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Trematode parasites of the mudsnail Ilyanassa obsoleta: An analysis of parasite communities at different scales

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TREMATODE PARASITES OF THE MUDSNAIL *ILYANASSA OBSOLETA*: AN ANALYSIS OF PARASITE COMMUNITIES AT DIFFERENT SCALES

BY

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DISSERTATION

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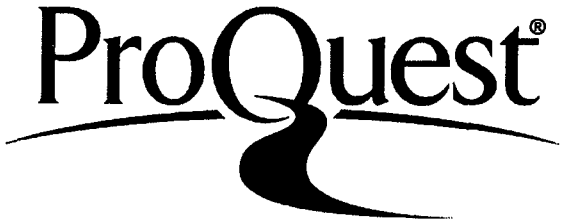

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DEDICATION

To TWD for taking the long way home.

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ABSTRACT

TREMATODE PARASITES OF THE MUDSNAIL *ILYANASSA OBSOLETA*: AN ANALYSIS OF PARASITE COMMUNITIES AT DIFFERENT SCALES

by

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University of New Hampshire, May, 2010

This research examines the ecological factors that shape trematode parasite communities of mudsnail *Ilyanassa obsoleta* at three different spatial scales. Nine species of trematode which obligately infect *I. obsoleta* during larval stages but use numerous estuarine species as second intermediate and definitive hosts are considered. The work provides the most geographically extensive examination to date of this trematode parasite community.

At the broadest scale, *I. obsoleta* trematodes were examined across their distributional range (Chapter 2) which includes both native and introduced populations. The results demonstrate that introduced trematode communities are characterized by lower abundance and diversity compared to native communities and therefore conform to the pattern predicted by the enemy release hypothesis. The ecological factors that contribute to the establishment of specific *I. obsoleta* trematodes in the introduced range are considered.

A regional scale analysis of *I. obsoleta* trematode communities is presented in Chapter 3. Trematode abundance and diversity along with a wide variety of biological, chemical, and physical factors was examined at fifteen salt marsh sites located throughout northern New England, USA. Although the abundance of numerous hosts were measured as part of this work, variables found to be most strongly correlated with trematode abundance and diversity at sites (revealed through multiple regression analysis) were of physical and chemical origin including sediment nitrogen, roads, trace metals and the distance of sites from the ocean. The results are explored in the context of a variety of candidate mechanisms.

Chapter 4 focuses on *I. obsoleta* trematodes at a local scale within a single salt marsh site. The work examines intra- and inter-annual patterns of trematode infection in snails associated with four distinct salt marsh habitat types. Experiments were conducted to assess the importance of key processes in determining infection patterns including acquisition of infection by *I. obsoleta*, mortality, movement, and demographics of the snail hosts. Results indicate that patterns of infection among the saltmarsh habitats are subject to strong shifts over time. Changing demographics and snail movement (but not infection input) are likely to be the strongest factors contributing to changing infection patterns across habitats in this system.

CHAPTER 1

INTRODUCTION

Trematode parasites of the mudsnail *Ilyanassa obsoleta*: An analysis of parasite community structure at different scales

Habitat provides an important role in the development of ecological communities because it serves as the template upon which communities develop (Southwood 1977). However, from a research standpoint identifying what constitutes essential habitat for most ecological communities is difficult as a myriad of variables are likely involved. Parasite communities are ideally suited for the study of community dynamics because their habitat is uniquely defined by the host which they rely on for numerous resources including food, shelter, and transport (Price 1990). In contrast, determining appropriate sampling units to examine free-living communities may be highly difficult. The unique association between parasites and their host habitats has led to the use of parasite communities to test theories that lie at the heart of community ecology such as species packing and niche theory (Lawton 1984, Bush and Holmes 1986, Stock and Holmes 1988). In addition to these local-scale processes which occur between individuals or

groups of individuals, processes that occur at regional and global scales are also recognized to play a critical role in determining the structure of communities (Gaston 2000, Godfray and Lawton 2001). Here again, parasites provide a particular research advantage since the effects of scale-dependent processes on community assemblage can be isolated from the effects of variable habitats.

This research examines the determinants of community structure in a group of digenetic trematode parasites at three spatial scales associated with their larval snail host. Digenetic trematodes (phylum: Platyhelminthes) commonly require three distinct hosts to complete their life cycle. Larval trematodes develop inside mollusc first intermediate hosts which, once infected, remain so for life. Inside this host, trematodes undergo asexual reproduction and when conditions are suitable, free-swimming stages of the parasite emerge into the environment and have a short period of time to seek out and infect an appropriate second intermediate host. Completion of the trematode life cycle occurs when a definitive vertebrate host acquires the parasite through ingesting an infected second intermediate host (Fig. 1.1). While trematodes exhibit high host specificity for first intermediate host, usually infecting only a single species of mollusc, specificity is less restricted for second intermediate hosts. For adult trematodes, the specificity of definitive vertebrate hosts is mainly determined by host feeding behavior (Graczyk 1997).

This research examines trematode communities at three different scales associated with their first intermediate host the mudsnail *Ilyanassa obsoleta*. A highly successful species, *I. obsoleta* exhibits an extensive geographical range. Native populations of the snail extend along the Atlantic coast of North America from Labrador to Florida

(Bousfield 1960, Abbott 1974). The mudsnail was also been introduced to the Pacific coast of North America where populations are currently established in three distinct bays (Carlton 1992). In both native and introduced populations snails are commonly found in dense aggregations than can range from hundreds to tens of thousands of snails per m^2 (Scheltema 1961, Brown 1969, Curtis and Hurd 1981, Race 1982, Norkko and Bondsdorff 1996). From ecological perspective the snail has been shown to exhibit a variety of effects on algal (Nichols and Robertson 1979, Novak et al. 2001), microbial (Pace and Darley 1979), and infaunal communities (Nichols and Robertson 1979, DeWitt and Levinton 1985, Hunt et al. 1987, Kelaher et al. 2003). Given the broad effects of trematode infection on snails including reproductive cessation and changes in growth and movement (Lafferty 1993, Mouritsen and Jensen 1994, Curtis 1995, Miller and Poulin 2001), there is good reason to believe that these parasites could play an important indirect role in mediating the impacts of this host in estuarine environments.

Many early studies describe individual *I. obsoleta* trematodes, however McDermott (1951) and later Stunkard (1983) were the first to provide a comprehensive description of this group including details about the morphology of larval stages and life cycles of the nine trematodes (Table 1.1). Ecological work on *I. obsoleta* trematodes include investigations of temporal (Sindermann 1960) and spatial patterns (McCurdy et al. 2000) of select trematode species from northern populations of *I. obsoleta* (Maine and Canada). However, the most extensive examinations of this group from a community analysis perspective (not including the research presented here) is found in the work of Curtis whose studies focus exclusively on populations of trematodes and their snail hosts in Delaware Bay, USA.

Much of Curtis' work focuses on organization of trematode communities at the level of an individual snail (i.e. the infracommunity sensu Esch et al. 1990). Observations at this scale suggest that interspecific competition among *I. obsoleta* trematodes is low when multiple species occur in a single snail host and that turnover of infecting trematode species within an individual host occurs infrequently (Curtis 1997, 2003). Thus it appears that interspecific competition between *I. obsoleta* trematodes is weaker than in many other trematode systems studied (Kuris 1990). Curtis' work tracking marked *I. obsoleta* in the field provides important information that helps characterize trematode communities at the scale of the snail host population (i.e. the component community sensu Esch et al. 1990). In Delaware snails, annual rates of infection are low (Curtis 1996, Curtis and Tanner 1999); however because snails are long lived (Curtis 1995, Curtis et al. 2000) and trematode infections are maintained over long time periods (Curtis 2003), high infection prevalence can be achieved in *I. obsoleta* populations. A number of Curtis's studies document strong spatial variability of infection in snails along Delaware sandflats (Curtis and Hurd 1983, Curtis 2007b). While snail movement seems to contribute to the maintenance of these spatial patterns, a rigorous test of the underlying causes and effects of such spatial heterogeneity was not been undertaken.

The work of Curtis and others provides a strong foundation for understanding *I. obsoleta* trematode communities. Some intriguing spatial and temporal patterns of infection in *I. obsoleta* have been documented, yet the majority of these studies either focus narrowly on single factor explanations related to snail host populations or fail to investigate any mechanisms at all. An understanding of how a diversity of ecological variables (including non-snail hosts, environmental conditions, and historical factors)

determine the structure of *I. obsoleta* communities is therefore lacking. Moreover, previous studies of this trematode community have been geographically restricted to a small portion of the range of *I. obsoleta*. Determining whether *I. obsoleta* trematode communities exhibit similar patterns across the extent of their range is critical to a more comprehensive understanding of this snail-trematode system.

Research Overview

The research detailed in the following chapters provides the most extensive examination of *I. obsoleta* trematode communities undertaken to date. The work is organized into three chapters, each corresponding to a different spatial scale associated with the mudsnail host.

Chapter 1 investigates *I. obsoleta* trematodes at the broadest spatial scale which encompasses nearly the full distributional range of this snail host including both native and introduced populations. Trematode communities at this scale are examined with the specific goal of determining whether human mediated introduction plays a significant role in determining parasite community structure. A regional scale analysis of *I. obsoleta* trematode communities is presented in Chapter 2. The research examines these parasite communities across fifteen saltmarsh sites located throughout northern New England, USA. The work investigates a wide variety of biological, chemical, and physical factors and their relationship to *I. obsoleta* trematode communities. Chapter 3 focuses on *I. obsoleta* at a local scale of a single saltmarsh site. The research examines the effects of variable habitat types on spatial distribution of *I. obsoleta* trematodes through time.

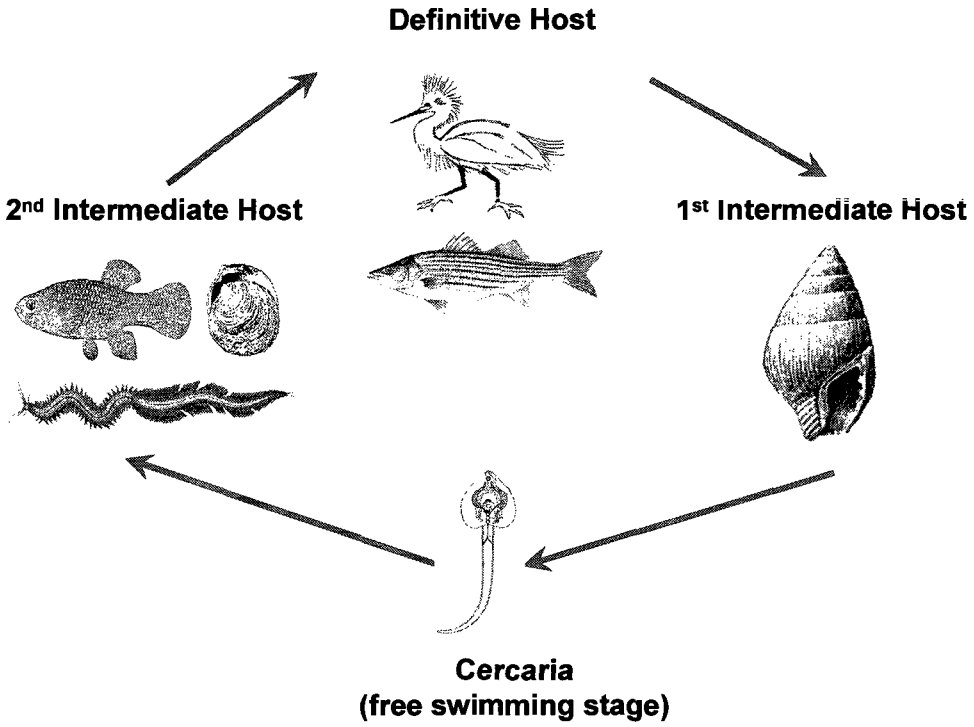

Figure 1. 1

Adult trematode parasites reside in definitive vertebrate hosts (commonly species of birds and fish) inside which they undergo sexual reproduction. Trematode eggs are passed with definitive host feces into the environment and become the infective agents to the mudsnail, *Ilyanassa obsoleta*, which acquires infection either through accidental ingestion of parasite eggs or by penetration of active larval stage called a miracidium. Once infected, the mudsnail remains so for life. Inside the mudsnail host, trematodes develop asexually and produce sporocysts or redia containing the free-swimming stage of the parasites called cercaria. Although both sporocysts and redia function to asexually reproduce cercaria, only redia can actively move and feed inside the snail host tissues. When environmental conditions are suitable (i.e. temperature, salinity, light conditions are appropriate), cercaria are shed from the snail into the surrounding water. Short-lived cercaria must then locate and infect a second intermediate host. Appropriate second intermediate hosts vary by trematode species, but for *I. obsoleta* trematodes include bivalves, planktivorous fish, polychaete worms, and crustaceans. Trematodes form metacercarial cysts inside tissues of second intermediate hosts. Cyst walls protect metacercaria from external environmental conditions and allow the parasite to withstand long periods during which transmission to definitive hosts is unsuitable. Transmission of the parasite to the definitive host is accomplished through ingestion when the vertebrate host consumes an infected second intermediate host.

Table 1. 1

Nine digenetic trematodes parasites that infect the mudsnail *I. obsoleta* during larval stages with information about their taxonomy and life history characteristics including a description of second intermediate and definitive hosts necessary to complete their life cycle. While trematodes in this group are highly host specific during larval stages and generally only infect *I. obsoleta*, a wider range of species may be used as hosts during later life stages.

Trematode Species	Oder: Family	Type	2nd Intermediate Host	Definitive Host
Austrobilharzia variglandis	Strigeiformes: Schistosomidatidae	sporocyst	none	bird
Diplostomum nassa	Strigeoidea: Diplostomatidae	redia	fish	bird likely
Gynaecotyla adunca	Plagiorchiformes: Microphallidae	sporocyst	crustacean	bird
Himasthla quissetensis	Echinostomatiform: Echinostomatidae	redia	bivalve	bird
Lepocreadium setiferoides	Lepocreadiiformes: Leprocreadiidae	redia	polychaete worm	fish
Pleurogonius malaclemys	Paramphistoformes: Pronocephalidae	redia	hard substrate (e.g. vegetation, snail opercula, other abiotic substrate)	terrapin
Stephanostomum dentatum	Plagiorchiformes: Acanthocolpidae	redia	fish	fish
Stephanostomum tenue	Plagiorchiformes: Acanthocolpidae	redia	fish	fish
Zoogonus rubellus	Plagiorchiformes: Zoogonidae	sporocyst	polychaete worm	fish

CHAPTER 2

GENERAL DETERMINANTS OF STRUCTURE IN TREMATODE COMMUNITIES ACROSS THE GEOGRAPHICAL RANGE OF THEIR SNAIL HOST, *ILYANASSA OBSOLETA*

Introduction

Parasites are abundant members of ecological communities that can have strong effects on host populations (Anderson and May 1978, Hudson et al. 1998), non-host populations (Poulin 1999, Wood et al. 2007, Grewell 2008), and ecological processes (Thomas et al. 1998, Mouritsen and Poulin 2005). Few studies, however, examine parasites across their geographical distribution. Understanding parasites at this scale provides a context for determining how their functional role may vary across a geographical landscape. In addition, large scale studies can test whether parasite communities exhibit similar patterns as free living organisms at comparable scales.

At their largest spatial scale, larval trematode communities of the eastern mudsnail *Ilyanassa obsoleta* encompass the geographic range occupied by their snail host which provides essential habitat to these parasites. During larval stages trematodes of this group are highly host specific and are generally only able to infect *I. obsoleta*. As a

result, the geographic range of this group of parasites is limited by the distribution of *I. obsoleta* which includes both native and introduced populations. The native distribution of *I. obsoleta* extends across much of the Atlantic coast of North America from the Gulf of St Lawrence, Canada to Florida, USA (Bousfield 1960, Abbott 1974). Introduced populations of the snail are established along portions of the Pacific coast of North America (Carlton 1992).

The process of human mediated introduction is hypothesized to play a strong role in determining the structure (i.e. the abundance and diversity) of *I. obsoleta* trematode communities across their geographical distribution because a common characteristic of introduced hosts is that they harbor fewer natural enemies (parasites, pathogens, and predators) than in the native range. This reduction is the result of two primary factors: (1) a low likelihood that enemies will accompany species into the introduced range (Delvinquier and Freeland 1988, Dobson 1988), and (2) an inability of enemies found there naturally to make up for this loss (Torchin et al. 2003, Torchin and Mitchell 2004). Escape from natural enemies may result in higher relative fitness of introduced species compared to native conspecifics and this mechanism, referred to as "the enemy release hypothesis", is often cited to explain their high success (Crawley 1997, Maron and Vila 2001, Keane and Crawley 2002).

Recent examinations demonstrate that a broad range of introduced host populations have left behind their native parasites (Mitchell and Power 2003, Torchin et al. 2003, Blakeslee and Byers 2008). While these studies focus on comparisons of native and introduced hosts on a coarse level, understanding geographical variability in parasite communities in more detail could provide additional insight into the history of species

introductions. For example, if infection rates and especially the diversity of parasites vary across the native range, it may be possible to determine specific subregions that are the source of introductions. Research on free-living species has often focused on matching genetic structure of introduced populations to likely source regions (Collins et al. 2002, Voisin et al. 2005, Kolbe et al. 2007) and a similar approach could be taken using parasites to track the source of introduced communities (Blakeslee and Byers 2008). Examining parasite escape in targeted source and introduced communities could also help elucidate whether some parasite species are more likely to become established than others either as a result of their abundance in native range or because of some other characteristic associated with the parasite's life cycle or the recipient community. Finally, little is known about the structure of parasite communities within introduced regions. Differences among introduced parasite communities may reflect differences in introduction histories, host communities, or the time since introduction.

This chapter examines *I. obsoleta* trematode parasite communities across the geographical scale of their snail host to determine whether introduced populations have escaped their native parasites. In addition to broad comparisons between native and introduced regions, a more refined examination is undertaken to assess whether community structure varies among a set of nested subregions. Native subregions include source and non-source populations of *I. obsoleta* and introduced subregions include the distinct areas where the snail has become established on the Pacific coast. The specific research questions examined are:

1) Have *I. obsoleta* trematode communities in the introduced region escaped trematode parasites i.e. do they exhibit reduced species richness and prevalence

(the proportion of hosts infected) compared to parasite communities in the native region?

2) Are there differences in parasite community structure within native and introduced subregions? If so, are native source communities more similar to introduced communities than other non-source subregions?
3) Do *I. obsoleta* trematode communities in the native region conform to large scale latitudinal diversity patterns observed for free-living organisms?

The trematode community associated with *I. obsoleta* represents a good case study to examine these questions because the history and source of this introduction is relatively well documented. In addition, populations of this snail host have been established in their introduced range in some areas for over 100 years (Carlton 1992) with the introduction vector eliminated for the majority of that time (~70 years). Introduced trematode populations therefore are no longer being subsidized by input from introduction vectors and therefore likely reflect equilibrium conditions.

Overview of *Ilyanassa obsoleta* Distribution and Introduction History

Native to the east coast of North America, *I. obsoleta* populations extend from the Gulf of St Lawrence, Canada to northern Florida, USA (Bousfield 1960, Abbott 1974). Snails are generally found in soft sediment, low energy, estuarine and marine environments and can reach extremely high abundance throughout their native range (Scheltema 1961, Brown 1969, Curtis and Hurd 1981).

Ilyanassa obsoleta was introduced to the Pacific coast of North America with commercial shipments of the eastern oyster, *Crassostrea virginica*, in the early 1900's

(Carlton 1992). Oysters were shipped from their native range to the Pacific coast for retail sale and to support the development of aquaculture. Commercial harvesting of oysters was extensive along the east coast of the US from Maine to Florida during the 19th century representing tens of millions of oysters harvested per year with catches valued at more than 14 million dollars annually during the industry's peak (Ingersoll 1881). Although the distribution of *C. virginica* extends across the Atlantic coast of the US, northern oysters were primarily targeted for transcontinental shipping as they were especially hardy and tolerant of long distance travel (Kochiss 1974). New York City served as the commercial center for the transcontinental transport of oysters (Miller 2000) and while oyster shipments received from bays located in close proximity to the city supplied the majority of the New York market, other areas of high production like Delaware and Massachusetts (Kochiss 1974) were also likely involved in long distance transport (A. W. Miller personal communication). Oysters from Chesapeake Bay, while not involved in direct transport to the Pacific coast, were still a likely strong indirect source of introduction. Extensive transplanting of oysters from this estuary were used to bolster stocks from northern bays which were depleted due to intense local harvesting (Miller 2000). As a result, substantial mixing of oysters (and likely associated fauna) occurred within northern bays. While harvesting and local sale of oysters in the southeastern US and Gulf coast was substantial, there is no evidence to suggest that these oysters were involved in transport to Pacific coast markets (Miller 2000).

Given the history of *C. virginica* transport, it is likely that the source region for *I. obsoleta* introduction to the Pacific coast were the areas around Long Island Sound and to a lesser extent bays located in close proximity from which oysters were shipped into New

York including Connecticut, Rhode Island, Massachusetts, and Delaware. The source region should also include Chesapeake Bay since transplantation of oysters from this area to more northern bays was extensive.

Oysters represented a strong vector for the introduction of non-indigenous species for a number of reasons. The harvesting of native oysters for export was both intense and sustained over a long period of time representing billions of individuals transported from 1869-1940 (Miller 2000). Dredging methods used to collect oysters were highly unselective (Ingersoll 1881) which contributed to many associated organisms being transported with the main harvest. Characteristics of transport also ensured that oysters arrived at their destination in good condition so that they could either be sold live at market or used to support growing aquaculture industries. These factors and others contributed to an abundance of oyster associated introductions to the Pacific coast including seven species of gastropod and five bivalves (Carlton 1992, Cohen 2005).

Established introduced populations of *I. obsoleta* are currently located in three Pacific coast bays. San Francisco Bay, California (SFB) represents the earliest documented introduction of the snail which was first observed there in 1907 (Carlton 1992). SFB *I. obsoleta* populations may reach extremely high abundances and have likely contributed to the decline of the native mudsnail in this area (Race 1982). *I. obsoleta* was documented in Willapa Bay, Washington (WB) in 1945 (Carlton 1992). While both SFB and WB received direct transport of eastern oysters from Atlantic ports, exchange between the two bays was also substantial (Barrett 1963). The sources of these introduced populations were therefore likely mixed. *I. obsoleta* was first documented in Boundary Bay, British Columbia (BB) in 1952 (Demond 1952). Little information exists to evaluate

whether introduced mudsnails arrived to this location from other Pacific coast bays or directly from Atlantic coast populations.

Methods

Ilyanassa obsoleta trematode communities were sampled across their geographical distribution using a nested sampling design. Samples were collected in two regions, representing native and introduced trematode communities. Sampling sites within the native region spanned a distance of 1,800 km extending from mid-coast Maine (43° N) to southern Georgia (31° N), which encompasses the majority of *I. obsoleta*'s native range. Sampling in the introduced region encompassed the three bays where introduced *I. obsoleta* populations are established: SFB (37° N), WB (46° N), and BB (49° N). Within each region, three subregions were selected for sampling. Native subregions sampled were the source, northern non-source, and southern non-source. Sites within the source subregion (n = 14) were located in Long Island Sound, and northern bays of Delaware and Massachusetts. Chesapeake Bay was also included in the source subregion because of its likely importance as a stepping stone for introduction into northern bays and subsequently the Pacific coast. The two native non-source subregions include sites that were not important in transport of oysters to the Pacific coast. Northern non-source sites were located in New Hampshire and Maine (n = 10). Sites in the southern non-source subregion extended from North Carolina to Georgia (n = 15). It should be noted that within the southern sampling region, eleven sites were specifically targeted to assess trematodes from areas of known variability with respect to terrapin abundance which are definitive hosts to a single rare *I. obsoleta* trematode species,

Pleurogonius malaclemys. Selection of these particular sites was motivated by a separate but related research question regarding *I. obsoleta* trematodes for which the data was also analyzed. The targeting of these sites with respect to terrapin abundance is not likely to introduce bias in the results because a range of terrapin abundance was sought and these definitive hosts inhabit marsh areas that are characteristic of the other sites selected for this study. In the introduced region, the three bays where *I. obsoleta* has been introduced also served as introduced subregions (SFB, n = 3; WB, n = 3; BB, n = 3).

Habitat type was controlled for as much as possible when selecting sampling sites in order to standardize environmental variables associated with parasite communities as well as potential presence and abundance of all other second intermediate and definitive hosts. All sampling took place in soft sediment, estuarine environments and whenever possible saltmarshes where *Spartina* spp. was present were selected for sampling. At each site adult snails were collected by hand while walking 100 m along the tidal channel or shoreline. Snails greater than 14 mm were collected haphazardly from native sites. In introduced sites slightly larger snails >18 mm were targeted for assessment of trematode infection to ensure the greatest likelihood of infection. Results therefore represent a conservative estimate of parasite escape because snails sampled in native regions were younger (smaller) on average compared to introduced older (larger) introduced snails which likely had a greater period of time to accumulate infections (assuming similar growth rates in native and introduced regions).

Snails were dissected in the lab and the presence of infection determined through visual inspection of snail gonad and digestive tissues beneath a stereo dissecting microscope. Trematode species identification was determined using a compound

microscope and with the aid of published and unpublished descriptions of *I. obsoleta* trematodes (McDermott 1951, Yamaguti 1975, Stunkard 1983). Total infection prevalence (i.e. the proportion of snails infected with any species of trematode) and the prevalence of individual trematode species (i.e. the proportion of snails infected with a particular trematode species) was calculated for each site and used in statistical analyses.

Species Richness Standardization and Estimation

Measuring species richness (and other diversity metrics that take richness into account) can be problematic because, for a diversity of taxa, the number of species increases with the number of individuals observed (Bunge and Fitzpatrick 1993). It is therefore critical to adequately account for differences in sampling effort when comparing diversity across samples (Gotelli and Colwell 2001). The number of sampling sites differed among the two regions in this study. Thus in order to determine whether sampling in the native and introduced region was sufficient to capture the underlying richness present in these regions, species accumulation and estimation curves were constructed using EstimateS 8.0 (Colwell 2006). EstimateS employs Monte Carlo resampling of the data (randomization of sample order over a specified number of runs) to determine the mean number of species (S_{obs}) and 95% confidence intervals at each level of sampling. Because species accumulation curves may not adequately capture underlying richness, especially if they have not reached an asymptote, species richness estimators can be used to provide additional and corroborative information (Gotelli and Colwell 2001). Chao2 (Chao 2004) is a nonparametric species richness estimator that is based on the idea that rare species encountered in the data provide information about

missing species not observed in samples (see Appendix A for a complete descript of this equation). In an evaluation of seven species richness estimators, Chao2 was found to be the least biased estimator for small sample sizes (Colwell and Coddington 1994) and has also been specifically recommended for use with parasite data (Walther and Morand 1998). Species accumulation curves were compared to Chao2 estimation curves to determine if more trematode species are likely to be encountered with additional sampling in the native and introduced region. Convergence of S_{obs} and Chao2 estimations at an asymptote provides strong evidence that sampling was sufficient to reveal the underlying species richness present across samples.

While species accumulation and estimation curves determine asymptotic species richness across native and introduced regions of *I. obsoleta*, additional methods were employed to standardize species richness at the site level. This was necessary because the number of snails dissected and hence the sampling effort varied across sites. To standardize sampling effort across sites, Monte Carlo resampling was performed at the site level using EstimateS. For each site the mean number of trematode species observed (S_{obs}) at a sampling effort of 83 snails (the minimum number of snails dissected at each site) was obtained. This measure of adjusted richness was then used in site-level analyses including ANOVA and regression methods.

Statistical Analyses

Two-factor nested ANOVA tests were performed to examine differences in total prevalence, prevalence of individual trematode species, and species richness (adjusted for sampling effort using methods described above) of trematodes between the two regions

(native and introduced) and among the nested subregions. Because subregions were selected specifically to examine differences in trematode communities based on the introduction history of *I. obsoleta*, they were considered fixed factors in the analysis. Prevalence data was anscombe transformed for statistical analyses as recommended for percent data (Zar 1996). When results of ANOVA indicated significant differences at the region and subregion level, posthoc Tukey's tests were performed for native and introduced subregions separately to determine which subregions were statistically distinct. When ANOVA results indicated a statistical difference at the subregion level only, a single Tukey's test was used to identify statistical differences among all possible subregion pairs.

Nonparametric multivariate analyses that take into account both richness and abundance of trematodes were performed using Primer v6 (Primer-E Ltd, Plymouth, UK). For each pair of sites the Bray-Curtis similarity index (Legendre and Legendre 1998) was calculated. This index uses both the number of species in a sample and the number of individuals observed within each species category to determine the degree of similarity among two communities (see Appendix B for a complete description of the equation). Because the Bray-Curtis for two samples with zero observations of species is undefined, a 'dummy species' was added to the species by sample matrix and populated with 1's for each sample (Clarke and Gorley 2006, Clarke et al. 2006). This allows for inclusion of sites with zero observations of trematodes in the analyses and results in these sites having 100% similarity. Bray-Curtis similarities were based on square root transformed prevalence data to minimize the importance of highly abundant species on the results. Two analyses were performed based on the pairwise Bray-Curtis similarity

matrix. Multidimensional scaling (MDS) plots provide a visual representation of similarity among sampled communities where the greater the distance between points in space, the more dissimilar are the two communities. A two-way nested analysis of similarity (ANOSIM) was used to test the null hypothesis that there is no difference in trematode community composition between the two regions or within the nested subregions. An approximate analogue to ANOVA, ANOSIM relies on a permutation/randomization test and makes a minimum of assumptions. The routine generates an R statistic which ranges from -1 to +1 and a significance test. An R statistic close to zero indicates the null hypothesis should not be rejected. The closer R is to -1 or +1, the greater dissimilarity there is among groups.

Results

In total 5,085 snails were examined for parasite infection across 39 sites in the native region. A total of 960 snails were examined for parasite infection from the introduced region collected across nine sites that span the three bays where *I. obsoleta* has been introduced (see Fig. 2.1 for map of collection sites). Across all sites, the average number of snails dissected was 127 (range = 83-216).

All nine species documented to infect *I. obsoleta* (Table 1.1) were observed across the native region. In order of decreasing average prevalence across the native range these were *Zoogonus rubellus*, *Stephanostomum tenue*, *Lepocreadium setiferoides*, *Stephanostomum dentatum*, *Himasthla quissetensis*, *Austrobilharzia variglandis*, *Gynaecotyla adunca*, *Pleurogonius malaclemys*, and *Diplostomum nassa*. The full component of species, however, was not observed across all native subregions. One

species, *P. malaclemys* was not found in the source and northern non-source subregions. In the southern non-source region, *D. nassa* was not observed (Table 2.1).

In the introduced region a total of five trematode species were observed to infect snails. Trematode species infecting introduced snails represent a subset of the species found in native snails (i.e. all trematodes found infecting introduced snails were species that were also observed to infect native snails). In order of decreasing average prevalence across the introduced range these were *H. quissetensis*, *A. variglandis*, *Z. rubellus*, *S. tenue*, and *L. setiferoides*. All five trematodes were observed in the subregion of SFB. *A. variglandis* and *H. quissetensis* were the only trematodes observed to infect snails in WB and BB (Table 2.1).

