

Large-scale spatial variation in parasite communities influenced by anthropogenic factors

IRIT ALTMAN^{1,3} AND JAMES E. BYERS²

¹Biology Department, Boston University, 5 Cummington Mall, Boston, Massachusetts 02215 USA

²Odum School of Ecology, University of Georgia, Athens, Georgia 30602 USA

Abstract. Parasites are integral members of natural communities, but large-scale determinants of their abundance and diversity, including the importance of biotic and abiotic factors, both natural and anthropogenic, are often not well understood. Here, we examine which factors best predict larval trematode communities in the mudsnail host *Ilyanassa obsoleta* across a regional landscape. At 15 salt marsh sites spanning 200 km, we quantified the diversity of trematodes and the prevalence (i.e., proportion) of infected hosts and sampled a broad array of potential parasite predictors including abundance of intermediate and definitive hosts, habitat, nutrients, metals, roads, and sediment characteristics. We identified the set of best performing models to explain variability associated with five metrics of trematode prevalence and diversity using an information-theoretic approach. Results indicate that several anthropogenic factors associate with this trematode community and that the direction of their influence differs. Road density around sites was a strong negative predictor of all trematode prevalence and species richness metrics. Nitrogen, another human influenced variable, was a strong positive predictor for the most abundant trematode species in the system. In addition, the abundance of definitive fish hosts was a positive predictor in several models, confirming the importance of this direct biological link to parasites. Other influential variables included sediment composition and heavy metals (arsenic, copper, lead, and zinc). We discuss possible direct and indirect mechanisms to explain these findings including that anthropogenic factors may be directly influencing free-living stages of trematodes, or be acting as proxies of hard-to-measure hosts.

Key words: biological indicators; community structure; disturbance; nitrogen; regional variability; roads; trematode parasites.

INTRODUCTION

Parasites are ubiquitous and integral members of natural communities that often impose strong influence on host populations. Although impacts on hosts provide an obvious starting point for understanding their effects on ecological systems, over the last decade a broader role of parasites in ecological communities has begun to be uncovered (Thomas et al. 2005). Research now demonstrates that parasites can influence community structure and biodiversity (Wood et al. 2007, Grewell 2008), food webs (Lafferty et al. 2006), and even the biomass and energy flows in natural systems (Kuris et al. 2008). An important step toward integrating parasites into a larger ecological context is to understand the range of factors that drive their abundance and diversity across scales. This includes characterizing the effects of natural factors and human-associated disturbance on parasite infection, the net result of which depends on complex interactions between parasites, hosts, and the

abiotic conditions to which both groups are exposed (Lafferty and Kuris 1999).

For multi-host parasites, natural variation in the abundance and diversity of definitive hosts (in which adult parasites mature) is often a key determinant of larval infections (Hechinger and Lafferty 2005, Byers et al. 2008). Environmental factors, including habitat features, may also affect parasites if they attract host populations (Skirnisson et al. 2004) or alter encounter rates between parasites and hosts (Caceres et al. 2006). Abiotic factors can have direct influence on parasites too, especially those that exhibit free-living stages during which they may be highly sensitive to environmental factors like temperature, salinity, and light (Pechenik and Fried 1995, Pietroock and Marcogliese 2003, Thielgtes and Rick 2006, Studer and Poulin 2012). Thus, natural variability in biotic and abiotic environment can influence parasites through direct and indirect pathways.

Anthropogenic activities are also known to influence parasites and, as with natural factors, their effects operate through a variety of pathways. Human activities may attract or deter definitive hosts with corresponding positive or negative consequences on parasites. For example, larval parasite infection was higher at fishing

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³ E-mail: altmani@bu.edu

sites that attract birds (Bustnes and Galaktionov 1999) and in association with roads that may act as movement corridors for mammal hosts in an otherwise undeveloped landscape (Urban 2006). In contrast, infection was lower at agricultural and urban sites (King et al. 2010) and in association with degraded wetlands (Huspeni and Lafferty 2004), habitats thought to be unfavorable for associated vertebrate hosts. Anthropogenic pollutants can also alter environmental conditions with negative effects on parasite survival and infectivity, underscoring the direct influence that these impacts have on transmission pathways (Pietroock and Marcogliese 2003, Koprivnikar et al. 2006).

Many studies explore the effect of natural and anthropogenic factors on parasites at small scales and this work contributes greatly to our understanding of mechanisms that structure parasite communities locally (Smith 2001, Hechinger and Lafferty 2005, Koprivnikar et al. 2007). Such examinations, however, do not weigh the relative importance of larger scale factors that may control a parasite's regional distribution. Of the few larger scale studies that do exist, some suggest both regional and local scale influences can be associated with parasites at different life stages (e.g., adult vs. larval [Schotthoefer et al. 2011]) and that groups of parasites respond differently to scale dependent influences (Poulin and Mouritsen 2003, King et al. 2010). More work, however, is needed to understand whether general patterns of parasite determinants exist at large scales. Investigations that examine metrics of a target population along with a wide range of potentially influential factors across broad spatial scales have provided key insights into the determinants of *free-living* species communities (Gotelli and Ellison 2002, Urbieta et al. 2008). We take a similar approach here by investigating how a variety of candidate factors, including ecological measures of hosts, environmental conditions, and metrics tied to human activities predict the proportion of hosts infected (i.e., prevalence) and diversity of trematode parasites in a community. By examining these factors across a regional landscape, our work provides a more synthetic and ecosystem-scale picture of the determinants of parasite abundance and diversity than has previously been available (Ostfeld et al. 2005).

Study system.—We focus on a community of larval trematode parasites associated with the mudsnail *Ilyanassa obsoleta*, a common inhabitant of low-energy estuarine environments along the east coast of the United States, where it is often found in high abundance. Trematodes obligately infect *I. obsoleta* during larval stages and snails are unable to rid themselves of trematodes once infected. The parasite produces free-swimming stages called cercariae, which are shed from the snail and have only limited time to seek out and infect a second intermediate host, which, depending on the trematode species could be a fish, bivalve, polychaete worm, or crustacean. Completion of the trematode life cycle occurs when definitive hosts

(mostly species of fish and birds) prey upon infected second intermediate hosts and thereby acquire the parasite. A total of nine trematode species infect *I. obsoleta* during larval stages and each depends on a unique combination of host to reach mature stages (Curtis 1997, Blakeslee et al. 2012).

