16	0.923	
	snail sex	1	14.46	14.46	2.78	0.101	
	Residuals	53	275.60	5.20			

*** Significant value at the <0.001 level

* Significant value at the 0.01 level

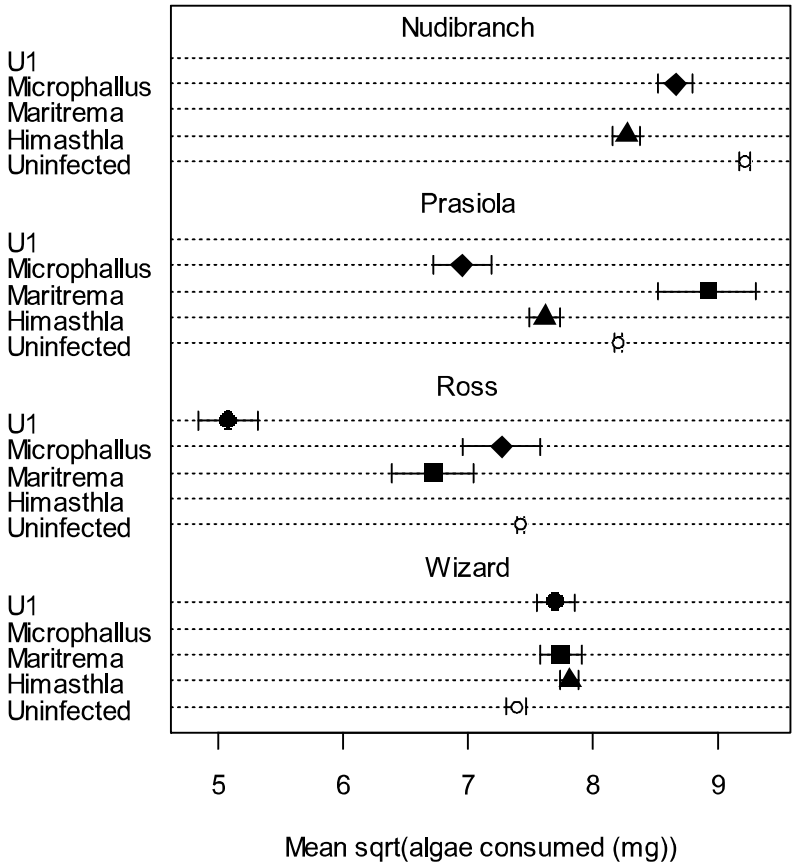

Figure 17. Feeding behaviour: Mean dry weight of *U. intestinalis* ± SE ingested by *L. sitkana* according to trematode species. Data collected under laboratory conditions in 2012. Dry weights of *U. intestinalis* consumed data were transformed to their square root values.

Snail size and trematode infection

Permutation t-tests from both years combined suggest that *L. sitkana* infected with trematodes are significantly larger than uninfected snails at Wizard Islet ($z = -4.68$, $P < 0.001$). However, shell height did not differ significantly between infected and uninfected snails in Prasiola Point ($z = -0.42$, $P = 0.68$), Nudibranch Point ($z = -1.61$, $P = 0.11$) and Ross Islet ($z = 0.65$, $P = 0.52$) (Fig. 15).

Sex ratio in samples

The samples from Nudibranch Point were very close to 1:1 sex ratio consisting of 56% females. Samples collected at Prasiola Point and Wizard Islet were female-biased with 74% females. Ross Islet was the most female-biased sample, consisting of 81% females.

Discussion

In this study, I show that trematode prevalence and species richness in the intertidal snail, *Littorina sitkana*, together with snail movement and feeding behaviour, vary significantly among localities that are geographically close to each other. This result is in keeping with previous suggestions that separate localities in a small geographical area might differ in trematode community composition (Kube *et al.*, 2002). Such differences among localities arise because trematode prevalence, intensity and species richness vary according to the species richness of definitive hosts within the same habitat (Hechinger and Lafferty, 2005). Different trematode communities might have differential effects on snail ecology depending on both biotic (e.g. invertebrate and vertebrate species richness and abundance) and abiotic components (e.g. wave exposure, substrate composition, temperature) present in their environment; hereafter I refer to these combined components as habitat composition. The majority of research on how trematodes affect their intermediate host's ecology and behaviour are focused on a particular population of intermediate hosts (Curtis, 1987; 1990; McCarthy *et al.*, 2000; Miller and Poulin, 2001; Miura *et al.*, 2006), and comparisons among different populations looking for more general patterns are lacking.