Species Richness Accumulation and Estimation

In the native region, species accumulation (S_{obs}) and the species estimator (Chao2) reached an asymptote at nine (S_{obs} CI = 9-9; Chao2 CI = 9-9; Fig 2.2a). In the introduced region both S_{obs} and Chao2 were found to asymptote at five species (S_{obs} CI = 2-8; Chao2 CI = 5-6; Fig 2.2b). For both regions, S_{obs} converges with the Chao2 estimate of species richness at the asymptote indicating that additional species are not likely to found with continued sampling (Fig. 2.2). Although species richness accumulation and estimations are derived from sample based data, graphs have been rescaled to describe the number of trematode individuals observed at each level of sampling in order to compare diversity across datasets where the number of individuals varies (Gotelli and Colwell 2001).

Adjusted Species Richness

Site-level adjusted species richness (obtained from EstimateS) was compared between the two regions and among the subregions using a nested ANOVA. Results indicate that adjusted species richness of trematodes is significantly greater in native compared to the introduced regions (native = 4 $\pm$ 0; introduced = 2 $\pm$ 1 [mean $\pm$ SE]; $F_{1,42} = 13.32$, $P < 0.01$). In addition a significant difference in adjusted species richness among nested subregions was found ($F_{4,42} = 4.23$, $P = 0.01$). Posthoc analysis using a Tukey's test found the southern non-source subregion to have significantly decreased adjusted richness compared to the source subregions. No significant differences were found in adjusted richness among the introduced subregions (Fig. 2.3).

Infection Prevalence

Results of nested ANOVAs for anscombe transformed prevalence are reported below. For simplicity, however, figures and tables depict raw prevalence values.

The results of nested ANOVA found the native region to have significantly greater prevalence than introduced region (native = 28.6 $\pm$ 2.6; introduced = 15.1 $\pm$ 4.8; [mean $\pm$ SE]; $F_{1,42} = 6.8$, $P = 0.01$; Fig. 2.4). No significant differences were found among the nested subregions.

For the five trematode species found in both introduced and native regions, prevalence of each individual species was tested using separate nested ANOVAs to determine whether significant differences exist between the two regions and among the nested subregions. The prevalence of *Z. rubellus* was found to be significantly greater in

native compared to introduced region (native = 16.6 ± 1.4; introduced = 5.3 ± 3.0; [mean ± SE]; $F_{1,42} = 12.7$, $P < 0.001$; Fig. 2.5a). No significant differences were found among the nested subregions. Similarly, the prevalence of *L. setiferoides* was significantly greater in native compared to introduced region (native = 11.6 ± 1.1; introduced = 4.7 ± 2.2; [mean ± SE]; $F_{1,42} = 7.9$, $P = 0.01$; Fig. 2.5b) with no significant differences among the nested subregions. *S. tenue* exhibited significantly greater prevalence in the native compared to introduced region (native = 8.8 ± 1.0; introduced = 4.7 ± 2.0; [mean ± SE]; $F_{1,42} = 4.8$, $P = 0.03$; Fig. 2.5c). A significant difference was also found among the nested subregions ($F_{4,42} = 4.3$, $P = 0.01$). Tukey's test revealed that southern non-source regions exhibited significantly lower prevalence than the other two native subregions (Fig. 2.5c). No differences were found among introduced subregions. *A. variglandis* was the only trematode species to exhibit significantly greater prevalence in the introduced region compared to native region (introduced = 6.8 ± 0.7; native = 4.5 ± 0.4; [mean ± SE]; $F_{1,42} = 7.95$, $P = 0.01$; Fig. 2.5d), with no significant differences observed among the nested subregions. While there was no significant difference in the prevalence of *H. quissetensis* in the native and introduced region ($F_{1,42} = 2.49$, $P = 0.12$), a significant difference was found among the nested subregions ($F_{4,42} = 3.6$, $P = 0.01$). Results of a Tukey's test reveal significantly higher prevalence in SFB compared to the southern non-source region (Fig. 2.5e).

Community Composition

For the non-parametric ANOSIM test, an R statistic close to one indicates the maximum dissimilarity among pairs of groups at a specified significance level. Results of

the nested ANOSIM demonstrate that there are no significant differences in community composition between the native and introduced regions (R = 0.93, $P = 0.10$). Because the nested ANOSIM uses subregions as samples (rather than sites) to test for differences among the two regions, only 10 permutations of the data were possible to create the null distribution for the R sample statistic. The ability of the test to determine significance was therefore limited by low sample size. However, the high value of R (which is close to one) suggests that native and introduced communities would be likely to exhibit significant differences were the sample size to be increased.

ANOSIM results did reveal a significant differences among subregions (R = 0.28, $P = 0.001$). A posthoc ANOSIM to examine pairwise differences among subregions was therefore performed. Results of this test indicate significant differences among southern non-source communities compared to the other two native subregions. In contrast, no significant differences were found among *I. obsoleta* trematode communities associated with introduced subregions (Table 2.2). The MDS plot illustrates these differences (Fig. 2.6) and exihibits low stress (0.15) indicating that the results of the community analysis are stable (Clarke and Gorley 2006).

Latitudinal Patterns

Site-level adjusted trematode species richness was found to significantly increase with latitude across the native range ($R^2 = 0.25$, $P = 0.002$; Fig. 2.7). In northern latitudes, the maximum site-level adjusted richness was eight species with an average of about five. In southern latitudes, a maximum adjusted richness of five species was observed at sites with an average of about 2.5.

Across the native range, a weak but significant positive relationship between overall prevalence of infection in *I. obsoleta* (anscombe transformed) and latitude was observed ($R^2 = 0.13$, $P = 0.03$; Fig. 2.8). The prevalence of three individual trematode species (anscombe transformed) also exhibited significant positive relationships with latitude. Two of these trematodes, *Gynaecotyla adunca*, and *H. quissetensis*, are bird using species ($R^2 = 0.12$, $P = 0.04$; $R^2 = 0.36$, $P < 0.0001$, respectively) while the third, *S. tenue*, uses fish as a definitive host ($R^2 = 0.26$, $P = 0.001$). *P. malaclemys* is unique among *I. obsoleta* trematodes in its use of a terrapin as definitive host; it was the only species whose prevalence (anscombe transformed) showed a significant negative relationship with latitude ($R^2 = 0.38$, $P < 0.0001$).

Because latitudinal gradients in definitive host taxa could affect prevalence, patterns were examined for trematodes categorized by functional groups according to the type of definitive host required (either fish, birds, or terrapin). While no significant relationship was observed between latitude and prevalence (anscombe transformed) of fish using trematodes ($R^2 = 0.08$, $P = 0.07$; Fig. 2.9a), strong latitudinal gradients were exhibited for both bird using trematode species ($R^2 = 0.45$, $P < 0.0001$; Fig. 2.9b) and the single species that uses a terrapin definitive host ($R^2 = 0.38$, $P < 0.0001$; Fig. 2.9c).

Discussion

1) Have *I. obsoleta* trematode communities in the introduced region escaped trematode parasites i.e. do they exhibit reduced species richness and prevalence (the proportion of hosts infected) compared to parasite communities in the native region?

Clear patterns of reduced richness and total prevalence of trematodes in introduced regions (Fig. 2.3; Fig 2.4) indicate that *I. obsoleta* has escaped many of the trematode parasites found in native populations and is also, therefore, meeting a critical expectation of the enemy release hypothesis. Adjusted species richness in introduced populations was significantly reduced by nearly 50% compared to native regions and this magnitude of parasite loss is consistent with other studies examining parasite escape (Torchin et al. 2003). Total prevalence was also significantly reduced in introduced snails and, in most cases, this was also true of the prevalence associated with individual trematode species. While no significant differences were detected between the two regions using community analyses (ANOSIM), increasing the sample size of introduced communities may reveal significant patterns.

The presence of more than half of *I. obsoleta*'s trematode species in the introduced range indicates that these parasites are able to complete their life cycles using second intermediate and definitive hosts found there. A review of the current literature as well as a search of relevant databases was conducted to determine which hosts of *I. obsoleta* trematodes that are documented in the literature are also found in the introduced range (Appendix C provides a detailed description of methodology associated with this

review; Table 2.3 summarizes the results). While this examination does not yield definitive patterns, it does suggest a number of host related factors that are likely important to the successful introduction of trematode species.

Three of the five introduced trematodes (*L. setiferoides*, *S. tenue*, and *Z. rubellus*) were found to have either no documented second intermediate or definitive hosts on the Pacific coast (Table 2.3). Thus, it appears that the presence of a documented host associated with each stage of the trematode's life cycle is not required for successful introduction. Because trematodes exhibit decreased specificity for non-snail hosts, some are likely able to infect suitable novel hosts found in introduced regions. However, the success of introduced *H. quissetensis* (especially in San Francisco Bay; Fig. 2.5d) along with the presence many species of second intermediate bivalve hosts and definitive bird hosts there (Table 2.3 and Appendix C), suggests that the when trematodes are able to use hosts with a shared evolutionary history (as opposed to hosts species that have only recently been encountered) it is advantageous to the completion of their life cycle.

All trematodes are highly adapted to their first intermediate host and generally are only able to infect a single snail species. Yet trematodes may vary in the degree of specialization required to infect second intermediate and definitive hosts (Graczyk 1997). In this context, trematode species that exhibit lower specificity for second intermediate and definitive hosts are likely to have a greater chance of becoming successfully established in introduced environments where they may encounter a variety of suitable hosts to which they are not specifically adapted. To examine whether introduced *I. obsoleta* trematodes are less specialized than species which were absent from the Pacific coast, the number of second intermediate and definitive hosts documented in the

literature (and presented in Appendix C) was used as a proxy for host specificity. Results of this comparison, while not significant, demonstrate a positive trend between successful introduction and number documented non-snail hosts (Appendix C, number of hosts associated with introduced trematodes = 10.4 $\pm$ 2.5, number of hosts associated with trematodes that have not been introduced = 4.8 $\pm$ 2.1, avg $\pm$ SE; $F_{1,7} = 2.82$, $P = 0.12$). While this pattern suggests that host specificity is inversely related to successful introduction, a number of assumptions need more rigorous testing before definitive conclusions can be made. First, no study has specifically tested the degree of host specificity associated with this trematode group and it is possible, therefore, that trematodes with greater number of documented hosts simply represent species that are better studied. Second, trematodes with few documented second intermediate and definitive host species may be able to infect a variety of hosts from a physiological standpoint, but for ecological reasons may be limited in the species of hosts they come in contact with. Thus, additional research is needed to determine the specificity required by a trematode to infect a second intermediate or definitive host.

Three of the five introduced trematodes species (*Z. rubellus*, *S. tenue*, and *L. setiferoides*) exhibited a significant decrease in prevalence across the introduced region (Figure 2.5a, 2.5b, 2.5c). A major factor contributing to the reduced prevalence of these species is that infections were limited to SFB with no infections found in either WB or BB. For *Z. rubellus*, species of fish definitive hosts, *Anguilla rostrata*, are only documented from California waters and this is likely a dominant factor in limiting the spread of the trematode to other bays where snail hosts have been introduced (Table 2.4). On the other hand, at least some documented second intermediate and definitive hosts of

S. tenue and *L. setiferoides* are found across *I. obsoleta*'s introduced range indicating that factors other than the presence of appropriate hosts have prevented the establishment of these trematodes (Table 2.4). In addition to their limited geographical distribution, prevalence of these trematode species in SFB was still much reduced compared to average prevalence of nearly all the native subregions. While hosts must be available in SFB for these parasites to complete their life cycle, a fundamental difference appears to exist here which makes infection more difficult compared to native subregions. The lower observed prevalence could be the result of reduced abundance of second intermediate and definitive hosts. In addition, some species encountered by trematodes in SFB may become infected but be less efficient at transmitting the trematode compared to host species in the native range that have had a shared evolutionary history with these trematodes. Less likely is the possibility that *I. obsoleta* snails are better protected from acquiring infection in the introduced range as a result of changes in behavior or increased genetic resistance to infection.

Two introduced trematode species exhibited unique trends with respect to prevalence in the introduced range. *A. variglandis* was the only trematode found to have significantly higher prevalence in the introduced region (Fig. 2.5e). While no significant difference in prevalence was found between the native and introduced regions for *H. quissetensis*, the highest prevalence observed across all sampled subregions was in introduced sites of SFB (Fig. 2.5d). Potential explanations for the high prevalence of these trematodes in introduced populations are explored below.

A. variglandis is unique among *I. obsoleta* trematodes for a number of reasons. First, this species is the only one to exhibit a truncated life cycle. Instead of encysting in a

second intermediate host, *A. variglandis* moves directly from a snail to a definitive bird host (Table 1.1). This reduction in the number of hosts needed may eliminate one challenge associated with successful establishment in introduced areas. Secondly, *A. variglandis* is unique because of reports that the parasite infects an additional snail species, *Littorina pintado*, during larval stages in Hawaii (Chu 1952). Moreover, an unidentified species in the same genus, referred to only as *Austrobilharzia* spp. has been documented to infect *Cerithidea californica*, the mudsnail native to the Pacific coast of North America (Sousa 1993). Reports of *A. variglandis* and *Austrobilharzia* spp. may reflect the presence of a single trematode species with a cosmopolitan distribution. In this case, the infection of introduced *I. obsoleta* may be the result of *A. variglandis* trematodes already present along the Pacific coast. Alternatively, these reports may reflect the presence of multiple species that exhibit a high degree of morphological similarity and are closely related (i.e. cryptic species). In this case, introduced *I. obsoleta* may be infected with a distinct species that is morphologically indistinguishable from *A. variglandis* found in Atlantic coast populations. Genetic analysis of *A. variglandis* from a number of geographically distinct snail populations is necessary to distinguish between these possibilities.

Himasthla quissetensis was the only introduced trematode to exhibit similar prevalence in native and introduced regions. Surprisingly, among all the subregions, introduced areas exhibited the highest prevalence, although in most cases differences were not statistically significant. High prevalence of *H. quissetensis* indicates that parasites which become established in introduced areas can obtain equal or greater prevalence as that found in native communities. In the case of *H. quissetensis* this may be

the consequence of abundant second intermediate and/or definitive host communities being present in introduced region. At least four species of bivalves that serve as second intermediate hosts for *H. quissetensis* have been introduced to the Pacific coast (Table 2.3) suggesting that this trematode species may be able to easily complete its life cycle in the introduced region.

Higher prevalence of *H. quissetensis* in introduced sites could also be associated with a release from interspecific competition. For example, if a subordinate trematode species becomes established in introduced communities that lack more dominant species, they may be able to reach higher abundance. While strong interspecific competition has been shown in other snail-trematode systems (Kuris 1990), some authors argue that this is not a strong factor in structuring *I. obsoleta* trematodes (Curtis 2002). Moreover, an examination focusing specifically on *H. quissetensis* found no evidence for changes in the distribution of the parasite within the snail host in the presence of another species *Z. rubellus* (Hendrickson and Curtis 2002). However, competitive effects between *H. quissetensis* and other trematode species have not been examined.

The primary vector for introduction of trematodes is *I. obsoleta* snails; however introduced non-snail hosts could provide secondary pathways for the transfer of trematodes. In particular, the introduction of definitive hosts could be an important route for transmitting the parasite directly to snails. For example, introduced striped bass, *Morone saxatilis*, infected with *S. tenue* could have been an important vector transporting this trematode species to the Pacific coast. Introduction of second intermediate and definitive hosts should therefore boost the likelihood that associated trematodes could become introduced.

Compared to native populations, *I. obsoleta* in the introduced range exhibits reduced diversity and prevalence of trematode infection. Moreover, even though the snail has been established in some portions of the eastern Pacific coast for over one hundred years, no evidence was found that new trematodes have adapted to use the snail as a first intermediate host. These findings strongly support the idea that introduced *I. obsoleta* have escaped many trematode parasites found in the native range. While not tested as part of this study, the benefits to *I. obsoleta* at the individual and population level as a result of parasite escape could be substantial. Given that infection by trematodes results in castration of their snail host, a reduced parasite burden could directly increase the reproductive capacity of a host individual. While no research has investigated the population level effects of parasite loss for introduced *I. obsoleta*, Race (1982) documented the high success of this snail species in SFB and Carlton (1992) described the species as "astronomically abundant" there. The results outlined here coupled with observations by other researchers suggest reduced infection by parasites may play an important role in the success of introduced *I. obsoleta* populations.

2) Are there differences in parasite community structure within native and introduced subregions?

The southern non-source subregion exhibited significantly lower diversity compared to other native subregions. While only *S. tenue* was found to have significantly lower prevalence in the southern non-source subregion, prevalence of all other trematode species as well as total prevalence exhibited a trend of decreasing prevalence in the south. In contrast, source communities and those located in the northern subregion were similar

with respect to diversity, total prevalence, and prevalence of individual trematode species. Results of ANOSIM test to distinguish among subregions confirm that southern non-source communities are distinct from the other two native subregions (Table 2.2).

What explains the higher diversity and prevalence of trematodes observed in northern and source subregions compared to trematode communities in the south? Many studies of trematodes emphasize that abundant host populations (especially definitive hosts which are the most direct link to infection in snails) support high prevalence (Smith 2001, Kube et al. 2002, Byers et al. 2008) and diversity (Huspeni and Lafferty 2004, Hechinger and Lafferty 2005) of trematode infection in snails. While host populations were not examined as part of this study, it seems unlikely that southern marsh systems, which are generally of much greater size than northern marshes, consistently support fewer birds, fish, and other estuarine organisms that serve as hosts for *I. obsoleta* trematodes. On the other hand, if expansive southern marshes provide a more heterogeneous landscape than northern marshes (or exhibit heterogeneity across a larger scale), host populations in the south may be more widely or heterogeneously distributed than in the north. Because site selection in this study was haphazard, the full range of trematode prevalence and diversity present in southern marshes may not have been captured.

Prevalence and diversity in native trematode communities located in the source and northern subregions strongly support the historical evidence that *I. obsoleta* trematodes were introduced from northern populations. Similar to other introduced parasites, many trematodes found to infect *I. obsoleta* on the Pacific coast were those that exhibited the highest prevalence in the source and northern subregions (Torchin et al.

2003). Of the five trematodes found in the introduced range, three exhibited the highest average infection prevalence in these native subregions (Table 2.1). This finding underscores the strong role that propagule pressure likely plays in determining which species become established in an introduced environment (Miller et al. 2007).

Among introduced subregions, consistent trends were found for increased diversity and prevalence of trematodes in SFB compared to both WB and BB. While the results of ANOSIM indicate that trematode communities in SFB are not significantly different from the other two introduced subregions, SFB is unique in that it is the only introduced subregion that does not exhibit significant differences when compared to the majority of native subregions (Table 2.2). SFB was the main receiving area for transcontinental shipments of eastern oysters which averaged 150 rail cars per year (the equivalent of >150 million pounds) during the industries peak from 1887-1909 (Carlton 1979). The extremely high propagule pressure that resulted from the transport of oysters and associated fauna likely contributed to the establishment of a greater number of *I. obsoleta* trematodes in this introduced subregion. That SFB appears to be a hotspot for introduced *I. obsoleta* trematodes is consistent with other studies that show this estuary to be one of the most invaded in the world (Cohen and Carlton 1998). Replacement of native fauna throughout SFB (Carlton 1979, Nichols and Thompson 1985, Matern et al. 2002), especially many species of estuarine infauna that serve as hosts for *I. obsoleta* trematodes in the parasites' native region, present introduced trematodes with a full complement of hosts necessary to complete their life cycle. The relatively high diversity of *I. obsoleta* trematodes established in this estuary emphasize that, given appropriate

environmental conditions and host communities, parasites can flourish in introduced communities.

3) Do *I. obsoleta* trematode communities in the native region conform to large scale latitudinal diversity patterns observed for free-living organisms?

Examinations of latitudinal diversity patterns across the native range reveal some significant patterns. A weak but significant positive relationship was found between overall prevalence and latitude (Fig. 2.8) and this relationship was driven primarily by species of trematodes that use birds as definitive hosts (Fig. 2.9b). Lower abundance of birds in southern marshes is an unlikely explanation for reduced prevalence of bird-using trematodes there since large southern marshes support abundant populations of definitive bird hosts. It is possible, however, that the distribution of birds in these marshes is more variable than in the north and this could lead to high spatial variability in trematode infection. The limited number of marshes from which snails were sampled (relative to the overall size and abundance of marshes in the south) might not therefore reflect the full variability of bird host distribution. Increased sampling across a variety of habitats in southern marshes is needed to determine if prevalence of bird using species is consistently low across marshes in southern latitudes.

A strong negative relationship between latitude and infection prevalence of the terrapin using trematode, *P. malaclemys*, is not surprising given that the geographical range of terrapins is restricted to south of Cape Cod and that the abundance of these definitive hosts increases in southern latitudes. In addition, sampling of some southern marshes targeted sites with high abundance of terrapin hosts increasing the likelihood that

at least some *P. malaclemys* infections would be found. Yet, even in the southern most study sites which are located squarely within the terrapin latitudinal range, infections by *P. malaclemys* were absent from all but a handful of sites. Furthermore, even when infections of this species were encountered, only extremely low prevalence was observed (note that the maximum prevalence obtained by this species was 6%, equivalent to anscombe transformed 10%). These findings demonstrate *P. malaclemys* to be a rare trematode across its distributional range. The underlying cause of low infection of *P. malaclemys* could be a function of definitive host density or it is possible that a trade-off exists where transmission at later stages in the parasite's life cycle exhibit high efficiency and therefore compensate for low levels of infection in snails.

A stronger latitudinal gradient was found for adjusted species richness which also increased significantly with latitude (Fig. 2.7). The latter finding, however, should be interpreted with caution since absolute species richness was equal across all the native subregions (Table 2.1). Lower site-level richness in southern latitudes is likely affected by the lower prevalence of some species in south, specifically those that use bird definitive hosts.

Increasing richness at higher latitudes runs contrary the classical patterns which commonly find higher richness at lower latitudes for many free-living organisms (Simpson 1964, Rahbek et al. 2007). While studies examining parasites across a latitudinal range are few, those that do exist present mixed evidence to support classical diversity patterns. In addition, the presence of latitudinal diversity patterns for parasites tends to vary strongly with the type of parasite under investigation. Even within a single study, different groups of parasites may display different patterns with respect to latitude

(Rohde and Heap 1998, Nunn et al. 2005, Merino et al. 2008). Differences in diversity patterns of parasites could be related to underlying patterns of host diversity (Nunn et al. 2005) as well differences in mobility of hosts which could affect the transport of parasites across latitudes (Merino et al. 2008).

The few studies to investigate latitudinal gradients in diversity of helminth parasites (a group which include trematodes) show inconsistent patterns. At least one investigation of latitudinal diversity focusing on fish helminthes suggests similar patterns to what was found here of increasing diversity in northern latitudes (Choudhury and Dick 2000). Patterns in that study were robust after controlling for the potentially confounding variables of sampling effort, host body size, and shared phylogenetic history (Poulin 2001). However other helminth studies show either classical patterns of increased diversity in tropical compared to temperate environments (Kennedy 1995) or no relationship between latitude and diversity (Rohde and Heap 1998).

Conclusions

In total, five trematode species were documented to infect introduced *I. obsoleta* and the majority of these represent clear cases that introduced parasite established on the Pacific coast. While no previous studies have examined *I. obsoleta* trematodes across their introduced range, the work of Grodhaus and Keh (1958) assessed trematode infection in a single *I. obsoleta* population located in San Francisco Bay more than fifty years ago. While that study found five trematodes infecting *I. obsoleta* in SFB, only one, *A. variglandis*, was identified. The findings presented here therefore corroborate and expand upon this previous study by indentifying all introduced trematodes of *I. obsoleta*

in SFB and exploring this trematode community across the full extent of *I. obsoleta*'s introduction.

Despite established populations of *I. obsoleta* being present in the introduced region for over one hundred years, there is no evidence that trematodes not found in the snail's native range have adapted to using this introduced species as a host. While trematode parasites are known to be highly specific to their snail hosts, a number of functionally similar mudsnail species who themselves are host to trematodes coexist with *I. obsoleta*, or have coexisted with it in the past (Race 1982). Despite the opportunity in time and space for Pacific coast trematode species to utilize the potentially available habitat represented by *I. obsoleta*, trematode species have not done so. This underscores the difficulty these parasites have in adapting to new host species.

This study is the first to comprehensively examine trematode communities of *I. obsoleta* across their geographical range. Given the widespread distribution of the mudsnail host and its important ecological role in estuarine soft sediment environments, documenting its trematodes parasites at this scale is a first step towards understanding the role of these parasites within the greater ecological community.

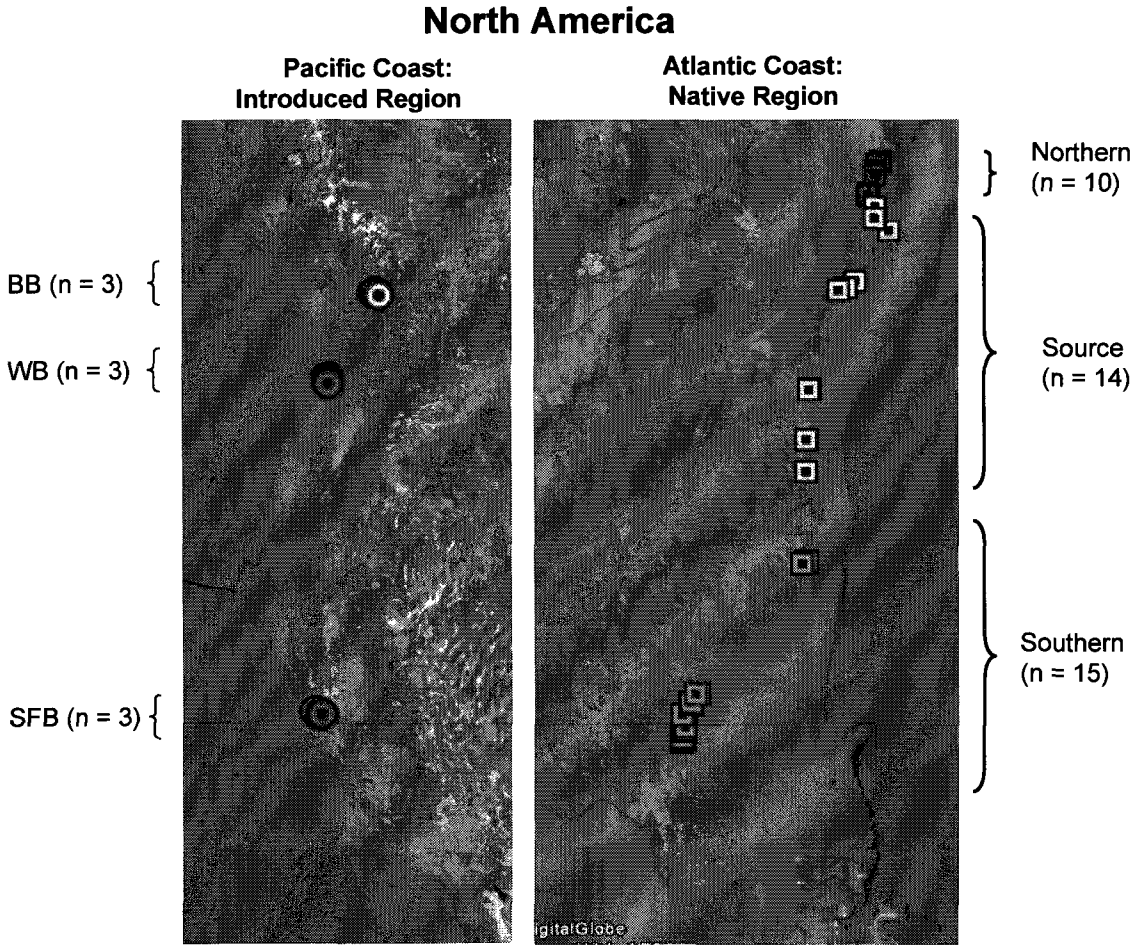

Figure 2. 1

Map of sampling sites for *I. obsoleta* trematodes in the native (Atlantic coast; squares) and introduced region (Pacific coast; circles) of their snail host. Nested subregions are also shown with number of sampling sites outlined in parentheses. Abbreviations for introduced subregions are: BB for Boundary Bay, British Columbia, WB for Willapa Bay, Washington, and SFB for San Francisco Bay, California.

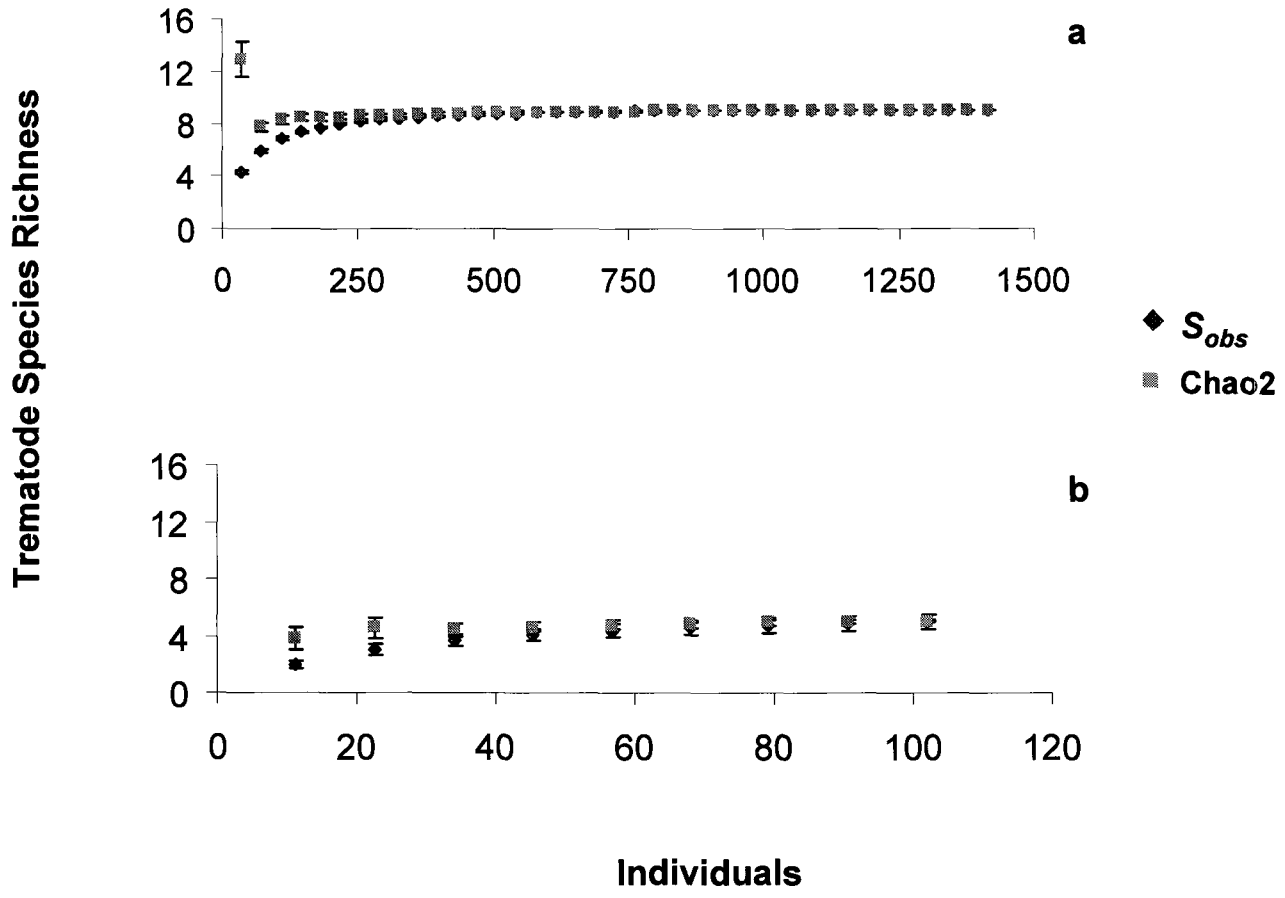

Figure 2.2

Species accumulation and estimation curves for native (a) and introduced (b) regions of *I. obsoleta*. The mean number of trematode species based on Monte Carlo resampling of the data (S_{obs}) is plotted at each sampling level along with standard error bars based on 500 runs in EstimateS (Colwell 2006). The species estimator (Chao2) is also plotted with standard error bars at each level of sampling. Although the data is sample based (i.e. trematode species were assessed from snails collected across a given number of sites), the X axis has been rescaled to show the number of the number of trematode individuals encountered in order to allow for comparison across communities with different numbers of these parasites (Gotelli and Colwell 2001).