Trematode parasites are an ideally suited group to examine how a set of potential drivers including those related to human activities influence parasite abundance and diversity. They are common in marine, estuarine, and freshwater environments, allowing for comparisons at broad scales and across a diversity of ecosystems. During free-living stages, cercariae are highly sensitive to environmental factors, both natural and anthropogenic (e.g., heavy metals and synthetic compounds) that can decrease survival and transmission to subsequent hosts (Bennett et al. 2003, Pietroock and Marcogliese 2003, Koprivnikar et al. 2006). These parasites are also readily studied during larval stages where infections in snails may consist of hundreds or more individual cercariae whose distinct morphological features allow for species-level identification. In contrast to parasites that only require a single host to complete their life cycles, digenean trematodes depend on a combination of species, each of which may be influenced by a unique set of biotic and abiotic factors.

MATERIALS AND METHODS

Sampling range and site selection.—Fifteen salt marsh sites were selected in northern New England, USA, spanning a distance of more than 200 km (42.1°–44.1° N; Fig. 1). To standardize the potential species pool across sites, sampling was restricted to marshes north of Cape Cod, a known biogeographic boundary for marine organisms in the western North Atlantic (Engles and Summers 1999). Sites were also selected to represent a wide range of biotic and abiotic conditions (for example, we included protected salt marsh sites located far from human developments as well as marsh sites located in close proximity to urban centers). Within a site, the sampling location was standardized to a single first- or second-order tidal channel where *I. obsoleta* was common. Unless otherwise indicated, samples were collected during the summer months (June–August) in 2004 along a 100-m transect placed at the mouth of the tidal channel and extending upstream. In the next several subsections, we describe the essential features of sampling methods used; Appendix A provides more detailed descriptions for some techniques.

Sampling of *Ilyanassa obsoleta* trematode community.—To assess trematode infection in *I. obsoleta*, an average of 134 (range 95–182) adult mudsnails 15–20 mm in shell height, were haphazardly collected along a 100-m stretch of tidal channel that served as the focal sampling area. Snails <20 mm have not reached maximum size (i.e., growth is not yet asymptotic) and shell height can therefore serve as a proxy for age. Snails also accumulate trematode infections through time

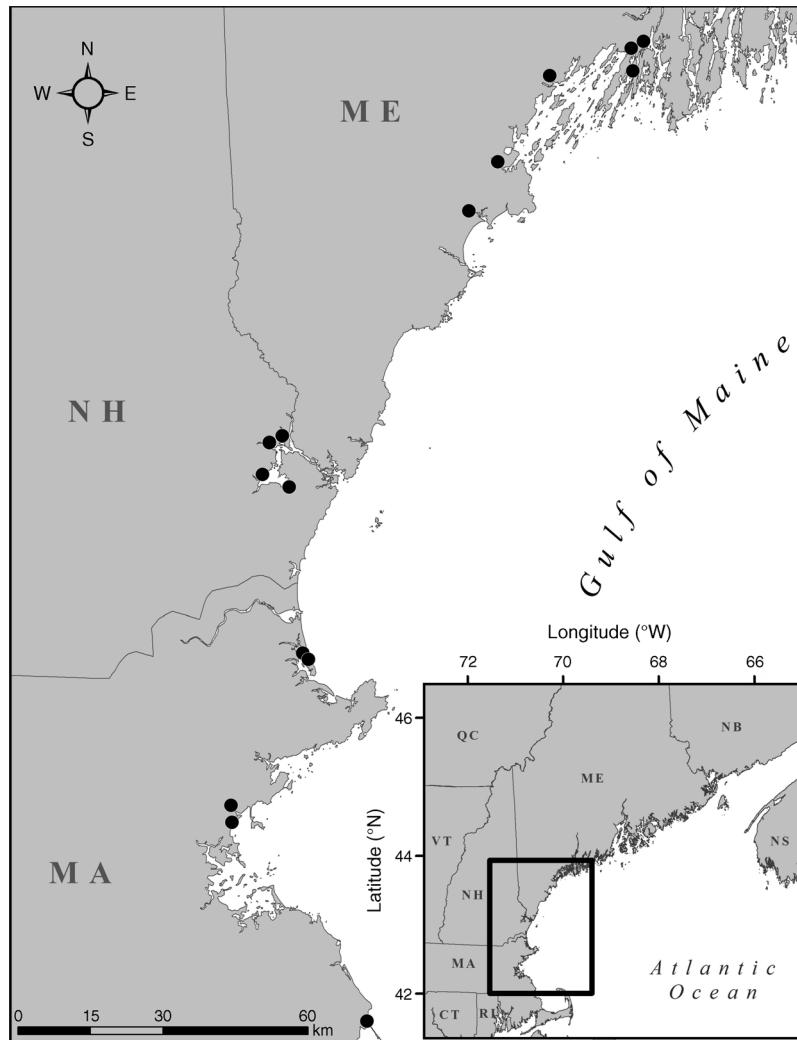


FIG. 1. A map of 15 salt marsh sites spanning 200 km along the Atlantic coast of North America, from which *Ilyanassa obsoleta* trematode communities and a wide range of biological, chemical, and physical characteristics were sampled. Abbreviations are ME, Maine; NH, New Hampshire; MA, Massachusetts; RI, Rhode Island; CT, Connecticut; VT, Vermont; QC, Quebec; NB, New Brunswick; NS, Nova Scotia.

(Curtis and Hurd 1983); thus selecting early adult snails from a narrow size range helps to minimize variation in the host's exposure time to parasite risk among sites and isolate infection processes occurring in the recent past. Snails were dissected in the laboratory and the digestive and gonadal tissues examined under a stereomicroscope (40 \times magnification) for the presence of trematode cercariae and the rediae or sporocyst in which they are contained. When infections were found, we examined morphological characteristics of trematode structures, especially cercariae, under a compound microscope (200 \times) and used a number of sources to confirm species identification (McDermott 1951, Stunkard 1961, Yamaguti 1975). We also photographed representative infections of each species encountered and used this photographic atlas to verify consistency in species identification across sites. Total infection prevalence,

or the proportion of snails infected, was calculated for each site along with species-specific prevalence for the most common trematodes encountered. To ensure equal sample sizes, species richness was calculated for each site by randomly selecting a subset of 95 observations.