Behaviour in infected vs. uninfected snails

Snail movement and feeding behaviours are phenotypic traits that are often affected by trematode infection (Lambert and Farley, 1968; Williams and Ellis, 1975; Curtis, 1987; 1990; 2007; Levri and Lively, 1996; McCarthy *et al.*, 2000; Miller and Poulin, 2001; Miura *et al.*, 2006; Wood *et al.*, 2007; Clausen *et al.*, 2008). In this study, a 3-way interaction among site, infection status, and snail sex was present for distance travelled in the field (Table 6). In both Nudibranch and Prasiola Points infected males and females differed in distance travelled from uninfected snails, but in Nudibranch infected males moved less while in Prasiola both infected males and females moved more. Similar, though smaller, trends were observed for snails from these two sites in the laboratory study (Fig. 7). In Ross and Wizard islets, males and females responded similarly to trematode infection, increasing distance travelled in Ross infected male snails but decreasing distance travelled in Wizard infected male snails (Fig. 7). Results from the

laboratory showed the reverse trend, with Wizard infected male snails moving more and Ross infected male snails moving less (Fig. 7). It seems unlikely that the discrepancy observed between laboratory and field data in the islets is due to human error, as the same methodology was used to test the four sites. Distance travelled by *L. sitkana* infected with trematodes might be dependent on some environmental cue that was missing during the movement trials conducted in the laboratory (e.g. predator cues, food, rocky substrate), while snails from Prasiola and Nudibranch Points could either have environmental cues replicable under laboratory conditions (which seems unlikely) or their movement patterns might be less dependent on environmental conditions than movement of infected snails from the islets.

My findings regarding shorter distances travelled by snails infected with trematodes are in agreement with previous research. Miller and Poulin (2001) suggested that decreased movement is simply a pathological response of the snail to trematode infection. This explanation is plausible for Nudibranch Point where male snails infected with trematodes travelled shorter distances. However, this explanation seems unlikely for Prasiola Point and Ross Islet in the field and Wizard Islet under laboratory conditions, where infected snails moved longer distances than uninfected snails, contrary to the behaviour expected as a pathological result. Mouritsen and Jensen (1994) proposed that trematode infection and crawling involve high energy costs to the snail. Thus a decrease in movement would be a way to overcome the energy demand caused by trematodes (Mouritsen and Jensen, 1994). This could be the case for infected snails sampled at Ross Islet that were more active in the field, but less active under laboratory conditions where food was not available.

The greater distance travelled of infected snails could be a mechanism of trematode larval dispersal if transmission to the next intermediate host, depends not on predation, but rather on an increased probability of contact between trematode larvae and the second intermediate host (e.g. Curtis, 1987). If cercarial release occurs while snails crawl from one place to another in the intertidal zone and cercariae use mucus trails to disperse, inhabiting a snail that travels longer distances would increase cercarial dispersal. Direction of distance travelled was not affected by trematode infection in this study, suggesting that, in this system, only the distance covered by infected snails is important

rather than where the infected snails move to, contrary to the suggestion of Miller and Poulin (2001). It is also possible that my study design lacked sufficient power to detect an effect of trematode infection on direction of snail movement, and that differences in habitat complexity play an important role on snail movement which my methodology was not able to detect. Further research is necessary in order to find the next intermediate host for the trematode species present in this study; this will be key to finding a plausible explanation for the behavioural differences between infected and uninfected snails. From previous research, second intermediate hosts most common for the trematode species found here are blue mussels (*Mytilus edulis*) and shore crabs (*Hemigrapsus oregonensis* and *H. nudus*) (Ching, 1991). Blue mussels and shore crabs are commonly found at my study sites; trematode cercariae access to these hosts by penetration of the host tissue rather than predation (Ching, 1991). However, one species of trematode found here (*Microphallus* sp.) does not shed cercariae, is believed to lack a second intermediate host, and is usually found as adult in the intestines of common eiders, ducks, sea gulls and sandpipers (Saville *et al.*, 1997). The transmission of *Microphallus* sp. would necessarily involve predation on snails by birds, and I found shells showing damage typical of beak action made while birds feed on snails. But I did not directly observe birds eating snails during this study. Bird exclusion experiments under field conditions testing if birds prey directly on *L. sitkana* are necessary to confirm that bird predation is taking place in my study sites or to propose an alternative pathway for trematode transmission in this case.