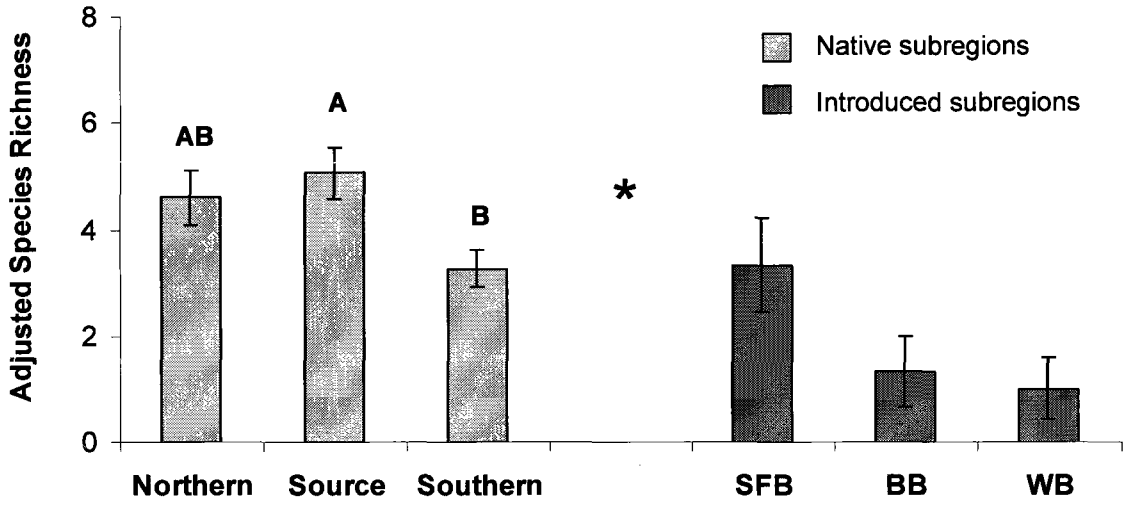

Figure 2. 3

Adjusted species richness of trematodes (obtained from EstimateS in order to standardize effort across all samples) across native (blue) and introduced (red) subregions. "*" indicates a significant difference in adjusted species richness between the native and introduced sampling regions based on the results of a nested ANOVA. Letters above bars represent the results of Tukey's test to determine differences among nested subregions. Bars with no letters in common indicate significant differences.

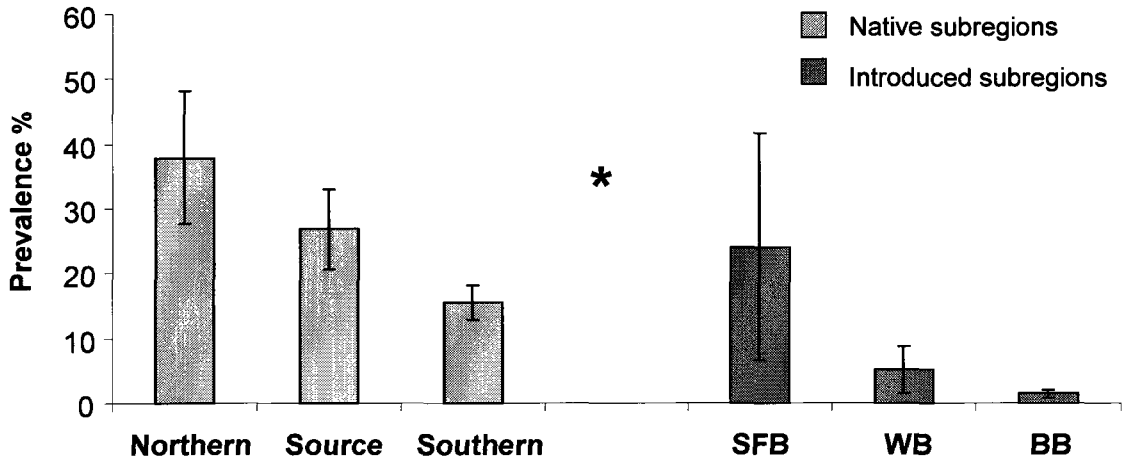

Figure 2. 4

Prevalence of trematode infection across native (blue) and introduced (red) subregions. "*" indicates a significant difference in prevalence between the native and introduced sampling regions based on results of a nested ANOVA.

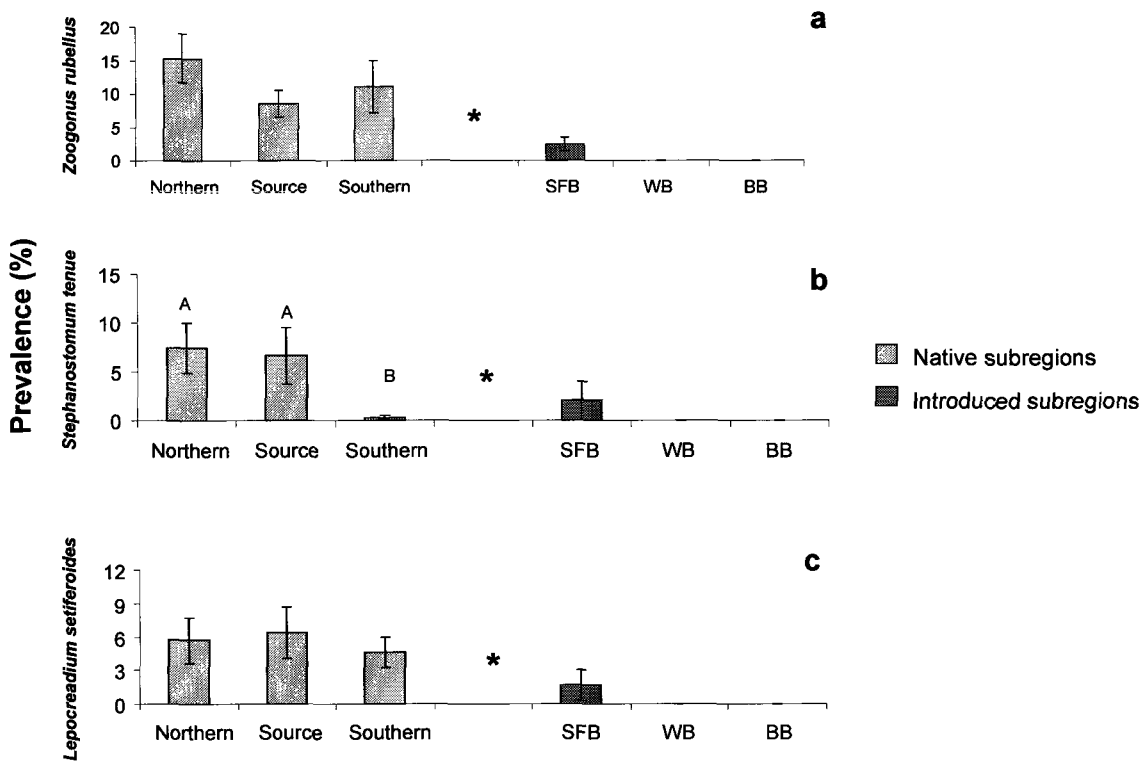

Figure 2. 5

Prevalence of individual trematode species (a) *Z. rubellus*, (b) *S. tenue*, (c) *L. setiferoides*, (d) *H. quissetensis*, and (e) *A. variglandis* across native (blue) and introduced (red) subregions. Note the differences in Y axis across the graphs. "*" indicates a significant difference in prevalence between the native and introduced sampling regions based on results of a nested ANOVA. "ns" indicates no significant difference between the two regions. When significant differences were found among regions and nested subregions, results of a Tukey's test to determine which nested subregions are significantly different are outlined with letters above the bars. In the case of *H. quissetensis* (d), significant differences were only found for subregions (but not for regions). In this case, pairwise comparisons were performed across all subregions with a Tukey's test the results of which are plotted using letters above the bars.

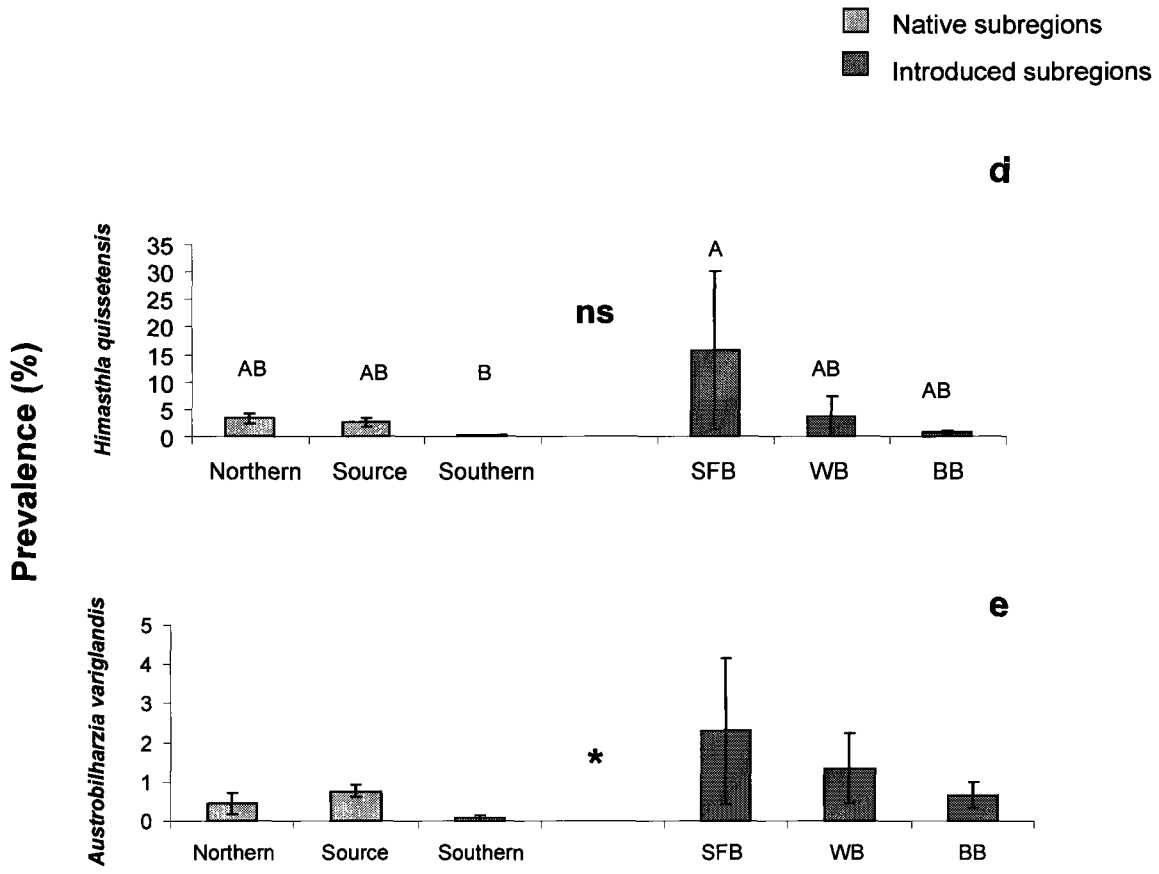

Figure 2.5 continued

Prevalence of individual trematode species (a) *Z. rubellus*, (b) *S. tenue*, (c) *L. setiferoides*, (d) H. quissetensis, and (e) *A. variglandis* across native (blue) and introduced (red) subregions. Note the differences in Y axis across the graphs. "*" indicates a significant difference in prevalence between the native and introduced sampling regions based on results of a nested ANOVA. "ns" indicates no significant difference between the two regions. When significant differences were found among regions and nested subregions, results of a Tukey's test to determine which nested subregions are significantly different are outlined with letters above the bars. In the case of H. quissetensis (d), significant differences were only found for subregions (but not for regions). In this case, pairwise comparisons were performed across all subregions with a Tukey's test the results of which are plotted using letters above the bars.

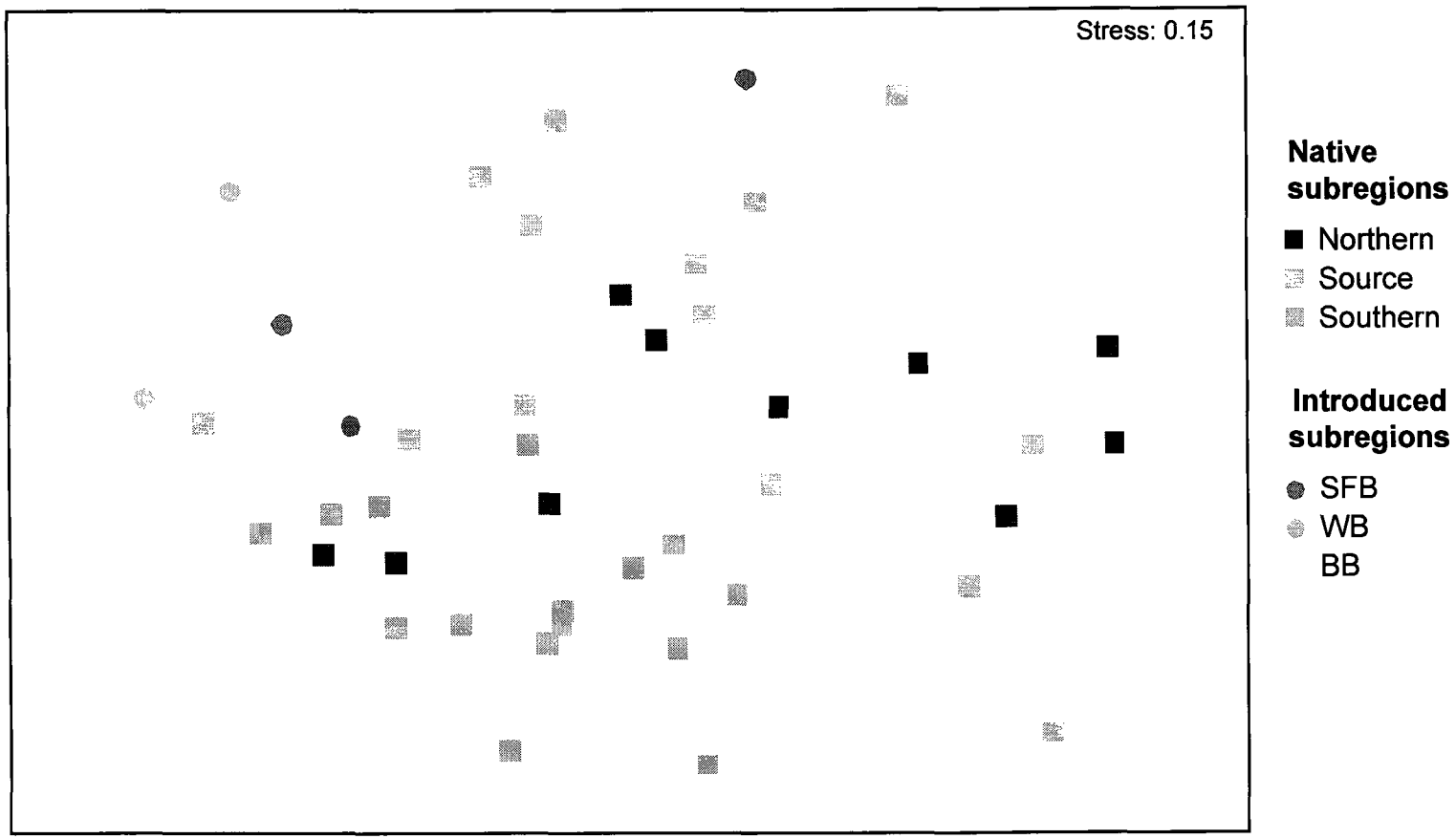

Figure 2. 6

Multidimensional scaling (MDS) plot based on Bray-Curtis similarity of trematode communities calculated for each pair of sites. The greater the distance between two points in the plot, the more dissimilar are the two trematode communities. Native sites (squares) and introduced sites (circles) are colored to reflect the nested subregions from which trematode communities were sampled.

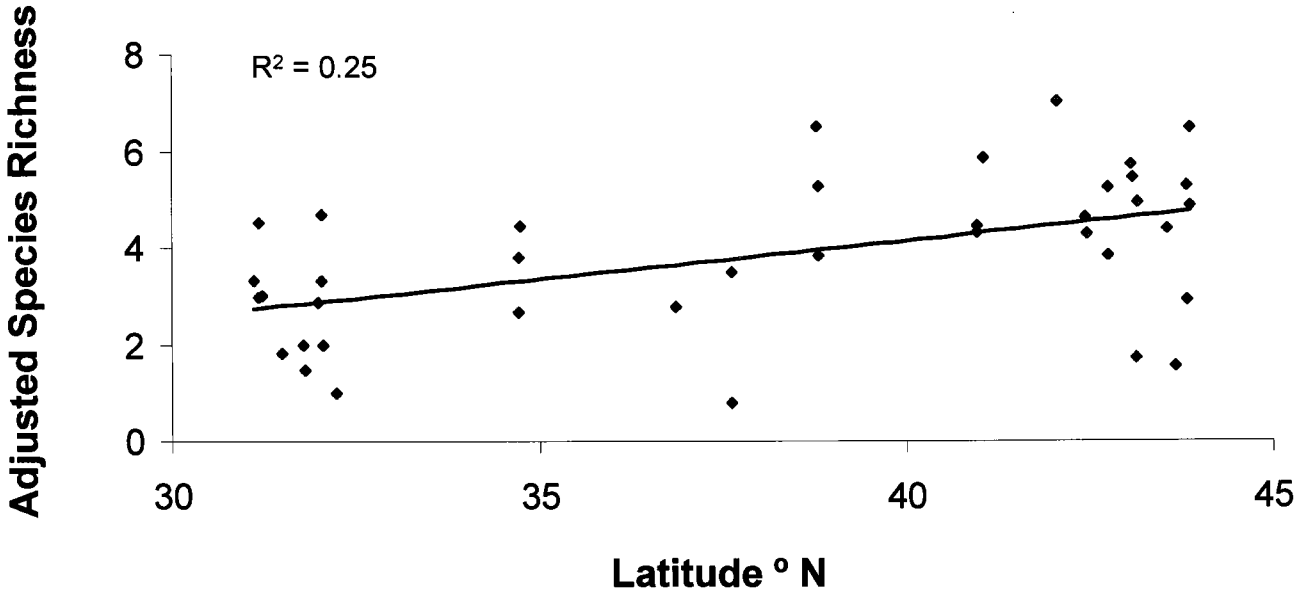

Figure 2. 7

Linear relationship between adjusted species richness of trematodes (obtained through EstimateS) and latitude for sites across the native region. Results of a simple linear regression indicate this relationship is significant $R^2 = 0.25$, $P < 0.01$.

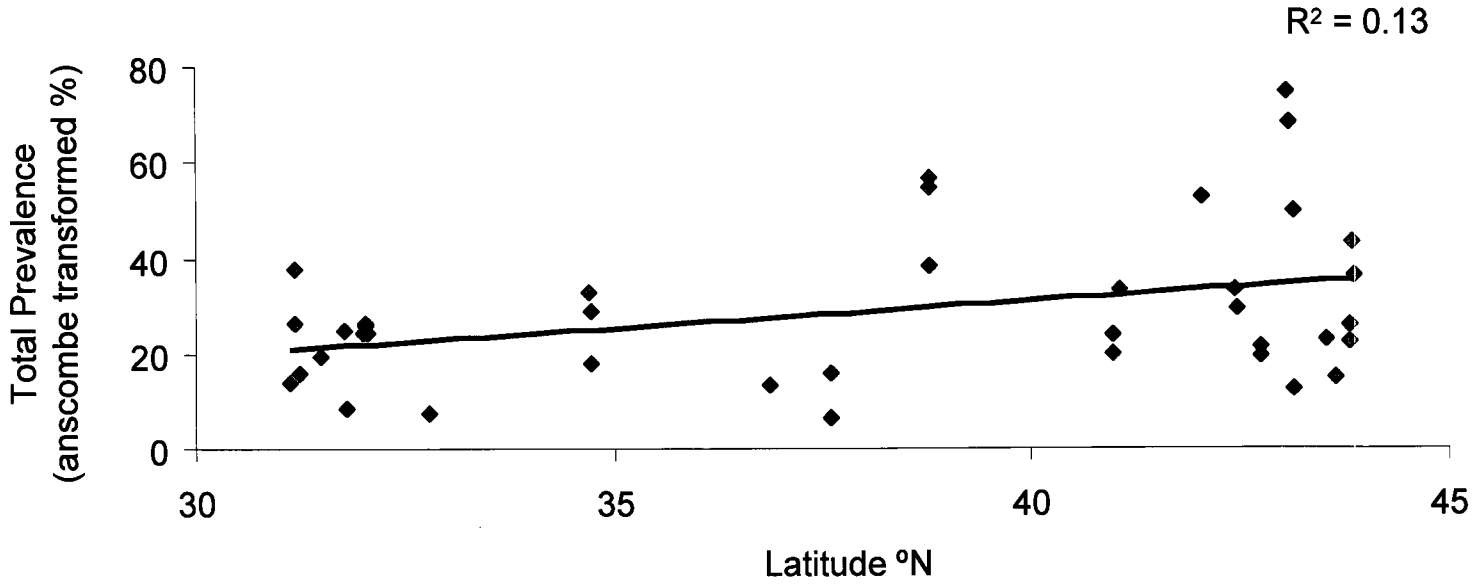

Figure 2. 8

Linear relationship between anscombe transformed infection prevalence and latitude for sites across the native region.Results of a simple linear regression indicate that the relationship, while weak ($R^2 = 0.13$), is significant, $P = 0.03$.

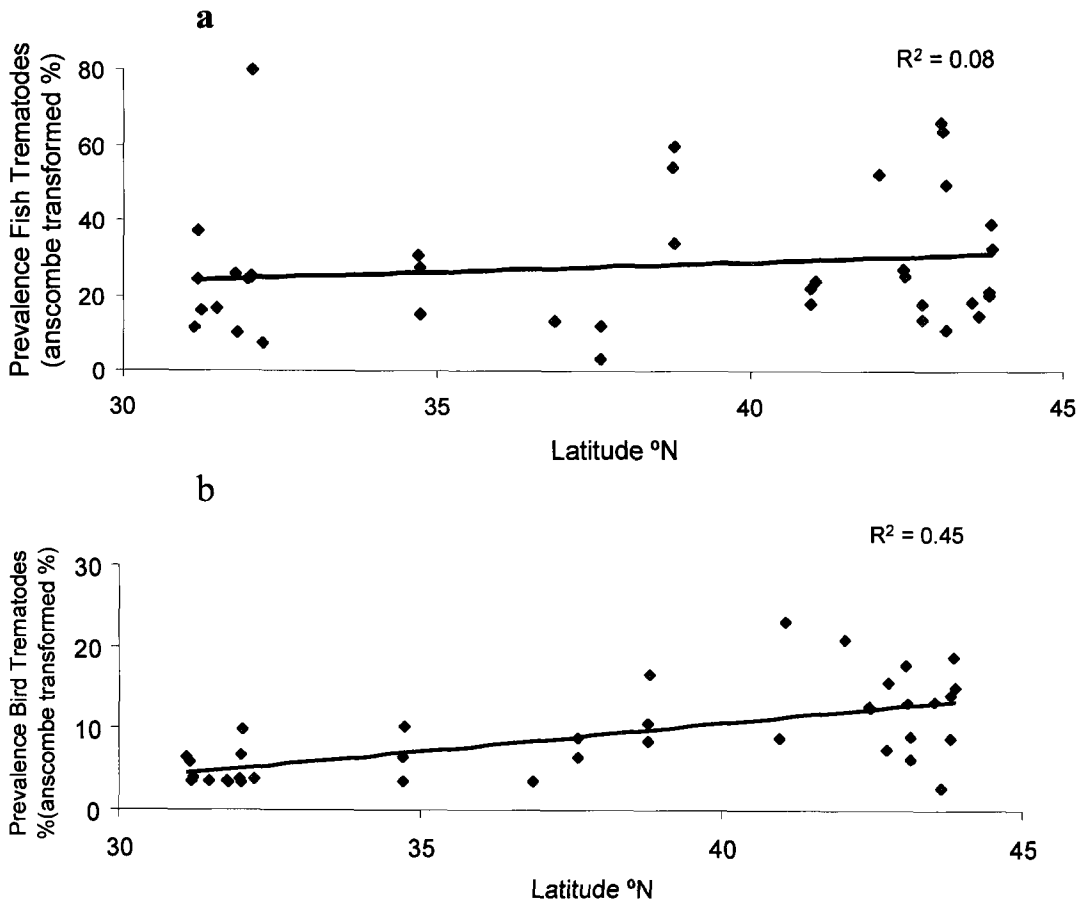

Figure 2. 9

Linear relationship between infection prevalence of trematode functional groups and latitude for sites across the native region. Trematode species are considered in the same functional group if they use similar definitive hosts. (a) Combined anscombe transformed prevalence of four trematode species (*L. setiferoides*, *S. dentatum*, *S. tenue*, and *Z. rubellus*) that use fish as definitive host ($R^2 = 0.08$, $F_{1,37} = 3.37$, $P = 0.07$), (b) combined anscombe transformed prevalence of four trematode species (*A. variglandis*, *D. nassa*, G.adunca, and *H. quissetensis*) that use birds as definitive host $R^2 = 0.45$, $F_{1,37} =$, $P < 0.0001$). Note difference in scale of Y axis.

c

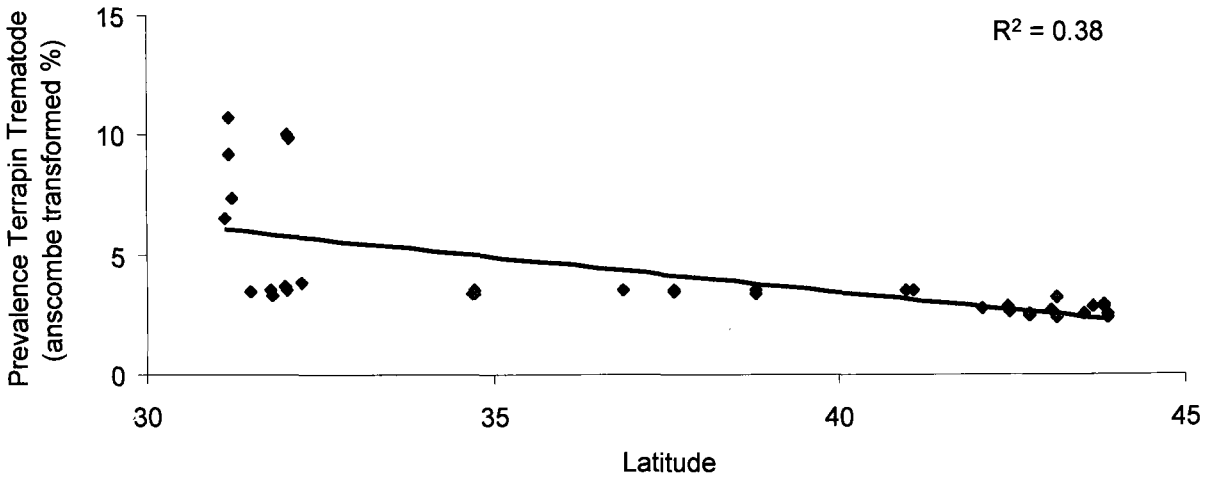

Figure 2. 9 continued

Linear relationship between infection prevalence of trematode functional groups and latitude for sites across the native region. Trematode species are considered in the same functional group if they use similar definitive hosts. (c) Anscombe transformed prevalence of *P. malaclemys* the only trematode species which uses a terrapin as a definitive host ($R^2 = 0.38$, $F_{1,37} = 22.73$, $P < 0.0001$). Note difference in scale of Y axis.

Table 2. 1

Average total prevalence (i.e. percent of snails infected with any trematode) and prevalence of individual *I. obsoleta* trematode species (i.e. percent of snails infected with a specific trematode species) across native (blue) and introduced (red) subregions ± SE. "—" indicates no snails were infected for a particular species. The number of sites sampled for each subregion is listed below the subregion heading.

	NATIVE			INTRODUCED		
Trematode Species	**Northern** (n =10)	**Source** (n = 14)	**Southern** (n = 15)	**SFB** (n = 3)	**WB** (n =3)	**BB** (n = 3)
Zoogonus rubellus	15.3 (± 3.6)	11.0 (± 3.9)	8.5 (± 2.0)	2.3 (± 1.0)	--	--
Stephanostomum tenue	7.4 (± 2.6)	6.6 (±2.9)	0.3 (± 0.2)	2.0 (± 2.0)	--	--
Lepocreadium setiferoides	5.7 (± 2.0)	6.4 (± 2.3)	4.6 (± 1.4)	1.7 (± 1.3)	--	--
Stephanostomum dentatum	5.0 (± 2.7)	4.8 (± 2.3)	0.8 (± 0.4)	--	--	--
Himasthla quissetensis	3.3 (± 0.9)	2.6 (± 0.7)	0.2 (± 0.1)	15.6 (± 14.4)	3.7 (± 3.7)	0.7 (± 0.3)
Austrobilharzia variglandis	0.4 (± 0.3)	0.8 (± 0.2)	0.1 (± 0.1)	2.3 (± 1.9)	1.3 (± 0.9)	0.7 (± 0.3)
Gynaecotyla adunca	1.0 (± 0.4)	0.5 (± 0.1)	0.2 (± 0.1)	--	--	--
Diplostomum nassa	0.1 (± 0.1)	0.2 (± 0.1)	--	--	--	--
Pleurogonius malaclemys	--	--	0.7 (± 0.3)	--	--	--
Total prevalence	37.8 (± 10.3)	26.7 (± 6.1)	15.5 (± 2.6)	24.1 (± 17.5)	5.0 (± 3.6)	1.3 (± 0.7)

Table 2. 2

Results of ANOSIM test to determine differences in the Bray-Curtis similarity of I. obsolete trematode communities across all pairs of subregions. "*" indicates that the R statistic associated with the test was significant at the level of $P < 0.05$. "ns" indicates no significant difference was found in trematode communities between the two subregions.