Sampling hosts and conspicuous community members.—Estuarine organisms that are known or likely hosts of *I. obsoleta* trematodes (Blakeslee et al. 2012), as well as other conspicuous species found at sites, were sampled using a variety of methods. To determine the relative abundance of the snail hosts, we measured the abundance of *I. obsoleta* within a 0.05-m² quadrat placed every 10 m along a transect that extended 100 m upstream from the mouth of the tidal channel. Infaunal bivalve abundance was assessed using a 0.075-m² core inserted into sediments to a depth of 20 cm. Contents from these cores were washed through a 3.75-mm sieve

TABLE 1. Trematode infection in *Ilyanassa obsoleta* across 15 salt marsh sites in northern New England.

Trematode species	Second intermediate host	Definitive host	Prevalence (%)			Sites where present (%)
			Mean	SD	Range	
<i>Stephanostomum</i> spp.	fish	fish	11.0	17.3	0–45.3	73.3
<i>Zoogonus rubellus</i>	polychaete worm	fish	9.2	11.7	0–34.5	66.7
<i>Lepocreadium setiferoides</i>	polychaete worm	fish	3.6	4.3	0–15.5	73.3
<i>Himasthla quissetensis</i>	bivalve	bird	2.3	2.8	0–7.6	60.0
<i>Gynaecotyla adunca</i>	amphipod	bird	0.7	1.6	0–5.8	26.7
<i>Austroilharzia variglandis</i>	none	bird	0.4	0.7	0–1.9	33.3
<i>Diplostomum nassa</i>	fish	bird likely	0.2	0.5	0–1.9	20.0
Unidentified			0.2	0.3	0–1.0	20.0
All species			27.3	32.0	0.7–92.8	

Notes: Mean 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 a handful of cases, however, snails were infected by multiple trematode species (Appendix A: Table A1). 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.

and all remaining bivalves identified and counted. Abundance of minnows (*Fundulus* spp.) was measured using two minnow traps spaced at least 50 m from each other, baited with mussels (*Geukensia* spp.), and set for 30 minutes on an incoming tide during September and early October 2004.

Birds and fish are definitive hosts for the majority of *I. obsoleta* trematodes (Blakeslee et al. 2012; Table 1), although one species found only in southeastern portions of the United States and outside the geographical range of this study uses a terrapin (Byers et al. 2011). The abundance and diversity of wetland birds that regularly include estuarine organisms in their diet was assessed within a 100 m radius circular zone around the sampling channel using 10-minute surveys. At least two bird surveys, and as many as six, were performed at sites over the course of the summer sampling season in 2004. Due to the intensive sampling effort required to characterize the assemblage of definitive fish hosts of *I. obsoleta* trematodes, these hosts could not be assessed concurrently with other metrics and were instead measured in the summer of 2005. We used fyke nets to determine relative abundance of fish species in tidal creeks from which trematode infection and other variables were measured the previous year. Fyke nets consisted of a funnel shaped cod end constructed of 6-mm mesh, to which a pair of wings constructed of 1.3-cm mesh were attached. Each wing can extend 14.3 m and was outfitted with floats along the upper edge and weights along the bottom to create a perpendicular barrier (measuring 1.2 m in height) across the water column on an outgoing tide. Total soak time for fyke nets was approximately six hours capturing a full outgoing tidal cycle, a time period that has been shown to be associated with low escape rates from this type of sampling net (Breen and Ruetz 2006).

Sampling of physical attributes.—The contiguous area of soft-sediment habitats is a natural measure of habitat expansiveness associated with trematode host communities. Using ArcMap v.9.3 (ESRI, Redland, California,

USA) and data available through U.S. Fish and Wildlife's National Wetlands Inventory (NWI) program, we calculated the contiguous area of intertidal soft-sediment habitats by summing the area of adjacent polygons associated with relevant habitat codes from Cowardin et al. (1979). Because many definitive fish hosts of trematodes make regular migrations between marine and estuarine habitats (e.g., striped bass, flounder, and American eels), we calculated the shortest swimming route from the sampling site to the open ocean using ArcMap. This metric may influence the likelihood of certain fishes inhabiting salt marsh sites and also reflects the degree of oceanic influence associated with sites. Sediment grain size is an important habitat feature that can influence the presence and abundance of many benthic species, some of which are trematode hosts. Grain size can also reflect flows at sites and affect turbidity, which could influence encounter rates between free-living trematode parasites and hosts. The proportion of sand, silt, and clay was assessed at sites from a core measuring 19.6 cm² and inserted to a depth of 5 cm at the midpoint of the sampling transect. Roads can act as barriers or corridors to wildlife that serve as trematode hosts. Roads can also fragment marsh habitats and influence environmental conditions by helping to introduce pollutants. Using ArcMap, we quantified the cumulative length of roads within a 1 km radius circular zone extending around each collection site. Road data was obtained through state transportation authorities of Maine, New Hampshire, and Massachusetts.

Sampling of chemical attributes.—High concentrations of some heavy metals are known to cause deleterious effects on free-living trematodes (Cross et al. 2001, Morley et al. 2005). Other chemicals, such as nitrogen, can indicate anthropogenic disturbance in estuarine and coastal waters and can have strong effects on benthic populations, trophic interactions, and abiotic conditions. We assessed the chemical milieu at sites from cores measuring 19.6 cm² and inserted to a depth of 5 cm

TABLE 2. Descriptive statistics for attributes measured across 15 salt marsh sites and used in multiple regression analysis to predict *I. obsoleta* trematode abundance and diversity.