It has been proposed that trematodes inhabiting a snail intermediate host vary their behavioural manipulation according to the availability of the second intermediate host and resources in the ecosystem (Curtis, 2007). In the case of Wizard Islet, infected snails might travel longer distances in the laboratory because they are constantly looking for food, as food was not supplied during movement trials. In contrast, in the field, microalgae and seaweeds are not limiting and moving shorter distances would save energy. Some of the behavioural differences I found among localities during this study could be explained if trematodes use different second intermediate hosts depending on the habitat composition of each locality. Trematode infection at Prasiola Point does not seem to have an important effect on distance travelled by *L. sitkana*. This could be because the next intermediate host in that site interacts with the snails on a regular basis

and contact between trematode cercariae and second intermediate host takes place regularly. If contact between swimming cercariae and second intermediate host is common in Prasiola Point, host manipulation in this system would be unfavourable due to its high costs for the parasite (Poulin, 1994; Poulin *et al.*, 2005). I observed several individuals of blue mussels, purple shore crabs, sculpins and amphipods sharing habitat with *L. sitkana* at Prasiola Point, all of them potentially suitable second intermediate hosts for trematodes. A project involving more extensive invertebrate species richness evaluation and/or dissection is necessary to propose potential second intermediate hosts for trematode larvae in both islets in order to understand the snail behavioural patterns observed here.

My results are comparable to previous work finding differential dispersal and movement of infected snails. Although my analysis suggests that snails infected with trematodes tend to be more active than uninfected individuals, infected snails tested here do not seem to react to rising and/or receding tide as has been proposed for other species of *Littorina* (McCarthy *et al.*, 2000). Locality once again is one of the main factors influencing vertical displacement of snails. There are differences between vertical displacement of snails observed directly in the field and snails tested under laboratory conditions. While trematode infection had a small effect on snail vertical displacement in the field, it did not show important effects on vertical displacement when tested under laboratory conditions. This might be related to some environmental cue that was missing during laboratory trials. The differential presence of an environmental cue cannot be tested using the data from this study, thus experimentation with a more natural setup is necessary to determine and resemble the role of environmental cues on vertical displacement of *L. sitkana*. The greatest difference between the results of both laboratory and field data is related to snail size. Whereas snail size plays an important role on vertical snail displacement in the laboratory, it does not seem to be relevant when tested in the field. One plausible explanation for this could be the large standard error of shell height from snails tested in the field (shell height was more variable for the snails used in field experiments as larger snails were kept in the laboratory because they are easier to handle), making it difficult to detect an effect of size classes on snail vertical displacement.

Mean algae consumed was less in infected than uninfected snails for three of the four sites studied; infection status of snails significantly affected snail algae consumption at the level of $p < 0.10$. Decreased feeding of trematode-infected individuals has been observed for other species of *Littorina* (Wood *et al.*, 2007; Clausen *et al.*, 2008). The decrease in algae consumption has been hypothesized to be a product of host castration, slow or stunted growth or damage to digestive tissue. It is not possible to distinguish among these possibilities with the data from this study, but all the identified species of trematodes found here are related to known parasitic castrators of their snail host (Levri, 1999; Sorensen and Minchella, 2001; Hechinger *et al.*, 2009), and are likely host castrators themselves as infections can be very heavy and all gonad tissue is replaced by trematode larvae. Furthermore, as all trematodes found in this study were found inhabiting the reproductive and digestive glands of their snail host, direct damage to the tissue of the snail is also a plausible explanation for lower feeding rates of infected snails. Damage caused to reproductive and digestive glands does not seem to lower snail survival (see results from Chapter 2). Snails can live for more than 48 hours without these glands once their shells are crushed (pers. obs.). Wizard Islet is the only site where snail grazing rate of infected snails did not differ when compared to uninfected snails. Interestingly, Wizard Islet showed the highest trematode prevalence, larger size of infected snails, and largest distances travelled in the laboratory among the four sites. This suggests that Wizard Islet is the most different of the four sites studied, though the reason(s) for these differences remain unclear. The lack of difference in algae consumed between infected and uninfected snails at Wizard Islet might be caused by the higher activity level of infected individuals; infected snails would have higher energetic demands allocated to crawling than uninfected individuals. The extra energy spent crawling might require these snails to graze more despite the tendency to graze less in snails infected by trematodes. If this is true, the combination of trematode infection and high activity levels could equalize the amount of algae consumed between infected and uninfected snails.