	Northern (Native)	**Source** (Native)	**Southern** (Native)	**SFB** (Introduced)	**WB** (Introduced)
Source (Native)	ns				
Southern (Native)	*	*			
SFB (Introduced)	ns	ns	*		
WB (Introduced)	*	*	*	ns	
BB (Introduced)	*	ns	*	ns	ns

Table 2. 3

Summary results of a literature and database search to determine the presence of second intermediate and definitive hosts for *I. obsoleta* trematodes in the introduced range of their snail host (see Appendix C for details on methodology, full results, and associated references). The table presents the total number of documented host species found in the snail's introduced range as well as the percentage that this number represents compared to the full list of documented hosts. Note that species of hosts documented from the literature often do not represent an exhaustive list of species that could serve as hosts for trematodes. Thus in the table, it is possible for introduced trematodes to be associated with zero documented hosts. When this is the case it strongly suggests that additional, undocumented hosts are being used by the parasite to complete its life cycle (for example, see definitive hosts of *L. setiferoides*). For this table the introduced range is treated monolithically. That is, host species were considered to be present in the introduced range if they were found in at least some portions of CA, OR, and/or WA. For more detailed information on the distribution of each host in *I. obsoleta*'s introduced range refer to table 2.4.

		Second Intermediate Hosts		Definitive Hosts	
Trematode species	**Present in introduced range?**	**# of documented host species in introduced range**	**% of documented host spp found in introduced range compared to native range**	**# of documented host spp in introduced range**	**% of documented host spp found in introduced range compared to native range**
A. variglandis	yes	na	na	2	100%
H. quissetensis	yes	5	45%	2	100%
L. setiferoides	yes	6	46%	0	0%
S. tenue	yes	0	0%	1	14%
Z. rubellus	yes	0	0%	1	33%
D. nassa	no	1	50%	unknown	unknown
G. adunca	no	0	0%	3	43%
P. malaclemys	no	na	na	0	0%
S. dentatum	no	0	0%	0	0%

Table 2. 4

Distributional overlap of trematode second intermediate and definitive hosts with relevant areas of introduced *I. obsoleta* populations on the Pacific coast. The information presented is based on methods outlined in Appendix C.

Trematode species	Host Designation	Host species	Pacific coast distribution		
			CA	OR	WA
A, variglandis	definitive	*Arenaria interpres interpres*	yes	yes	yes
		Aythya affinis	yes	yes	yes
H. quissetensis	second intermediate	*Crepidula fornicata*	yes		yes
		Mercenaria mercenaria	yes		yes
		Modiolus demissus = Geukensia demissa	yes		
		Mya arenaria	yes	yes	yes
		Mytilus edulis	yes	yes	yes
	definitive	*Larus argentatus*	yes	yes	yes
		Sterna hirundo	yes	yes	yes
L. setiferoides	second intermediate	*Chaetozone setosa Malmgren*			yes
		Eteone longa			yes
		Heteromastus filiformis	yes		yes
		Pygospio elegans	yes	yes	yes
		Scoloplos armiger			yes
		Streblospio benedicti	yes	yes	yes
S. tenue	definitive	*Morone/Roccus saxatilis*	yes	yes	yes
Z. rubellus	definitive	*Anguilla rostrata*	yes		

CHAPTER 3

DETERMINANTS OF *ILYANASSA OBSOLETA* LARVAL TREMATODE PARASITE COMMUNITIES AT A REGIONAL SCALE IN NORTHERN NEW ENGLAND

Introduction

Parasites are ubiquitous members of ecological communities representing more than half of the animal species on Earth (Price 1980). Effects of parasites have been widely documented especially with respect to hosts whose abundance they can depress (Keymer 1981, Scott and Dobson 1989, Fredensborg et al. 2005), or cause to fluctuate cyclically and in some cases go locally extinct (Hudson et al. 1992, Kohler and Wiley 1992, Hudson et al. 1998). Although difficult to determine experimentally (Gregory and Hudson 2000), the strong influence of parasites on hosts suggest that they can regulate host populations in nature (Hochachka and Dhondt 2000). In addition to their effects on hosts, indirect effects of parasites can have community wide consequences (Kohler and Wiley 1992, Mouritsen and Poulin 2002, Hatcher et al. 2006, Wood et al. 2007, Grewell 2008). Given their importance in ecological systems, uncovering the determinants of

parasite community structure has strong implications for a broader understanding of community ecology.

Much evidence supports the idea that high abundance of hosts leads to an abundance of parasites (Bustnes and Galaktionov 1999, Smith 2001, Huspeni and Lafferty 2004, Byers et al. 2008). The richness and relative abundance of organisms that are not primary hosts of parasites can also play an important role in disease transmission in some cases enhancing (Ostfeld and Keesing 2000) and in others reducing the abundance of parasites (Dobson et al. 2006, Ostfeld et al. 2006, Hall et al. 2009, Thieltges et al. 2009). In addition to biological factors, environmental conditions (e.g. pH, salinity, temperature) can strongly influence parasites directly (Poulin 1992, Pietrock and Marcogliese 2003, Koprivnikar et al. 2006, Soliman 2009). Abiotic factors may also act through indirect pathways. For example, when parasite transmission is dependent on the density of hosts, abiotic conditions that support abundant host communities can indirectly affect parasites (Skirnisson et al. 2004, Caceres et al. 2006, Rogowski and Stockwell 2006).

While there is strong evidence that a variety of biological and abiotic factors can affect parasites, because most studies examine individual factors in isolation, the relative importance of their roles in shaping parasite community structure is obscured. To provide a more synthetic picture, this study investigates how a large number of potentially influential variables including those of biological, chemical, and physical origin affect a specific community of digenetic trematode parasites across a regional landscape. Trematode parasites are an ideally suited group for this examination because they rely on multiple host species to complete their life cycle (Fig. 1.1) and therefore provide an

opportunity to determine the importance of hosts at different points in the life cycle. Free-living stages of trematodes also make them strong candidates to examine how abiotic variables, including the presence of contaminants, affect parasite communities (McCallum et al. 2004). Finally, because trematode parasites are easily studied and common in marine, estuarine, and freshwater environments, they allow for comparisons at broad scales and across a diversity of ecosystems.

Methods

Sampling range and site selection

Fifteen saltmarsh sites were selected in northern New England extending from north of Cape Cod, MA to mid coast ME spanning a distance of ~200 km (42.1-44.1°N). Sampling was targeted to marshes north of Cape Cod as this has been shown to be an important biogeographic boundary for marine organisms in the western North Atlantic (Gosner 1971, Hutchins 1974, Engles and Summers 1999). Sampling was limited in the north to Brunswick, ME as rocky coastlines in latitudes higher than this region generally prevent the formation of extensive saltmarsh habitat until the Bay of Fundy (Fig. 3.1). Within this region, sites were selected to ensure that a wide range of biological, chemical, and physical conditions were represented. For example, protected saltmarsh sites located far from human population centers were selected for sampling in addition to sites located in close proximity to likely sources of disturbance like roads and industrial centers.

While biotic and abiotic characteristics surrounding sites varied considerably, sampling location within sites was standardized to a single second or third order intertidal

channel that extended at least 100 m landward into the marsh and where *I. obsoleta* was common. Measurements of parasite communities and associated independent variables were conducted within this focal channel. The majority of samples were collected from June to early October, 2004 along 100 m transect placed at the mouth of a tidal channel and extending upstream. Due to the intense sampling effort required to characterize definitive host fish communities, however, these hosts could not be assessed concurrently with other metrics and had to be measured in 2005.

Sampling of *Ilyanassa obsoleta* Trematode Community

To assess trematode infection in *I. obsoleta*, mudsnails were haphazardly collected along 100 m stretch of a tidal channel that served as the focal sampling area at each site. Adult snails 15-20 mm in shell height were targeted for collection. This narrow size class was sought in order to minimize differences in the host's exposure to parasite risk among sites since snails accumulate trematode infections through time, and larger (older) snails are generally more infected than smaller (younger) individuals (Whitlatch 1974, Hughes and Answer 1982, Curtis and Hurd 1983).

Snails were dissected in the laboratory and the digestive and gonadal tissues examined for the presence of trematode infection. Trematode species identification was confirmed using multiple sources (McDermott 1951, Yamaguti 1975, Stunkard 1983). Total infection prevalence, or the proportion of snails infected, was calculated for each site. The prevalence of individual trematode species (i.e. the proportion of snails infected with a specific trematode species) was also calculated for trematodes across sites. Species richness and the Shannon index of diversity (H') which takes into account both species

richness and evenness of trematodes, was calculated for a random subset of 95 snails from each site selected from the larger dataset in order to ensure equal sample sizes of snails (Bunge and Fitzpatrick 1993).

Sampling of First and Second Intermediate Hosts

Estuarine organisms that are documented hosts of *I. obsoleta* trematodes (Table 1.1) were targeted for sampling using a variety of methods. The abundance of *I. obsoleta* was measured along a 100 m transect that extended from the mouth of the intertidal channel upstream. At every 10 m along this transect a 0.05 m^2 quadrat was placed at a random width along the channel. Within quadrats *I. obsoleta* were collected to a depth of 2 cm and washed on 0.5 mm sieve. All snails were counted and measured to nearest 0.1 mm. Infaunal bivalve abundance was assessed using a 0.075 m^2 core which was inserted into sediments to a depth of 20 cm. The contents of the core were washed through a 3.75 mm sieve and remaining bivalves were identified, counted, and measured. Abundance of minnows (*Fundulus* spp.) was measured using minnow traps baited with mussels (*Gukensia* spp.) during September and early October, 2004. Traps were placed in the middle of the channel on an incoming tide at least 50 m from one another for 30 minutes. All fish captured within traps were counted and identified and a subset measured for total length.

Sampling of Definitive Hosts

Birds and fish are definitive hosts for the majority of *I. obsoleta* trematodes, although one species uses a terrapin (Table 1.1). Bird abundance and diversity was

assessed within a standardized area around the focal sampling channel using ten minute long surveys. Surveys were always conducted before other types of sampling began at sites to ensure that the activities of samplers did not interfere with typical bird behavior. With the aid of binoculars, all birds present within a 100 m radius of the midpoint of the focal sampling channel were identified and counted. At least two bird surveys, and as many as six, were performed at sites over the course of the summer sampling season in 2004.

During the summer of 2005, fyke nets were employed to assess definitive host fish communities at sites. Compared to other methods used to sample fish, fyke nets allow for the sampling of a wide variety of species over a large area (Weaver et al. 1993, Clark et al. 2007). The method was therefore particularly well suited for this study which aimed to characterize a potentially broad variety of definitive host fish species (e.g. flounder, striped bass, eel). Fyke nets consist of a funnel shaped cod end which extended 4.6 m and was constructed of 6 mm mesh. A pair of wings constructed of ½ inch mesh was attached to this catchment end to channel in fish for collection. When the net was actively fishing, the bottom of wings were staked into sediments and tops of wings, armed with floats, rose to the water's surface creating a perpendicular barrier to fish movement. Fish blocked by the nets were drawn into and trapped at the cod end. Logistical constraints did not allow us to sample definitive host fish concurrently with other metrics sampled in this study. However, trematode infections in *I. obsoleta* under natural conditions are maintained for many years (Curtis 2003) and annual infection rates are generally low (Curtis 1996, Curtis and Tanner 1999, Altman unpublished data). Infection prevalence therefore likely remains fairly stable from year to year. Thus while

simultaneous sampling of host parasite relationships is certainly ideal, the longevity of larval trematode communities should provide a buffer when samples cannot be collected concurrently.

Fyke nets were set during low tide at sites by anchoring the cod end in the middle of the sampling channel where parasites and related variables had been sampled the previous year. Fyke nets were oriented such that the opening of the net faced upstream. Wings were extended from either side of the cod end to span the entire width of the channel and meet the edge of the vegetated marsh. During an incoming tide, wings were secured flat against the bottom so that fish and other nekton had unrestrained movement into the channel. At slack tide, the stakes used to temporarily secure wings to the sediment were removed. The fyke net then created a barrier to fish movement across the channel, trapping fish on the outgoing tide. After the tide had fully receded from the sampling channel, all captured fish were identified and counted. Total soak time for fyke nets was approximately six hours, a time period which has been shown to be associated with low escape rates from this type of net (Breen and Ruetz 2006).

Sampling of Physical Attributes

The areal extent of saltmarsh habitat around sampling sites was determined using ArcMap v.9.3 and saltmarsh data available through US Fish and Wildlife's National Wetlands Inventory (NWI) program. NWI data is based on digitized, orthorectified areal images of saltmarshes across the sampling sites which have been categorized by habitat type according to the methods of Cowardin et al (1979). For each site the area of estuarine intertidal unvegetated habitat was determined by summing polygons classified

as 'unconsolidated bottom' and 'aquatic bed habitat' according to Cowardin et al (1979). To standardize the calculation of wetland area for each site, the main estuarine subtidal channel at each site was identified and the contiguous area of unvegetated intertidal habitat associated with this channel calculated.

Some definitive fish hosts of trematodes are temporary residents in saltmarshes and make regular migrations between marine and estuarine habitats (e.g. striped bass, flounder, and eels). For these species a metric was sought to characterize the likelihood of fish inhabiting saltmarshes sites located at different distances from the marine environment. Using ArcMap v9.3 and NWI data, the swimming distance from the ocean was therefore calculated by measuring the shortest track from the sampling site to marine environment through aquatic habitats.

To quantify physical characteristics of the benthic environment at sampling sites, sediment grain size was assessed. A single core measuring 19.6 cm^2 was placed at the midpoint along the 100 m sampling transect which followed the length of the channel and inserted to a depth of 5 cm. To standardize the placement of the core with respect to water flow, the core was placed midway between the two shores of the channel which was generally the area of maximum water flow at sites. Inorganic material from this core was sieved and the proportion of clay (-0.5-<4 phi), silt (4-<8 phi), and sand (>8 phi) determined.

The density of roads located in close proximity to sites was determined with GIS analysis using ArcMap version 9.1. Road data was obtained through state transportation authorities from Maine, New Hampshire and Massachusetts. The cumulative length of roads was quantified within a 1km radius extending around each collection site.

Latitude of sites was obtained using a handheld Garmin GPS unit.

Sampling of Chemical Attributes

Sediment cores measuring 19.6 cm^2 were inserted into sediments to a depth of five cm every ten m along a 100 m length of the channel. These samples were then pooled, homogenized, and a subsample removed for analysis of trace metals. Standard protocol US EPA method 6010b (US EPA 1996) was used to assess concentration of the following 12 metals in saltmarshes sediments: aluminum, arsenic, cadmium, chromium, copper, iron, lead, manganese, nickel, silver, tin and zinc. Concentration of mercury in sediments was assessed using standard protocol US EPA method 7470a. All analyses for metals were conducted by Resource Laboratories Inc. (Portsmouth, NH).

Three additional cores were collected at random from the sampling area measuring 19.6 cm^2 and inserted into the sediments to a depth if 5 cm. These samples were frozen at -40 C and later analyzed for nitrogen, carbon, hydrogen content using a Perkin Elmer 2400 Series II CHN Elemental Analyzer at the University of New Hampshire Water Quality Lab (Durham, NH).

Data Reduction of Predictor Variables

Principle component analysis (PCA) was used to reduce the subset of 13 trace metals because no *a priori* hypotheses were made about how specific metals affect *I. obsoleta* trematode parasites. PCA yields a new set of uncorrelated, derived variables that effectively index the overall chemical milieu at sites. PCA was performed on a correlation matrix because the variance of these variables strongly differed. Principal

components were retained as derived variables for multiple regressions analysis if their associated eigenvalues were greater than one.

The analysis of multivariate data in ecological studies may be difficult because of high multicollinearity (Graham 2003). To assess multicollinearity among independent variables ($n = 13$) the Pearson product-moment correlation (r) was examined for all possible pairs of predictor variables. For pairs of strongly correlated variables with an absolute value $r > 0.60$ where a functional relationship could reasonably be assumed, the residuals of the relationship between the putative dependent variable and the putative independent variable was used in multiple regression (Graham 2003). Essentially, this allows for the de-trending of one predictor variable from its strong association with another predictor variable in order to obtain an independent measure of its relationship to the response variable. Two host variables, the abundance of wetland birds and the abundance of bivalves exhibited a strong correlation with latitude. The residuals of this relationship were therefore used in multiple regression analysis. For simplicity throughout the remainder of the text, these variables for which the residuals with latitude were taken are referred to without explicit mention that they were de-trended prior to analyses.

After de-trending correlated variables for which functional relationships were likely, only two variables, nitrogen and metal PC1, exhibited an r greater than the threshold of 0.60 (r between nitrogen and metal PC1 = 0.62). However, given that 0.6 is a fairly conservative threshold for multicollinearity, both variables were allowed inclusion in multiple regression models.

Statistical Analysis

Multiple linear regression analysis was used to determine which variables best describe the variation in prevalence and diversity of *I. obsoleta* trematode infection across sites. Both total prevalence (i.e. the proportion of snails infected by any species of trematode) and the prevalence of individual trematode species (i.e. the proportion of snails infected with a particular trematode species) were used as response variables in the models. Prior to model construction all measures of prevalence were anscombe transformed as recommended for percent data (Zar 1996). Species richness and Shannon diversity index (H') were also used as response variables in models. Because the number of snails dissected per site varied, species richness and H' were calculated for a random subset of 95 snails (the lowest number of snails dissected across sites) for each site in order to standardize sampling effort (Bunge and Fitzpatrick 1993).

In total, 13 independent variables describing host abundance, chemical, and physical attributes of sites were used in multiple regression analyses (Table 3.1). For independent variables where replicate measurements were obtained, the mean of these measurements was used in model construction. All variables were examined to determine whether they met assumptions of normality and log transformations were performed when necessary (Table 3.1 provides details about which independent variables were transformed). For simplicity throughout the text, however, variables are not referred to with explicit mention of whether they were transformed. For four variables (clay and metal PC 1, definitive fish hosts and bivalve abundance) log transformations were either not possible (because of negative values) or did not result in normal distributions.

However, because multiple regression analysis is robust to minor violations of normality these variables were left untransformed in the final analyses (Underwood 1997). All statistical analysis was performed using JMP v7.0 (SAS 2007). Stepwise forward multiple regression was selected from the Fit Model platform and variables were allowed to enter at $P < 0.05$.

Because entrance into forward regression models was based on significance level, model results contain the independent variables that describe the response with the greatest certainty. However, variables that are the most certain predictors based on low P values are not necessarily those that explain the greatest amount of variance. In order to determine whether a different model explains a greater amount of variation in the response, the full model (obtained through forward regression techniques) was compared to a reduced model where the variable that contributed least to explaining the variance, as measured by the standardized beta coefficient, was removed. The change in variation explained by the full model compared to the reduced model where a specific predictor variable has been removed is a measure of the importance of that predictor (Quinn and Keough 2006). The proportional reduction in variation of Y when a variable X_j is added to the model is measured by the coefficient of partial determination of X_j.

Results

Trematode Abundance and Diversity

An average of 134 snails were dissected at sites to determine prevalence and diversity of *I. obsoleta* infection by larval trematodes (range = 95 -182). Infection

prevalence across sites was 27.3 ± 32.0% (average ± SD) and ranged from 0.7-92.8%. All except for one of the nine trematode species documented to infect *I. obsoleta* was observed in this study. The missing species, *Pleurogonius malaclemys*, was not expected to be observed in sampling sites of northern New England which is largely outside the range of its terrapin definitive host. The prevalence of the two *Stephanostomum* species was pooled within sites because immature infections were sometimes unable to be distinguished. Pooling these species should have little effect on model results as the two are closely related (i.e. they share the same genus) and use a similar set of second intermediate and definitive hosts to complete their life cycle (*Fundulus* spp. as second intermediate hosts and piscivorous fish as definitive hosts; Table 1.1)

In most cases the trematode species most frequently observed across sites were also the ones that exhibited the highest individual species prevalence (Table 3.1). These were *Stephanostomum* spp. (11.0 ± 17.3%), *Zoogonus rubellus* (9.2± 11.7%; avg ± SD), and *Lepocreadium setiferoides* (3.6 ± 4.3%; avg ± SD) all of which were encountered at over half the sites sampled. In addition to dominating *I. obsoleta* infections, these trematodes are also ones that reach maturity in definitive fish hosts (Table 1.1). Of the four trematode species that use birds as definitive hosts, *Himasthla quissetensis* exhibited the highest prevalence across sites (2.3 ± 2.8%; avg ± SD). The three remaining bird using trematodes, *Gynaecotyla adunca*, *Austrobilharzia variglandis* and *Diplostomum nassa*, were observed rarely exhibiting an average prevalence of less than 1% across sites.

Trematode species richness (calculated from a random subset of 95 snails to standardize sampling effort) was 3.3 ± 2.4 (average ± SD) and ranged from 0-7 species.

Although prevalence was always greater than zero across sites, because trematode species richness is based on a random subset of snails to standardize sampling effort, the range includes zero.

Biological, Chemical, Physical Variables

Table 3.1 and Table 3.3 provide summary statistics for biological, chemical and physical attributes measured across sites.

Ilyanassa obsoleta—The abundance of first intermediate host mudsnails, *I. obsoleta*, ranged from 18-556 individuals per m^2 across sites, with an average of 190 $\pm$ 155/m^2 (avg $\pm$ SD). Mean shell height of quadrat sampled snails across sites was 13.05 $\pm$ 1.171 mm (avg $\pm$ SE) and the maximum shell height observed was 30.55 mm.

Fundulus spp.—Mummichogs, *Fundulus heteroclitus* and striped killifish, *Fundulus majalis* were captured in minnow traps. Distinguishing between these two species is difficult during juvenile stages; we therefore report here on abundance measures for *Fundulus* spp. Abundance of *Fundulus* spp. from minnow traps ranged from 0-273 individuals per 30 minute soak time, with an average of 59 $\pm$ 80 ($\pm$ SE) individuals per 30 minute soak time.

Bivalves—Two species of bivalve, *Macoma balthica* and *Mya arenaria*, were encountered in large sediment cores at sites. Abundance of total bivalves was highly variable. The average number of individuals collected of both species was 10,094 $\pm$ 1,3818/ m^3 ($\pm$ SE) and ranged from 0-51,362/ m^3.

Definitive Fish Hosts—Four species of definitive host fish were collected using fyke nets fished on an outgoing tide (approximately 6 hours). These were American eel

(*Anguilla rostrata*), smooth flounder (*Liopsetta putnami*), winter flounder (*Pseudopleuronectes americanus*), striped bass (*Morone saxatilis*) and white perch (*Morone americana*). Average abundance of definitive fish host species collected from fyke nets was 9 ± 16 (avg ± SD) and ranged from 0-58.

Bird Definitive Hosts—Birds observed during standardized surveys which are documented or likely definitive hosts for *I. obsoleta* trematodes were species of gull, plover, swallow, cormorant, duck, egret, heron, sandpiper, whimbrel, and willet. The abundance of birds counted within 100 m^2 sampling area over 10 minutes was 10 ± 13 (avg ± SD) and ranged from 0-42.

Nitrogen—Total nitrogen analyzed from estuarine sediments was 3.7 ± 2.0 μg/g (avg ± SE) and ranged from 2.1-10.2 μg/g.

Sediment Grain Size—Inorganic sediment composition was measured as percent sand, silt and clay of saltmarshes sediments. Among sites the average percent sand, silt and clay was 8.7 ± 14.8%, 55.9 ± 13.3%, 35.4 ± 14.6% (± SD) respectively.

Latitude—Location of sampling sites ranged from 42.2-44.1° north.

Roads—Cumulative length of roads within a 1km radius area surrounding each site averaged 6,778 ± 6,116 km (± SD) and ranged from 2,287-25,094 km.

Distance from the ocean—The distance of sampling sites from the ocean (measured through aquatic habitat) averaged 9.5 ± 7.4 km (± SD) and range from 0.9-24.4 km.

Unvegetated intertidal habitat—The area of unvegetated intertidal mudflat (i.e. mudflat) associated with sites averaged 523 ± 688 km^2 (± SD) and ranged from 0-2,056 km^2.

Trace Metals—The average concentration of 13 trace metals measured from sediments at sites is outlined in Table 3.3. Long et al.(1995) established informal, non-regulatory benchmark values for metals referred to as "Effect Range Low" (ERL) in estuarine sediments below which adverse effects of sediment dwelling infauna would be expected infrequently. When not exceeded, ERL are highly predictive of non-toxicity. Five metals sampled in this study for which ERL have been established were found to exceed ERL in at least one site; these were arsenic, chromium, lead, mercury and nickel. Concentrations of the 13 metals detected in this study were not found to exceed "Effect Range Median" (ERM, Long et al. 1995), a value above which adverse effects are likely to occur for sediment dwelling infauna.

Principle Component Analysis

Results of PCA performed on the 13 metals found the first two principle components to be associated with eigenvalues greater than one. Together these two components explained a total 82.5% of the variance associated with metal concentrations across sites (Table 3.4). Numerous heavy metals exhibited positive loadings on PC1 including zinc, lead, and copper. PC2 exhibited particularly strong positive loadings from manganese, iron, and nickel. Scores associated with these first two principle components were used as derived variables in multiple regression analyses.

Multiple regression models

A total of six response variables were used to construct multiple regression models in order to understand the factors that best predict prevalence and diversity of *I.*

obsoleta trematodes. Total prevalence is simply the proportion of snails infected with any species of trematode at sites. In the absence of multiple infections (where two or more trematode species are found to infect a single snail), the total prevalence is equivalent to the sum of individual species' prevalence. The prevalence of individual trematode species was modeled for trematodes which exhibited the highest average prevalence in order to ensure adequate resolution. These were *Stephanostomum* spp., *Z. rubellus*, and *L. setiferoides* (Table 3.2). Two diversity measures were modeled, species richness and Shannon diversity index.

Comparing the results of full models, obtained through forward regression techniques, and reduced models, where the variable with lowest absolute standardized beta coefficient was removed, revealed that for all response variables, forward regression models contained predictor variables that explained greater than 30% of the variation associated with the response variables (Table 3.5). Moreover, for all the models a lower AIC (a measure of relative goodness of fit for competing models where the lower value indicates a better model) was obtained with the full regression models indicating these models represent the best fit of the data. These findings support the conclusion that full models represent the best fit of the data for all response variables.

Total prevalence—Results of forward regression models explained 93% of the variance in total prevalence which was the highest adjusted R^2 obtained among all the models examined. A total of five predictor variables were included in the model (Table 3.6a). In decreasing order of importance based on their standardized beta coefficient these variables were clay (+), roads (-), distance from the ocean (+), nitrogen (+), definitive bird host abundance (-). While model results indicate a high level of significance

associated with the first four predictor variables (among these four variables the average $P = 0.01$), definitive bird host abundance was found to have only marginal significance ($P = 0.05$). Comparing the results of the full model with a reduced model excluding the definitive bird host abundance indicates that this variable explains an additional 38% of the variation in total prevalence (Table 3.5). Moreover, excluding this variable resulted in an increase of the AIC from 58.5 to 63.6, indicating that despite the higher number of predictor variables included, the full model is the best fit.

Stephanostomum spp. prevalence—The forward regression model explained 69% of the variation in *Stephanostomum* spp. The three significant predictor variables in the model were nitrogen (+), roads (-), and distance from the ocean (+; Table 3.6b).

Zoogonus rubellus prevalence—The forward regression model for prevalence of *Z. rubellus* included two predictor variables which together explained 78% in the variance in this response variable. In decreasing order of importance based on the absolute value of standardized beta coefficient these were metal PC2 (+) and distance from the ocean (+; Table 3.6c).

Lepocreadium setiferoides prevalence—The forward regression model explained 64% of the variation in *L. setiferoides* prevalence and included the two predictor variables clay (+) and roads (-; Table 3.6d).

Species richness—The forward regression model explained 66% in the variance in species richness of trematodes. The two significant variables included in the model were roads (-) and the area of unvegetated intertidal habitat (-; Table 3.6e).

Shannon diversity—The model for the Shannon diversity (H') contained only one variable, roads (-) which explained 48% of the variation in this response variable (Table 3.6f).

Discussion

In total, six different response variables associated with *I. obsoleta* trematode communities were modeled. Five of the six models explained greater than half the variation in the trematode response variable based on the adjusted R^2. In general, models of trematode prevalence explained a greater proportion of variation than models of trematode diversity (prevalence models average adjusted $R^2 = 0.76$, diversity models average adjusted $R^2 = 0.57$). Models of infection prevalence also tended to include more predictor variables compared to diversity models (predictor variables for prevalence models 3 ± 1.4; predictor variables for diversity models 1.5 ± 0.7; avg $\pm$ SD). Results from multiple regression models demonstrate that a core group of physical and chemical factors are strong predictors of trematode prevalence and diversity in this system including roads, distance from the ocean, clay, PC2 and nitrogen. Predictor variables may act directly on trematode parasites or could be associated with other factors which themselves shape trematode communities. Our methods did not experimentally test the effects of predictor variables on trematode communities, however, some mechanisms are hypothesized to be at the root of these patterns.

Impact of roads on trematodes

Roads in close proximity to sites (around a 1km radius) were a strong negative predictor of total prevalence as well as the prevalence of *Stephanostomum* spp., *L. setiferoides*, species richness and H' diversity. Roads likely affect trematodes through two non-mutually exclusive pathways. First, roads may be associated with environmental degradation that interferes directly with trematode transmission and survival. The data show that roads are positively related to increased concentrations of some heavy metals at sites. For example, at a scale of 1km, the concentration of copper in saltmarshes sediments exhibited a significant positive correlation with roads ($R^2 = 0.34$, $P = 0.02$). Although the response of *I. obsoleta* trematodes to copper has not been documented, decreased swimming ability and reduced longevity has been shown for cercariae of another trematode species, *Cryptocotyle lingua*, after exposure to copper and other heavy metals (Cross et al. 2001). Roads may also be associated with other types of environmental degradation not examined in this study. Freshwater runoff from impervious services associated with developed watersheds can have strong effects on salinity (Lerberg et al. 2000), a factor known to effect the emergence of free-swimming trematodes from snails in estuarine and marine environments (Rees 1948, Sindermann 1960, Sindermann and Farrin 1962, Koprivnikar and Poulin 2009). In addition, runoff from roads likely contributes to conditions of high turbidity and reduced light at marsh sites. Light can affect the emergence of free-swimming trematode stages from snails in some cases stimulating emergence (Wagenbach and Alldredge 1974, Lewis et al. 1989, McCarthy 1999) and in others inhibiting the process (Craig 1975). While the response of

most *I. obsoleta* trematode species to light has not been examined, two species *L. setiferoides* and *H. quissetensis*, are likely to benefit from conditions of reduced light (Roman et al. 2000, Roman et al. 2002). For these species, negative relationships with roads can not be explained by turbidity since this would predict a response in the opposite direction than what was observed. However, turbidity may be a factor contributing to decreased prevalence of other *I. obsoleta* trematode species.