Attribute	Type	Mean	SD	CV	Min	Max
<i>Ilyanassa obsoleta</i>	biological: first intermediate host	190	155	0.8	18	556
<i>Fundulus</i> spp.	biological: second intermediate host	59	80	1.4	0	273
Definitive fish hosts, DF	biological: definitive hosts	9	16	1.9	0	58
Wetland birds, WB	biological: some are definitive hosts	10	13	1.3	0.2	42
PC-Cu,Pb,Zn	chemical	0	1.71	na	-1.7	5.7
Arsenic, As	chemical	6.03	2.94	0.5	1.9	11
Nitrogen, N	chemical	3.7	2	0.5	2.1	10.3
Sand	physical	55.9	13.3	0.2	35.1	93.3
Roads	physical	6778	6117	0.9	2288	25094
Distance from the ocean, OD	physical	10	7	0.8	0.9	24.4
Soft-sediment habitat, SSH	physical	523	688	1.3	0	2056

Notes: Summary statistics (mean, SD, CV, minimum [min], and maximum [max]) are presented across sites. Metal PC-Cu,Pb,Zn is a unitless variable that reflects the scores of the first principal component associated with concentration of copper, lead, and zinc found in marsh sediments. For some variables, transformations were applied prior to model construction. The abbreviation na stands for not applicable.

every 10 m along a 100 m length of the channel. Samples were pooled, homogenized, and a subsample removed for analysis of heavy metals. Standard protocols (U.S. EPA method 6010b and 7470a [U.S. EPA 1996]) were used to assess the concentration of the following 13 metals at sites: aluminum, arsenic, cadmium, chromium, copper, iron, lead, manganese, mercury, nickel, silver, tin, and zinc. We also assessed the concentration of total nitrogen from three sediment cores measuring 19.6 cm² collected at random from the main sampling channel and inserted into the to a depth of 5 cm. These samples were frozen at -40°C and later analyzed using a Perkin Elmer 2400 Series II CHN Elemental Analyzer (Perkin Elmer, Waltham, Massachusetts, USA).

Statistical analysis.—Prior to analyses, all measures of prevalence were transformed using Anscombe arcsine-square-root methods; the transformation is appropriate when data are based on proportions in order to meet normality assumptions (Zar 1996). We used linear regression analyses to determine which variables best predict *I. obsoleta* trematode infection and verified that residuals of all trematode response metrics conformed to normality expectations. We modeled total prevalence (i.e., the proportion of snails infected by any species of trematode), the prevalence of infection for three individual trematode species (or species' group) most commonly observed in this system, and trematode species richness.

Data reduction of predictor variables.—Sets of related variables characterizing (1) metals and (2) grain size, were found to strongly covary across sites. To avoid problems of collinearity and overparameterization, we took steps to reduce these variables to subsets that reflect the strongest potential relationship with trematodes. For metals, we first examined cross correlations between observed concentrations of 13 metals and five trematode response metrics at sites. Results indicated that only a small handful (arsenic, copper, lead, and zinc) exhibit strong relationships with *I. obsoleta* trematodes and only these were therefore retained for

additional analyses (see Appendix B for full description). To determine the extent of collinearity within this metal subset, we next examined the pairwise cross correlations for the group. Results revealed that only arsenic exhibited unique patterns across sites and for this reason it was preserved in its measured form in subsequent analyses (Appendix B). Using principal component analyses, we reduced values associated with copper, lead, and zinc to a single principal component (PC) score that reflects their high correlation among sites. We refer to this metric as PC-Cu,Pb,Zn. For grain size, an initial exploration of relationships among the measured variables (sand, silt, and clay) revealed that the proportion of sand reflects the most independent measure of a site's sediment conditions; we therefore only retained this measure of grain size in subsequent analyses.

For all regression models we used the total abundance of fish species that are documented definitive hosts of any *I. obsoleta* trematode and those that are closely related both taxonomically and ecologically to known hosts. For a full account of the fish species included in this measure and discussion of an alternative (but less supported) way of grouping fish, see Appendix C.

We examined the distribution all independent variables to determine whether they met assumptions of normality using site-level averages when replicate measures were taken. For most non-normal variables, a log-transformation (either $\ln[x]$ or $\ln[x + 1]$ when zero values were included) resulted in a normal fit (Table 2). In the case of definitive fish host abundance and PC-Cu,Pb,Zn, log transformations did not achieve normality, however we still included them because they were close to normal and regression methods are robust to deviations from normality (Underwood 1997).

Multicollinearity among all pairs of sampled independent variables was assessed using the Pearson product-moment correlation (r). In the vast majority of cases, strong correlations were not found among pairs of independent variables (using threshold of $r = 0.70$);

TABLE 2. Extended.

Unit	Transformation
no. snails/m ²	none
no. fish/30-min soak time	natural log
no. caught in fyke net/outgoing tide (~6 h)	natural log
no. individuals·0.3 km ⁻² ·10-min count	natural log
na	none
µg/g sediment	none
µg/g sediment	natural log
percentage of sediment grains	natural log
km roads/3.14 km ²	natural log
km	natural log
km ²	natural log

however, a single exception was identified for the case of $\ln(\text{nitrogen})$ and $\ln(\text{bivalves})$. To ensure that models of trematode prevalence and diversity were not confounded by collinearity associated with independent variables, we considered which of the two variables provided the strongest potential to explain variation across trematode prevalence and diversity metrics. While bivalves are a documented secondary intermediate host for one trematode in this system, *Himasthla quissetensis*, this species was less commonly encountered in our work (Table 1). Moreover second intermediate hosts (unlike definitive hosts) are not direct links to trematodes in snails. On the other hand, nitrogen may be indicative of broader, site-level, food web dynamics given its strong influence on bottom-up processes. Based on these factors, we chose to remove $\ln(\text{bivalves})$ from further analysis while retaining $\ln(\text{nitrogen})$ as a potential explanatory variable. In total, 11 independent variables describing host abundance and chemical and physical attributes of sites were used in multiple regression analyses (Table 2).

For each trematode response metric, we identified all possible models and examined the Akaike information criterion corrected for small sample sizes (AIC_c); the metric provides a relative measure of model performance for a given set of data and is designed to tradeoff complexity (i.e., the number of model parameters included) and goodness of fit. We calculated the difference between AIC_c for a given model i and the minimum AIC_c value in the set (ΔAIC_c), where $i = 1, 2, \dots, R$. Models were then ranked using (relative) likelihood (l_i), a formal strength-of-evidence measure defined by the equation $l_i = \exp(-[1/2]\Delta AIC_c)$, where likelihood values closer to 1 indicate greater confidence (Burnham et al. 2011). We examined how much better the top model is compared to others in the set using an evidence ratio, this metric is calculated as the ratio between the likelihood of a given model and that of the overall best-performing model (Burnham et al. 2011). For each trematode response metric, we considered the top model (i.e., lowest AIC_c), as well as models with

evidence ratios that were less than three for interpretation. As an additional index of performance, we ranked models according to their Akaike weight (w_i), which is the model likelihood normalized by the sum of all model likelihoods within a set; values close to 1 indicate greater confidence in the selection of the best model. We used relative variable importance (RVI), to rank all explanatory variables associated with a set of models from low to high importance, where RVI is calculated as the sum of the Akaike weights (w_i) of all models that contain a given predictor (Pinsky et al. 2013). Finally, we identified the best single-variable model for each trematode response using the criterion of lowest AIC_c . All statistical analysis was performed using JMP v10 (SAS Institute, Cary, North Carolina, USA).