Infected snails are bigger than uninfected snails at Wizard Islet, in accordance with previous findings in marine snails (Mouritsen and Jensen, 1994; Miller and Poulin, 2001; McCarthy *et al.*, 2004). Different explanations have been proposed to explain larger shell

sizes of infected snails. Freshwater snails infected with trematodes can grow faster and get to larger sizes than uninfected individuals (a process known as gigantism) (Rothschild, 1936), but this explanation lacks convincing support in the marine system (but see Miura *et al.*, 2006). Thus, here I adopt the explanation proposed by Miller and Poulin (2001) that larger snails are simply older than smaller snails, have therefore been exposed to trematodes for longer, and are thus more likely to have contracted parasites. This difference of snail size could also be a factor influencing the similarity of foraging rates in infected and uninfected snails at Wizard Islet. Larger snails tend to eat more than smaller snails. The lowering effect of trematode infection on consumption rate of *L. sitkana* might also be obscured by the increasing effect of size on grazing rates of the snails at Wizard Islet, resulting in similar algae consumption rates between infected and uninfected snails. It is necessary to calculate the energetic cost that snail size, crawling and trematode infection pose on *L. sitkana* to test this hypothesis, and further research involving trematode/snail physiology and energetic flows are in order.

Differences among trematode species

Host behavioural manipulation by trematodes often depends on the species of parasite inhabiting a certain intermediate host (Poulin and Forbes, 2012). Trematode species differently affected vertical displacement of *L. sitkana* under laboratory conditions for both Nudibranch Point (where snails infected with *Microphallus* sp. displace further vertically than snails infected with any other trematode species) and Wizard Islet (where snails infected with *Maritrema laricola* displace the furthest vertically), suggesting that at least two trematode species are important behavioural manipulators at these sites. These trematode species have been noted to use birds as definitive hosts in nature (Ching, 1963; Yamaguti, 1975). *Microphallus* is only found in sporocyst and metacercariae stages inside snail hosts, lacking a free swimming cercarial stage for transmission and dispersal. For this reason, it has been suggested that *Microphallus* transmission to the definitive host requires direct predation of the snail host by the bird definitive host (Saville *et al.*, 1997). If this is true in my study system, *Microphallus* sp. transmission would be enhanced if infected snails were in closer contact with birds (i.e. climbing further upwards where birds are more likely to see them) as has been proposed for *L. saxatilis*

infected with *Microphallus piriformes* (McCarthy *et al.*, 2000). I did not see evidence of direct predation of *L. sitkana* by birds in any of my study sites, probably due to the short time I spent working in the intertidal zone and also because my presence in the sites while I was working would have disturbed birds and affected their normal behaviour. More bird observation and feeding behaviour studies or bird exclusion experiments in the field are necessary in order to support or refute this hypothesis.

Ching (1963) described the life cycle of *Maritrema laricola* in Vancouver, British Columbia and found this trematode species as adult form in sea gulls, as cercariae in *L. sitkana* and encysted metacercariae in *Hemigrapsus nudus*; all of these hosts are present in my study sites, suggesting the same life cycle could be taking place here. Cercarial transmission from snail to crab in this case is thought to be achieved by cercarial release from the snail. After leaving the snail, cercariae swim freely until finding a shore crab and penetrating soft tissue. In this case, trematode transmission does not involve predation. Purple shore crabs have been observed to travel to the supratidal zone to feed on amphipods and seaweed debris (Lewis *et al.*, 2007). This suggests that purple shore crabs spend long periods of time at relatively high tide zones. Moreover, during this study at Wizard Islet, I observed several purple shore crabs hiding in rock crevices located above tide pools where marked snails were released. Without a larger sample size it is not possible to verify if the movement pattern of snails infected with *M. laricola* is expressed in the field in the same way I saw them under laboratory conditions and if the vertical distance displaced by infected snails gets them closer to purple shore crabs located in the rock crevices. Although, if these conditions are met, cercarial release from the snail higher in the intertidal zone and closer to purple shore crabs would increase the probability of trematode transmission success. Field experiments involving a larger number of infected snails mixed with cercariae trapping systems at intertidal zones where purple crabs are found are necessary to confirm this or to propose an alternative explanation for the larger vertical distances displaced by snails infected with *M. laricola* from Wizard Islet.