The second way roads are likely to negatively affect trematodes is through environmental changes that decrease host abundance and thus have negative consequences for trematodes. Increased roads around sites are more likely to be associated with culverts, dikes, or other restrictions that limit the abundance or change the behavior of definitive fish hosts which were found to dominate trematode communities (Fig. 3.2). Although simple linear regression between roads and the definitive fish host abundance revealed no evidence for a relationship between these two variables ($R^2 = 0.07$, $P = 0.33$), estimates of fish abundance were constrained to a single measure of the community at one time point and therefore may not adequately capture the true abundance of fish at sites. While more extensive sampling of fish communities was not possible as part of this work, other studies indicate that tidal restrictions associated with roads can have a negative impact on both the abundance and diversity of nekton assemblages (2006). We found no evidence for a relationship between roads and the abundance of birds, the other definitive hosts used by *I. obsoleta* trematodes. Furthermore, no relationships were observed between roads and the abundance of snail hosts or any of the second intermediate hosts measured at sites (*I. obsoleta* $R^2 = 0.01$, $P =$

0.69; abundance of *Fundulus* spp. $R^2 = 0.03$, $P = 0.51$; abundance of bivalves $R^2 = 0.07$, $P = 0.65$).

In another study examining trematode infection in freshwater snails, Urban (Urban 2006) found infection prevalence increased with closer proximity to the Dalton Highway in Alaska. The contrasting relationship with roads found in my study is likely related to two factors. First, roads should effect the movement and abundance of various definitive host taxa differently. For terrestrial hosts (mammals and birds) that were the focus of Urban's work, roads likely act as a corridor to movement and sites located in close proximity to roads therefore may be associated with a greater number of these definitive hosts. On the other hand, roads are likely to restrict the movement of the fish definitive hosts important in *I. obsoleta* trematode communities since they are often associated with culverts, dikes, and other construction that limits their natural movement patterns. Second, the highway which was the focus of Urban's investigation was a single road of relatively recent construction associated with few other impacts (Stunkard 1983). Roads in this study, however, have been present for many decades and are associated with human population centers (for example, the sites with the highest road densities are located in large urban areas of Boston, MA and Portland, ME). These roads may therefore be acting as a proxy for other disturbances associated with human development and/or pollution that negatively affects trematode transmission and survival.

Impact of nitrogen on trematodes

Nitrogen in sediments was the strongest predictor of *Stephanostomum* spp. prevalence, yet it was not found to be a significant predictor of other individual trematode

species. The unique effects of nitrogen on the two species included in this group, *S. tenue* and *S. dentatum*, may be attributed to the effects of increased nutrients on the second intermediate hosts of these trematodes which are planktivorous fish (LaBrecque et al. 1996). Simple linear regression demonstrates a significant increase in the abundance of second intermediate host *Fundulus* spp. with nitrogen at sites ($R^2 = 0.29$, $P = 0.04$). Moreover, this relationship was strengthened when the site with highest nitrogen value (more than three times the average value found at other sites and nearly double the value found at the next highest site) was excluded from linear regression analysis ($R^2 = 0.46$, $P < 0.01$). In contrast, no relationship was found between the abundance of bivalves, the other second intermediate hosts measured in this study, and nitrogen at sites.

The relationship observed between nitrogen and *Fundulus* spp. is consistent with other studies showing an increase in the abundance of *Fundulus heteroclitus* in bays along a gradient of increasing nitrogen (Mattson 1980). Higher abundance of *Fundulus* spp. should provide increased habitat for trematodes that rely on these fish as second intermediate hosts. Data from this study also suggest that abundant *Fundulus* spp. populations may attract piscivorous fish that are definitive hosts of these trematode species (linear regression of definitive fish host abundance on abundance of *Fundulus* spp., $R^2 = 0.26$, $P = 0.05$). Given the limited ability to sample definitive fish in this study, the exact strength of this relationship is difficult to ascertain, but assuming any sampling deficiencies were unbiased across sites, this relationship likely represents a minimum of the describable variability.

In addition to the positive effects of nitrogen on fish abundance, higher growth rates of *Fundulus* spp. have been reported with increased nitrogen loading (McGladdery

et al. 1990) suggesting increased food consumption in response to higher nutrients. Because *Stephanostomum* spp. infections are transmitted when fish consume free-swimming stages of these trematodes (2003), increases in consumption rates could also contribute to increased transmission of these trematodes at sites.

Impact of sediment characteristics on trematodes

Clay and metal PC2 were found to be unique predictors of prevalence for two trematode species that rely on sediment dwelling organisms as second intermediate hosts, *L. setiferoides* and *Z. rubellus*. Although the two predictor variables are distinct, they both are a measure of sediment conditions at sites. Second intermediate hosts of *L. setiferoides* are tube-dwelling spionid worms (*Polydora* spp.) for which a high composition of clay in sediments may represent appropriate habitat conditions. Alternatively, increases in clay content of sediments may result from high abundance of tube-dwelling worms at sites. Bolam and Fernandez (2008) found increases in silt/clay fraction of sediments in the presence of high densities of Polydora elegans, a documented host of *L. setiferoides*. The authors suggest that this could be caused by a reduction in passive deposition associated with decreased water velocity in the presence of high densities of tube structures.

Zoogonus rubellus is unique among all of *I. obsoleta* trematodes in that the cercariae of this species are unable to swim and instead crawl on sediments in search of second intermediate hosts. Given their behavior, *Z. rubellus* may be more sensitive to sediment characteristics than other trematodes examined in this study. The strongest predictor of prevalence for this species was metal PC2, a variable characterized by high

contributions of manganese and iron. To determine whether observed concentrations of these metals exceed commonly used toxicity standards we compared measured values to common benchmarks for metals in estuarine sediments outlined Buchman (see Buchman 2008). Although iron and manganese are not included in most of the established toxicity standards for metals in estuarine sediments, levels have been set for the Apparent Effect Threshold (AET), a standard based on empirical effects of metals on benthic communities and above which toxic effects were observed (Mitsch and Gosselink 1993). Concentration of iron at sites never exceeded the AET. This was also true for manganese at all but one site. These findings suggest that the levels of iron and manganese observed in this study were not associated with strong contamination at sites and likely reflect natural conditions.

Both iron and manganese are important in anaerobic metabolic processes that occur in estuarine sediments. In the absence of oxygen, both metals serve as terminal electron receptors for microbial respiration (Martino and Able 2003). Our analytical methods were not designed to determine whether elevated levels of these metals were associated with different rates of anaerobic respiration. Nevertheless, it is likely that high concentrations of manganese and iron are related to natural processes at sites and that metal PC2 is therefore an indication of similarity among sites with respect to biogeochemical processes. It is possible that *Z. rubellus* may favor sediments characterized by high iron and/or manganese or that the parasite might benefit from conditions that are associated with these characteristics. In addition, sediment dwelling polychaetes (*Nereis* spp.) that are documented second intermediate hosts for this parasite may be positively associated with sediments characterized by these metals.

Impact of physical characteristics on trematodes

The distance from the ocean was a significant positive predictor of *Stephanostomum* spp., *Z. rubellus*, and total trematode prevalence. Numerous gradients are likely to be associated with distance from the ocean including salinity, pH, and temperature (Martino and Able 2003) and these factors may have important direct effects on trematodes. However, a more likely mechanism for the relationship with prevalence is that distance from the ocean is positively associated with species of definitive host fish abundance at sites. While sampling of fish using fyke nets provides a coarse measurement of definitive fish host (due to our limited ability to replicate sampling at sites), results of this sampling do support a significant positive relationship between abundance of definitive fish hosts and distance from the ocean ($R^2 = 0.33$, $P = 0.02$). In general, fish species are likely to exhibit different patterns with respect to this variable depending on their specific life history characteristics and tolerance to abiotic factors (2003). However, species that are important definitive hosts for *Z. rubellus* and *Stephanostomum* spp. may show particularly strong patterns of increasing abundance away from the ocean since both trematode species rely on diadromous fish species that migrate between freshwater and marine habitats during portions of their life cycle.

Definitive hosts of *Stephanostomum* spp. are *Morone saxatilis* and *Morone americana* both of which return from marine or brackish environments to spawn in freshwater. In a multiyear study of fish assemblages across ocean, estuarine, and riverine sites spanning 40 km, Martoni and Able (Smogor et al. 1995) found these species were only present at riverine stations which were located approximately 15-25 km from the

ocean. This is consistent with our findings that *Morone* spp. were only present in fyke net samples from estuarine sites located >17km from the ocean. This suggests that estuarine sites which support these fish may be located at a threshold distance from the ocean and in close proximity to riverine outputs.

Z. rubellus uses the catadromous *Anguilla rostrata* as a definitive host. Densities of small and medium eels were found to decrease with increasing distance from the ocean in Virginia streams (Ford and Mercer 1986) and to the landward side of marshes in Massachusetts (Laffaille et al. 2004). However, differences in habitat use between small and large eels have also been exhibited (Morrison et al. 2003, Cairns et al. 2004, Lamson et al. 2006) and would be predicted based on complex migrations occurring at different life history stages. Thus, in contrast to patterns displayed by smaller eels, larger individuals show mixed patterns of habitat use, either remaining in lower portions of rivers and estuaries or migrating upstream (Graczyk 1997). Despite the variability of habitat use, at a larger spatial scale older eels appear to be associated with freshwater river flows whether they prefer to inhabit areas upstream or at the confluence of estuaries. Because our study sites were restricted to estuarine habitats, it is likely that those located in close proximity to freshwater river outflows (and consequently farther from the ocean) were associated with higher abundance of eels.

Definitive fish hosts may include more species than what has been documented especially since definitive hosts are far less specialized compared to larval hosts of trematodes (Dagg and Whitledge 1991, Grimes and Finucane 1991, St John and Pond 1992, Mackas and Louttit 1998). It is possible, therefore, that fish species in addition to the ones outlined above are influenced by factors associated with distance from the

ocean. Such factors that are likely to support high abundance of fish include the proximity to river plume-fronts which are areas associated with high plankton production (MacGregor and Houde 1996) and may also attract fish that benefit from these high resource areas. Predators and other sources of mortality may also decrease with increasing distance from the ocean (Menge 1978, Rilov et al. 2005).

Ecological processes underlying observed patterns

Mechanisms suggested by individual predictor variables acting independently were explored above. While these represent plausible scenarios, some are based on indirect relationships to trematodes, often operating through host populations. On the other hand, consideration of relationships among variables can help suggest underlying ecological processes that represent more parsimonious explanations of trematode abundance and diversity. For example, two of the strongest positive predictors of total trematode prevalence at sites were clay and distance from the ocean. Taken together these variables could describe sites protected from wave energy associated with a more inland environment where a higher proportion of clay particles would be expected. Trematode eggs deposited in sites like these may be better retained in the system and could thereby contribute to higher infection prevalence. Protected sites could also be associated with higher predation rates by definitive bird and fish hosts. Although no studies have examined predation rates by fish and birds (definitive hosts of trematodes) as a function of wave energy, increased predation by invertebrate predators in protected compared to exposed sites has been demonstrated (Bustnes and Galaktionov 1999, Smith 2001, Huspeni and Lafferty 2004, Byers et al. 2008). If similar processes act on fish and bird

hosts of trematodes this could lead to increased infection at sites because transmission of most *I. obsoleta* trematodes is dependent on predation by these hosts on infected prey. Mechanisms based on single predictor variables and those related to ecological processes have the potential to explain trematode infection in snails equally well. The most parsimonious explanation should therefore guide our understanding of which explanations most likely underlie observed patterns.

Conclusion

Although many studies find host communities (and especially definitive hosts) to be strong determinants of trematode infection in snails (Skirnisson et al. 2004, Koprivnikar et al. 2007), direct support for such a link is lacking in this work. Instead, these results suggest many physical and chemical variables are strong predictors of the prevalence and diversity of *I. obsoleta* trematodes across northern New England. One likely reason for this discrepancy is the difficulty in accurately characterizing the definitive fish hosts that are so important in the *I. obsoleta* trematode system. In contrast, trematode species that have been the focus of many other studies are primarily those that use birds as definitive hosts, which are much more easily sampled across the appropriate spatial and temporal scales necessary to observe strong relationships with trematodes. For example, bird surveys are less labor intensive, can be replicated more easily, and are less likely to alter the behavior of hosts than techniques used to measure fish communities.

While definitive hosts were rarely significant predictors of *I. obsoleta* trematodes, many of the variables that did exhibit strong patterns with prevalence and diversity are hypothesized to correlate strongly with definitive host individuals, especially fish which

were the predominant definitive hosts associated with this trematode system. For example, the negative effects of roads on trematodes are proposed to act by limiting fish movement and/or changing fish behavior. Distance from the ocean is also thought to be related to fish host use of saltmarsh sites. Thus, while definitive hosts themselves were not found to be strong predictors in models, many of the variables that were important in describing these parasites may represent those that affect the fish hosts themselves, and are thus valuable proxies for the hard-to-measure fish. Additional research would prove highly valuable in testing the mechanistic underpinnings of many of these findings. Strong relationships between trematodes and numerous abiotic factors (roads, clay, and distance from the ocean) highlight the potential importance of non-host related variables as determinants of trematode communities. The majority of previous studies examining determinants of trematode community structure focus on relationships between host abundance and diversity with little regard for the role of key environmental variables (Kube et al. 2002, Hechinger and Lafferty 2005, Hechinger et al. 2007). In contrast, this work represents one of only a handful of studies to examine such a broad range of variables including the abundance of non-host organisms, physical and chemical factors that could affect trematodes (Skirnisson et al. 2004, Koprivnikar et al. 2007). Moreover, the results of this work demonstrate that many strong, non-intuitive relationships between trematodes and these factors exist in natural environments. Additional research to uncover whether these variables act directly or indirectly on trematodes will help to determine the relative importance of host versus non-host variables in shaping trematode communities

In addition to contributing to a broader understanding of trematode parasite ecology, these findings provide preliminary support for the use of *I. obsoleta* trematodes as biological indicators of wetland condition because at least some of the relationships with key environmental variables are strong. Relationships between trematodes and roads, nitrogen, and trace metals demonstrate the potential power of this parasite community to predict conditions at sites that may in turn influence a variety of saltmarshes organisms. The use of trematodes as an applied tool to assess host populations is supported by previous studies (Huspeni et al. 2004) and expanding their use to assess environmental conditions has been proposed (Anderson and May 1978, Shaw et al. 1998, Hassell 2000, Ostfeld et al. 2005). However, this is the first study to document the broad potential of these parasites to predict a suite of important environmental factors within a natural setting at a large/regional scale.

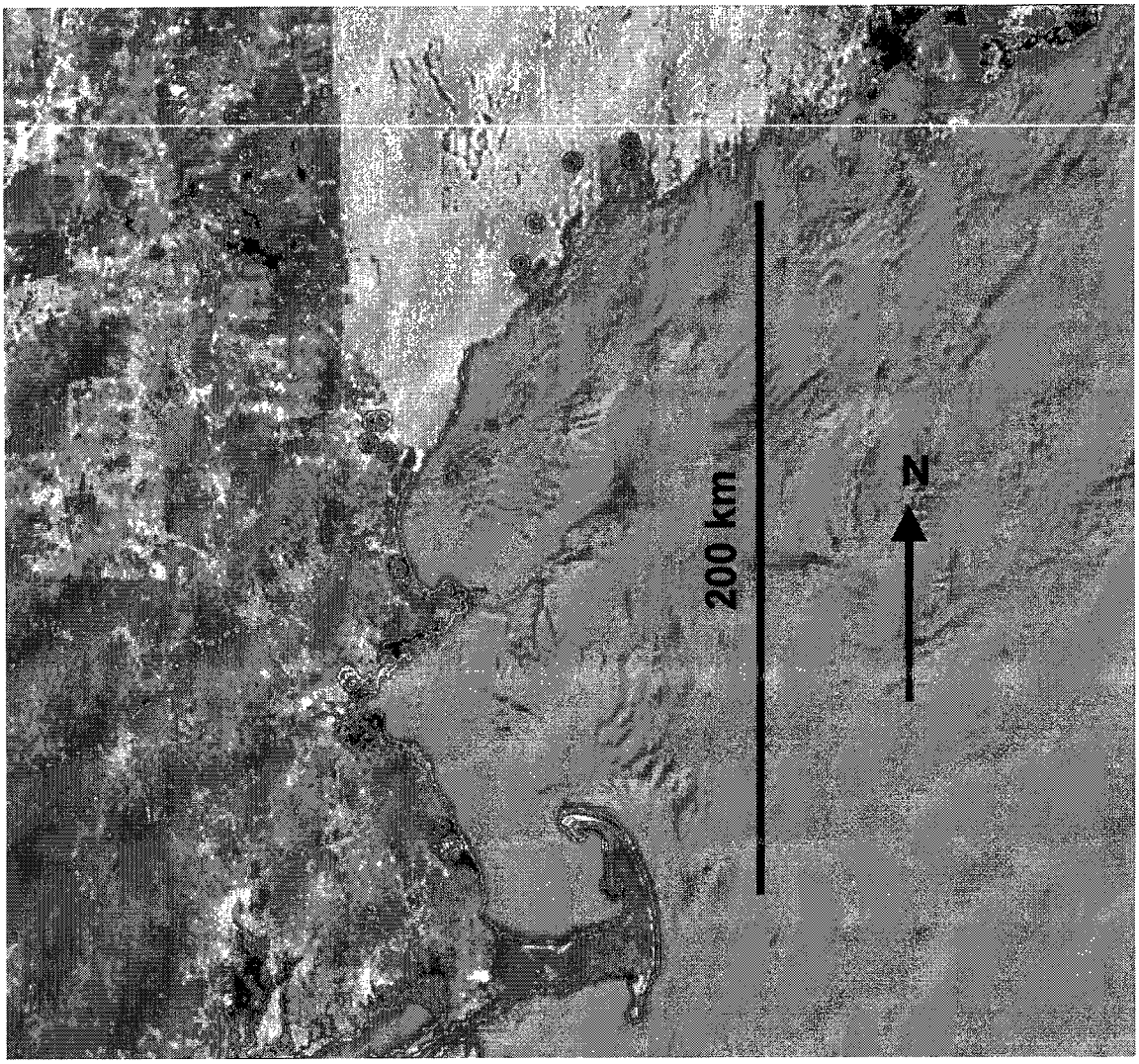

Figure 3. 1

Saltmarshes sites in northern New England, USA. Fifteen sites spanning 200 km along the Atlantic coast of North America from which *I. obsoleta* trematode communities and a wide range of biological, chemical, and physical characteristics were sampled

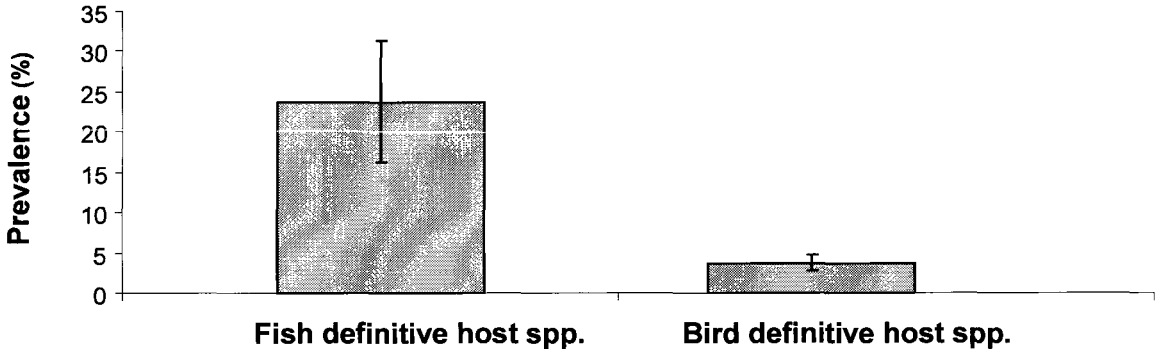

Figure 3. 2

Average site-level prevalence infection in *I. obsoleta* by by trematode species that use either fish or birds as definitive hosts. Fish species include infection by *L. setiferoides*, *S. tenue*, *S. dentatum*, and *Z. rubellus*; bird species includes infection by A.variglandis, *D. nassa*, *Gynaecotyla adunca* and *H. quissetensis*. Significant difference between prevalence of these two groups of trematodes was found, $F_{1, 28} = 6.26$, $P = 0.01$

Table 3. 1

Summary statistics for attributes measured in saltmarshes sites that were used in multiple regression analysis.

Attribute	Avg	SD	Min	Max	Unit	Transformation
Ilyanassa obsoleta	190	155	18	556	#/ m^2	none
Fundulus spp	59	80	0	273	#/ 30 min soak time	natural log
Bivalves	10094	13818	0	51362	#/ m^3	none
Definitive fish hosts	9	16	0	58	# caught/ outgoing tide	none
Definitive bird hosts	10	13	0	42	#/ 100m sampling area	natural log
Metal PC1	0.0	1.5	-3.2	2.4	na	none
Metal PC2	0.0	2.9	-3.9	8.6	na	none
Sediment Nitrogen	3.7	2.0	2.1	10.2	ug/ g sediment	natural log
Clay	35.4	14.6	0.9	56.4	%	none
Latitude	43.3	0.6	42.2	44.1	° North	none
Roads	6778.1	6116.6	2287.6	25094.3	km/ 3.14 km^2	natural log
Distance from the ocean	9.5	7.4	0.9	24.4	km	natural log
Unvegetated intertidal habitat	522.8	688.4	0.0	2056.1	km^2	natural log

Table 3. 2

Trematode infection in *I. obsoleta* across fifteen saltmarshes sites in northern New England. Infection prevalence for each trematode species and percent of sites at which each species was encountered (presence/absence) is presented. *Stephanostomum* spp. consists of infection by two species in this genus: *S. dentatum* and *S. tenue*. In the majority of snails examined, single species infections were observed. In some cases, however, snails were infected by multiple trematode species. Due to the presence of these multiple infections, the average total prevalence (i.e. snails infected with any species of trematode) is slightly lower than the summed prevalence of infection for individual trematode species.

Trematode Species	Avg Prevalence	SD	Range	% of Sites Present
Stephanostomum spp.	11.0%	17.3%	0 - 45.3%	73.3%
Zoogonus rubellus	9.2%	11.7%	0 - 34.5%	66.7%
Lepocreadium setiferoides	3.6%	4.3%	0 - 15.5%	73.3%
Himasthla quissetensis	2.3%	2.8%	0 - 7.6%	60.0%
Gynaecotyla adunca	0.7%	1.6%	0 - 5.8%	26.7%
Austrobilharzia variglandis	0.4%	0.7%	0 - 1.9%	33.3%
Diplostomum nassa	0.2%	0.5%	0 - 1.9%	20.0%
Unidentified	0.05%	0.2%	0 - 0.7%	6.7%
All species	27.3%	32.0%	0.7 - 92.8%	--

Table 3.3

Average and max Concentration of trace metals measured from sediments among saltmarshes sites. ERL is the "Effect Range Low", an informal (i.e. non-regulatory) benchmark established to aid interpretation of chemical data and based primarily on synoptic marine sediment chemistry and toxicity bioassay studies. ERL value is intended as a threshold below which adverse effects of sediment dwelling infauna would be expected infrequently. When ERL is not exceeded it is highly predictive on nontoxicity . "—" indicates that ERL has not been established for a specific analyte.

Metal	Avg ug/g	± SE ug/g	Max* ug/g	ERL ug/g	# Sites > ERL
Aluminum	25000.0	15725.3	79000.0	--	--
Arsenic	6.0	2.9	11.0	8.2	3
Cadmium	0.4	0.2	0.8	1.2	0
Chromium	59.5	32.3	110.0	81.0	6
Copper	18.6	7.4	32.0	34.0	0
Iron	20780.0	5791.4	29000.0	--	--
Lead	34.9	15.8	54.0	47.0	5
Manganese	195.3	44.2	270.0	--	--
Mercury	0.1	0.1	0.3	0.2	2
Nickel	16.1	5.2	24.0	21.0	3
Silver	0.4	0.1	0.4	1.0	0
Tin	4.1	1.5	6.0	--	--
Zinc	84.9	29.9	130.0	150.0	0

Table 3.4

Variable loadings from Principle Component Analysis performed on concentrations of trace metals found in sediments among fifteen saltmarshes sites sampled for this study. The two principle components in the table cumulatively explain 83% of the variance of these data. Principal component scores associated with metal PC1 and PC2 were used as independent variables in multiple regression analyses.

	Loadings	
Metal	**PC 1**	**PC 2**
Zinc	0.34	-0.04
Lead	0.32	-0.17
Copper	0.32	-0.17
Silver	0.31	-0.18
Tin	0.31	-0.25
Cadmium	0.31	-0.25
Chromium	0.30	0.00
Mercury	0.29	-0.12
Arsenic	0.28	0.19
Nickel	0.23	0.41
Iron	0.22	0.48
Manganese	0.18	0.53
Aluminum	-0.09	-0.22
Eigenvalue	8.41	2.37
Variance Explained (%)	64.70	18.25

Table 3.5

Comparison of full and reduced multiple regression models to explain prevalence and diversity of *I. obsoleta* trematode communities. Full models are the result of forward regression techniques (see methods for full description) and reduced models exclude the independent variable (Xj) associated with lowest standardized beta coefficient (absolute value). The coefficient of partial determination describes the proportional increase in explanatory variation attributed to the addition of the variable Xj.

Response variable	Independent variable removed	Full model SS Regression	Reduced model SS Regression	Coefficiant of partial determination	AIC Full	AIC Reduced
Total Prevalence	Definitive bird host abundance	7038.28	6837.49	0.38	58.5	63.6
Prevalence *Stephanostomum spp*	Distance from the ocean	2326.76	1872.65	0.38	66.4	71.6
Prevalence *Zoogonus rubellus*	Distance from the ocean	1552.03	786.12	0.68	53.6	68.8
Prevalence *Lepocraedium setiferoides*	Roads	340.20	128.40	0.58	40.7	48.5
Species Diversity	Unvegetated intertidal habitat	54.77	37.68	0.43	12.1	20.5
H'	Roads	2.57	0.00	0.51	-23.2	-14.5

Table 3.6

Best fit multiple regression models for response variables associated with *I. obsoleta* trematode communities. For each model, independent variables are listed in order of importance as determined by the absolute value of their standardized beta coefficient.

Model	Dependent Variable	Model input	Regression Coefficient	Standardized Beta Coefficient	F ratio	Prob > T
a	Total Prevalence	Clay	(+)	0.46	32.4	0.00
	(F 5, 9 = 37.94, P <0.0001, R^2 adj = 0.93)	Roads	(-)	-0.43	31.2	0.00
	AIC = 58.54	Distance from ocean	(+)	0.43	33.2	0.00
		Nitrogen	(+)	0.38	23.9	0.00
		Definitive bird hosts	(-)	-0.19	5.4	0.05
b	Prevalence *Stephanostomum sp*	Nitrogen	(+)	0.52	11.43	0.01
	(*F* 3,11 = 11.57, *P* = 0.001, R^2 adj = 0.69)	Roads	(-)	-0.41	7.48	0.02
	AIC = 66.42	Distance from ocean	(+)	0.40	6.78	0.02
c	Prevalence *Zoogonus rubellus*	PC2	(+)	0.66	28.22	0.00
	(*F* 2,12 = 26.43, *P <0.0001*, R^2 adj = 0.78)	Distance from ocean	(+)	0.65	27.20	0.00
	AIC = 55.98					
d	Prevalence *Lepocreadium setiferoides*	Clay	(+)	-0.66	10.95	0.01
	(*F* 2,12 = 13.42, *P* = 0.0009, R^2 adj = 0.64)	Roads	(-)	0.53	16.71	0.00
	AIC = 40.74					
e	Species Richness	Roads	(-)	-0.56	12.03	0.00
	(F 2,12 = 14.57, P < 0.0006, R^2 adj = 0.66)	Unvegetated intertidal habitat	(-)	-0.49	9.09	0.01
	AIC = 12.12					
f	H' Diversity	Roads	(-)	-0.72	13.69	0.00
	(*F* 1,13 = 13.69, *P* = 0.0027, R^2 adj = 0.48)					
	AIC = -23.25					

CHAPTER 4

SPATIOTEMPORAL VARIABILITY IN *ILYANASSA OBSOLETA* TREMATODE INFECTION ACROSS A SALTMARSH LANDSCAPE

Introduction

Spatiotemporal variation is a common feature of disease (Dronen 1978, Esch et al. 1997, Smith 2001, Hechinger and Lafferty 2005, Herrmann and Sorensen 2009) which can have important consequences for host populations as well as for the populations of disease agents themselves. Variability in recruitment of trematodes associated with aggregated patterns of definitive host abundance in both space and time (Mouritsen and Jensen 1994, McCarthy et al. 2000, Miller and Poulin 2001) can be an important factor driving infection in first intermediate hosts. Spatial and temporal differences in mortality or migration of infected and uninfected hosts can also result in infection heterogeneity. This may be especially true if infection itself influences movement patterns of hosts. For example, differential movement can cause infected individuals to distribute non-randomly (Dronen 1978, Curtis 1996, Esch et al. 1997, Esch et al. 2001, Kube et al. 2002). Temporal heterogeneity may be strongly affected by host life history and demographics (Dronen 1978, Kube et al. 2002, Poulin 2006) as well as seasonal changes

in environmental conditions, especially temperature (Halpin 1997, Fry et al. 2003, Minello et al. 2003).

The focus of this work is to examine spatial and temporal patterns of *Ilyanassa obsoleta* trematode infection across a saltmarsh landscape. Saltmarshes are composed of a complex mosaic of habitats defined by differences in tidal height, vegetation, flow rates, temperature and other abiotic factors that provide advantages and disadvantages to estuarine organisms with regard to food availability and growth (Halpin 1997, Micheli 1997), protection from predators (Stolen et al. 2007), and presence of suitable environmental conditions. Differences in habitat can affect the distribution and abundance of estuarine organisms which serve as hosts for *I. obsoleta* trematodes within a saltmarshes including birds (Baltz et al. 1993, Peterson and Turner 1994, Rozas and Minello 1998), fishes (Peterson and Turner 1994, Rozas and Minello 1998), and crustaceans (Batchelder 1915). The distribution of second intermediate and definitive hosts across habitats could have important consequences for trematode infection in *I. obsoleta* since snails are more likely to become infected if they are in close proximity to these hosts. Distribution patterns of snail hosts themselves could also lead to spatial heterogeneity in infection. From a temporal perspective, *I. obsoleta* are believed to make seasonal migrations from intertidal to subtidal areas of the marsh where they overwinter. Observations of snails moving down the marsh with the onset of colder temperatures (Sindermann 1960) and discoveries of dense aggregations of snails buried in subtidal sediments (Curtis and Hurd 1983) supports the idea that snails migrate seasonally. Thus, any spatial patterns observed at one time point may be subject to a seasonal redistribution of snails that occurs as a result of migrations.