RESULTS

Descriptive patterns

Trematode abundance and diversity.—Trematode-infected snails were found at every site and all except one of the nine trematode species documented to infect *I. obsoleta* were observed. The unobserved species, *Pleurogonius malaclemys*, is not expected in our study area because northern New England is largely outside the range of its terrapin definitive host (Byers et al. 2011). The average infection prevalence across sites was $27.3\% \pm 32.0\%$ (mean \pm SD) and ranged from 0.7% to 92.8% (Table 1). Although the great majority of infections could be identified at the species level, a small handful associated with the *Stephanostomum* genus (there are two congeners found in this system) were observed at an early stage of development and could not be identified to species. In our analyses, we pool infections across *Stephanostomum* spp. in order to provide the least biased estimate of infection associated with this group (see Appendix A for additional details).

The trematode species most frequently observed across sites were also the ones that exhibited the highest individual species prevalence (Table 1). These were *Stephanostomum* spp. ($11.0\% \pm 17.3\%$), *Zoogonus rubellus* ($9.2\% \pm 11.7\%$), and *Lepocreadium setiferoides* ($3.6\% \pm 4.3\%$) each of which was encountered at more than 65% of sampling sites. In addition to dominating *I. obsoleta* infections, these trematodes are ones that reach maturity in definitive fish hosts (Table 1). Of the four trematode species that use birds as definitive hosts, *Himasthla quissetensis* exhibited the highest prevalence across sites ($2.3\% \pm 2.8\%$). The three remaining bird-using trematodes, *Gynaecotyla adunca*, *Austroilharzia variglandis*, and *Diplostomum nassa*, were observed rarely, each exhibiting an average prevalence across sites of less than 1%. Average trematode species richness was 3.3 ± 2.4 and ranged from 0 to 7 species. Although prevalence was always greater than zero (Appendix A: Table A1), because trematode species richness was based on a random subset of 95 snails to standardize sampling effort across sites, the calculated range of species richness includes zero.

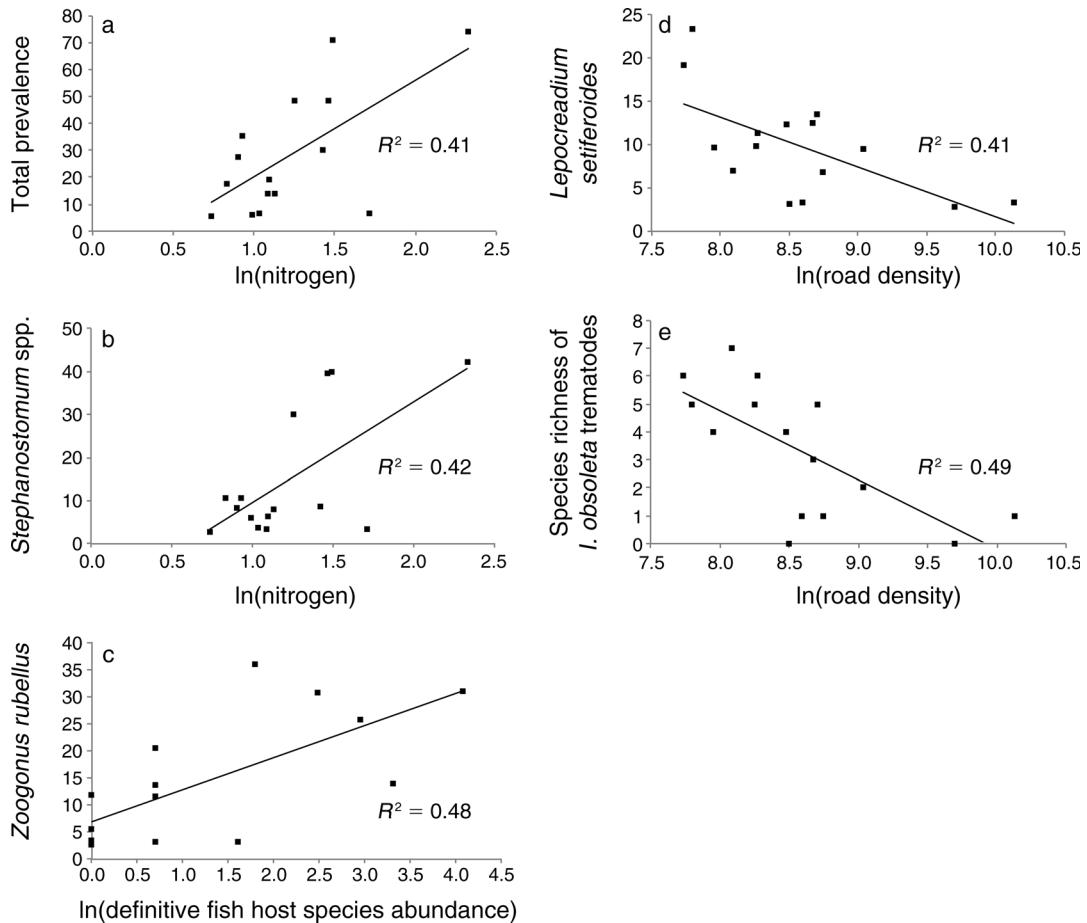


FIG. 2. *I. obsoleta* trematode prevalence and diversity compared to the single best predictor variable (selected from 11 candidate predictors presented in Table 2) to explain variability of trematode metrics across salt marsh sites ($n = 15$). Note that while raw prevalence values are plotted (a–d), all statistics are based on Anscombe transformed prevalence metrics (see *Materials and methods*). (a) Total prevalence is the percentage of *I. obsoleta* snails infected by any species of trematode ($F_{1,13} = 9.04$, $P = 0.01$). Nitrogen was measured as $\mu\text{g N/g}$ sediment. Three metrics of prevalence associated with specific trematode species are shown: (b) *Stephanostomum* spp. ($F_{1,13} = 9.32$, $P = 0.01$), (c) *Zoogonus rubellus* ($F_{1,13} = 12.10$, $P < 0.01$; fish abundance was measured as number of individuals per fyke net catch), and (d) *Lepocreadium setiferoides* ($F_{1,13} = 9.01$, $P = 0.01$; road density was measured as $\text{km road}/3.14 \text{ km}^2$). In addition, (e) species richness ($F_{1,13} = 12.35$, $P < 0.01$) is also presented.