Despite the differences in vertical displacement observed under laboratory conditions, I could not detect important variation in snail distance travelled or amount of algae consumed related to trematode species. It was also not possible to test for effects of

maturity of infection on *L. sitkana* movement and feeding behaviours because of the few snails carrying immature infections. Trematode prevalence in mudsnails diminishes over winter months (Kube *et al.*, 2002). If trematode infection also diminishes during winter at my study sites, further research taking place earlier in the year might be more successful in finding more immature infections than the present study, making it possible to look for effects of maturity of infection on snail behaviour.

Snail trait effects

Sex - Snail gender affected distance travelled of *L. sitkana* at Prasiola and Nudibranch Points in opposite ways: male snails travelled longer distances than female snails at Prasiola Point, but females moved more than male snails at Nudibranch Point. Results from Prasiola Point are in accordance with previous findings from other species of freshwater snails (Michel *et al.*, 2007; Valentine-Darby *et al.*, 2011). One proposed hypothesis explaining this difference in movement states that male snails spend more time moving and looking for potential mates to increase their reproductive success and thus travel longer distances to enhance their probability of encounter with receptive females, whereas females do not need to look for potential mates to increase their reproductive success (Valentine-Darby *et al.*, 2011). This seems improbable in Prasiola Point because the samples tested had a female-biased sex ratio of 76%, suggesting male competition for mates is not that strong. However, it has also been proposed that in some species of snails, females store sperm and reject male attempts to mate, increasing male competition and causing them to move more to find receptive females (Michel *et al.*, 2007). *L. sitkana* has a sperm storage organ (Buckland-Nicks and Chia, 1990), suggesting females might reject male attempts to mate and therefore increase male competition. It has also been noted that male *Littorina* tend to reject females that have had recent sexual encounters to maximize male reproductive success (Zahradnik *et al.*, 2008). Whether the combination of both female and male rejection of potential mates can increase male competition enough to counteract the effects of female-biased sex ratio remains to be tested.

In Nudibranch Point, female snails travelled longer distances than did males. The most logical explanation for this result is that females move longer distances simply because

they are bigger than males at this site. This explanation would be in agreement with Miller and Poulin (2001). Further, the almost 1:1 (i.e. 56% females) sex ratio observed in the samples from Nudibranch Point suggests increased competition between males, this may explain increased movement in females if they are subject to male harassment. It has been documented in fish that male harassment can induce male-avoidance behaviours in females (Weir, 2013); this has not been studied in snails but would help to explain why females travel longer distances than males in the only population sampled in this study that was not strongly female-biased. Snail sex does not seem to be an important factor influencing distance travelled in either of the islets. Ross Islet was female-biased at approximately 81% and there was no significant difference on shell height between the sexes. On the other hand, Wizard Islet was female biased at 74% and male snails were slightly larger than females. I could not find a general pattern to describe distance travelled that was common among the four sites studied, reinforcing the idea that snail movement is highly dependent on locality and its habitat composition and highly complex to analyze without knowing more variables that might have an influence on snail displacement.

Body size - Larger snails from both Nudibranch Point and Wizard Islet displace further vertically when tested in the laboratory and this might reflect anti-predatory responses in the snail. Larger snails are preferred prey for predatory crabs (e.g. *Cancer productus*) and larger snails tend to move further above the water level to avoid predation by crabs that feed only when totally submerged (Rochette and Dill, 2000). This idea could be taken further to explain why snail vertical displacement at Nudibranch Point was the shortest among the four sites if abundance of predatory crabs is also lowest at that site. Another potential explanation for shorter vertical displacement at Nudibranch Point could be presence of predatory birds at that site that would make snails avoid going too high in the intertidal zone to avoid predation. Studies assessing predator crab and bird presence and abundance in each of the four sites studied here are required to test the hypothesis that differential predator presence among sites is linked to differential anti-predatory response.