A number of previous studies have demonstrated spatial heterogeneity in *I. obsoleta* trematode communities across a fairly homogenous sandflat environment (Curtis 2007b, a), or in association with predetermined areas of high infection (Curtis 1987). These studies set the stage for a more targeted investigation to determine whether heterogeneity is a common feature of *I. obsoleta* populations found in other localities, whether heterogeneity is associated with discrete habitat types, and whether spatial heterogeneity is maintained through time. The goals of this work are to examine inter and intra-annual patterns in infection prevalence of *I. obsoleta* across four predefined saltmarshes habitats. The ecological processes that can cause prevalence to increase and decrease in the system (i.e. acquisition of trematode infection in the snail hosts, differences in rates of movement of infected and uninfected snails, and snail host vital rates) are also considered to determine which most strongly drive observed patterns. While some processes are examined directly by the research, others are considered in the context of previous studies as well as a more general knowledge of trematode-snail interactions.

Methods

Site description and habitat designation

This research was conducted in Bellamy River Wildlife Management Area, a 400 acre protected reserve located in the Great Bay estuary, New Hampshire. Saltmarshes line the banks of this river and extend shoreward with associated intertidal channels. Previous

sampling of *I. obsoleta* indicated that snails at the site exhibit high prevalence and diversity of infecting trematodes. In addition, because the uplands surrounding the site are protected, this site represents an area of low anthropogenic disturbance in which to examine heterogeneity in this parasite community. All sampling efforts were conducted within four intertidal saltmarshes habitat types located along a tidal gradient in which *I. obsoleta* is found in abundance. The habitats are described below in order of increasing tidal height.

Mudflat. The mudflat habitat was distinguished by extensive areas of unvegetated, soft sediment, intertidal habitat which borders the Bellamy River. The mudflat is located adjacent to the subtidal portions of the river and at a 0 m low tide the area extends shoreward approximately 40 m.

Mudflat Edge. The mudflat edge was designated as a narrow, 10 m band where the mudflat meets the vegetated marsh zone. This habitat was specifically targeted for sampling because of previous studies that indicate *I. obsoleta* infected with one species of trematode are induced by the parasite to move from low intertidal areas to shoreward banks, an area preferred by the parasite's second intermediate hosts, a semi-terrestrial amphipod (Minello et al. 1994, Minello et al. 2003). Edge habitats in the marsh (where vegetated zones meets unvegetated zones) have also been shown to be areas of high abundance and diversity of fish species, which are definitive hosts of many *I. obsoleta* trematodes (MacKenzie and Dionne 2008).

Channel. Channel habitat focused on a single intertidal channel that feeds into the Bellamy River and extends approximately 0.5 km upstream. The channel is surrounded on either side by vegetated marsh. At low tide the majority of water empties from this

channel leaving exposed the muddy substrate. Sampling in the channel took place approximately 100 m from its convergence with the Bellamy River. At this location the average width of the channel extending between the vegetated marsh is 15.9 m $\pm$ 6.1 (avg $\pm$ SD).

Pools. Pool habitats were found in vegetated marsh areas dominated by *Spartina alterniflora*. They ranged in size from approximately 4-14 m^2 (avg = 11.5 m^2) and appeared as shallow depressions on the surface of the saltmarshes peat. Although surrounded by *S. alterniflora*, pools lacked any standing vegetation and always contained water at low tide. *Fundulus* spp., especially juveniles, were often observed in high abundance within pools at low tide. Importantly, pools that are the focus of this research differ from high marsh pools associated with *Spartina patens* vegetation and described in a recent study of saltmarshes located within a similar geographical region (McDermott 1951, Yamaguti 1975, Stunkard 1983).

Assessment of trematode infection prevalence among habitats: inter and intra-annual patterns

To determine patterns of infection across habitat types, snails were collected from 0.07 m^2 quadrats placed at random intervals along a fixed 50 m transect in the mudflat, mudflat edge, and channel. A slightly altered approach was used to sample snails from pools since this habitat, unlike the others sampled, does not represent a continuous area that can support the placement of a 50 m transect. At the start of the study, ten pools in which *I. obsoleta* was observed were assigned numbers. Trematode infection from pool

snails was assessed by randomly selecting a pool and haphazard placement of quadrat within this collection pool.

At least five quadrats were collected from each habitat. More quadrats were collected when necessary to obtain a sufficient sample size (~100 snails) for assessing trematode infection. For each habitat, snails collected from quadrats were pooled and a random subset of approximately 100 snails dissected to identify the presence and species of infecting trematodes. Only snails measuring greater than 10 mm were dissected because previous work showed that at this site, smaller snails are very rarely infected. Snails were dissected in the lab and their gonad and digestive tissues examined beneath a stereo-dissecting microscope to determine the presence of trematode infection. Trematode species identification was aided by the use of a compound microscope as well as published and unpublished descriptions of these trematodes (Batchelder 1915).

Patterns of infection in habitats were examined across two time scales. Inter-annual patterns were determined by collection of snails during August 2007, June 2008, and June 2009. Intra-annual patterns in infection were determined during 2008 in the months of June and October.

Determining the mechanisms underlying infection patterns

A number of potential mechanisms were explored to determine the causes of inter and intra-annual infection patterns across saltmarshes habitats. Whenever possible candidate mechanisms were tested directly (or in some cases indirect proxies were measured) and these methods are described below. In addition to these tests, inference and logic was also used to determine the most likely explanations for infection patterns.

Acquisition of trematode infection among habitats. A multiyear field experiment was conducted to determine whether the risk of acquiring infection differs among habitat types in Bellamy marsh. The experiment relied on sentinel snails, collected from an area of low infection prevalence, which were placed in cages deployed in different habitats and allowed to accumulate infection over time.

Sentinel snails were collected in August 2007 from the mudflat habitat which previous examination had revealed to be a low prevalence environment. To further ensure that these snails were minimally infected, only smaller individuals measuring 10-14 mm in shell height (12.39 $\pm$ 1.07 mm; avg $\pm$ SD) were used in experiments. Prior to placement in experimental cages, the shells of all sentinel snails were marked using Testors® enamel. This marking ensured that any snails able to gain access to the insides of cages could be easily distinguished from sentinels and removed. Prior to the start of experiment, a random subset of 100 snails was removed from the larger collection and dissected to determine the starting infection prevalence of sentinel snails.

Six circular, bottomless cages were deployed in August 2007 in each of four habitat types in Bellamy marsh. Cages were constructed of stainless steel, 6 mm square mesh and enclosed an area of 0.14 m^2. In the mudflat, mudflat edge, and channel the six experimental cages were placed 10 m apart in an area adjacent to the fixed transect used to determine patterns of infection. Six pools of similar dimensions were randomly selected for placement of pool cages. In all habitats, cages were inserted into the sediments to a depth of 15 cm and all ambient snails removed. Lids, constructed of same material as the cage itself, were secured onto cages using zip ties and allowed to sit for 24 hours in order to allow sediments to settle. After this time period, 50 sentinel snails

selected at random from the larger collection were placed into each cage. Lids were then secured with zip ties to prevent sentinel snails from escaping (Fig. 4.1).

Sentinel snails from four cages deployed in August 2007 were collected in November of the same year. After all snails were picked from the mud surface, sediments were hand sifted to reveal any buried individuals. All live snails and empty snail shells were removed from the cages and placed in separate mesh bag for transport to the Jackson Estuarine Lab (Durham, NH). Bagged sentinel snails were then placed in a single flow-through tank for the duration of the winter (November 2007-May 2008). Under natural conditions, snails are thought to migrate subtidally during colder months (Sindermann 1960) to avoid exposure to harsh winter conditions found in intertidal areas. Holding sentinel snails in the lab during this time therefore likely reduced their risk of possible mortality from exposure. In addition, winter weather conditions subject cages to high risk of destruction. As trematode accumulation does not occur in the winter months (Curtis 1987, Levin 1999), the removal of sentinel snails from field conditions during this time should not compromise the estimate of natural infection rates.

In May 2008, snails were returned to the cages from which they had been removed the previous autumn. Although the mortality of snails held in laboratory flow-through tanks over the winter months was minimal (mortality per cage = 1.9 ± 1.4, avg $\pm$ SD) to ensure that a standardized density of snails was returned to field cages in the spring of 2008, additional *I. obsoleta* were added to cages to reproduce a starting density of 50 individuals per cage (the same as what was established at the start of the experiment in August 2007). Individuals added for the purposes of standardizing snail density were marked separately and not included in estimates of final infection. The experiment was

then allowed to run until August of 2008, at which time snails were once again collected from cages. Thus, sentinel snails which overwintered in the lab were exposed to natural habitat conditions for a total of 7.5 months. All snails were dissected in the lab and their gonad and digestive tissues examined for presence and identity of infecting trematodes.

Sentinel snails in two of the six cages were left in the field during winter months to be exposed to natural habitat conditions. These snails, deployed in August 2007, were not collected until 12 months later in August 2008 after which they were dissected to determine infection.

Survival among habitats. Differential survival could also affect heterogeneous pattern of trematode prevalence. Caged snails used to assess the acquisition of infection rates among habitats (described above) were also used to determine survival over two time periods. Survival during the summer and fall months, August-November 2007, was assessed from those same caged individuals which were collected in the fall and brought into the lab to overwinter. Survival of these snails was determined immediately upon their collection in November 2007 and therefore does not reflect the overwintering period during which individuals were housed in laboratory flow through tanks. Survival of snails was also assessed over a 12 month period using data from snails caged in intertidal habitats for a total of 12 months, from August 2007-August 2008.

Growth Rates. Habitats may differ with respect to snail host growth and development and this could have important consequences for the distribution of trematode prevalence since these parasites inhabit the gonad tissue of mature snails. Thus higher growth rates could lead snail hosts to mature more quickly and provide greater resources to infecting trematodes. In addition, if snail hosts prefer habitats where they

experience high rates of growth, they may be more likely to immigrate into and less likely to emigrate out of these preferred habitats. Growth rate was measured in each habitat and used to determine the quality of or accessibility to food resources there. Again, using the same sentinel snails from two of the four cages deployed to assess trematode recruitment among habitat types (described above), a numbered bee tag was affixed to the shell of each using Krazy Glue® to individually mark snails. Snails marked in this way were carefully measured at the beginning and end of the experiment as well as during some intermediate time points. Growth rates provide an indirect measure of which habitats might be preferred by *I. obsoleta.*

Movement of snails among habitats. A movement experiment was conducted to understand the role that short term movements of *I. obsoleta* has on shifting patterns of infection across saltmarshes habitats. Marked snails were followed in July 2008 to determine the extent of snail movement in the four habitats. Within each habitat three release points were designated which snails were collected, marked, and subsequently released in order to follow their movement. Release points in the mudflat, mudflat edge, and channel were located 20 m apart. For pool habitats, the three release points were designated as the center of three separate pools (also separated by approximately 20 m) of similar dimensions and within which snails were observed be common.

Snails for this movement study were collected July 10 and 11, 2008 using 0.53 m^2 quadrats placed at each release point. Snails were collected from at least one quadrat and up to three in order to obtain a minimum of 75 snails. When collection of snails from additional quadrats was necessary, quadrats were placed end to end at the release point.

In the lab, 75 snails greater than 10 mm in shell height were selected at random from all the snails collected at each release point. The shells of these snails were marked using different colors of Testors® enamel to distinguish the habitat from which they were collected. In addition, a colored bee tag was affixed to the shell of each snail to distinguish which of the three release point it was collected from. Snails were released within the habitat and release point from which they were originally collected on July 16 and 17, 2008.

Marked snails were searched for by walking a standardized path. Beginning at the release point, a 1 m radius circle was walked around the release point. This was followed by a walking a circular path with a 2 m radius. Concentric circles, each with a radius 1 m greater than the circle walked previously, were traveled until a total area of 314 m^2 (i.e. a 10 m radius circle) was searched around each release point. This walking pattern ensured that a standardized area was covered and that experimental markings on snails were most likely to be observed. Whenever a marked snail was encountered during the search, its habitat and release point of origin were identified and the distance back to this point measured with the aid of laser range finder (Bushnell Yardage Pro). Movement of marked snails was determined at 3 and 16 days post release. After 16 days the sediments surrounding all release points up to a distance of 2 m were collected to a depth of 2 cm. To determine whether marked snails bury beneath the sediments, sediments were sieved across 6 mm square mesh filter and any additional marked snails recovered.

Results

Inter-annual infection among habitats

In 2007, total infection prevalence (i.e. infections aggregated across all trematode species) was found to be highest in pools (13.2%) and decreased across habitats in the following order: mudflat edge (3.1%), channel (3.1%), and mudflats (2.2%). The average prevalence across habitats during this year was 5.4 $\pm$ 5.2% (avg $\pm$ SD). The rank order of prevalence among habitats in 2008 and 2009 was similar. In both years the highest prevalence was found in the mudflat edge (77.3% and 52.8% in 2008 and 2009, respectively) with the next highest prevalence found in the channel habitat (64.8% and 49.1% in 2008 and 2009, respectively). In 2008, prevalence further decreased in mudflat (32.0%) with the lowest prevalence found in pools (20.8 %). In 2009, the prevalence decreased in the pools (23.7%) with the lowest prevalence found in the mudflat (16.0%). Average prevalence across habitats in 2008 and 2009 was 48.7 $\pm$ 26.7% and 35.4 $\pm$ 18.3% (avg $\pm$ SD), respectively (Fig. 4.2).

A chi-square test was performed to determine whether the distribution of infected snails across habitat types differed significantly across the three sampling years. The number of snails sampled for estimation of inter-annual prevalence sometimes varied. Therefore, in order to obtain equal sample size for all habitats in all years for this test, individual observations were randomly removed to obtain a sample size of 89 individuals (the lowest number of snails dissected within a habitat for all years). Results of chi-square test indicate a significant difference in the distribution of infected individuals across habitat types among the sampling years ($X^2 = 24.13$, $P < 0.001$). Subsequent chi-

square tests performed only on sampling year 2007 and 2008 indicate no significant difference between these two years ($X^2 = 2.71$, $P = 0.44$). The results of these tests therefore indicate that while infection distribution was similar in 2008 and 2009, both years were significantly different from 2007.

An ANOVA test was performed on the anscombe transformed prevalence to determine whether infection across all habitats varied among the different sampling years. Results of this test reveal significant difference in infection prevalence among the sampling years. A Tukey's test determined that prevalence in 2007 was significantly lower compared to the other two sampling years. No significant differences were found in overall prevalence between 2008 and 2009 (Fig. 4.3).

Six species of trematode were observed to infect *I. obsoleta* from inter-annual samples. *Zoogonus rubellus* was the most commonly encountered trematode in all sampling years and in 2008 and 2009 was over four times more common than the next most abundant species. The trematodes *Himasthla quissetensis*, *Lepocreadium setiferoides*, *Stephanostomum tenue*, and *Stephanostomum dentatum* were observed at intermediate frequencies across the sampling years. In most years, *Gynaecotyla adunca* was the rarest trematode species observed reaching a maximum prevalence of only 1.7% in 2009. A chi-square test of homogeneity revealed no difference in the distribution of infections among the sampling years ($X^2 = 10.64$; $P = 0.39$; Fig. 4.4).

Examining the distribution of infections based on functional groupings of these trematodes could reveal patterns that are otherwise muted when infections are considered in aggregate. Trematode species were categorized according to the type of host needed for transmission from *I. obsoleta* to the next life history stage. The focus on type of

second intermediate hosts required was motivated by fact that some trematodes are thought to alter behavior or movement of snails in order to increase contact rates with second intermediate hosts (Sindermann and Farrin 1962) and this could bias their distribution among habitats. In addition, trematodes that use similar second intermediate hosts may be more closely related from a phylogenetic standpoint and therefore have similar physiological effects on snail host behavior. Species were placed in functional groups according to similarities in the second intermediate hosts used. Infaunal species are those that rely on either polychaete worms or bivalves as second intermediate hosts and include *Z. rubellus*, *L. setiferoides*, and *H. quissetensis*. Fish species infect planktivorous fish during intermediate stages and include the two *Stephanostomum* species, *S. tenue* and *S. dentatum*. *Gynaecotyla adunca* was placed in its own functional group of crustacean species since this is the only *I. obsoleta* trematode that relies on an amphipod or crab to complete its life cycle. Results of chi-square tests of homogeneity for each of the functional groups reveal no significant difference in their distribution among sampling years (infaunal species $X^2 = 10.42$, $P = 0.12$; fish species $X^2 = 12.06$, $P = 0.06$; crustacean species $X^2 = 3.81$, $P = 0.70$; Fig. 4.5).

Intra-annual infection among habitats

In June and October, 2008 highest prevalence was found in snails from mudflat edge. Apart from this similarity, prevalence decreased across habitats in a different order between the two months. In June 2008 the rank order of prevalence among habitats decreased in the following order: mudflat edge (79.2%), channel (66.7%), mudflat (32.3%), and pools (22.9%). In October 2008 the highest infection prevalence was found

in the mudflat edge (57.4%) and decreased across habitats in the following order: pools (42.9%), channel (32.1%), and mudflat (8.3%; Fig. 4.6a).

A chi-square test was performed to determine whether the distribution of infected snails across habitat types differed significantly between the sampling months in 2008. The number of snails sampled for estimation of intra-annual prevalence sometimes varied. Therefore, in order to obtain equal sample size for all habitats in all years for this test, individual observations were randomly removed to obtain a sample size of 96 individuals (the lowest number of snails dissected within a habitat for both months). Results of a chi-square test found a significant difference in the distribution of infection among habitats during August and October of 2008 ($X^2 = 26.95$, $P < 0.0001$). In contrast, results of an ANOVA indicate no significant difference between average prevalence in the two sampling months ($F_{1,6} = 0.75$, $P = 0.42$).

One species, *Austrobilharzia variglandis*, was observed in snails sampled from October 2008 which was absent from all other samples. Otherwise, the same complement of six species previously described for inter-annual samples was observed in the snails examined for intra-annual patterns. The distribution of infections across the trematode species categories for the two months was significantly different ($X^2 = 439.7$, $P < 0.001$). While *Z. rubellus* was by far the most common species observed in both June and October of 2008, the rank order of the five other species observed was not consistent across the two months (Fig. 4.7).

Additional chi-square tests were conducted to determine whether functional groupings (described above in inter-annual section of results) of trematodes associated with similar second intermediate hosts exhibited consistent intra-annual distributions. For

infaunal trematodes (those species that use either polychaete worms or bivalves as second intermediate hosts), distributional patterns across habitats varied significantly between the two sampling months ($X^2 = 32.5$ $P < 0.001$; Fig. 4.8a). On the other hand, species of trematode that use fish as second intermediate hosts (whose prevalence was much lower than that of the infaunal trematode species group) exhibited no significant difference in their distribution across the two sampling months ($X^2 = 3.12$, $P = 0.4$; Fig. 4.8b). *Gynaecotyla adunca* is the only trematode that requires the use of a crustacean during intermediate life stages. The species was observed rarely and only in the mudflat and mudflat edge habitats. A significant difference was found in the distribution of *Gynaecotyla adunca* among the sampling months ($X^2 = 8.83$, $P < 0.01$; Fig. 4.8c).

Acquisition of trematode infection among habitats

Infection prevalence of sentinel snails at the start of the experiment determined from a subset of randomly selected snails (n = 102) was 6.9%. Sentinel snails that were held in laboratory flow through tanks during the winter months were exposed to natural field conditions (within the treatment habitats) for a total of 7.5 months. Recovery of sentinel snails at the end of this time period was high for most cages. However, two cages, one located in the mudflat edge and one in a pool, exhibited low recovery of only 18% and 2%, respectively. Given the low recovery from these cages, snails from these cages were excluded from analyses. After excluding these two cages, recovery of sentinel snails averaged 72% $\pm$ 26% ($\pm$ SD; range = 26-98%).

No difference was found in final infection prevalence of sentinel snails among the different habitats ($F_{3,12} = 0.14$, $P = 0.94$). Across all treatments the average prevalence at

the end of the experiment was 9.2 $\pm$ 3.7% ($\pm$ SD). Infection prevalence therefore increased by 2.3% on average across all habitats during the experiment (Fig. 4.9).

Survival among habitats

Survival of caged snails was assessed for two time periods: over the summer months in 2007 (August-November) and over a 12 month period from August 2007-August 2008. Recovery of live sentinel snails from cages is a positive indication that snails can survive in a given habitat across the experimental time period. Likewise, recovery of marked empty shells is a positive indication of snail mortality as a result of environmental factors (as opposed to predation) within a given habitat. Missing snails, on the other hand, are not positive indicators of mortality but likely indicate that snails were able to escape cage conditions.

From August-November, 2007 average recovery of live snails was 96.3 $\pm$ 2.7% (avg $\pm$ SD), indicating snails exposed to environmental conditions in all habitats had very high survival during the summer. Marked, empty shells were not recovered, suggesting that unrecovered snails either escaped cages or were overlooked during collections. Personal observations of marked, empty snails shells found in sediments three months after the original deployment of snails suggests that decomposition over this time is an unlikely explanation for unrecovered snails. The results of an ANOVA indicate no significant difference in survival of sentinel snails among habitats ($F_{3,12} = 1.41$, $P = 0.29$; Fig.4.10)

Compared to survival across the summer months, recovery of live snails caged in intertidal habitats for 12 months (August 2007-August 2008) was much lower. The

mudflat was the only habitat in which both cages remained present for the duration of the experiment. In the two mudflat cages, 84.0% and 50.0% of sentinel snails were recovered after 12 months. No empty shells were recovered from mudflat cages. A single cage remained standing in the mudflat edge in August 2008 in which 58.0% of the sentinel snails were recovered. No empty shells were recovered from these mudflat edge cages. One cage was also found in the channel habitat at the end of the experiment in which 40% of the sentinel snails were recovered alive. This cage was the only one in which empty marked shells were found. Based on the number of shells found, positive mortality of sentinel snails in the channel was 10%. No cages were found from pool habitats at the end of the winter, thus survival of sentinel snails in this habitat over the year can not be assessed (Fig. 4.10).

Growth Rates

Significant differences in growth from August-November 2007 were found among snails caged in different habitats ($F_{3, 174}$= 70.99, P <0.001). Tukey's test revealed that the pool habitat exhibited the highest growth rate (1.68 mm $\pm$ 0.60; avg $\pm$ SD) and was significantly different from all other habitats. The second highest growth rate was from snails caged in the channel habitat (0.82 mm $\pm$ 0.54; avg $\pm$ SD), this habitat was also significantly different from all others. The lowest growth was found in the mudflat edge habitat (0.37 mm $\pm$ 0.24; avg $\pm$ SD) and mudflat (0.28 mm $\pm$ 0.58; avg $\pm$ SD). While these two habitats were significantly different from all the others, they were not significantly different from each other (Fig. 4.11).

Movement of snails among habitats

Recovery of marked snails occurred at 3 and 16 days after their release. At both time points the average recovery of snails was less than 50%, with significantly lower proportion of marked snails found after 16 days (35% $\pm$ 5%; avg $\pm$ SE) than after 3 days (19% $\pm$ 5%; avg $\pm$ SE; results of ANOVA, $F_{1,22} = 6.6$, $P = 0.02$).

Significant differences were found in the proportion of recovered snails among habitats at 3 days post release (results of ANOVA, $F_{3,8} = 15.7$, $P = 0.001$). The results of a Tukey's test indicate that highest recovery was in pools and channels and that both these habitats exhibited significantly higher recovery than the mudflat edge. While recovery trends are similar after 16 days, high variability was observed especially in pool habitats and no significant differences were found based on ANOVA results (Fig.4.12). No additional marked snails were found from sieving sediments around the release points after 16 days.

Discussion

Heterogeneity in time and space was found to be a prominent feature of trematode infection in *I. obsoleta* across Bellamy saltmarshes habitats. Strong differences in infection prevalence were found across habitats at inter-annual and intra-annual time scales demonstrating that different habitats exhibited hot spots of infection at different times. The ecological processes that govern changes in prevalence through time and space are those through which infections are gained (acquisition of new infections, movement of infected snails into the system, loss of uninfected snails through mortality,

movement, or changing demographics) and lost (mortality of infected snails, movement of infected snails out or movement of uninfected snails in, or changing demographics). While some of these processes were examined directly as part of this research, others must be inferred from additional studies or from a general understanding of the key factors that shape trematode-host dynamics.

Inter-annual patterns

Marsh-wide prevalence was found to increase sharply and significantly from 5.4% in 2007 to 48.7% in 2008 with no significant change between the latter two sampling years, 2008 and 2009 (Fig. 4.3). In theory, the strong increase in infection in 2008 could result from high trematode recruitment to uninfected snails over that year. Direct evidence from infection rates of caged snails, however, indicates that this explanation is largely unsatisfactory since over the course of the summer, the ecologically relevant time period during which snails become infected (1996), acquisition of infection was very low. Over the 7.5 month period during which caged snails were exposed to field conditions (a time period which was coincident with the observed increase in marsh-wide infection), prevalence in snails increased by only 2.3% with no difference across the habitats. The observed change in infection prevalence was therefore over 17 times the increase measured from caged snails and does not account for the dramatic increase between sampling years.

It is possible that the experimentally determined rate of infection is an underestimate because of potential effects of cages on the contact rate between snails and relevant trematode stages (eggs and miracidia). Cages were constructed of mesh with

openings sufficient to allow movement of trematode eggs and miracidia inside, however the presence of cage structures could alter flow rates and thereby limit movement of infective stages from reaching snails. Restricted movement of caged snails themselves could also decrease their contact with trematode eggs and miracidia compared to what would be experienced under natural conditions. While these cage effects could misrepresent infection rates, other studies lend support to the results observed here. Curtis (Curtis) followed marked, uncaged snails over the summer and found a similarly low infection rate of 1.6%. The monthly infection rate between the two studies was therefore equivalent at 0.3% and suggests that, in fact, infection of caged snails provides an accurate measure of the natural infection risk. Moreover, because *I. obsoleta* is a long lived snail (a lifespan of 30-40 years has been proposed, Curtis 1997, 2001), low annual infection rates could easily account for the prevalence of 50% or more in adult snails observed in this and other studies (Curtis 1996).

Examination of demographic patterns indicates that the presence of a great number of small snails in 2007 likely contributed to the low prevalence observed in this year since smaller, younger snails are less likely to be infected compared to larger, older individuals that have had more time to acquire trematode parasites (Perez et al. 2009). Size frequency histograms of snails show that in 2007 the abundance of individuals in the smallest size class assessed for infection, 10-12 mm, was more than ten times higher than 2008 and 2009 (Fig. 4.13). Similarly, 12-14 mm snails were over three times more abundant in 2007 than the next two sampling years. The high proportion of small snails in 2007 is also reflected in the smaller average size of snails dissected for assessment of trematodes in this year (compare shell heights across years in Fig. 4.3).

Demographic patterns suggest a high recruitment year just prior to 2007 with relatively low recruitment in the following two years. In addition, the cohort of small snails observed in 2007 is conspicuously missing from the sampled population in 2008 and 2009. While it is possible that differential mortality of small snails could contribute to their absence in the latter sampling years, results from snails caged over the summer months does not support this explanation as survival was extremely high during this time (average survival = 96.3%; Fig. 4.10 blue bars). Mortality is more likely to occur in the winter than the summer months, however, and this is supported by observations of snails caged over a longer, 12 month period, which demonstrate lower survival and suggest that small snails are particularly sensitive to harsh winter conditions (Fig. 4.10 maroon bars). While not examined in this study, mortality from predation could also contribute to the differential loss of small individuals since relevant predators often target smaller individuals as sources of prey (Vernberg and Vernberg 1963, McDaniel 1969, Fredensborg et al. 2005).

To control for changes in demographics and determine whether the presence of many small individuals in 2007 is the only factor driving down prevalence in this year, infection was examined for snails across narrow size classes. This essentially has the effect of standardizing comparisons of infections for snails of similar sizes (ages). Results of this examination reveal a marked lower prevalence for all size classes of snails in 2007 compared to the latter two sampling years (Fig. 4.14) and demonstrate that another factor (in addition to the presence of small snails) must be contributing to reduced prevalence in this year. A possible explanation for the population-wide low prevalence is that many more infected snails were present at the site in 2007, however due to some environmental

factor, these snails exhibited different behavior resulting in their being excluded from sampling. In other studies snails infected with trematodes have been shown to exhibit lower tolerance to high temperatures (Lackner 1980), physical, thermal, and osmotic stress (McDaniel 1969, Tallmark and Norrgren 1979, Lackner 1980), and in theory this could lead snails to change their behavior to avoid or ameliorate these conditions. Environmental conditions, especially temperature, may be a particularly relevant factor to understanding these patterns given that sampling in 2007 occurred during the latter part of the summer in August, while in 2008 and 2009 snails were sampled in June.

Temperature data from a scientific buoy stationed near the Bellamy site in Great Bay was obtained from the Great Bay National Estuarine Research Reserve to explore whether conditions during sampling in 2007 were markedly different from those in 2008 and 2009. Average weekly temperature was determined from data collected every 15 minutes across the summer months (June, July, and August). Trends within this data demonstrate that sampling in August 2007 corresponded to temperatures that were slightly higher than in the June sampling month of 2008 and 2009. In addition, the greatest temperatures observed in this data set were taken in early August of 2007 (see data point corresponding to week 9 in August 2007, Fig. 4.15). It is possible that higher temperatures associated with the August 2007 collection caused infected snails to distribute differently in this year. Environmental cues that occur late in the summer could also induce some behavior response in snails, such as the onset of migration. If such cues affect snails differently depending on infection, this could also play a role in shifting distribution of snails.

A significant difference was found in the distribution of infected snails (aggregated across all species) among habitats in 2007; however this difference was removed when infections were considered among different trematode functional groups. Similar inter-annual patterns of infection were observed for infaunal trematode species (Fig. 4.5a) and fish trematode species (Fig. 4.5b) and these two functional groups contributed most greatly to patterns observed in total prevalence (Fig. 4.3). On an annual basis, trematodes included in both these functional groups exhibited higher prevalence in the mudflat edge and channel habitats during the mid summer sampling period. It is possible that these habitats represent ones where relevant second intermediate hosts are found in abundance and that infected snails therefore target these areas in order to increase the probability of transmission to the next host. Directional movement of *I. obsoleta* towards preferred habitats could also play a role in the spatial distribution of trematodes that occurs annually. While this study did not directly examine whether directional movement of snails occurs, some indirect lines of evidence supports this idea. Significantly higher growth of snails caged in pools and channels (Fig. 4.11) suggests that these habitats are advantageous to snails. In addition, movement experiments provide support for the idea that pools and channel are preferred because snails exhibited lower emigration out of these habitats (Fig. 4.12). If snails move from overwintering subtidal habitats (because of reduced movement rates caused by infection, Lambert and Farley 1968, Mouritsen and Jensen 1994, Miller and Poulin 2001) towards high intertidal pool and channel habitats during the early part of the summer, but infected snails show some lag time in their movement (because of reduced movement rates caused by infection (Curtis 1987) a greater proportion of infected snails could end up in habitats located at

mudflat edge and channel habitats that are located at intermediate tidal heights. This could result in higher infection prevalence of snails in these habitats as observed in both 2008 and 2009.