Biological, chemical, and physical variables.—Table 2 provides summary statistics for biological, chemical and physical attributes measured across sites (for site-level summary see Appendix A: Table A2). Most of the measured independent attributes exhibited very high variability and this was especially true of biological hosts (see high CV of host attributes in Table 2). We compared concentrations of heavy metals at sites to the informal (i.e., non-regulatory) toxicity benchmark: effect range low (ERL; Long et al. 1998), a threshold above which effects in sediment-dwelling infauna are known to occur. Of the four metals included in trematode regression models, copper and zinc each exceeded ERL at just one site. Lead and arsenic exhibited concentrations greater than ERL at five and three sites, respectively. A full description of findings related to metals and ERL is presented in Appendix B: Table B2.

Principal component analysis.—Results of PCA performed on the three strongly collinear metals (copper, lead, zinc) showed that the first principal component explained 97.7% of the variance across sites (Appendix B: Table B1). Scores of all three metals also contributed strongly to the loading on the first principal component, indicating that patterns of variability are highly consistent.

Statistical models

Overall, single predictor models of *I. obsoleta* trematode prevalence and diversity exhibit very high explanatory capacity (Fig. 2). The adjusted R^2 of bivariate relationships associated with these models ranged from 0.41 for the model of total prevalence to 0.49 for the model of species richness (Fig. 2). The variable $\ln(\text{roads})$ was the strongest predictor of *L. setiferoides* and species richness, exhibiting a negative

TABLE 3. The set of competing models for each trematode response variable.

Response variable and model rank	Predictor variables													As	PC-Cu, Pb,Zn
	R^2_{adj}	AIC _c	ΔAIC_c	l_i	$1/l_i$	w_i	ln(N)	ln(WB)	ln(sand)	ln(DF)	ln(roads)	ln(OD)	ln(SSH)		
a) Total prevalence															
A	0.75	128.05	0.00	1.00	1.00	0.27	0.80	-0.52	-0.40						
B	0.81	128.32	0.27	0.87	1.14	0.23	0.46		-0.29	0.40	-0.38				
C	0.73	128.69	0.65	0.72	1.38	0.19	0.48			0.36	-0.49				
D	0.73	128.76	0.71	0.70	1.43	0.19	0.51				-0.49	0.35			
E	0.79	129.56	1.51	0.47	2.13	0.12	0.71	-0.44	-0.39			0.24			
RVI							1.00	0.39	0.62	0.42	0.61	0.31			
b) <i>Stephanostomum</i> spp. prevalence															
A	0.73	115.57	0.00	1.00	1.00	0.45	0.52		-0.40			0.42			
B	0.80	115.88	0.31	0.86	1.17	0.39	0.50		-0.34		-0.28	0.40			
C	0.69	117.66	2.08	0.35	2.83	0.16	0.52				-0.41	0.40			
RVI							1.00		0.84		0.55	1.00			
c) <i>Z. rubellus</i> prevalence															
A	0.57	112.27	0.00	1.00	1.00	0.19				0.59				-0.40	
B	0.56	112.64	0.37	0.83	1.20	0.16	0.72	-0.68							
C	0.65	112.78	0.50	0.78	1.29	0.15	0.60	-0.56				0.34			
D	0.55	113.02	0.75	0.69	1.46	0.13						0.56	-0.50		
E	0.53	113.50	1.22	0.54	1.84	0.11				0.66	-0.35				
F	0.44	113.59	1.31	0.52	1.93	0.10				0.69					
G	0.61	114.09	1.82	0.40	2.48	0.08				0.58	-0.26		-0.33		
H	0.61	114.14	1.86	0.39	2.54	0.08						0.57	-0.55	0.29	
RVI							0.31	0.31		0.48	0.18	0.36	0.48	0.08	
d) <i>L. setiferoides</i> prevalence															
A	0.60	94.20	0.00	1.00	1.00	0.27					-0.48			0.67	-0.60
B	0.68	95.18	0.98	0.61	1.63	0.17				-0.37	0.76	-0.49	-0.60		
C	0.36	95.22	1.02	0.60	1.66	0.17						-0.64			
D	0.57	95.33	1.13	0.57	1.76	0.16					0.68	-0.63	-0.54	0.85	-0.63
E	0.57	95.49	1.29	0.53	1.90	0.14			-0.43						
F	0.42	96.38	2.18	0.34	2.98	0.09				0.31	-0.61				
RVI										0.31	0.42	0.86	0.32	0.42	0.42
e) Species richness															
A	0.74	59.83	0.00	1.00	1.00	0.61			-0.33		-0.43		-0.52		
B	0.66	60.69	0.86	0.65	1.53	0.39					-0.56		-0.49		
RVI									0.61		1.00		1.00		

Notes: The best performing (i.e., lowest Akaike information criterion corrected for small sample sizes, AIC_c) model is shown in boldface text. For all models, columns indicate adjusted R²; corrected Akaike information criterion score (AIC_c); ΔAIC_c (i.e., the change in AIC_c compared to the lowest AIC_c observed in the set); relative likelihood (l_i , a formal strength of evidence measure for each model); evidence ratio ($1/l_i$, a metric to quantify the magnitude of improvement for the best model compared to the alternative presented); and Akaike weight (w_i). For predictor variables, standardized beta coefficient scores are presented. See Table 2 for explanation of predictor variable abbreviations. Along the bottommost row of each model set, the relative variable indicator (RVI) score is also presented for each independent variable.

relationship with both. The variable ln(nitrogen) was the best predictor of total prevalence (which combines all trematode species observed) and the prevalence of *Stephanostomum* spp., exhibiting a positive relationship with these trematode metrics. Finally, ln(abundance of definitive fish hosts) was the single best predictor of *Z. rubellus* prevalence exhibiting a positive relationship with the trematode. All variables associated with single predictor models were also included in the best-performing multivariate model.