Foraging behaviour - Feeding behaviour of *L. sitkana* in this study depended mainly on locality where snails were collected, snail size and sex. This outcome is in accordance

with previous findings (Clausen *et al.*, 2008). Larger snails eat more than smaller individuals and the most plausible explanation is intuitive: larger individuals need more food to fulfill their energy demands than smaller individuals. Females feeding more than males of the same size also makes biological sense, as females invest more resources in the production of eggs. However, male snails from Prasiola Point ate more algae than females, in contrast with Nudibranch Point, the two islets and previous studies using *Littorina* species (Clausen *et al.*, 2008). An explanation for higher feeding rates of males in Prasiola Point is not immediately apparent. When observing patterns in the results, males travel greater distances than females at Prasiola Point. The combined results can be explained from two perspectives: 1) Males need more food resources because they are more active than females; or 2) Males are more active because they are more frequently looking for food. This question cannot be answered with the methodology followed in this project and movement trials where tested snails are provided with unlimited food would help to distinguish cause from effect.

Other potentially important factors for energy requirements of male snails are body size and/or reproductive effort. Shell height is slightly smaller in male snails at Prasiola Point, suggesting snail size hypothesis is not likely for this site. Reproductive effort could be a reasonable explanation for excessive energy demands in males at Prasiola Point, as sex ratio was female-biased (74% female), giving male snails the opportunity to maximize number of mates and increase their reproductive success with the cost of increased sperm production. Direct male competition for females could be increased if male rejection takes place at Prasiola Point, forcing males to spend more energy competing directly with other males for a suitable mate. Males of *L. subrotundata* express aggressive competitive behaviours when placed together with a single female (Zahradnik *et al.*, 2008). If the same kind of male competition is present in *L. sitkana*, aggressive encounters among males would help to explain the higher energetic demands of males.

Conclusions

Trematode infection in snails has been proposed as driver of ecological communities in the intertidal zone due to differential grazing activity of infected individuals (Wood *et al.*,

2007). At both Prasiola and Nudibranch Points, snails infected with trematodes tend to eat smaller amounts of algae than uninfected snails. This difference in grazing rates in addition to the relatively high trematode prevalence that I found in these sites suggest that trematode infection might have an important effect on the algal composition of the intertidal zone in these localities. Wood *et al.* (2007) demonstrated that *L. littorea* infected with trematodes tend to eat less algae than uninfected snails, and through field experiments showed that the algal diversity in the intertidal zone was higher on patches containing infected snails than on patches with uninfected snails. These authors observed that trematode presence in the intertidal zone changed the availability of edible algae also consumed and/or used as shelter by other organisms, thus potentially influencing the intertidal invertebrate community as well. However, this effect is most likely not important at Ross Islet where trematode prevalence was so low. Despite the high trematode prevalence present at Wizard Islet, the effects of trematode infection might be irrelevant to algal diversity at that site as infected and uninfected snails tend to eat similar amounts of seaweed. Further field experiments of snail feeding behaviour at these sites is necessary to ensure that results in the field match those observed under laboratory conditions, something that was not true for distance travelled in both islets.

Trematode infection in *L. sitkana* varies depending on the habitat composition of the locality studied; habitat composition also modulates the effect of trematode infection on the snail intermediate host. It is therefore important to bear in mind that host manipulation by parasites is not a process that happens under every circumstance, but rather that it depends on the environmental composition of the study site, and the identity and availability of the second intermediate host in the system (Poulin, 2010; Thomas *et al.*, 2005). This thesis suggests that variation in host manipulation stands even among close geographic sites in the presence of very similar trematode communities inhabiting the same snail host species. It is also noteworthy that snail dispersal proved to be a complicated behaviour with many unknown potential components that are independent of trematode infection; this study shows that dispersal behaviour varies depending on environmental conditions and habitat composition.

Chapter 4: Conclusions and future research

In this thesis, I studied the effects of trematode infection on survival and behaviour of their intermediate host, the intertidal snail *Littorina sitkana* and potential effects of infection on the ecology of this snail species. I found that trematode species richness and prevalence differed significantly among the four sites studied. This result was somewhat expected based on previous studies stating trematode communities vary with locality (Kube *et al.*, 2002; Faltynkova *et al.*, 2008). My results were also surprising, as I was expecting sites located on mainland to be more similar between them when compared to the islands and vice versa. However, I found that trematode communities were most different between the two islets: Wizard Islet had the highest trematode prevalence but the lowest trematode species richness among the four sites studied, while Ross Islet had the lowest trematode prevalence among the four sites but higher species richness than Wizard Islet. Trematode communities also differ between sites as similar in abiotic components and as close to each other as Prasiola and Nudibranch Points, which are located only 550 m apart.