Distribution patterns of the trematode *Gynaecotyla adunca*, which relies on crustaceans as second intermediate host, differed from the other functional groups examined. This rare trematode was almost always encountered in mudflat and mudflat edge habitats (Fig. 4.5c) which are areas where it may come in higher contact with one of its documented host, a semi-terrestrial amphipod. Curtis conducted detailed studies of *I. obsoleta* infected with *Gynaecotyla adunca* and showed that this species induces snails to move to closer to the terrestrial edge of sandy beaches in Delaware where it is more likely to encounter appropriate second intermediate hosts (Lambert and Farley 1968, Mouritsen and Jensen 1994, Miller and Poulin 2001).

Intra-annual patterns

Marsh-wide prevalence was not found to differ at an intra-annual time scale in 2008; however patterns in total infection across habitats exhibited significant variation across different sampling months (Fig. 4.6a). This pattern was primarily driven by trematodes that use infaunal species as second intermediate hosts as infection by this group were the most commonly encountered (observe the similarity in infection patterns between Fig. 4.6a and Fig. 4.8a). Differential input of infection cannot explain intra-annual infection patterns given the low marsh-wide rates of infection exhibited by snails. Differential mortality of infected and uninfected snails is also an unlikely explanation given that the process would have to be operating in different directions within each

habitat to provide a comprehensive explanation (e.g. higher mortality of uninfected snails in pools, higher mortality of infected snails in channel). On the other hand, high rates of movement and differences in the movement of infected and uninfected snails could explain the observed patterns.

Experiments demonstrate that *I. obsoleta* exhibits high rates of movement out of local areas supporting the general idea that changes in infection patterns could result from snail movement processes. While differential movement of infected and uninfected snails was not examined as part of experiments, other studies indicate reduced movements of snails infected with trematodes (Batchelder 1915). During the October sampling period, snails are likely to have begun migrating from higher intertidal areas towards subtidal overwintering habitats (Stunkard 1938, McDermott 1951, Hanna 1966, Magendanzt 1969, Schell 1970, Rosenblum and Niesen 1985, Palacios et al. 1994, McCurdy et al. 2000, Palacios et al. 2000, McCurdy 2001, Desclaux et al. 2004, Thompson and Lowe 2004, Dudas et al. 2005, Curtis 2007a, Strasser and Barber 2009). If infected snails lag behind uninfected individuals during this migration, prevalence in lower intertidal habitats would be expected to decrease as a result of the higher proportion of uninfected snails moving into these habitats. On the other hand, prevalence in higher intertidal habitats would increase as infected snails were left behind. Infection patterns consistent with this scenario were observed. Prevalence in pools, the highest intertidal habitat, was shown to increase from June to October which is consistent with uninfected snails emigrating from that habitat towards subtidal areas of the marsh. In addition, influx of uninfected snails could explain the decrease in prevalence observed in the lower intertidal habitats (channel, mudflat edge, and mudflat) in October. Changes in snail density among

habitats during the relevant sampling months also lend some support for this explanation. A gradient of increasing snail density is exhibited from the lower to higher intertidal habitats in June with the highest density of snails present in pools. In October, however, snail densities are more evenly distributed across the habitats suggesting that some snails have moved out of pools and channels and into formerly lower density habitats (i.e. mudflat edge and mudflat habitats; Figure 4.16).

In contrast to the trematode species that use infaunal organisms as second intermediate hosts, fish using trematodes exhibited a consistent distribution among habitats in both months (Fig. 4.8b). This difference emphasizes the potential importance of considering the species specific effects of infection on snails since not all trematodes may have a similar effect on their host. This point is also underscored by comparing the intra-annual distribution of *Gynaecotyla adunca*. Distinct from the other trematode functional groups examined, both intra- and inter-annual samples found snails infected by this rare species were restricted to mudflat and mudflat edge habitats in the marsh.

Conclusion

Changing patterns of infection in I. obsoleta populations across space and time in Bellamy saltmarsh are thought to occur as a result of a limited number of key processes which are summarized below. Low annual infection rate and similar rates of infection among habitats were measured directly in this study and indicate that infection input cannot explain changing patterns in prevalence across habitats over the course of a few years. Moreover, even if differences in infection rates among habitats occurred over

longer time periods than what was measured experimentally, the migratory movements of snails would outweigh the potential importance of these differences.

Changing demographics was found to be a key factor which contributed strongly to differences in inter-annual infection prevalence. Since trematode infection in snail hosts is an age dependent process (older snails are much more likely to be infected than younger ones), loss of specific snail cohorts from the population can dramatically alter infection prevalence in the population. The loss of many small (young) snails in the population after 2007, for example, contributed strongly to the higher prevalence in 2008 and 2009.

Growth of I. obsoleta was also found to differ significantly among habitat types. While growth itself does not directly affect infection prevalence in snail populations, habitats in which snails exhibit high growth may indicate preferred areas which are targeted by snails. If snails exhibit directional movement with respect to these high growth habitats, this could help explain how a regular spatial pattern of infection can emerge as occurred in 2008 and 2009.

Snail movement is likely to be one of the most important factors in explaining shifting patterns of infection prevalence in this system. High emigration of snails in most habitats was found in this study and indicates that snail hosts are highly mobile throughout the saltmarsh. Although not measured as part of this work, differences in rates of movement between infected and uninfected snails could contribute strongly to changing infection patterns across the saltmarsh landscape. Over longer time scales, subtidal winter migrations of snails is also likely to contribute to the redistribution of infection on an annual basis. Understanding movements of infected and uninfected snails,

especially in relation to migration is likely the key to understanding patterns of spatial and temporal variability in trematode infection in this system.

From an evolutionary standpoint, high movement rates exhibited by snails and seasonal migrations likely make it difficult for I. obsoleta trematodes to become adapted to specific habitats which increase their transmission success (e.g. habitats with high abundance of second intermediate hosts or favorable environmental conditions that lead to transmission). On the other hand, the wide use of saltmarshes habitats by I. obsoleta may increase the probability that trematodes come into close contact with next hosts at some point during larval stages.

Figure 4. 1

Pictures detailing a cage experiment using low infection, sentinel, snails to determine the risk of trematode infection across different habitats in Bellamy saltmarshes, NH. (A) Low infection snails were marked and (B) placed in cages inserted into the sediments. Replicate cages were deployed in the following habitats: mudflat (not shown), mudflat edge (not shown), (C) channel, and (D) pools.

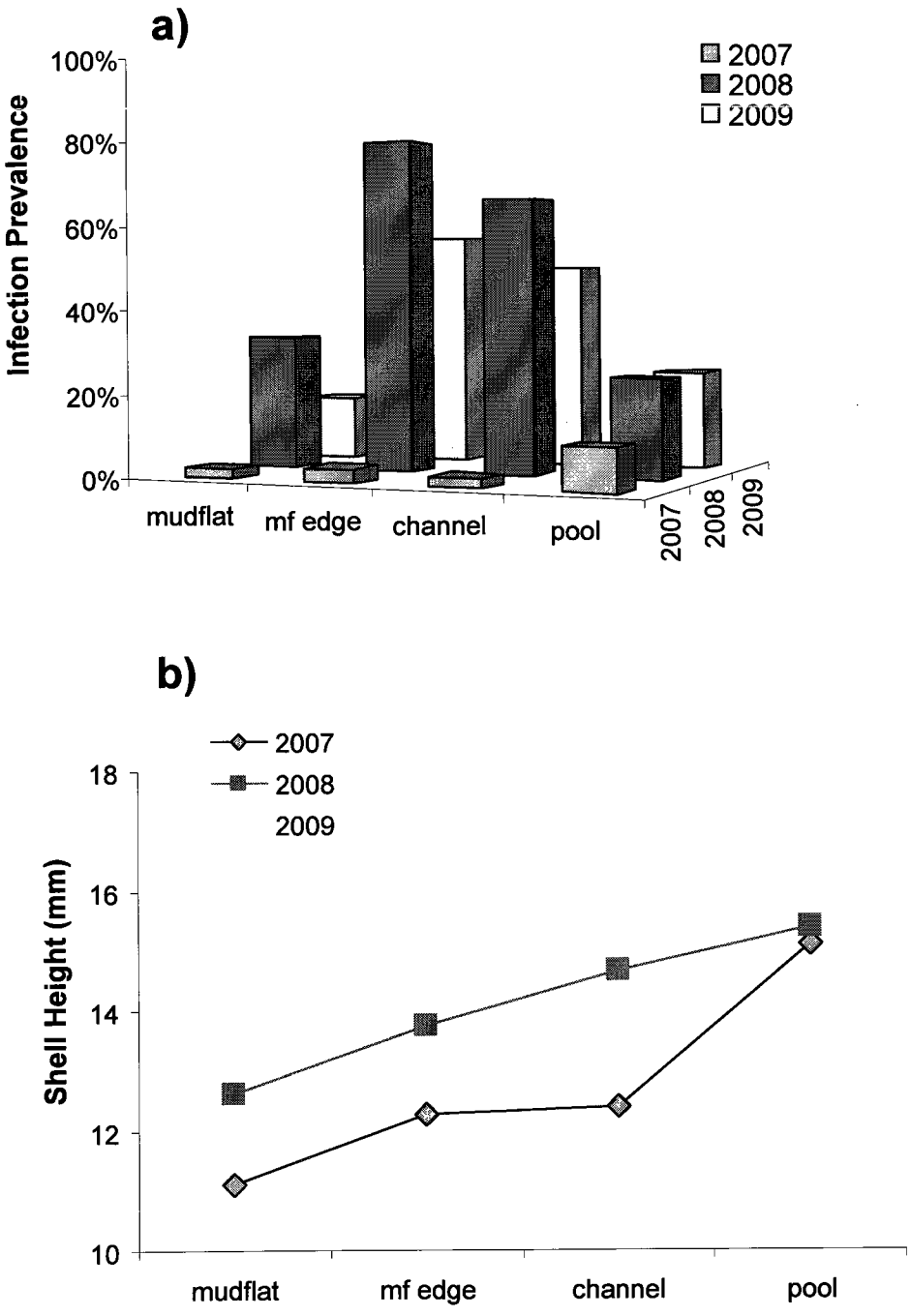

Figure 4. 2

(a) Inter-annual patterns of infection prevalence across saltmarshes habitats, (b) average shell heights of quadrat collected snails used to assess infection prevalence

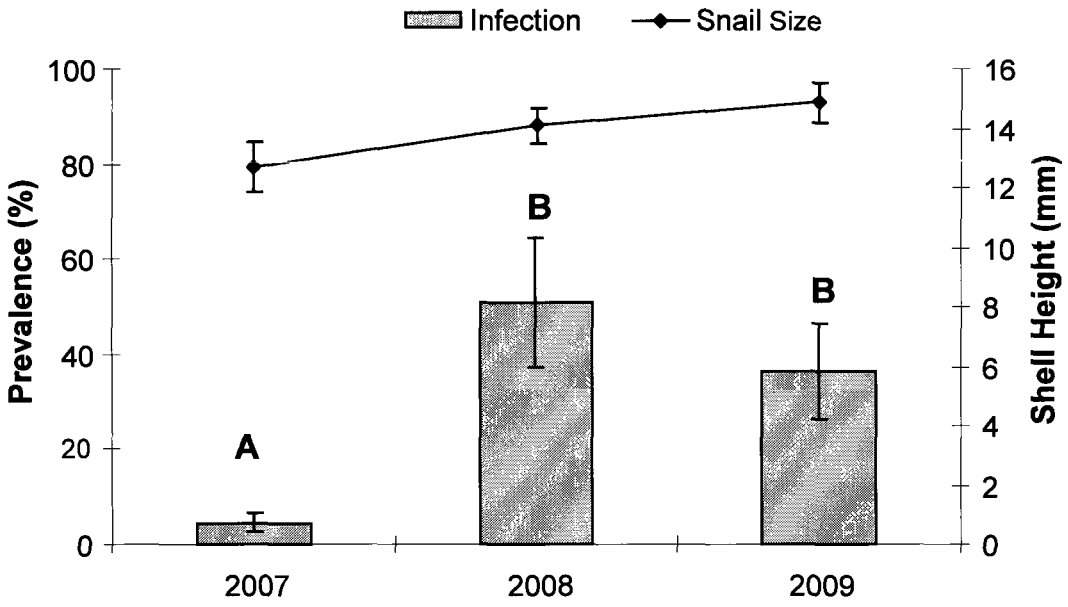

Figure 4. 3

Average infection prevalence for all saltmarshes habitats across three sampling years (bars). Approximately 100 snails were used to determine infection prevalence within a given habitat for each year (minimum n = 89; maximum n = 124). Bars with different letters indicate which years were observed to have significantly different prevalence. The average shell height of dissected snails used to determine infection is also shown (line).

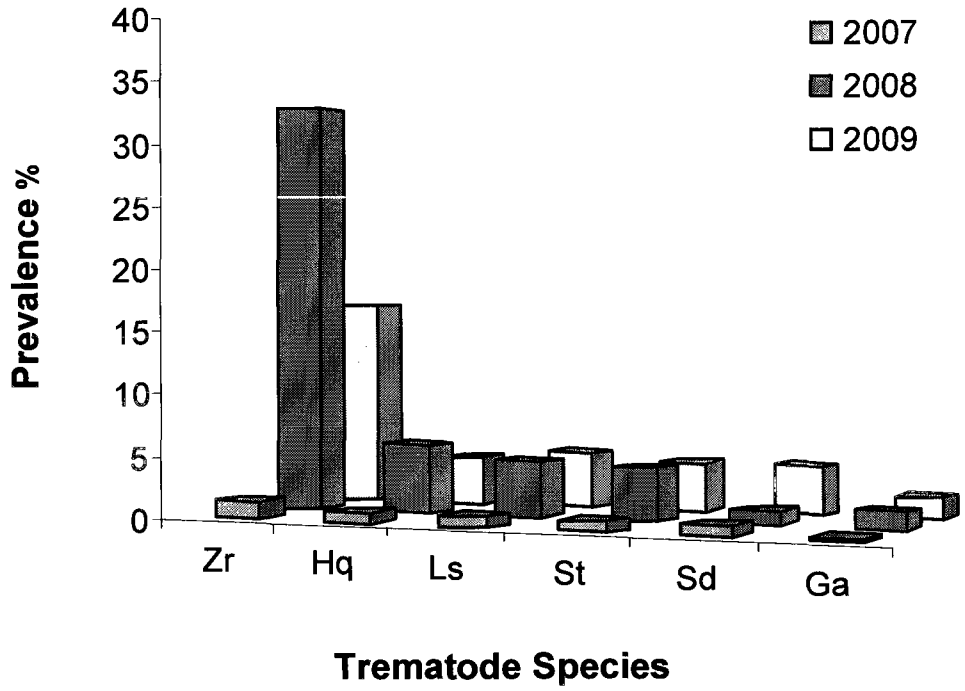

Figure 4. 4

Distribution of trematode infections among species categories for each of three sampling years. Results of a chi-square test of homogeneity demonstrate no significant difference in the distribution of infection (X^2 = 10.64; df = 10; P = 0.39). Abbreviations for trematode species along x axis are as follows: Zr = *Zoogonus rubellus*, Hq = *Himasthla quissetensis*, Ls = *Lepocreadium setiferoides*, St = *Stephanostomum tenue* , Sd = *Stephanostomum dentatum*, Ga = *Gynaecotyla adunca*.

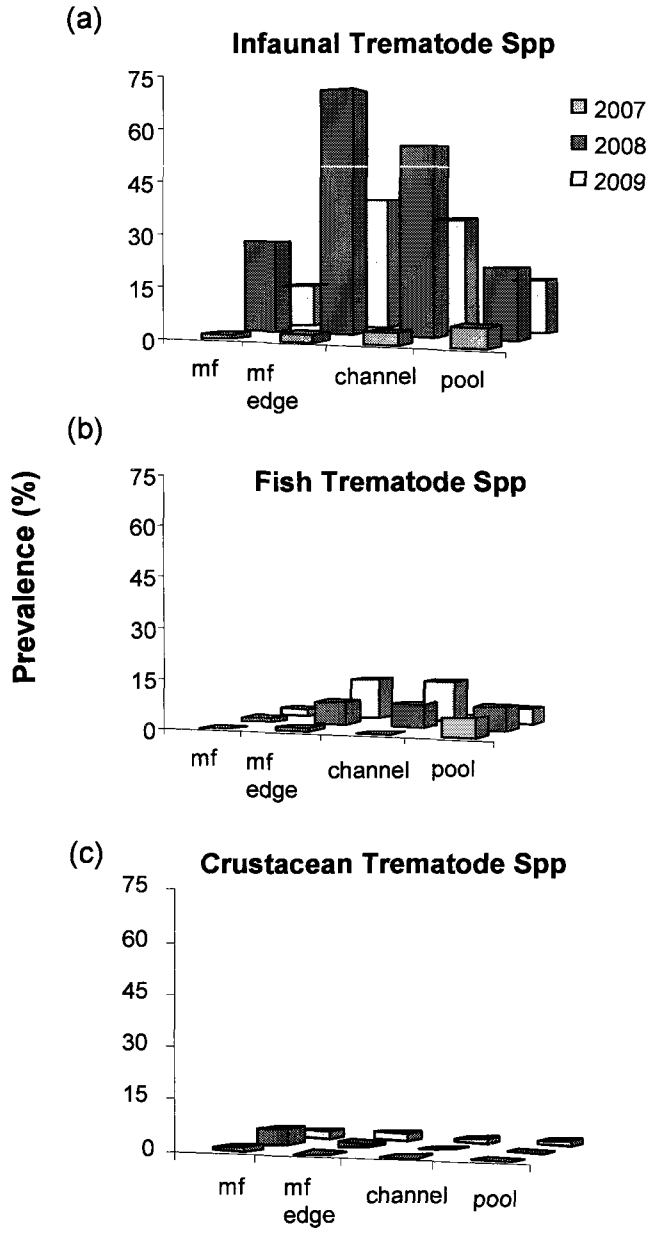

Figure 4. 5

Inter-annual patterns of infection for functional groups of trematode species based on similarities in second intermediate hosts used. (a) No significant difference was found in the distribution of infection for infaunal trematode species which consist of those that use polychaete worms or bivalves as second intermediate hosts and includes *Z. rubellus*, *L. setiferoides*, and *H. quissetensis* ($X^2 = 10.42$, $P = 0.12$). (b) Fish trematode species are the two *Stephanostomum* spp., *S. tenue* and *S. dentatum*, which require infection in planktivorous fish as second intermediate hosts. No significant difference was found in the inter-annual distribution of infection of this functional group($X^2 = 12.06$, $P = 0.06$). (c) Crustacean species includes only the species *Gynaecotyla adunca* which uses amphipods or crabs during second intermediate larval stages. No significant difference was found in the inter-annual distribution of this species ($X^2 = 3.81$, $P = 0.70$).

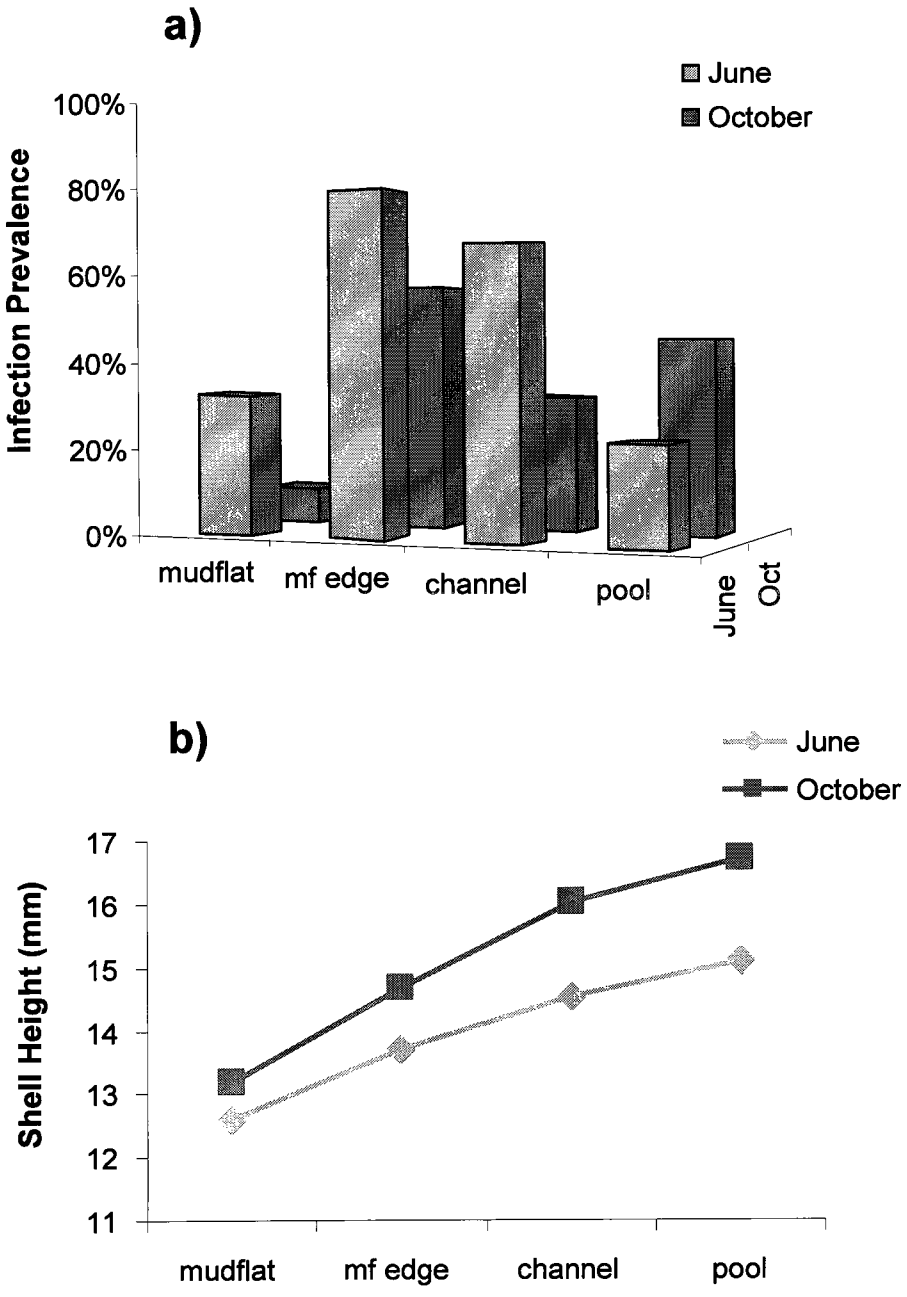

Figure 4. 6

a) Intra-annual patterns of infection prevalence across saltmarshes habitats in 2008, (b) average shell heights of quadrat collected snails used to assess infection prevalence.

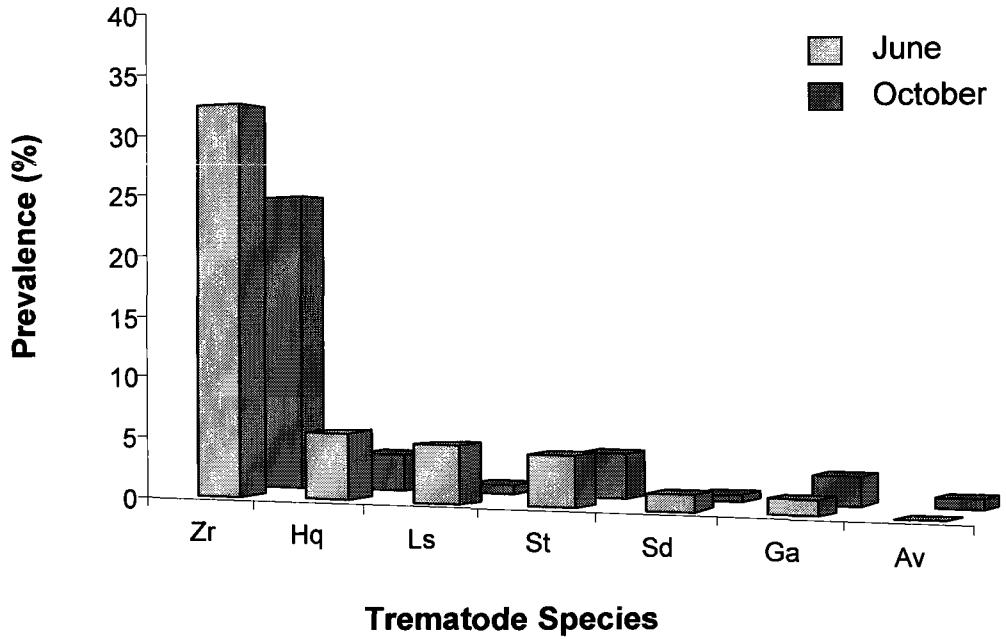

Figure 4. 7

A significant difference in the distribution of trematode infections among species categories was found between the two sampling months in 2008 (X^2 = 439.7, df = 6, P <0.001). Abbreviations for trematode species along x axis are as follows: Zr = *Zoogonus rubellus*, Hq = *Himasthla quissetensis*, Ls = *Lepocreadium setiferoides*, St = *Stephanostomum tenue* , Sd = *Stephanostomum dentatum*, Ga = *Gynaecotyla adunca*, and Av = *Austrobilharzia variglandis*.

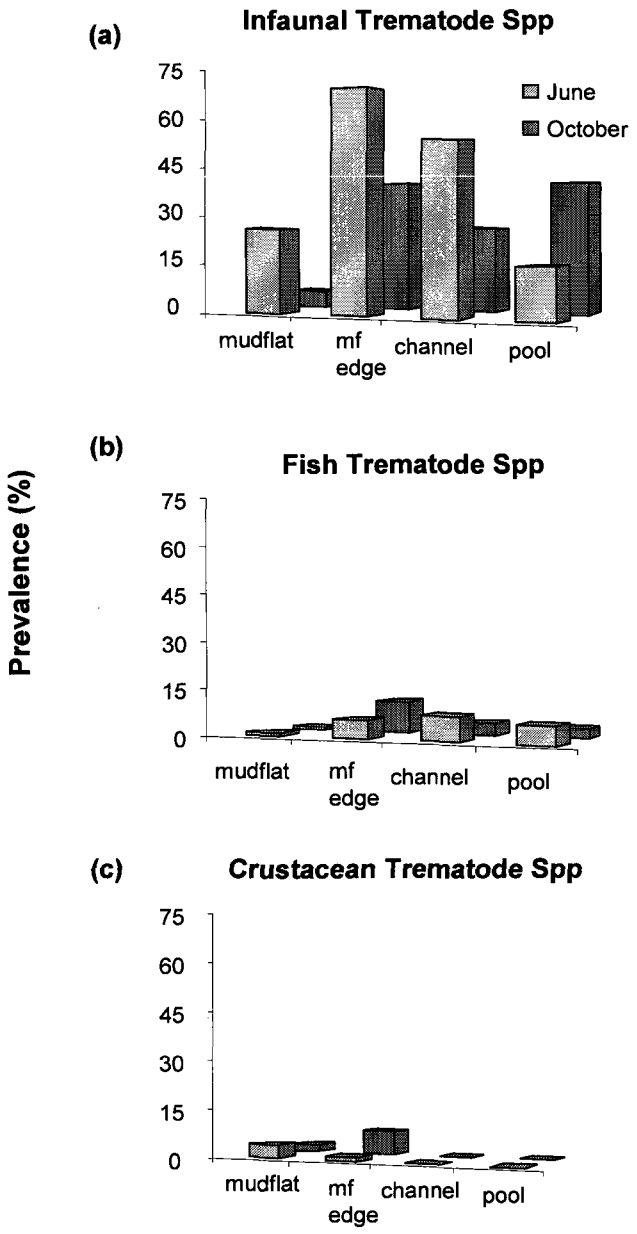

Figure 4. 8

Intra-annual patterns of infection for functional groups of trematode species based on similarities in second intermediate hosts used. (a) A significant difference was observed in the intra-annual distribution of infaunal trematode species which consists of those that use polychaete worms or bivalves as second intermediate hosts and includes *Z. rubellus*, *L. setiferoides*, and *H. quissetensis* ($X^2 = 32.5$, df = 4, $P < 0.001$). (b) Fish species are the two *Stephanostomum* spp., *S. tenue* and *S. dentatum*, which require infection in planktivorous fish as second intermediate hosts. No significant difference was observed in the distribution of trematodes in this functional group ($X^2 = 3.12$, df = 4, $P = 0.4$). (c) A significant difference was found in the distribution of crustacean species which includes only the species *Gynaecotyla adunca* which uses amphipods or crabs during second intermediate larval stages ($X^2 = 8.83$, df = 2, $P < 0.01$).

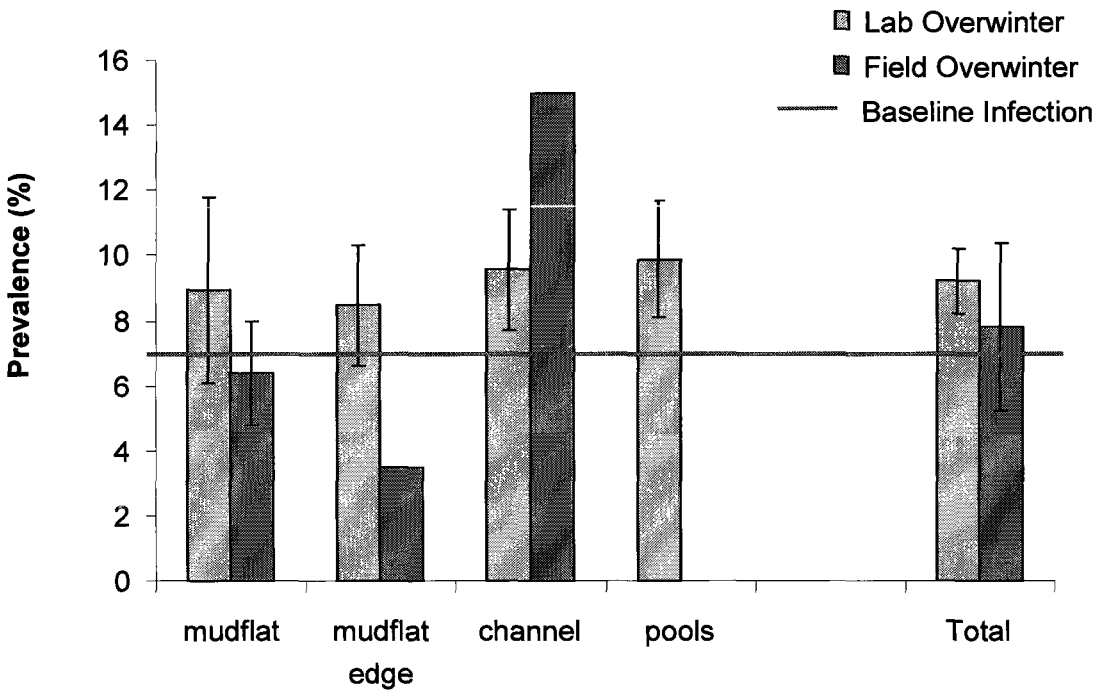

Figure 4. 9

Final prevalence of sentinel snails caged in different habitats to determine the risk of acquiring trematode infections. Blue bars indicate the prevalence of snails that were held in laboratory flowthrough tanks during winter months ("Lab Overwinter") and exposed to field conditions for a total of 7.5 months. No significant differences were found in the final infection prevalence for lab overwintering snails. Maroon bars indicate the prevalence of snails left in to field during winter months ("Field Overwinter") and exposed to field conditions for a total of 12 months. Baseline infection (red line) indicates the starting infection prevalence of sentinel snails which was determined by dissection of a random subset of snails at the beginning of the experiment. Error bars are $\pm$ SE.