Total prevalence.—A total of five competing models were found to explain total trematode prevalence (Table 3a). The adjusted R² across the model set ranged from 0.73 to 0.81. Across the models, there is strong evidence that ln(nitrogen) (positive predictor; RVI = 1), ln(sand) (negative predictor; RVI = 0.62), and ln(roads) (negative predictor; RVI = 0.61) are the strongest determinants of

infection. The best performing model for total prevalence (i.e., lowest AIC_c) also includes ln(wetland birds) as a negative predictor, however the associated RVI score indicates less overall support for this predictor of trematode infection (RVI = 0.39).

Stephanostomum spp. prevalence.—The best performing model of *Stephanostomum* spp. prevalence includes predictor variables that are strongly and consistently supported across the set of three competing models identified (Table 3b); these are ln(nitrogen) (positive predictor, RVI = 1.00), ln(distance from the ocean) (positive predictor; RVI = 1.00), and ln(sand) (negative predictor; RVI = 0.84). Adjusted R² across the model set ranged from 0.69 to 0.80.

Zoogonus rubellus.—Eight competing models were found for *Z. rubellus* prevalence. Adjusted R² across the model set ranged from 0.44 to 0.65. Two predictor

variables associated with the best performing model were also the ones most strongly supported across the set; these were $\ln(\text{definitive fish hosts})$ (positive predictor; RVI = 0.48) and $\ln(\text{soft sediment habitat})$ (negative predictor; RVI = 0.48; Table 3c).

Lepocreadium setiferoides.—Six competing models for prevalence of *L. setiferoides* were found (Table 3d). Adjusted R^2 across the model set ranged from 0.36 to 0.68. The best performing model includes variables that were well supported across the full set, these were $\ln(\text{roads})$ (negative predictor; RVI = 0.86), PC-Cu,Pb,Zn (negative predictor; RVI = 0.42) and arsenic (positive predictor; RVI = 0.42). Although not associated with the best performing model, $\ln(\text{definitive fish hosts})$ was also well supported as a predictor variable when considering the full set of competing models (positive predictor; RVI = 0.42).

Species richness.—Two competitive models were found for species richness (Table 3e). The best performing model exhibited an adjusted $R^2 = 0.74$ and included as negative predictors $\ln(\text{roads})$ (RVI = 1) and $\ln(\text{habitat})$ (RVI = 1). $\ln(\text{sand})$ was also included as a positive predictor (RVI = 0.61). The alternative model exhibited an adjusted R^2 of 0.66 and included only $\ln(\text{roads})$ and $\ln(\text{habitat})$ as negative predictors.

DISCUSSION

Our results reveal a strong capacity for physical and chemical variables, including many that are related to anthropogenic activities (e.g., roads, nitrogen, and heavy metals) to explain prevalence and diversity of trematode parasites across a large regional scale. Other field studies demonstrate strong effects of particular anthropogenic factors on parasites (Bustnes and Galaktionov 1999, Johnson et al. 2007, Shea et al. 2012), however the wide diversity of influential factors in this system is striking and suggests multiple, simultaneous pathways through which human activities may influence parasites.

Road density surrounding sites was a ubiquitous negative predictor for all trematode response metrics. It was the only independent variable included in the set of best performing models for every trematode response, and often showed relatively high capacity to explain parasite variability (Table 3). The frequency with which roads emerges across trematode models underscores that this human derived feature, which is known to have significant impacts on free-living communities (Hourdequin 2000), also plays an important role in structuring parasites. Roads may influence trematodes if they affect densities of definitive host populations (e.g., as proposed by Urban [2006]). The most abundant trematode species observed in our sampling mature in estuarine fish hosts (Table 1), which may be sensitive to roads that fragment marshes or are associated with culverts that block or limit their movement (Brown and Hartman 1988, Eberhardt et al. 2011). Although we found only very weak direct evidence that roads negatively affect fish hosts in this system (see correlations between roads and

fish in Appendix D: Table D1), we emphasize that higher resolution fish sampling, particularly for species that serve as definitive hosts and are especially difficult to quantify, may be necessary to detect such a relationship.

Road density may also influence environmental conditions at sites through runoff that contains pollutants like insecticides, herbicides, and hydrocarbons—contaminants not specifically examined in this study. In general, pollutants are likely to have negative effects on a broad range of parasites since chemicals can cause depressed immunological responses in hosts (Khan 1990, McDowell et al. 1999) as well as impact parasites directly (Koprivnikar et al. 2006). Abiotic conditions like salinity, light, and temperature, which may also be affected by runoff, are known to cause behavior changes in trematodes including reduced emergence from snails (Thieltges and Rick 2006, Studer and Poulin 2012) and lower transmission success to the next host (Pechenik and Fried 1995) when the parasites are exposed to levels outside a narrow optimal range. Whatever the mechanism, the negative association between this anthropogenic feature and trematodes demonstrates that parasites are yet another taxonomic group that can be adversely affected by roads (Trombulak and Frissell 2000) with important consequences to ecosystem level processes (van der Ree et al. 2011).

Another human-associated factor, nitrogen, was found to be a strong positive predictor for both total prevalence and the prevalence of *Stephanostomum* spp. infection. In estuaries, elevated nitrogen can lead to increased production and, in high concentrations, cause eutrophication. In parasite systems, nitrogen is shown (Johnson et al. 2007) or suggested (McKenzie 2007) to increase densities of larval snail hosts and lead to increased contact rates between hosts and parasites. Abundance of *I. obsoleta* in our study was not positively associated with nitrogen nor was snail host abundance included as a predictor in any of our top models. However, if nitrogen is tied to increased production or quality of benthic micro- and macroalgae that are food sources for snails, increased within-snail production of cercariae that transmit infection to the next host is possible (Johnson et al. 2007). In general, the findings contribute to a growing body of literature demonstrating how parasites benefit from elevated nutrients and eutrophication (Beer and German 1993, Valtonen et al. 1997, Johnson and Chase 2004).