Previous research shows trematode infection decreases snail host survival by increasing predation risk on the snail, decreasing heat tolerance of the host, damaging host tissue or by creating immunological compatibilities, or because infected snail hosts have an increased energy demand to support themselves and their trematode parasites (McDaniel, 1969; Minchella, 1985; Sousa and Gleason, 1989; Huxham *et al.*, 1993; Sorensen and Minchella, 2001; Sandland and Minchella, 2003; Fredensborg *et al.*, 2005; Gorbushin and Iakovleva, 2008; Koprivnikar and Walker, 2011). I tested these assumptions by correlating snail survival under laboratory and field conditions with trematode species prevalence at each study site. Based on the differences observed on trematode community composition among my study sites, and presuming trematode presence and prevalence are negatively correlated with snail survival, I expected Wizard Island to have the lowest snail survival and Ross Islet to have the highest snail survival in both experimental designs during this study. As expected, Wizard Islet showed the lowest snail survival under field conditions. Nevertheless, Ross Islet had slightly higher survival than Wizard

Islet, while the sites located on the mainland had highest snail survival from the four sites. On the other hand, snails kept under laboratory conditions showed significantly higher survival when compared to snails in the field, and no significant difference among sites was observed. These findings suggest that trematode infection has no direct negative effect on snail survival.

Trematode parasites are known to alter their snail host's behaviour to increase trematode transmission success and complete their life cycles (Badie *et al.*, 1973; Johnson *et al.*, 2001; 2002; Manga-González *et al.*, 2001; Stopper *et al.*, 2002; Goodman and Johnson, 2011; Szuroczki *et al.*, 2012). Some species of trematodes alter dispersal of their snail host to increase the probability of encounter between free swimming cercariae released from the snail and the trematode's next host (Curtis, 1987; 1990; Miura *et al.*, 2006). This seems to be the case for *Maritrema laricola* because snails infected with this trematode travel further vertically than either uninfected individuals or snails infected with other trematode species at Wizard Islet. Trematode species that develop into metacercariae inside their snail host manipulate the snail's behaviour to move closer to their bird definitive host, increasing predation risk of infected snails by foraging birds (McCarthy *et al.*, 2000). This may be the explanation for behavior of snails infected with *Microphallus* sp. at Nudibranch Point, which travel further vertically than uninfected snails or snails infected with other trematode species at this site.

Snails infected with trematodes tend to eat less algae than uninfected individuals according to previous research (Wood *et al.*, 2007; Clausen *et al.*, 2008). My findings are in accordance with this hypothesis; in Prasiola and Nudibranch Points and Ross Islet, infected snails fed less than uninfected individuals under laboratory conditions. Littorinid snails are very abundant in rocky intertidal ecosystems, and can regulate algal diversity of their community by feeding preferentially on some algae species over others. From this perspective, trematode infection in *L. sitkana* might have ecological consequences at Prasiola and Nudibranch Points where over 15% of the snail population is infected with at least one species of trematode.

The majority of studies on trematode effects on their host ecology are based at a local scale and rarely compared with other localities looking for more general patterns. With this thesis, I showed that trematode infection and its effects on snail hosts vary according

to locality and can seldom be generalized, suggesting that environmental characteristics may play a more important role in intertidal snail ecology than trematode infection on its own, and have to be taken into account when studying effects of trematode infection on their intermediate host before drawing conclusions on the system.

Snails collected at the two Islets were significantly smaller than snails collected on the mainland. This fact might be related to the lower survival rates observed at the Islets and could be caused by differential wave action, temperature, humidity, landscape formation or substrate texture, as any of these factors or a combination of them might play an important role on survival of larger snails.

In this thesis I focused on getting high quality estimates of survival at multiple field sites. This compromised my ability to measure additional environmental variables that in hindsight would have been useful. Future research involving measurements of wave exposure, temperature and humidity are necessary for finding an explanation for my results. Further research involving thorough trematode surveys in different invertebrate taxa are required to find the next intermediate host for the trematode species found at my study sites. It is also necessary to make vertebrate species richness and abundance surveys to locate potential definitive hosts for the trematode species found at my four study sites. By doing this, the life cycle of these trematode species could be traced and the hypothesis of host manipulation as means of enhanced trematode transmission tested. These studies are necessary, as my results suggest that changes in snail host behaviour caused by trematodes, and survival of *L. sitkana* are highly dependent on environmental conditions and second intermediate host/definitive host availability.

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