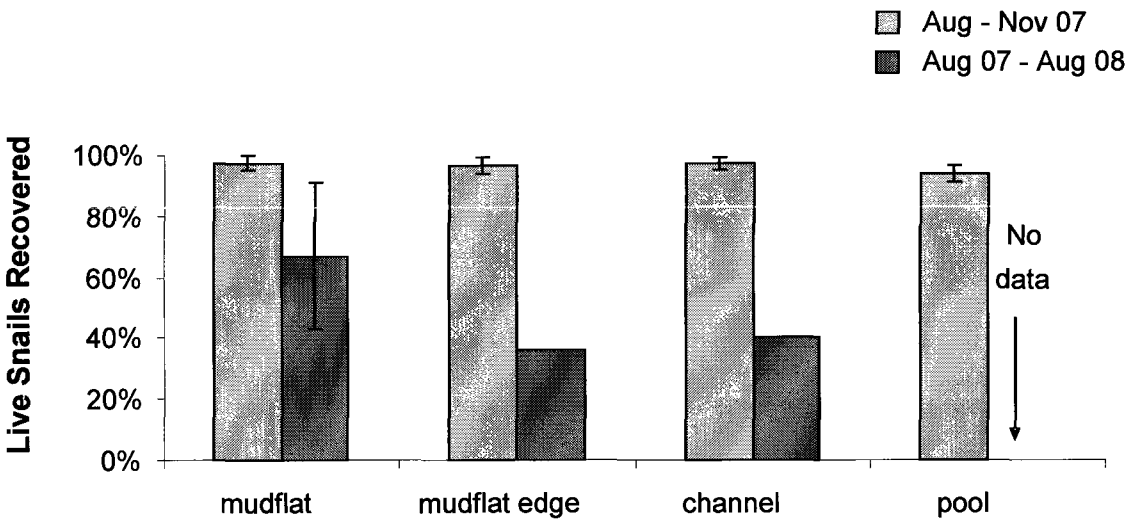

Figure 4. 10

Proportion of sentinel, caged snails found alive across different periods of exposure. High survival was found for snails exposed to field conditions from August-November 2007. Lower survival was found for snails exposed to field conditions from August 2007 to August 2008. Error bars are ± SD. Note: no cages were recovered from pool habitats after 12 months, therefore survival from this habitat could not be determined from August 2007 - August 2008

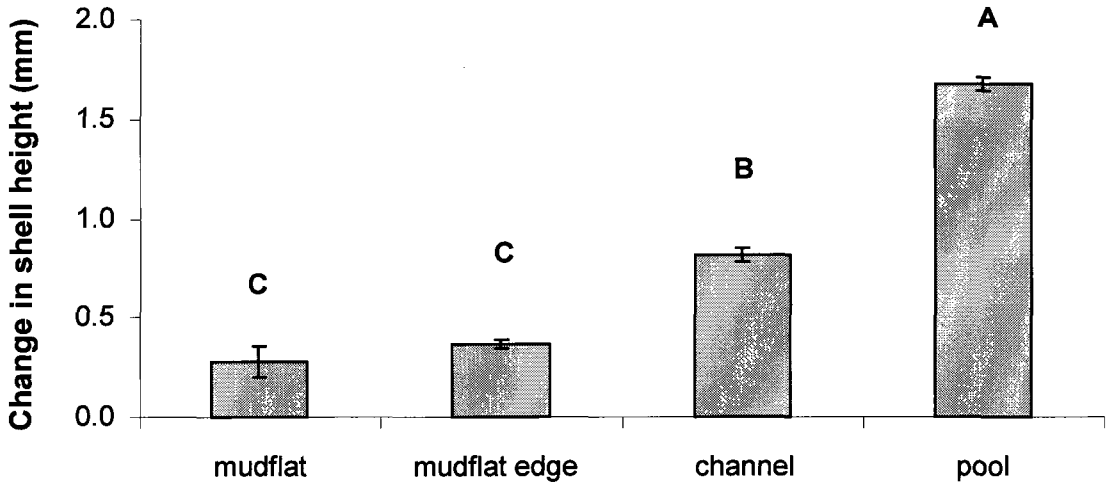

Figure 4. 11

Growth of sentinel, caged snails exposed to habitat conditions from August-November 2007. Letters above bars indicate which habitats exhibited significantly different growth of caged snails.

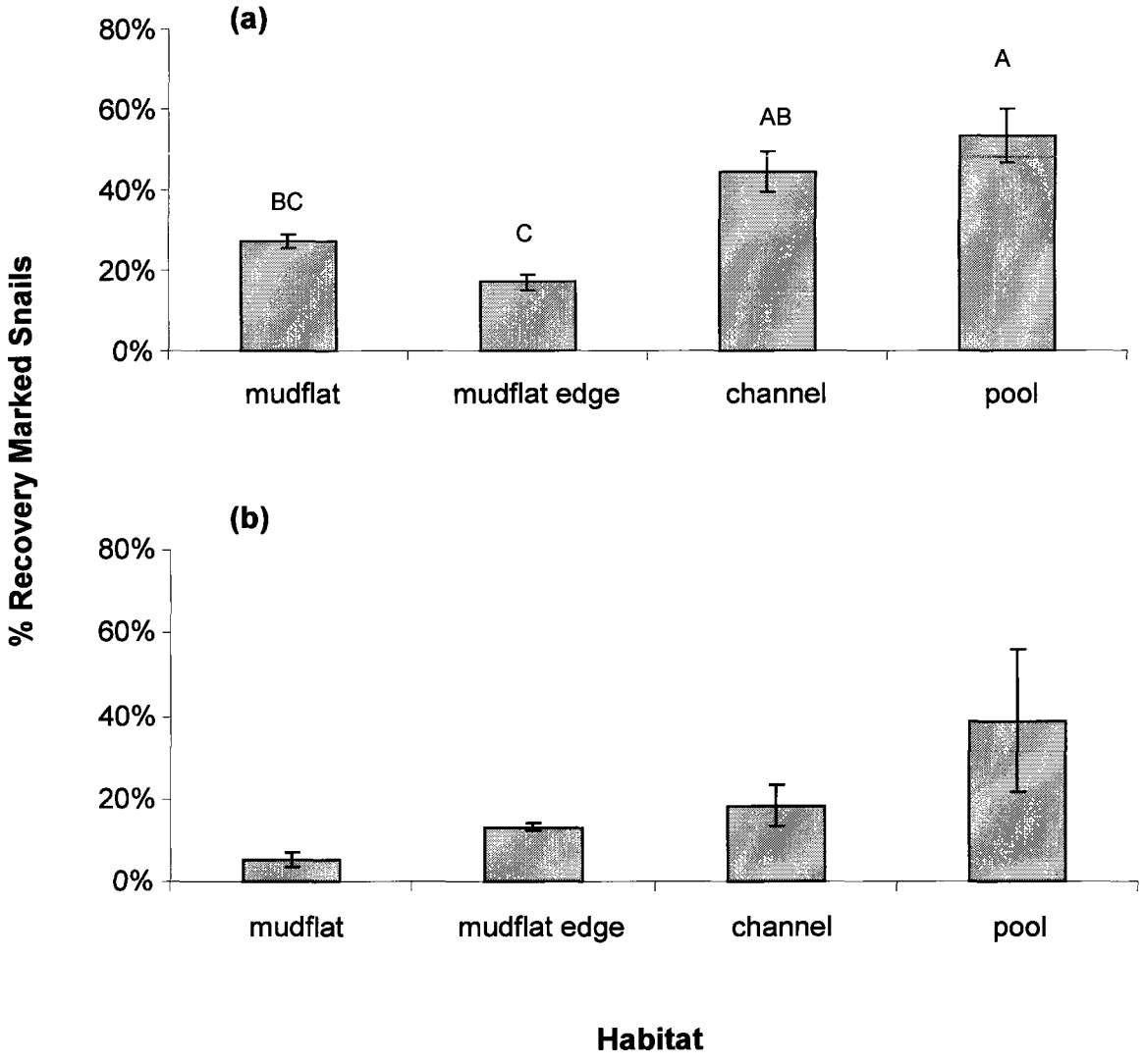

Figure 4. 12

Recovery of marked snails within a 314 m^2 area around release points (a) 3 days after their release and (b) 16 after their release. Significant differences in recovery among habitats after 3 days is indicated by different letters displayed above the bars in (a). No significant differences were found in percent of marked snails recovered after 16 days, although trends are similar to (a). Error bars are $\pm$ SE.

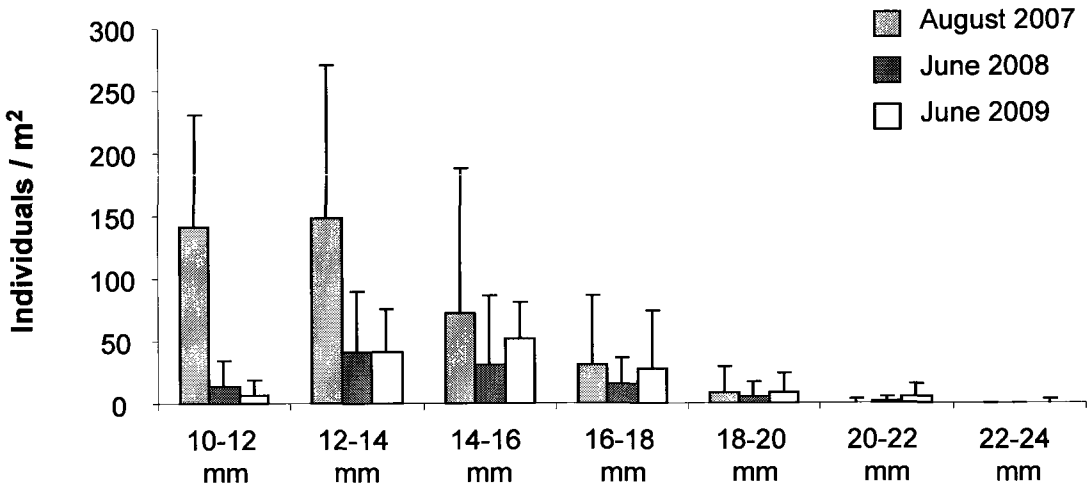

Figure 4. 13

Size frequency distribution of snails (averaged across all habitats) for three sampling years over which snails were assessed for infection. Error bars are $\pm$ 1 SD

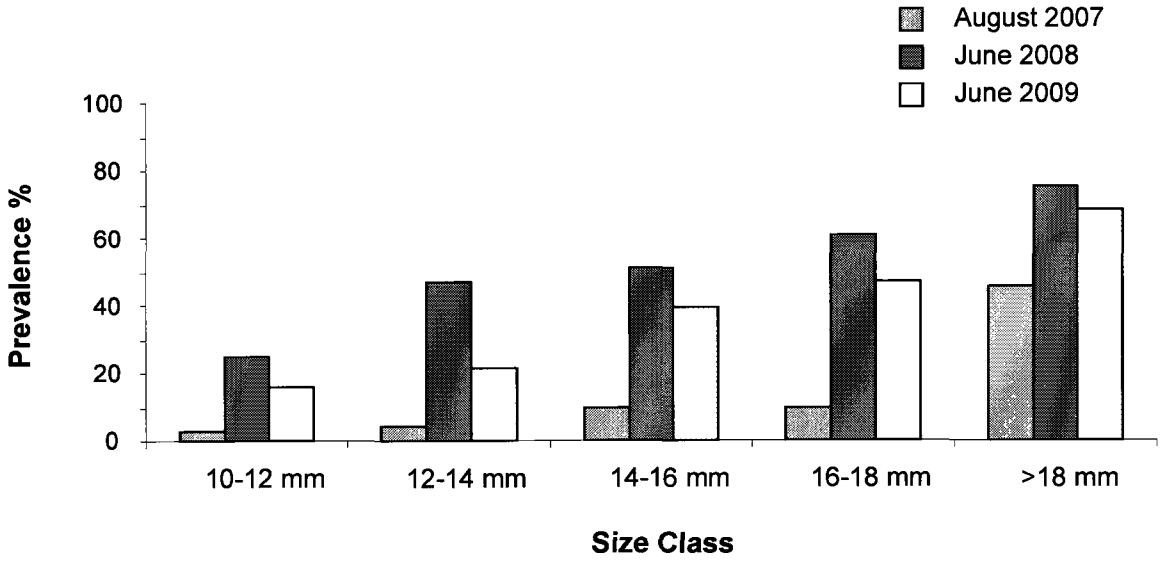

Figure 4. 14

Marsh-wide prevalence broken down according to size classes of snails for each of three sampling years examined

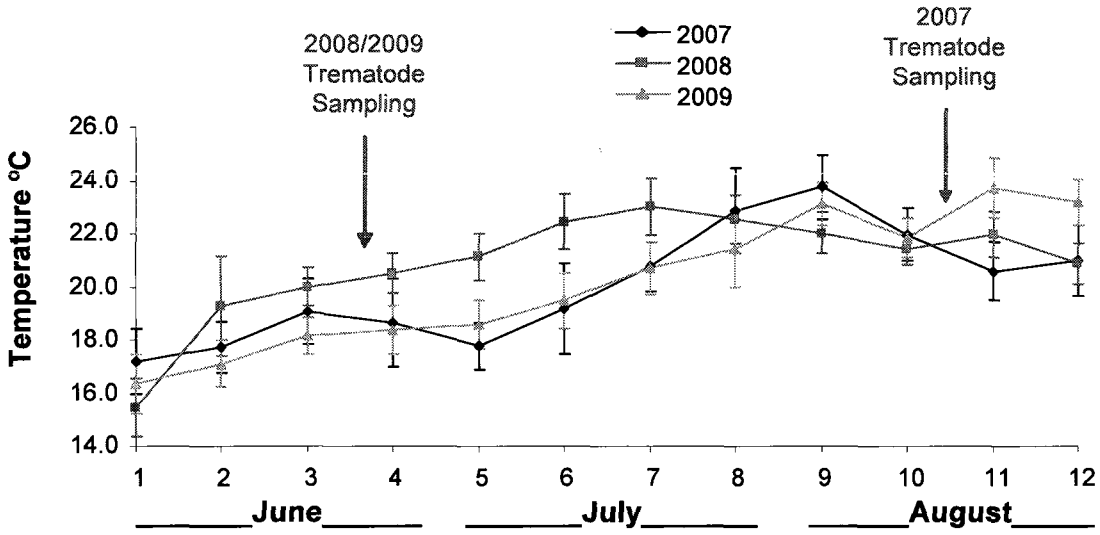

Figure 4. 15

Mean (± SD) weekly water temperatures during the summer months as measured from a monitoring buoy stationed in Great Bay, NH. Data was collected every 15 minutes over the course of the summer. Weeks are labeled numerically starting with June 1 and continuing until the end of August, week 12. Data from years in which snails were sampled to determine trematode infection are shown. Red arrows indicate the approximate time point from which snails were collected each year.

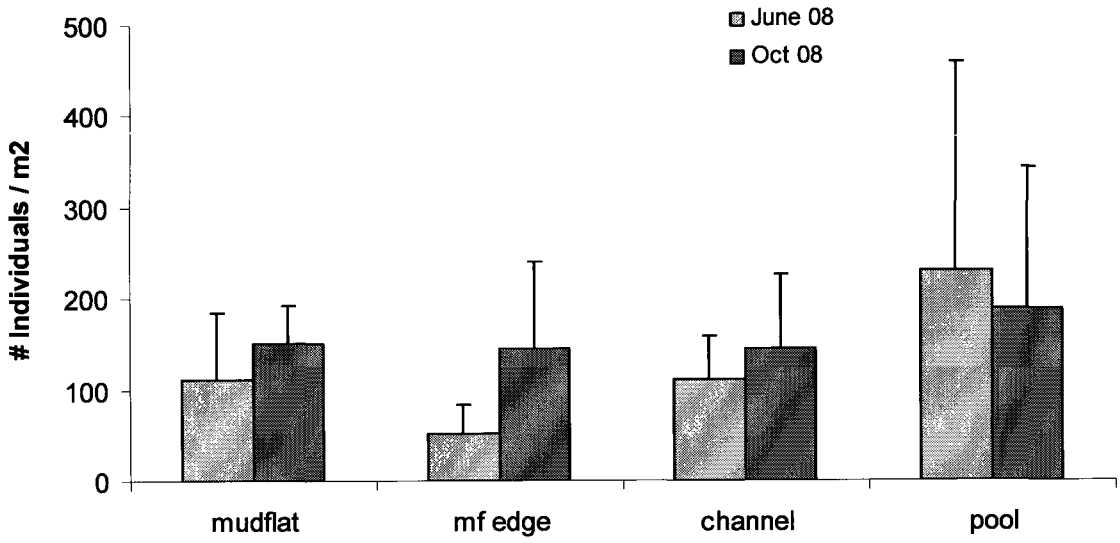

Figure 4. 16

Density of *I. obsoleta* across habitats during two sampling months from which infection was assessed in 2008. Habitats are listed from left to right in order of increasing tidal height. Error bars are + SD.

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APPENDICES

Appendix A: Description of Chao2

Chao2 = S_{obs} + $Q_1^2/2Q_2$, where S_{obs} is the number of species observed in the sample, Q_1 is the number of species that occur in just one sample (uniques) and Q_2 is the number of species that occur in exactly two samples (duplicates). Thus, relative to S_{obs} the value of Chao2 becomes increasing large when the number of unique species is much greater than the number of duplicates. However, if no unique species are observed in the samples, Chao2 and S_{obs} are equal.

Appendix B: Description of Bray-Curtis Similarity Index

The Bray Curtis Similarity Index (BC) is defined samples 1 and 2 as:

$$Bray\ Curtis = 100\left(1 - \frac{\sum_i |yi1 - yi2|}{\sum_i yi1 + \sum_i yi2}\right)$$

Where $yi1$ is the count for the ith (of p) species from sample 1 and $\sum_i$ is the summation of all species. BC values range from 100 (indicating a perfect match in species composition and species specific abundance between the two samples) and 0 (indicating there are no common species between sample 1 and 2)

Appendix C: Presence of *I. obsoleta* trematode hosts on the Pacific Coast of North America

Species that serve as second intermediate and definitive hosts of all nine *I. obsoleta* trematodes were identified from the literature and are presented in the Appendix C Results table below. Hosts listed in this table include both natural hosts (i.e. those found to be infected under natural field conditions) and experimental hosts (i.e. those shown to be successful hosts when exposed to infective stages of trematodes in the lab). Host information for trematode species that have been introduced to the Pacific coast is listed first in the table and shown in shaded boxes for easy identification. Portions of the table without shading present information for trematode species that are not found in the introduced region. The presence and introduced/native status of each host species in *I. obsoleta*'s introduced range was determined by searching the literature and two relevant databases: The Enclyopedia of Life (http://www.eol.org) and The Nonindigenous Aquatic Species database (http://nas.er.usgs.gov). For each host species a literature search was conducted using Web of Science and the following search criteria: topic =genus name and species name of the host in question, topic = San Francisco OR Willapa Bay OR Boundary Bay. Results of the literature search were carefully reviewed for information on the host species' introduced/native status in the Pacific coast bays as well as any information regarding its abundance. When no evidence was found suggesting a host species is present in *I. obsoleta*'s introduced range, it was designated as "absent". For hosts present in the introduced region, (n) indicates that the host species is native on the Pacific coast, (i) indicates that the host species has been introduce to the Pacific coast, (?) indicates that the origin of the host species is unclear. References for this table are presented in Appendix D.

Trematode Species	Second Intermediate Host		Definitive Host	
	Host Species	Status in *I. obsoleta* introduced range	Host Species	Status in *I. obsoleta* introduced range
Austrobilharzia variaglandis	None	not applicable	*Arenaria interpres interpres*	present (n)
			Aythya affinis	present (n)
Himasthla quissetensis	*Crepidula fornicate*	present (i)	*Larus argentatus*	present (n)
	Mercenaria mercenaria	present (i)	*Sterna hirundo*	present (n)
	Modiolus demissus = Geukensia demissa	present (i)		
	Mya arenaria	present (i)		
	Mytilus edulis	present (n)		
	Agropecten irradians	absent		
	Cerastoderma edule	absent		
	Cumingia tellimoides	absent		
	Ensis directus	absent		
	Modiolus modiolus	absent		

Trematode Species	Second Intermediate Host		Definitive Host	
	Host Species	Status in *I. obsoleta* introduced range	Host Species	Status in *I. obsoleta* introduced range
Lepocreadium setiferoides	*Chaetozone setosa Malmgren*	present (?)	*Hippoglossoides platessoides*	absent
	Eteone longa	present (?)	*Liopsetta putnami*	absent
	Heteromastus filiformis	present (?)	*Myoxocsphalus ocridecimspinosus*	absent
	Pygospio elegans	present (?)	*Pseudopleuronectes americanus*	absent
	Scoloplos armiger	present (?)		
	Streblospio benedicti	present (?)		
	Childia spp.	absent		
	Chrysaora quinquecirrha	absent		
	Euplana gracilis	absent		
	Polydora ciliate	absent		
	Polydora ligni	absent		
	Procerodes warreni	absent		
	Stylochus ellipticus	absent		

Trematode Species	Second Intermediate Host		Definitive Host	
	Host Species	Status in *I. obsoleta* introduced range	Host Species	Status in *I. obsoleta* introduced range
Stephanostomum tenue	*Fundulus heteroclitus*	absent	*Morone/Roccus saxatilis*	present (i)
	Menidia menidia notata	absent	*Ammodytes americanus*	absent
			Hemitripterus americanus	absent
			Menticirrhus saxatilis	absent
			Morone Americana	absent
			Opsanus tau	absent
			Sphoeroides maculates	absent
Zoogonus rubellus	*Acmaea intestinalis*	absent	*Anguilla rostrata*	present (i)
	Arabella opalina	absent	*Opsanus tau*	absent
	Bdelloura candida	absent	*Tautoga onitis*	absent
	Hydroides spp.	absent		
	Lumbrinereis hebes	absent		
	Nereis virens	absent		

Trematode Species	Second Intermediate Host		Definitive Host	
	Host Species	Status in *I. obsoleta* introduced range	Host Species	Status in *I. obsoleta* introduced range
Zoogonus rubellus (cont.)	*Notoacmaea intestinalis*	absent		
	Scoloplos robustus	absent		
Diplostomum nassa	*Mugil cephalus*	present (i)	Unknown	unknown
	Fundulus heteroclitus	absent		
Gynaecotyla adunca	*Corophium volutator*	absent	*Larus argentatus smithsonianus*	present (n)
	Talorchestia longicornis	absent	*Rhynchops nigra nigra*	present (n)
	Talorchestia megalopthalmia	absent	*Sterna hirundo*	present (n)
	Uca pugilator	absent	*Ammospiza maritime*	absent
			Charadrius wilsonia wilsonia	absent
			Larus atricilla	absent
			Sterna albifrons antillarum	absent

Trematode Species | Second Intermediate Host | Definitive Host

	Host Species	Status in *I. obsoleta* introduced range	Host Species	Status in *I. obsoleta* introduced range
Pleurogonius malaclemys	*none; encysts on hard substrate*	not applicable	*Malaclemys centrata*	absent
			Malaclemys terrapin	absent
Stephanostomum dentatum	*Menidia menidia*	absent	*Paralichthys dentatus*	absent
			Sphoeroides maculates	absent

Appendix D: References for *I. obsoleta* trematode hosts

References for species that are second intermediate and definitive hosts of *I. obseleta* trematodes presented in Appendix C. Note species f definitive hosts for *D. nassa* are unknown and are therefore not listed.

Trematode species	Host type	Host species	Reference(s) for host of *I. obsoleta*
A. variaglandis	2nd intermediate	none used	
	definitive	*Arenaria interpres interpres*	(Schell 1970)
		Aythya affinis	(McDermott 1951)
D. nassa	2nd intermediate	*Fundulus heteroclitus*	(Stunkard 1973)
		Mugil cephalus	(McDemott1951)
G. adunca	2nd intermediate	*Corophium volutator*	(McCurdy et al 2000, McCurdy 2001)
		Talorchestia longicornis	(McDermott 1951, Schell 1970, Stunkard 1983, Curtis 1987)

G. adunca	2nd intermediate	*Talorchestia megalopthalmia*
		Uca pugilator
	definitive	*Ammospiza maritime*
		Charadrius wilsonia wilsonia
		Larus argentatus smithsonianus
		Larus atricilla
		Rhynchops nigra nigra
		Sterna albifrons antillarum
		Sterna hirundo
H. quissetensis	2nd intermediate	*Agropecten irradians*
		Cerastoderma edule
		Crepidula fornicata
		Cumingia tellimoides
		Ensis directus
H. quissetensis	2nd intermediate	*Mercenaria mercenaria*
		Modiolus demissus = *Geukensia demissa*
		Modiolus modiolus

(Curtis 1987)

(Yamaguti 1958, Curtis 1987)

(Hunter 1952, Yamaguti 1958)

(Hunter 1952, Yamaguti 1958)

(McDermott 1951, Hunter 1952, Yamaguti 1958)

(Hunter 1952, Yamaguti 1958)

(Yamaguti 1958)

(Yamaguti 1958)

(Yamaguti 1958)

(Curtis 2007a)

(Desclaux et al 2004)

(Stunkard 1938. Schell 1970)

(Stunkard 1938, Schell 1970)

(Stunkard 1938, Schell 1970)

(Curtis 2007)

(Stunkard 1938, McDermott 1951)

(Stunkard 1938, Schell 1970)

		Mya arenaria
		Mytilus edulis
		Pecten irradians
	definitive	*Larus argentatus*
		Sterna hirundo
L. setiferoides	2nd intermediate	*Chaetozone setosa Malmgren*
		Childia spp
		Chrysaora quinquecirrha
		Eteone longa
		Euplana gracilis
L. setiferoides	2nd intermediate	*Heteromastus filiformis*
		Polydora ciliate
		Polydora ligni
		Procerodes warreni
		Pygospio elegans
		Scoloplos armiger
		Streblospio benedicti

(Stunkard 1938, McDermott 1951)

(Stunkard 1938, Schell 1970)

(Stunkard 1938, Schell 1970)

(McDermott 1951, Yamaguti 1958)

(Yamaguti 1958)

(McCurdy et al 2000)

(McDermott 1951, Curtis 2007)

(McDermott 1951)

(McCurdy et al 2000)

(McDermott 1951)

(McCurdy et al 2000)

(McDermott 1951)

(McDermott 1951, Magendanzt 1969)

(Yamaguti 1958, Schell 1970)

(McCurdy et al 2000, McCurdy 2001)

(McCurdy et al 2000)

(McCurdy et al 2000)

		Stylochus ellipticus	(McDermott 1951)
	definitive	*Hippoglossoides platessoides*	(McDermott 1951)
		Liopsetta putnami	(Magendanzt 1969)
		Myoxocsphalus ocridecimspinosus	(Magendanzt 1969)
		Pseudopleuronectes americanus	(McDermott 1951, Magendanzt 1969)
P. malaclemys	2nd intermediate	*encysts on hard substrate*	(McDermott 1951)
P. malaclemys	definitive	*Malaclemys terrapin*	(McDermott 1951)
S. dentatum	2nd intermediate	*Menidia menidia*	(McDermott 1951, Yamaguti 1958, Stunkard 1983)
	definitive	*Paralichthys dentatus*	(McDermott 1951)
		Sphoeroides maculatus	(Stunkard 1961, Stunkard 1983)
S. tenue	2nd intermediate	*Fundulus heteroclitus*	(Stunkard 1961)
		Menidia menidia notata	(McDermott 1951, Yamaguti 1958, Schell 1970)
	definitive	*Ammodytes americanus*	(McDermott 1951)
		Hemitripterus americanus	(McDermott 1951)
		Menticirrhus saxatilis	(McDermott 1951)

		Morone americana
		Morone/Roccus saxatilis
		Opsanus tau
S. tenue	definitive	*Sphoeroides maculates*
Z. rubellus	2nd intermediate	*Acmaea intestinalis*
		Arabella opalina
		Bdelloura candida
		Hydroides spp
		Lumbrinereis hebes
		Nereis virens
		Notoacmaea intestinalis
		Scoloplos robustus
	definitive	*Anguilla rostrata*
		Opsanus tau
		Tautoga onitis

(McDermott 1951)

(McDermott 1951, Schell 1970)

(McDermott 1951)

(Yamaguti 1958)

(McDermott 1951)

(Shaw 1933)

(Shaw 1933)

(Shaw 1933)

(Shaw 1933)

(Shaw 1933, Stunkard 1938, McCurdy and Moran 2004)

(Curtis 2007)

(Shaw 1933)

(Stunkard 1938, McDermott 1951, Schell 1970)

(McDermott 1951, Schell 1970)

(Curtis 2007)

UNIVERSITY of NEW HAMPSHIRE

May 5, 2004

Byers, James
Zoology
Spaulding Life Science Center
Durham, NH 03824

IACUC #: 040205
Approval Date: 02/24/2004
Review Level: B

Project: Using Trematode Parasites as Bioindicators of Salt Marsh Health

The Institutional Animal Care and Use Committee (IACUC) reviewed and approved the protocol submitted for this study under Category B on Page 4 of the Application for Review of Vertebrate Animal Use in Research or Instruction - *the study involves either no pain or potentially involves momentary, slight pain, discomfort or stress.*

Approval is granted for a period of three years from the approval date above. Continued approval throughout the three year period is contingent upon completion of annual reports on the use of animals. At the end of the three year approval period you may submit a new application and request for extension to continue this study. Requests for extension must be filed prior to the expiration of the original approval.

Please Note:

1. All cage, pen, or other animal identification records must include your IACUC # listed above.
2. Use of animals in research and instruction is approved contingent upon participation in the UNH Occupational Health Program for persons handling animals. Participation is mandatory for all principal investigators and their affiliated personnel, employees of the University and students alike. A Medical History Questionnaire accompanies this approval; please copy and distribute to all listed project staff who have not completed this form already. Completed questionnaires should be sent to Dr. Gladi Porsche, UNH Health Services.

If you have any questions, please contact either Van Gould at 862-4629 or Julie Simpson at 862-2003.

For the IACUC,

Roger Wells, D.V.M.
Vice Chair

cc: File