Prominent anthropogenic variables included in *I. obsoleta* trematode models were often observed to influence the parasites in opposing directions. Road density was negatively associated with prevalence and diversity while parasitism increased with nitrogen. Different metals also influenced trematodes in opposite directions: copper, lead, and zinc (as indicated through the principal component variable PC-Cu,Pb,Zn) was a negative predictor of *L. setiferoides* prevalence, but arsenic exhibited a positive influence on the same species

(Table 3d). All four metals were observed above a toxicity threshold in at least one site, suggesting their concentration was elevated by anthropogenic activity (Appendix B: Table B2). The findings therefore suggest that metals may be acting as pollutants in the system and causing differential effects on parasites compared to hosts. Whereas the effects of copper, lead, and zinc are consistent with direct negative effect on free-living stages of parasites (Cross et al. 2001, Bennett et al. 2003), the positive influence associated with arsenic could indicate that host immune response is relatively more impacted than are parasites exposed to this analyte. Opposing directions of influence from anthropogenic factors emphasize that the direction in which disturbance effects parasite communities is not universal (Lafferty and Kuris 1999, Lafferty and Holt 2003).

Perhaps of greatest interest was that anthropogenic impacts often exerted stronger influence than measures of definitive host abundance. While measures of definitive fish hosts were important predictors for some trematode metrics, their abundance often exhibited a similar or lower capacity to explain parasites than non-host variables. From a life history standpoint, this may be surprising given that definitive hosts provide the direct biological link to infection in snails and are known to be strong determinants of larval trematode prevalence and diversity in other systems (Smith 2001, Hechinger and Lafferty 2005, Byers et al. 2008). The lower relative importance of definitive fish host metrics compared to non-host variables (which appear more frequently and with higher strength in trematode models; Table 3) in part reflects the fact that these fish hosts were quantified on a single occasion and in a different year than other samples for this study. This snap shot and temporally incongruent measure could limit our ability to detect strong and consistent relationships with trematodes; however, we hypothesize that some non-host variables may be included in our final models because of their influence on, or covariance with, fish abundance. Birds are also definitive hosts for some *I. obsoleta* parasites, yet infections by bird-using species were observed far less frequently than fish-using trematodes (Table 2). The inclusion of bird abundance as a negative predictor for two trematode response metrics (Table 3a and c) may, therefore, reflect their role as predators that drive down second intermediate hosts abundance at sites. Additional research is needed to better understand the negative influence of birds in this system.

Other environmental variables of natural origin like distance from the ocean and sediment composition (i.e., $\ln[\text{sand}]$) exhibited important associations with trematode abundance metrics (Table 3). The former may be an indicator of some definitive fish hosts in the system as suggested by the positive and somewhat strong cross correlation between the two variables (Appendix D: Table D1). Species that migrate between ocean and estuarine environments like striped bass, white perch, and American eel are documented

definitive hosts of *I. obsoleta* trematodes (Appendix C: Table C1) and may exhibit higher densities as they travel further away from ocean environments and become funneled into estuarine corridors. Taken together, distance from the ocean and sand could also reflect a set of linked processes that contribute to trematode success. For example, salt marsh sites located farther from the ocean are likely lower energy environments than those found nearer to the coast. The lower proportion of sand at sites (and associated higher proportion clay and silt) often indicates lower flow rates and less advection, factors that could aid successful transmission of free-swimming trematodes to the next host and/or increase the capture of infected prey by definitive hosts.

Anthropogenic factors that strongly predicted trematodes, (roads, nitrogen, and metal contaminants) also cause impacts to biological communities and ecosystem function; in fact, we suspect that some of their effects on trematodes operate through their influence on fish host abundance. By extension, our findings cautiously suggest that *I. obsoleta* trematodes themselves may provide information about impacted fish host populations in an applied context. One *I. obsoleta* trematode (whose geographical distribution is outside the range of this study) has already been demonstrated as an indicator of hard-to-measure terrapin hosts (Byers et al. 2011). Other studies suggest that trematode prevalence and diversity may be good predictors of definitive bird hosts and wetland condition (Huspeni and Lafferty 2004, Hechinger and Lafferty 2005, Shea et al. 2012). Ours is the first study to demonstrate links between fish-using trematodes and anthropogenic-associated factors.

Two facets of our work were likely important in capturing relationships between non-host variables and parasites. First, the group of parasites examined may be particularly sensitive to abiotic factors because during free-living stages they are highly susceptible to environmental conditions (Pietrock and Marcogliese 2003). Trematodes also rely on a variety of host taxa, which themselves may be dependent on a broad range of environmental factors. Second, the large spatial scale of our sampling likely helped uncover relationships between non-biological factors and parasites. This is because the range and variability of observed values for many factors (e.g., roads, nitrogen, metals) are likely to be much higher at a regional scale, rather than the local one often targeted in parasite studies. Even if the variables found here to be strong trematode determinants are operating through effects on host species, their identification as proxies is likely only possible at larger spatial scales over which sufficient variation occurs. Overall, our work emphasizes the importance of a variety of non-host factors whose strong influence in structuring parasite communities at large scales has previously been underappreciated.

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SUPPLEMENTAL MATERIAL

Appendix A

Additional details on sampling methodologies as well as site-level results of trematode infection and biological/chemical/physical measurements. A discussion regarding pooling infections of *Stephanostomum tenue*, *Stephanostomum dentatum*, and trematodes of the *Stephanostomum* genus that could not be identified to the species level is also included ([Ecological Archives E095-165-A1](#)).

Appendix B

Full description of methods to reduce metal data prior to analytical modeling of trematode prevalence and diversity and comparisons of metal concentrations at sites to the informal toxicity benchmark: Effect Range Low ([Ecological Archives E095-165-A2](#)).

Appendix C

Analysis and discussion of relationships between prevalence of *Ilyanassa obsoleta* trematodes that mature in fish hosts and abundance of definitive fish hosts measured from salt marsh sites ([Ecological Archives E095-165-A3](#)).

Appendix D

Cross correlations among biological, chemical, and physical variables that are potential determinants of trematode prevalence and abundance ([Ecological Archives E095-165-A4](#)).